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In Silico Molecular Docking Studies of Medicinal Arabic Plant-Based Bioactive Compounds as a Promising Drug Candidate against COVID-19

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Abstract:- COVID-19, a new strain of coronavirus (CoV), has affected more than 200 countries and received worldwide attention. Till now, there is no specific therapies or vaccines are available, and investigations concerning COVID-19 treatment are lacking. The aim behind our study is to evaluate the efficacy of several medicinal plant-based bioactive compounds against COVID-19 main protease, and these selected plants are frequently used in the Arabic area for treating viral infections, that might directly inhibit 2019 novel coronavirus. The method is based on a docking analysis that used to test whether the compound had the potential for direct COVID-19 protein interaction. Molecular docking investigations were achieved using Autodock 4.2 to analyze the inhibition probability of these compounds against COVID-19. COVID-19 Mpro was docked with 36 compounds, and the binding energies were obtained from the docking of (PDB ID: 6LU7: Resolution 2.16 Å) with the native ligand (N3). According to obtained results, Betulinic acid (-10.0 Kcal/mol), Silibinin (-9.13 Kcal/mol), Oleanolic acid (-9.08 Kcal/mol), epigallocatechin-3-gallate (-8.51 Kcal/mol), , showed higher binding affinity to 6LU7 than N3 (-8.42 Kcal/mol), chloroquine (-7.12 Kcal/mol), and hydroxychloroquine (-7.35 Kcal/mol).This study concluded that Arabic herbal, which classically used for treating viral infection, might contain compounds with potential therapy against COVID-19.

I. INTRODUCTION

Viral diseases are still a serious threat to public health and remain a significant problem all over the world. Coronavirus is one of the major pathogens which primarily targets the human respiratory system. In the last years, we continue to fight both previous outbreaks of coronaviruses (CoVs), include the severe acute respiratory syndrome (SARS)-CoV and the Middle East respiratory syndrome (MERS)-CoV, which have been previously characterized as agents that are a great public health threat(Bloom and Cadarette, 2019). Now a day, the novel coronavirus disease 2019 (COVID-19), is in the midst of worldwide panic and global health concern since December 2019(Vellingiri et al., 2020). Early reports on the COVID-19 outbreak situations that available on the WHO website by the first of May 2020: confirmed cases rise to (3 525 116) and (243 540)deaths over the world(WHO, 2020). Due to the gravity of the situation, critical and complementary efforts from researchers are necessary to find therapeutic agents and new preventive methods.

Coronaviruses comprise a genome composed of a long RNA strand, one of the largest of all RNA viruses. This genome acts as messenger RNA when it infects a cell, that will direct the synthesis of two long polyproteins which include the machinery that the virus needs to replicate new viruses(Fehr and Perlman, 2015). These proteins include a replication/transcription complex that makes more RNA, several structural proteins that construct new virions, and two proteases. The proteases play essential roles in cutting the polyproteins into all of these functional pieces. It is a dimer of two identical subunits that together form two active sites. The protein fold is similar to serine proteases like trypsin, but a cysteine amino acid and a nearby histidine perform the protein-cutting reaction, and an extra domain stabilizes the dimer. This structure has a peptide-like inhibitor bound in the active site. Bioinformatics is one

of the most important and innovative approaches in the design and manufacture of new drugs. Due to the high cost of clinical and laboratory trials, the time consuming, and the possibility of error, various bioinformatics techniques are nowadays used in the design of new drugs. Molecular docking, simulation, target point determination, and chemical stability studies are the most important bioinformatics methods used in drug design. In the meantime, molecular docking of a special place in the process of designing new drugs, examining and comparing their efficacy. One of the novel therapeutic strategies for virus infection apart from the design and chemical synthesis of protease inhibitors is the search for inhibitors of this enzyme among natural compounds in order to obtain drugs with minimal side effects(Neda, 2020, Narges and Neda, 2020).

In the past few decades, natural compounds have been an important source of potential drug hits and leads(Petrovska, 2012, Ivanova et al., 2018). Regarding a World Health Organization (WHO) report, between 65% to 80 % of the population in developing countries extremely depend on traditional plants for health requirements(Robinson and Zhang, 2011). According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants(Mukhtar et al., 2008). Many drugs correlated to diseases such as malaria, human immunodeficiency virus (HIV), tuberculosis, cancer, cardiac diseases, diabetes, inflammatory diseases, etc. have been derived from natural compounds(Choudhary et al., 2018). In this work, we have screened the virtual library of phytochemicals that reported in selected medicinal plants that grow in Arabic area against protein targets of COVID-19 virus which is COVID-19 mean protease (3CL-protease structure) and compare them to proposed drugs such as chloroquine and hydroxychloroquine; this will provide other researchers with important investigation way to identify new COVID-19 treatment.

II. MATERIALS AND METHODS

A. Computational Materials

Avogadro version 1.2, with force field type MMFF94 was used to optimize the chosen compounds. Autodock 4.2 was used to prepare the proposed compound and further prediction the binding mode inside the active site and to estimate their binding affinity. Discovery studio 4.0 was used for the final visualization of the docked structure.

B. Computational Methods

- *Preparation of the Proposed compounds*

Based on a literature survey, thirty-six phytochemical compounds that reported in selected medicinal plants that were growing in the Arabic area and reported to have antiviral properties in addition to two FDA-approved antimalarial drugs were selected for the study. The 3-dimensional (3D) structures of the chosen compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). AutoDock Tools Version 1.4.5 was used to merge non-polar hydrogen, assigning the torsions and Gasteiger charges to each compound. Finally, these compounds were saved in PDBQT format.

- *Preparation of the 3CL-protease structure*

The crystal structure of COVID-19 mean protease (3CL-protease structure) was downloaded from Protein Data Bank ((https://www.rcsb.org/), PDB code: 6LU7) with a resolution of 2.16 Å, which corresponds to COVID-19 main protease in complex with the inhibitor N3. Then it was prepared for further processing using the AutoDock Tools Version 1.4.5 where polar hydrogens and Kollman charges were added. Finally, the 3D structure was saved as PDBQT.

- *Molecular Docking and Scoring*

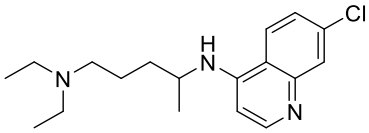
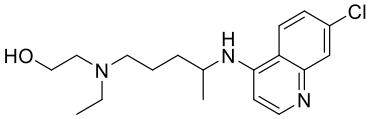
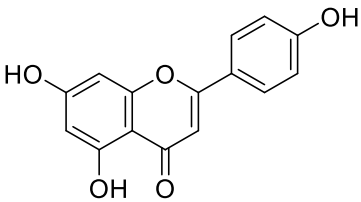
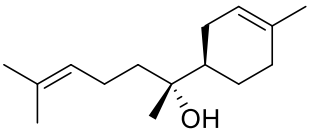
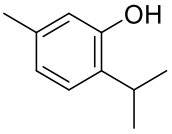
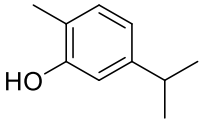
The grid map was constructed as the following: 60 x 60 x 60 points with a spacing of 0.375 Å and within the coordinates of the position are X: 10.995, Y: 12.899, and Z: 68.39. Molecular docking was achieved using Lamarckian Genetic Algorithm (LGA) within autodock 4.2. using the default parameters except for the number of docking runs, which was increased up to 100 runs for each compound.

