

# Identification of Bioactive Phytoconstituents from the Plant *Euphorbia hirta* as Potential Inhibitor of SARS-CoV-2: an *In-Silico* Approach

Ghanshyam Parmar<sup>1</sup> , Ashish Shah<sup>1,\*</sup> , Sapan Shah<sup>2</sup> , Avinash Kumar Seth<sup>1</sup> 

<sup>1</sup> Drug Discovery Lab, Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara, Gujarat, India

<sup>2</sup> Department of Pharmaceutical Chemistry, Priyadarshini J L College of Pharmacy, Nagpur, Maharashtra, India

\* Correspondence: [shah\\_ashishpharmacy@yahoo.co.in](mailto:shah_ashishpharmacy@yahoo.co.in) (S.S.);

Scopus Author ID 57214919105

Received: 10.05.2021; Revised: 4.06.2021; Accepted: 6.06.2021; Published: 9.06.2021

**Abstract:** Currently, the entire globe is under the deadliest pandemic of Covid-19 caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). At present, no specific treatment is available to combat COVID-19 infection. *Euphorbia hirta* (Euphorbiaceae) have been reported for a variety of biological activities, including antiviral. The present investigation aimed to identify potential phytoconstituents of the plant *E. hirta* from the category flavonoids and coumarins against the SARS-CoV-2 using in silico approach. The molecular docking studies were performed using two different targets of SARS-CoV-2, namely Main protease (M<sup>Pro</sup>; PDB ID: 6M2N) and RNA-dependent RNA polymerase (RdRp; PDB ID: 7BW4). Based on the molecular docking study in comparison with standard drug, four compounds, namely Euphorbianin, Quercetin, 3-o-alpha-rhamnoside, Isoquercitrin, and rutin, were screened against the target M<sup>Pro</sup>. Three phytoconstituents, euphorbianin, myricetin, and rutin, were screened against the target RdRp. In the *in silico* toxicity studies of screened phytoconstituents, except myricetin all were predicted safe. Results of euphorbianin and rutin were found more interesting as both compounds had high binding affinity against both targets. Finally, we want to conclude that euphorbianin, quercetin 3-o-alpha-rhamnoside, isoquercitrin, and rutin could be further explored rapidly as they may have the potential to fight against COVID-19.

**Keywords:** in silico study; *Euphorbia hirta*; flavonoids; coumarins; M<sup>Pro</sup>; RdRp.

© 2021 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

At the beginning of 2020, the emergence of a global public health emergency was noted due to novel coronavirus disease 2019 (COVID-19). Later, it was determined that Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the primary cause of COVID-19 [1,2]. As of 10th May 2021, the virus has caused 3 288 455 deaths and 157 973 438 confirmed cases globally [3]. SARS-CoV-2 is a single-stranded RNA virus that belongs to the genus Beta coronavirus. This group also contains SARS-CoV and MERS-CoV, responsible for triggering the pneumonia epidemic in 2003 and 2012, respectively [4,5]. COVID-19 infection can lead to numerous respiratory, hepatic, enteric, and neurological conditions. The SARS-CoV-2 is primarily transmitted through respiratory droplets, aerosol particles, and personal contact [6]. It can enter into respiratory cells by coupling its spike protein to the angiotensin-converting enzyme 2 (ACE-2) receptors present over the pneumocytes in the lungs, which then induces

the activation of inflammatory and immune reactions. Physiological response to these reactions causes tachypnea, increased cough production, and a rise in body temperature [7].

At present, no specific treatment is available to combat COVID-19 infection. Existing treatment protocols mainly involve using certain repurposed medications, such as Chloroquine/ Hydroxychloroquine, Lopinavir/ Ritonavir, Azithromycin, and Umifenovir. Additionally, certain drugs like, Remdesivir, Favipiravir, and Tocilizumab are currently in the clinical trial phase and are thought to cure COVID-19 [5,8,9]. Besides these synthetic drug products, several vaccines are also in the clinical trial phase, and it will probably take a year or more for these therapies to be launched in the market for community people. Meanwhile, an alternative to testing existing antiviral natural/ herbal products could be explored to find a cure for the current pandemic. Also, according to a World Health Organization (WHO) report, 80% of people in developing countries rely on herbal remedies for their health management [10].

The family Euphorbiaceae is unique in that it contains highly reputed plants useful in various diseases. This genus has diverse chemical entities with a lot of structural variation sources. Such plants like Amla (*Embelica officinalis*), Bhoiamli (*Phyllanthus fractus*), Arendmul (*Racinus communis*), Dudhi (*Euphorbia hirta*) and Ratidudhi (*Euphorbia thymifolia*), etc., are medicinally valuable in the treatment of some chronic disease such as diabetes, hemorrhoid asthma inflammation, etc. [11]. Complementary and alternative medicine (CAM) covers a heterogeneous spectrum of ancient to new-age approaches that intend to prevent or treat disease. A traditional Indian system of medicine has represented a few medications from the traditional indigenous plants in the treatment of liver disease [12]. The *Euphorbia hirta* L (Euphorbiaceae), commonly known as dudheli in the vernacular language (Gujarati), commonly grows widely in most parts of India and other tropical countries, especially on roadsides and on wasteland [13, 14]. The *E. hirta* (EUH) is amply found as a weed plant in roadside and open grasslands. It is erect, slender plant or sometimes found diffused through the soil, leaves are opposite, elliptical, or lanceolate, with a toothed margin. The flowers are cymes, smaller in size [15]. The milky white juice is exhausted upon cutting of stem [16,17]. Traditionally *E. hirta* is used in respiratory diseases like asthma, bronchitis, and hay fever, due to its analgesic and anti-inflammatory activity [10, 18].

Flavonoids are termed polyphenolic compounds present in the plant parts, which own different biological activities [19]. Based on the literature survey, many flavonoids have been seen as effective against infectious viral diseases by restricting the growth of these deadly viruses at a molecular level [20]. The mechanism behind these antiviral properties of flavonoids is inhibiting molecular receptors and enzymes and interfering with viral replication and translation process [21]. COVID-19 main protease (Mpro) and RNA-dependent RNA polymerase (RdRp) play a vital role in viral replication, translation, and transcription processes in Covid-19. Replication of SARS-CoV-2 could be blocked by targeting the main protease and RdRp. However, other targets are important for the life-cycle of various, but due to vital role in viral replication, translation and transcription process both targets are front line choice for the researchers [22,23]. In this study, we performed in silico analysis of phytoconstituents from the category flavonoids and coumarins, obtained from the plant *E. hirta* to find potential candidates against the SARS-CoV-2. The phytoconstituents are screened based on molecular docking, drug-likeness, and *in silico* toxicity evaluation.

