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Molecular Docking Unmasks Potent Phytoligands against SARS-CoV-2 Spike Glycoprotein, Main Protease, Papain-like Protease, and RNA-dependent RNA Polymerase

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ABSTRACT

The recent coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has offered a unique challenge for human survival. However, there is no available known prophylaxis, therapeutic intervention, and vaccine candidate against SARS-CoV-2 to date. We aimed towards identifying novel phytoligands from widely available botanical resources which could serve as potential inhibitors against SARS-CoV-2. Based on literature review, database search, ADMET, and drug-likeness, 55 phytoligands and 8 synthetic repurposing drugs were screened and tested against SARS-CoV-2 spike glycoprotein, main protease, papain-like protease, and RNA-dependent RNA polymerase using molecular docking and protein-ligand interaction. All phytoligands and repurposing drugs showed binding affinity based inhibitory potential against the viral proteins. The highest binding affinities of phytoligands towards antiviral targets were exhibited by colchicine and oleic acid, and that of repurposing drugs was shown by saquinavir and nelfinavir. Capsaicin, oleic acid, azithromycin, nelfinavir, remdesivir, and saquinavir were acted as plausible broad-spectrum inhibitors. Hydrogen bonds and hydrophobic interactions of amino acids were varied significantly within the conserved domain along with glutamic acid richness. Further investigation should be carried out to obtain the synergistic effect using cell-based assays, animal models, and clinical trials to discover novel phytomedicine against SARS-CoV-2.

Keywords: COVID-19; SARS-CoV-2 viral proteins; Phytoligands; Repurposing drugs; Molecular docking; Protein-ligand interaction; Phytomedicine

1. INTRODUCTION

A recent outbreak of the global pandemic coronavirus disease 2019 (COVID-19) caused by a deadly virus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has offered extreme health threats extending the plight of human survival[1-2]. The stark reality comes with global death toll crossing 4,00,857, with 69,31,000 laboratory-confirmed positive cases by June 8, 2020 and the toll is climbing day by day[3]. This unprecedented event of rapid disease spread and fatality all over the globe have forced many countries to initiate prudent decision of complete lockdown for reducing the rate of infection and subsequent deaths. Presently, the global case fatality rate is around 4% which is varying in different countries[4]. The World Health Organisation (WHO) could not lensed to recommend any antiviral medicine and vaccine candidate for the prevention and treatment of this disease to date. Therefore, the scientists, clinicians, and all other researchers are desperately working towards inventing the potential antiviral components against SARS-CoV-2.

Several phytochemicals and natural products showed antiviral properties during the previous two coronavirus outbreaks (SARS-CoV in 2013 and MERS-CoV in 2012), influenza virus-induced seasonal epidemics and against dengue virus[5-6]. It was reported that preventive antiviral therapies with phytochemicals and other drugs like chloroquine, hydroxychloroquine, azithromycin, lopinavir/ritonavir, remdesivir, flavipiravir, teicoplanin , etc. for management of COVID-19[7]. However, the roadmaps to get full proof of experimental results of the clinical trials for effective COVID-19 treatment are still under process. Therefore, the prediction of potential inhibitors and target molecules to COVID-19 with a structure-based computational approach is urgently required to rebuild human lives and survival. To achieve this, several studies have been initiated in recent time to examine the potential of numerous botanical secondary metabolites in inhibiting SARS-CoV-2 target molecules which need further investigations for the development of successful drug[8]. Among these viral proteins, the spike glycoprotein, main protease, papain-like protease, and RNA-dependent RNA polymerase play crucial role in viral entry, attachment, processing, replication, and transcription that cause cellular damage and death. However, the *in-silico* inhibitory potential of phytoligands against these prime viral proteins remains to be investigated with detail to date. Authors hypothesis was to predict whether these botanical natural products could be effective against COVID-19 and how these might provide the opportunity to develop prophylactic or therapeutic strategies to combat this pandemic.

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Hence, authors aimed to study the role of antiviral phytoligands from the commonly available traditional botanical resources against SARS-CoV-2 which could lead to the discovery of future prophylactics and therapeutic strategies for management of COVID-19 and related unshielded expenses viral diseases. Recent reports from our group showed the medicinal and therapeutic properties of botanical resources from different phytogeographical regions of India which could be used as a prophylactic and therapeutic agent against various human maladies including viral diseases like COVID-19[9-11]. In the present study, several phytoligands found abundantly in various medicinal plants were selected after extensive literature review, rigorous screening of major databases, and ethnopharmacological claims[12]. Authors aimed towards identifying novel phytoligands from widely available botanical resources by the computational approach which could serve as potential inhibitors against the antiviral targets of SARS-CoV-2.

To achieve this, authors looked at the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties, molecular docking based binding affinities, and protein-ligand interaction profile of virtually screened phytoligands and few recommended synthetic repurposing drugs against SARS-CoV-2 spike glycoprotein, main protease, papain-like protease, and RNA-dependent RNA polymerase. Finally, we proceed to examine which phytoligand(s) could serve as specific as well as broad-spectrum inhibitor(s) against these most significant antiviral targets and to provide the foundation of drug discovery. The high degree of positive results coupled with these phytoligands from the common medicinal plants could provide novel, affordable, and safe drug or natural prophylactic against the newly emerging and highly contagious COVID-19.

2. MATERIALS AND METHODS

2.1 Data Mining for Phytoligands and Viral Proteins

Antiviral phytoligands from very common plant resources having various traditional uses in the Indian subcontinent were screened from various databases such as Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT)[13]; ZINC database[14]; Traditional Knowledge Digital Library (TKDL) (http://www.tkdl.res.in); Dr Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search/list); Pubchem (https://pubchemdocs.ncbi.nlm.nih.gov/covid-19); and DrugBank (https://www.drugbank.ca/covid-19, https://www.drugbank.ca/categories/DBCAT000066). Initially, from 14532 potential compounds, a total of 55 phytoligands were selected after rigorous screening based on ADMET properties and drug-likeness, literature survey, and traditional botanical knowledge with wide availability in the Indian subcontinent. We also included 8 commonly recommended synthetic antiviral drug candidates which are being tested against SARS-CoV-2 as the control for this study.

The three-dimensional (3D) structures of all phytoligands were retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and DrugBank (https://www.drugbank.ca/covid-19) in structured data format (SDF) and Protein data bank (PDB) formats, respectively. The phytoligands were then converted into PDB format from SDF format by OPENBABEL (http://www.vcclab.org/lab/babel/). The crystal structures of SARS-CoV-2 spike glycoprotein (PDB: 6VYB)[15], main protease (PDB: 6Y2E)[16], papain-like protease (PDB: 6W9C)[17], and RNA-dependent RNA polymerase (PDB: 7BTF)[18] were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) database PDB (https://www.rcsb.org/)[19].

2.2 Evaluation of ADMET Properties and Drug-likeness

The test for ADMET properties and drug-likeness of phytoligands based on Lipinski's rule was performed using ADMETsar[20] and SwissADME[21]. The predicted toxicity profile of all phytoligands and the control drugs were also determined using the PROTOX platform[22].

