Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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A computational approach for the screening of potential antiviral compounds against SARS-CoV-2 protease: Ionic liquid vs herbal and natural compounds

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ABSTRACT

The current scenario across the globe shows unprecedented healthcare and an economic crisis due to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Recently, the World Health Organization (WHO) has declared a pandemic stage worldwide because of the high mortality and morbidity rate caused by novel infection disease. There have been several clinical trials and identification underway to find a treatment of this novel virus. For the treatment of severe infection involves the blocking of the replication of its CoV-2 protein. Hydroxychloroquine and remdesivir has been used on an emergency basis for its treatment. The uncontrolled infection and increasing death rate underline the emergence to develop the antiviral drug. In our study, the blind docking of various classes of compounds including control antiviral drugs (*abacavir, acyclovir, quinoline, hydroxyquinoline*), antimicrobial drugs (*levofloxacin, amoxicillin, cloxacin, ofloxacin*), natural compounds (*lycorine, saikosaponins, myricetin, amentaflavone*), herbal compounds (*silymarin, palmatine, curcumin, eugenin*) available in *Indian Ayurveda* was done. Besides, we have also performed the blind docking of various ionic liquids (ILs) such as *pyrrolidinium, piperidinium, pyridinium, imidazolium based ILs* against CoV-2 protease as they have recently emerged as a potential antimicrobial agent. Further, the pharmacokinetic properties and cytotoxicity of the compounds were determined computationally. The docking results showed successful binding to the active site or near a crucial site. The present computational approach was found helpful to predict the best possible inhibitor of protease and may result in an effective therapeutic agent against COVID-19.

1. Introduction

Currently, the whole world is looking finding the alternative against the rapid outbreak of novel Coronavirus disease (COVID-19). The epicentre of the novel COVID-19 was in Wuhan, China which infected humankind worldwide [1]. According to the World Health Organization (WHO) report of 28th July 2020 (while writing the manuscript), 14,348, 858 active cases and 6,03,691 death have been reported worldwide which expected to increase exponentially [2]. A novel virus strains severe acute respiratory syndrome (SARS-CoV-2) causes infectious disease with several characteristic symptoms such as dry cough high-temperature other lungs related diseases such as pneumonia or acute [3,4] respiratory damages, etc. The patients with such symptoms are named as symptomatic patients. On the contrary, patients with no such symptoms have been noticed and are called asymptomatic patients [5]. Earlier studies revealed that person with low immunity and pre-existing health issues such as cancer, diabetes, hypertension, and respiratory-related diseases, etc. are more prone towards severe infection [6].

The polypeptide backbone is generated by RNA inside the cell which is cleaved prototypically by M_{Pro}/$3CL_{Pro}$ like protease at 11 different sites which produce necessary proteins required for the replication of the virus. To stop the replication process of the virus, the activity of M_{Pro} protease is to be inhibited. Till now no other human protease is known for this specific cleavage. Therefore, M_{Pro} as protein becomes the most important target protein to study the target drug that may play a major role in the inhibition of protein activity [7]. Coronavirus strains are more likely to spread through insects and animals such as a bat. The human to human transmission may progress through water droplets either by nose or mouth generally. The water droplets being heavy it stays in the air and simultaneously falls on the surface where it could reside for an hour or so. The one who passes by or touches the surface gets infected and this is how the multiplication of virus takes place from one person to the other [8]. In this current pandemic scenario, it is believed that this is more likely to affect a large number of communities with a huge economic breakdown [9,10]. Till now no medication has been known for the

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treatment of severe disease [11–13]. As per the government advisory, medical doctors and researchers, the only way to break the chain of spread is to wear facemask, use hand sanitizer, and increase immunity by consuming herbs [14–16]. Hydroxychloroquine and other antiviral drugs are being used to treat mild and light infection occurring due to coronavirus [17]. Therefore, it becomes an urgent need to find out the strategy or therapeutic agent which may target the coronavirus.

The antiviral activities of natural products and herbs are been extensively studied and are proven to show the remarkable activity against coronavirus [18–21]. Hence, keeping in mind the current situation we have focussed on various classes of the compound such as antiviral drugs (*abacavir, acyclovir, quinoline, hydroxyquinoline*), antimicrobial drugs (*levofloxacin, amoxicillin, cloxacillin, ofloxacin*), natural compounds (*lycorine, saikosaponin, myricetin, amentoflavone*), herbal

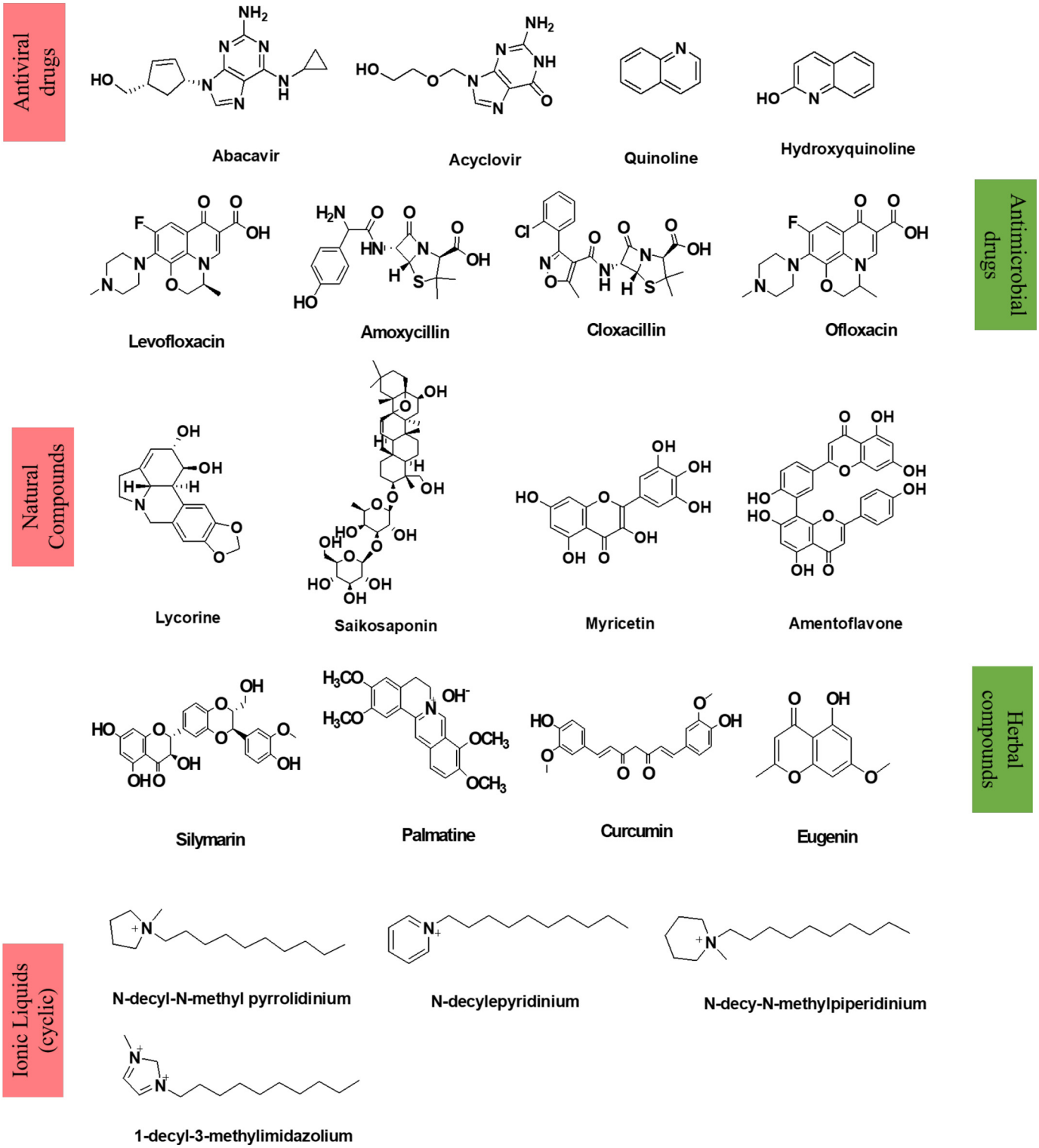


Fig. 1. Chemical structures of selected class of ligands used in molecular docking.

compounds (*silymarin, palmatine, curcumin, eugenin*) available in *Indian Ayurveda.* More recently, ionic liquids (ILs) have emerged as a potential antimicrobial, therefore, we have also performed and compared the docking results of various classes of antiviral compounds with the results obtained from molecular docking of CoV protease with ILs containing cyclic ring (*pyrrolidinium, piperidinium, pyridinium, imidazolium*) to explore the applicability of ILs as antiviral compounds. ILs are formed by the combination of various organic/inorganic cation and anion, feebly attached by the non-covalent interactions [22], provides a range of properties that makes ILs a potential candidate for diverse applications such as catalyst/solvent in organic chemistry [23], electrochemical industries [24], drug delivery system [25], pharma industries [26], and self-assembled nanostructure formation [27–29]. The tunable nature of ILs makes them a desirable molecule for various applications. The recent finding showed the antimicrobial activity (antibacterial, antifungal, antiviral, and antibiotics activities) of ILs where ILs were found to kill microorganisms such as bacteria, virus, and fungi [30–32]. Lot of work has been done where ILs, especially imidazolium based and pyrrolidinium based ILs are found to be active against several bacterial strains. Hydrophobicity and dispersed charge on ILs make them a potential candidate for the antibacterial application [33–35]. The antiviral activity of ILs are also known but much information regarding the antiviral activity of ILs is still missing [36]. Different and most reliable class of antiviral agents nowadays, herbs was focussed in our study due to their biological activity and their utility as an antiviral agent. *Silymarin* isolated from *Milk thistle* is a biologically active compound and is used as an herbal medicine for centuries. *Palmatine* is found and is extracted from various medicinal plants such as *Coptis chinensis*, *Rhizoma coptidis*, *Corydalis yanhusuo*, *Radix tinosporae.* Curcuminoids are pharmaceutically important compounds isolated from the herb *Curcuma longa.* Eugenin is isolated from buds of *Syzyium aromatica (Myrtaceae)* and *Paeonia suffruticosa (Paeoniaceae)* [20].

In the present study, blind molecular docking of selected class of potential therapeutics was done and was compared with the conventionally available antiviral drugs. Antimicrobial agents and ILs were also screened for their antiviral behaviour. Later, the toxicity and absorption efficiency of all classes of compounds were determined to evaluate the best activity in terms of absorption, solubility, cytotoxicity, and carcinogenicity etc.

