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Screening and druggability analysis of some plant metabolites against SARS-CoV-2: An integrative computational approach

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ABSTRACT

The sudden outbreak of novel coronavirus has caused a global concern due to its infection rate and mortality. Despite extensive research, there are still no specific drugs or vaccines to combat SARS-CoV-2 infection. Hence, this study was designed to evaluate some plant-based active compounds for drug candidacy against SARS-CoV-2 by using virtual screening methods and various computational analyses. A total of 27 plant metabolites were screened against SARS-CoV-2 main protease proteins (MPP), Nsp9 RNA binding protein, spike receptor binding domain, spike ecto-domain and HR2 domain using a molecular docking approach. Four metabolites, i.e., asiatic acid, avicularin, guajaverin, and withaferin showed maximum binding affinity with all key proteins in terms of lowest global binding energy. The crucial binding sites and drug surface hotspots were unravelled for each viral protein. The top candidates were further employed for ADME (absorption, distribution, metabolism, and excretion) analysis to investigate their drug profiles. Results suggest that none of the compounds render any undesirable consequences that could reduce their drug likeness properties. The analysis of toxicity pattern revealed no significant tumorigenic, mutagenic, irritating, or reproductive effects by the compounds. However, withaferin was comparatively toxic among the top four candidates with considerable cytotoxicity and immunotoxicity. Most of the target class by top drug candidates belonged to enzyme groups (e.g. oxidoreductases hydrolases, phosphatases). Moreover, results of drug similarity prediction revealed two approved structural analogs of Asiatic acid i.e. Hydrocortisone (DB00741) (previously used for SARS-CoV-1 and MERS) and Dinoprost-tromethamine (DB01160) from DrugBank. In addition, two other biologically active compounds, Mupirocin (DB00410) and Simvastatin (DB00641) could be an option for the treatment of viral infections. The study may pave the way to develop effective medications and preventive measure against SARS-CoV-2. Due to the encouraging results, we highly recommend further *in vivo* trials for the experimental validation of our findings.

1. Introduction

The sudden outbreak of novel coronavirus (SARS-CoV-2) infection emanated from Wuhan, China and spread throughout the world excepting a few countries to date [1]. The virus is responsible for causing novel disease, which WHO officially called COVID-19. As of April 23, 2020, World Health Organization (WHO) estimated that new coronavirus touched 213 countries, areas or territories [2,3]. The infection rate is increasing. However, the fatality rate of SARS-CoV-2 (3.4%) estimated by WHO is lower than previous fatal diseases SARS and MERS, which had 9.6% and 35% death rates, respectively [4,5].

Coronaviruses are enveloped, positive single-stranded RNA viruses with large genome size ranging from 26 kb to 32 kb. These viruses are representative of four subfamilies, which include alpha-, beta-, gamma- and delta-coronaviruses. SARS-CoV-2 showed more sequence similarity with SARS-CoV than MERS-CoV when genome sequences of these mentioned viruses were compared [6]. But they also exhibit dissimilarities that can influence their process of pathogenesis [7,8]. SARS-CoV-2 infects humans through the same entry point of the ACE receptor which is expressed in the respiratory tract [9,10]. However,

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Table 1
List of plant metabolites used in the study with respective source and activities.

Metabolites	PubChem CID	Class	Source	Activities	References
Allicin	65036	S-containing compound	*Allium sativum*	Antimicrorial, antiviral Antioxidant, anti-cancer activity	[25]
Andrographolide	5318517	Diterpenoid labdane	*Andrographis paniculata*	antioxidant, anti-inflammatory, and anti-cancer	[26]
Apigenin	5280443	Flavonoid	Vegetable and fruit	Effective in cancer, depression, diabetes & Alzheimer's disease,	[27]
Asiatic acid	119034	Aglycone type pentacyclic triterpenoids	*Centella asiatica*	Antioxidant, cardioprotective, anti-inflammatory, antitumor, neuroprotective, antimicrobial	[28]
Avicularin	5490064	quercetin-3-a-L arabinofuranoside (flavonoid)	*Psidium guyava, Lespedeza cuneata*	anti-inflammatory, anti-oxidant, hepatoprotective activity	[29]
Capsaicin	1548943	Alkaloid	*Capsicum genus*	Pruritis, pain relief, non-steroidal anti-inflammatory drug induced gastritis	[30]
Chavibetol	596375	Phenylpropanoid	Piper betle	immunomodulatory, radical scavenging	[31]
Cinnamic acid	444539	Aromatic carboxylic acids	*Cinnamomum* species	Antibacterial, antifungal, antimalarial, antitubercular	[32]
Curcumin	969516	Polyphenolic compound	*Curcuma longa*	antibacterial, anti-inflammatory antiviral, antioxidant, anti-arthritis & anti-cancer activity	[33]
Eugenol	3314	Phenylpropanoid	*Ocimum tenuiflorum, Eugenia caryophyllata*	antimicrobial, anti-inflammatory, analgesic and antioxidant	[34]
Arjunone	14034821	Flavonoids	*Terminalia arjuna*	Arjunone and other compounds have role in antioxidant, antiatherogenic, anti-inflammatory, anti-carcinogenic activity	[35]
Galangin	5281616	Flavonol	Honey, *Alpinia officinarum*, propolis	Anti-cancer, anti-mutagenic, anti-oxidative, radical scavenging etc.	[36]
Gentisic acid	3469	Phenolic acid	*Gentiana*, *Citrus*, *H. rosa-sinensis*, *O. europaea, S. indicum*	Antioxidant, neuroprotective, antiinflammatory, hepatoprotective, antimicrobial activities	[37]
Guajaverin	5481224	Flavonoid	*Psidium guyava*	Anti-plaque activity	[38]
Kaempferol	5280863	Flavonoid aglycone	Vegetable and fruit	Anti-inflammatory, antioxidant, antimicrobial, antitumor, cardioprotective, and antidiabetic activities	[39]
Luteolin	5280445	Flavonoid	Carrots, celery peppers, olive peppermint	Anticancer, antioxidant, antimicrobial, anti-inflammatory, and activities	[40]
m-Coumaric acid	637541	Phenolic acid	*Solanum nigrum*	Role in pharmacological activities	[41]
Piperic acid	5370536	Alkaloid	*Piper nigrum*	No known function	[42]
Piperine	638024	Alkaloid	*Piper* spp.	Anticancer, antimicrobial, antimalarial	[42]
Quercetine	5280343	Flavonoid	Diverse plant species	Antioxidant, cardiovascular, antiviral, anti-inflammatory, anticancer, antimicrobial	[43]
Swertiamarin	442435	Secoiridoid glycoside	*Swertia chirata*	Anti-arthritic, anti-diabetic Cardio-protective, Anticancer, Anti-hepatitis, Antibacterial, anti-atherosclerotic	[44]
Swertinin	5491517	Secoiridoid glycoside	*Swertia chirata*	Role in pharmacological activities	[45]
Thymoquinone	10281	Monoterpene	*Nigella sativa*	Anti-oxidant and anti-inflammatory properties, Anti-microbial, Anti-arthritic, anti-cancer efficacy	[46]
Vincamine	15376	Alkaloid	*Catharanthus roseus, Vinca minor*	Cerebral disorders, antiulcer activity, cerebrovascular insufficiencies	[47]
Vitexin	5280441	Apigenin flavone glucoside	*Crataegus species*	Anti-inflammatory effects, anti-oxidant effects, anti-carcinogenic effects, anti-viral effects	[48]
Withaferin	265237	Steroidal lactone	*Withania somnifera*	Anti-cancer, adaptogenic, anti-stress, immunomodulatory, anti-inflammatory, anti-tumor, cardioprotective, and neuroprotective activities.	[49]
Zingiberene	92776	Isoprenoids	Zingiber Officinale	Anti-ulcer, antibacterial, cytoxic effect	[50]

among various proteins, four proteins are commonly found in the structure of all coronaviruses representing spike (S), envelope (E), membrane (M), and nucleocapsid (N) [8]. The initial and important stage of viral entry into host cell is receptor recognition [11]. The assembly of viral particle involves M protein and E protein, while virus binding and entrance into host cell take place by S protein with the assistance of SARS-CoV-2 angiotensin-converting enzyme [10,12].