2.3 Molecular Docking and Visualisation

Molecular docking of selected phytoligands and repurposing drugs with the targeted proteins were carried out and visualised using Hex 8.0.0 docking software[23]. The best docking site was considered using the binding free energies (high negative value). Hex uses spherical polar fourier (SPF) correlations. The docking was performed by adjusting appropriate parameters like correlation type-shape+electrostatics, FFT mode-3D, grid dimension-0.6, receptor range-180, ligand range-180, twist range-360, and distance range-40. The resultant binding energies (kJ/mol) were further tabulated[24-25]. The docked structures were visualised using The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC (https://pymol.org/2/).

2.4 Protein-ligand Interaction Study

The interaction study of the targeted proteins with the selected phytololigands and repurposing drugs was carried out using the LIGPLOT program of Ligplot+ suite (Verion v.2.2)[26-27].

2.5 Conserved Domain Search

The conserved domains of the four viral proteins were retrieved using the conserved domain database (CDD), which were the source of protein annotation of NCBI Entrez query and retrieval system (https://www.ncbi. nlm.nih.gov/ Structure/cdd/wrpsb.cgi).

2.6 Active Site Identification

The probable active sites or binding pockets of the four viral proteins were identified with CASTp[28]. The active sites were identified to find out whether the phytoligands and repurposing synthetic drugs are allosteric, orthosteric or bitopic in nature.

3. RESULTS

3.1 ADMET Properties and Drug-likeness

The ADMET properties and drug-likeness profile of the 55 phytoligands and 8 repurposing synthetic drugs have been

depicted in Table 1. All phytoligands and repurposing drugs under the investigation were found to have suitable ADMET values and satisfy the suitable drug-likeness properties.

3.2 Molecular Docking Analysis

The binding energy values (E-total) of 55 phytoligands against SARS-CoV-2 spike glycoprotein (6VYB) were ranged between −102.51 kJ/mol (tyrosol) to −544.15 kJ/mol (colchicine) and that of repurposing synthetic drugs were ranged between −201.03 kJ/mol (favipiravir) to −846.66 kJ/mol (saquinavir). The E-total values of 55 phytoligands against SARS-CoV-2 main protease (6Y2E) were ranged between −157.57 kJ/mol (tyrosol) to − 712.63 kJ/mol (colchicine) and that of repurposing drugs were ranged between − 129.38 kJ/mol (favipiravir) to − 1120.70 kJ/mol (saquinavir). The binding energy values of 55 phytoligands against SARS-CoV-2 papain-like protease (6W9C) were ranged between −137.61 kJ/mol (tyrosol) to −346.54 kJ/mol (oleic acid) and that of repurposing drugs were ranged between −198.21 kJ/mol (favipiravir) to −323.32 kJ/mol (nelfinavir). The binding energy values of 55 phytoligands against SARS-CoV-2 RNA-dependent RNA polymerase (7BTF) were ranged between −144.68 kJ/mol (tyrosol) to −627.24 kJ/mol (oleic acid) and that of repurposing synthetic drugs were ranged between −266.50 kJ/mol (favipiravir) to −1100.66 kJ/mol (saquinavir).

Authors determined a specific threshold limit of binding energy (E-total) concerning higher negative value for the final selection of phytoligands and repurposing synthetic drugs because the high negative value is required for better binding stability which may have better potential to develop anti-COVID-19 prophylaxis and therapy. The threshold binding energy of 55 phytoligands against spike glycoprotein, main protease, papain-like protease, and RNA-dependent RNA polymerase of SARS-CoV-2 were > −400 kJ/mol, > −500 kJ/mol, > −250 kJ/mol, and > −500 kJ/mol, respectively. Similarly, the threshold binding energy values of 8 repurposing drugs against spike glycoprotein, main protease, papain-like protease, and RNA-dependent RNA polymerase were > −400 kJ/mol, > −400 kJ/mol, > −250 kJ/mol, > −500 kJ/mol, respectively. Seventeen phytoligands and 7 repurposing synthetic drugs showed optimum binding affinities against the viral proteins above these threshold limits. These 17 phytoligands were andrographolide, apigenin, artemisinin, berberine, capsaicin, colchicine, emodin, glabridin, harmaline, harmine, kaempferol, niranthin, oleic acid, phyllanthin, rosavin, withaferin A, and withanolide A; and those of 7 repurposing synthetic drugs were azithromycin, chloromethyl ketone, chloroquine, hydroxychloroquine, nelfinavir, remdesivir, and saquinavir. The docked complexes of the four viral proteins taken into consideration with their respective screened

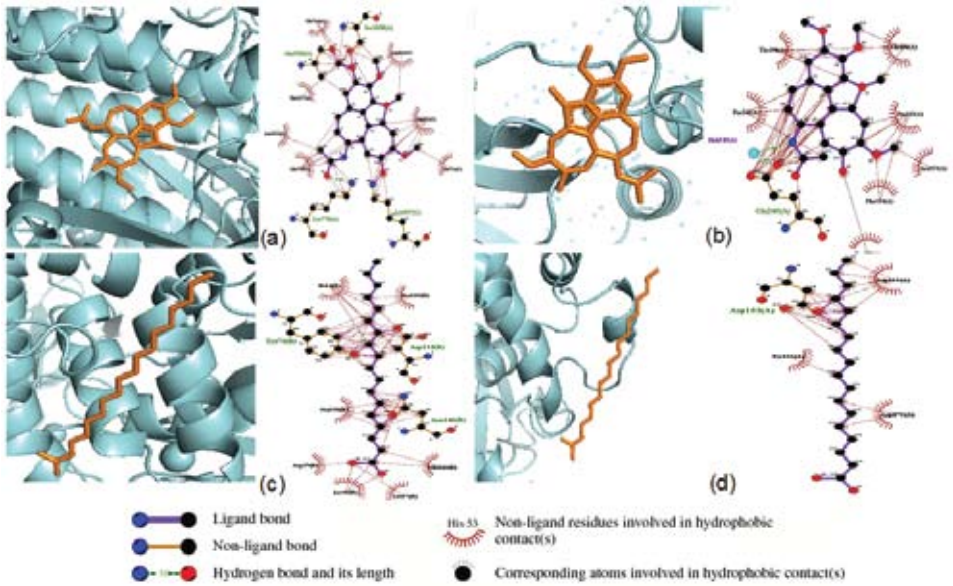

Figure 1. The minimum docked poses and corresponding Ligplot+ 2D interaction plot of phytoligands having highest binding affinities with SARS-CoV-2 viral proteins. (a) spike glycoprotein (6VYB) vs colchicine, (b) main protease (6Y2E) vs colchicine, (c) papain-like protease (6W9C) vs oleic acid, (d) RNA-dependent RNA polymerase (7BTF) vs oleic acid.