2. Materials and methods

In silico studies, the best tool for the study of the interaction between drug and target molecules (biomolecules). The computational approach helps in determining the behaviour (toxicity, solubility, etc.) and molecular interactions without compromising the chances of failure during the identification of drug molecules.

2.1. Structure and preparation of target protein (M_{Pro}) for molecular docking studies

The recently reported three-dimensional structure of the novel COVID-19 target protein (PDB ID: 6Y84) was retrieved from Protein Data Bank (PDB) from https://www.rcsb.org/ [7]. The water molecules and co-crystallized ligands were removed from the protein PDB file using PyMOL Geowall 3D.

2.2. Ligands preparation

The two-dimensional (2D) structures of 20 compounds, antiviral drugs (*abacavir, acyclovir, quinoline, hydroxyquinoline*), antimicrobial

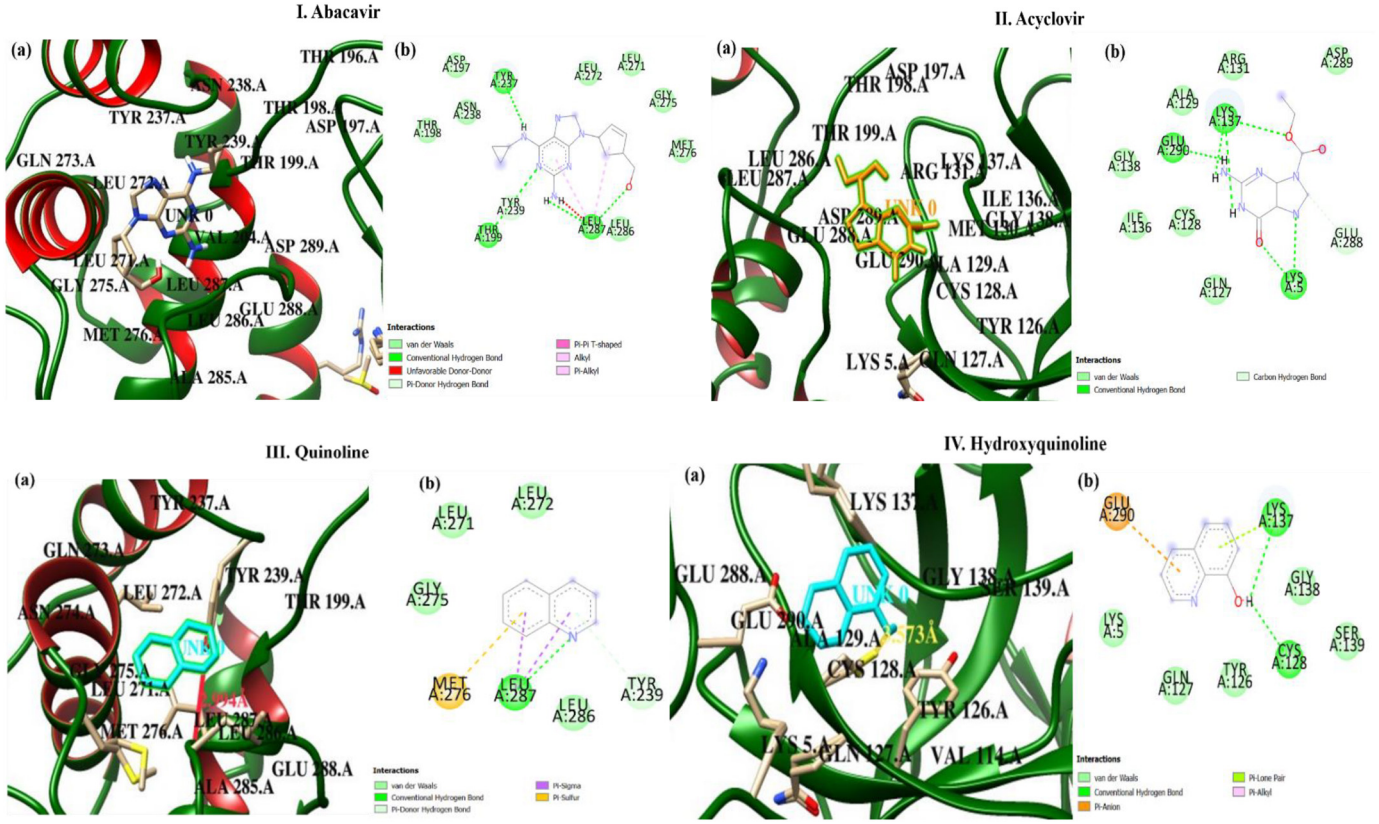


Fig. 2. The minimum docked poses of the control drugs along with their corresponding 2D plots within the active site of SARS-CoV-2.

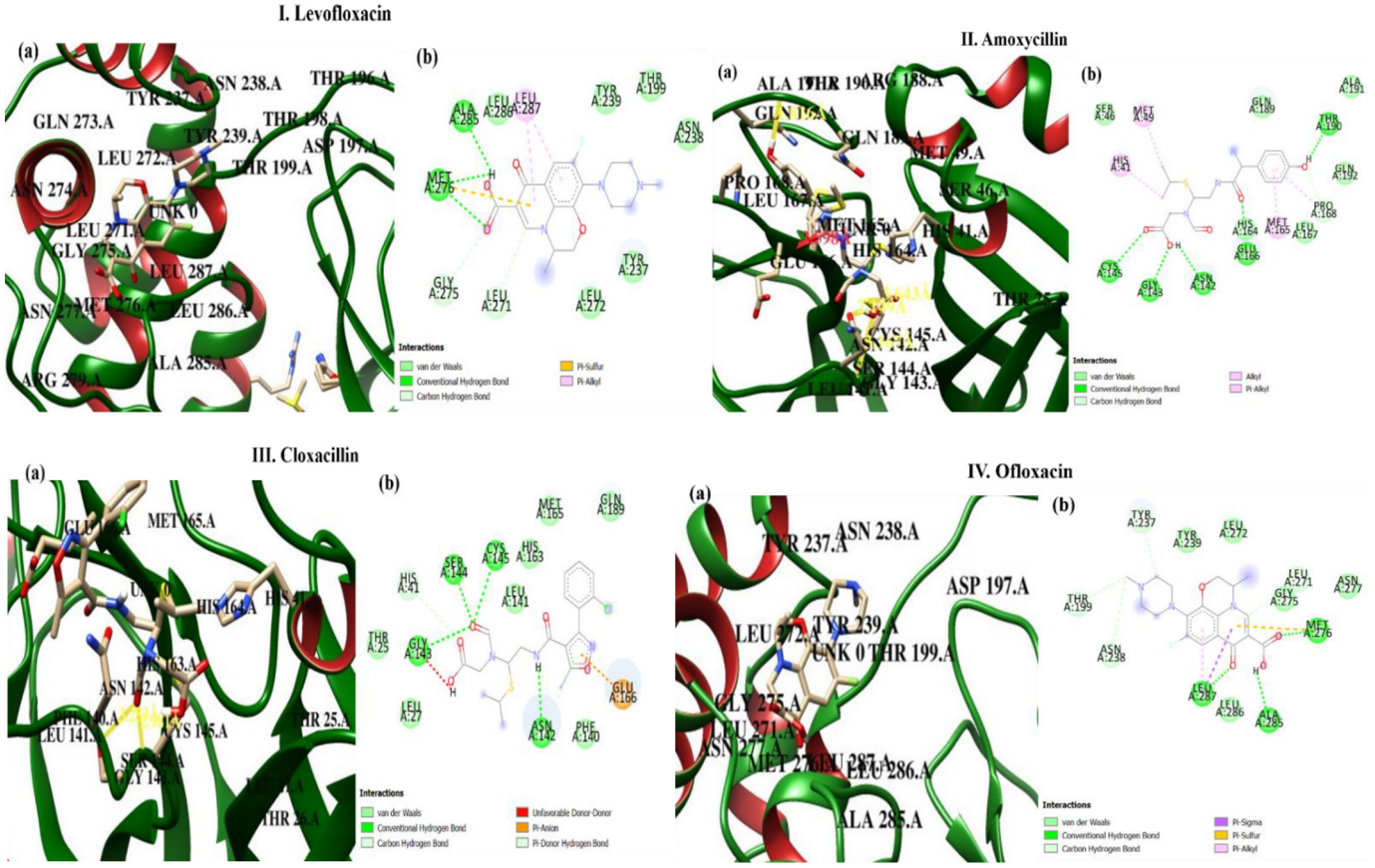


Fig. 3. The minimum docked poses of the antimicrobial drugs along with their corresponding 2D plots within the active site of SARS-CoV-2.