The coronavirus (SARS-CoV-2) belongs to the family of Beta-coronaviruses, which are responsible for causing severe human respiratory syndrome [3,13]. The virus is spread mainly through community transmission, while SARS and MERS affect people via nosocomial spread [14]. It can transmit from one individual to other by respiratory droplets. SARS-CoV-2 infected patients have general signs and symptoms, suffering initially from common flu-like fever, sputum production, dyspnoea, headache, sore throat/pharyngalgia, and diarrhoea, which may further lead to express life-threatening symptoms including fatal pneumonia [15]. COVID-19 affected patients, either symptomatic or asymptomatic, were detected with the nose area containing a higher viral load than in throat [16]. A critically ill patient has a series of complexities with progression of disease.

The efficacy and safety of antivirals require evaluation by clinical trial [3]. There is no efficient, safe, and specific potential therapeutic to be approved for rapid remedy of this new respiratory syndrome to date [17,18]. Clinical trials of some drugs have been started, yet till now, only a few candidates have shown some efficacy in *in vitro* studies [19]. Not many have progressed to randomized animal or human trials; hence they may have limited use to counter infection [19]. Many countries and some pharmaceutical companies announced their headway and programs to develop vaccines (e.g. subunit, mRNA, DNA, live-vector vaccine) against the virus. But the developmental process of making human vaccine from concept to licensure may require years [20]. As the epidemic is still spreading, medicinal plants may be alternatively used in making drugs as early as possible. Several scientific researchers reported the helpfulness of plants due to their medicinal value and therapeutic uses as drugs from the ancient times [21]. Plant-derived active compounds of different plant parts are useful for treating diseases including diarrhoea, headache, and inflammation, and bacterial and fungal infections. From prehistoric times, traditional people utilized these plants for the remedial purposes of health deteriorating diseases because of the existence of numerous phytochemicals [22]. Various limitations are associated with modern treatment options including drug-resistance, severe side effects, adverse toxicity profiles, complicated medication etc. Natural products have the potential to form the basis of holistic health care [23]. The properties of antioxidants render medicinal plants

Table 2
Analysis of global binding energy and interaction sites of the screened top 4 metabolites.

Macromolecules	Ligands	Global Energy	ACE	Score	Area	Ligand binding residues
6W63	α-ketoamide (Control)	−56.92	−16.84	4560	526.40	Asp197, Leu272, Gly275, Leu286, Leu287, Asp289
	Asiatic acid	−53.05	−15.26	4916	577.10	His41, Met49, Tyr54, Asn142, Met165
	Avicularin	−48.62	−18.50	4694	532.10	Thr25, Thr26, His41, Cys44, Ser46, Met49, Gly143, Cys145
	Guajaverin	−48.48	−15.12	4450	497.50	Thr25, His41, Cys44, Met49, Asn142, Cys145, Met165, Asp187, Arg188
	Withaferin	−48.46	−14.08	4984	597.40	His41, Met49, Met165, Pro168, Ala191
6W4B	α-ketoamide (Control)	−48.60	−16.39	4458	504.60	Phe41, Trp54, Ile66, Thr68, Glu69
	Asiatic acid	−50.04	−16.37	4998	564.20	Met13, Gly39, Arg40, Phe41, Val42, Phe57, Pro58, Ile66
	Withaferin	−47.95	−13.30	4896	570.40	Arg40, Val42, Phe57, Pro58,Lys59, Ser60, Ile66
	Guajaverin	−42.72	−10.63	4548	641.40	Asn1, Asn2, Glu3, Gln50, Pro72, Pro73
	Avicularin	−39.83	−23.80	4556	514.50	Met49, Met165, Glu166, Thr190
6VYB	α-ketoamide (Control)	−63.94	−17.32	5728	705.10	Thr547, Gly548, Thr549,Asp745, Val976
	Asiatic acid	−60.68	−22.33	6276	771.50	Phe338, Ala363, Tyr365, Leu368, Cys379, Pro384, Leu387, Leu390, Phe392, Val395, Cys432, Ile434, Leu513, Val524
	Withaferin	−60.19	−20.49	5760	793.10	Ile410, Pro412, Leu425, Pro426, Cys432, Val433, Phe464, Val512, Leu513
	Guajaverin	−55.24	−17.51	5208	659.20	Ile410, Pro412, Lys424, Gly431, Cys432, Val433, Val512
	Avicularin	−52.93	−17.15	5474	683.30	Ala411, Pro412, Leu425, Cys432, Val433, Val512
6LVN	α-ketoamide (Control)	−25.52	−2.71	4318	564.20	Ile16, Asn20, Lys24, Asn27, Glu28
	Guajaverin	−28.73	−2.13	3696	443.50	Asp17, Arg18, Glu21, Lys24
	Withaferin	−28.11	−1.24	4376	507.70	Lys14, Lys24, Arg18
	Asiatic acid	−27.58	−1.12	4366	500.30	Lys14, Lys24, Asp17, Arg18, Glu21
	Avicularin	−26.48	−1.22	3986	465.10	Asp17, Arg18, Glu21, Asn20
6M0J	α-ketoamide (Control)	−60.50	−9.34	5374	655.40	Lys94, Tyr196, Asp206, Glu208, Val209, Asn210
	Guajaverin	−47.34	−11.22	4554	575.60	Leu95, Gln98, Ala99, Glu208, Asn210, Ala396, Lys562, Trp566
	Withaferin	−46.84	−11.13	5598	640.50	Leu95, Gln98, His195, Tyr196, Lys562
	Asiatic acid	−45.69	−13.09	5978	691.70	Leu95, Gln98, Ala99, Tyr202, Asp206, Glu208, Val209, Ala396, Lys562, Pro565, Trp566
	Avicularin	−43.13	−11.09	5232	604.20	Lys94, Leu95, Tyr196, Val209, Asn210, Ala396, Lys562
6LU7	α-ketoamide (Control)	−56.13	−15.07	4578	492.00	Asp197, Lys236, Tyr237, Leu272
	Avicularin	−54.04	−14.77	4584	520.60	His41, Met49, His164, Met165, Glu166, Pro168, Thr190, Asp187, Arg188, Gln189
	Guajaverin	−51.69	−12.92	4182	515.50	Met49, Phe140, His163, His164, Met165, Glu166, Arg188, Gln192
	Withaferin	−47.08	−14.06	4708	560.60	Thr24, Met49, His41, Cys145, Met165, Arg188
	Asiatic acid	−43.52	−13.90	5050	562.20	Met49, Leu141, Asn142, Ser144, Cys145, His163, Glu166

to be effective in treating life-threatening diseases such as cancer, Alzheimer's disease, diabetes, malaria, and cardiac diseases (Table 1) while minimizing drug toxicity [24].

The expansion of natural product as new medicine or drug to resist the emerging virus SARS-CoV-2 could be done to bypass the side effects of synthetic drugs. Therefore, the study aimed at evaluating some plant-based active compound for drug candidacy against SARS-CoV-2 through virtual screening methods and various computational investigations.

2. Material and methods

2.1. Retrieval of SARS-CoV-2 proteins/protein-domains and plant metabolites

The 3D structures of SARS-CoV-2 main proteases (6W63, 6LU7), Nsp9 (Non-structural protein-9) RNA binding protein (6W4B), spike receptor binding domain (6M0J), spike ecto-domain (6VYB), and HR2 Domain (6LVN) were retrieved from the RCSB Protein Data Bank [51]. A total 27 plant metabolites belonging to different classes were extracted from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDS (3D) format (Table 1) [52]. The structures were further converted into the PDB format by Open Babel v2.3 [53].

2.2. Screening of plant metabolites against SARS-CoV-2 proteins/protein-domains

Molecular docking is an effective approach for screening suitable therapeutics against specific drug target of deadly pathogens [54]. This powerful tool is used to model the interaction between small ligands and macromolecules, thereby paving the way for drug discovery [55]. The binding affinity of 27 plant metabolites with different SARS-CoV-2 proteins/protein domains (drug targets/macromolecules) were determined by using the PatchDock server [56]. Recently, alpha-ketoamide (CID 6482451) has been suggested as a SARS-CoV-2 MPP inhibitor by experimental study [57]. The ligand was used as a positive control for the present study, and employed for docking analysis against all six macromolecules. The docked complexes were further refined via the FireDock refinement tool [58]. The ligand bond complexes were visualized by Discovery Studio v3.1 and PyMOL v2.0 [59,60].