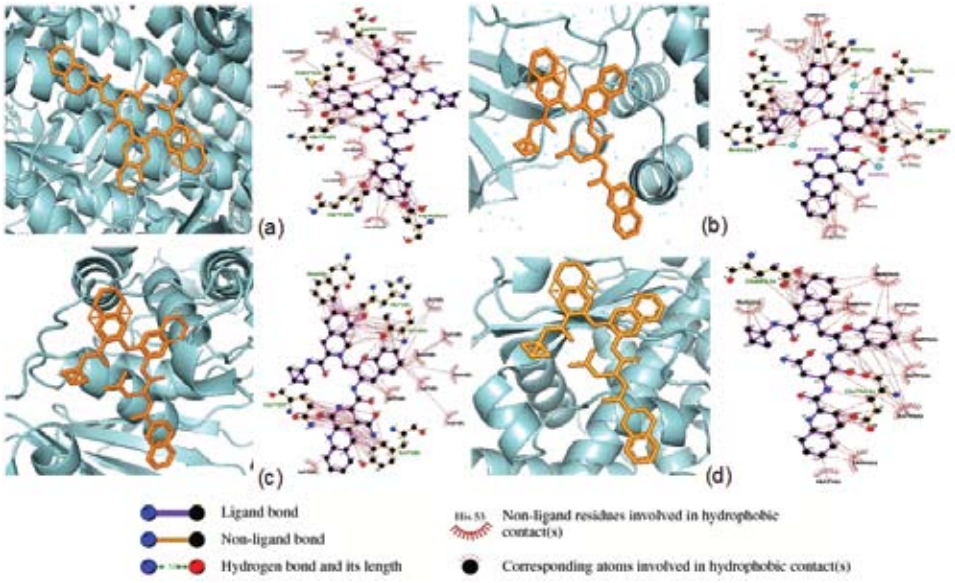

Figure 2. The minimum docked poses and corresponding Ligplot+ 2D interaction plot of repurposing synthetic drug saquinavir having the highest binding affinities with SARS-CoV-2 viral proteins. (a) spike glycoprotein (6VYB), (b) main protease (6Y2E), (c) papain-like protease (6W9C), (d) RNA-dependent RNA polymerase (7BTF).

Table 1. The absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of phytoligands and repurposing synthetic drugs

S. No.	Phytoligand	Pubchem CID	ADME properties (Lipinki's rule of five)						Topological Polar Surface Area (Å²)	Bioavailability score	Toxicity
			Molecular weight (<500 Da)	AlogP (<5)	H-Bond acceptor (<10)	H-Bond donor (<5)	Rotatable Bonds	Drug-likeness			
1	Andrographolide	5318517	350.46	1.96	5	3	3	Yes	86.99	0.55	5000
2	Apigenin	5280443	270.24	2.58	5	3	1	Yes	90.90	0.55	2500
3	Artemisinin	68827	282.34	2.39	5	0	0	Yes	53.99	0.55	4228
4	Berberine	2353	336.37	3.10	4	0	2	Yes	0.00	0.55	1000
5	beta-Caryophyllene	5281515	204.36	4.73	0	0	0	Yes	0.00	0.55	5000
6	beta-Maaliene	101596917	204.36	4.56	0	0	0	Yes	0.00	0.55	3700
7	Caffeine	2519	194.19	−1.03	6	0	0	Yes	61.82	0.55	127
8	Capsaicin	1548943	305.42	3.79	3	2	9	Yes	58.56	0.55	47
9	Catechin	73160	290.27	1.55	6	5	1	Yes	110.38	0.55	10000
10	Colchicine	6167	399.44	2.87	6	1	5	Yes	83.09	0.55	6
11	Coumarin	323	146.14	1.79	2	0	0	Yes	30.21	0.55	196
12	Curcumin	368.39	368.39	3.37	6	2	8	Yes	93.06	0.55	2000
13	Cyperene	99856	204.36	4.56	0	0	0	Yes	0.00	0.55	4690
14	Diosgenin	99474	414.63	5.71	3	1	0	Yes	38.69	0.55	8000
15	Emodin	3220	270.24	1.89	5	3	0	Yes	94.83	0.55	5000
16	Fisetin	54758660	285.23	1.65	6	3	1	Yes	113.96	0.56	159
17	Genistein	5280961	270.24	2.58	5	3	1	Yes	90.90	0.55	2500
18	Glabridin	124052	324.38	4.00	4	2	1	Yes	58.92	0.55	500
19	Harmaline	3564	214.27	2.54	2	1	1	Yes	37.38	0.55	480
20	Harmane	5281404	182.23	3.02	1	1	0	Yes	28.68	0.55	1230
21	Harmine	5280953	212.25	3.03	2	1	1	Yes	37.91	0.55	500
22	Harmol	68094	198.23	2.32	1	2	0	Yes	48.65	0.55	2000
23	Hederagenin	73299	472.71	6.21	3	3	2	Yes	77.76	0.56	2000
24	Isolongifolen-9-one	14286857	218.34	3.74	1	0	0	Yes	17.07	0.55	2450
25	Isorhamnetin	5281654	316.26	2.29	7	4	2	Yes	120.36	0.55	5000
26	Kaempferol	5280863	286.24	2.28	6	4	1	Yes	111.13	0.55	3919
27	Liquiritigenin	114829	256.26	2.80	4	2	1	Yes	66.76	0.55	2000
28	Longiverbenone	530428	218.34	3.59	1	0	0	Yes	17.07	0.55	2300
29	Luteolin	5280445	286.24	2.28	6	4	1	Yes	111.13	0.55	3919
30	Myricitin	5281672	318.24	1.69	8	6	1	Yes	151.59	0.55	159
31	Niranthin	13989915	432.51	3.75	7	0	12	Yes	64.61	0.55	214
32	Ocotillol	102058355	460.74	6.74	3	2	2	Yes	49.69	0.55	2280

