In silico screening of potent bioactive compounds from honey bee products against *COVID-19* target enzymes

Moataz A. Shaldam[1], Galal Yahya[2,3,] Nashwa H. Mohamed[4], Mohamed M. Abdel-Daim[5,6] , Yahya Al Naggar[5,6]*

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kafr EL Sheikh University, Kafr El Sheikh, 33516, Egypt.

[2]Microbiology and Immunology Department, Faculty of Pharmacy, Zagazig University 44519, Al Sharqia, Egypt.

[3]Department of Molecular Genetics, Faculty of Biology, Technical University of Kaiserslautern, Paul-Ehrlich Str. 24, Kaiserslautern 67663, Germany.

[4]Hospitals of Zagazig University, Al Sharqia, Egypt.

[5]Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

[6]Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt

[7]Zoology Department, Faculty of Science, Tanta University31527, Tanta, Egypt.

[8]General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher weg 8, 06120 Halle (Saale), Germany.

***Correspondence:**

Dr Y. Al Naggar, General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany

E-mail: yehia.elnagar@science.tanta.edu.eg

Telephone: +49-345 5526511

FAX: +49-345 5527428

Abstract

From the early days of the COVID-19 pandemic, side by side to immense investigates to design specific drugs or to develop a potential vaccine for the novel coronavirus. Myriads of FDA approved drugs are massively repurposed for COVID-19 treatment based on molecular docking of selected protein targets that play vital for the replication cycle of the virus. Honey bee products are well known of their nutritional values and medicinal effects. Antimicrobial activity of bee products and natural honey have been documented in several clinical studies and was considered a good alternative for antiviral medications to treat some viral infections. Bee products contain bioactive compounds in the form of a collection of phenolic acids, flavonoids and terpenes of natural origin. We revealed by molecular docking the profound binding affinity of 14 selected phenolics and terpenes present in honey and propolis (bees glue) against the main protease (M^{pro}) and RNA dependent RNA polymerase (RdRp) enzymes of the novel 2019-nCoV coronavirus. Of these compounds, *p*-coumaric acid, ellagic acid, kaemferol and quercetin has the strongest interaction with the 2019-nCoV target enzymes, and they may be considered as an effective 2019-nCoV inhibitors.

Key words: COVID-19, Honey Bee products, Phenolic compounds, Molecular docking, Drug repurposing, Natural products.

Introduction

Owing to increased global travel and rapid urbanization, viruses are responsible for a variety of human pathologies. In December 2019, several cases of pneumonia of unknown cause were detected in Wuhan, province of Hubei in China. Most patients shared similar symptoms of dry cough, fever, and fatigue, then they developed into dyspnea quickly, ending up with acute respiratory distress syndrome (ARDS) (Chen et al. 2020, Chan et al. 2020, Zhu et al. 2020, Huang et al. 2020, Zhou et al. 2020). By whole-genome sequencing of samples obtained from lower respiratory tract of the patients, a new coronavirus was detected, further investigations revealed that the novel corona virus is different from severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) coronavirus (Zhu et al. 2020). On February 11, 2020, World Health Organization (WHO) named it coronavirus disease-19 (COVID-19) officially, then declared it a pandemic on March 12, 2020 (World Health Organization 2020).

As of June 06, 2020, the cumulative number of cases diagnosed with COVID-19 in the world was more than 7 million, while the cumulative number of cured cases was more than 3,4 million whereas more than 400,000 cases died (2020b). As a direct effect of the outbreak of COVID-19 , more than 160 countries are fighting to combat the spread of COVID-19 and taking protective measures to save their citizens from the pandemic, at the same time research institutes, drug corporations, biotechnology institutes, research groups in different universities all over the world are racing to develop effective drugs or potential vaccines for COVI-19. Internationally by June 2020, there are over 159 vaccine candidates (Sharpe et al. 2020, Thanh Le et al. 2020) and more than 300 potential therapies for COVID-19 disease in various stages of preclinical or clinical research (Pooladanda et al. 2020, Hachfi and Ben Lasfar 2020, 2020a, Mullard 2020).

As a fast track to save time needed for safety and approval studies, researchers started to massively repurpose already FDA approved drugs for Covid19 treatment (Kandeel and Al-Nazawi 2020, Harrison 2020). Computational based techniques like molecular modeling and virtual screening represent magic tools to understand the molecular aspects of protein ligand interactions during rational drug design process (Murgueitio et al. 2012). Virtual screening has been encountered in structure-based drug design against emerging and fatal diseases of viral origin. (Sirois et al. 2004, Elhefnawi et al. 2012, Raj and Varadwaj 2016, Zhou et al. 2008, Plewczynski et al. 2007).

