Molecular Docking of *Olea europaea* and *Curcuma Longa* Compounds as Potential Drug Agents for Targeting Main-Protease of SARS-nCoV2

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Abstract

One of the main reasons of rapidly growing cases of COVID-19 pandemic is the unavailability of approved therapeutic agents. Therefore, it is urgently required to find out the best drug/vaccine by all means. Aim of the current study is to test the anti-viral drug potential of many of the available olive and turmeric compounds that can be used as potential inhibitors against one of the target proteins of SARS-nCoV2 named Main protease (M^{pro}/$3cl^{pro}$). Molecular docking of thirty olive and turmeric compounds with target protein was performed using Molecular Operating Environment (MOE) software to determine the best ligand-protein interaction energies. The structural information of the viral target protein M^{pro}/$3CL^{pro}$ and ligands were taken from PDB and PubChem database respectively. Out of the thirty drug agents, 6 ligands do not follow the Lipinski rule of drug likeliness by violating two or more rules while remaining 24 obey the rules and included for the downstream analysis. Ten ligands from olive and four from turmeric gave the best lowest binding energies, which are *Neuzhenide, Rutin, Demethyloleoeuropein, Oleuropein, Luteolin-7-rutinoside, Ligstroside, Verbascoside, Luteolin-7-glucoside, Cosmosin, Curcumin, Tetrehydrocurcumin, Luteolin-4'-o-glucoside, Demethoxycurcumin* and *Bidemethoxycurcumin* with docking scores of -10.91, -9.49, -9.48, -9.21, -9.18, -8.72, -8.51, -7.68, -7.67, -7.65, -7.42, -7.25, -7.02 and -6.77 kcal/mol respectively. Our predictions suggest that these ligands have the potential inhibitory effects of M^{pro} of SARS-nCoV2, so, these herbal plants would be helpful in harnessing COVID-19 infection as home remedy with no serious known side effects. Further, in-silico MD simulations and in-vivo experimental studies are needed to validate the inhibitory properties of these compounds against the current and other target proteins in SARS-nCoV2.

Keywords Main Protease · SARS-nCoV2 · Molecular Docking · *Olea europaea* · *Curcuma longa* · MOE software

1 Introduction

Corona viruses (CoVs) are group of positive sense RNA viruses that cause upper respiratory tract infection, hepatic diseases, multiple organ failure and gastrointestinal disorder in both animals and humans [1-4]. In December 2019, patients with new kind of disease having symptoms like pneumonia were reported in Wuhan, Hubei Province of China [5,6]. This infectious agent was recognized as a new strain of corona virus because it shares 70% similarity with SARS-CoV-1 (severe acute respiratory syndrome) and was temporarily given a name 2019-nCov [7]. Virus has a characteristic human to human transmission and causes respiratory tract infection that ultimately leads to multiple organ failure [3,8,9]. World Health Organization (WHO) officially named the virus as SARS-nCoV2 (disease COVID-19) and on March 31, 2020 declared the disease a pandemic [10]. Until July 2020, COVID-19 caused more than 696,147 deaths and 18,354,342 confirmed cases worldwide [11]. SARS-nCoV2 (COVID-19) belongs to the family of *Coronaviridae* and is the seventh member of genus *Betacoronavirus* [12].

SARS-nCoV2 infects the host cell by attaching to ACE-2 receptor through its spike protein [3]. After binding, the virus moves into the cell and starts its replication. Besides spike protein, M^{pro} (main protease) / $3CL^{pro}$ and PL^{pro} (recognized as potential drug targets) also play main role in viral replication [13]. Until now, there is no approved drug against COVID-19; however, supporting drugs like remedsivir, nelfinavir and hydroxychloroquine provide immunomodulatory action and prevents organ damage [13]. The main target of drugs against which scientists are focusing these days is M^{pro} (main protease), because main protease of SARS-nCoV2 shares 96% similarity with SARS-CoV-1 [9]. COVID-19's main target, M^{pro}/ $3CL^{pro}$, has been successfully crystallized, submitted and repositioned in PDB (PDB ID: 6M2N) by Su *et al*. (2020). This protein represents a potential drug target and its inhibition results in the blockage of replication and infectious cycle of Corona virus [14].

Due to lack of specific drug against COVID-19, there is an ongoing trend of usage of herbs and herbal extracts because these are used as conventional antiviral medicines [15]. In present study, we will investigate the compounds of *Olea europaea* (olive) and *Curcuma longa* (turmeric) as the potential inhibitors of COVID-19 by computer-aided drug design (CADD) [16]. Molecular operating environment is used in this in-silico studies. This investigation will provide other researchers the opportunity to identify best drugs to treat COVID-19.

2 Material and Methods

2.1 Selection of Medicinal Herbs

2.1.1 *Olea europaea* (Olive)

Olea europaea, which is known for its great therapeutic potential, is widely recommended for treating COVID-19 infection because of its compounds, like *oleuropein,* that have antiviral properties [17]. For centuries, it is being used in North African and Asian areas as a food and conventional medication due to Islamic conviction [18]. *Olea europaea* extracts have antiviral, antiepileptic, antioxidant, anti-erythrogenic, germicide, cancer preventing, gastroprotective, wound mending, immunosuppressive, blood glucose lowering and pain relieving properties [19]. Nearly its 25 bioactive compounds have been reported in olive extract as cited in different literatures [20,21,19]. The structure of *Olea europaea* compounds are given in Figure 1.