S. No.	Phytoligand	Pubchem CID	ADME properties (Lipinki's rule of five)						Topological Polar Surface Area (Å²)	Bioavailability score	Toxicity
			Molecular weight (<500 Da)	AlogP (<5)	H-Bond acceptor (<10)	H-Bond donor (<5)	Rotatable Bonds	Drug-likeness			
33	Oleanane	9548717	412.75	9.28	0	0	0	Yes	0.00	0.55	1200
34	Oleic acid	637517	282.47	6.11	1	1	15	Yes	37.30	0.55	48
35	Phyllanthin	358901	418.53	4.03	6	0	13	Yes	55.38	0.56	1300
36	Picroside I	6440892	492.48	−1.33	11	5	7	Yes	167.67	0.55	2000
37	Piperine	638024	285.34	3.00	3	0	3	Yes	38.77	0.55	330
38	Plumbagin	10205	188.18	1.72	3	1	0	Yes	54.37	0.55	16
39	Podophyllotoxin	10607	414.41	2.41	8	1	4	Yes	92.68	0.55	100
40	Protopanaxadiol	9920281	460.74	6.50	3	3	4	Yes	60.69	0.55	3400
41	Protopanaxatriol	9847853	476.74	5.47	4	4	4	Yes	80.92	0.55	3450
42	Pterostilbene	5281727	256.30	3.58	3	1	4	Yes	38.69	0.55	1560
43	Quercetin	5280343	302.24	1.99	7	5	1	Yes	131.36	0.55	159
44	Resvaretrol	445154	228.25	2.97	3	3	2	Yes	60.69	0.55	1560
45	Rhein	10168	284.22	1.57	5	3	1	Yes	111.90	0.55	5000
46	Rosavin	9823887	428.43	−2.02	10	6	7	Yes	158.30	0.55	5000
47	Rosin	5280656	296.32	−0.48	6	4	5	Yes	99.38	0.55	5000
48	Rosmarinic acid	5281792	360.32	1.76	7	5	6	Yes	144.52	0.56	5000
49	Safficinolide	85152699	344.41	3.51	5	1	3	Yes	80.67	0.55	5000
50	Sageone	6481824	300.40	4.31	3	2	1	Yes	57.5	0.55	302
51	Salidroside	159278	300.31	−1.25	7	5	5	Yes	119.61	0.55	5000
52	Tinosporin A	122206355	406.43	1.17	8	2	3	Yes	118.73	0.55	280
53	Tyrosol	10393	138.17	0.93	2	2	2	Yes	40.46	0.55	870
54	Withaferin A	16760705	470.61	3.35	6	2	2	Yes	96.36	0.55	7
55	Withanolide A	11294368	470.61	3.50	6	2	2	Yes	96.36	0.55	7
	Repurposing synthetic drug										
1	Azithromycin	447043	748.98	1.52	14	5	7	Yes	180.08	0.17	2000
2	Chloromethyl ketone	439647	351.85	3.77	4	1	7	Yes	71.62	0.55	2500
3	Chloroquine	2719	319.87	4.62	2	1	8	Yes	28.16	0.55	270
4	Favipiravir	492405	157.10	−0.57	4	2	1	Yes	88.84	0.55	555
5	Hydroxychloroquine	3652	335.87	3.59	3	2	9	Yes	48.39	0	1240
6	Nelfinavir	64143	567.78	4.37	5	4	12	Yes	127.20	0.55	600
7	Remdesivir	121304016	602.58	2.21	12	4	14	Yes	213.36	0.17	1000
8	Saquinavir	441243	670.84	2.71	7	5	16	Yes	166.75	0.17	500

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phytoligands and repurposing synthetic drugs having the highest binding affinities have been shown in Fig. 1-2.

3.3 Study of Protein-ligand Interaction

The viral protein-phytoligand/repurposing drug interaction was performed using Ligplot+. The interactions of the screened phytoligands and repurposing drugs with the four viral proteins along with the detailed depiction of the amino acid residues involved in forming hydrogen bonds and hydrophobic interactions and the total number of hydrophobic interactions and hydrogen bonds of each ligand with respective protein has been shown in Fig. 3. The information regarding the chain number and residue number were provided therein. The total number of hydrophobic interactions and hydrogen bonds for each of the screened phytoligands and synthetic drugs were graphically represented in Figs. 3 (a) - 3(d). The number of hydrophobic interactions exceeded the number of hydrogen bonds in all cases. In case of spike glycoprotein, glutamine showed the maximum bias towards forming hydrogen bonds and hydrophobic interactions followed by glutamic acid, alanine, aspartic acid, and leucine. The least common residue involved was arginine. All other residues were resided in between (Fig. 3(a)). Considering the main protease, there was threonine richness in bond formation followed by proline, asparagine, and glutamic acid. The least common residues for interaction and bond formation were tryptophan< valine< isoleucine< methionine (Fig. 3(b)). For papain-like protease, there was tyrosine bias in hydrophobic interactions followed by proline, asparagine, and glutamic acid. However, in this case, the hydrogen bond formation showed a bias towards glutamic acid, followed by threonine, proline, and asparagine. Cysteine was the least involved residue for any kind of interface (Fig. 3(c)). For RNA-dependent RNA polymerase, glutamic acid richness was found in the interactions. Overall, the number of hydrogen bond formation, here, was less as compared to the three other viral proteins. Glutamic acid bias was followed by aspartic acid, alanine, threonine, and tryptophan. Glutamine was the least biased residue in forming interfaces in the case of RNA-dependent RNA polymerase (Fig. 3(d)). In all the four viral proteins taken into consideration, the results showed a commonality in glutamic acid bias towards forming interactions with selected phytoligands and repurposing synthetic drugs.

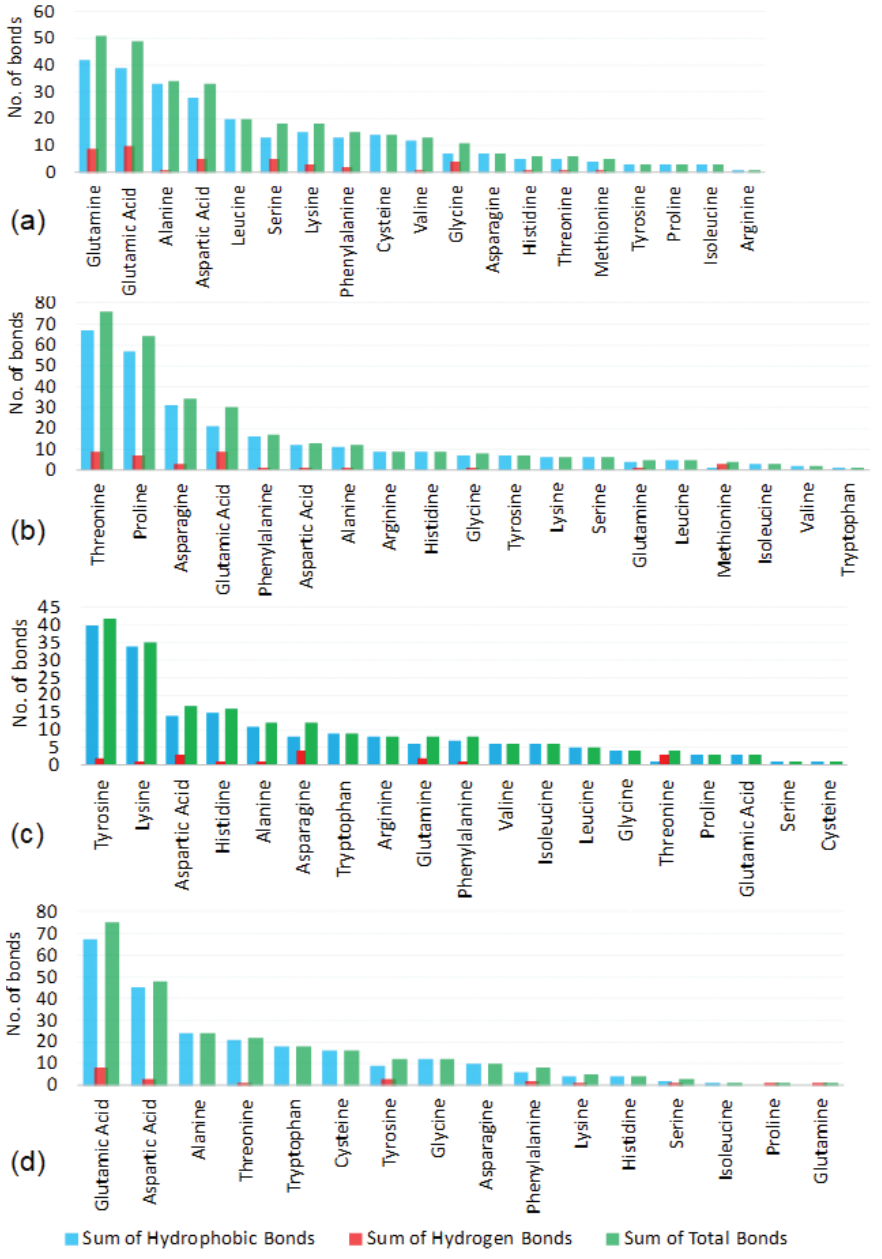

Figure 3. Hydrogen bonds and hydrobhobic interactions of SARS-CoV-2 proteins with phytoligands and repurposing drugs. (a) spike glycoprotein (6VYB), (b) main protease (6Y2E), (c) papain-like protease (6W9C), and (d) RNA-dependent RNA polymerase (7BTF).