2.3. Analysis of drug surface hotspot and ligand binding pocket prediction

The drug surface hotspot of SARS-CoV-2 proteins was analysed by investigating the docked complexes with the top metabolites using LigPlot+, Discovery Studio and PyMOL v.2.0 software [59,60]. Binding patterns of asiatic acid, avicularin, guajaverin, and withaferin with six macromolecules were allowed for comparative structural analysis. Moreover, interaction of Alpha-ketoamide with the studied proteins were also investigated.

2.4. Drug profile analysis of top metabolites

Absorption, distribution, metabolism, and excretion (ADME) are four major criteria that influence the drug levels and kinetics of drug exposure to the tissues within an organism. The pharmacological activity and performance of a drug is largely controlled by these parameters [61]. The SwissADME server was used to assess the ADME properties of top four metabolites [62]. The BOILED-Egg model was employed to

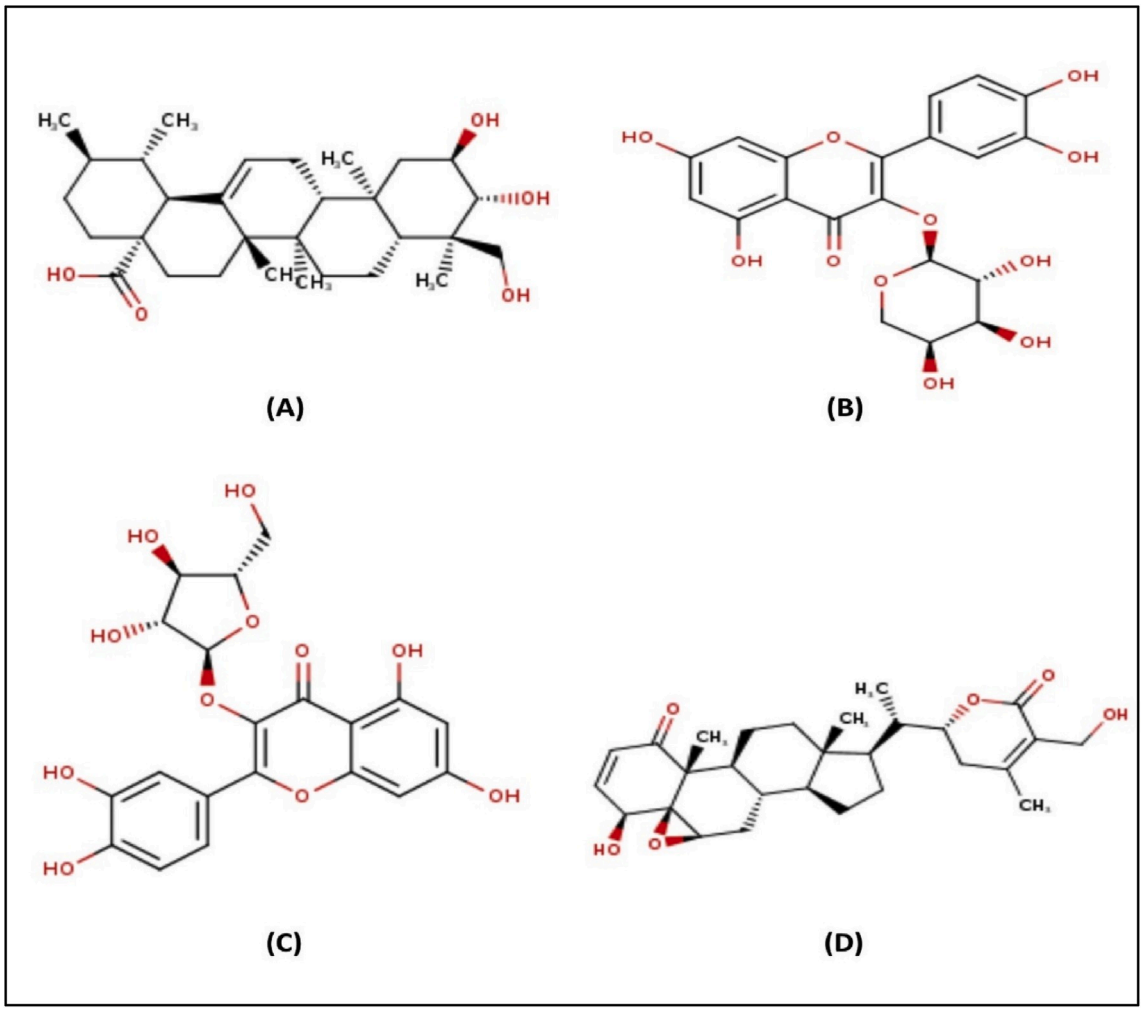

Fig. 1. Chemical structures of asiatic acid (A), guajaverin (B), avicularin (C) and withaferin (D).

calculate the blood-brain barrier (BBB) in the studied compounds [63]. The relative toxicity of top drug candidates were analysed via the ProToxII server [64]. This popular webserver efficiently predicts various toxicity endpoints by incorporating molecular similarity, fragment tendency and fragment similarity methods. The server also predicted the oral toxicity based on the analysis of two-dimensional (2D) similarity to compounds with a known median lethal doses (LD50). The set used for the prediction consists of approximately 38,000 unique compounds with known oral LD50 values measured in rodents [65]. Additionally, OSIRIS Property Explorer were employed to investigate the undesired effects of these compounds [66].

2.5. Prediction of drug targets and available drug molecules from DrugBank

SwissTargetPrediction was utilized to estimate the possible macromolecular targets of predicted drug candidates [67]. The server predicts based on a combination of 2D and 3D similarity with a library of 370000 known bioactive compounds on approximately 3000 proteins. Moreover, the SwissSimilarity web tools were used to identify potential drug molecules against SARS-CoV-2 based on homology screening of predicted top drug candidates. The server allowed ligand-based virtual screening of several libraries of small molecules to find approved, experimental, or commercially available drugs from DrugBank using different approaches including FP2 fingerprints, electroshape, spectrophores, and align-IT [68].

3. Results

3.1. Screening of plant metabolites against SARS-CoV-2

All of the retrieved structures of SARS-CoV-2 proteins/protein-domains (macromolecules) and plant metabolites (ligands) were optimized and employed for molecular docking to predict the affinity between the above-mentioned ligands and the macromolecules. The metabolites were ranked based on global binding energy and the results depict that the top four scorers (metabolites) were the same for each of the macromolecules in terms of minimum binding energy (Table 2 and Supplementary File 1). In each case, asiatic acid, avicularin, guajaverin, and withaferin showed the best binding interactions with six studied macromolecules (Fig. 1 and Table 2). Moreover, asiatic acid exhibited the highest binding affinity with SARS-CoV-2 main protease (−53.05 kcal/mol), Nsp9 RNA binding protein (−50.04 kcal/mol), and spike ectodomain (60.68 kcal/mol) (Fig. 2 and Table 2), while guajaverin bound with the spike receptor binding domain and HR2 Domain with a binding energy of −47.34 kcal/mol and −28.73 kcal/mol, respectively (Fig. 3 and Table 2).