## 2. Materials and Methods

### 2.1. Preparation of ligand library.

Twenty-seven different phytoconstituents of *E. hirta* from the class flavonoid and coumarins reported in various literature were used to prepare the ligand library [24]. All structures were downloaded in the SDF format from the PubChem database. The LigPrep module was used for the preparation of the library. The geometry of all structures was optimized using the OPLS3 force field, and their specific chirality was retained; the generation of tautomer was restricted to 32.

### 2.2. Drug-likeness screening and *in silico* toxicity prediction of phytoconstituents.

All the phytoconstituents were screened for their drug-likeness properties. The drug-likeness properties were evaluated using the Lipinski rule of five. Additionally, other parameters like no rotatable bond, GI absorption, and LogS were also predicted. The evaluation of drug-likeness properties was done using the Schrodinger QuikProp module, where the LigPrep file was used as input. The *In silico* toxicity prediction of the finally screened compound (based on docking and drug-likeness) was done using VEGA-QSAR. The various toxicological properties like mutagenicity, carcinogenicity, skin sensitization etc., were predicted

### 2.3. Protein preparation and receptor grid generation.

Our study selected two proteins of SARS-CoV-2, namely main protease (M<sup>PRO</sup>) and RNA-dependent RNA polymerase (RdRp). Both proteins are essential for the viral life cycle. Inhibition of these proteins may lead to blockage of further viral life cycle and viral growth. The crystal structures of both protein M<sup>PRO</sup> and RdRp were downloaded from the protein data bank in PDB format. Protein pre-preparation includes assigning bond orders, the addition of formal charges, the addition of hydrogen atoms, and missing chain residues added. The water molecules beyond 5Å distance from the hetero atom were removed, and a possible ionization state was generated. Finally, after pre-preparation, proteins were energy minimized. The receptor grid was generated after energy minimization. For M<sup>PRO</sup> and RdRp, the grid dimensions of protein were grid box dimension around the centroid of ligand was 10Å<sup>o</sup> x 10Å<sup>o</sup> x 10Å<sup>o</sup>.

### 2.4. Molecular docking studies.

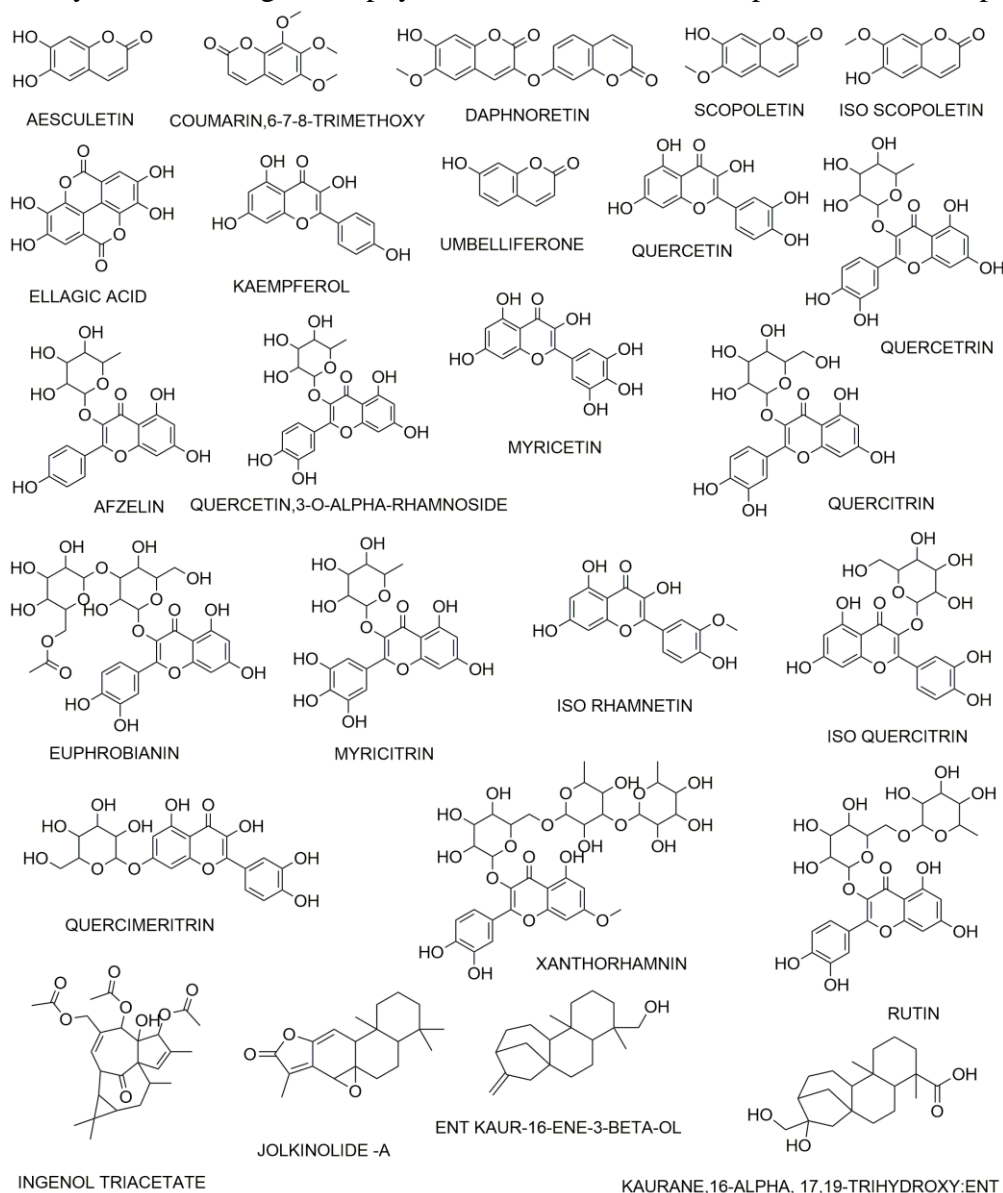
To find ligand-protein interaction and binding affinity, we performed molecular docking studies using the glide module of Schrodinger. The glide docking of selected phytoconstituents was carried using both the protein receptor grid and LigPrep file. The docking calculations were performed in extra precisions (XP) mode. Docking scores of all compounds and ligand-receptor interactions were collected upon completion of docking protocol. The docking methodology was validated by removing co-crystallized ligand and redocked into the active site.

