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Terpenoids from *Centella asiatica*, a novel inhibitor against RNA-dependent-RNA polymerase activity of NSP12 of the SARS CoV-2 (COVID-19)

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Covid-19, the first case of which was reported in Wuhan (China) in December 2019 was found to be a strain of coronaviruses like SARS and MERS reported earlier. These viruses are positive strain RNA viruses composed of both structural as well as non-structural proteins. The enzyme RdRp (RNA dependent RNA polymerase) stands responsible for catalyzing the replication of this virus within the host cell. A disruption in the core catalytic subunit composed of nsp12, nsp7, and nsp8 may inhibit the replication of the same. Different drugs targeting different sites on the virus have been developed. In this context, some of the natural products of the plant *Centella asiatica* was lead for further drug development against the target proteins of RdRp protein (PDB ID: 6NUR) through molecular docking. These compounds are 2,3-dihydroxyurs-12-en-28-oic acid, corosolic acid and pomolic acid which are triterpenoids and have highest binding affinity against RdRp protein thereby arresting the viral replication. Several previous studies showed triterpenoids as pertinent mediators implicated in the *in vitro* immune response.

Keywords: *Centella asiatica*, Covid-19, Molecular docking, RdRp, Terpenoids.

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Introduction

The outbreak of an unknown cause leading to pneumonia in December 2019 took place in Wuhan (China); initially designated as 2019-nCoV was later identified as a new strand of coronaviruses. These viruses have been associated with infectious epidemics (Severe Acute Respiratory Syndrome CoV-SARS-CoV in 2000-2004 and Middle East Respiratory Syndrome-MERS in 2013) hazardous to mankind. According to the WHO, as of 11 November 2020 there are 296,496,809 confirmed cases and 5,462,631 fatalities worldwide[1-3].

Coronaviruses (CoVs) belong to the family Coronaviridae and order Nidovirales. The structural features of viruses belonging to the order Nidovirales include being positive sense single stranded RNA virus with 5'cap and 3'poly A tail envelope. The distinguishing characteristic of the same includes the presence of spikes projecting out from its surface[4,5].

Structural analysis of the genome of coronaviruses reveals its association with four major structural proteins: Spike (S) protein which is responsible for viral entry; Membrane (M) protein and Envelope (E) protein which assist in the assembly of the virus; Nucleocapsid (N) protein which is helically symmetrical nucleocapsid. The non-structural protein (undergoing transcription) part on the other hand is constituted by ORF3a, ORF1ab, ORF6, ORF10a, ORF7a, and ORF8. Cleavage of ORF1a and ORF1b yield a set of non-structural proteins carrying out processes such as viral transcription and replication. A total of ten ORFs are present in coronaviruses out of which ORF1ab encodes the replicase polyprotein 1 ab, the remaining however encode for viral structural proteins including ORF2 encodes spike; ORF3 encodes envelope; ORF4 encodes for membrane; ORF5 encodes nucleocapsid while the remaining code for other auxiliary proteins. These ORFs are found to encode the replicase proteins. These proteins, however, are encoded from the 5'- 3' end of the viral genome[5,6].

The non-structural part of the SARS-CoV2 viruses includes RdRp (RNA dependent RNA polymerases) and other proteases such as nsp3 and nsp5 out of which RdRp is categorised as a crucial viral enzyme for replication of RNA viruses within the host cell and is, therefore targeted in case of numerous viral

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infections. The RdRp active site is a highly conserved region encompassing the two surface-accessible and successive aspartates in a beta-turn structure[7].

This RdRp is also known as nsp12. The nsp12 structure mimics a 'cupped' right hand structure comprising of the palm (amino acids 466-511 and 565-699), finger (amino acids 180-465), and thumb (700-803) domains[8]. On infection of the host cell, this RdRp forms complexes with other factors and participates in genome replication machinery formation. This RdRp is said to initiate as well as govern RNA strand elongation that includes multiple nucleotide addition. Participation of nsp7 and nsp8 in this RdRp catalysed RNA replication machinery is of utmost importance. The nsp12 being the core catalytic unit of RdRp gets further stimulated by the cofactors nsp7 and nsp8 thereby, enhancing the RdRp catalysed RNA replication machinery. A drug designed to interact and thereby, suppress the activity of this RdRp protein or associated residues may prove beneficial against replication of Covid-19. Apart from coronaviruses, drugs developed against Zika Virus (ZIKV) and Hepatitis C virus (HCV) also target their RdRp[8-10].