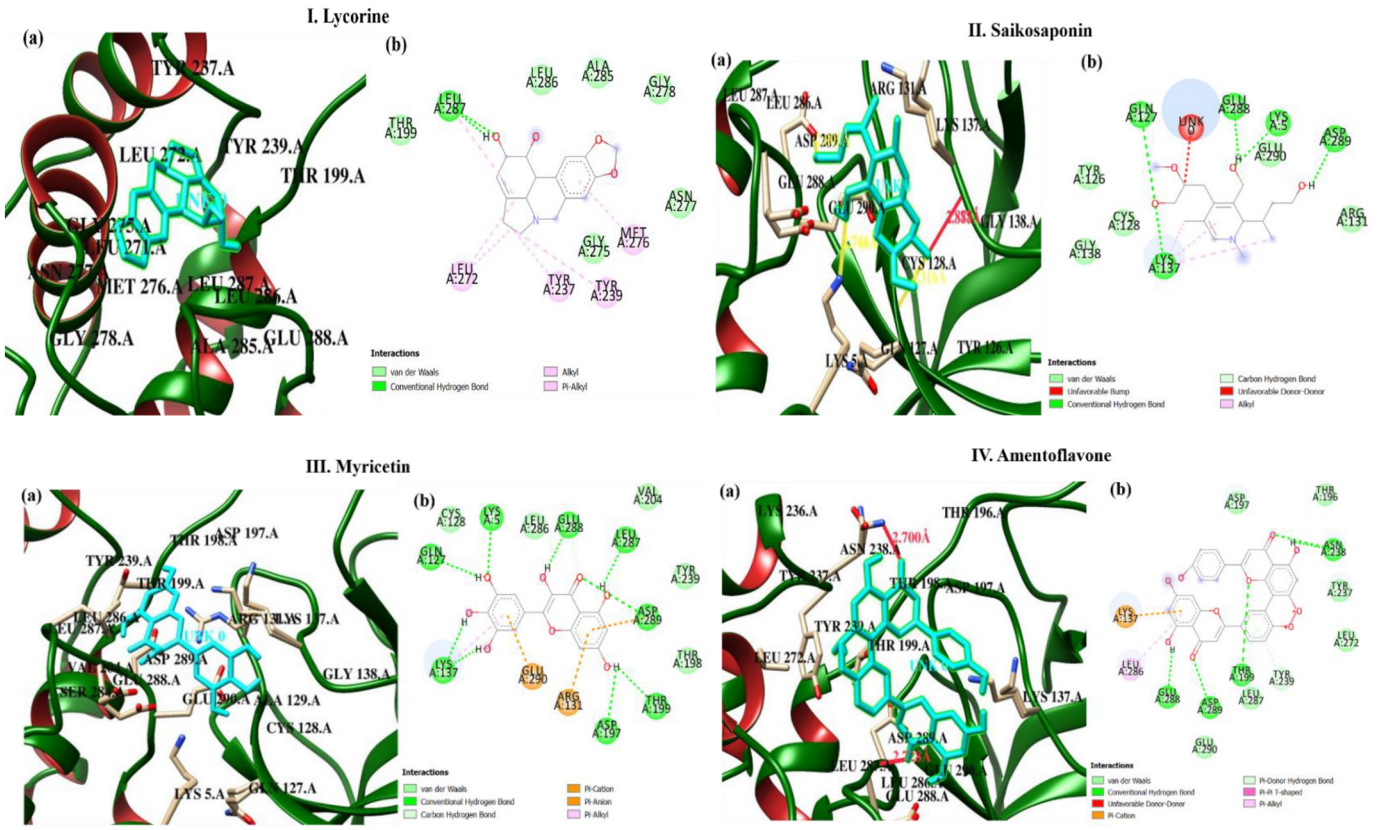


Fig. 4. The minimum docked poses of the natural compounds along with their corresponding 2D plots within the active site of SARS-CoV-2.

drugs (*levofloxacin, amoxicillin, cloxacillin, ofloxacin*), natural compounds (*lycorine, saikosaponin, myricetin, amentoflavone*), herbal compounds (*silymarin, palmatine, curcumin, eugenin*) available in *Indian Ayurveda*, ionic liquids (IL) containing cyclic ring (*pyrrolidinium, piperidinium, pyridinium, imidazolium based ILs*) were formulated using ChemDraw Ultra 12.0. Further, the 2D structure was converted to PDB file format using ChemDraw 3D Ultra 12.0. For energy optimization of structure SPDV.EXE software 4.10 was employed [3].

2.3. Molecular docking protocol

Identification of mode of binding and interactions of a selected class of compounds and main protease was done using AutoDock Tool 1.5.6 software [37]. Based on adaptive local method search, Lamarckian genetic algorithm (LGA) tool of the AutoDock Tools (ADTs) produced different ligand conformers. The molecular docking was performed by setting the grid size (Table S1) along x, y, z axes with a grid spacing and grid centre. Firstly, generated the grid map for various atoms of the ligand and protein by running the AutoGrid. After the generation of the grid, maps run the AutoDock. In docking the total of 100 numbers of runs were carried out, and then minimum energy conformers were picked according to ranking and scoring and were further visualized using various software [38].

2.4. Docking analysis and visualization

The results obtained from molecular docking were visualized using PyMOL and Discovery Studio, 2016. The docked pose with the highest stability having minimum energy were further analyzed for their ADMET properties and toxicity. To obtain the docked pose in 3D view and the hydrogen bond with bond distances, UCFS Chimera 1.12 software was employed as a molecular visualization tool [39]. Further, Discovery Studio Visualizer was used to obtain the docked poses and 2D interaction plots (BIOVIA, 2016) [40].

2.5. Absorption, distribution, metabolism, and excretion (ADMET) property analysis

To predict the pharmacokinetics properties such as adsorption, distribution, metabolism, and toxicity of all selected class of compounds as listed above, ADMET properties analysis was employed. SwissADME software (database) was used to predict the pharmacokinetic parameters for a selected class of compounds which is freely available online http://www.swissadme.ch/index.php [41].

2.6. Toxicity prediction

Toxicity issues remain the important aspect to be taken care of, therefore, the toxicity profile in terms of various parameters such as hepatotoxicity, immunogenicity, mutagenicity, carcinogenicity, etc.) of the selected class of compounds were determined using the free online available software named Pro-Tox-II software at http://tox.charite.de/tox [42].

3. Results

The importance of natural compounds as an antiviral agent has earlier been reported. In addition to the control drugs for treating SARS-CoV-2, in the present study we performed the molecular docking study on the antimicrobial drugs, naturally occurring compounds (alkaloids), herbs, and ILs whose chemical structure are shown in Fig. 1. The binding modes and binding sites of ligand on protein targets were

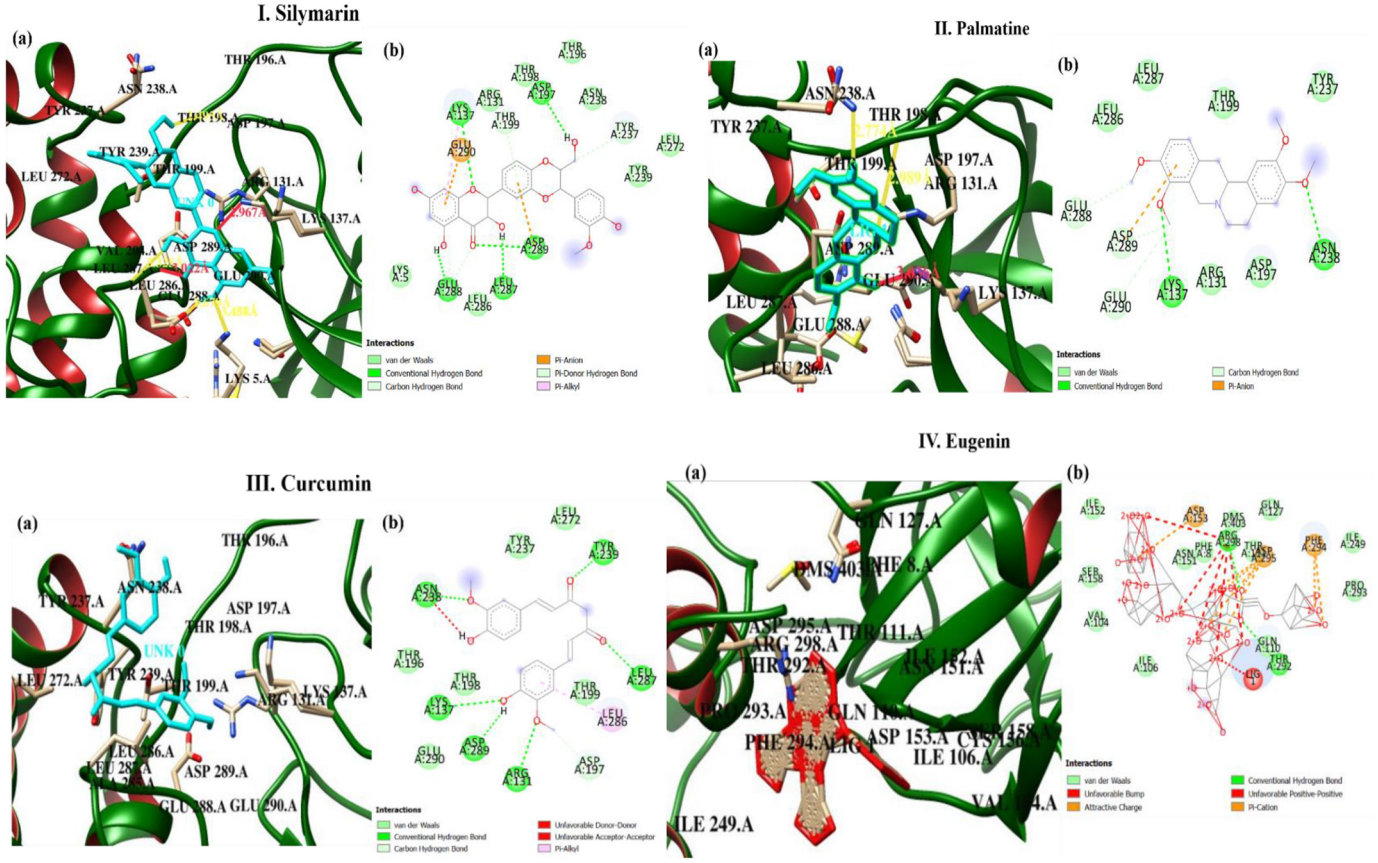


Fig. 5. The minimum docked poses of herbal compounds along with their corresponding 2D plots within the active site of SARS-CoV-2.