## 3. Results and Discussion

There is an urgent need for therapeutics for controlling coronavirus infections [25]. Replication of SARS-CoV-2 could be blocked by targeting the main protease (3CL<sup>PRO</sup> or

M<sup>Pro</sup> [26] and crucial replicase RNA-dependent RNA polymerase (RdRp) [27]. Traditional therapeutics have shown valuable effects from usage in patients infected with novel SARS-CoV-2 [28]. *Euphorbia hirta* extracts were shown significant antiretroviral activities on the MT4 human T lymphocyte cell line [29]. Therefore, the present study aimed to screen active chemical constituents of *Euphorbia hirta* to highly conserved proteins, M<sup>Pro</sup> and RdRp of SARS-CoV-2, therefore we performed molecular docking studies of all phytoconstituents of *E. hirta* followed by the study of interacting amino acid residues. Selected phytoconstituents showing best fit are further evaluated for pharmacokinetics and toxicity results to propose them as potential drug candidates.

All the prepared ligands (Figure 1) were docked (XP module) on the prepared proteins (Main protease, PDB ID: 6m2n) and (RdRp, PDB ID: 7BW4) successfully, and the XP docking score was analyzed. The XP docking score for all the ligands is listed in Table 1 and Table 2 and their interactions with amino acid residues. Visual examination of the computationally docked optimal binding poses of chemical constituents of *E. hirta* on 6m2n and 7BW4 revealed the significant involvement of various types of interactions viz. hydrogen bonding and hydrophobic interactions, including ( $\pi$ - $\pi$  stacking,  $\pi$ -cation, and  $\pi$ - $\sigma$ ) and charged interactions in the stability of the binding of the phytoconstituents to the main protease and RdRp.



**Figure 1.** Two-dimensional structures of phytoconstituents from *E. hirta*.

### 3.1. Molecular docking of selected phytoconstituents with main protease (6m2n).

All the 26 chemical compounds were docked to the active site of 6m2n. The docking score of all selected phytoconstituents was compared with standard drug lopinavir. As compared to the standard four compounds (Table 1, Figure 2) euphrobianin, Quercetin, 3- $\alpha$ -rhamnoside, Isoquercitrin and rutin had a higher binding affinity.

It was observed that euphrobianin had shown the highest binding energy value of  $-8.4$  kcal/mol forming various H-bonded and nonbonded interaction contact. The ligand is properly positioned into the binding pocket constructed by polar LEU 141D, ASN 142D, GLY143D, SER144D, GLU166D, ARG188D with hydrophobic MET 165D ( $\pi$ -alkyl), CYS 145D ( $\pi$ -sulfur), HIS 41D ( $\pi$ - $\pi$  T-shape) and GLN189D amino acids. Similarly, compound quercetin, 3- $\alpha$ -rhamnoside interacted with 13 amino acids in the active site with 3 hydrogen bonds THR 26D, GLY 143D, GLU 166D and hydrophobic interactions with THR 25D, HIS 41D, MET 49D, TYR 54D, ASN 142D, CYS 145D, HIS 164D, MET 165D, ASP 187D, ARG 188D amino acid residues. Hydrogen bonding is more enhanced by the interaction of O6 and O9 atoms of the ligand with N-atom of THR 26D (distance of 3.25 Å) and GLU 166D (distance of 2.92 Å) residues, respectively. ARG 188D forms a conventional carbon-hydrogen bond between the carboxyl group and the O7 atom of ligand. Whereas HIS 41D ( $\pi$ - $\pi$  stacked), CYS 44D, CYS 145D ( $\pi$ -alkyl), and MET 165D (alkyl) involved in strengthening hydrophobic junction.

The isoquercitrin and rutin showed binding affinity of  $-7.9$  kcal/mol and  $-7.6$  kcal/mol, respectively. Isoquercitrin forms polar interactions with HIS 41D, LEU 141D, GLY 143D, GLU 166D, GLN 192D, and hydrophobic contacts with THR 25D, HIS 41D, VAL 42D, CYS 44D, MET 49D, PHE 140D, LEU 141D, ASN 142D, GLY 143D, SER 144D, CYS 145D, HIS 163D, MET 165D, GLU 166D, ARG 188D, GLN 189D, GLN 192D amino acids. Further enhancement in binding due to the formation of conventional carbon-hydrogen bonding of O2 atoms (chromene ring), C1, C4, C5, C6 (3, 4-dihydroxyphenyl ring), and MET 165D residue. Compound Isoquercitrin forms  $\pi$ - $\pi$  T-shape interactions with HIS 163D,  $\pi$ -alkyl interactions with CYS 145D residues.

Rutin forms multiple contact points, including various H-bonded and nonbonded contacts within a radius of 4 Å with the main protease of SARS-CoV-2. Hydrogen bond formation involves THR 26D, HIS 41D, PHE 140D, GLY 143D, GLU 166D amino acid residues. Further hydrophobic contacts with SER 1B, THR 25D, THR 26D, LEU 27D, HIS 41D, CYS 44D, THR 45D, MET 49D, PHE 140D, LEU 141D, ASN 142D, GLY 143D, SER 144D, CYS 145D, HIS 164D, MET 165D, GLU 166D amino acids give the complex its stability.