Centella asiatica, popularly known as Gotu Kola is an ethnomedicinal plant belonging to the Apiaceae family and is found in countries having a warm climate. This plant is described as Mandukaparni by the Indian Ayurvedic system of medicine. Phytoconstituents of the plant includes pentacyclic triterpenes, saponins along with other primary constituents proven to show wound healing[11], anti-inflammatory[12], antioxidant[13], antibacterial[13], antitumor[13], anti-anxiety[14], antiviral[15], properties along with other beneficial uses. Commercial preparations of the same have proven beneficial in skin diseases, improving venous insufficiency of the lower limbs, wound healing[16] and anxiety. The skin conditions covered under its healing properties include psoriasis[13], leprosy[13], eczema[13], lupus[13], varicose ulcers[13], amenorrhea[11], fever[11], diarrhoea[13]. Apart from this, other conditions such as female genitourinary tract disease and cognition improvement are also covered under its properties. The active principles such as asiaticoside (I), asiatic acid (II), madecassoside (III), and madecassic acid (IV) contribute majorly to the above properties of the plant[16]. The secondary metabolites however are synthesized through isoprenoid pathway to produce hydrophobic triterpenoid structure. The medicinal properties of this plant analysed and thus utilized against the activity of Covid-19 virus include its antioxidant[13], anti-inflammatory[12], and antiviral properties[15]. Covid-19 being a virus is shown to cause inflammation and oxidative stress generation at its site of invasion. Therefore, the antioxidant, anti-inflammatory, and antiviral activities of this plant seem to prove beneficial for curing patients infected with Covid-19.

RdRp is the enzyme responsible for catalysing the replication of SARS CoV2 viral strain. The work done emphasizes the RdRp enzyme composed of nsp12 as the catalytic subunit which is composed of palm, thumb, and finger domains forming a complex with nsp7 and nsp8 forming the active binding pockets for CASTp via molecular docking. The phytoconstituents of the plant *C. asiatica* were docked against the respective PDBIDs of the RdRp enzyme. The best-docked inhibitors can further be used for preclinical trials to halt viral replication.

Materials and Methods

Physicochemical properties

The physicochemical properties of the selected compounds were predicted by freely available online SwissADME software. Toxicity is important to evaluate in drug designing as it helps in determining the toxic dose in animal model studies and lessens the number of animal model studies[17-19].

Molecular docking: Receptor and ligand preparation

The 3D structures of ~100 phytoconstituents from *C. asiatica* were downloaded from PubChem online server (which is freely available software). The structures were then converted into mol2 format with the help of Chem3D Ultra (another package of Chem Office and PDBQT file converted by the PyRx tool)[20-22].

Target selection and preparation

Earlier reported three-dimensional (3D) structure of the crystal structure of the nsp12 (nsp7 and nsp8) complex structure (PDB ID: 6M71, 6NUR, and 7BTF) was retrieved from RCSB PDB. The water molecules and co-crystallized ligands were deleted from the PDB file and polar hydrogen bonds were added[23,24].

Docking protocol

Before moving to the molecular docking, the topological analysis of the protein structures including active binding site/pocket was determined by using CASTp 3.0[25]. Some of the residues are responsible for predicted ligand-binding site in the protein where the ligand can reversibly bind. To determine the binding

mode and interaction of the selected compounds and target, docking studies were performed using AutoDock/vina[23,26]. However, other amino acid residues of the protein were providing correct orientation and confirmation[27]. The pdbqt files of the receptor protein and *C. asiatica* compounds along with the grid box getting at the active site of the receptor for compounds binding was done through Auto Dock GUI program. The grid size boundaries along the X, Y, and Z axis were scaled at 40 Å with a grid spacing of 1 Å to allow proper binding flexibility at the docked site. The output pdbqt files were therefore inscribed into a configuration (conf) file. The best-fitting conformation with respect to the receptor complex; the ligands were kept flexible while the receptor was treated as a rigid entity. Solutions of docking thus generated were clustered and only those with root mean square deviation (RMSD) value <1.0 Å were considered. The ligand's most stable conformation with respect to the receptor was assigned to the ligand with the lowest binding affinity.

Protein-ligand interaction studies

The interaction studies of the protein-ligand complexed file visualization were done by PyMOL software 1.3 version. This PyMOL software is shown to yield a high-quality 3D image of small molecules and proteins. Visualisation of the non-polar as well as polar (hydrogen bond) interactions between receptor and small ligand(s) was also done using the PyMOL software. The binding analysis of the 2D interaction on the other hand was done by Discovery Studio 2019 (BIOVIA)[26,27].

Results

***C. asiatica* phytoconstituent(s)**

C. asiatica phytoconstituents were used to target RdRp enzyme of SARS-COV-2 with the aim to discover COVID-19 drug. The flowchart for the used Computational approaches methodology to predict the binding affinity of *C. asiatica* plant's phytoconstituents against SARS COV-2 (RdRp protein) is described in (Fig. 1).

The reported literature was utilized to identify 140 phytocompounds of *C. asiatica* and a database was created of these compounds by downloading their structures from the PubChem database. All these 140 phytoconstituents of the *C. Asiatica* were analysed via molecular docking against the PDBIDs of the RdRp enzyme of Covid-19 (Table 1).