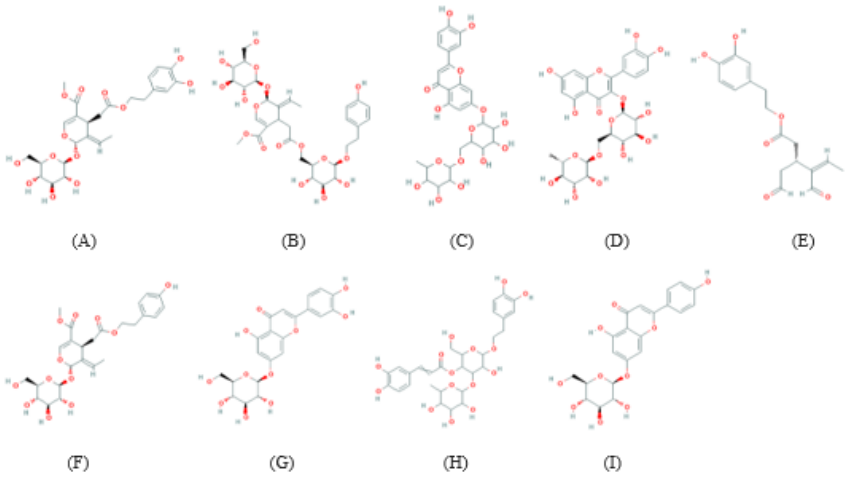

Fig. 1 Chemical structure of major compounds of *Olea europaea*. (A) Oleuropein (B) Neuzhenide (C) Luteolin 7-rutinoside (D) Rutin (E) Deacetoxyoleuropein aglycone (F) Ligstroside (G) Cynaroside (Luteolin-7-glucoside) (H) Verbascoside (I) Cosmosin

The chemical properties of compounds were taken from PubChem. Different scientist performed various experiments to obtain a set of properties and then submitted those in PubChem and other similar chemical databases. The chemical properties of *Olea europaea compounds* are given in Table 1.

Table 1 The chemical parameters of *Olea europaea* compounds. TPSA= topological polar surface area, H-donn= hydrogen bond donor, H-acc= hydrogen bond acceptor, RB= rotatable bond

Ligands	Molecular Weight (g/mol)	PubChem CID	Toxicity	Log P	H-donn	H-acc	RB	TPSA	Molecular Formula
Oleuropein	540.5	5281544	NO	-0.4	6	13	11	202Å²	$C_{25}H_{32}O_{13}$
Neuzhenide	686.7	6440999	NO	-2.2	8	17	14	261 Å²	$C_{31}H_{42}O_{17}$
luteolin 7rutinoside	594.5	12315422	NO	-1.1	9	15	6	245 Å²	$C_{27}H_{30}O_{15}$
Rutin	610.5	5280805	irritant	-1.3	10	16	6	266 Å²	$C_{27}H_{30}O_{16}$
Demethyloleoeuropein	526.5	6450302	NO	-0.8	7	13	10	213 Å²	$C_{24}H_{30}O_{13}$
Ligstroside	524.5	14136859	NO	-0.1	5	12	11	181 Å²	$C_{25}H_{32}O_{12}$
Verbascoside	624.6	354009	NO	-0.5	9	15	11	245 Å²	$C_{29}H_{36}O_{15}$
Cosmosin (apigenin 7-glucoside)	432.4	5280704	NO	-0.1	6	10	4	166 Å²	$C_{21}H_{20}O_{10}$
luteolin	286.24	5280445	irritant	1.4	4	6	1	107 Å²	$C_{15}H_{10}O_6$
luteolin7glucoside	448.4	5280637	NO	0.5	7	11	4	186 Å²	$C_{21}H_{20}O_{11}$
Deacetoxyoleuropein aglycone	320.3	101102227	NO	1.1	2	6	10	101 Å²	$C_{17}H_{20}O_6$
Chlorogenic acid	354.31	1794427	irritant	-0.4	6	9	5	165 Å²	$C_{16}H_{18}O_9$
luteolin-4'-o-glucoside	448.4	5319116	NO	0.5	7	11	4	186 Å²	$C_{21}H_{20}O_{11}$
Apigenin	270.24	5280443	irritant	1.7	3	5	1	87 Å²	$C_{15}H_{10}O_5$
Quercetin	302.23	5280343	Irritant Toxic	1.5	5	7	1	127 Å²	$C_{15}H_{10}O_7$
Ferulic Acid	194.18	445858	Irritant	1.5	2	4	3	66.8Å²	$C_{10}H_{10}O_4$
Flavylium	207.25	145858	NO	0	0	0	1	1 Å²	$C_{15}H_{11}O^+$
Sinapic Acid	224.21	637775	irritant	1.5	2	5	4	76 Å²	$C_{11}H_{12}O_5$
Homovanillic Acid	182.17	1738	irritant	0.4	2	4	3	66.8Å²	$C_9H_{10}O_4$
Cinamic Acid	255.4	5372020	NO	-0.6	2	5	6	114 Å²	$C_{11}H_{13}NO_2S_2$
Vanillic Acid	168.15	8468	irritant	1.4	2	4	2	66.8Å²	$C_8H_8O_4$

Tyrosol	138.16	10393	irritant	0.4	2	2	2	40.5Å²	$C_8H_{10}O_2$
Protocatehuic acid	154.12	72	Irritant	1.1	3	4	1	77.8Å²	$C_7H_6O_4$
Hydroxytyrosol	154.16	82755	Irritant	-0.7	3	3	2	60.7Å²	$C_8H_{10}O_3$
4-Hydroxy Benzoic Acid	138.12	135	Irritant	1.6	2	3	1	57.5Å²	$C_7H_6O_3$

2.1.2 *Curcuma longa* (Turmeric)

Curcuma longa is known as a powerful natural healer. For quite a long time, it is being utilized in Asia as a traditional medicine [22]. Compounds in olive have antiviral, antineoplastic, antiprotozoal, microbicidal, fungicidal, COX-inhibitor, antioxidant and antivenin properties [22]. Five active compounds are present in turmeric [23,24] and their structures are given in Figure 2.