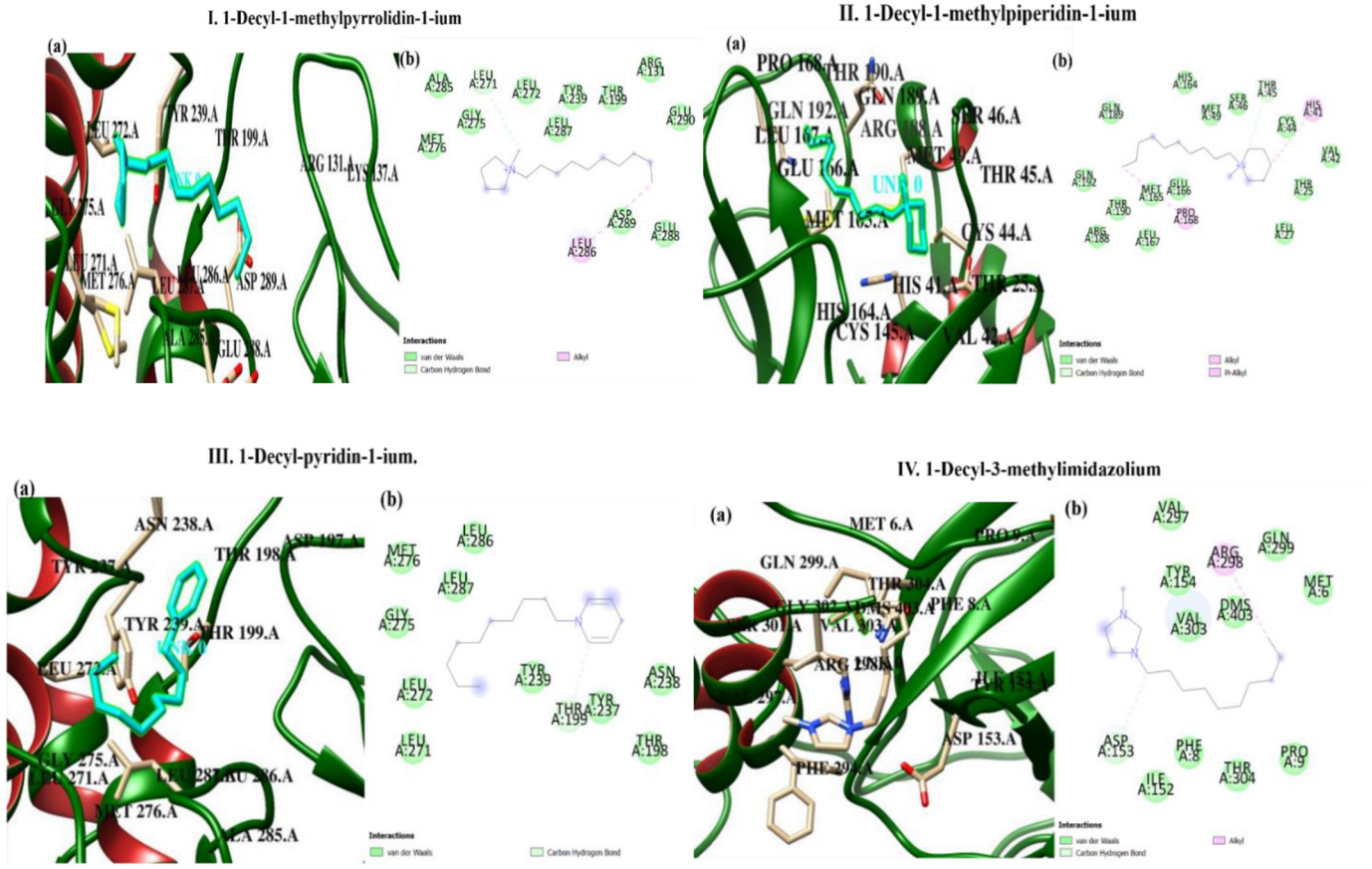


Fig. 6. The minimum docked poses of ILs (cyclic) along with their corresponding 2D plots within the active site of SARS-CoV-2.

determined. The best possible stable conformations were ranked according to their highest binding energy and the result obtained were further utilized to rationalize the findings one by one [43].

3.1. Molecular docking

3.1.1. Antiviral drugs (control drugs)

The molecular docking results based on the minimum energy (full fitness score) the conformers for four antiviral (control) drugs, namely, *abacavir, acyclovir, quinoline,* and *hydroxyquinoline* along with their corresponding 2D interaction plots are depicted in Fig. 2. The docked poses demonstrate that the drugs molecules bind within the active site of the SARS-CoV-2 (target protein). Fig. 2(I) shows that *abacavir* binds through van der Waal (ASP-197, THR-198, ASN-238, TYR-239, LEU-271, LEU-272, GLU-275, MET-276, LEU-286) and conventional hydrogen bonds with residues THR-199, TYR-237, LEU-287 on COVID-19 target protein. The binding energy involved in complex formation was found to be negative which suggested the spontaneous complex formation between the two. The magnitude of the binding energy was −6.91 kcal/mol. Besides, the other interaction such as pi-cation interaction was found to be occurring between the target protein and *abacavir*. The docking result of *acyclovir* showed that it binds through van der Waal interaction where GLN-127, CYS-128, ALA-129, ARG-131, ILE-136, GLY-138, ASP-289 were involved and conventional hydrogen bond where LYS-5, LYS-137, GLU-288, GLU-290 as shown in Fig. 2(II). The binding energy was found to be −5.72 kcal/mol. Likewise, a molecular docking study suggests that van der Waal interaction played a major role in complex formation. The residues involved while binding of *quinoline* with target protein are *TYR*-239, LEU-271, LEU-272, GLY-275, MET-276, LEU-286, LEU-287 as shown in Fig. 2(III). For *hydroxyquinoline* the residues, LYS-5, TYR-126, GLN-127, CYS-128, LYS-137, GLY-138, SER-139, GLU-290 are involved in the binding as shown in Fig. 2(IV). The binding energy of *quinoline* and *hydroxyquinoline* with target protein was found to be −6.09 kcal/mol and − 1.19 kcal/mol, respectively.

Table 1
Binding energy obtained from Docking of all classes of compounds with COVID-19 target protein.

S. No.	Classification	Ligands	Binding energy (kcal/mol)
1	Antiviral drug	Abacavir	−6.91
2		Acyclovir	−5.72
3		Quinoline	−6.09
4		Hydroxyquinoline	−1.15
5	Antimicrobial drugs	Levofloxacin	−7.27
6		Amoxicillin	−8.01
7		Cloxacillin	−7.51
8		Ofloxacin	−7.22
9	Natural compounds	Lycorine	−7.54
10		Saikosaponin	−7.71
11		Myricetin	−7.98
12		Amentoflavone	−10.76
13	Herbal compounds	Silymarin	−9.43
14		Palmatine	−7.67
15		Curcumin	−8.79
16		Eugenin	−19.93
17	ILs cyclic ring	Pyrrolidinium	−6.52
18		Piperidinium	−7.05
19		Pyridinium	−6.31
20		Imidazolium	−5.62

Table 2
Best five molecules selected from screening results of molecular docking based on ranking score.

S. No.	Ligands	Binding energy (kcal/mol)
1	Eugenin	−19.93
2	Amentoflavone	−10.76
3	Silymarin	−9.43
4	Curcumin	−8.79
5	Amoxicillin	−8.01

3.1.2. Antimicrobial drugs

The molecular docking results based on the minimum energy (full fitness score), the conformers for four antimicrobial drugs, namely, *levofloxacin, amoxycillin cloxacillin, and ofloxacin* along with their corresponding 2D interaction plots are shown in Fig. 3. The docked structure demonstrate that the drug molecules bind within the active site of the SARS-CoV-2 (target protein). The docked figure of *levofloxacin* is shown in Fig. 3(I). The docking figure shows that *levofloxacin* binds through van der Waal interaction (amino acids involved were THR-199, TYR-237, ASN-238, TYR-239, LEU-271, LEU-272, GLY-275, LEU-286) with the COVID-19 protein. The binding energy involved in complex formation was found to be negative which suggested the spontaneous complex formation between the two. The magnitude of the binding energy was −7.27 kcal/mol. Besides this other interaction such as pi-cation interaction (LEU 287) and conventional hydrogen bonding interactions (MET 276) was found to be occurring between the target protein and *levofloxacin*. The docking result of *amoxycillin* suggests that it binds mainly through van der Waal interaction where SER-46, LEU-167, PRO-168, GLN-189, ALA-191, GLN-192 were involved in binding. Despite, conventional hydrogen bonding between the drug and target protein was found where GLY-143, CYS-145, ASN-147, HIS-164, MET-165, GLU-166, THR-190 were involved. Besides these interactions pi-cation interactions were also found to be participating in the binding process where MET-49, HIS-41 residues were involved as shown in Fig. 3(II). The binding energy was found to be −8.01 kcal/mol. Likewise, the molecular docking study of *cloxacillin* and *ofloxacin* suggests that van der Waal interaction played a major role in binding with complex formation. The amino acids involved while binding of *cloxacillin* with target protein is THR-25, LEU-27, HIS-41, PHE-140, LEU-141, ASN-142, GLY-143, SER-144, CYS-145, HIS-163, MET-165, GLU-166, GLN-189 as shown in Fig. 3(III). For *ofloxacin*, THR-199, TYR-237, ASN-238, TYR-239, LEU-271, LEU-272, GLY-275, MET-276, ASN-277, LEU-286, LEU-287, ALA-285 residues of CoV-2 protein were involved in the binding process as shown in Fig. 3(IV). The binding energy of *cloxacillin* and *ofloxacin* with target protein was found to be −7.51 kcal/mol and − 7.22 kcal/mol, respectively.

3.1.3. Natural compounds

The molecular docking results based on the minimum energy (full fitness score), the conformers for four natural compounds, namely, *lycorine, saikosaponin, myricetin,* and *amentoflavone* along with their corresponding 2D interaction plots are shown in Fig. 4. The docked poses demonstrate that the drugs molecules bind within the active site of the SARS-CoV-2. Fig. 4(I) suggests that *lycorine* binds through the van der Waal interaction in which THR-199, TYR-237, TYR-239, GLY-275, ASN-277, GLU-278, ALA-285, LEU-286 residue on target

Table 3
ADMET properties of best five compounds and IL screened by molecular docking.

Properties	Eugenin	Amentoflavone	Silymarin	Curcumin	Amoxycillin	IL
Class	Herbal	Natural	Herbal	Herbal	Antimicrobial	Piperidinium IL
Molecular weight g/mol	918.5	542.49	482.44	355.43	365.4	240.45
Lipophilicity (logP)	2.08	4.35	1.71	2.52	−0.68	4.4
Water Solubility (ESOL)	Moderately soluble	Poorly soluble	Moderately soluble	Moderately soluble	Very soluble	Moderately soluble
GI absorption	Low	Low	Low	High	Low	High
BBB permeant	Yes	Yes	No	Yes	No	Yes

Table 4
The properties of potential inhibitors of Sars-CoV-2 obtained from SwissADME analysis.

S. No.	Compounds/Classification	Molecular formula	Lipinski's rule of five	
1.	Eugenin (Herbal)	$C_{41}H_{10}O_{26}$	Molecular weight (<500 Da)	918.5
			H-Bond donor (<5)	0
			H-Bond acceptor (<10)	26
			Violations	2
2.	Amentoflavone (Natural)	$C_{30}H_{22}O_{10}$	Molecular weight (<500 Da)	542.49
			H-Bond donor (<5)	6
			H-Bond acceptor (<10)	10
			Violations	2
3.	Silymarin (Herbal)	$C_{25}H_{22}O_{10}$	Molecular weight (<500 Da)	482.44
			H-Bond donor (<5)	5
			H-Bond acceptor (<10)	10
			Violations	0
4.	Curcumin (Herbal)	$C_{21}H_{25}NO_4$	Molecular weight (<500 Da)	355.43
			H-Bond donor (<5)	0
			H-Bond acceptor (<10)	5
			Violations	0
5.	Amoxycillin (Antimicrobial)	$C_{16}H_{19}N_3O_5S$	Molecular weight (<500 Da)	365.4
			H-Bond donor (<5)	4
			H-Bond acceptor (<10)	6
			Violations	0
6.	Piperidine (IL)	$C_{16}H_{34}N$	Molecular weight (<500 Da)	240.45
			H-Bond donor (<5)	0
			H-Bond acceptor (<10)	0
			Violations	0