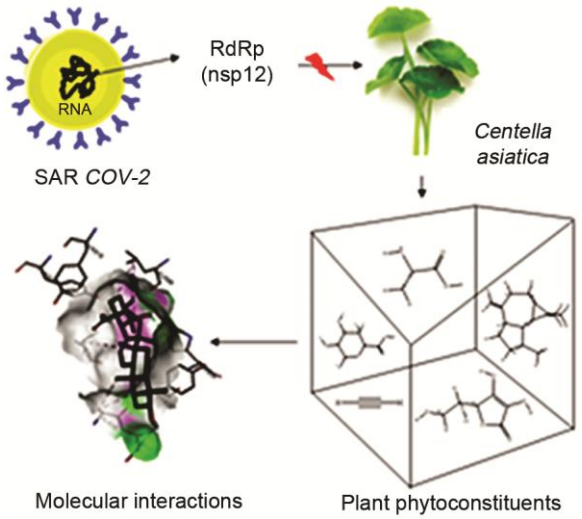

Fig. 1 — Methodology to predict the binding affinity of *Centella asiatica* plant's phytoconstituents against SAR COV-2 (RdRp protein) via Computational approaches.

The phytoconstituent of *C. asiatica* pharmacokinetics analysis was done by the Swiss ADME server where the molecular weight (MW) is a significant characteristic in a curative way of the drug action. The molecular weight of natural compounds from the plant *C. asiatica* ranged within ≤500. Therefore, as compared to those with high molecular weight; compounds with molecular weight lower than or equal to 500 Daltons is transported, diffuse and absorbed without any obstacle as per the RO5 rule. By compound analysis according to the RO5 rule; the next step is based upon the number of accepted hydrogen bonds (O and N atoms) from 0-10 and the number of donor's hydrogen bonds (NH and OH) 0-5 respectively within the Lipinski's allowed limit. Lipophilicity (log P) and topological polar surface area (TSPA) values are crucial properties for the estimate of the oral liability of drug molecules. According to the RO5 rule, the acceptable range of log P is 0-5; most of the compounds from *C. asiatica* plant range from 0.94-5.00 (≤5) which is within the acceptable limits and helps the drug to penetrate the bio-membrane. TPSA is precisely associated with the hydrogen bonding potential of the compound. *C. asiatica* phytoconstituents were found to possess the best drug-like properties (Table 2).

Docking studies

The prediction of the active binding site/pocket for (PDB ID: 6M71, 6NUR, and 7BTF) were shown using CASTp 3.0 server (Fig. 2). Molecular docking was done by AutoDock/vina using a rigid docking

Table 1 — List of phytoconstituents of the plant *Centella asiatica* analysed via molecular docking against the PDBIDs of the RdRp enzyme of Covid-19.

S. No	Name of the Phytoconstituent	Type of content	PubChem ID
1	Asiaticoside	triterpenic saponin	11954171
2	Brahmic acid	triterpenic acid	73412
3	Saponin	saponin	198016
4	Asiatic acid	triterpenic acid	119034
5	Brahmoside	triterpenic saponin	395762
6	Brahminoside	triterpenic saponin	395761
7	Mesoinositol	isomer of glucose	892
8	Centellose	oligosaccharide	NA
9	Glucose	monosaccharide	5793
10	Polyacetylene	polyacetylene	6326
11	α-pinene	monoterpene	440968
12	β-pinene	monoterpene	14896
13	Myrcene	monoterpene	31253
14	γ-terpinene	monoterpene	7461
15	Bornyl acetate	sesquiterpene	6448
16	α-copaene	sesquiterpene	19725
17	β-elemene	sesquiterpene	6918391
18	β-caryophyllene	sesquiterpene	1742210
19	Trans-β-farnesene	sesquiterpene	5281517
20	Germacrene-d	sesquiterpene	91723653
21	Bicycloelemene	sesquiterpene	56842786
22	Campesterol	triterpenic steroid	173183
23	Stigmasterol	triterpenic steroid	5280794
24	Sitosterol	triterpenic steroid	222284
25	unidentified terpenic acetate	triterpenic steroid	NA
26	Asiaticoside-a	triterpenic saponin	45356919
27	Asiaticoside-b	triterpenic saponin	74962739
28	6β-hydroxyasiatic acid	triterpenic acid	433986382
29	Terminolic acid	triterpenic acid	12314613
30	Aspartic acid	amino acid	5960
31	Glutamic acid	amino acid	33032
32	Leucine	amino acid	6106
33	Iso-leucine	amino acid	6306
34	Valine	amino acid	6287
35	Methionine	amino acid	6137
36	Lysine	amino acid	5962
37	Histidine	amino acid	6274
38	Tyrosine	amino acid	6057
39	Phenylalanine	amino acid	6140
40	Alanine	amino acid	5950
41	Threonine	amino acid	6288
42	Glycine	amino acid	750
43	Madecassic acid	triterpenic acid	73412
44	11-oxoheneicosanyl cyclohexane	polyacetylene	129882177
45	Dotriacont-8-en-1-oic acid	polyacetylene	NA
46	3-o-*α-l- arabinopyranosyl+ 2α, 3β, 6β, 23-α tetrahydroxyurs-12-ene-28-oic acid	triterpenoid glycoside	NA
47	Centellasaponin b	triterpene oligoglycoside	85411973
48	Centellasaponin c	triterpene oligoglycoside	85348461
49	Centellasaponin d	triterpene oligoglycoside	101103168
50	Asiaticoside b	triterpenic saponin	91618002

(*Contd*.)