3.2. Analysis of drug surface hotspot and ligand binding pocket prediction

The structural conformation of the docked complex was analysed to unravel the drug surface hotspot of studied SARS-CoV-2 proteins. The ligand binding pattern and interacting residues with their respective positions were investigated (Table 2). Results revealed that the amino

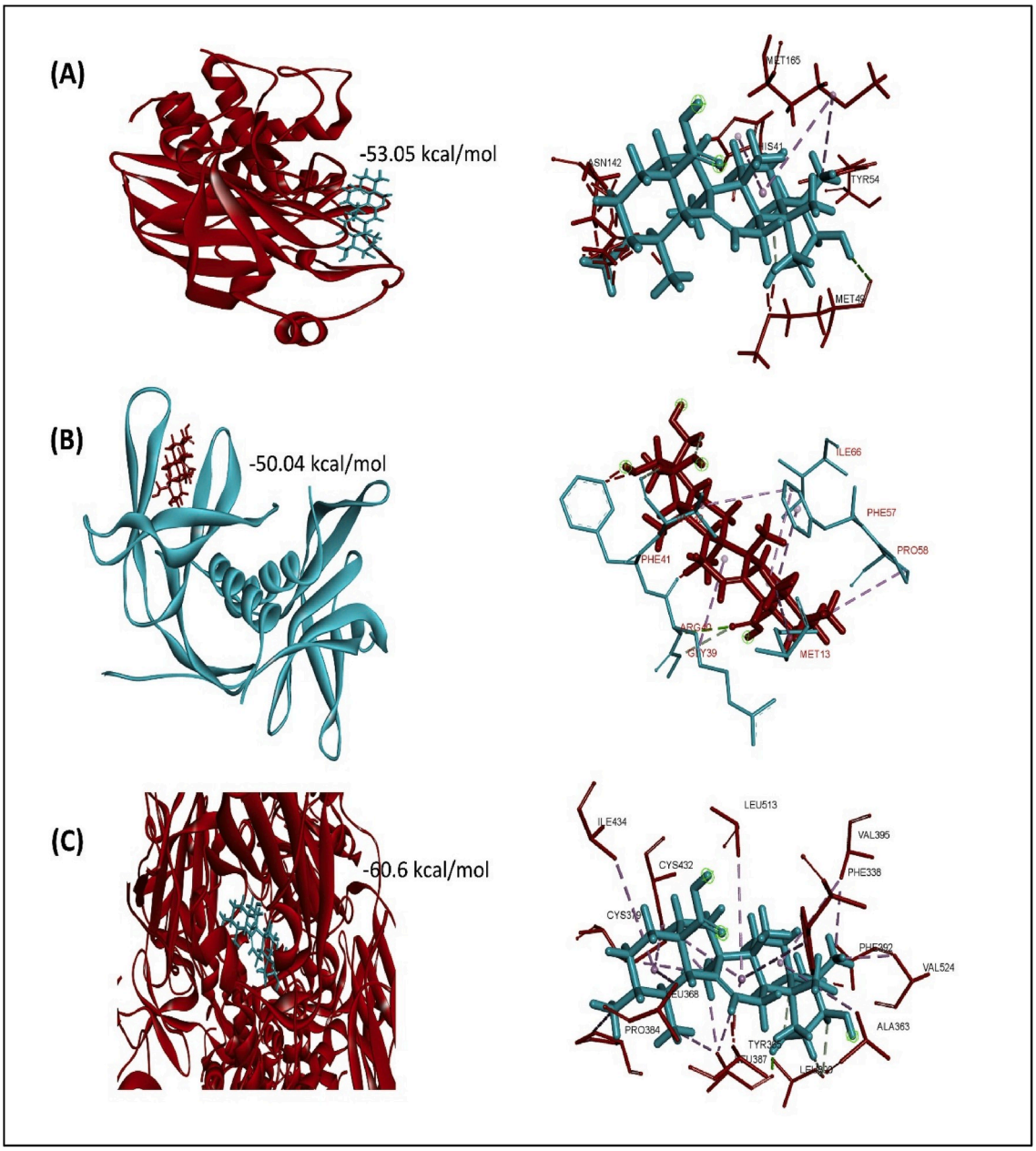

Fig. 2. Molecular interaction of asiatic acid with SARS-CoV-2 main protease (A), Nsp9 RNA binding protein (B), and spike ecto-domain (C).

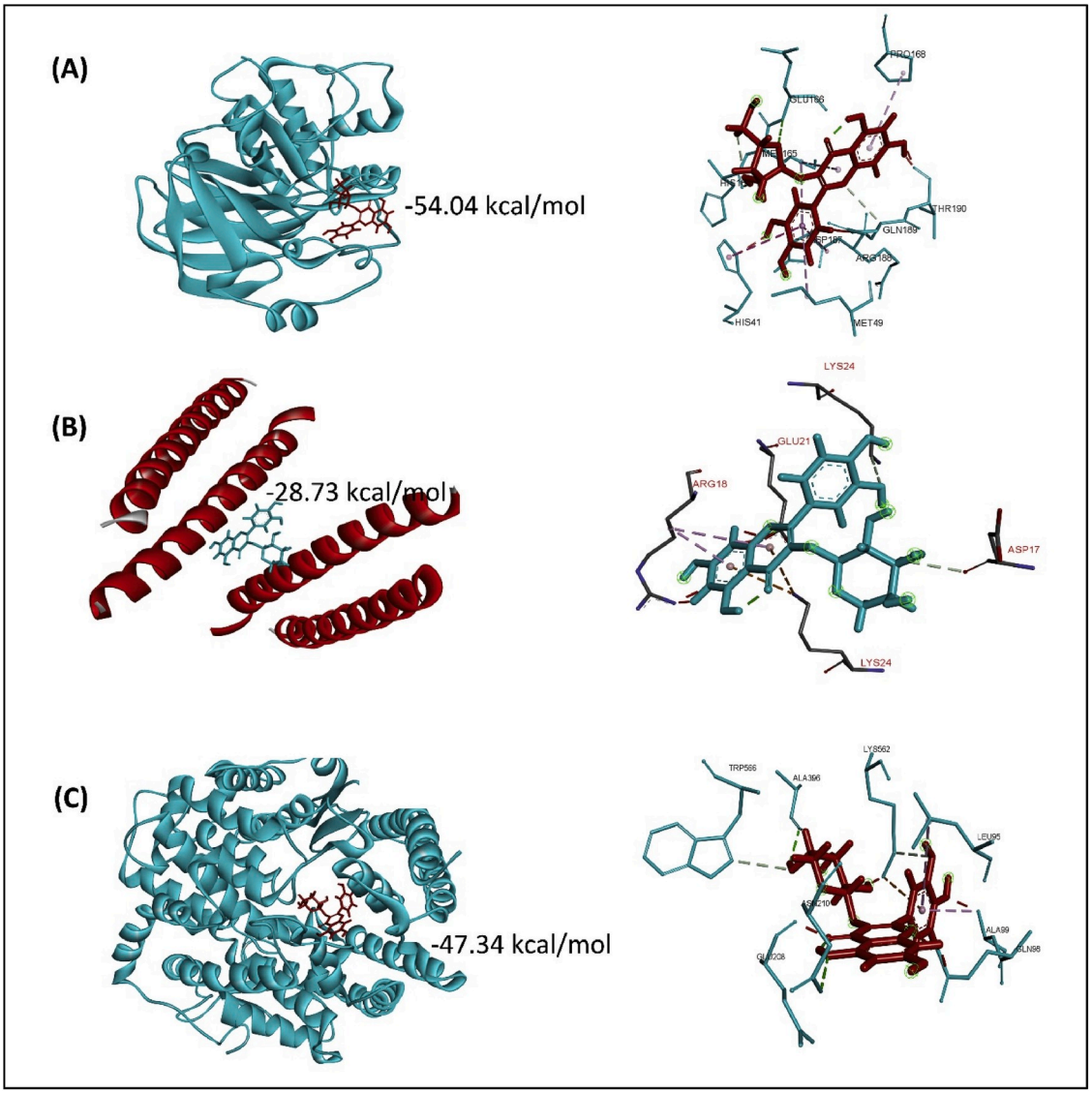

Fig. 3. Molecular interaction of SARS-CoV-2 main protease with avicularin (A), HR2 domain with guajaverin (B), and spike receptor-binding domain with guajaverin (C).