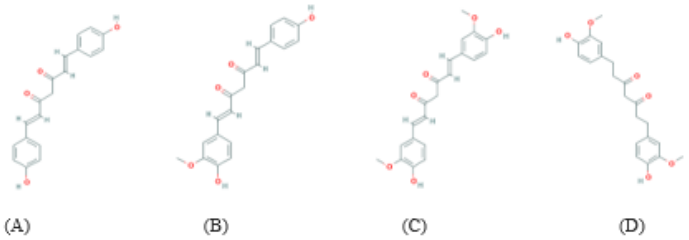

Fig. 2 The chemical structures of major compounds of *Curcuma longa*. (A) Bisdemethoxycurcumin (B) Demethoxycurcumin (C) Curcumin (D) Tetrahydrocurcumin

As *Curcuma longa* compounds are recognized for reducing effect of inflammation causing cytokinin (such as interleukin 6) but this herb is also reported for causing dermatitis. To predict drug potential, their properties are taken from PubChem database. The chemical properties of *Curcuma longa* compounds are given in Table 2.

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Table 2 Chemical properties of Turmeric compounds. TPSA= topological polar surface area, H-donn= hydrogen bond donor, H-acc= hydrogen bond acceptor, Env= environment, Haz= hazard

Ligands	Molecular Weight (g/mol)	PubChem CID	Toxicity	Log P	H-donn	H-acc	Biological Activity	TPSA	Molecular Formula
Curcumin	368.4	969516	irritant	3.2	2	6	Antibacterial, Antiviral	93.1Å²	$C_{21}H_{20}O_6$
Bisdemethoxycurcumin	308.3	5315472	irritant	3.3	2	4	Antioxidant	74.6Å²	$C_{19}H_{16}O_4$
Demethoxycurcumin	338.4	5469424	Env. Haz.	3.3	2	5	Antioxidant	83.8Å²	$C_{20}H_{18}O_5$
Tetrahydrocurcumin	372.4	124072	NO	2.8	2	6	Anti-inflammatory	93.1Å²	$C_{21}H_{24}O_6$
Ar-turmeron	216.32	160512	irritant	4	0	1	Antivenom	17.1Å²	$C_{15}H_{20}O$

2.2 Selection of Targeted Protein

2.2.1 Main Protease

M^{pro}/$3cl^{pro}$ (PDB ID: 6M2N) is the key enzyme in SARS-nCoV2 that has a main role in viral replication and transcription [25]. This enzyme is involved in producing Nsps (non-structural proteins) which then assemble the viral protein. So by targeting M^{pro}, viral replication can be halted [26].

Table 3 Crystallographic properties of enzyme (Mpro)

Enzyme	PDB ID	Classification	Virus	Expression system	Resolution	Method	Total structure weight (DA)	Chain	Atom count	Active site residues
Main protease	6M2N	Viral Protein	SARS-nCOV2 (Severe acute respiratory syndrome)	Escherichia coli BL21	2.20 Å	X-RAY Diffraction	136.38 kDa	A, B, C, D	9544	THR24, THR26, PHE140, ASN142, GLY143, CYS145, HIS163, HIS164, GLU166, HIS172

2.3 Molecular Docking

The molecular docking was performed by Molecular Operating Environment (MOE) software. It is a drug discovery software that can be used for checking protein-ligand interactions and for drug likeness analyses. MOE is a platform that incorporates visualization of results, modeling, simulations of structures and methodology development in one package [27].

2.4 Preparation of Ligand

Several databases are available to obtain the desired ligand e.g. *PubChem, ChEMBL, DrugBank, Zinc, Merck, Asinex, Enamine* etc. [28-32]. These ligands can either be downloaded in sdf format or can be sketched in MOE interface by using *Builder Mode*. After sketching, the partial charges were added by using *compute* in MOE. Once the charges were added the prepared ligand is then saved as *MDB file*.

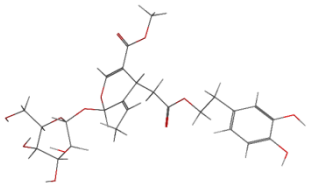


Fig. 3 Prepared ligand of *Oleuropein*

2.5 Preparation of Proteins

Protein data bank is the source of our target protein. PDB file 6M2N was downloaded and opened in MOE [33].

2.5.1 Removal of water, Inhibitor and Repeated Chains

The already attached ligand was removed to make active site accessible for new ligand. The water molecules were also removed from the protein surface so that the interacting region would not be hidden during docking. The repeated chains of M^{pro} were also removed to avoid complications during docking.

2.5.2 Correction of Protein Structure

Errors and missing atoms in structure were then corrected and added by using the feature of *structure preparation* in MOE. For correction of structure, first Protein module of MOE was selected. Afterwards, by selecting *structure and preparation* option, the new window appeared and from that *protonate 3D* module was selected and in the last step, the *correction* option corrected our desired protein's structure.