Table 1 — List of phytoconstituents of the plant *Centella asiatica* analysed via molecular docking against the PDBIDs of the RdRp enzyme of Covid-19. (*Contd*.)

S. No	Name of the Phytoconstituent	Type of content	PubChem ID
51	Sceffoleoside a	triterpenic saponin	NA
52	Linalool	monoterpnene	6549
53	Neophytadiene	diterpene	10446
54	α-humulene	sesquiterpene	5281520
55	(e)-b-farnesene	sesquiterpene	5281517
56	δ-cadinene	sesquiterpene	441005
57	epi-bicyclosesquiphellandrene	sesquiterpene	91747125
58	Epiglobulol	sesquiterpene	11858788
59	1-decano	sesquiterpene	8174
60	β-acoradiene	sesquiterpene	20055537
61	Pectin	polysaccharide	441476
62	Arabinogalactan	polysaccharide	24847856
63	Quercetin	flavonoid	5280343
64	Kaempferol	flavonoid	5280863
65	Asiaticoside c	triterpenoid glycoside	101103169
66	Asiaticoside d	triterpenoid glycoside	102212084
67	Asiaticoside e	triterpenoid glycoside	102212085
68	Asiaticoside f	triterpenoid glycoside	53317001
69	Scheffuroside b	triterpenoid	102212086
70	α-thjuene	monoterpene	NA
71	α-terpinene	monoterpene	7462
72	ρ-cymene	monoterpene	10908223
73	Limonene	monoterpene	22311
74	Terpinolene	monoterpene	11463
75	Terpinen-4-ol	monoterpene	11230
76	Methyl thymol	monoterpene	6989
77	Pulegone	monoterpene	442495
78	Methyl carvacrol	monoterpene	80790
79	3-nonen2-one	monoterpene	NA
80	Menthone	monoterpene	26447
81	Chrysanthenyl acetate	monoterpene	162747
82	Bornyl acetate	monoterpene	6448
83	Camphene	monoterpene hydrocarbons	6616
84	α-phellandrene	monoterpene hydrocarbons	443160
85	Bicycloelemene	sesquiterpene	56842786
86	Aromadendrene	sesquiterpene	91354
87	Allo-aromadendrene	sesquiterpene	42608158
88	Germacrene d	sesquiterpene	5317570
89	γ-curcumene	sesquiterpene	12304273
90	Bicyclogermacrene	sesquiterpene	13894537
91	Germacrene a	sesquiterpene	5835162
92	Germacrene b	sesquiterpene	5281519
93	Caryophyllene oxide	sesquiterpene	1742210
94	Humulene epoxide	sesquiterpene	5352470
95	Isopauthenol	sesquiterpene	NA
96	Spathulenol	sesquiterpene	92231
97	Viridiflorol	sesquiterpene	11996452
98	Mintsulfide	alcohol sulphide sesquiterpenoid	14564587
99	Arabinogalactan	polysaccharide	24847856
100	Urosolic acid	triterpenic acid	64945
101	Pomolic acid	triterpenic acid	382831
102	2α, 3αdihydroxyurs-12-en-28-oic acid	triterpenic acid	155934

(*Contd*.)

Table 1 — List of phytoconstituents of the plant *Centella asiatica* analysed via molecular docking against the PDBIDs of the RdRp enzyme of Covid-19. (*Contd*.)