Based on their crucial role in the life cycle of SARS CoV2 , COVID -19 RNA-dependent RNA polymerase (RdRp) (Gao et al. 2020) and the main protease (Mpro) (Jin et al. 2020) have been extensively docked to design or distinguish effective drugs for COVID-19. Bioactive compounds from natural origin are currently screened by molecular docking to *in silico* test their affinity to molecular targets of COVID-19 taking the advantage that natural product are free from toxic or side effects (Mani et al. 2020, Sayed et al. 2020, Gurung et al. 2020). Of the natural products that recently acquired increasing prophylactic importance to combat viral infections in general and COVID-19 in particular are honey bee products.

Honey bees are the "Golden insects" that produce honey and other vital honeybee products. Their products have a long history in medicine. All cultures have traditions of folk medicine which include the use of honey bee products, i.e. honey, bee pollen, propolis, royal jelly, beeswax, and bee venom. It was found that these products display anti-inflammatory, anti-bacterial , anti-fungal, anti-viral, antioxidant activities and neuroprotection (Pasupuleti et al. 2017, El-Seedi et al. 2020). Recently, honey has been proposed as a potential compatible antiseptic prophylaxis to help protect against the COVID-19 based on biocidal effect of

hydrogen peroxide that produced in most traditional honeys (Al Naggar et al. 2020, in press). However, the potential bioactive compounds derived from honey and other bee products are not identified yet and deserve more attention.

Therefore, the aim of the present study is to perform deep virtual screening via molecular docking to test binding affinity of various selected bioactive compounds such as terpenes and flavonoids of honey and propolis as inhibitors against COVID-19 essential enzymes: RNA-dependent RNA polymerase and the main protease.

Docking methodology

The crystal structure of COVID -19 RNA-dependent RNA polymerase (RdRp) (PDB code: 6M71) (Gao et al. 2020) and the main protease (M^{pro}) (PDB code: 6LU7) (Jin et al. 2020) were retrieved from Protein Data Bank. This study was carried out on 14 compounds (Fig. 1) from honey and propolis into the receptor active site using AutoDock Vina (Trott and Olson 2010). Ligand structures were drawn into Marvin Sketch V19.12 (2020c) and the most energetically favored conformer was exported as (*.pdb) file format. AutoDockTools package (Morris et al. 2009) was used to assign Gasteiger atomic partial charges and all the rotatable bonds in ligands were set to be flexible. For receptor preparation, all water molecules were removed, the co-crystalized ligand was removed, Gasteiger atomic partial charges were assigned and all receptors and ligands were converted to the PDBQT format using AutoDockTools package for docking process. In the AutoDock Vina configuration files, the parameter num modes was set to 10 and exhaustiveness to 14. The grid boxes of center (x= 118.23, y= 103.32 and z= 118.37) with size (x=17, y=25, z=17) for the RNA-dependent RNA polymerase and center (x= -10.71, y= 12.41 and z= 68.83) with size (x=16, y=18, z=16) for the main protease were used to define the active site. AutoDock Vina was executed. Pymol (2020d) was used for

3D visualization and the 2D schematic presentation was generated using LigPlot+ V1.4.5 (Laskowski and Swindells 2011).

Results and discussion

Honey bee products contain minor amounts of flavonoids, phenols, phenolic acids, carotenoids and terpenes **(Fig. 1).** These phenolic compounds and terpenes found to possess variable medicinal effects including wound healing, antioxidant, antimicrobial, antiviral, anti-inflammatory, cardioprotective, and neuroprotective activities (Biesalski et al. 2009; Küçük et al. 2007; El-Seedi et al. 2020). Recently many drugs that are designed and clinically implicated for other medicinal aspects have been repurposed for COVID-19 treatment (Kandeel and Al-Nazawi 2020, Oliveira et al. 2020). Therefore, honey bee products represent a natural pharmacy that harbor collection of remedies of broad medicinal effects and might be repurposed against COVID-19.