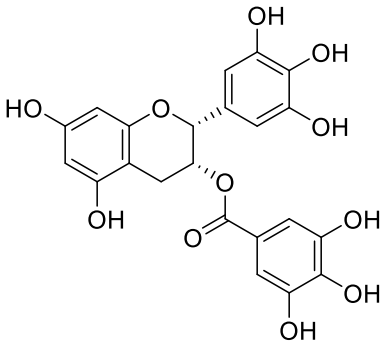
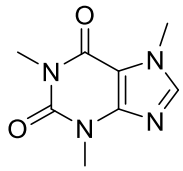
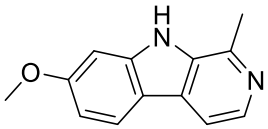
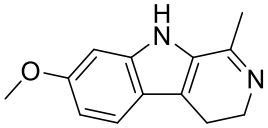
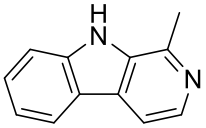
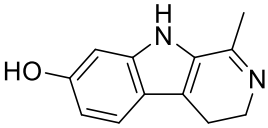
III. RESULTS AND DISCUSSION

Twenty medicinal plants and its main active compounds that reported to have antiviral activities, as seen in the table (1), were selected to study their theoretical probability for binding into 3CL protease active site and further serve as inhibitors for covid-19 virus replication. Additionally, two FDA-approved old antimalarial drugs, Chloroquine, and Hydroxychloroquine recommended for treating COVID–19 patients (Gautret et al., 2020) were used as positive docking controls.

No	Plant	Common name	Family	Active compounds	Rf
1	Matricaria chamomilla	Chamomile	Asteraceae	Apigenin and bisabolol	(Das et al., 2019)
2	Thymus vulgaris L	Thyme	Lamiaceae	Thymol and Carvacrol	(Behravan et al., 2011) (Pilau et al., 2011)
3	Camellia sinensis	Green tea	Theaceae	epigallocatechin-3-gallate and caffeine	(Mahmood et al., 2016)
4	Peganum harmala L.	Harmal	Zygophyllaceae	harmine, harmaline, harman, harmalol vasicine and vasicinone	(Farhad et al., 2017)
5	Glycyrrhiza glabra	Irik Sus	Leguminosae	glycyrrhizin	(Fiore et al., 2008) (Anagha et al., 2014)
6	Silybum marianum	Milk thistle	Asteraceae	silibinin	(Liu et al., 2019)
8	Atropa belladona (L.),	Belladonna	Solanaceae	Atropine	(Özçelik et al., 2011)
9	Allium sativum	Garlic	Amaryllidaceae	diallyl thiosulfinate (allicin) and ajoene	(Weber et al., 1992)
10	Allium cepa	Onion	Amaryllidaceae	Allicin and quercetin	(Chen et al., 2011)
11	Ziziphus spina-christi	Christ thorn	Rhamnaceae	Betulinic acid	(Hong et al., 2015a)
12	Olea europaea	Olives	Oleaceae	oleuropein	(Omar, 2010)
13	Punica granatum	Pomegranate	punicaceae	Punicalagin	(Houston et al., 2017)
14	Pimpinella anisum	anise oil	Apiaceae	trans-Anethole and eugenol	(Choi, 2018)
15	Salvia officinali	sage oil	Lamiaceae	Linalayl acetate, Linalool, 1,8-Cineole, and Camphor	(Choi, 2018) (Khalil and Li, 2010)
16	Hedera helix	ivy	Araliaceae	Hederasaponin B	(Al-Snafi, 2019)
17	Artemisia annua L	Artemisia	Asteraceae	Artemisinin and artesunate	(Li et al., 2018)
18	Melissa officinalis	Lemon balm	Lamiaceae	Rosmarinic acid	(Tóth et al., 2003)
19	Rosmarinus officinalis	Rosemary	Lamiaceae	Oleanolic Acid	(Khwaza et al., 2018)
20	Zingiber officinale	Ginger	Zingiberaceae	6-gingerol, 6-shogaol, and 6-paradol	(Chang et al., 2013)

Table 1:- selected plants and its main antiviral compounds

		
Chloroquine	Hydroxychloroquine	Apigenin
		
bisabolol	Thymol	Carvacrol

		
epigallocatechin-3-gallate	caffeine	harmine
		
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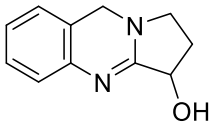
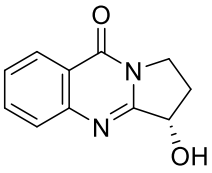
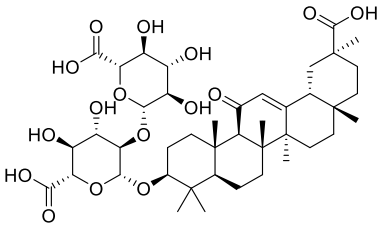
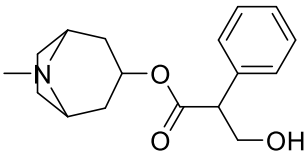
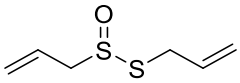
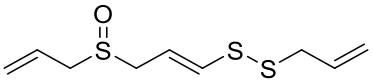
		
vasicine	vasicinone	glycyrrhizin
		
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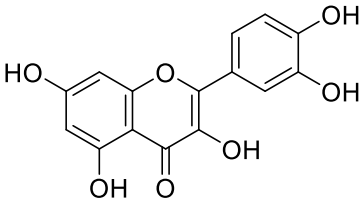
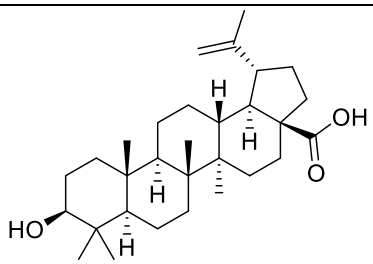
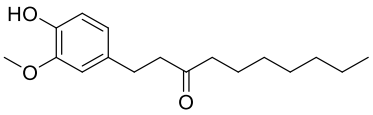
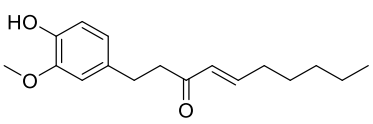
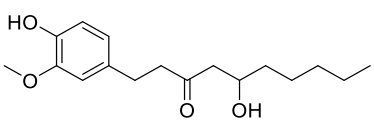
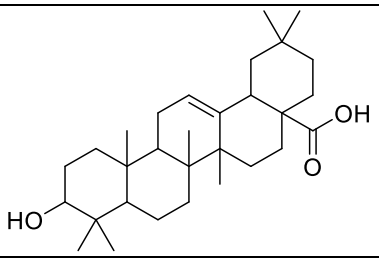
		