S. No	Name of the Phytoconstituent	Type of content	PubChem ID
103	3-epi-maslinic acid	triterpenic acid	25564831
104	Corosolic acid	triterpenes polyphenol	6918774
105	Rosmarinic acid	triterpenes polyphenol	5281792
106	2α, 3β, 20, 23- tetrahydroxyurs-28-oic acid	triterpenoid glycoside	NA
107	Cadiyenol	polyacetylene c	17757599
108	8-acetoxycentellynol	c15-polyacetylene	NA
109	Chlorogenic acid	phenol	1794427
110	3,5-di-o-caffeoyl quinic acid	phenolic acid	6474310
111	1,5-di-o-caffeoyl quinic acid	phenolic acid	NA
112	3,4-di-o-caffeoyl quinic acid	phenolic acid	NA
113	4,5-di-o-caffeoyl quinic acid	phenolic acid	NA
114	Kaempferol-3-o-b-d-glucoside	flavonoid	135653722
115	Quercetin-3-ob-d-glucoside	flavonoid	NA
116	Centellin	NA	NA
117	Asiaticin	NA	NA
118	Centellicin	NA	NA
119	2α, 3β, 23-trihydroxyurs-20-en-28-oic acid	triterpenic saponin	NA
120	2α, 3β, 23-trihydroxyurs-20-en-28-oic acid oα-l-rhamnopyranosyl- (1→4) - o-β-dglucopyranosyl-(1→6)-o-β-d- glucopyranosyl ester	triterpenic saponin	NA
121	Ascorbic acid	vitamin tetraterpene	54670067
122	β-carotene	vitamin tetraterpene	5280489
123	Castilliferol	flavonoid polyphenol	10526707
124	Castillicetin	flavonoid polyphenol	102394640
125	Isochlorogenic acid	flavonoid polyphenol	6436237
126	Tannin	tanin	NA
127	Phlobatannin	tanin	NA
128	Saponin	saponin	198016
129	Terpenoid	terpenoid	5281400
130	Ouabain (Cardiac Glycoside)	triterpene	439501
131	Flavonoid	flavonoid	NA
132	Alkaloid	alkaloid	NA
133	23-o-acetylmadecassoside	triterpene glycoside	NA
314	23-o-acetylasiaticoside b (oleanane)	triterpene glycoside	NA
135	Sitosterol 3-o-β-glucoside	triterpenic steroid	NA
136	Stigmasterol 3-o-β-glucoside	triterpenic steroid	NA
137	Querectin-3-o-β-d-glucuronide	flavonoid glycoside	NA
138	α-copaene	sesquiterpene	19725
139	β-elemene	sesquiterpene	6918391
140	β-caryophyllene	sesquiterpene	5281515

Table 2 — Physicochemical properties of selected natural compounds

Compound	Molecular weight	miLogP	natoms	H-bond acceptor	H-bond donor	nVio	nrotb
Ouabain (Cardiac Glycoside)	584.65	2.67	41	12	8	No (3)	4
2α, 3α dihydroxyurs-12-en-28-oic acid	472.70	3.13	34	4	3	Yes (1)	1
3-epi-maslinic acid	472.70	3.31	34	4	3	Yes (1)	1
Castillicetin	464.38	1.83	34	10	6	Yes (1)	5
Corosolic acid	472.70	3.60	34	4	3	Yes (1)	1
Pomolic acid	472.70	3.65	34	4	3	Yes (1)	1
Terminolic acid	504.70	3.05	36	6	5	Yes (1)	2
β-carotene	536.87	7.89	40	0	0	No (2)	10

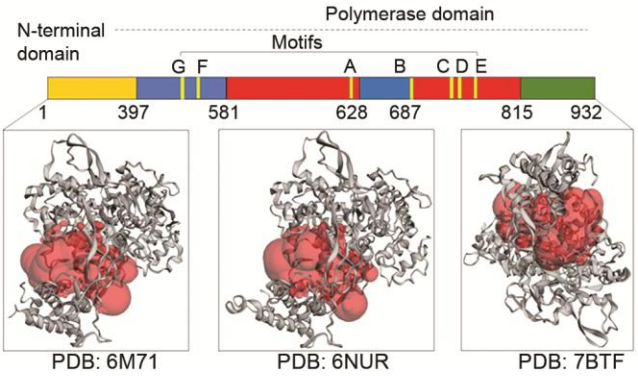

Fig. 2 — Structural representation of the SARS COV2 RdRp (nsp12) protein: An overall representation showing the nsp12 protein sequence (1-932) Linear schematic divided in domain, here thumb (green), palm (red) and fingers (blue) subdomains and the motifs (A-F) are shown in (yellow). The cartoon representation of the nsp12 (nsp7 and nsp8) complex structure (PDB ID: 6M71, 6NUR and 7BTF) inside the dot represented in (red) predicting the active binding pockets by CASTp.

method which was used because of the finding inhibitor and each step and binding must be examined. The grid box getting at the active site of the receptor for compounds binding was done through Auto Dock GUI program. Grid was generated, the centre of the grid box PDB ID: 6M71 (X-115.178, Y-119.032, and Z-121.999) else the dimensions of the grid box (X-38, Y-34, and Z-76); PDB ID: 6NUR (X-148.869, Y-150.808, and Z-145.436) else the dimensions of the grid box (X-82, Y-84, and Z-88) and PDB ID: 7BTF(X-129.523, Y-129.748, and Z-135.794) else the dimensions of the grid box (X-40, Y-40, and Z-42) for the prepared proteins. For the crystal structure of the nsp12 (nsp7 and nsp8) complex, the grid was generated around active sites. The docked PDB ID: 6NUR selected top hits and PubChem IDs: 155934, 6918774, and 382831. Terpenoids (155934, 6918774, and 382831) has a docking score -9.4 kcal/mol and against PDB ID: 6NUR (Fig. 3).