Bioinformatics is one of the most important and innovative approaches to design new drugs (Li *et al.*, 2020). Due to the high cost of clinical and laboratory trials, the time consuming and the possibility of error, different bioinformatics techniques are nowadays used in the design of new drugs (Shaghaghi, 2020). In the current study, computational docking was implemented to predict the binding mode of 14 compounds from honey and propolis with two different targets from COVID-19; RNA-dependent RNA polymerase (RdRp) (PDB code: 6M71) and the main protease (M^{pro}) (PDB code: 6LU7). We revealed that the bioactive compounds; ellagic acid, hesperetin, and kaempferol are the most promising compounds on COVID-19 RdRp while artepillin C, ellagic acid, hesperetin, kaempferol and quercetin were the most active on the main protease (Mpro). The binding scores for each compound into the two targets are shown in **Table 1.** The binding mode for ellagic acid to COVID-19 RdRb site was attributed to H-bond

interaction with Gly808, pro809, His816, Thr817 and Tyr 831, while amino acid residues Trp617, Asp760 and Asp761 are positioned at distance of H-bond with hesperetin, and also kaempferol interacts with Glu811 and Asp761 by H-bond. Furthermore, the aromatic ring system of ellagic acid, hesperetin and kaempferol make π-ion hydrophobic interaction with Lys798 **(Fig. 2).** We spot the light on the high affinity bioactive compounds like phenolic and flavonoids of honey as potent inhibitors of viral replication.

From the docking of all identified compounds into the active site of SARS-CoV-2 main protease (M^{pro}) in the current study, artepillin C showed H-bond interaction with Cys145, Arg188, Thr190 and Gln192, while amino acid residues His41, Gly143 and Arg188 are positioned at distance of H-bond with ellagic acid **(Fig. 3).** In addition, hesperetin interacts with Gly143 by H-bond while amino acid residues Tyr54, Leu141, Ser144, Asp 187 and Gln189 are positioned at distance of H-bond with kaempferol, and also quercetin makes H-bond with Tyr54, Leu141, Ser144, His163, and Gln189. Furthermore, the aromatic ring system of artepillin C, ellagic acid, hesperetin, kaempferol and quercetin make π-ion hydrophobic interaction with either Met165 or Glu166 **(Fig. 4).** Taken together we propose phenolic acids and flavonoids from honey bee products as potential inhibitor of the main protease of SARS-CoV-2 (COVID-19).

In the same context, our promising candidates like *p*-coumaric acid, ellagic acid, kaemferol and quercetin were previously found to have potential antiviral activity against the common cold human rhinovirus which is RNA virus like SARS-CoV2. Surprisingly the mentioned bioactive compounds were suggested in the same study to block or reduce the viral entry into the cells to protect the cells from the virus cytopathic effects and subside virus replication (Kwon et al. 2019), supporting our virtual screening. Moreover, quercetin and its derivatives were previously confirmed to inhibit the SARS-CoV proteases (Nguyen et al. 2012).

and other coronaviruses including SARS-CoV proteases (3CLpro and PLpro) as well as the Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) 3CLpro protease. Quercetin was also able to inhibit both enzymes *in vitro* in micromolar doses (Park et al. 2017).

Conclusions

Theoretical studies through molecular docking of collection of bioactive compounds of honey bee products against selected targets of COVID-19 including Mpro and RdRb enzymes of the 2019-nCoV virus have distinguished promising bioactive compounds of natural origin that exhibited profound binding to the respective COVID- 19 targets. Among the investigated bioactive compounds derived from honey and propolis, *p*-coumaric acid, ellagic acid, kaemferol and quercetin are the most promising compounds on 2019-nCoV active sites (RdRb and Mpro) These potent bioactive compounds were also found to have potential antiviral activity against the common cold human rhinovirus which is RNA virus like SARS-CoV2. Taken all together and based on our theoretical studies supported by previous *in vitro* confirmatory studies, we recommend further *in vivo* investigations to assess the predicted affinity of the selected compounds against the novel coronavirus (COVID-19) target enzymes.

Acknowledgments

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Figure legends

Figure 1. Chemical structure of important bioactive compounds in honey, propolis, and royal jelly.

Figure 2. The docking complex of (a) **Ellagic acid**, (b) **Hesperetin** and (c) **Kaempferol** (green) with the X-ray structure of **6M71;** SARS-CoV-2 RNA-dependent RNA polymerase (left, Tint) that showed hydrogen bond (blue) interaction and 2D schematic diagram of the interaction (right).