quercetin	Betulinic acid	Paradol
		
Shogaol	Gingerol	Oleanolic acid

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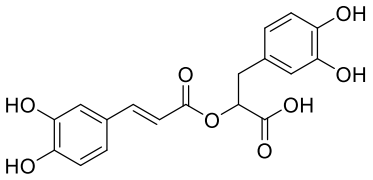
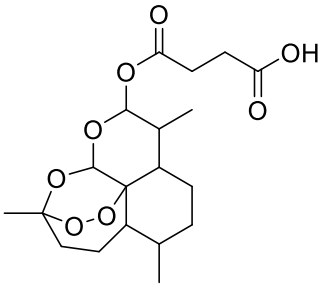
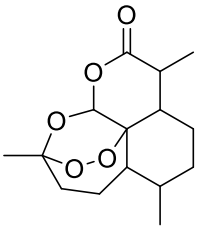
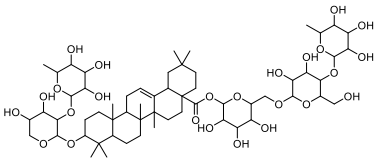
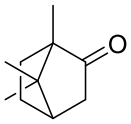
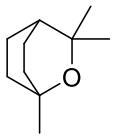
		
Rosmarinic acid	Artesunate	Artemisinin
		
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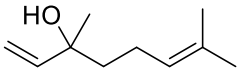
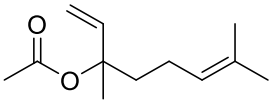
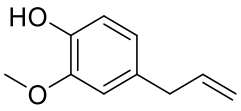
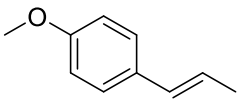
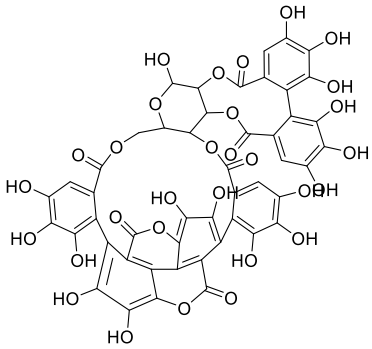
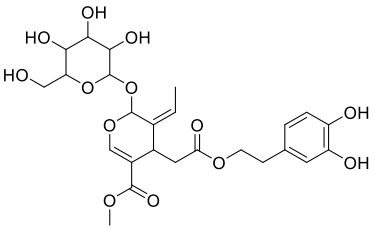
		
Linalool	Linalyl acetate	Eugenol
		
Anethole	Punicalagin	Oleuropein

Table 2:- Structures of the studied antiviral compounds

The binding mode with least binding energy is regarded as best mode of binding as it is most stable for the ligand. The least binding energy which indicates better fit for all the proposed compounds and 2D interactions obtained from docking 6LU7 with the native ligand (N3) in addition to the proposed compounds are illustrated in table (3).

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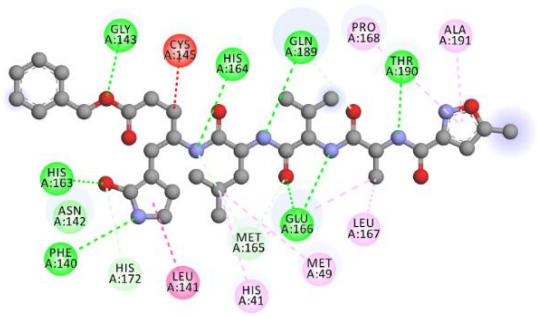
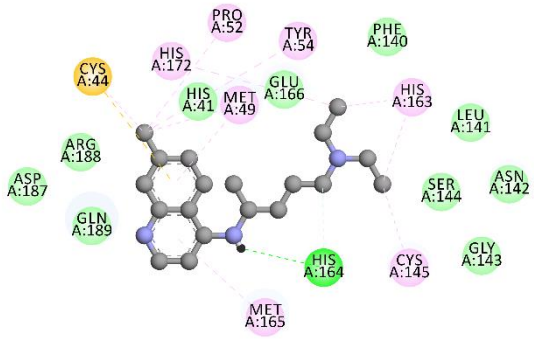
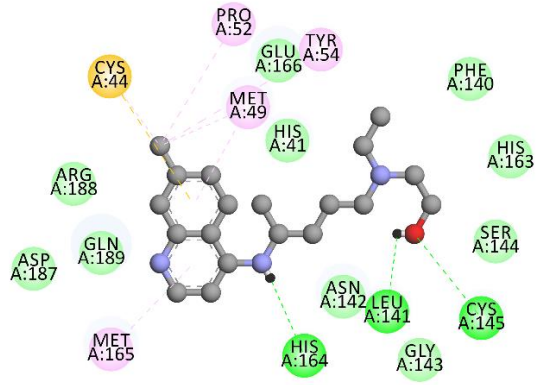
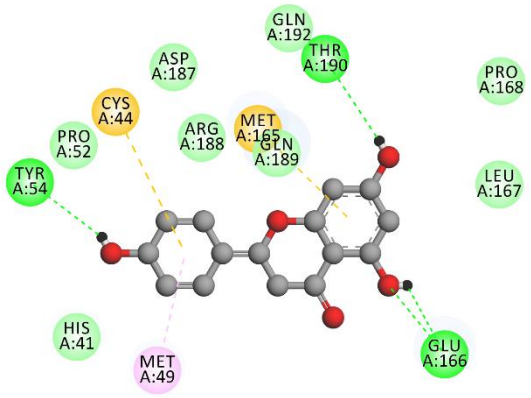
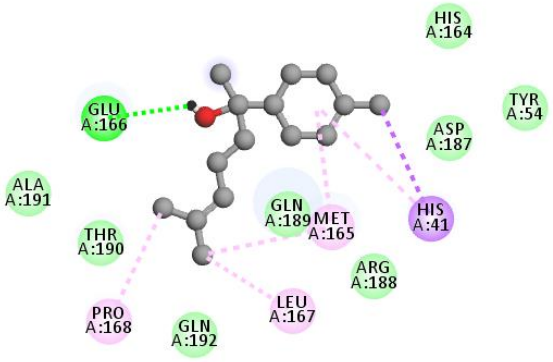
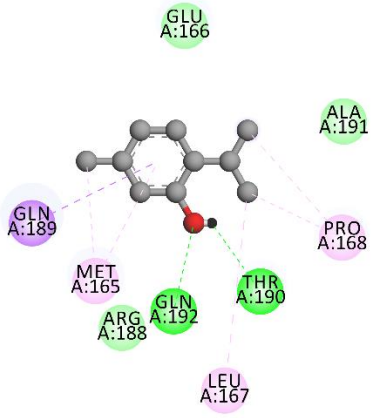
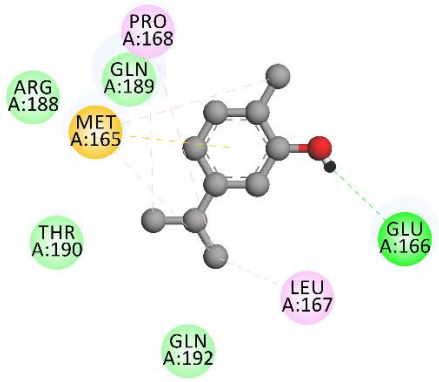
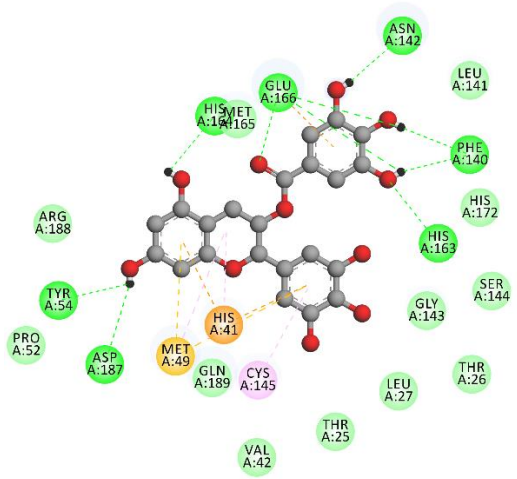
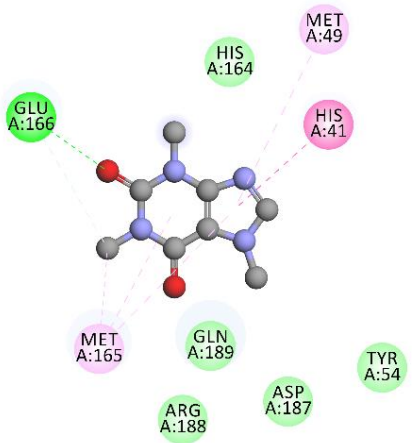
Compounds	Score (Kcal/mol)	Inhibition constant (µM)	2D interaction
Native ligand (N3)	-8.42	0.672	
chloroquine	-7.12	6.04	
hydroxychloroquine	-7.35	4.13	