Protein-ligand complex interaction

After docking analysis, the protein-ligand complex file analysing the interacting protein binding sites (amino acid), the phytoconstituents of *C. asiatica* against SARS COV-2 (RdRp) PDB ID: 6NUR reveals, all the ligands binding active sites (amino acid residues) occupy active pockets of the proteins (Fig. 2). In this study of ligand-receptor interactions, SARS COV-2 (RdRp protein) target protein is having the highest interaction (Fig. 4). The ligand-receptor complex resolves the important challenge for

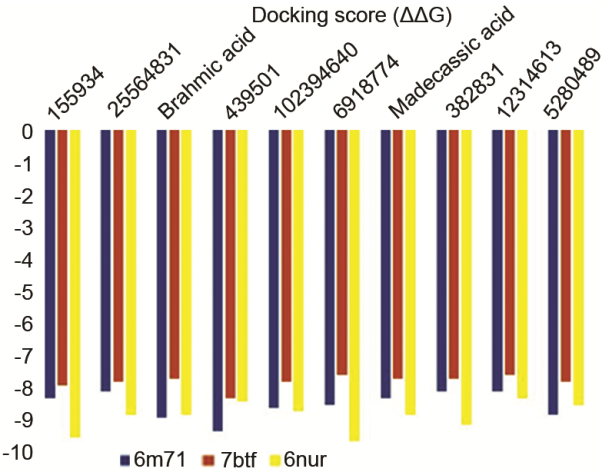

Fig. 3 — Molecular docking (AutoDock Vina) selected top 10 hit: In docking analysis calculated top binding energies (docking scores) of natural compounds against PDB ID: 6m71 in (blue), 7btf in (maroon) and 6nur in (yellow). The description of the PubChem IDs: 155934 (2,3-Dihydroxyurs-12-en-28-oic acid), 25564831 (3-epi-maslinic acid), 439501 (Ouabain), 102394640 (Castillicetin), 6918774 (Corosolic acid), 382831 (Pomolic acid), 12314613 (Terminolic acid) and 5280489 (β-carotene).

which residues are significant for protein stabilization (Table 3).

The compounds including 2, 3-dihydroxyurs-12-en-28-oic acid, corosolic acid and pomolic acid were found to be potential targets against the RdRp protein. The structures of these compounds are given in (Fig. 5).

Discussion

The outbreak of pneumonia that took place in December 2019 in Wuhan (China) and later came to be known as SARS-CoV-2 or Covid-19 ruled in the whole world within a short time. This disease turned out to be so infectious that in no time it was declared to be pandemic i.e., health hazard for mankind across the globe. Patients who died as a result of this infection; pathological studies of the same revealed bilateral diffuse alveolar damage (DAD) along with cellular fibro myxoid exudates. The presence of this DAD in samples therefore, indicates the presence of acute respiratory distress syndrome (ARSD); suggesting increased cytokine levels via janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway that may contribute to the progression of Covid-19. Drugs or phytoconstituents targeting and therefore inhibiting this cytokine storm are employed for treatment against Covid-19[28]. Oxidative stress leading to the formation of reactive oxygen species (ROS) when in