Figure 3. The docking complex of (a) **Artepillin C** and (b) **Ellagic acid** (green) with the X-ray structure of **6LU7;** SARS-CoV-2 main protease (M^{pro}) (left, Tint) that showed hydrogen bond (blue) interaction and 2D schematic diagram of the interaction (right).

Figure 4. The docking complex of (a) **Hesperetin** (b) **Kaempferol** and (c) **Quercetin** (green) with the X-ray structure of **6LU7;** SARS-CoV-2 main protease (M^{pro}) (left, Tint) that showed hydrogen bond (blue) interaction and 2D schematic diagram of the interaction (right).

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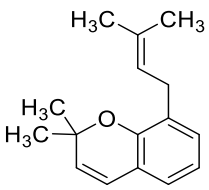
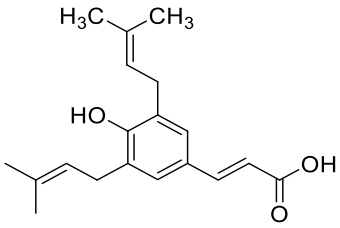
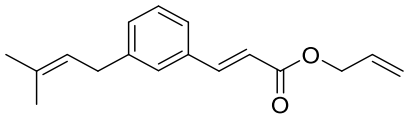
340 **Fig.1**



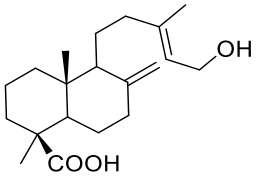
2,2-Dimethyl-8-prenylchromene



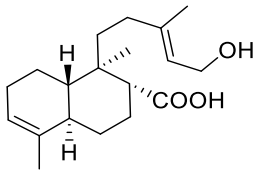
4-Hydroxy-3,5-diprenyl cinnamic acid (Artepillin C)



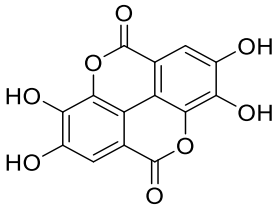
3-Prenyl cinnamic acid allyl ester



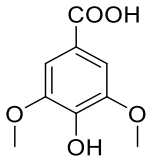
Isocupressic acid



13C-symphyoreticulic acid



Ellagic acid



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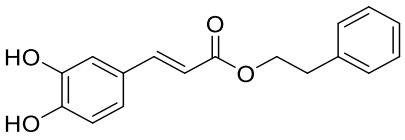
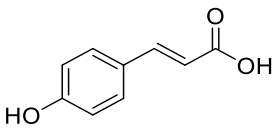
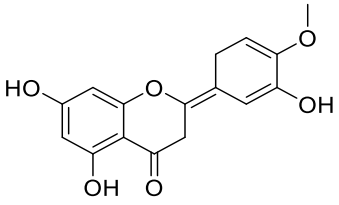