3.4 Active Site Analysis

Among the binding pockets evaluated by CASTp for the viral proteins, only the largest pocket for each protein was considered. The probe radius used for pocket identification was 1.4Å as shown in Fig. 4. The amino acids present in the probable active sites of all the four viral proteins were compared with the amino acid residues docked with the proteins in the results obtained from LIGPLOT.

It was found that out of the 55 phytoligands and 8 repurposing synthetic drugs, some showed allosteric properties while few other showed orthosteric features. Interestingly, few ligands also showed bitopic properties. As, in our study, the screening of 17 out of 55 phytoligands and 7 out of 8 repurposing synthetic drugs were mainly based on their binding affinities toward respective viral proteins, the binding properties (whether orthosteric, allosteric or bitopic) of these 24 ligands were primarily taken into consideration. We found that most of the phytoligands

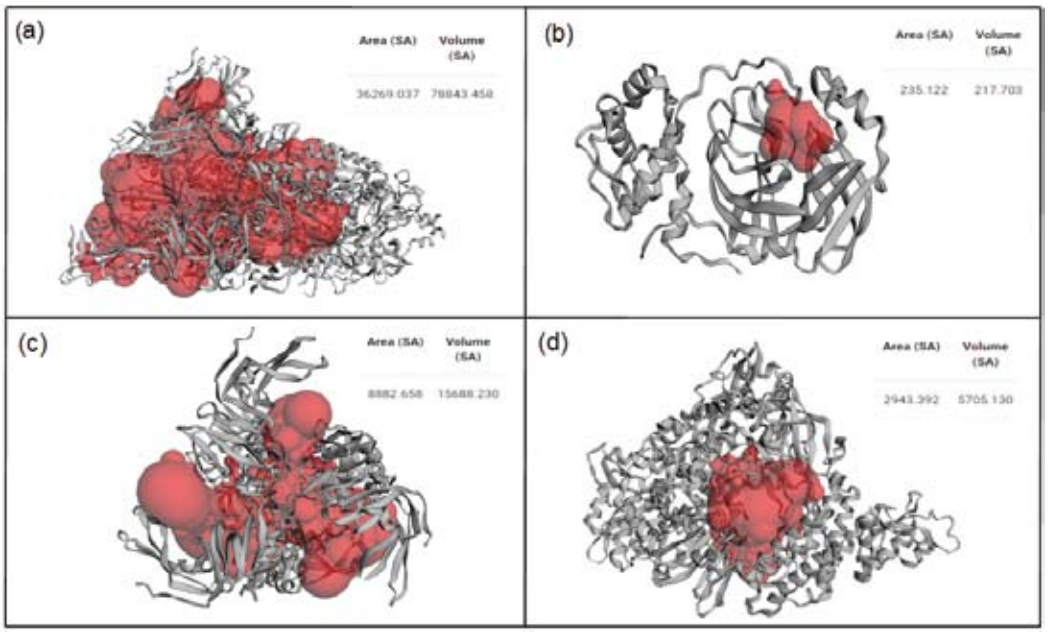

Figure 4. Putative active sites of SARS-CoV-2 proteins showing area and volume. (a) spike glycoprotein, (b) main protease, (c) papain-like protease, and (d) RNA-dependent RNA polymerase.

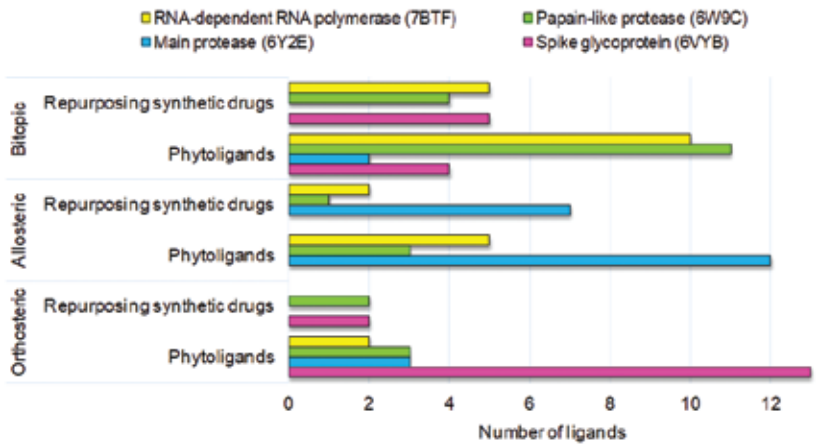

Figure 5. Bar graph showing the binding properties (orthosteric, allosteric and bitopic) of phytoligands and repurposing synthetic drugs for SARS-CoV-2 proteins.

and repurposing drugs may act as allosteric or orthosteric or bitopic, depending upon the protein they were binding to (Fig. 5). For example, artemisinin was orthosteric for spike glycoprotein, allosteric for main protease and papain-like protease, but it was acting as bitopic against RNA-dependent RNA polymerase. There were no allosteric phytoligands and repurposing synthetic drugs in case of spike glycoprotein.

4. DISCUSSION

Similar to SARS and MERS β-coronaviruses, SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus, but with a much higher rate of spreading efficiency than the other coronaviruses. We carried out this investigation to show the inhibitory potential of phytoligands and repurposing synthetic drugs in terms of binding affinity towards the spike glycoprotein (that helps the virus to attach with the host cell receptor and fuse with the cell membrane thereby facilitates the entry of viral genes to host cell to be copied, and viral multiplication), main protease (that helps in processing of the polyproteins translated from viral RNA to produce functional viral proteins), papain-like protease (proteolytic and deubiquitinating function, the de-ISGylating capacity of protein conjugates, and helps in viral evasion of the host innate immune responses), and RNA-dependent RNA polymerase (the central enzyme of viral replication), the most prominent antiviral targets of SARS-CoV-2. Targeting these viral proteins with antiviral molecules would be of great significance in not only inhibiting viral entry, processing of translated proteins, and replication but also restraining the dysregulation of cellular signaling events infected cells that might cause cell death in the adjacent, uninfected cells. From our results, it was evident that all 55 phytoligands and 8 repurposing drugs showed inhibitory potential (binding affinity) against the viral proteins. The highest binding affinities of phytoligands towards the antiviral targets were shown by colchicine and oleic acid, and that of repurposing drugs were exhibited by saquinavir and nelfinavir. Especially, 17 phyloligands *viz.* andrographolide, apigenin, artemisinin, berberine, capsaicin, colchicine, emodin, glabridin, harmaline, harmine, kaempferol, niranthin, oleic acid, phyllanthin, rosavin, withaferin A, withanolide A; and 7 repurposing drugs namely azithromycin, chloromethyl ketone, chloroquine, hydroxychloroquine, nelfinavir, remdesivir, and saquinavir showed significantly higher binding affinities towards the viral proteins. The positive ADMET properties and drug-likeness profile of all phytoligands under this investigation warrant their probable use for prophylactic and/or therapeutic interventions. We also determined 2 phytoligands (capsaicin and oleic acid) and 4 repurposing drugs (azithromycin, nelfinavir, remdesivir, and saquinavir) acting as broad-spectrum inhibitors against these antiviral targets putatively. Protein-ligand interaction