2.5.3 Active Site Finder

MOE main interface was used to open *compute* and after that *Site Finder* was selected. We chose *apply* option from new interface which gave number of different chains that could be the possible active site of target protein. From literature survey or Pymol , we selected the chain which had the sequence of active site residues. If the sequence of active site is unknown, then *blind docking* will prefer (it is better to use first 3 chains). *No Centers* and *atoms and backbone* option were selected from *Render* and *isolate* module respectively. Then by clicking *dummies* option, the dummy atoms were created.

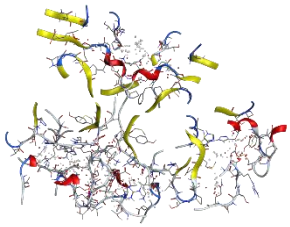


Fig. 4 Prepared protein (M-pro)

3 Docking and Surface Maps

Docking was performed to determine the possible interaction between ligand and active site of target protein 6M2N [34]. A new dock window was opened when we selected *dock* from compute option in MOE interface. *Dummy atoms* in *site* module was selected (from which the docking was performed). We uploaded ligand file from *ligand mdb file* module which was previously prepared. In the end *command* was run. *Surface and maps* module in MOE focused and isolated the point where ligand attached to protein with minimum energy.

4 Results

4.1 Docking Scores of *Olea europaea*

Molecular docking was done to estimate the ligand-protein interaction between different compounds of herbs and protein (main protease). The chance of ligand to be an effective drug increases with decrease of binding energy. The docking score of Olea europaea is given in Table 4.

Table 4 Docking score Olive's compounds with 6M2N

SN	Ligands	Docking score (kcal/mol) with 6M2N
1	Neuzhenide	-10.9176493
2	Demethyloleoeuropein	-9.48762321
3	Rutin	-9.49832058
4	Oleuropein	-9.21493816
5	Luteolin 7-rutinoside	-9.18656158
6	Ligstroside	-8.72711468
7	Verbascoside	-8.5100832
8	Luteolin-7-glucoside	-7.68533516
9	Cosmosin	-7.67128038
10	luteolin-4'-o-glucoside	-7.25527763
11	Chlorogenic acid	-6.8014946
12	Deacetoxyoleuropein aglycone	-6.75398064
13	Leutolin	-6.27251291
14	Apigenin	-6.2212038
15	Quercetin	-6.00290871
16	Cinamic acid	-5.72288179
17	Sinapic acid	-5.69604254
18	Ferulic acid	-5.44703674

19	Homovanillic acid	-5.18638182
20	Flavylium	-5.05841064
21	Vanillic acid	-4.89071226
22	Hydroxytyrosol	-4.70743608
23	4-hydroxybenzoic	-4.65060616
24	Protocatehuic acid	-4.69090509
25	Tyrosol	-4.5343833

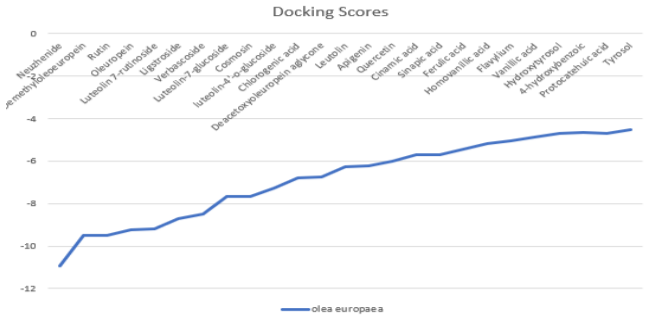


Fig. 5 Graphical representation of scores

4.2 Docking Scores of *Curcuma Longa*

The docking scores of *curcuma longa* compounds are given in Table 5. Curcumin gave the highest score with the binding energy of -7.6 kcal/mol. It is followed by Tetrahydrocurcumin and Demethoxycurcumin having energies of -7.4 and -7.02 respectively. Criteria based on docking scores selects the best compounds.

Table 5 Docking score Turmeric's compounds with 6M2N

SN	Ligands	Docking score (kcal/mol) with 6M2N
1	Curcumin	-7.65329599
2	Tetrehydrocurcumin	-7.42297649
3	Demethoxycurcumin	-7.02905893
4	Bidemethoxycurcumin	-6.77281666
5	Ar-Turmerone	-5.70936966

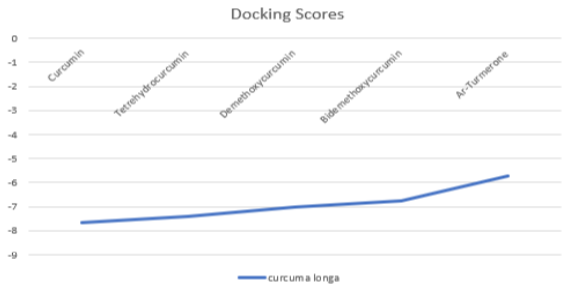


Fig. 6 Graphical representation of scores

4.3 Relationship of Lipinski's Rule and Ligand

According to Lipinski's rule of 5, the ligand which follows 2 or more rules can be considered as a good drug [35]. We used the SwissADME tool (http://www.swissadme.ch/index.php) to determine how many ligands (which we used) in docking study were following Lipinski' rule. The ligands shown in Table 6 follow the Lipinski's rule, and ligands which don't follow this rule are given in Table 7.