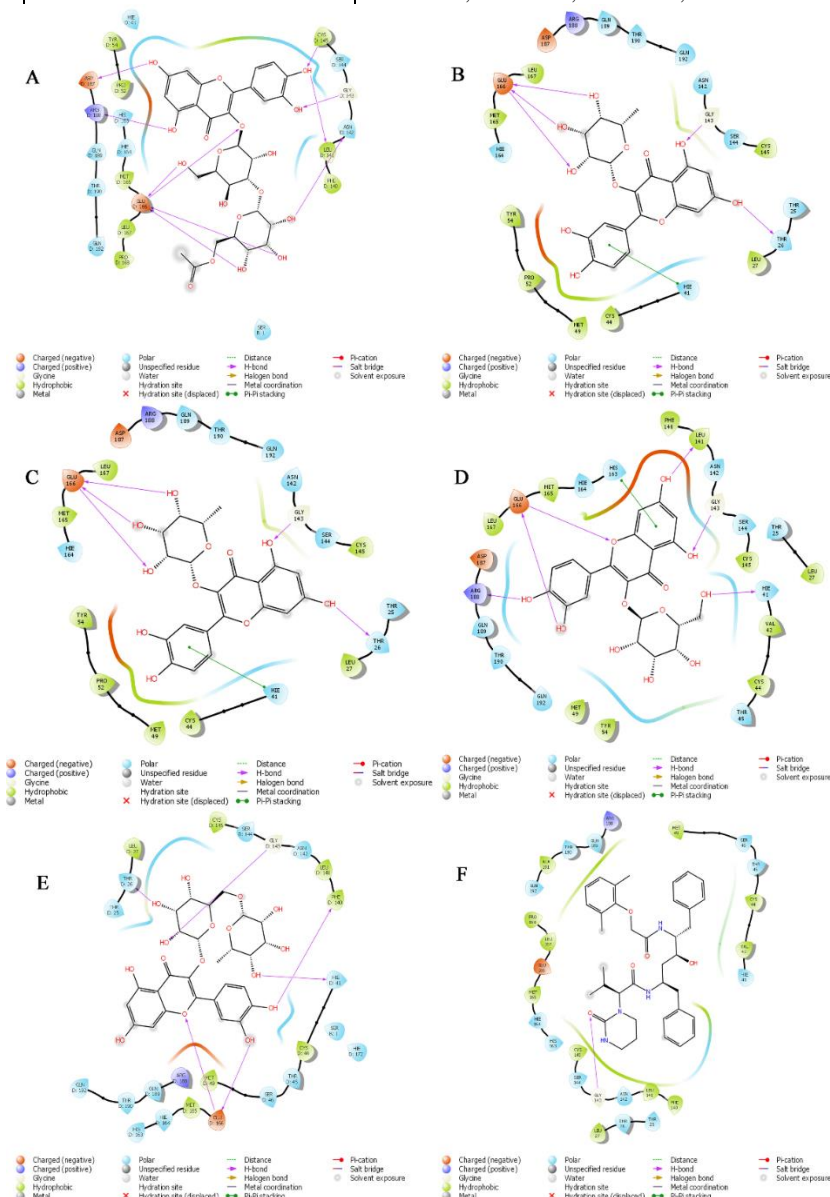
### 3.2. Molecular docking of selected phytoconstituents with RdRp (7BW4).

All phytochemicals were also docked against RdRp (7BW4) of SARS-CoV-2, and it was observed that the compounds exhibited substantial binding affinity with relatively important interactions. The docking score of all selected phytoconstituents was compared with standard drug remdesivir. As compared to the standard three compounds (table 2, Figure 3), euphrobianin, myrecetin and rutin had a higher binding affinity.



**Table 1.** Binding interactions of ligands with the binding site of the main protease of SARS-CoV-2 (PDB ID: 6m2n).

Ligand with docking score (kcal/mol)	H-bonding	Non-bonding
Euphrobianin (-8.4)	LEU 141D, ASN 142D, GLY143D, SER144D, GLU166D, ARG188D	HIS 41D, TYR 54D, LEU141D, ASN 142D, GLY 143D, SER 144D, CYS 145D, HIS 164D, MET 165D, GLU166D, PRO168D, ASP 187D, ARG188D, GLN 189D
Quercetin,3-o-alpha-rhamnoside (-8.1)	THR 26D, GLY 143D, GLU 166D	THR 25D, THR 26D, HIS 41D, MET 49D, TYR 54D, ASN 142D, GLY 143D, CYS 145D, HIS 164D, MET 165D, GLU 166D, ASP 187D, ARG 188D
Isoquercitrin (-7.9)	HIS 41D, LEU 141D, GLY 143D, GLU 166D, GLN 192D	THR 25D, HIS 41D, VAL 42D, CYS 44D, MET 49D, PHE 140D, LEU 141D, ASN 142D, GLY 143D, SER 144D, CYS 145D, HIS 163D, MET 165D, GLU 166D, ARG 188D, GLN 189D, GLN 192D
Rutin (-7.6)	THR 26D, HIS 41D, PHE 140D, GLY 143D, GLU 166D	SER 1B, THR 25D, THR 26D, LEU 27D, HIS 41D, CYS 44D, THR 45D, MET 49D, PHE 140D, LEU 141D, ASN 142D, GLY 143D, SER 144D, CYS 145D, HIS 164D, MET 165D, GLU 166D
Lopinavir (-7.3)	GLY 143D	THR 25D, THR 26D, PHE 140D, LEU 141D, LEU 27D, SER 143D, CYS 145D, HIS 163D, HIE 164D, MET 265D, LEU 167D, PRO 168D, GLN 192D, ALA 191D, THR 190D, MET 49D, SER 46D, THR 45D, CYS 44D, VAL 42D, HIE 41D