data showed significant variation in the number of hydrogen bonds and hydrophobic interactions of amino acid residues that are included within the conserved domain along with commonality in glutamic acid richness towards forming interactions of all viral proteins with selected phytoligands and repurposing synthetic drugs.

Any known prophylaxis, therapeutic intervention, and vaccine candidates against COVID-19 are still unavailable. Several clinical therapies were approved and are at full swing following various approaches such as repurposing anti-influenza and anti-retroviral drug candidates with different combinations, immunomodulation and/or immunosuppression-derived therapeutic intervention, combinational plasma therapy, monoclonal antibody treatment, etc. for the treatment of COVID-19[29-32]. However, these medications were found to produce a negative outcome and very less number of patient recoveries due to the severe complications and side effects associated with these therapies and drug candidates, which lead to increased mortality across the globe[33-34].

Medicinal plants have achieved increased attention among researchers and medical practitioners all over the world for its enormous therapeutic and health-promoting properties. At the turn of twenty-first century, scientists and pharmacologists around the globe have put great efforts to discover the most efficient and safe phytomedicines against various health ailments using different botanical resources. Various Indian medicinal plants were reported to possess antiviral properties, but their pharmacological validations remain to be explored with detail[12]. The Indian context of the healthcare system in particular is also comprised of various ethnobotanical and/or traditional knowledge-based components such as Ayurveda, Unani, Siddha, Sowa-Rigpa, etc. However, very few research investigations have been conducted to validate the pharmacological efficacy, mechanism of action, and safety profile of these phytomedicines[9-12]. Conducting *in vivo* trials using animal models and human volunteers to validate the efficacy of diverse bioactive ingredients from natural sources require huge investment, ethical constrain, and extended time. Therefore, virtual screening of active ingredients, prediction of drug-likeness, pharmacological efficacy, and specificity of botanical active principles with computational tools would be of great significance for discovering novel phytomedicines. The repurposing drugs were also found to be partially successful in a small number of cases due to a lack of specificity and efficacy against SARS-Cov-2. The development of an efficient vaccine against this virus having a high frequency of mutation, the peculiar mode of infection, and multi-factorial damage to its host would be a herculean task for the scientists all over the globe. Therefore, the discovery of prophylaxis with optimum efficacy against SARS-Cov-2 would be great hope to combat the disease. Development of combination therapies using synthetic drugs and potential anti-SARS-Cov-2 phytoligands would not only provide higher synergistic action but also lower the severity

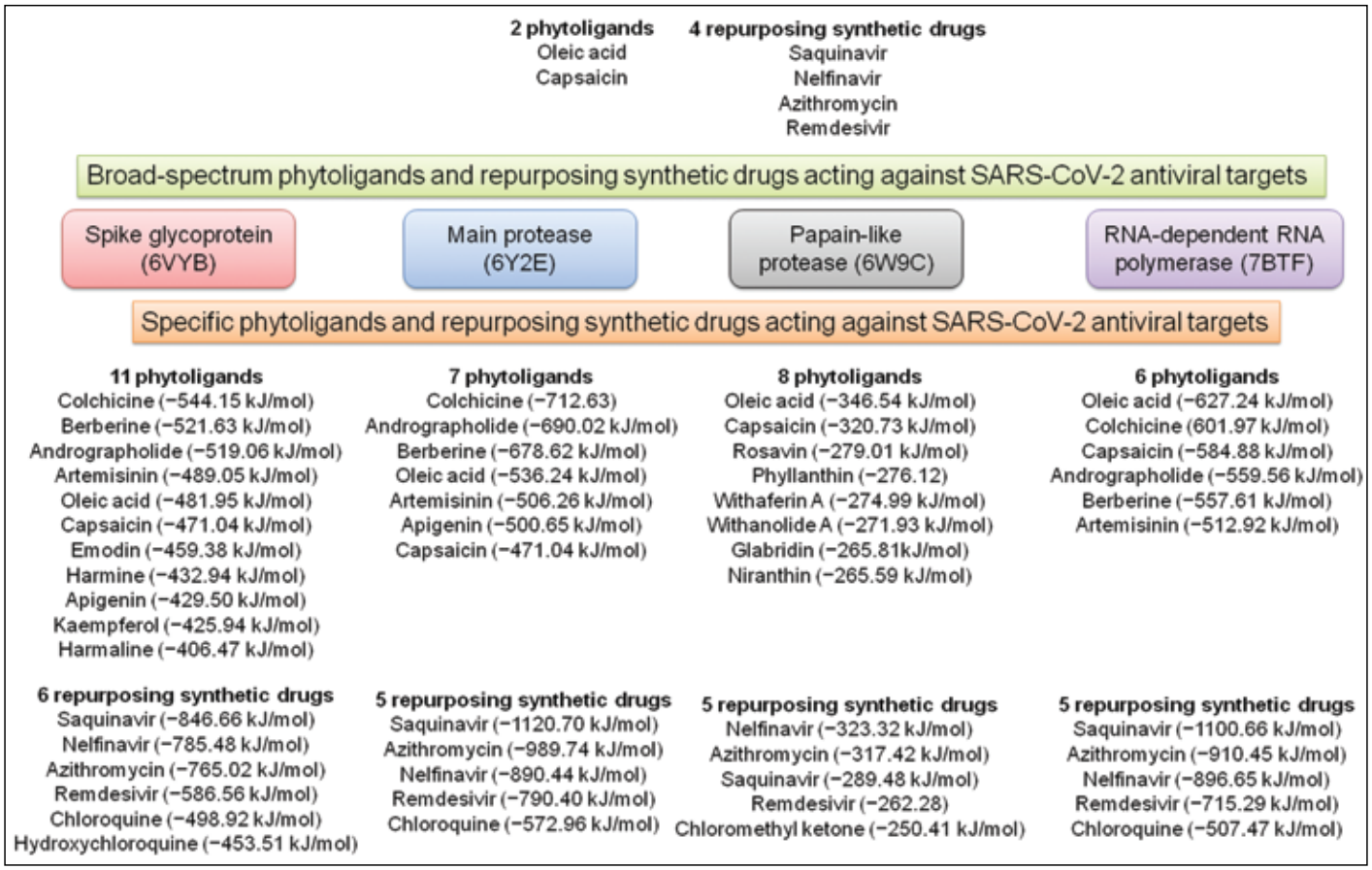

Figure 6. Specific and broad-spectrum phytoligands and repurposing synthetic drugs with higher binding energy (E-total) against the four antiviral targets under investigation.

of side effects caused by the repurposing antiviral therapies and pave the way of discovering novel prophylaxis and therapeutic intervention against COVID-19.