Apigenin	-7.99	1.4	
bisabolol	-6.86	9.44	
Thymol	-5.33	123.5	

Carvacrol	-5.31	127.45	
epigallocatechin-3-gallate	-8.51	0.582	
caffeine	-4.61	420.86	

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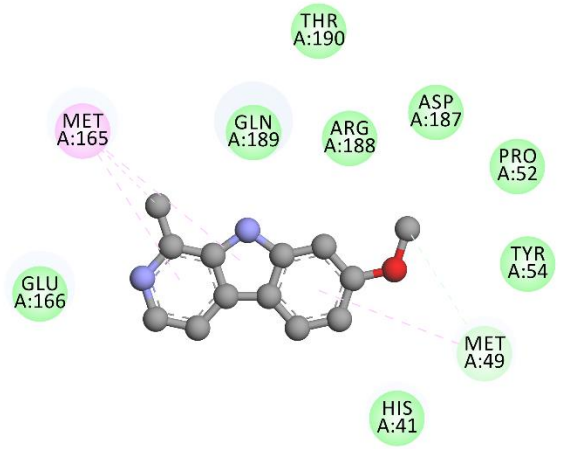
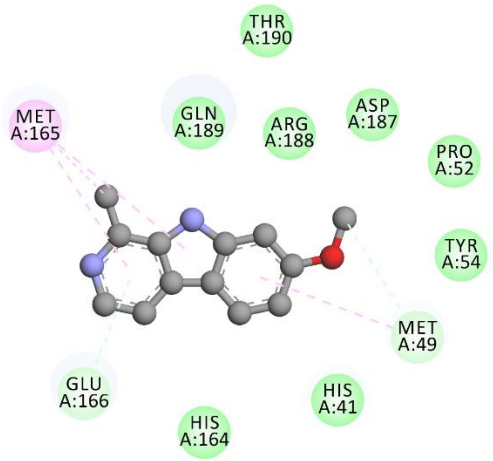
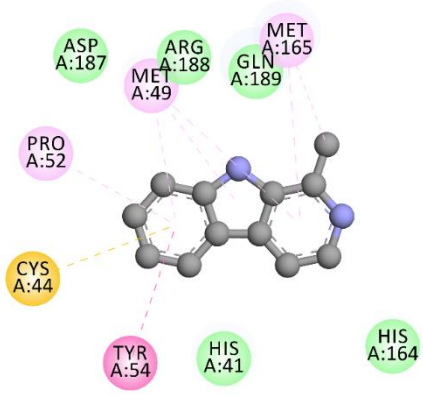
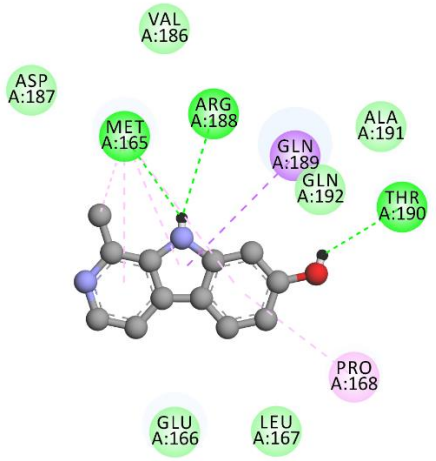