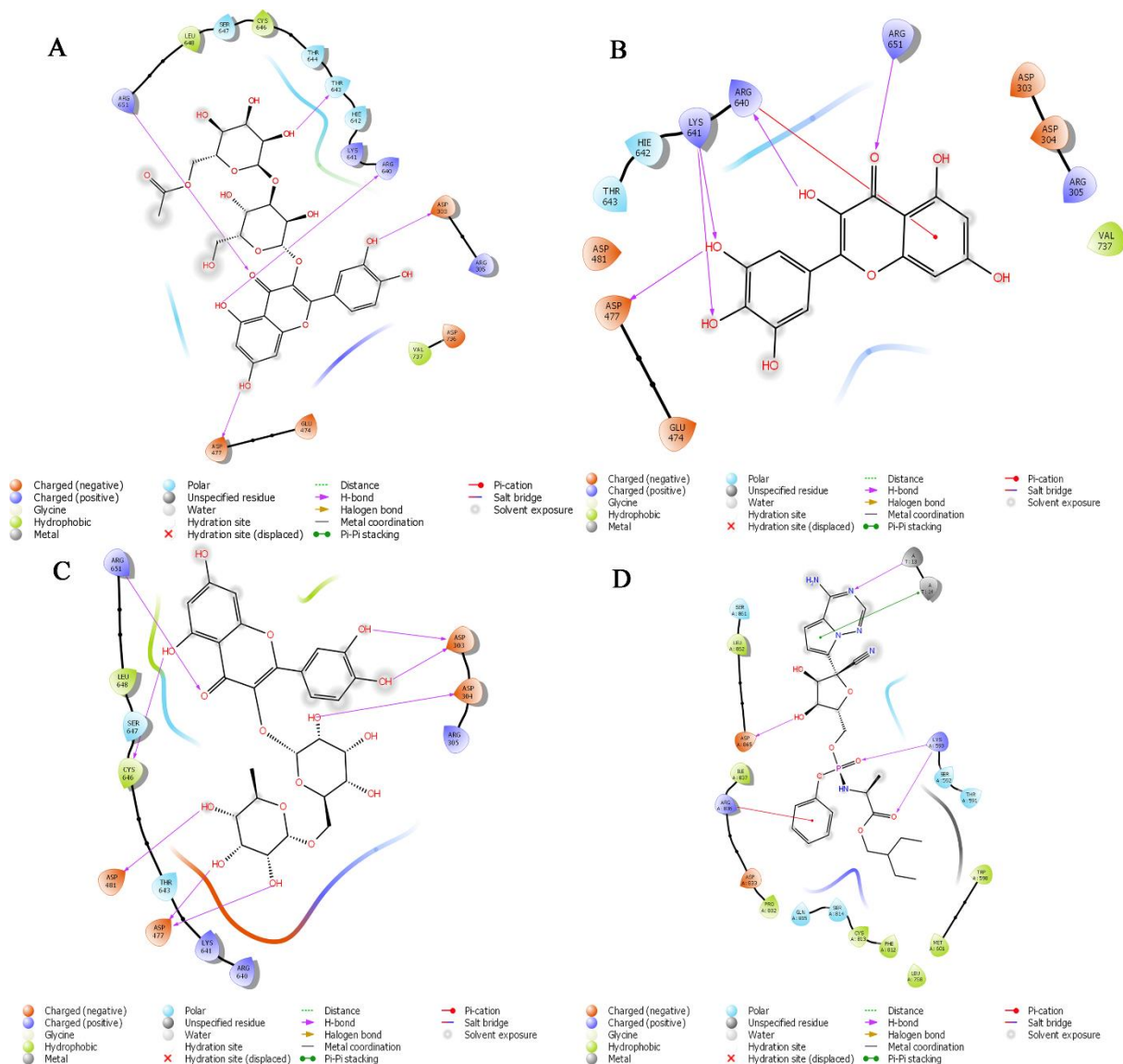


**Figure 2.** The binding interaction of (A) Euphrobianin; (B) Quercetin,3-O-Alpha-Rhamnoside; (C) Quercetin-3-O-Rhamnopyranoside; (D) Isoquercitrin; (E) Rutin; (F) Lopinavir with the binding site of SARS-CoV-2 main protease ( $M^{Pro}$ ) (PDB ID: 6m2n).

Out of the all compounds, the Euphorbianin showed the highest binding energy value of  $-6.1$  kcal/mol founding polar hydrogen-bonding interactions with ASP 477A, ARG 640A, THR 643A, ARG 651A, hydrophobic with ASP 303A, ARG 305A, ASP 477A, ARG 640A, HIS 642A, THR 643A, CYS 646A, SER 647A, LEU 648A, ARG 651A, VAL 737A amino acids.

Hydrophobic interactions include characteristics of charged interaction with ASP 304A ( $\pi$ -anion) and ARG 640A ( $\pi$ -cation). CYS 646A and SET 647A amino acid residues from conventional hydrogen bonding with C3, C5, and C7 atoms of euphorbianin.

Myricetin and rutin have binding energy  $-5.7$  and  $-5.5$  kcal/mol, respectively. Both phytochemicals, myricetin and rutin, commonly form hydrogen bonding with ASP 477A and ARG 651A, hydrophobic interactions with ASP 477A, ASP 481A, LYS 641A, THR 643A, ARG 651A, charged contacts ASP 304A ( $\pi$ -anion), ARG 640A ( $\pi$ -cation) amino acids. Rutin also shows polar contacts with ASP 303A, ASP 304A, ASP 481A, CYS 646A, and hydrophobic with SER 647 A, LEU 648A in contrast to compound 11 showing polar LYS 641A, hydrophobic GLU 474A, HIS 642A, THR 643A, ARG 651A, VAL 737A amino acids.



**Figure 3.** The binding interaction of (A) Euphorbianin; (B) Myricetin; (C) Rutin; (D) Remdesivir with the binding site of RdRp of SARS-CoV-2 (PDB ID: 7BW4).

**Table 2.** Binding interactions of ligands with the binding site of RdRp of SARS-CoV-2 (PDB ID: 7BW4)

Ligand with docking score (Kcal/mol)	H-bonding	Non-bonding
Euphorbianin (-6.1)	ASP 477A, ARG 640A, THR 643A, ARG 651A	ASP 303A, ARG 305A, ASP 477A, ARG 640A, HIS 642A, THR 643A, CYS 646A, SER 647A, LEU 648A, ARG 651A, VAL 737A
Myricetin (-5.7)	ASP 477A, LYS 641A, ARG 651A	ASP 304A, GLU 474A, ASP 477A, ASP 481A, ARG 640A, LYS 641A, HIS 642A, THR 643A, ARG 651A, VAL 737A
Rutin (-5.5)	ASP 303A, ASP 304A, ASP 477A, ASP 481A, CYS 646A, ARG 651A	ASP 303A, ASP 304A, ASP 477A, ASP 481A, ARG 640A, LYS 641A, THR 643A, CYS 646A, SER 647 A, LEU 648A, ARG 651A
Remdesivir (-5.2)	LYS 593A, ARG 836A, ASP 865A	SER 593A, THR 591A, TRP 598A, MET 601A, PHE 812A, CYS 813A, SER 814A, GLN 815A, PRO 832A, ASP 833A, ILE 837A, SER 861A, LEU 862A

### 3.3. Screening of drug-likeness and *in silico* toxicity studies.

Druglikeness evaluation of all selected phytoconstituents was performed using the Schrodinger QuikProp module. Out of 26 selected phytoconstituents, 13 had passed the Lipinski rule of five. Other compounds had up to two violations which are acceptable for natural products (Table 3). Toxicological evaluation of selected phytoconstituents was performed by the QSAR modeling method using VEGA-QSAR (Table 4). The results represented as applicability domain index (ADI) scores calculated by algorithm incorporated in VEG-QSAR software. Positive results of ADI > 0.5 are considered as indicators of reliability effect. All phytoconstituents found to be non-mutagenic (SarPy/IRFMN, ISS and KNN/Read-Across, assessment and prediction) except myricetin that may have mutagenicity according to KNN/Read-Across model [30]. Also, all chemical constituents do not have carcinogenicity (CAESAR model, assessment, and prediction) [31]. Phytochemicals euphorbianin and rutin do not show developmental toxicity (PG model assessment and prediction) [32], and all selected compounds are found to be inactive for Thyroid hormone receptor  $\alpha/\beta$  (NRMEA, assessment, and prediction). There is very low reliability for myricetin to have skin sensitivity. Conversely, other chemical constituents do not have skin sensitivity (CAESAR model, assessment, and prediction) [33]. However, with low reliability of the IRFMN (assessment and prediction) model suggests that all constituents should be considered for their hepatotoxic potentials. Thus, overall euphorbianin and rutin can be suitable for further *in vitro* and *in vivo* assessment for its inhibitory potential against SARS-CoV-2.