We observed significant variation of binding affinities in different phytoligands and repurposing drugs targeted against the 4 antiviral targets. Based on the ADMET properties, binding energy, and protein-ligand interaction profile, we finally identified the plausible 17 potent phytoligands *viz.* andrographolide, apigenin, artemisinin, berberine, capsaicin, colchicine, emodin, glabridin, harmaline, harmine, kaempferol, niranthin, oleic acid, phyllanthin, rosavin, withaferin A, and withanolide A to inhibit the 4 viral proteins studied under this investigation. On the other hand, the 7 repurposing synthetic drugs *viz.* azithromycin, chloromethyl ketone, chloroquine, hydroxychloroquine, nelfinavir, remdesivir, and saquinavir showed optimum antiviral properties by inhibition of 4 viral components under investigation (Fig. 6). From these results, we also identified the putative specific and broad-spectrum phytoligands and repurposing synthetic drugs acting against the 4 antiviral targets under investigation (Fig. 6). Among all phytoligands, oleic acid and capsaicin were found to exhibit broad-spectrum binding affinity towards these antiviral targets of SARS-CoV-2. Among the repurposing drugs, saquinavir, nelfinavir, azithromycin, remdesivir showed broad-spectrum binding affinity towards all antiviral targets. Four phytoligands such as colchicine, berberine, andrographolide, and artemisinin along with one repurposing drug chloroquine were also found to possess potential binding affinities towards 3 antiviral targets except for papain-like protease. The phytoligand apigenin was found to have an optimum binding affinity towards the spike glycoprotein and the main protease of SARS-CoV-2 (Fig. 6).

Colchicine showed the highest binding energy of −544.15 kJ/mol and −712.63 kJ/mol against the spike glycoprotein (6VYB) and main protease (6Y2E), respectively. Oleic acid exhibited the maximum binding affinity with −346.54 kJ/mol and −627.24 kJ/mol, against papain-like protease (6W9C) and RNA-dependent RNA polymerase (7BTF), respectively. Among the repurposing drugs under investigation, saquinavir showed the highest binding energy of −846.66 kJ/mol, −1120.70 kJ/mol, −323.32 kJ/mol, and −1100.66 kJ/mol against spike glycoprotein (6VYB), main protease (6Y2E), papain-like protease (6W9C) and RNA-dependent RNA polymerase (7BTF), respectively. Our results were in corroboration with previous reports where saquinavir was found to produce optimum inhibition against viral main protease[35].

The overall interaction data indicated that both the phytoligands and the repurposing synthetic drugs rely more on hydrophobic interactions for binding with the target proteins as compared to hydrogen bonds. The bias towards some amino acids may indicate some distinctive patterns. The glutamic acid bias in the interfaces helps us to conclude that glutamic acid-rich interactions possibly will lead to viral protein solubility change that in turn may affect the viral lethality towards the host[36]. We also found aspartic acid richness. Aspartic acid is important in proper receptor functioning that in turn may stabilise the protein-ligand complexes[37]. The bias towards glutamine in the case of spike glycoprotein-ligand/synthetic drug interaction may increase the stability of the complexes[38]. Tyrosine bias in papain-like protease was observed. Tyrosine was also found to facilitate molecular interactions and may affect the binding affinity and specificity[39]. Threonine is also essential for viral assembly and stability[40]. We observed threonine richness primarily in main protease-ligand/repurposing drug interaction and also more or less in the case of the other three viral proteins. These interactions, thus, may affect the normal viral structural conformation and also their activities.

Conserved domain analysis of viral proteins revealed the following findings. For spike glycoprotein, the phytoligands and repurposing drugs interacted with the residues that were included within the Corona_S2 superfamily conserved domain. A similar observation was found for the main protease. The conserved domain betaCoV_Nsp5_Mpro was in the interval of 4-300 and all the residues that interacted with the respective ligands were within this range. Viral_protease superfamily of papain-like protease was within the interval of 1-315. The residues within these intervals were involved in protein-ligand interaction. For RNA-dependent RNA polymerase, the SARS-CoV-like_RdRp (interval 5-932), the same results were concluded as above. These results may help us to get insights into the functional pattern of the conserved domains with and without drug interactions. Further characterisation of the interaction of the SARS-Cov-2 protein domains with phytoligands and repurposing synthetic drugs may help in drug target identification.

Andrographolide, apigenin, artemisinin, berberine, capsaicin, emodin, harmine, harmaline, kaempferol, and oleic acid, were found to produce therapeutic action against zoonotic respiratory viral threats such as H1N1, H9N2, H5N1, H3N2, and SARS coronaviruses which may be effective against the new and emerging infectious disease like COVID-19[41-44]. Our results were in agreement with previous report where the plausible anti-SARS-CoV-2 potential of lipid-soluble alkaloid colchicine was recommended and under clinical trial for the prevention of cytokine storm and fibrosis in COVID-19 due to its anti-inflammatory and anti-fibrotic properties[45]. Bioactive lipid, like oleic acid and other botanical active ingredients like emodin, harmine, and harmaline was also hypothesised to be the potent lead molecules for anti-COVID drug development[46]. Along with capsaicin and oleic acid, which were associated with high binding affinities towards the 4 SARS-CoV-2 antiviral targets, few other phytoligands namely glabridin, niranthin, phyllanthin, rosavin, withaferin A, and withanolide A were also found to show potential inhibition against papain-like protease of SARS-CoV-2. Glabridin, niranthin, phyllanthin, rosavin, withaferin A, and withanolide A have been reported for their antiviral properties and may have the potential to inhibit the antiviral targets of SARS-CoV-2 with high specificity and efficiency[11,47]. However, validation of the pharmacological efficacy of these 17 phytoligands showing higher binding affinity and residue interaction (Fig. 6) against the potential antiviral targets of SARS-CoV-2 are yet to be explored with detail. Therefore, the *in vivo* inhibitory potential of these phytoligands along with repurposing drugs with various combinations should be tested against COVID-19 which may produce improved synergistic effect.

Similar approach was followed by previous investigators

from different parts of the globe to discover potential repurposing drugs and phytomolecules against SARS-CoV-2 by virtual screening and computational methods[4,32,48-50]. However, no data is available to date to show the inhibitory potential of broad-spectrum phytoligands and repurposing synthetic antiviral drugs which might be effective against the 4 prime antiviral targets of SARS-CoV-2 included in our study. We carried out this investigation with SARS-CoV-2 protein structures retrieved RSCB PDB which were recently resolved by electron microscopy and X-ray diffraction. However, in contrast with our study, most of the previous research investigators showed the *in silico* inhibitory potential of phytomolecules against homology-based predicted structures of SARS-CoV-2 proteins which may not hold conclusive remarks. Also, very less information is available to demonstrate the insight of protein-ligand interaction profile and its relation to developing possible inhibitors against SARS-CoV-2 viral proteins studied in this investigation. Therefore, to the best of our knowledge, this is the first study to demonstrate the in-depth protein-ligand interaction profile of SARS-CoV-2 viral proteins and phytoligands/repurposing synthetic drugs to discover the plausible specific and broad-spectrum novel phytomedicne or prophylactic against COVID-19.