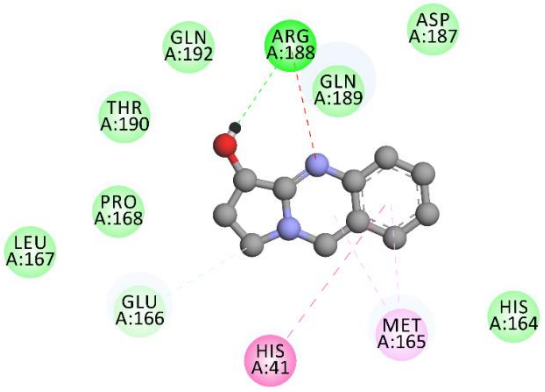
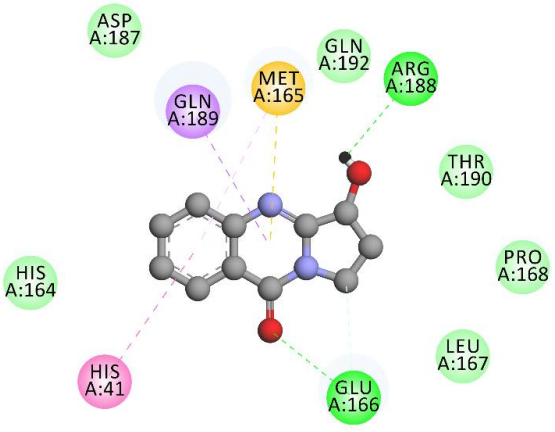
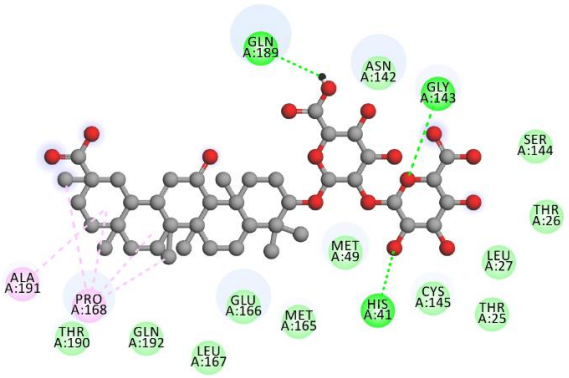
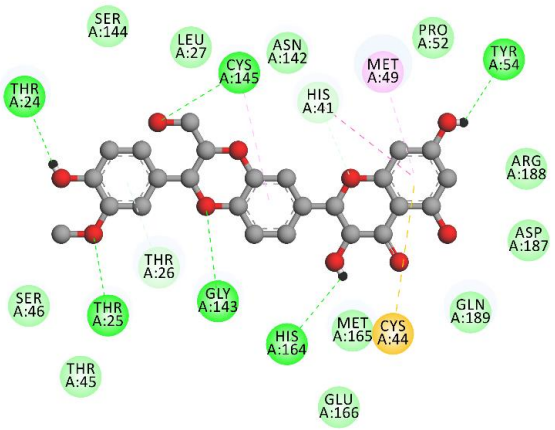
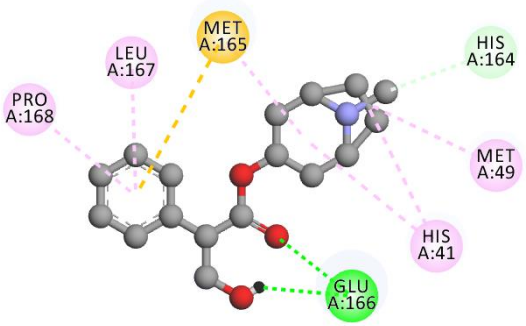
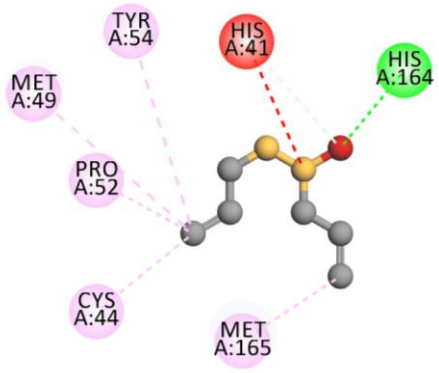
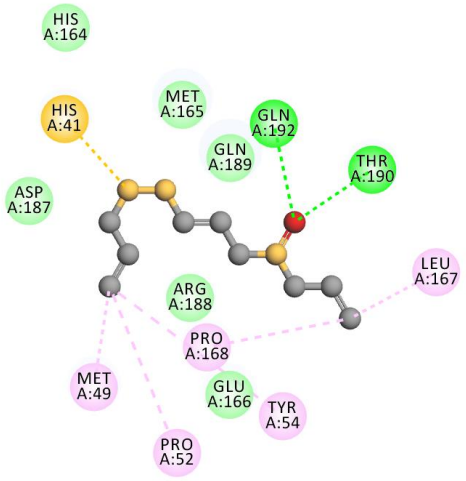
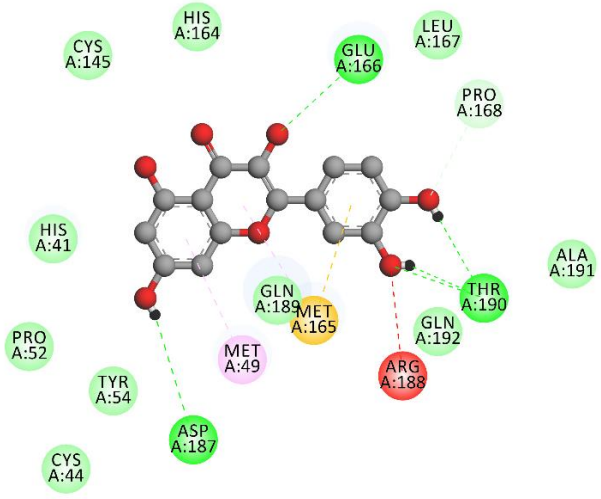
harmine	-6.41	19.9	
harmaline	-6.53	16.47	
harman	-5.93	45.38	