**Table 3.** Drug-likeness profile of selected phytoconstituents.

Sr. no	Name of Compound	MW (g/mol)	No. of rotatable bond	H-accepter	H-Donor	LogP	LogS	GI permeability
1	Aesculetin	178.14	0	4	2	1.25	-2.28	High
2	Coumarin,6-7-8-Trimethoxy	236.22	3	5	0	2.46	-2.69	High
3	Daphnoretin	352.29	3	7	1	2.87	-4.44	High
4	Ellagic Acid	302.19	0	8	4	0.79	-2.94	High
5	Scopoletin	192.17	1	4	1	1.86	-2.46	High
6	Isoscoletin	192.17	1	4	1	1.86	-2.46	High
7	Umbelliferone	162.14	0	3	1	1.44	-2.46	High
8	Afzelin	432.38	3	10	6	1.84	-3.47	Low
9	Euphorbianin	668.55	9	18	10	2.22	-2.88	Low
10	Kaempferol	286.24	1	6	4	1.70	-3.31	High
11	Myricetin	318.24	1	8	6	1.08	-3.01	Low
12	Myricitrin	464.38	3	12	8	0.92	-3.20	Low



Sr. no	Name of Compound	MW (g/mol)	No. of rotatable bond	H-accepter	H-Donor	LogP	LogS	GI permeability
13	Quercetin	302.24	1	7	5	1.63	-3.16	High
14	Quercetin,3-O-Alpha-Rhamnoside	448.38	3	11	7	0.49	-3.20	Low
15	Quercetin-3-O-Rhamnopyranoside	448.38	3	11	7	0.49	-3.20	Low
16	Quercitrin	448.38	3	11	7	0.49	-3.20	Low
17	Quercimeritrin	464.379	4	12	8	-0.54	-2.92	High
18	Quercitrin	432.381	3	10	6	1.52	-3.41	Low
19	Iso Quercitrin	464.379	4	12	8	-0.54	-3.00	High
20	Iso Rhamnetin	316.265	2	7	4	2.29	-3.11	High
21	Rutin	610.521	6	16	10	-1.69	-2.91	Low
22	Xanthorhamnin	770.69	9	20	11	-2.53	-2.90	Low
23	Ingenol Triacetate	474.55	4	8	1	2.53	-5.01	High
24	Jolkinolide A	314.425	0	3	0	4.14	-4.96	High
25	Ent Kaur-16-Ene-3-Beta-Ol:	288.475	1	1	1	4.95	-4.50	High
26	Kaurane,16-Alpha, 17,19-Trihydroxy:Ent	336.472	2	3	3	3.21	-3.47	High

**Table 4.** Toxicity Assessment selected active phytoconstituents (QSAR Models).

Toxicity Test	Euphrobianin	Myricetin	Quercetin,3-O-Alpha-Rhamnoside	Iso quercitrin	Rutin
Mutagenicity (Ames test) model (SarPy/IRFMN) - assessment	0.925***	0.567*	0.744**	0.695**	0.932***
Mutagenicity (Ames test) model (SarPy/IRFMN) - prediction	NON-Mutagenic	NON-Mutagenic	NON-Mutagenic	NON-Mutagenic	NON-Mutagenic
Mutagenicity (Ames test) model (KNN/Read-Across) - assessment	0.80**	0.847**	0.717**	0.717**	0.807**
Mutagenicity (Ames test) model (KNN/Read-Across) - prediction	NON-Mutagenic	Mutagenic	NON-Mutagenic	NON-Mutagenic	NON-Mutagenic
Carcinogenicity model (CAESAR) - assessment	0.729**	0.793**	0.636**	0.631**	0.721**
Carcinogenicity model (CAESAR) - prediction	NON-Carcinogen	NON-Carcinogen	NON-Carcinogen	NON-Carcinogen	NON-Carcinogen
Developmental/Reproductive Toxicity library (PG) - assessment	Outside domain	0.874**	0.877**	0.874**	Outside domain
Developmental/Reproductive Toxicity library (PG) - prediction	NON-Toxicant	Toxicant	Toxicant	Toxicant	NON-Toxicant
Thyroid Receptor Alpha and Beta effect (NRMEA) - assessment	0.962***	0.932***	0.923***	0.93***	1***
Thyroid Receptor Alpha and Beta effect (NRMEA) - prediction	Inactive	Inactive			Inactive
Skin Sensitization model (CAESAR) - assessment	Outside domain	0.431*	Outside domain	0.359*	Outside domain
Skin Sensitization model (CAESAR) - prediction	NON-Sensitizer	Sensitizer	NON-Sensitizer	NON-Sensitizer	NON-Sensitizer
Hepatotoxicity model (IRFMN) - assessment	0.549*	0.775**	0.54*	0.537*	0.556*
Hepatotoxicity model (IRFMN) - prediction	Toxic	Toxic	Toxic	Toxic	Toxic

#### 4. Conclusions

We performed a detailed in silico study of twenty-six phytoconstituents obtained from the plant *E. hirta* to find potential candidates against SARS-CoV-2. All the phytoconstituents belong to either category of flavonoid or coumarins. The molecular docking studies were performed using two different targets, namely M<sup>Pro</sup> and RdRp. Based on the molecular docking

study compared with the standard drug, we screened four compounds, namely euphorbianin, quercetin 3-o-alpha-rhamnoside, isoquercitrin, and rutin, against the target M<sup>Pro</sup> while three phytoconstituents euphorbianin, myricetin and rutin were screened against the target RdRp. Euphorbianin and rutin were found common against both targets. All the screened phytoconstituents based on docking score were evaluated for *in silico* toxicity profiles, and except myricetin all were predicted safe. Results of euphorbianin and rutin were found more interesting as both compounds had high binding affinity against both targets. Finally, we want to conclude that euphorbianin, quercetin, 3-o-alpha-rhamnoside, isoquercitrin, and rutin could be rapidly further explored as they may have the potential to fight against COVID-19.