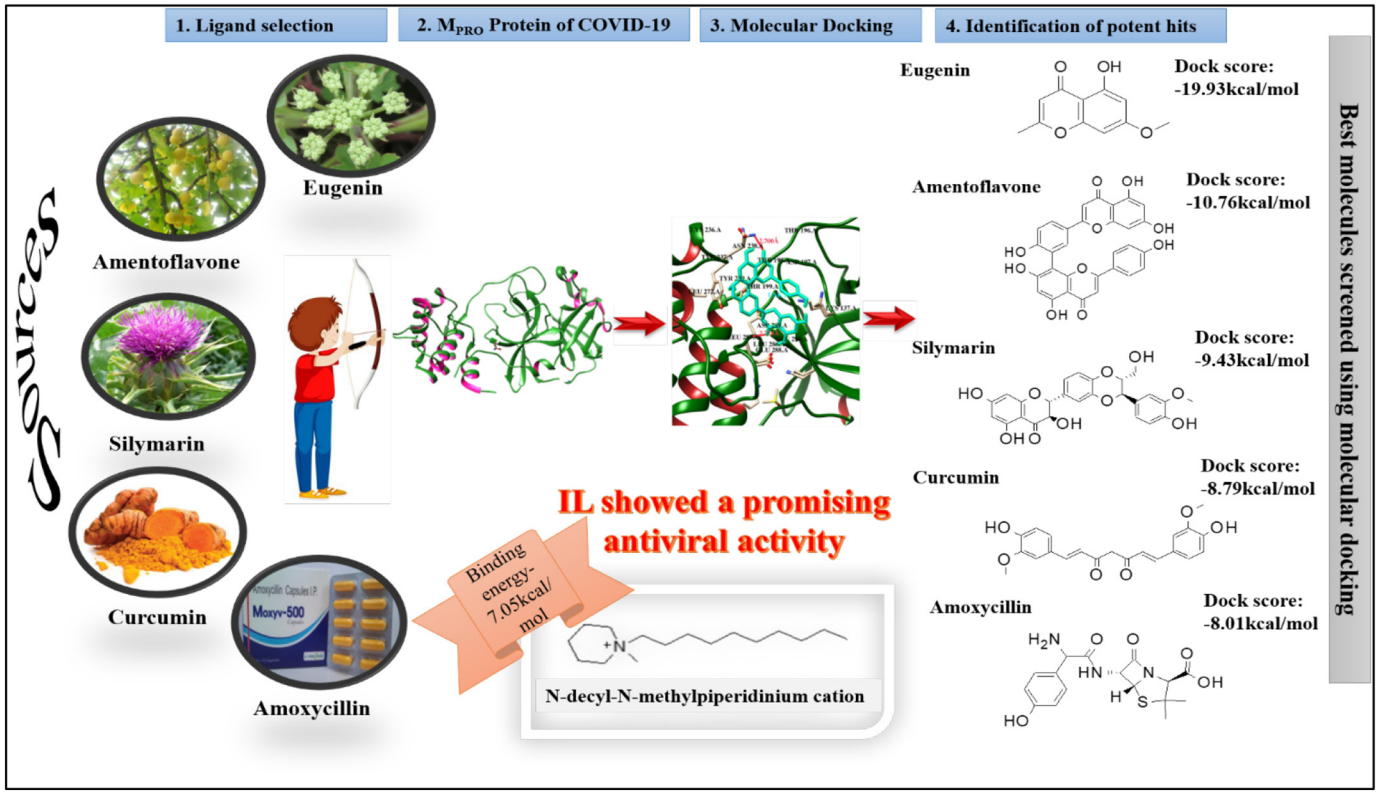
Table 5
Toxicity prediction using ProTox-II prediction software.

S. No.	Ligands	Classification	Hepatoxicity probability	Carcinogenicity probability	Cytotoxicity probability	Predicted LD_{50}(mg/kg)
1.	Eugenin	Herbal	Inactive (0.64)	Inactive (0.53)	Inactive (0.69)	1000
2.	Amentoflavone	Natural	Inactive (0.73)	Inactive(0.68)	Inactive (0.81)	2000
3.	Silymarin	Herbal	Inactive (0.78)	Inactive (0.71)	Inactive (0.77)	1000
4.	Curcumin	Herbal	Inactive (0.61)	Inactive (0.84)	Inactive (0.88)	2000
5.	Amoxicillin	Antimicrobial	Inactive (0.84)	Inactive (0.80)	Inactive (0.60)	15,000
6.	Piperidine	IL	Inactive (0.61)	Inactive (0.56)	Inactive (0.64)	1300

protein were involved in the complex formation. The binding energy involved in complex formation was found to be negative which suggesting the spontaneity of the complex formation. The magnitude of the binding energy was −7.54 kcal/mol. Besides this other interaction such as pi-cation interaction (LEU 272, MET 276) and conventional hydrogen bonding interactions (LEU 286) was found to be occurring between the target protein and *lycorine*. The docking result of *Saikosaponin* suggests that it binds mainly through van der Waal interaction where TYR-126, CYS-128, ARG-131, GLY-138, GLU-290. were involved and conventional hydrogen bond where LYS-5, GLN-127 LYS-137, GLU-288, ASP-289 were involved as shown in Fig. 4(II). The binding energy was found to be −7.71 kcal/mol. Likewise, the molecular docking study of *myricetin* and *amentoflavone* suggests that van der Waal interaction played a major role in binding with complex formation. The amino acids involved in the binding of *myricetin* with target protein, LYS-5, GLN-127, CYS-128, ARG-131, LYS 137, ASP-197, THR-198, THR-199, VAL-204, TYR-239, LEU-286, LEU-287, GLU-288, ASP-289, GLU-290 as shown in Fig. 4(III). For *amentoflavone,* LYS-137, THR-196, ASP-197, THR-199, TYR-237, ASN-238, TRY-239, LEU-272, LEU-286, LEU-287, GLU-288, ASP-289, GLU-290 residues are involved in binding as shown in Fig. 4(IV). The binding energy of *myricetin* and *amentoflavone* with target protein was found to be −7.98 kcal/mol and −10.78 kcal/mol, respectively.

3.1.4. Herbal compounds

The molecular docking results based on the minimum energy (full fitness score) the conformers for four herbal compounds, namely, *silymarin, palmatine, curcumin, and eugenin* along with their corresponding 2D interaction plots are shown in Fig. 5. The docked poses demonstrate that the drugs molecules bind within the active site of the SARS-CoV-2. The docked figure of *silymarin* is shown in Fig. 5(I). The docking figure shows that *silymarin* binds with the target protein through van der Waal interactions in which LYS-5, ARG-131, THR-196, THR-198, THR-199, TYR-237, ASN-238, TYR-239, LEU-272, LEU-286, GLU-288 were involved in the complex formation. The binding energy involved in complex formation was found to be negative which suggested the spontaneous complex formation between *silymarin* and CoV-2 protein. The magnitude of the binding energy was −9.43 kcal/mol. Besides this other interaction such as pi-anion interaction (GLU-290) and conventional hydrogen bonding interactions (LYS-137, ASP-197, LEU-287, GLU-288, ASP-289) was found to be occurring between the target protein and *lycorine*. The docking result of *palmatine* suggests that it binds mainly



Scheme 1. A summary of the screening result obtained from the molecular docking technique.

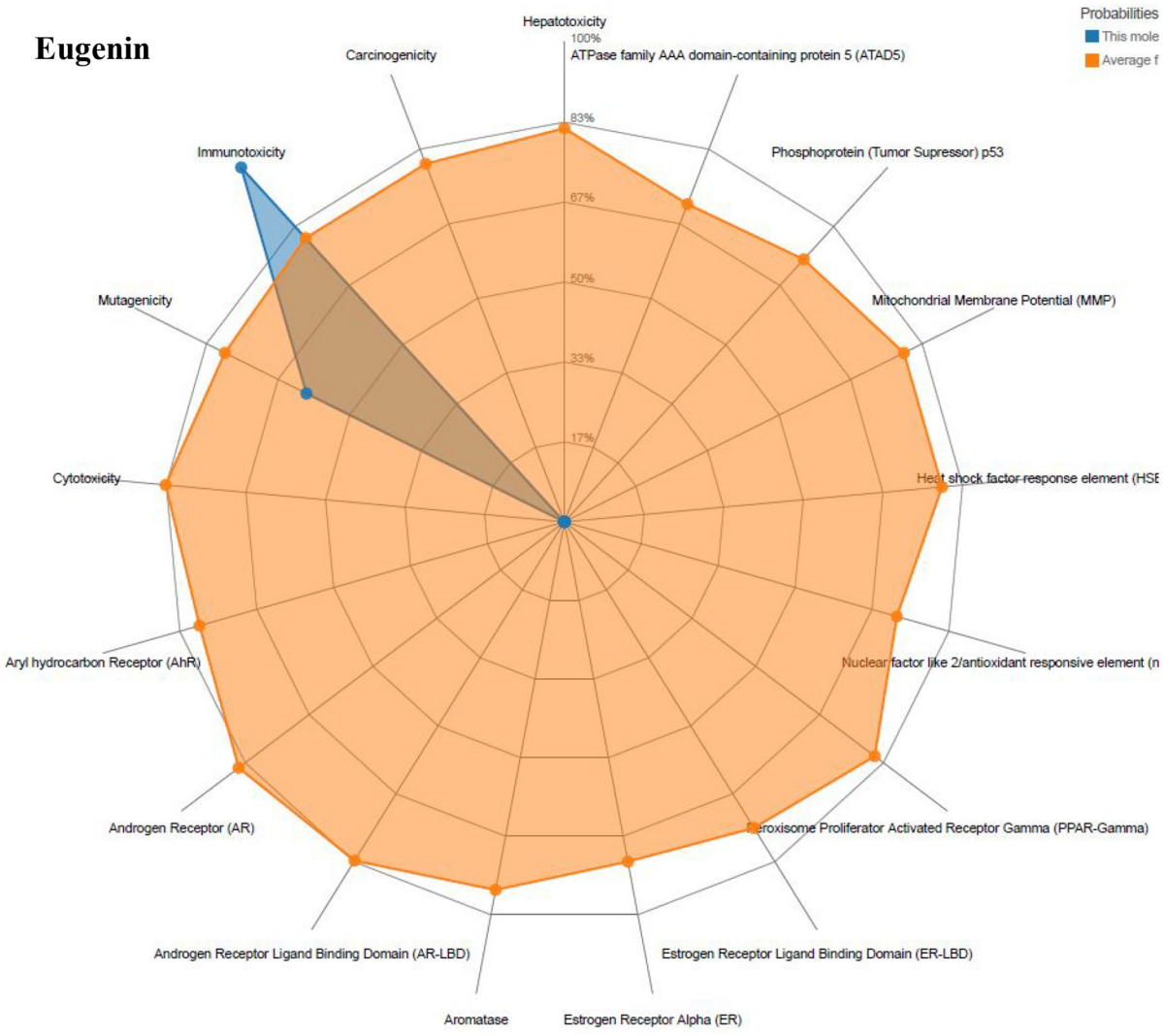


Fig. 7. The toxicity radar chart of best fit five potential antiviral molecules.

through van der Waal interaction where ARG-131, ASP-197, THR-199, TYR-237, LEU-286, LEU-287, GLU-288, ASP-289, GLU-290 were involved and conventional hydrogen bond where LYS-137, ASN-238 were involved as shown in Fig. 5(II). The binding energy was found to be −7.67 kcal/mol. Likewise, the molecular docking study of *curcumin* and *eugenin* suggests that van der Waal interaction played a major role in the complex formation. The amino acids involved in binding of *curcumin* with target protein were ARG-131, LYS-137, THR-196, ASP-197, THR-198, THR-199, TYR-237, ASN-238, TYR-239, LEU-272, LEU-286, LEU-287, ASP-289, GLU-290 as shown in Fig. 5(III). For *eugenin* van der Waal interaction was found to be more prominent in the complex formation. The amino acids involved were PHE-8, VAL-104, ILE-106, GLN-110, THR-111, GLN-127, ASN-151, ILE-152, SER-158, ILE-249, THR-292, PRO-293, PHE-294, ARG-298 as shown in Fig. 5(IV). The binding energy of *curcumin* and *eugenin* with target protein was found to be −8.79 kcal/mol and − 19.93 kcal/mol, respectively.