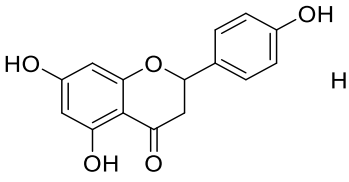
Caffeic acid phenethyl ester



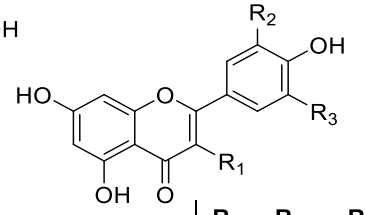
p-Coumaric acid



Hesperetin



Naringenin



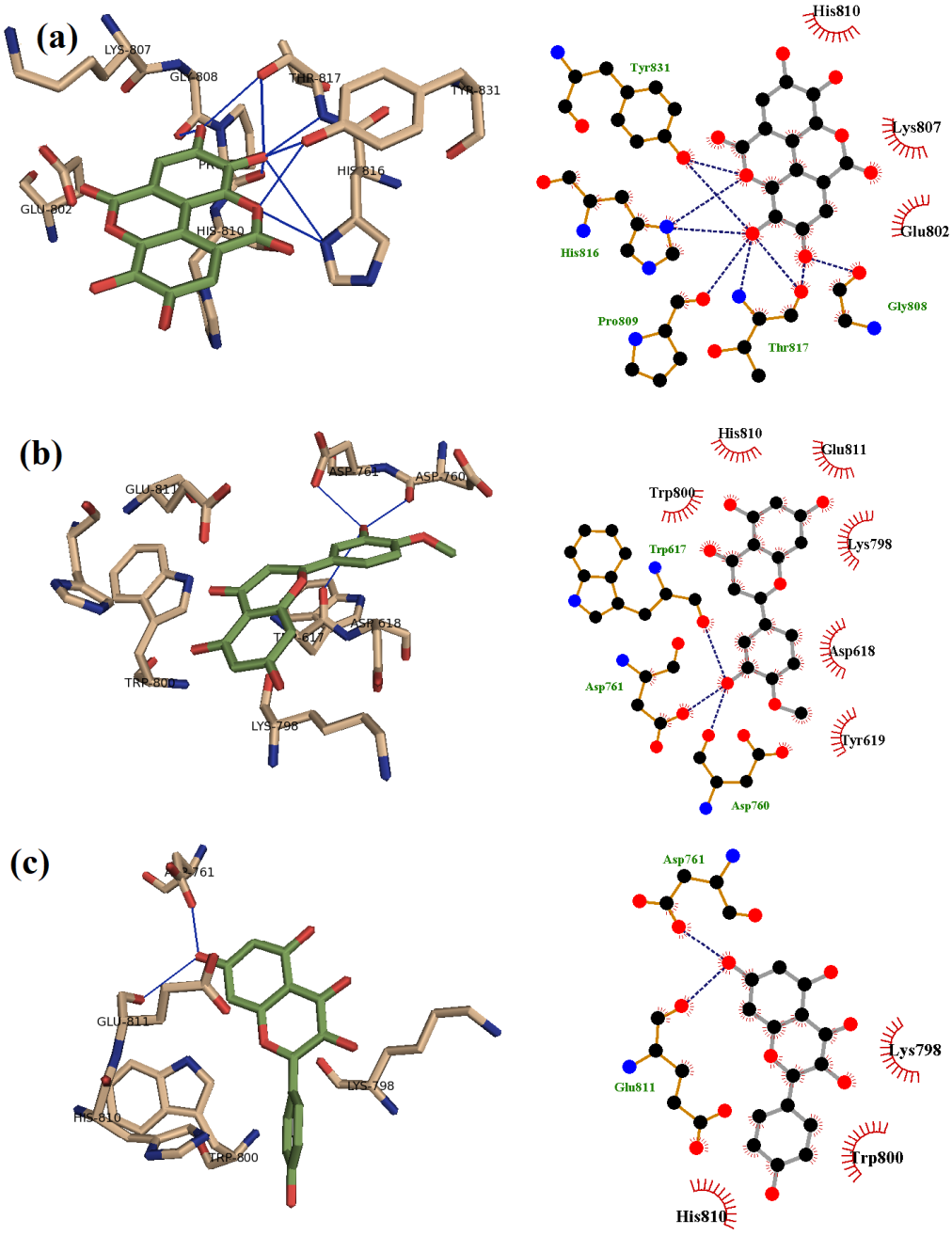
	R_1	R_2	R_3
Kaempferol	OH	H	H
Quercetin	OH	H	OH
Chrysin	H	OCH_3	OCH_3

341

342

343

Fig.2


(a)
LYS-807
GLU-808
THR-817
TYR-831
HIS-816
GLU-802
HIS-810
His810
Tyr831
Lys807
Glu802
His816
Gly808
Pro809
Thr817
(b)
ASP-761
ASP-760
GLU-811
ASP-618
TRP-800
LYS-798
His810
Glu811
Trp800
Trp617
Lys798
Asp618
Asp761
Tyr619
Asp760
(c)
GLU-811
LYS-798
HIS-810
TRP-800
Asp761
Lys798
Glu811
Trp800
His810