harmalol	-6.77	10.97	
vasicine	-5.7	65.99	
vasicinone	-6.12	32.45	

glycyrrhizin	-5.63	75.21	
Silibinin	-9.13	0.204	
Atropine	-8.01	1.34	

allicin	-4.06	1050	
ajoene	-5.62	76.16	
Quercetin	-7.85	1.76	

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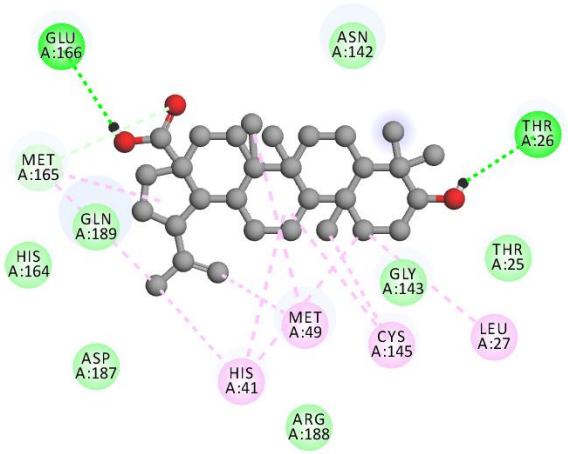
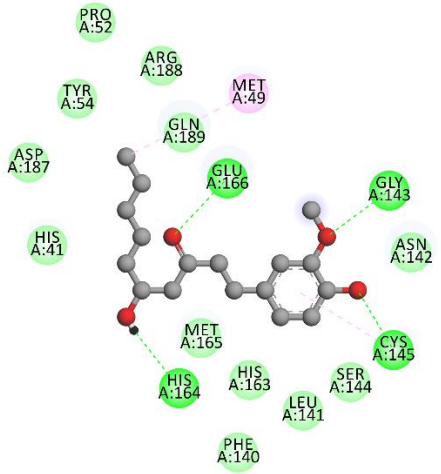
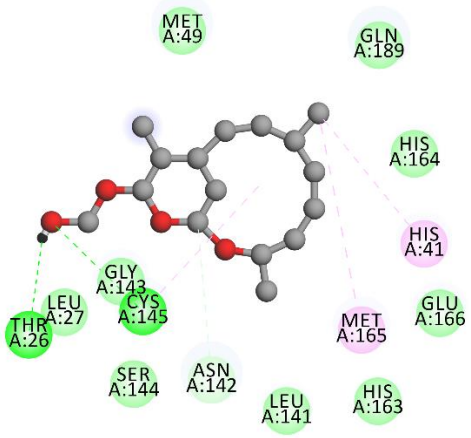
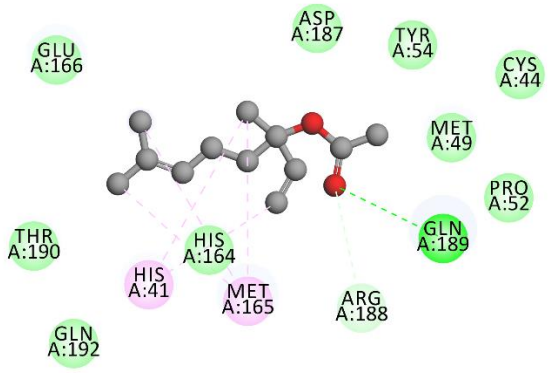
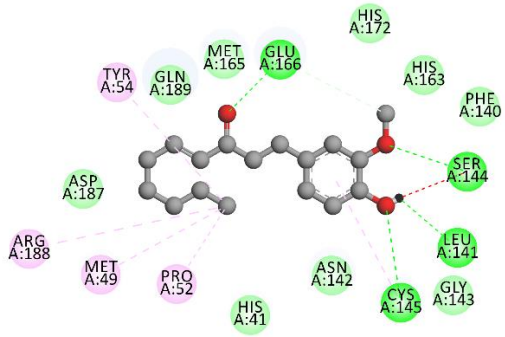
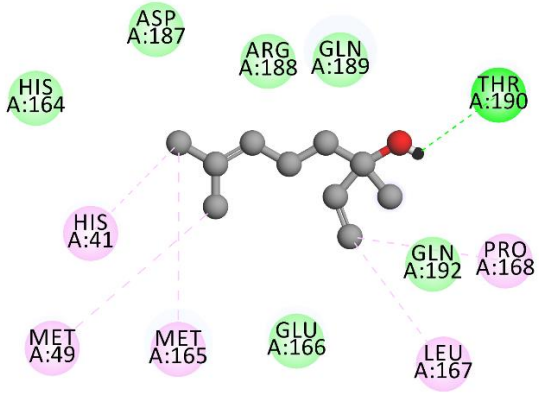
Betulinic acid	-10.0	0.046	
Gingerol	-6.38	20.95	
Artesunate	-6.46	18.40	

Linalyl acetate	-5.58	81.03	
Shogaol	-7.36	4.00	
Linalool	-5.36	118.45	

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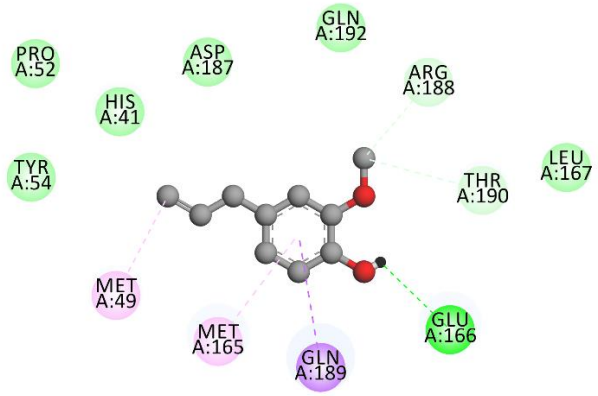
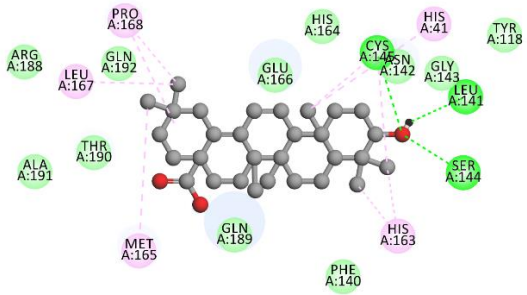
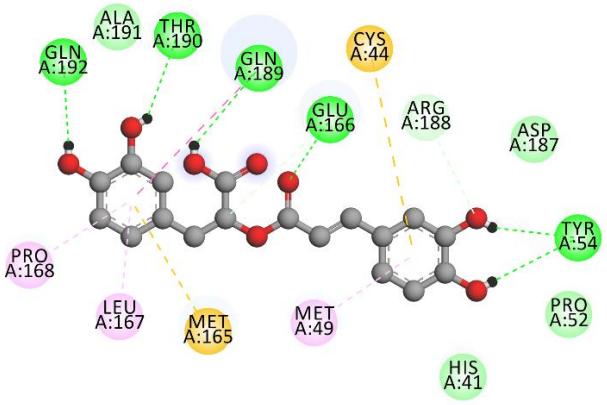
Eugenol	-5.06	194.81	
Oleanolic acid	-9.08	219.96	
Rosmarinic acid	-7.07	6.55	