4.3.1 Negation to Lipinski's Rule

There are total 7 ligands (Neuzhenide, Demethyloleoeuropein, Rutin, Oleuropein, Luteolin 7-rutinoside, Verbascoside) which violate 3 rules, but their energies range from -10 to -8.5 (-10.9176493, -9.48762321, -9.49832058, -9.21493816, -9.18656158, -8.5100832 respectively).

Table 6 Ligands which do not follow Lipinski's rule

PubChem ID	Ligands	Molecular weight (<500Da)	LogP (<5)	H-Bond donor (5)	H-bond acceptor (<10)	Violations	Docking score
6440999	Neuzhenide	686.7	-2.2	8	17	3	-10.9176493
6450302	Demethyloleoeuropein	526.49	-0.8	7	13	3	-9.48762321
5280805	Rutin	610.5	-1.3	10	16	3	-9.49832058
5281544	Oleuropein	540.5	-0.4	6	13	3	-9.21493816
12315422	Luteolin 7-rutinoside	595.5	-1.1	9	15	3	-9.18656158
354009	Verbascoside	624.5	-0.5	9	15	3	-8.5100832

4.3.2 Ligands under Lipinski's Rule

There are total 24 ligands which follow Lipinski's rule of 5 and their energies range from -8.7 to -4.5. The drug scanning results show that all tested compounds in this study were accepted according to Lipinski's rule of five (Table 7).

Table 7 Ligands which follow Lipinski's rule

PubChem ID	Ligands	Molecular weight (<500Da)	LogP (<5)	H-Bond donor (5)	H-bond acceptor (<10)	Violations	Docking score
14136859	Ligstroside	524.5	-0.1	5	11	2	-8.72711468
5280637	Luteolin-7-glucoside	448.4	0.5	7	11	2	-7.68533516
5280704	Cosmosin	432.4	-0.1	6	10	1	-7.67128038
5319116	luteolin-4'-o-glucoside	448.4	0.5	7	11	2	-7.25527763
1794427	Chlorogenic acid	354.31	-0.4	6	9	1	-6.8014946
101102227	Deacetoxyoleuropein aglycone	320	1.1	2	6	0	-6.75398064
5280445	Leutolin	286.23	1.4	4	6	0	-6.27251291
5280443	Apigenin	270.24	1.7	3	5	0	-6.2212038
5280343	Quercetin	302.23	1.5	5	6	0	-6.00290871
5372020	Cinamic acid	255.54	-0.6	2	5	0	-5.72288179
637775	Sinapic acid	224.21	1.5	2	5	0	-5.69604254
445858	Ferulic acid	194.18	1.5	2	4	0	-5.44703674
1738	Homovanillic acid	182.17	0.4	2	4	0	-5.18638182
145858	Flavylium	207.25	0	0	1	0	-5.05841064
8468	Vanillic acid	168.15	1.4	2	4	0	-4.89071226
82755	Hydroxytyrosol	154,16	-0.7	3	3	0	-4.70743608
135	4-hydroxybenzoic	138.12	1.6	2	3	0	-4.65060616
72	Protocatehuic acid	154.12	1.1	3	4	0	-4.69090509
10393	Tyrosol	138.16	0.4	2	2	0	-4.5343833
969516	Curcumin	368.4	3.2	2	6	0	-7.65329599
124072	Tetrehydrocurcumin	372.4	2.8	2	6	0	-7.42297649
5469424	Demethoxycurcumin	338.4	3,2	2	5	0	-7.02905893
5315472	Bidemethoxycurcumin	308.4	3.3	2	4	0	-6.77281666
160512	Ar- turmerone	216.32	4	0	1	0	-5.70936966

4.4 Ligands with Best Binding Energies

When docked, the ligand attached to the active site of $3CL^{pro}/M^{pro}$ and can be visualized by ligand and interaction module for 2D structure and surface and maps module for 3D structure of MOE. Docking results from table 6 and 7 show Neuzhenide from olive and Curcumin from turmeric give the lowest energy i.e. (-10.9176493 Kcal/mol) and (-7.65329599) respectively. Through MOE *ligand interactions* module, the binding pattern can be visualized.

4.4.1 Best Ligands of *Olea europaea*

Neuzhenide, when docked with 6M2N, showed two hydrogen possible interactions with amino acid LEU B282 (H-donor) with distance of $2.85A^{o}$ and energy of -1.4 and amino acid GLU B288 (H-donor) with distance of $2.89A^{o}$ and energy of -0.9 kcal/mol.

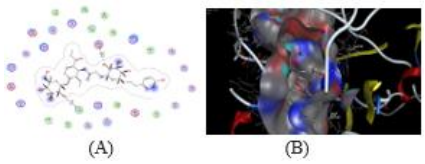
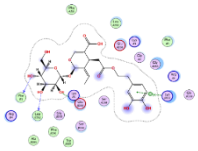
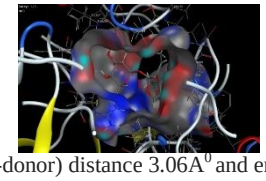
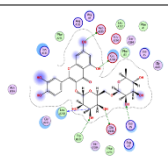
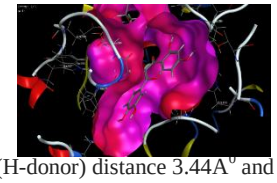
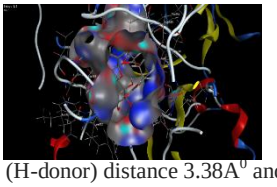


Fig. 7 (A) Ligand interaction of 6M2N with Neuzhenide (B) 3D diagram of pocket 6M2N with Neuzhenide

The interaction usually describes that how much a ligand can form a stabilized bond with target protein. Low binding energy leads to the formation of stabilized bond which give possibility for ligand to be an effective inhibitor. Interaction of other major ligands of *Olea europaea* whom energies range from -10 to -6 are given in Table 8.