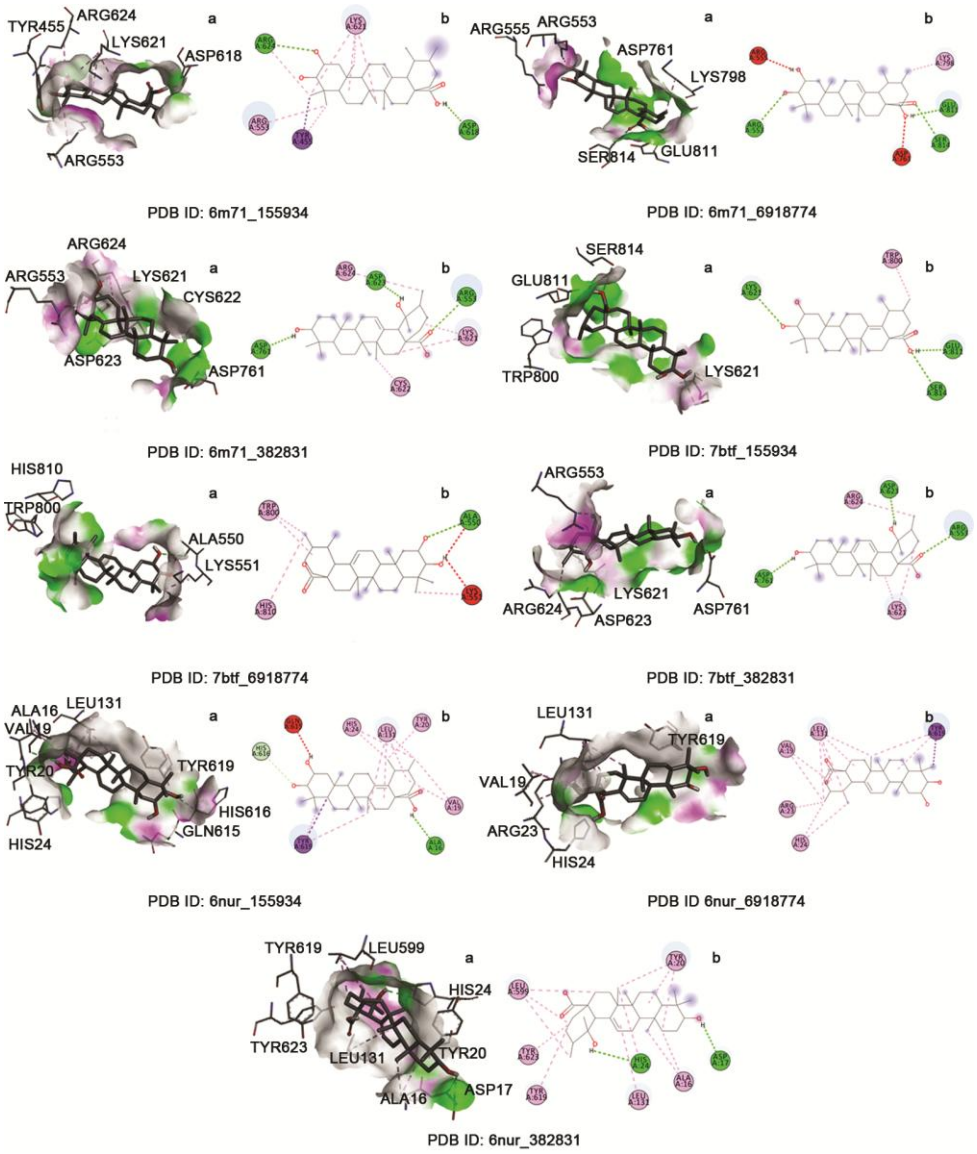

Fig. 4 — Docked pose of rigid ligand docking of RdRp (nsp12) protein, a) showing molecular interactions- hydrogen and hydrophobic bonds as green and pink/purple dashed lines, respectively; b) 2D plot of interactions between Ligand Receptor generated by BIOVIA.

Table 3 — Detailed molecular interactions obtained following the rigid ligand docking of PDB ID: 6m71, 6nur, and 7btf.

Protein ligand	Interacting residues	Category	Type
	TYR455	Hydrophobic	Pi-Alkyl
6M71_155934	ARG553	Hydrophobic	Pi-Sigma
	ASP618	H-Bond	Conventional
	LYS621	Hydrophobic	Pi-Alkyl
	ARG624	H-Bond	Conventional
	ARG553	H-Bond	Conventional
6M71_6918774	ARG555	Donor	Unfavourable
	ASP761	Donor	Unfavourable
	LYS798	Hydrophobic	Pi-Alkyl
	GLU811	H-Bond	Conventional
	SER814	H-Bond	Conventional

(*Contd*.)

Table 3 — Detailed molecular interactions obtained following the rigid ligand docking of PDB ID: 6m71, 6nur, and 7btf. (*Contd*.)

Protein ligand	Interacting residues	Category	Type
	ARG553	H-Bond	Conventional
6M71_382831	LYS621	Hydrophobic	Pi-Alkyl
	CSY622	Hydrophobic	Pi-Alkyl
	ASP623	H-Bond	Conventional
	ARG624	Hydrophobic	Pi-Alkyl
	ASP761	H-Bond	Conventional
7BTF_155934	LYS621	H-Bond	Conventional
	TRP800	Hydrophobic	Pi-Alkyl
	GLU811	H-Bond	Conventional
	SER814	H-Bond	Conventional
7BTF_6918774	ALA550	H-Bond	Conventional
	LYS551	Donor	Unfavourable
	TRP800	Hydrophobic	Pi-Alkyl
	GLU810	Hydrophobic	Pi-Alkyl
7BTF_382831	ARG553	H-Bond	Conventional
	LYS621	Hydrophobic	Pi-Alkyl
	ASP623	H-Bond	Conventional
	ARG624	Hydrophobic	Pi-Alkyl
	ASP761	H-Bond	Conventional
	ALA16	H-Bond	Conventional
6NUR_155934	VAL19	Hydrophobic	Pi-Alkyl
	TYR20	Hydrophobic	Pi-Alkyl
	HIS24	Hydrophobic	Pi-Alkyl
	LEU131	Hydrophobic	Pi-Alkyl
	GLN615	Donor	Unfavourable
	HIS616	Carbon H-Bond	Conventional
	TYR619	Hydrophobic	Pi-Sigma
	VAL19	Hydrophobic	Pi-Alkyl
6NUR_6918774	ARG23	Hydrophobic	Pi-Alkyl
	HIS24	Hydrophobic	Pi-Alkyl
	LEU131	Hydrophobic	Pi-Alkyl
	TYR619	Hydrophobic	Pi-Sigma
	ALA16	H-Bond	Conventional
6NUR_382831	ASP17	Hydrophobic	Pi-Alkyl
	TYR20	Hydrophobic	Pi-Alkyl
	HIS24	H-Bond	Conventional
	LEU131	Hydrophobic	Pi-Alkyl
	LEU599	Hydrophobic	Pi-Alkyl
	TYR619	Hydrophobic	Pi-Alkyl
	TYR623	Hydrophobic	Pi-Alkyl

excess is said to cause oxidation of proteins, DNA, and lipids that may later act as damage-associated molecular patterns (DAMPs) thereby fostering inflammation and tissue damage. A continuation of the same may result in viral replication that may facilitate oxidative stress[29-31]. The invasion of the SARS-CoVs-2 within the human respiratory tract causes inflammation via the JAK-STAT pathway apart from causing the generation of ROS as a result of oxidative stress generated in response to the viral invasion.