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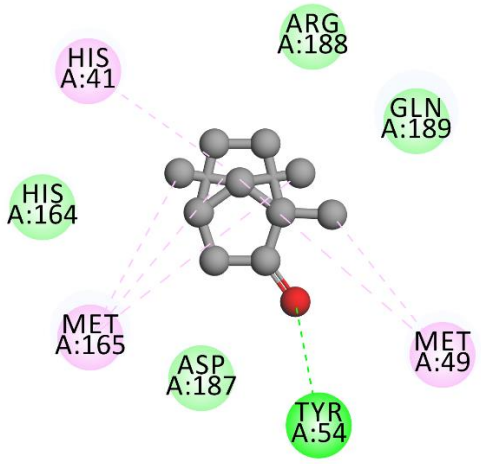
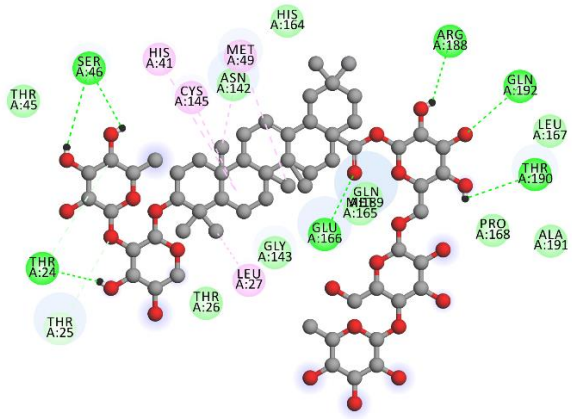
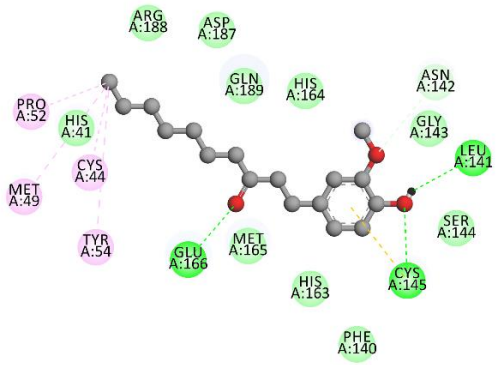
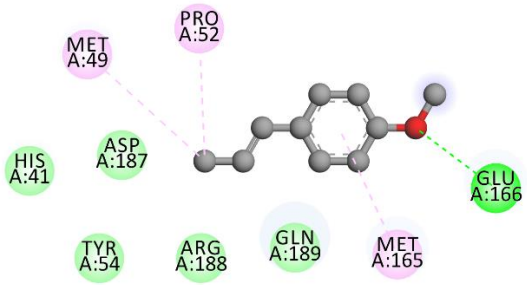
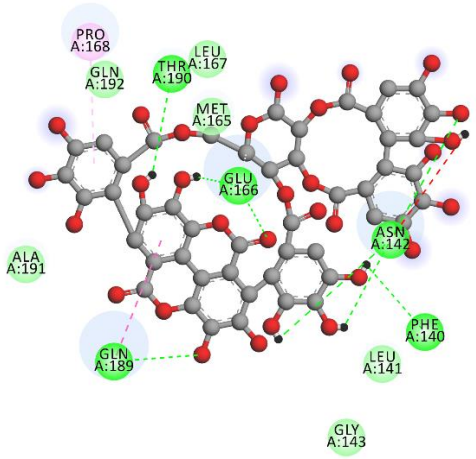
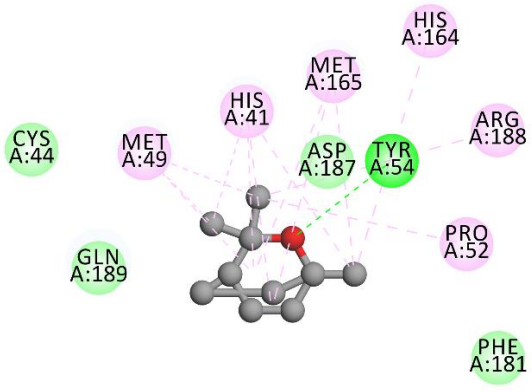
Camphor	-5.59	79.35	
Hederasaponin B	-6.89	8.92	
Paradol	-6.94	8.22	

Anethole	-4.94	241.05	
Punicalagin	-5.39	112.35	
1,8-Cineole	-5.78	57.62	

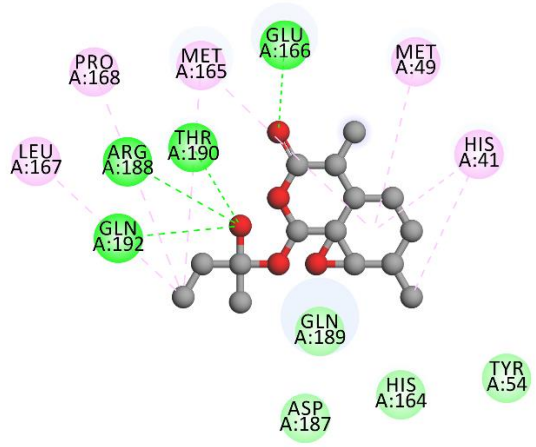
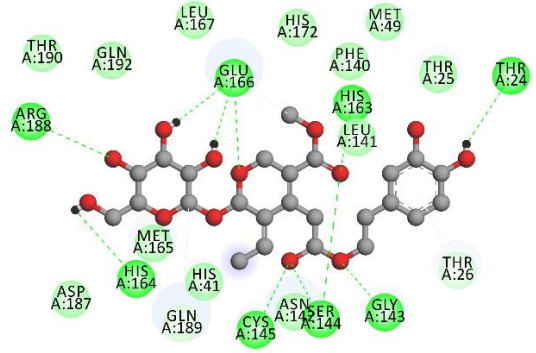
Artemisinin	-7.78	1.97	
Oleuropein	-7.81	1.88	

Table 3:- Results of the docking of the antiviral compounds on the crystal structure of COVID-19 main protease

Based on the X-ray structure of COVID-19 main protease in complex with its inhibitor N3 (PDB: 6LU7), seven conventional hydrogen bonds were observed between N3 and Thr 190, Gln 189, His 164, Gly 143, His 163, Phe 140, and Glu 166 residues. Other noticeable interactions are amide-pi stacking with Leu 141 residue, pi-alkyl interaction with Ala 191, Pro 168, His 41, and Met 49 residues, and alkyl interactions with Met 46, Met 165 and Leu 167. Unfavorable bump interaction with Cys 145 is also observed.

Chloroquine and hydroxychloroquine are two antimalarial drugs with reported antiviral activity were also included in this study. The interaction of chloroquine with 6LU7 active site attributed mainly by one hydrogen bond with His 164, sex pi-alkyl interactions with His 172, His 163, Met 165, Met 49, and Tyr 54, one pi-sulfur bond with Cys 44, in addition to a large number of amino acids involved in van der Waals interaction including His41, Phe 140, Glu 166, Leu 141, Ser 144, Asn 142, Gly 143, Gln 189, Asp 187, and Arg 188. On the other hand, the hydroxychloroquine showed similar binding mode to its derivatives chloroquine with a slightly higher binding affinity (-7.35 and -7.12, respectively). The extra hydroxyl group of the hydroxychloroquine was involved in two hydrogen bonds with Cys 145 and Leu 141 residues, which may explain the increase in its binding affinity over chloroquine.