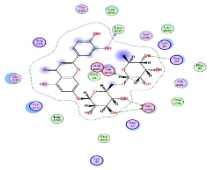
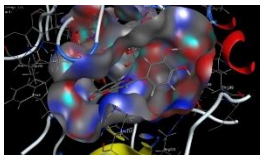
Table 8 Interaction of major ligands of Olive

Demethyloleoeuropein
  Four hydrogen interaction are possible (a) Amino acid PHE 3 (H-donor) distance $3.06A^{0}$ and energy of -0.9kcal/mol (b) Amino acid LEU 282 (H-donor) distance $2.97A^{0}$ and energy of -2.1kcal/mol (c) Amino acid GLU 288 (H-donor) distance $2.87A^{0}$ and energy of -1.7kcal/mol (d) Amino acid LYS 137 (π-H) distance $3.84A^{0}$ and energy of -0.7kcal/mol
Rutin
  Six hydrogen interactions are possible (a) Amino acid LEU 282 (H-donor) distance $3.44A^{0}$ and energy -0.7kcal/mol (b) Amino acid GLU 288 (H-donor) distance $2.80A^{0}$ and energy -4.1kcal/mol (c) Amino acid ASP 289 (H-donor) distance $3.06A^{0}$ and energy -2.3kcal/mol (d) Amino acid GLU 288 (H-donor) distance $2.75A^{0}$ and energy -1.5kcal/mol (e) Amino acid TRP 207 (H-acceptor) distance $3.13A^{0}$ and energy -1.3kcal/mol (f) Amino acid LYS 5 H-acceptor distance $2.80A^{0}$ and energy -1.7kcal/mol
Oleuropein

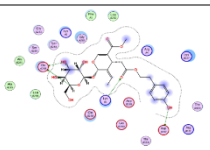
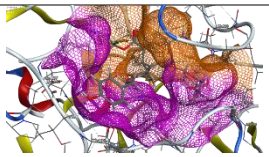
Three hydrogen interactions are possible (a) Amino acid GLU 288 (H-donor) distance 3.38A^0 and energy -1.0kcal/mol (b) Amino acid PHE 3 (H-donor) distance 2.63A^0 and energy -2.7kcal/mol (c) Amino acid TRP 207 (H-acceptor) distance 2.87A^0 and energy -1.8kcal/mol

Luteolin 7-rutinoside

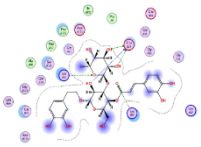
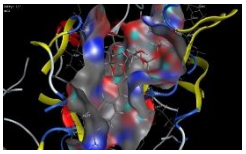
Five type of hydrogen interactions are possible (a) Amino acid LEU 287 (H-donor) distance 2.83A^0 energy of -0.7kcal/mol (b) Amino acid GLU 288 (H-donor) distance 2.93A^0 energy of -1.4kcal/mol (c) Amino acid GLU 288 (H-donor) distance 2.80A^0 energy of -3.4kcal/mol (d) Amino acid GLU 288 (H-donor) distance 3.00A^0 energy of -1.4kcal/mol (e) Amino acid ARG 4 (H-acceptor) distance 3.34A^0 energy of -1.2kcal/mol

Ligstroside

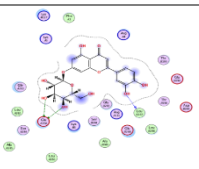
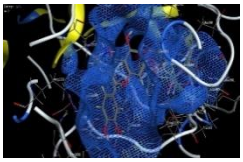
Four types of hydrogen interactions are possible (a) Amino acid GLU 288 (H-donor) distance 3.26A^0 energy of -0.8kcal/mol (a) Amino acid GLU 288 (H-donor) distance 2.87A^0 energy of -4.0kcal/mol (b) Amino acid ASP 197 (H-donor) distance 3.11A^0 energy -1.3kcal/mol (c) Amino acid LYS 5 (H-acceptor) distance 3.04A^0 energy -4.7kcal/mol

Verbascoside

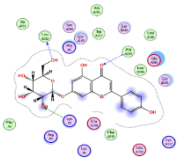
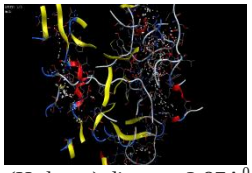
Five types of hydrogen interactions are possible (a) Amino acid GLU 14 (H-donor) distance 3.06A^0 energy of -1.5kcal/mol (b) Amino acid GLU 14 (H-donor) distance 2.75A^0 energy of -4.5kcal/mol (c) Amino acid GLU 14 (H-donor) distance 2.83A^0 energy of -2.0kcal/mol (d) Amino acid LYS 12 (H-acceptor) distance 3.23A^0 energy of -3.4kcal/mol (e) Amino acid LYS 97 (H-acceptor) distance 3.24A^0 energy of -1.2kcal/mol