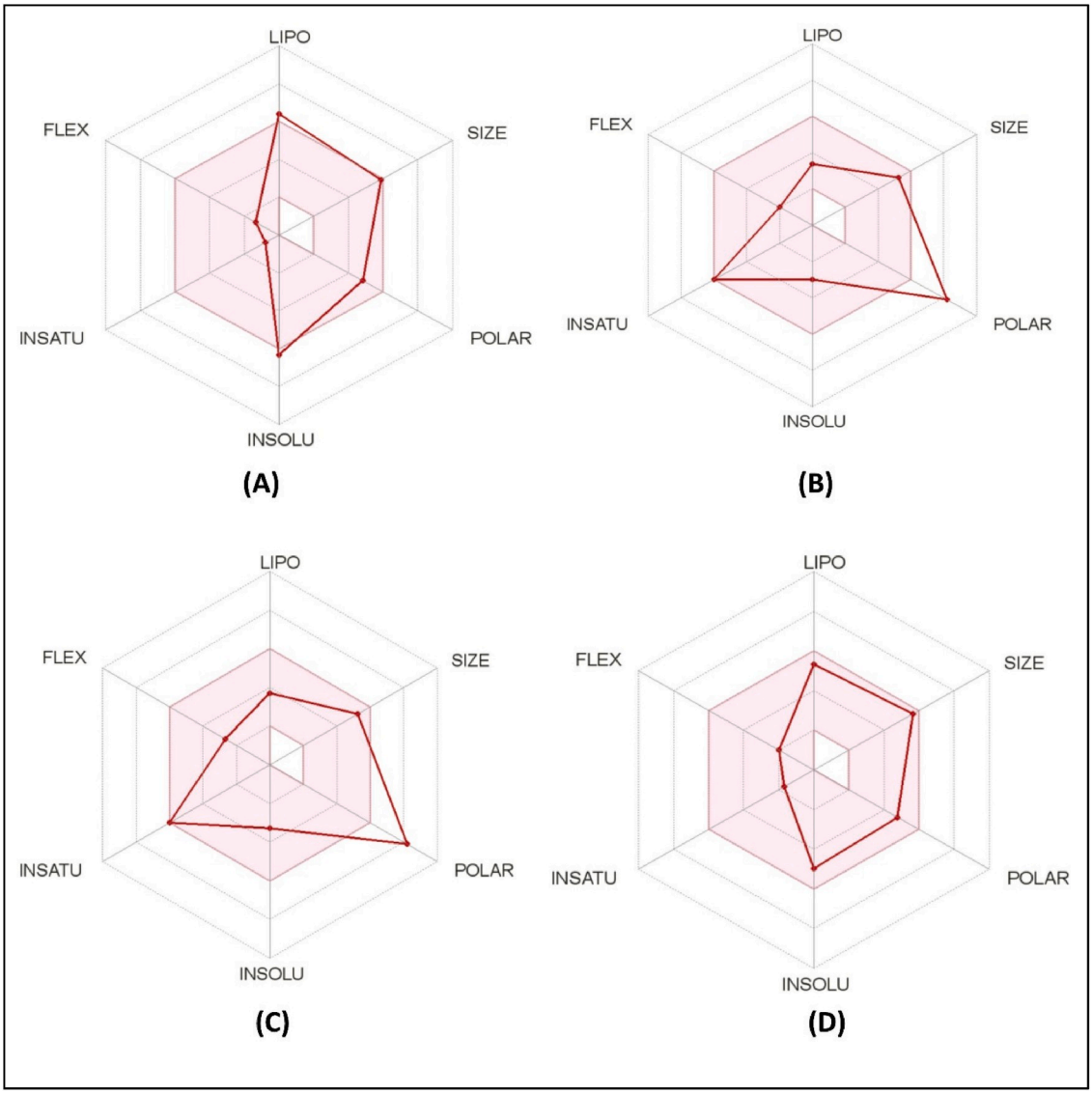

Fig. 4. ADME analysis of top four metabolites; A: Asiatic acid, B: Guajaverin, C: Avicularin, and D: Withaferin.

Table 3
Drug profile and ADME analysis of the top four metabolites.

Parameter		Top Main Protease Protein Inhibitors of SARS-CoV-2			
		Asiatic acid	Guajaverin	Avicularin	Withaferin
Physicochemical parameters	Formula	C30H48O5	C20H18O11	C20H18O11	C28H38O6
	Molecular weight	488.70 g/mol	434.35 g/mol	434.35 g/mol	470.60 g/mol
	No. H-bond acceptor	5	11	11	6
	No. H-bond donors	4	7	7	2
	Molar Refractivity	139.24	104.19	104.19	127.49
	TPSA	97.99 Å^2	190.28 Å^2	190.28 Å^2	96.36 Å^2
Lipophilicity	Log $P_{o/w}$ (iLOGP)	2.95	1.77	1.86	3.24
	Log $P_{o/w}$ (XLOGP3)	5.70	0.43	0.98	3.83
	Log $P_{o/w}$ (WLOGP)	5.03	0.10	0.10	3.35
	Log $P_{o/w}$ (MLOGP)	4.14	−2.06	−2.06	2.75
	Log $P_{o/w}$ (SILICOS-IT)	3.96	−0.10	0.06	3.93
	Consensus Log $P_{o/w}$	4.36	0.03	0.19	3.42
Pharmacokinetics	GI absorption	High	Low	Low	High
	BBB permeant	No	No	No	No
	P-gp substrate	Yes	No	No	Yes
	CYP1A2 inhibitor	No	No	No	No
	CYP2C19 inhibitor	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No
	CYP3A4 inhibitor	No	No	No	No
	Log K_p (skin permeation)	- 5.23 cm/s	−8.64 cm/s	−8.25 cm/s	−6.45 cm/s
Water Solubility	Log *S* (ESOL)	−6.33	−2.99	−3.27	−4.97
	Solubility	2.29e-4 mg/ml; 4.69e-7 mol/l	4.47e-01 mg/ml; 1.03e-03 mol/l	2.34e-01 mg/ml; 5.39e-04 mol/l	5.01e-03 mg/ml; 1.07e-05 mol/l
	Class	Poorly soluble	Soluble	Soluble	Moderately soluble
	Log *S* (SILICOS-IT)	−4.28	−1.94	−2.07	−3.79
	Solubility	2.59e-2 mg/ml; 5.31e-05 mol/l	4.96e+00 mg/ml; 1.14e-02 mol/l	3.71e+0 mg/ml; 8.55e-3 mol/l	7.54e-02 mg/ml; 1.60e-04 mol/l
	Class	Moderately soluble	Soluble	Soluble	Soluble
Medicinal Chemistry	Leadlikeness	No; 2 violations: MW > 350, XLOGP3>3.5	No; 1 violation: MW > 350	No; 1 violation: MW > 350	No; 2 violations: MW > 350, XLOGP3>3.5
	Bioavailability Score	0.56	0.17	0.17	0.55
	PAINS	0 alert	1 alert: catechol_A	1 alert: catechol_A	0 alert
	Synthetic accessibility	6.56	5.05	5.04	6.83

acids from 41 to 54 and 142–190 positions were crucial for the binding interactions of SARS-CoV-2 main protease protein (6W63). Moreover, His41, Cys44, Met49, Asn142, Cys145, Met165 were involved in maximum cases to form the docked complexes. The ligands showed highest binding affinity for 39–73 and 142–166 regions of Nsp9 RNA binding protein (6W4B). Again, the residues from 94 to 99 and 563–566 regions were identified as top surface hotspots for spike receptor binding domain (6M0J) where the position Lys94, Leu95, Tyr196, Lys562, Trp566 were most dominant (Table 2).

3.3. ADME analysis of top drug candidates

Different ADME properties, i.e., physicochemical parameters, pharmacokinetics, lipophilicity, water solubility, medicinal chemistry of top drug candidates were estimated to evaluate their drug profiles (Fig. 4 and Table 3). Analysis of inhibition effects with different CYP isoforms (CYP1A2, CYP2D6, CYP2C9, CYP2C19, CYP3A4) revealed that none of the candidates had such an interaction possibility with any cytochromes P450 isoforms. GI absorption was found higher for asiatic acid and withaferin, while lower for guajaverin and avicularin. Moreover, blood-brain barrier (BBB) permeation was calculated by the BOILED-Egg model, which revealed no BBB permeant among the studied top drug candidates. Each candidate was water soluble from a moderate to high level, while guajaverin and avicularin showed maximum solubility (Table 3).

3.4. Toxicity pattern analysis of top drug candidates

Prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets were analysed (Table 4). Results revealed that guajaverin and avicularin fell in the category of toxicity class 5, while the predicted toxicity group for Asiatic acid and withaferin were 4 and 2 respectively (the lower the class the higher the toxicity). Estimated LD50 for asiatic acid, avicularin, guajaverin and withaferin were 2000, 5000, 5000 and 7 mg/kg respectively. The toxicity radar in Fig. 5 illustrates the confidence of positive toxicity results compared to the average of its class. None of the compounds showed any undesired effects such as tumorigenicity, mutagenicity, irritating, or reproductive effects. Withaferin, however, was found to be relatively toxic among the four candidates, with considerable cytotoxicity and immunotoxicity (Fig. 5).