3.1.5. Ionic liquids (ILs): containing cyclic ring as cation moiety

The molecular docking results based on the minimum energy (full fitness score) the conformers for four ILs with varied cation moiety, namely, *1-decyl-1-methylpyrrolidin-1-ium, 1-decyl-1-methyl-piperidin-1-ium, 1-decyl-pyridin-1-ium, 1-decyl-3-methylimidazolium* along with their corresponding 2D interaction plots are depicted in Fig. 6. The docked poses demonstrate that the drugs molecules bind within the active site of the SARS-CoV-2. The docked figure of *1-decyl-1-methylpyrrolidin-1-ium* is shown in Fig. 6(I) which suggests that *1-decyl-1-methylpyrrolidin-1-ium* binds through van der Waal (ARG-131, THR-199, TYR-239, LEU-271, LEU-272, GLY-275, MET-276, ALA-285, LEU-286, LEU-287, GLU-288, ASP-289, GLU-290) in COVID-19 protein. The binding energy involved in complex formation was found to be negative which suggested the spontaneous complex formation between the two. The magnitude of the binding energy was −6.52 kcal/mol. Besides this other interaction such as conventional hydrogen bonding interaction was found to be occurring between the target protein and *1-decyl-1-methylpyrrolidin-1-ium*. The docking result of *1-decyl-1-methyl-piperidin-1-ium* suggests that it binds through van der Waal interaction where THR-25, LEU-27, HIS-41, VAL-42, CYS-44, THR-45, SER-46, MET-49, HIS-164, MET-165, GLU-166, LEU-167, PRO-168, ARG-188, GLN-189, THR-190, GLN-192 amino acids were involved as shown in Fig. 6(II). Besides, conventional hydrogen bonding interaction also played a role in the binding process. The binding energy was found to be −7.05 kcal/mol. Likewise, molecular docking study of *1-decyl-pyridin-1-ium,* and *1-decyl-3-methylimidazolium* suggests that van der Waal interaction played a major role in binding with complex formation. The amino acids involved in binding of *1-decyl-pyridin-1-ium* with target protein are TYR-198, THR-199, TYR-237, ASN-238,

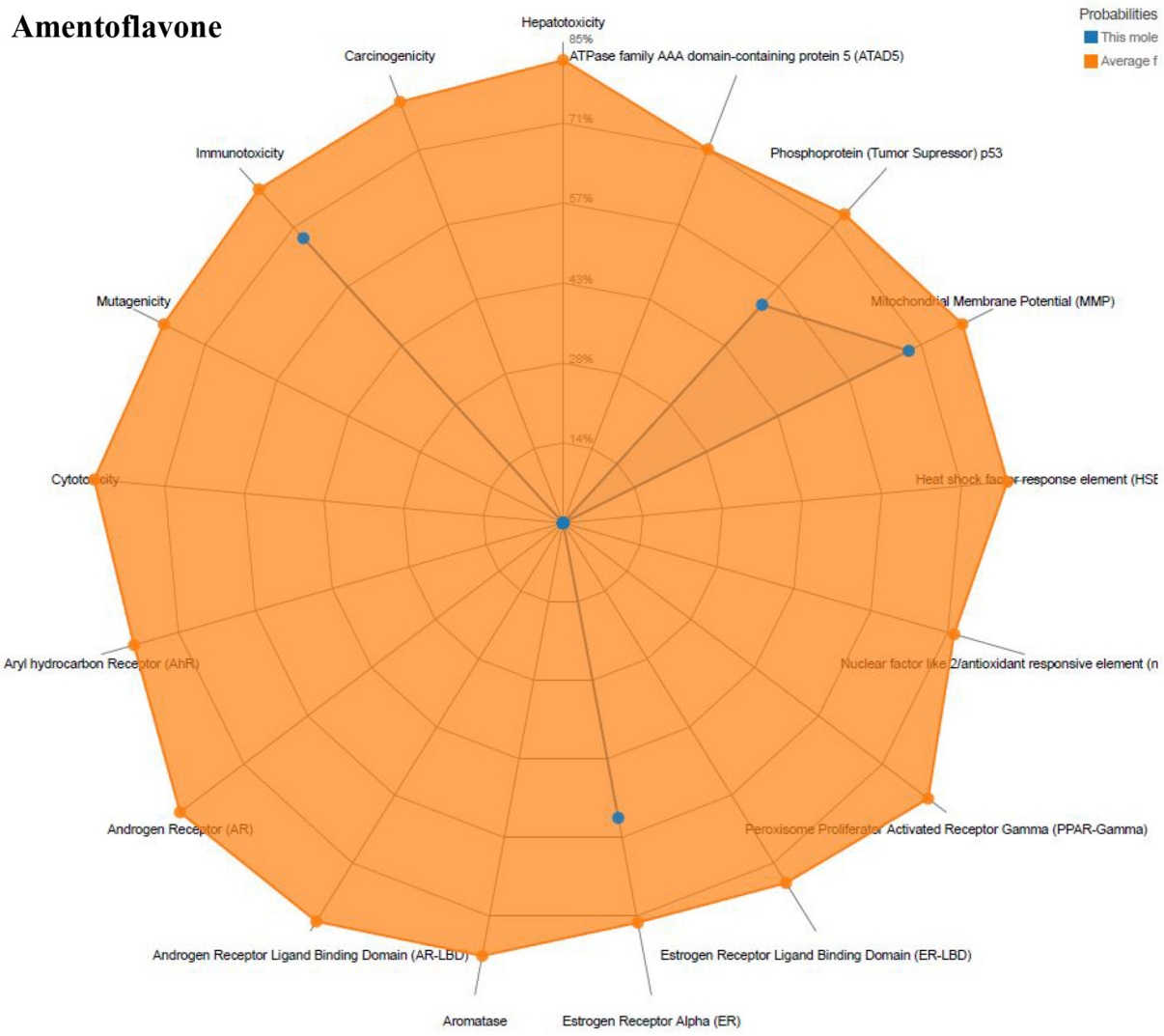


Fig. 7 (*continued*).

TYR-239, LEU-271, LEU-272, GLY-275, MET-276, LEU-286, LEU-287 as shown in Fig. 6(III). For *1-decyl-3-methylimidazolium* based IL MET-6, PHE-8, PRO-9, ILE-152, ASP-153, TYR-154, VAL-297, ARG-298, GLN-299, VAL-303 as shown in Fig. 6(IV). The binding energy of *1-decyl-pyridin-1-ium* and *1-decyl-3-methylimidazolium* with target protein was found to be −6.31 kcal/mol and − 5.62 kcal/mol, respectively. Based on binding energy (listed in Table 1) the compounds were ranked and were shortlisted as potential antiviral compounds. The screened molecules with their binding energy (kcal/mol) are listed in Table 2. Further, the best fit molecules were screened for their ADMET properties and toxicity profiling.

3.2. ADMET properties analysis

The drug likeliness was evaluated using the ADMET properties analysis study. Based on most used drug-like databases such as Comprehensive Medical Chemistry (CMC), Derwent World Drug Index (WDI), and Modern Drug Data Report (MDDR), the results were summarised in Table S2. The "dug-likeliness" parameter is dependent on the mode of administration of the concerned compound. The rule of five (RO5) deals with the dependency of active compounds and defines four simple pharmacokinetics parameter named as molecular weight, log P, H-bond donors, H-bond acceptors. These pharmacokinetics parameters are associated with acceptable aqueous solubility and intestinal permeability and comprise the first steps in oral bioavailability. The RO5 helps in predicting the medicinal and combinatorial chemistry of the selected class of compounds. The predicted values of various properties categorized as size, lipophilicity, polarity, etc. whilst the value of molecular weight describes the size of the molecule. The value of log P corresponds to the lipophilicity of the molecules which is related to the solubility of the drug molecule in the aqueous medium. Higher is the solubility, higher is the activity of therapeutic agents. Another parameter, H-bond donor and H-bond acceptor suggest the quantification of all atoms (O, N) and their efficiency in the formation of the hydrogen bond. While drug designing, the rule of 5 suggests low absorption is more when there are more than 5H-bond donors, 10H-bond acceptors, the molecular weight (MWT) is greater than 500 and the calculated log P is greater than 5 [10].