Luteolin-7-glucoside

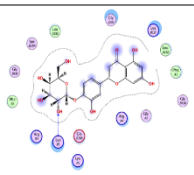
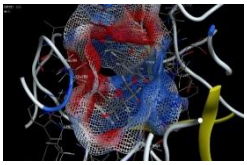
Three types of hydrogen interaction are possible (a) Amino acid GLU 288 (H-donor) distance 2.81A^0 energy of -4.4kcal/mol (b) Amino acid GLU 288 (H-donor) distance 3.14A^0 energy of -2.4kcal/mol (c) Amino acid LEU 287 (H-donor) distance 2.94A^0 energy of -1.0kcal/mol

Cosmosin

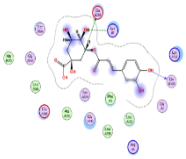
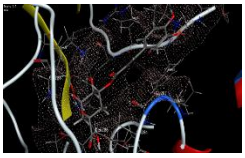
Three hydrogen interactions are possible (a) Amino acid LEU 282 (H-donor) distance 2.87A^0 energy of -1.2kcal/mol (b) Amino acid ALA 285 (H-acceptor) distance 3.41A^0 energy of -0.9kcal/mol (c) Amino acid LYS 5 (H-acceptor) distance 3.34A^0 energy of -0.5kcal/mol

Luteolin-4'-o-glucoside

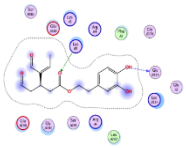
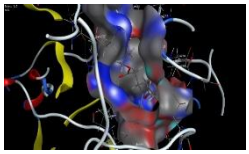
One type of hydrogen interaction is possible (a) Amino acid LYS 5 (H-acceptor) distance 3.29A^0 energy of -1.1kcal/mol

Chlorogenic acid

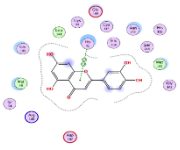
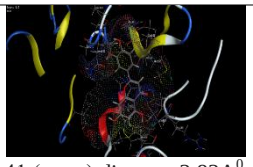
Three types of hydrogen interactions are possible (a) Amino acid GLU 288 (H-donor) distance 3.59A^0 energy of -0.7kcal/mol (b) Amino acid GLY 138 (H-donor) distance 3.06A^0 energy of -2.5kcal/mol (c) Amino acid LYS 5 (H-acceptor) distance 3.27A^0 energy of -0.6kcal/mol

Deacetoxyoleuropein aglycone

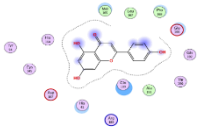
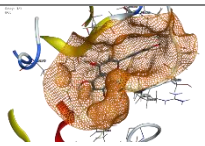
Two types of hydrogen interactions are possible (a) Amino acid GLY 138 (H-donor) distance 3.11A^0 energy of -2.4kcal/mol (b) Amino acid LYS 5 (H-acceptor) distance 3.06A^0 energy of -1.6kcal/mol

Leutolin

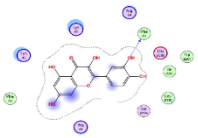
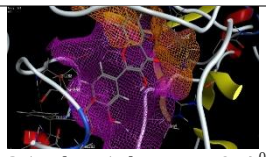
one type of hydrogen interaction is possible (a) Amino acid HIS 41 ($\pi - \pi$) distance 3.83A^0 energy of -0.0kcal/mol

Apigenin

No perceptible interactions, only electrostatics exist (Van der Waals)

Quercetin

one type of hydrogen interaction is possible (a) Amino acid PHE 3 (H-donor) distance 2.92A^0 energy of -1.7kcal/mol

4.4.2 Best Ligands of *Curcuma longa*

Curcumin, when docked with 6M2N, showed one hydrogen possible interaction with amino acid GLU C290 with distance of 2.83A^o and energy of -0.8 kcal/mol.

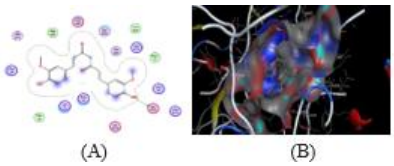
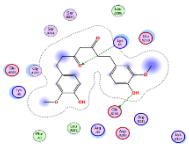
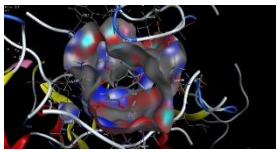
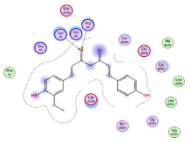
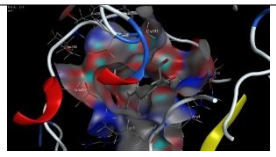
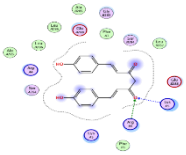
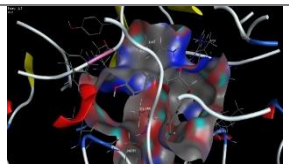


Fig.8. (A) Ligand interaction of 6M2N with Curcumin (B) 3D diagram of pocket 6M2N with Curcumin

Curcuma longa ligands gave the lower score as compared to major *Olea europaea* compounds. But these ligands have great potential to inhibit viral activity of SARS-nCOV2. The interactions of major ligands of *Curcuma longa* (other than curcumin) whom energies are from -7 to -6 are given in Table 9.