3.5. Prediction of drug targets and available drug molecules from DrugBank

Most of the target class belonged to enzymes, kinase proteins, oxidoreductases (i.e. aldose reductase, aldo-keto reductase), phosphatases and lyases (i.e. carbonic anhydrase) (Fig. 6 and Table 5). Ligand-based virtual screening was performed to predict biologically active small compounds against SARS-CoV-2 from DrugBank. Two approved drugs, Hydrocortisone (DB00741) and Dinoprost-tromethamine (DB01160) were found analogous to asiatic acid with prediction scores of 50.52 and 50.53, respectively. Moreover, results revealed the similarity of Mupirocin (DB00410) and Simvastatin (DB00641) with withaferin, with a high prediction score (Table 6). The findings suggest that these could be potential drug candidates against SARS-CoV-2, thus requiring further experimental trials.

Table 4
Toxicity model reports of the top four drug candidates.

Classification	Target	Prediction and Probability			
		Asiatic Acid	Aviculerin	Guajaverin	Withaferin
Organ toxicity	Hepatotoxicity	Inactive (0.91)	Inactive (0.80)	Inactive (0.80)	Inactive (0.93)
Toxicity end points	Carcinogenicity	Inactive (0.70)	Inactive (0.79)	Inactive (0.79)	Inactive (0.55)
Toxicity end points	Immunotoxicity	Active (0.77)	Active (0.68)	Active (0.93)	Active (0.99)
Toxicity end points	Mutagenicity	Inactive (0.81)	Inactive (0.73)	Inactive (0.79)	Inactive (0.79)
Toxicity end points	Cytotoxicity	Inactive (0.73)	Inactive (0.72)	Inactive (0.69)	Active (0.87)
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	Inactive (0.99)	Inactive (0.85)	Inactive (0.90)	Inactive (0.98)
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	Inactive (0.59)	Inactive (0.92)	Inactive (0.96)	Inactive (0.63)
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive (0.51)	Inactive (0.98)	Inactive (0.97)	Inactive (0.54)
Tox21-Nuclear receptor signalling pathways	Aromatase	Inactive (0.91)	Inactive (0.98)	Inactive (0.97)	Inactive (0.80)
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	Inactive (0.73)	Inactive (0.85)	Inactive (0.92)	Inactive (0.60)
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive (0.97)	Inactive (0.99)	Inactive (0.99)	Inactive (0.98)
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ)	Inactive (0.97)	Inactive (0.93)	Inactive (0.94)	Inactive (0.91)
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element	Inactive (0.89)	Inactive (0.91)	Inactive (0.94)	Inactive (0.86)
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive (0.89)	Inactive (0.91)	Inactive (0.94)	Inactive (0.86)
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	Inactive (0.85)	Inactive (0.89)	Inactive (0.89)	Inactive (0.80)
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	Inactive (0.93)	Active (0.55)	Inactive (0.72)	Inactive (0.75)
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive (0.96)	Inactive (0.96)	Inactive (0.96)	Inactive (0.94)

4. Discussion

Excessive infection rates and mortality of SARS-CoV-2 led the researchers to concentrate immensely on developing strategies for combating infections caused by the pathogen [69–71]. Regardless of this praiseworthy initiative, there are still no specific drugs or approved vaccines that could treat SARS-CoV-2 infected patients [72,73]. Though some candidates are in the investigational stages, many of them raise controversial issues [74,75]. Plant-derived natural products play a significant role by being a lead molecule in the development of drug candidates [76]. Hence, in the present study, attempts were taken to evaluate some plant-derived metabolites as inhibitory agents of SARS-CoV-2 based on their binding affinities to the key proteins of the pathogen.

The contribution of computational biology has accelerated the pace of drug discovery [77]. It is now used in the biopharmaceutical industry to discover and develop new lead compounds against many infectious pathogens [77,78]. By this route, one can visualize the possibilities of binding of potential small molecules as ligands/inhibitors [76]. Phytomolecules like Baicalein, Luteolin, Quercetin, and Kaempferol are potential antiviral agents against a wide range of important viruses including Dengue, HIV, H5N1 influenza A virus, Coxsackie virus, CHIKV, and Japanese encephalitis virus [79]. Recent studies have focused on MPP inhibitors of SARS-CoV-2 i.e. alpha-ketoamide, Hydroxy, Remdesivir, Chloroquine and Favipiravir to evaluate their potency as drugs [80,81]. Several *in silico* strategies were also adopted to screen putative drug candidates against SARS-CoV-2 [82,83]. However, all these experiments used either main protease proteins or RNA-dependent RNA polymerase of SARS-CoV-2 as probable drug targets. In this study, we screened some natural metabolites against SARS-CoV-2 main proteases (6W63, 6LU7), Nsp9 (Non-structural protein-9) RNA binding protein (6W4B), spike receptor binding domain (6M0J), spike ecto-domain (6VYB), and HR2 domain (6LVN) using a molecular docking approach [84–86]. The polyproteins of coronavirus are cleaved and transformed in mature non-structural proteins (Nsp) by proteases [87]. As a putative component in the replication complex, Nsp9 may possibly have an RNA binding activity. Viral replication complexes are frequently membrane associated and Nsp9 helps in this case. The entry of coronavirus into host cells, on the contrary, is mediated by the transmembrane spike glycoprotein that forms homotrimers protruding from the viral surface. S protein comprises two functional subunits responsible for binding to the host cell receptor (S_1) and fusion of the viral and cellular membranes (S). After the attachment of the receptor-binding subunit to the receptor, the HR1 and HR2 domains in the membrane fusion subunit interact with each other and form a six-helix bundle, and this conformational change results in a close apposition of the fusion peptide, leading to virus-cell membrane fusion [88]. Thus, all of these proteins represent an attractive pharmacological target for SARS-CoV-2.

Results revealed that asiatic acid had highest binding affinity with SARS-CoV-2 main protease (−53.05 kcal/mol), Nsp9 RNA binding protein (−50.04 kcal/mol) and spike ecto-domain (60.68 kcal/mol) (Fig. 2 and Table 2). Remarkably, four metabolites i.e. asiatic acid, avicularin, guajaverin and withaferin scored best for each six macromolecules and bound with minimum global binding energy (Table 2 and Supplementary File 1). The scores of top candidates were either close or in some instances lower than alpha ketoamide, a positive control used in the present study (Table 2). Asiatic acid, a triterpenoid derivative from *Centella asiatica*, displayed antioxidative, anti-inflammatory, and protective properties against neurotoxicity induced by glutamate- or b-amyloid previously [89]. Bian et al. also reported the inhibitory activities of asiatic acid and effectivity against fibroproliferative disorders (Keloids) through blocking the TGF-β/Smad pathway [90]. Withanolides are nature-derived secondary metabolites produced in *Withania*