Among the investigated compounds: epigallocatechin-3-gallate (-8.51 Kcal/mol), Oleanolic acid (-9.08 Kcal/mol), Silibinin (-9.13 Kcal/mol), Betulinic acid (-10.0 Kcal/mol), showed higher binding affinity to 6LU7 than N3 (-8.42 Kcal/mol), chloroquine (-7.12 Kcal/mol), and hydroxychloroquine (-7.35 Kcal/mol). On the other hand, Atropine (-8.01 Kcal/mol), Apigenin (-7.99 Kcal/mol), Quercetin (-7.85 Kcal/mol), and Shogaol (-7.36 Kcal/mol) showed lower binding energy than both chloroquine and hydroxychloroquine but higher than the N3.

Epigallocatechin-3-gallate in complex with 6LU7 showed ten hydrogen bonds with amino acids (Tyr 54, Asp 187, His 163, Phe 140, Asn 142, Glu 166, and His 164). Interactions in the form of pi-sulfur, pi-anion, and pi-cation were also observed with Met 49, Glu 166, and His 41, respectively.

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Oleanolic acid also showed a significant binding affinity with the COVID-19 main protease (-9.08 Kcal/mol). Its hydroxyl group was able to form three hydrogen bonds with the amino acids Cys 145, Leu 141, Ser 144. The remaining observed intermolecular bonds were in the form of hydrophobic alkyl and pi-alkyl bonds with the Pro 168, Leu 167, Met 165, His 41, and His 163 residues.

The result obtained by docking of silibinin with COVID-19 main protease showed binding affinity of -9.13 Kcal/mol. Silibinin, significantly, showed formed sex conventional hydrogen bonding with amino acids: Thr 24, Thr 25, Gly 143, Cys 145, His 164, and Tyr 54. Other interactions include Pi-pi stacking between the benzene ring of chromanone group and His 41, pi-alkyl interactions between benzene rings of benzodioxane moiety and Cys 145, and pi- donor bond between benzene ring (attached to benzodioxane moiety) and Thr 25.

Although the betulinic acid in complex with 6LU7 showed only two conventional hydrogen bonds, it ranked in the top among the investigated compounds panel. The hydroxyl group of the betulinic acid interacts with Thr 26 at a distance of 1.95 Å with a hydrogen bond, whereas the hydroxyl group of the carboxylic acid moiety formed a second hydrogen bond with Glu 166 at a distance of 2.32 Å. The other predominant form of interaction was the hydrophobic interactions where ten pi-alkyl bonds formed between betulinic acid and amino acids: Leu 27, Cys 145, Met 49, His 41, and Met 165.

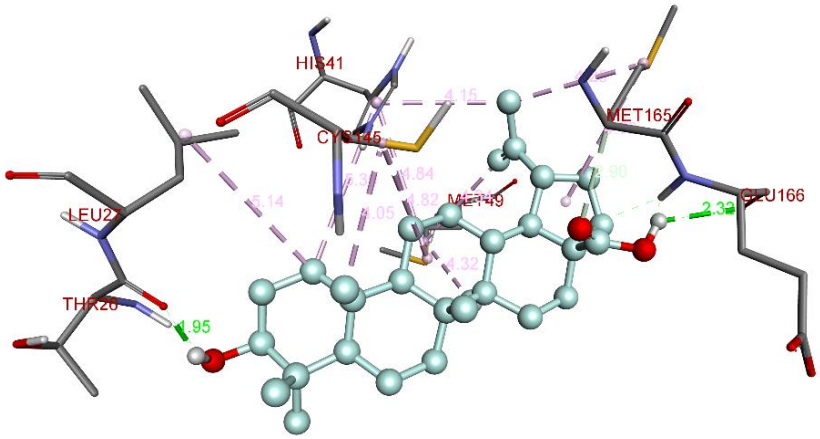

Fig 1:- Betulinic acid docked in COVID-19 main protease. Ligand is presented as a scaled ball and stick. Amino acid residues involved in the interaction with the ligand is presented as sticks. Binding interaction of the ligand with the amino acid residues are presented as dash lines.

Betulonic acid is a pentacyclic triterpene concentrated in Ziziphus spina-christi. It has been known for a wide range of biological and medicinal properties such as antibacterial, antimalarial, antihelmintic, antinociceptive, anti-inflammatory, and anticancer activities. Many research reported its antiviral properties against some enveloped and non-enveloped viruses(Pavlova et al., 2003, Hong et al., 2015b).

Silibinin, flavonolignans, which is one of the primary bioactive components of Silymarin, an extract from the seed of the milk thistle plant (Silybum marianum). It is well-known as antioxidative, anti-inflammatory, and hepatoprotective agent also many researches documented its antiviral activities against several viruses, including the togaviruses (Chikungunya virus and Mayaro virus), flaviviruses (hepatitis C virus and dengue virus), hepatitis B virus, influenza virus, and human immunodeficiency virus(Liu et al., 2019).

Oleanolic acid is a pentacyclic triterpenoid isolated from almost 2000 plant species such as Rosmarinus officinalis (rosemary) and Olea europaea (the olive). This compound and its derivatives possess several interesting pharmacological activities, such as antioxidant, anti-inflammatory, anticancer, and hepatoprotective effects. Also, oleanolic acid and its analogs have antiviral activities against several viruses, which include HIV, hepatitis B and C viruses, influenza virus, and herpes viruses(Khwaza et al., 2018, de Oliveira et al., 2019).

Epigallocatechin-3-O-gallate is the major catechin component of Cameria sinensis plant (green tea). It has received much attention because of its biological activities such as antimicrobial and anticancer activities also it has been reported to possess a broad spectrum of antiviral activities against DNA viruses such as herpes simplex, adenovirus, human papilloma virus, and hepatitis B virus, and against (+)-RNA viruses such as hepatitis C virus, Zika virus, dengue virus West Nile viruses, Chikungunya virus, and Porcine Reproductive and Respiratory virus, and (−)-RNA viruses such as human immunodeficiency, Ebola virus, and influenza virus(Kaihatsu et al., 2018).

Regarding to the obtained results and by reviewing the literature, it is suggested that Betulinic acid, Silibinin, Oleanolic acid, and Epigallocatechin-3-gallate may have the potential therapeutic agent for COVID-19 virus infection

IV. CONCLUSION

In conclusion, thirty-six compounds from natural sources were tested as inhibitors of in COVID-19 main protease via molecular docking. From docked compounds, we propose for the first time that Betulinic acid, Silibinin, Oleanolic acid, and Epigallocatechin-3-gallate demonstrated high binding affinity to the main protease and Betulinic acid ranked in the top among the investigated compounds panel, this is based on the lowest free energy value of (-10.0 Kcal/mol) on COVID-19 main protease. Further studies must be conducted on these compounds using molecular dynamics simulations in order to get more reliable data; also, we recommend more studies on the mechanisms of action of these compounds of interest, as well as experimental demonstrations of the possible antagonistic effect in vitro on COVID–19.

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