374 **Fig.3**

375

376

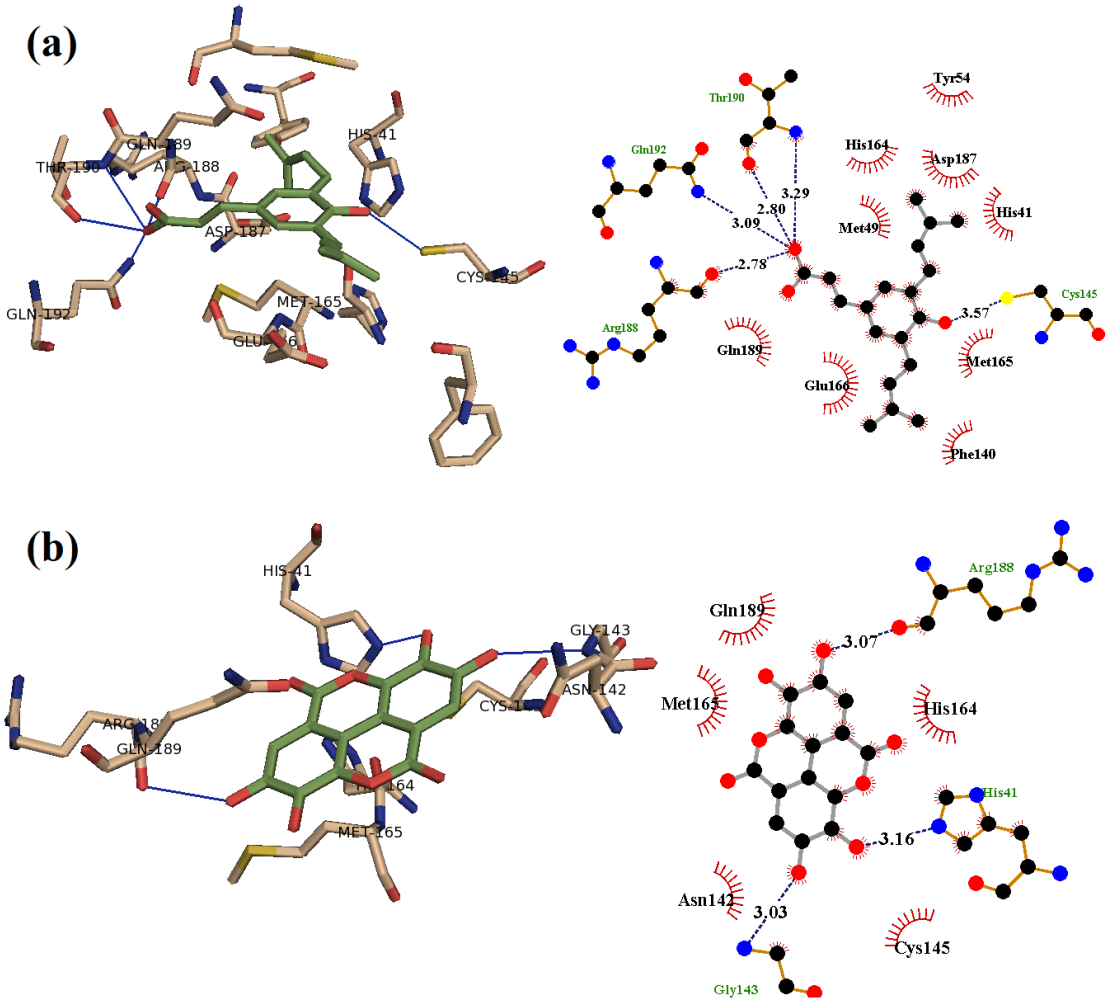
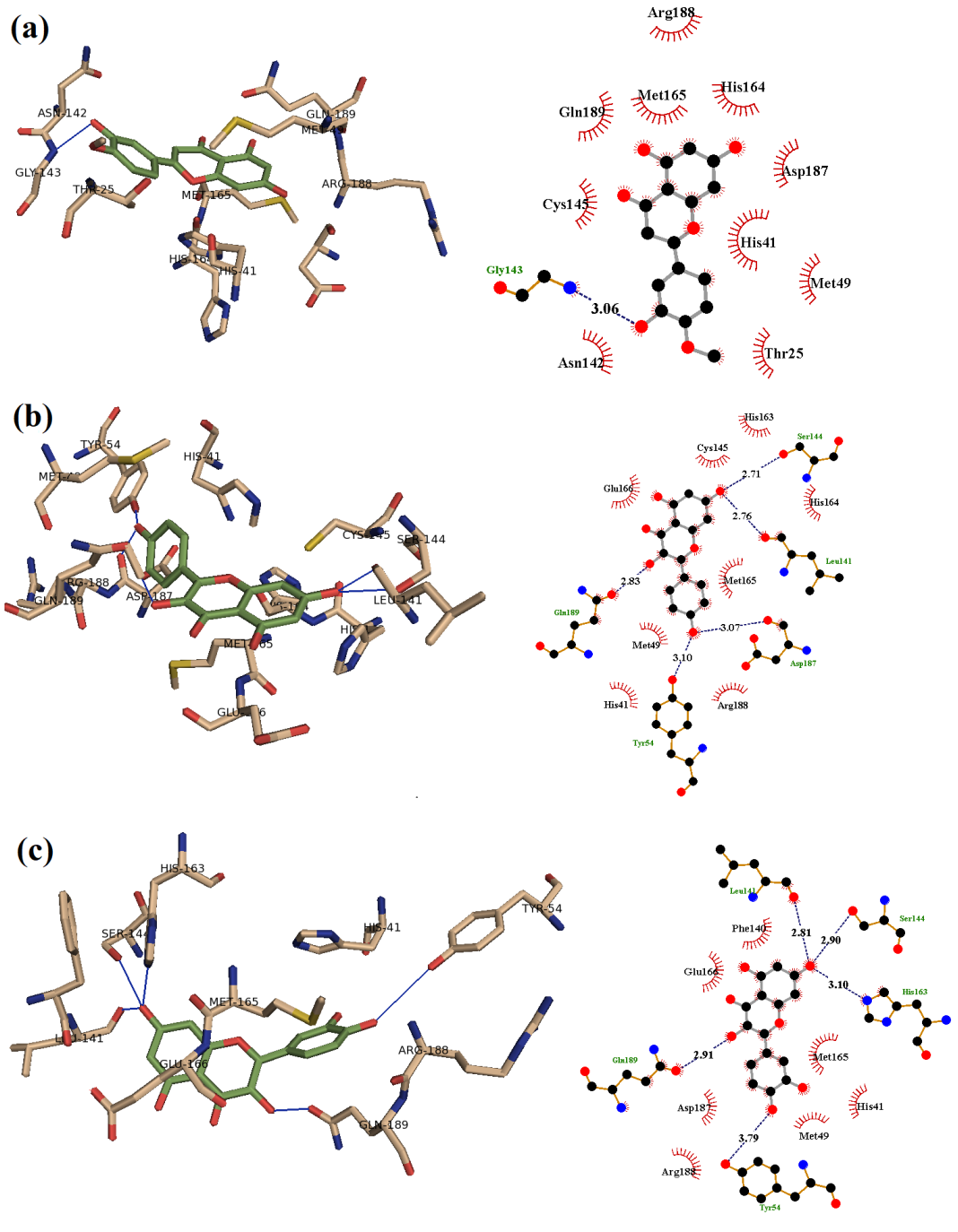