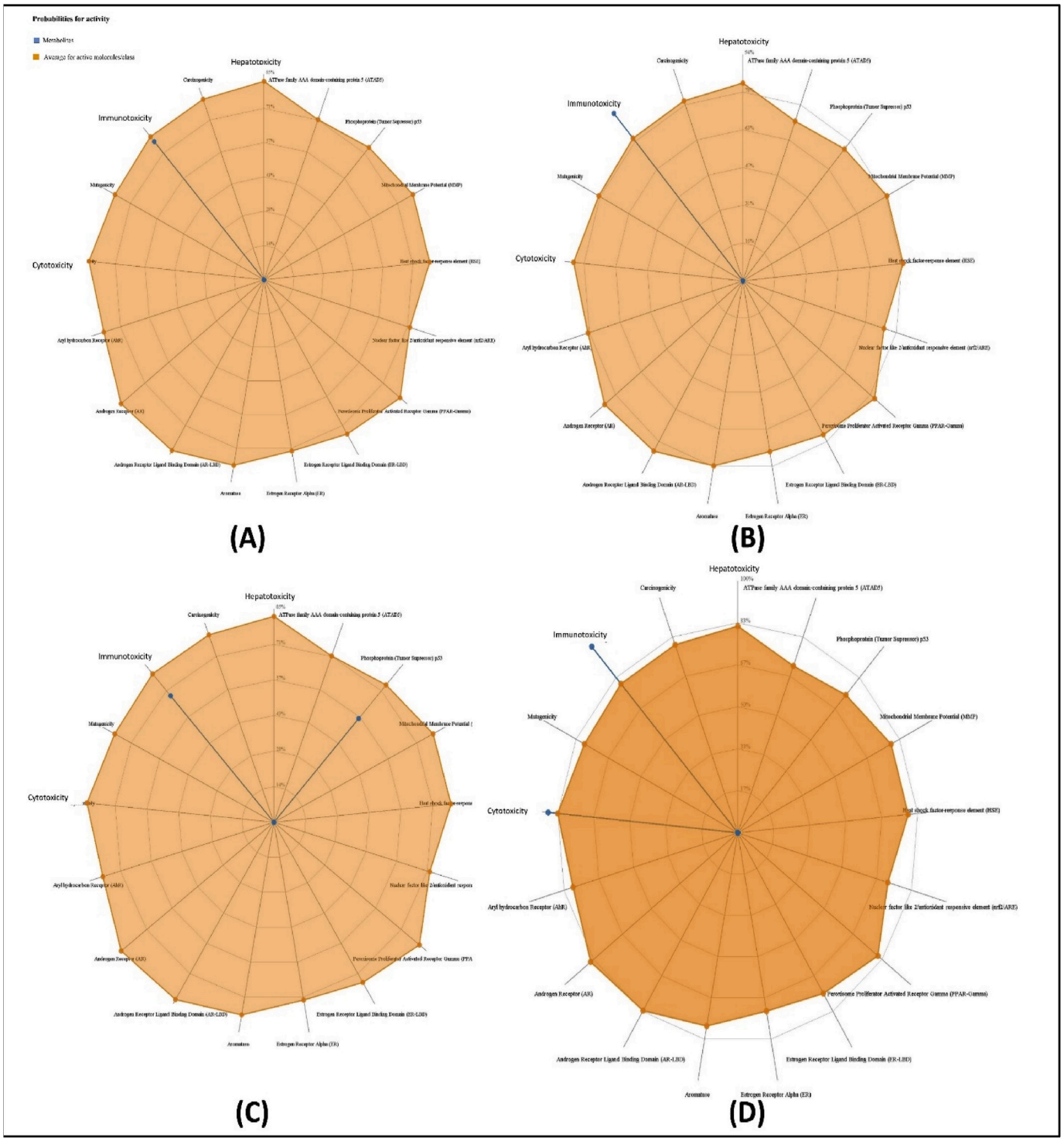

Fig. 5. Toxicity patterns of the top four drug candidates; A: Asiatic acid, B: Guajaverin, C: Avicularin, and D: Withaferin.

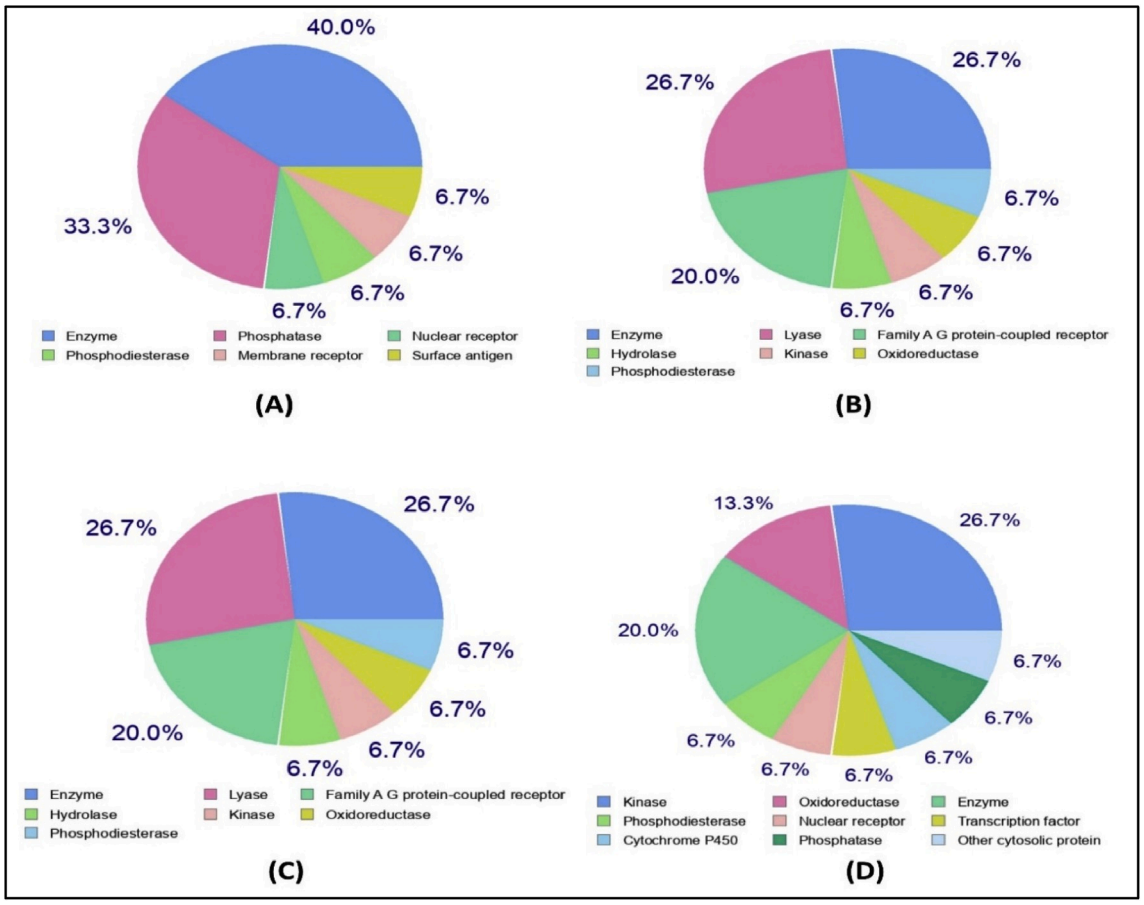

Fig. 6. Prediction of drug targets for asiatic acid (A), guajaverin (B), avicularin (C), and withaferin (D).

somnifera via oxidation of steroids, which have medicinal value like anti-inflammation, anti-cancer, adaptogenic and anti-oxidant effects [91]. Withaferin, a steroidal lactone from this group, suppresses HIV-1 LTR transcription and viral replication and also has a vital function to inhibit herpes simplex virus [92,93]. It has anti-inflammatory properties and also shows neuro-protective activity against Aβ neurotoxicity [94,95]. A molecular docking and simulation study revealed the vital function of withaferin to attenuate the neuraminidase of H1N1 influenza virus [96].

Guajaverin (Quercetin 3-arabinopyranoside) and avicularin (quercetin- 3-O-α-L-arabinofuranoside) are the main bioactive components of guava leaves with hypoglycemic properties and inhibitory capacity against free fatty acid release [97]. Previously, anti-plaque activity of guajaverine was attributed to its microbicidial activity against the growth of Strep [38]. Avicularin, a flavonoid of plants, displayed diverse pharmacological properties such as anti-inflammatory effects and anti-infectious effects against pathogens [98,99]. Lee et al. reported the effective anti-oxidant potentiality of Avicularin from *Lespedeza cuneata* [100]. Researchers also identified hepatoprotective activity of avicularin extracted from the aerial parts of *Lespedeza cuneata* against lesions caused by t-BHP in HepG2 cells [101]. It has also been suggested to inhibit activation of ERK signaling pathways through LPS-stimulated overproduction of pro-inflammatory mediators and cytokine [98]. Avicularin may suppress the inflammatory response, and causes apoptosis in human RA synovial cells through obstructing the activation of the MEK/NF-κB pathway, thus preventing rheumatoid arthritis (RA) *in vitro* [102].