3.2.1. Antiviral drugs

Based on the predicted log *P* value from SwissADME software, the compounds selected showed to be lipophilic (value of log P for *abacavir, acyclovir, quinoline,* and *hydroxyquinolin*e are given in Table S2) indicating that all the known drug are soluble. Based on log *P* value, the

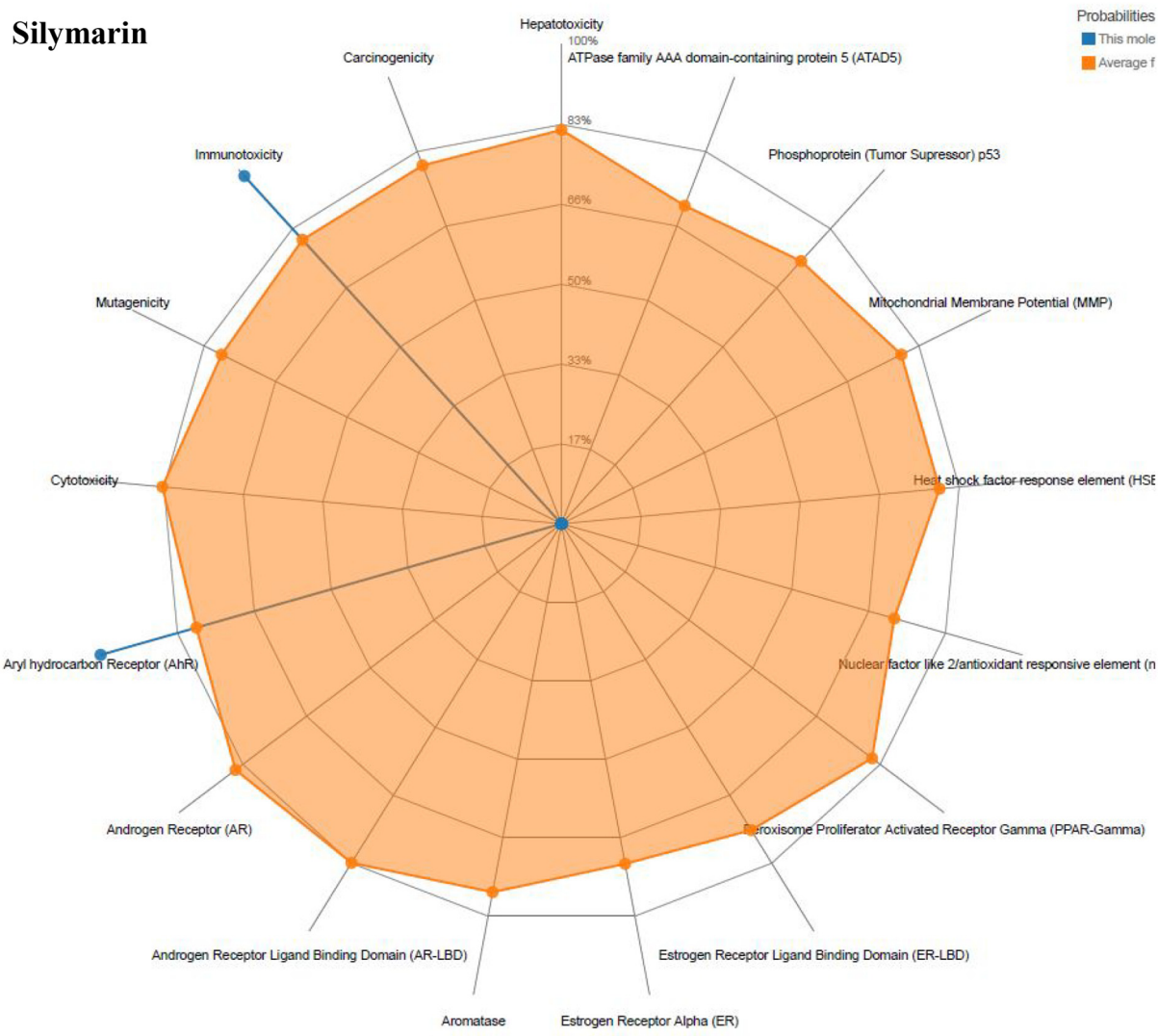


Fig. 7 (*continued*).

solubility comparison among the four antiviral drugs *acyclovir* (log $P = -2.51$) showed the highest solubility.

3.2.2. Antimicrobial drugs

Based on the predicted log *P* value from SwissADME software, the compounds selected showed to be lipophilic (value of log P for *levofloxacin, amoxycillin cloxacillin, and ofloxacin* are given in Table S2) indicating that all the known drug are soluble. Based on log P value, the solubility comparison among the four antimicrobial drugs amoxycillin (log $P = -0.68$) showed the highest solubility.

3.2.3. Natural compounds

Based on the predicted log P value from SwissADME software, the compounds selected showed diverse lipophilicity (value of Log P for *lycorine, saikosaponin, myricetin,* and *amentoflavone* are given in Table S2) indicating that all the natural compounds are soluble except *amentoflavone* (logP = 4.35). Among the four-natural compound, *Saikosaponin* (log $P = 0.01$) showed highest solubility.

3.2.4. Herbal compounds

Based on the predicted log *P* value from SwissADME software, the compounds selected showed to be lipophilic (value of log P for *silymarin, palmatine, curcumin, and eugenin* are given in Table S2) indicating that all the herbal compounds are moderately soluble. Based on log P value, the among the four-herbal compound, *silymarine* showed the comparatively highest solubility (log $P = 1.71$).

3.2.5. ILs (containing cyclic moiety)

Based on the predicted log P value from SwissADME software, the compounds selected showed to be lipophilic (value of log P for *1-decyl-1-methylpyrrolidin-1-ium, 1-decyl-1-methyl-piperidin-1-ium, 1-decyl-pyridin-1-ium, 1-decyl-3-methylimidazolium* based ILs are given in Table S2) indicating that pyrrole (log $P = 4.01$) and imidazolium-based (log $P = 3.04$) ILs are soluble. Whereas piperidine (log $P = 4.40$) and pyridine (log $P = 4.48$) were found to be moderately soluble.

The comparative data of ADMET properties of all the compounds are summarised in Table S2. Based on the log P value the results showed that the selected compounds are soluble and are proved to have promising adsorption in the gastrointestinal tract making it a good potential candidate for the oral route administration. Further, the value of violation (Lipinski's violation) (in Table S3) was found to be negligible. The ADMET properties calculations for the best five molecules screened out based on the binding energy criteria are listed in Table 3 and 4 suggests that the compounds selected are feasible to serve as a future potential antiviral candidate to treat COVID-19 infection.

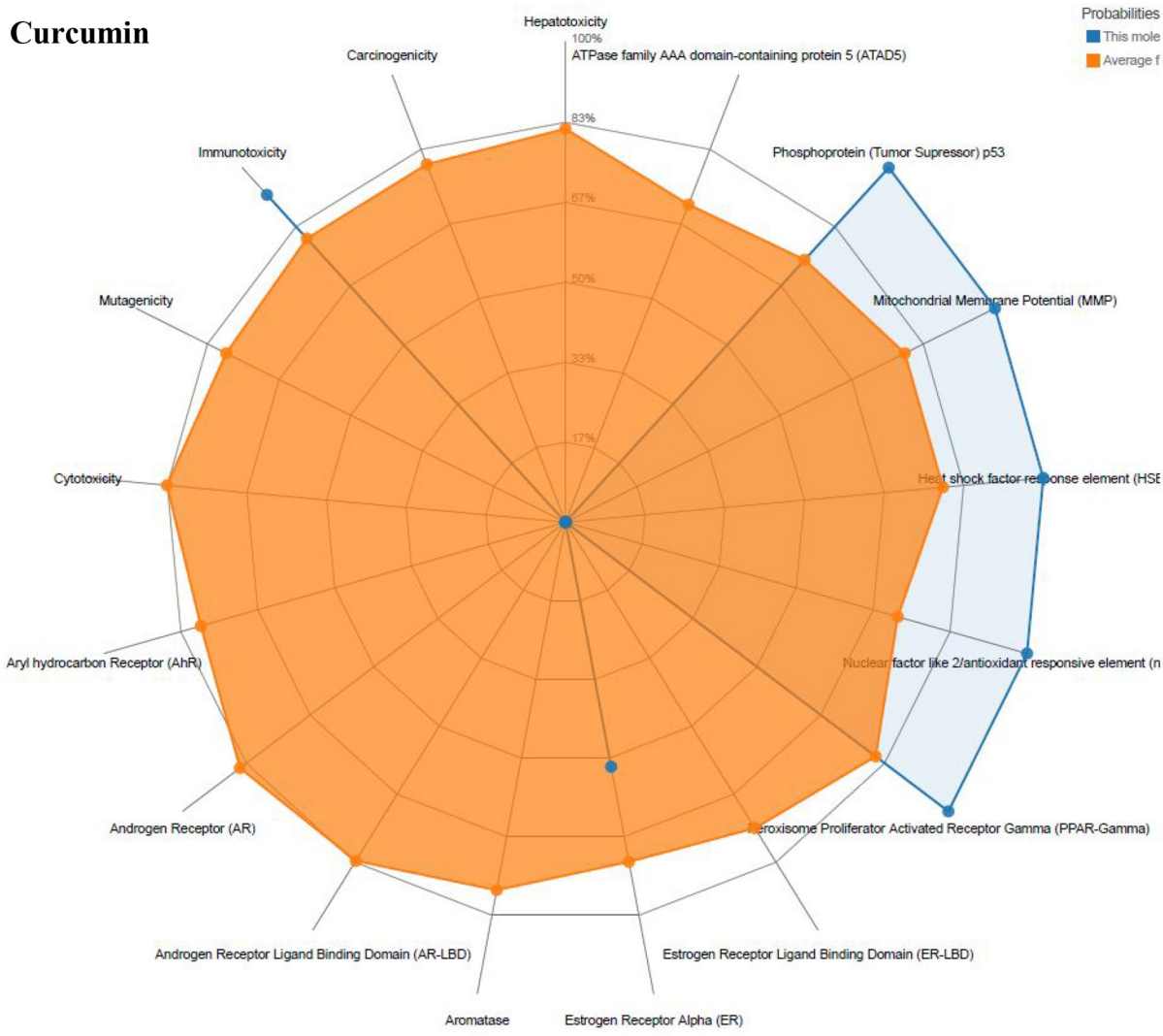


Fig. 7 (*continued*).

3.3. Toxicity analysis

The toxicity determination of selected class of compounds is another important component in drug designing. The computational approach to evaluate toxicity helps in determining the toxic level of doses in the animal model [44]. It helps in reducing the risk of failure in the experimental procedure and curtail the number of animal model trials. The ADMET properties data obtained from SwissADME analysis and the toxicity prediction obtained from ProTox-II software collectively showed the biological activity of the selected class of compounds [7].