In the present study, the phytoligands and repurposing drugs showed differential binding properties such as orthosteric, allosteric or bitopic functions against SARS-CoV-2 viral proteins. Orthosteric drugs binds to the active site of the target protein while allosteric drugs are binds to the protein surface other than the active site. Orthosteric drugs act by blocking the active site so that the native substrate becomes unable to bind to it. Conversely, allosteric drugs result in conformational change in the protein structure that in turn affects protein activity. Allosteric drugs, thus, are able to modify the protein activity unlike orthosteric drugs that focus on blocking the active site. The drugs available to us are commonly orthosteric. But, there are some disadvantages of orthosteric drugs. As they bind to the active site of the target protein, there are probabilities that they may also bind to other homologous proteins or proteins that belong to the same family as active sites are highly conserved. On the other hand, as allosteric drugs bind to the protein surfaces, which are much less conserved compared to the active site and non-competitive to endogenous ligands; hence, even if the target protein is bound to an endogenous ligand, allosteric drugs can be influential to the target protein by modulating its activity[51].

Several studies revealed that allosteric drugs have lesser side effects as compared to orthosteric drugs. Nevertheless, orthosteric drugs have many advantages, mainly, as they directly target the active site and hence, influence protein activity in a much faster way and stop it entirely. Mostly, the drugs available are categorised into these two types, but there is a new class of ligand, called bitopic ligands that have dual characteristic features, that is they can bind to both orthosteric as well as allosteric sites of the same protein at the same time[52]. Till date, the studies were mostly inclined towards designing of allosteric drugs or bitopic drugs. Hence, a combinatorial approach of applying multidrug therapy towards a target protein may open new avenues in drug discovery.

As, authors screening of 17 phytoligands and 7 repurposing synthetic drugs was based on their binding energies while docking with the viral proteins, the binding properties (orthosteric, allosteric or bitopic) of these ligands were mainly focussed on. Artemisinin, berberine, capsaicin, colchicine, emodin, harmaline, harmine, niranthin, rosavin among phytoligands and azithromycin, chloromethyl ketone and remdesivir among repurposing drugs were found to show all three types of binding properties (i.e. orthosteric, allosteric or bitopic). Bitopic and allosteric properties were shown by andrographolide, apigenin, kaempeferol phytoligands and hydroxychloroquine, nelfinavir, saquinavir repurposing drugs. Glabridin, withaferin A and withanolide A were found to exhibit orthosteric as well as bitopic properties. Oleic acid exhibited both allosteric and bitopic properties whreas phyllanthin showed orthosteric and allosteric properties.

In authors investigation, most of the selected ligands exhibited all the three types of binding properties. In recent time, drug discovery research focused on modifying orthosteric drugs into allosteric drugs and vice versa and also orthosteric and allosteric ligands into bitopic one. From our study, the ligands under investigation could be effective as orthosteric, allosteric or bitopic drugs. Depending upon the binding energies of the ligand-viral proteins docked complexes along with considering their nature of binding properties *viz.* orthosteric, allosteric or bitopic, a multi-drug therapy combinatorial approach may prove phenomenal in fighting against SARS-CoV-2. In this context, we need to select those drugs that will have the strongest binding energies with least side effects and fast approach towards deactivating protein functions. Our study is plausibly the first of its kind in reporting the multi-drug therapy against SARS-CoV-2 based upon binding energies and binding properties. This approach may provide a novel strategy against this deadly virus and possibly will solve the problem of drug resistance and can become a prodigious approach for next generation pharmacology.

In recent time, few clinical trials have already been approved and set in motion with medicinal plants to develop anti-COVID-19 phytomedicines and prophylaxis[8]. However, the anti-SARS-CoV-2 inhibitory properties of Indian medicinal plants still remain to be investigated with details. To the best of our knowledge, this is the first report where we showed the antiviral potential of these phytoligands obtained from widely available botanical resources against the 4 major SARS-CoV-2 proteins like spike glycoprotein, main protease, papain-like protease, and RNA-dependent RNA polymerase. Especially, the 17 potential phyloligands found in the common and traditionally used medicinal plants with promising ADMET properties and prominent inhibitory action against the most significant antiviral targets, if utilised in combination with the synthetic repurposing medicines such as azithromycin, chloromethyl ketone, chloroquine, hydroxychloroquine, nelfinavir, remdesivir, saquinavir, etc. may provide improved synergistic effect and novel lead for developing alternative therapy against COVID-19 until the discovery of a successful vaccine against the virus. Therefore, our results will definitely

provide new avenues to explore the plausible anti-SARS-CoV-2 potential of bioactive ingredients from the widely available plant sources.

5. CONCLUSIONS

Out of 55 phytoligands under investigation, 17 phytoligands *viz.* andrographolide, apigenin, artemisinin, berberine, capsaicin, colchicine, emodin, glabridin, harmaline, harmine, kaempferol, niranthin, oleic acid, phyllanthin, rosavin, withaferin A, and withanolide A showed optimum inhibitory potential in terms of higher binding affinities and interactions with the antiviral targets namely spike glycoprotein (6VYB), main protease (6Y2E), papain-like protease (6W9C), and RNA-dependent RNA polymerase (7BTF) of SARS-CoV-2. On the other hand, out of 8 repurposing synthetic drugs, 7 drug candidates namely azithromycin, chloromethyl ketone, chloroquine, hydroxychloroquine, nelfinavir, remdesivir, and saquinavir exhibited higher binding affinities and interactions against these antiviral targets. Hence, these molecules could prevent the attachment, replication, and processing of SARS-CoV-2 viral particles within the host cell. Therefore, these phytoligands along with synthetic drugs should be studied in different combinations to obtain the optimum antiviral synergistic effect in cell-based assays, animal systems, and clinical trials which may lead to the discovery of novel, safe, and affordable phytomedicine and/or prophylactic therapy with highest efficiency and specificity. In the process of finding solutions to the pandemic crisis, we are taking notes of the most abundant natural sources. In such a time, initiatives must be taken constantly to find ways from the common medicinal plants around us. With heightened senses, we have begun discovering antiviral pharmaceutical ingredients from quality raw materials and ensure that these active ingredients should act with optimum specificity and efficacy against SARS-CoV-2.

It is obvious to our vision that to settle the figuring, production, and generation of medicinal plants as the layer of our existence. At a stretch, it will open a new guideline and edge to the tedious pharma industry. So, this investigation may bring new hope of drug discovery by showing the plausible utilisation of widely available phytoligands with long lines of medicinal plants and safe repurposing drugs in combination against COVID-19. The extension of thrust to the usage of medicinal plants has been seemingly pointed out the length of safety, empowerment, and industrial application that could supply objectivity to any pandemic crisis.

Conflict of Interest: None

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