Fig.4


(a)
ASN-142
GLY-143
THR-25
MET-165
ARG-188
HIS-164
HIS-41
GLN-189
Arg188
Met165
His164
Gln189
Asp187
Cys145
His41
Gly143
Met49
3.06
Asn142
Thr25
(b)
TYR-54
HIS-41
MET-49
CYS-145
SER-144
ARG-188
GLN-189
ASP-187
LEU-141
MET-165
His163
Ser144
Cys145
2.71
Glu166
His164
2.76
Leu141
2.83
Met165
Gln189
Met49
3.07
3.10
Asp187
His41
Arg188
Tyr54
(c)
HIS-163
SER-144
HIS-41
TYR-54
MET-165
LEU-141
GLU-166
ARG-188
GLN-189
Leu141
Phe140
2.81
2.90
Ser144
Glu166
3.10
His163
Gln189
2.91
Met165
Asp187
His41
3.79
Met49
Arg188
Tyr54

407 **Table 1.** The binding scores for each compound into the two target enzymes of SARS-CoV-

408 2 RNA-dependent RNA polymerase (RdRp) and the main protease (M^{pro})

409

Bioactive compounds	SARS-CoV-2 RNA-dependent RNA polymerase	SARS-CoV-2 main protease (M^{pro})
2,2-Dimethyl-8-prenylchromene	**-5.6**	**-6.8**
Artepillin C	**-5.9**	**-7.5**
3-Prenyl cinnamic acid allyl ester	**-5.3**	**-6.2**
Isocupressic acid	**-5.8**	**-6.4**
13C-symphyoreticulic acid	**-5.7**	**-6.9**
Ellagic acid	**-6.4**	**-7.5**
Syringic acid	**-5.5**	**-5.6**
Caffeic acid phenethyl ester	**-5.4**	**-7.0**
***p*-Coumaric acid**	**-5.3**	**-5.6**
Hesperetin	**-6.3**	**-7.4**
Naringenin	**-6.0**	**-6.5**
Kaempferol	**-6.2**	**-7.8**
Quercetin	**-6.1**	**-7.4**
Chrysin	**-6.1**	**-7.2**