Table 9 Interaction of major ligands of Turmeric

Tetrehydrocurcumin
 
Two types of hydrogen interactions are possible (a) Amino acid GLU 290 (H-donor) distance 2.86A^0 energy -2.6kcal/mol (a) Amino acid LYS 5 (H-acceptor) distance 3.30A^0 energy -1.4kcal/mol
Demethoxycurcumin
 
Two types of hydrogen interactions are possible (a) Amino acid LYS A5 (H-accepter) distance 2.53A^0 energy of -1.3kcal/mol (b) Amino acid ARG B4 (H-accepter) distance 2.3A^0 energy of -1.2kcal/mol
Bidemethoxycurcumin
 
Two types of hydrogen interactions are possible (a) Amino acid LYS B5 (H-accepter) distance 2.1A^0 energy of -0.9kcal/mol (b) Amino acid LYS B4 (H-accepter) distance 1.9A^0 energy of -1.4kcal/mol

5 Discussion

To our knowledge, this kind of drug finding research on COVID-19 is limited. A number of barriers have been identified and out of these barriers, the most prominent one is mutation [36], as SARS-nCoV2 is RNA virus that mutates very quickly making its drug or vaccine less affective [37]. The present study focuses on finding potential drug of SARS-nCoV2 using docking study. The main protein target here is SARS-nCoV2 main protease (M^{pro}/ 3cl-protease) which is required for viral replication and maturation. By blocking this protein, the further replication of virus can be halted.

Major findings are neuzhenide from natural source of olive and curcumin from turmeric, which gave the top docking scores of -10 kcal/mol and -7.6 kcal/mol with M-protease of SARS-nCoV2 respectively, in comparison with other compounds that form stable protein-ligand complex with lowest energy by accurately fitting in active site of M-protease and forming the maximum hydrogen bonds. These findings are important because these compounds can be the potential inhibitors of SARS-nCoV2, as molecular docking predicts protein-ligand interaction and is used in computer aided drug designing.

Drugs which are currently recommended in COVID-19 (nelfinavir, remedsivir and hydroxychloroquine) show the ligand-protein interaction docking score of -9.18 kcal/mol, -6.3 kcal/mol and -5.7 kcal/mol respectively in different articles [38,39]. The ligands of olive and turmeric gave high docking score and are more stable in comparison to these recommended drugs.

The Neuzhenide, Oleuropein and Demethyloleoeuropein are nontoxic when their properties were checked from SwissADME and can be used without any harmful effects. Oleuropein has antiviral property and is currently used to treat infectious mononucleosis, epidemic jaundice, diarrheal disease, bovine rhinovirus infection, canine parvovirus infection and feline leukemia [40]. Pharmacological properties of Oleuropein include anti-irritant, antiangiogenic, anti-malignancy, antimicrobic and cytoprotective. Rutin, a flavonoid compound, has several biological activities like antiallergic, antitumor, reduce inflammation and antiangiogenic [41]. Luteolin 7-rutinoside has a number of different properties and the most promising ones are antiallergic, antimicrobial, antimutagenic and anticarcinogenic activities [42]. *Curcuma longa* compound Curcumin is a phytopolylphenol pigment, which blocks the formation of reactive-oxygen species and possesses antineoplastic and anti-inflammatory properties [43].

There are few limitations related to Lipinski's rule of drug likeness. As given in table 8 and 9, there are 6 ligands that do not follow Lipinski's rule, but their scores are between -10 and -7 kcal/mol while 24 ligands follow Lipinski's rule, scoring from -7 to -4 kcal/mol. But several articles reported that Lipinski's rule does not apply on natural products and semisynthetic natural drugs [44]. Furthermore, the recommended drug remedsivir and many other drugs that are currently being used in COVID-19 do not follow Lipinski's rule.

As research in this field is lacking and there is a desperate need to design an effective drug against COVID-19 in this pandemic, so Neuzhenide, Rutin, Oleuropein, Demethyloleoeuropein, Luteolin 7-rutinoside, Ligstroside, Verbascoside, Luteolin-7-glucoside, Curcumin, Tetrehydrocurcumin and Demethoxycurcumin can be the potential inhibitors of COVID-19 as they gave the best docking scores. As a result, these olive and turmeric ligands are recommended for future research.

6 Conclusions

In the current scenario of COVID-19 pandemic, where more than half million people died and more than 15 million people are affected till now, there is no approved drug against COVID-19. Computer-aided drug designing (CADD) can help to overcome this situation through ligand-protein interaction (docking) studies. The aim of this study was to examine compounds from olive and turmeric that can be used to inhibit SARS-nCoV2 by acting on one of its enzymes, Main protease (M-pro), which is essential for viral replication. Molecular docking results show that Neuzhenide, Demethyloleoeuropein, Rutin, Oleuropein, Luteolin 7-rutinoside, Ligstroside, Verbascoside, Luteolin-7-glucoside, Cosmosin, Luteolin-4'-o-glucoside, Curcumin, Tetrehydrocurcumin and Demethoxycurcumin gave the

lowest score from olive and turmeric and are the most recommended ones against COVID-19. These suggested inhibitors are necessary to be investigated in further research and clinical trials in order to determine their action against SARS-nCoV2.

Compliance with Ethical Standards

This manuscript required no ethical statement. Data was taken from public databases.

Conflict of Interest:

The authors declare no conflict of interest.

Acknowledgement:

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Authors Contribution:

Rashid Saif (RS) envisaged the idea, involved in critical thinking, analysis of data, editing, proofread and correspondence with journal. Muhammad Hassan Raza (MHR) involved in software features understanding, analysis of data, initial write-up and editing. Talha Rehman (TR) helping in data analysis, software features understanding and initial write-up. Muhammad Osama Zafar (MOZ) helping in data analysis, software features understanding and initial write-up.

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