In the present study, we revealed the molecular interactions of top drug candidates with SARS-CoV-2 key proteins (Fig. 2 and 3 and Table 2). The binding sites for each ligand occupied the catalytic domain of SARS-CoV-2 main protease protein [103]. Among the common binding residues, His41 and Cys145 form the catalytic dyad and act as a substrate recognition site [103,104]. The top candidates were well fitted into the active pocket of MPP where several hydrophobic amino acid residues including Met49, Gly143, Cys145, Met165, Pro168, Ala191 compose a relatively hydrophobic environment, which may help to stabilize its conformation [104]. The crucial binding sites of Nsp9 protein (39–73 region) are characterized by positively charged, glycine rich β-loops, which were proposed to be involved in RNA binding [105]. Moreover, we targeted three distinct domains of SARS-CoV-2 spike protein, all of which play essential roles in the mechanism of viral entry into the host cell [106]. The investigation may be useful to unravel the main drug target hotspot and medicinal chemistry of the investigational drugs currently under trials against SARS-CoV-2. ADME data, whether experimentally measured or computationally predicted, provide key insights into how a drug will ultimately be treated or accepted by the body. Hence, while a drug lead may exhibit phenomenal efficacy *in vitro*, poor ADME results often invariably terminate its development [107]. Computational methods play a key role in anticipating potential ADME and toxicity problems and reducing the number of experiments that involve animal testing. Therefore, the topmost drug candidates were employed for ADME analysis to investigate their drug profiles. None of the metabolites, however, showed any undesirable consequences that could reduce their drug likeness properties. SARS-CoV-2 appears as a severe acute respiratory disease not a neuro disease [108]. Thus, there is no need to permeate the blood brain barrier (BBB) for being an effective molecule against SARS-CoV-2. However, no BBB permeants were found among the top drug candidates. Most of the target class for the top drug candidates belonged to the categories of enzymes (e.g. oxidoreductases, hydrolase, phosphatases, lyases (Table 5). The major protease proteins

Table 5
Predicted drug targets for asiatic acid, guajaverin, aviculerin, and withaferin.

Metab-olites	Drug Targets	Common Name	Uniprot ID	ChEMBL ID	Target Class	Probability
Asiatic Acid	Aldo-keto reductase family 1 member B10	AKR1B10	O60218	CHEMBL5983	Enzyme	
	Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase	
	11-β-hydroxysteroid dehydrogenase 1	HSD11B1	P28845	CHEMBL4235	Enzyme	
	DNA polymerase beta	POLB	P06746	CHEMBL2392	Enzyme	
	T-cell protein-tyrosine phosphatase	PTPN2	P17706	CHEMBL3807	Phosphatase	
	Phospholipase A2 group 1B	PLA2G1B	P04054	CHEMBL4426	Enzyme	
Guajaverin & Aviculerin	Aldose reductase	AKR1B1	P15121	CHEMBL1900	Enzyme	
	Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	
	Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase	
	Carbonic anhydrase XII	CA12	O43570	CHEMBL3242	Lyase	
	Carbonic anhydrase IV	CA4	P22748	CHEMBL3729	Lyase	
	NADPH oxidase 4	NOX4	Q9NPH5	CHEMBL1250375	Enzyme	
	Adrenergic receptor alpha-2	ADRA2C	P18825	CHEMBL1916	Family A G protein-coupled-receptor	
	Acetylcholinesterase	ACHE	P22303	CHEMBL220	Hydrolase	
	Quinone reductase 2	NQO2	P16083	CHEMBL3959	Enzyme	
	Ribosomal protein S6 kinase alpha 3	RPS6KA3	P51812	CHEMBL2345	Kinase	
	Neuromedin-U receptor 2	NMUR2	Q9GZQ4	CHEMBL1075144	Family A G protein-coupled receptor	
Withaferin	Protein kinase C alpha	PRKCA	P17252	CHEMBL299	Kinase	
	Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase	
	Isoleucyl-tRNA synthetase	IARS	P41252	CHEMBL3235	Enzyme	
	Protein kinase C delta	PRKCD	Q05655	CHEMBL2996	Kinase	
	HMG-CoA reductase	HMGCR	P04035	CHEMBL402	Oxidoreductase	
	Phosphodiesterase 4D	PDE4D	Q08499	CHEMBL288	Phosphodiesterase	
	Telomerase reverse transcriptase	TERT	O14746	CHEMBL2916	Enzyme	
	Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor	
	Protein kinase C epsilon	PRKCE	Q02156	CHEMBL3582	Kinase	
	Proto-oncogene c-JUN	JUN	P05412	CHEMBL4977	Transcription factor	
	Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase	

(protein hydrolase) of SARS-CoV-2 thus can be a specific target for these natural metabolites. Guajaverin and avicularin are two isomers and derivatives of quercetin with a glycoside substituent in their chemical structure [109,110]. The significant similarity between these two polar compounds in terms of structure, chemical formula, molar mass, and other physicochemical parameters (Table 3) may be responsible for covering the same targets by these two flavonoids (Table 5). The toxicity of drug impurities is closely related to their structure. Structure-activity relationships (SARs) have been widely used in Europe and the United States to predict toxicity by computer [111]. The toxicity prediction results in the present study revealed negligible tumorigenic, mutagenic, irritating, or reproductive effects by the drug candidates, though withaferin was found to be comparatively toxic among the top four compounds. Despite having medicinal importance [112,113], the ability of withaferin to inhibit cell growth and induce apoptosis in *in vitro* and *in vivo* models were reported [114,115]. Moreover, dose dependent toxicity and other adverse effects such as elevation of liver enzymes, skin rash, fever etc. were observed by researchers [115,116]. Our study also revealed the hepatotoxic and cytotoxic nature of withaferin through computational investigations.

However, drug similarity prediction identified two approved structural analogs of withaferin, Mupirocin (DB00410) and Simvastatin (DB00641) which could be alternative choices, and therefore require further *in vivo* investigations. Ligand-based virtual screening using asiatic acid predicted two other biologically active compounds, Hydrocortisone (DB00741) and Dinoprost-tromethamine (DB01160) from DrugBank. Interestingly, Hydrocortisone, a cortisone based drug, was previously used during the SARS-CoV-1 and MERS outbreak [117]. Diosmin, on the contrary, is used as a supplementary drug and is found in various natural plants [118]. Myricetin showed the potential to inhibit reverse transcriptase of the RLV and HIV viruses, while characterized as having antioxidative and prooxidative properties. It is also a potent anticarcinogen and antimutagen [119]. The most significant finding of this study is Simvastatin, which can block downstream molecules (key factors in virus infectivity) and can control severe influenza and pneumonia through prevention of excess cytokine release [120]. The results suggest that all these compounds could be potential drug candidates against SARS-CoV-2. The study may pave the way to develop effective medications and preventive measures against SARS-CoV-2 in the future.

Table 6
Predicted bioactive molecules from DrugBank.

Metabolites	Drug bank id	Name	Score	Status
Asiatic acid	DB00741	Hydrocortisone	0.539	Approved
	DB01160	Dinoprost Tromethamine	0.529	Approved
	DB07886	(11alpha,14beta)-11,17,21-trihydroxypregn-4-ene-3,20-dione	0.539	Experimental
	DB07209	(8R,9Z,12Z)-8-hydroxy-6-oxooctadeca-9,12-dienoic acid	0.510	Experimental
Guajaverin	DB08995	Diosmin	0.280	Approved
	DB02375	Myricetin	0.236	Experimental
Withaferin	DB00410	Mupirocin	0.481	Approved
	DB00641	Simvastatin	0.447	Approved
	DB08224	hexahydro-7-methyl-8-[2-[(2r,4r)-tetrahydro-4-hydroxy-6-oxo-2h-pyran-2-yl]ethyl]-1-naphthalenol	0.501	Experimental
	DB04775	Reidispongiolide C	0.479	Experimental
Avicularin	DB08995	Diosmin	0.249	Approved
	DB02375	Myricetin	0.210	Experimental

5. Conclusion

The results suggest that asiatic acid, avicularin, and guajaverin could be options to treat SARS-CoV-2 associated infections. Furthermore, two biologically active structural analogs from DrugBank i.e. Hydrocortisone and Simvastain may be effective and show potency against the viral pathogen. However, all the investigational drugs of SARS-CoV-2 are under strict regulation by the World Health Organization. Due to the encouraging results, we highly recommend further *in vivo* trials for the experimental validation of our findings.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2020.100367.

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