Using *in silico* methods, by molecular docking the authors investigated the *C. asiatica* phytoconstituents against RNA-Dependent RNA Polymerase of Coronavirus[9,10]. *C. asiatica* is an ethnomedicinal plant consisting of saponins, pentacyclic triterpenes, and other primary constituents proven effective against inflammation, wound healing, anxiety along with other medicinal properties[11,13,14,16]. The phytoconstituents of the plant *C. asiatica* (that are reported to possess anti-inflammatory, antioxidant, and antiviral properties amongst others) were analyzed to inhibit different pathways followed by the Covid-19 thereby, inhibiting its replication within the host and therefore terminating its impact upon the same. This article emphasizes the impacts of different

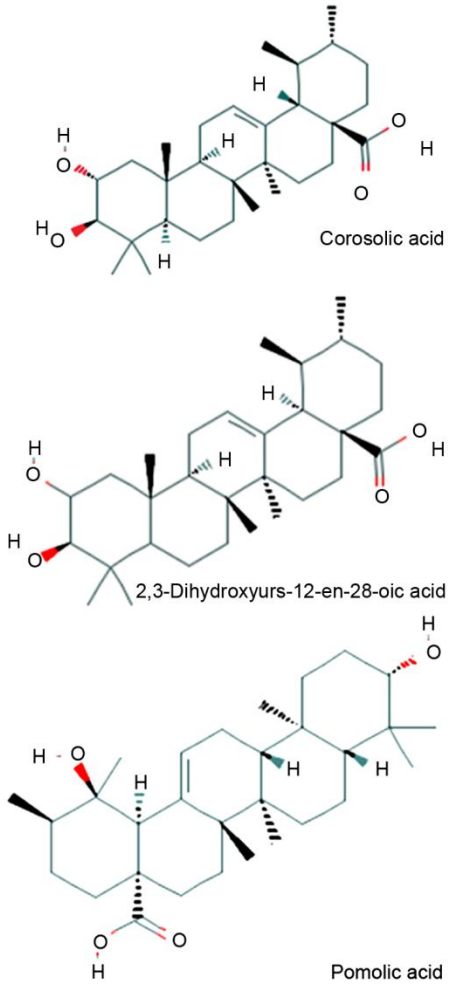

Fig. 5 — Structure of potential compounds against the RdRp protein.

phytoconstituents of the plant *C. asiatica* proven effective against the PDBIDs of the protein target RdRp enzyme in the SARS-CoV-2 using molecular docking[7,8]. Before proceeding with the process of molecular docking, predicting the best binding pocket via CASTp 3.0 server for appropriate ligand binding affinity through docking analysis using Auto Dock vina was done[21]. Post the docking procedure, the result analysis of the same led to the selection of top hits PubChem IDsi.e. 155934, 6918774, and 382831 based on high affinity. These natural compounds i.e.2, 3-dihydroxyurs-12-en-28-oic acid, corosolic acid and pomolic acid (155934, 6918774, and 382831) are terpenoids whose docking score is above -9.0 kcal/mol and against PDB ID: 6nur. For a drug, a good bioavailability is more likely for compounds following Lipinski's rule of five including properties such as ≤10 rotatable bonds and TPSA of ≤140Å, miLogP, number of atoms, and molecular weight. All the physicochemical properties of these terpenoids (2,3-Dihydroxyurs-12-en-28-oic acid, corosolic acid and pomolic acid), have been shown to satisfy the above and therefore are expected to show good bioavailability[17,20]. The ligand-receptor complex structure revealed the interactions between target proteins (RdRp). This study is challenging for the substantial steadiness of the protein which reveals the crucial role of significant amino acid modification of protein conformation[26,27].

The phytoconstituents including 2, 3-dihydroxyurs-12-en-28-oic acid, corosolic acid and pomolic acid were found to potential target against the RdRp protein and therefore it inhibits the effect of the enzyme thereby, arresting the replication of SARS-CoV-2 or Covid-19. Therefore, this plant proves to be an effective remedy in treating patients infected with Covid-19. These studies, therefore, establish evidence for further *in-vitro* and *in-vivo* investigations.

Conclusion

The study includes targeting the RdRp enzyme with phytoconstituents from the plant *C. asiatica* via molecular docking. The analysis of docking results revealed three phytoconstituents (terpenoids) 2, 3-dihydroxyurs-12-en-28-oic acid, corosolic acid and pomolic acid that might arrest the RdRp enzyme. Therefore, it can be concluded that *C. asiatica* may be used in the treatment against Covid-19.

Conflict of interest

There is no conflict of interest.

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