## Funding

Non-funded.

## Acknowledgments

We are thankful to Sumandeep Vidyapeeth Deemed to be University for supporting us to conduct this research work.

## Conflicts of Interest

There are no conflicts of interest.

## References

1. Sohrabi, C.; Alsafi, Z.; O'Neill, N.; Khan, M.; Kerwan, A.; Al-Jabir, A.; Iosifidis, C.; Agha, R. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). *International Journal of Surgery* **2020**, *76*, 71-76, <https://doi.org/10.1016/j.ijsu.2020.02.034>.
2. Wu, D.; Wu, T.; Liu, Q.; Yang, Z. The SARS-CoV-2 outbreak: What we know. *International Journal of Infectious Diseases* **2020**, *94*, 44-48, <https://doi.org/10.1016/j.ijid.2020.03.004>.
3. In *Coronavirus disease (COVID-19) Situation Report – 175*, <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>.
4. Guo, G.; Ye, L.; Pan, K.; Chen, Y.; Xing, D.; Yan, K.; Chen, Z.; Ding, N.; Li, W.; Huang, H.; Zhang, L.; Li, X.; Xue, X. New Insights of Emerging SARS-CoV-2: Epidemiology, Etiology, Clinical Features, Clinical Treatment, and Prevention. *Frontiers in Cell and Developmental Biology* **2020**, *8*, 1-22, <https://doi.org/10.3389/fcell.2020.00410>.
5. Tu, Y. F.; Chien, C. S.; Yarmishyn, A. A.; Lin, Y. Y.; Luo, Y. H.; Lin, Y. T.; Lai, W. Y.; Yang, D. M.; Chou, S. J.; Yang, Y. P.; Wang, M. L.; Chiou, S. H. A review of sars-cov-2 and the on going clinical trials. *International Journal of Molecular Sciences* **2020**, *21*, <https://doi.org/10.3390/ijms21072657>.
6. Wang, L.; Wang, Y.; Ye, D.; Liu, Q. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. *International Journal of Antimicrobial Agents* **2020**, *55*, 105948, <https://doi.org/10.1016/j.ijantimicag.2020.105948>.
7. Mirzaie, A.; Halaji, M.; Dehkordi, F. S.; Ranjbar, R.; Noorbazargan, H. A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). *Complementary Therapies in Clinical Practice* **2020**, *40*, 101214, <https://doi.org/10.1016/j.ctcp.2020.101214>.
8. Sanders, J. M.; Monogue, M. L.; Jodlowski, T. Z.; Cutrell, J. B. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA - Journal of the American Medical Association* **2020**, *323*, 1824-1836, <https://doi.org/10.1001/jama.2020.6019>.
9. Wu, R.; Wang, L.; Kuo, H. C. D.; Shannar, A.; Peter, R.; Chou, P. J.; Li, S.; Hudlikar, R.; Liu, X.; Liu, Z.; Poiani, G. J.; Amorosa, L.; Brunetti, L.; Kong, A. N. An Update on Current Therapeutic Drugs Treating COVID-19. *Current Pharmacology Reports* **2020**, *6*, 56-70, <https://doi.org/10.1007/s40495-020-00216-7>.
10. Parmar, G.; Pundarikakshudu, K.; Balaraman, R.; Sailor, G. Amelioration of anaphylaxis, mast cell degranulation and bronchospasm by *Euphorbia hirta* L. extracts in experimental animals. *Beni-Suef*

- University Journal of Basic and Applied Sciences* **2018**, *7*, 127-134, <https://doi.org/10.1016/j.bjbas.2017.11.001>.
11. Mwine, J. T.; van Damme, P. Why do euphorbiaceae tick as medicinal plants? a review of euphorbiaceae family and its medicinal features. *Journal of Medicinal Plants Research* **2011**, *5*, 652-662, [https://www.researchgate.net/publication/228478254\\_Why\\_do\\_Euphorbiaceae\\_tick\\_as\\_medicinal\\_Plants\\_A\\_review\\_of\\_Euphorbiaceae\\_family\\_and\\_its\\_medicinal\\_features](https://www.researchgate.net/publication/228478254_Why_do_Euphorbiaceae_tick_as_medicinal_Plants_A_review_of_Euphorbiaceae_family_and_its_medicinal_features).
  12. Kemper, K. J.; Lester, M. R. Alternative asthma therapies: An evidence-based review. *Contemporary Pediatrics* **1999**, *16*, 162-195, [https://www.researchgate.net/profile/Kathi-Kemper-2/publication/264957106\\_Alternative\\_asthma\\_therapies\\_An\\_evidence-based\\_review/links/55242daa0cf2caf11bfcc253/Alternative-asthma-therapies-An-evidence-based-review.pdf](https://www.researchgate.net/profile/Kathi-Kemper-2/publication/264957106_Alternative_asthma_therapies_An_evidence-based_review/links/55242daa0cf2caf11bfcc253/Alternative-asthma-therapies-An-evidence-based-review.pdf).
  13. Aquil, M. Euphorbianin, a new glycoside from *Euphorbia hirta* Linn. *Global Journal of Pure and applied science* **1999**, *5*, 371.
  14. Yusuf, M.; Wahab, M. A.; Yousuf, M. D.; Chowdhury, J. U.; Begum, J., Some tribal medicinal plants of Chittagong hill tracts, Bangladesh. *Bangladesh Journal of Plant Taxonomy* **2007**, *14* (2), 117-128..
  15. Parmar, G. R.; Pundarikakshudu, K., Comparative pharmacognostic and phytochemical standardization of *Euphorbia hirta* L. and *Euphorbia thymifolia* L. *American Journal of Pharmtech Research* **2017**, *7*, 351-544.
  16. Anjaria, J.; Parabia, M.; Bhatt, G.; Khamar, R., A glossary of selected indigenous medicinal plants of India. **1997**, [https://www.researchgate.net/publication/324978847\\_Comparative\\_Pharmacognostic\\_and\\_Phytochemical\\_Standardization\\_of\\_Euphorbia\\_hirta\\_L\\_and\\_Euphorbia\\_thymifolia\\_L](https://www.researchgate.net/publication/324978847_Comparative_Pharmacognostic_and_Phytochemical_Standardization_of_Euphorbia_hirta_L_and_Euphorbia_thymifolia_L).
  17. Capasso, F.; Gaginella, T.S.; Grandolini, G.; Izzo, A.A. Phytotherapy: a Quick Reference to Herbal Medicine. *Springer-Verlag Berlin Heidelberg: Berlin, Germany* **2010**, *9*, 164, <http://www.ethnopharmacologia.org/bibliotheque-ethnopharmacologie/phytotherapy-a-quick-reference-to-herbal-medicine>.
  18. Shah, A. P.; Parmar, G. R.; Sailor, G. U.; Seth, A. K. Antimalarial Phytochemicals Identification from *Euphorbia Hirta* against Plasmeprin Protease: an In Silico Approach. *Folia medica* **2019**, *61*, 584-593, <https://doi.org/10.3897/folmed.61.e47965>.
  19. Karak, P. J. I. J. o. P. S. Research, Biological activities of flavonoids: an overview. *Int J Pharm Sci & Res* **2019**, *10* (4), 1567-1574, [https://doi.org/10.13040/IJPSR.0975-8232.10\(4\).1567-74](https://doi.org/10.13040/IJPSR.0975-8232.10(4).1567-74).
  20. Jo, S.; Kim, S.; Shin, D. H.; Kim, M.-S. Inhibition of SARS-CoV 3CL protease by flavonoids. *Journal of enzyme inhibition and medicinal chemistry* **2020**, *35*, 145-151, <https://doi.org/10.1080/14756366.2019.1690480>.
  21. Das, A.; Pandita, D.; Jain, G. K.; Agarwal, P.; Grewal, A. S.; Khar, R. K.; Lather, V. Role of phytoconstituents in the management of COVID-19. *Chem Biol Interact* **2021**, *341*, 109449-109449, <https://doi.org/10.1016/j.cbi.2021.109449>.
  22. Khodadadi, E.; Maroufi, P.; Khodadadi, E.; Esposito, I.; Ganbarov, K.; Espoito, S.; Yousefi, M.; Zeinalzadeh, E.; Kafil, H. S. Study of combining virtual screening and antiviral treatments of the Sars-CoV-2 (Covid-19). *Microbial Pathogenesis* **2020**, *146*, 104241-104241, <https://doi.org/10.1016/j.micpath.2020.104241>.
  23. Kirchdoerfer, R. N.; Ward, A. B. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nature Communications* **2019**, *10*, 2342, <https://doi.org/10.1038/s41467-019-10280-3>.
  24. Farnsworth, N. R. NAPRALERT database. Chicago, University of Illinois at Chicago, IL, March: 1995.
  25. Figueroa, J. P.; Bottazzi, M. E.; Hotez, P.; Batista, C.; Ergonul, O.; Gilbert, S.; Gursel, M.; Hassanain, M.; Kim, J. H.; Lall, B.; Larson, H.; Nanche, D.; Sheahan, T.; Shoham, S.; Wilder-Smith, A.; Strub-Wourgaft, N.; Yadav, P.; Kang, G. Urgent needs of low-income and middle-income countries for COVID-19 vaccines and therapeutics. *The Lancet* **2021**, *397*, 562-564, [https://doi.org/10.1016/S0140-6736\(21\)00242-7](https://doi.org/10.1016/S0140-6736(21)00242-7).
  26. Shah, S.; Chaple, D.; Arora, S.; Yende, S.; Mehta, C.; Nayak, U. Prospecting for *Cressa cretica* to treat COVID-19 via in silico molecular docking models of the SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics* **2021**, 1-9, <https://doi.org/10.1080/07391102.2021.1872419>.
  27. Huang, J.; Song, W.; Huang, H.; Sun, Q. Pharmacological Therapeutics Targeting RNA-Dependent RNA Polymerase, Proteinase and Spike Protein: From Mechanistic Studies to Clinical Trials for COVID-19. *Journal of clinical medicine* **2020**, *9*, 1131, <https://doi.org/10.3390/jcm9041131>.

28. Yang, Y.; Islam, M. S.; Wang, J.; Li, Y.; Chen, X. Traditional Chinese Medicine in the Treatment of Patients Infected with 2019-New Coronavirus (SARS-CoV-2): A Review and Perspective. *International journal of biological sciences* **2020**, *16*, 1708-1717, <https://doi.org/10.7150/ijbs.45538>.
29. Gyuris, A.; Szlávik, L.; Minárovits, J.; Vasas, A.; Molnár, J.; Hohmann, J. Antiviral activities of extracts of *Euphorbia hirta* L. against HIV-1, HIV-2 and SIVmac251. *In vivo (Athens, Greece)* **2009**, *23*, 429-32, <https://pubmed.ncbi.nlm.nih.gov/19454510/>.
30. Votano, J. R.; Parham, M.; Hall, L. H.; Kier, L. B.; Oloff, S.; Tropsha, A.; Xie, Q.; Tong, W. Three new consensus QSAR models for the prediction of Ames genotoxicity. *Mutagenesis* **2004**, *19*, 365-377, <https://doi.org/10.1093/mutage/geh043>.
31. Fjodorova, N.; Vracko, M.; Novic, M.; Roncaglioni, A.; Benfenati, E. New public QSAR model for carcinogenicity. *Chemistry Central journal* **2010**, *4 Suppl 1*, S3-S3, <https://doi.org/10.1186/1752-153X-4-S1-S3>.
32. Simms, L.; Rudd, K.; Palmer, J.; Czekała, L.; Yu, F.; Chapman, F.; Trelles Sticken, E.; Wieczorek, R.; Bode, L. M.; Stevenson, M.; Walele, T. The use of human induced pluripotent stem cells to screen for developmental toxicity potential indicates reduced potential for non-combusted products, when compared to cigarettes. *Current Research in Toxicology* **2020**, *1*, 161-173, <https://doi.org/10.1016/j.crttox.2020.11.001>.
33. Chaudhry, Q.; Piclin, N.; Cotterill, J.; Pintore, M.; Price, N. R.; Chrétien, J. R.; Roncaglioni, A. Global QSAR models of skin sensitizers for regulatory purposes. *Chemistry Central journal* **2010**, *4 Suppl 1*, S5-S5, <https://doi.org/10.1186/1752-153X-4-S1-S5>.