The druglike properties of the selected classes of compounds to be a potential antiviral therapeutic agent such as Blood-brain barrier (BBB) penetration, gastrointestinal absorption (GI), carcinogenicity, and rat acute toxicity (LD_{50}) were evaluated. Blood-brain barrier and gastrointestinal absorption values are listed in Table S2. The problem associated with drug delivery is the BBB (transmission of substance across the barrier). BBB occurs by a variety of mechanism and plays an important role in the rate of acting drug. In the present study, the BBB results suggest that all studied compounds may not cross the blood brain barrier, however, the compounds falling under herbal and ILs class may cross under the blood-brain barrier implies that herbs and ILs as future antiviral agent may play a crucial role in drug development. Also, the gastrointestinal absorption data in the Table S2 showed permeability for all studied compounds except for 2, 6, 11, 12, 13, 16 in the Table S1. Suggesting the higher absorption of herbal and ILs. As per the reported values of LD_{50} values in the literature the acute oral toxicity model classified under four categories (category 1, $LD_{50} \leq 50$ mg/kg; category 2, 50 mg/kg < LD50 ≤ 500 mg/kg; category 3, (500 mg/kg < LD50 ≤ 5000 mg/kg and category 4, LD50 > 5000 mg/kg) [44]. LD_{50} value in Table S4 shows that all the compounds studied were non-toxic except 1, 9, 10. The toxicity data for the best five molecules screened out based on the binding energy criteria are listed in Table 5 suggests that the compounds selected are feasible to serve as a future potential antiviral candidate to treat COVID-19 infection. The toxicity results also showed the non-toxicity of natural and herbal with ILs, suggesting the strong applicability of these classes of drugs as antiviral compounds to treat COVID-19.

4. Discussion

The rapid outbreak of severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2) leads global healthcare, physical, economical threat for which WHO declared the public health emergency worldwide for six months [45,46]. This is the biggest emergency known till now. The CoV-2 virus belongs to the homogeneous family (spike protein). Spike in the protein contains spikes made up of 1300 amino acids which interact with the target cell viz. bronchial and pulmonary

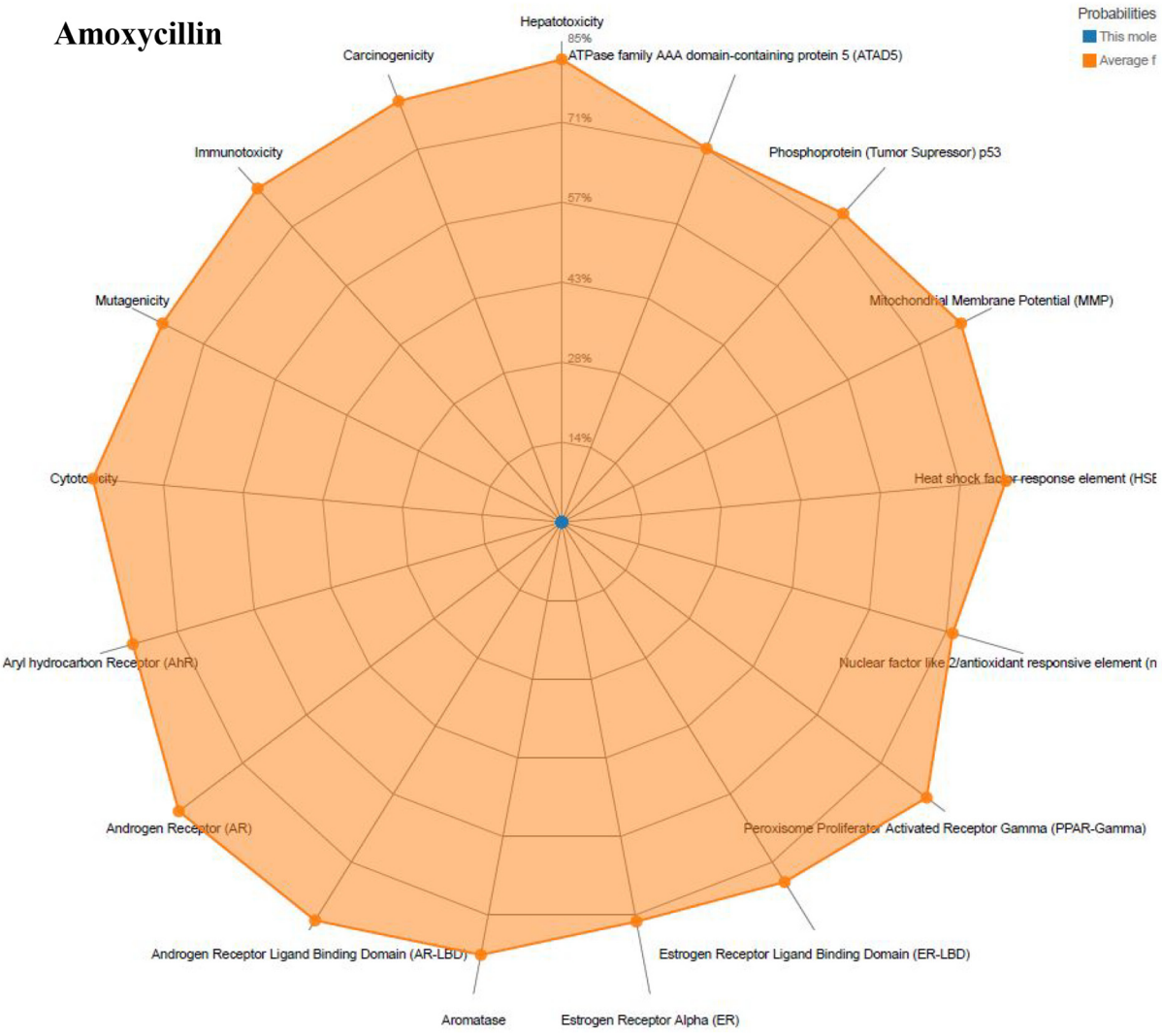


Fig. 7 (*continued*).

cells [47]. This interaction of S-protein with the target cell helps the CoV virus to enter via the cell membrane. The virus targets the enzyme in the epithelial cell which is known as angiotensin-converting enzyme 2 (ACE2) and this is how the virus starts circulating within the human circulatory system [48]. The scenario around the world came to a halt and each doctor and scientist are working hard to find the treatment. Till now, the patients suffering from coronavirus are quarantined and are treated with some conventional antiviral drugs such as *hydroxychloroquine, remdesivir*, etc. [17,48]. Although these drugs showed some improvement in the recovery time no significant effect was found during the treatment, therefore, the requirement for new specific and effective drugs against the CoV-2 virus is still needed. Several groups of researchers are working on vaccine and some are under human trial phase, however, designing drug molecule, synthesis, licensing, large production, and marketing will need ample time. Thus, the computational approach to identify the drug molecule which could specifically target the virus life cycle tender the faster and cost-effective way to design the potential candidate against CoV-2 virus and appears to be the best possible strategy [44].

We performed the molecular docking, ADMET study, and toxicity analysis to identify the potential molecule which could bind with the S-protein so that the interaction of the virus with target epithelial cells narrows down and will render the progress of the virus to grow within the human circulatory system [48]. Recently reported computational studies showed that the interaction occurring between molecules and target protein which indicated the disruption of the S-protein - ACE2 membrane and maybe an inviting target for structure-based drug discovery [44]. Earlier, the study showed the application of natural products and herbs to increase immunity [4,49]. Therefore, we choose natural compounds and herbal products and compared them with conventional antimicrobial and antiviral drugs with ILs having antimicrobial activity. The binding study showed that all the compounds were active against CoV-2 protease. Based on binding energy (Table 2), the molecules were arranged, and it was found that *eugenin, amentoflavone, silymarin, curcumin, myricetin* showed the maximum binding energy among the selected compounds on complexation with CoV-2 protease. All these molecules belong to the herbal and natural compound's category strongly suggests the utility of them as a potential candidate against coronavirus (shown in Scheme 1). Further, we reached to the novel findings when we took various ILs and studied for their antiviral activity. The binding results showed remarkable bonding between target protein and ILs. The pyrrolidinium, piperidinium, pyridinium, and imidazolium based ILs having decyl carbon chain length were found to be active against

coronavirus and could behave as a promising antiviral agent in the future. The following scheme summarises the screening result obtained from the molecular docking technique.

Further pharmacokinetic properties of the compounds were studied using ADMET property analysis. Earlier studies showed that the Lipinski rule (rule of 5) helped a lot in the last twenty years for determining the pharmacokinetic properties of drug molecules associated with permeability and solubility. In the last twenty years the "Rule of 5" has become a key for the researchers and pharma industrialists with drug-like properties and helped in designing the drug molecule [1]. Here in the present study, the ADMET analysis showed the solubility and permeability of all the selected class of compounds as shown in Table S2 & Table S3. The best drug molecule (based on highest binding energy) showed good to moderate solubility and excellent permeability across the cell membrane as shown in Tables 3 & 4. Additionally, toxicity profiling of the compounds was done and the data for the same is shown in Table S4. The data of the best-fitted molecule in Table 5 suggests that out of 5 molecules herbal molecule, *eugenin, amentoflavone,* and *curcumin* easily crosses the blood-brain barrier while *amoxycillin* and *silymarin* fails. However, GI adsorption for all the selected was found to be good (Table 5). The ADMET cytotoxicity, carcinogenicity, and hepatoxicity (shown in Table 5) results showed the non-toxicity of the best fit molecule screened out after molecule docking result analysis. The LD_{50} for all the molecule was non-toxic except *abacavir, lycorine, and saikosaponin*. The results in Table 5 for best-selected molecules strongly support the ability of the selected compounds to act like a drug that is non-carcinogenic and non-toxic. The toxicity radar chart of best five molecule is also shown in Fig. 7.

Despite screening the already available molecules we performed the same experiments with the ILs having cyclic moiety having decyl carbon chain length attached to it. The binding energy obtained from molecular docking was comparably like the conventional antiviral drugs as shown in Table 2. The ADMET cytotoxicity, carcinogenicity, and hepatoxicity showed good gastrointestinal absorption which favours good transport through the membrane, and this was also supported by the blood-brain barrier data in the Table 3. After all the screening process we reached to the novel finding that ILs could behave as a potential antiviral candidate against life-threatening virus. Tunability, the versatile feature of ILs will further open the floor to alter the activity and pharmacokinetic properties of the ILs.

5. Conclusion

In the present study, we performed blind molecular docking to propose the potential inhibitor of CoV-2 using the various classes of compounds viz. antimicrobial agents, natural compounds, herbal compounds, ILs, and the results were compared with the conventional antiviral drugs. We aimed to stop the replication of virus protein by inhibiting the mutarotation of CoV-2 protein which will ultimately decrease the spread of global threats occurring due to severe infection. The molecular docking study showed the importance of herbal and natural compounds preferably. The binding energy of *eugenin, amentoflavone, silymarin,* and *curcumin* was found to be highest as compared to the other compounds studied. Also, antimicrobial compounds, amoxycillin was found to be active against the virus protein. Additionally, during the screening process, we reached a novel finding where we found that ILs possess significant potency against virus protein which opens the floor to further design ILs with increased antiviral activity against virus protein. Moreover, toxicity profiling suggested the *eugenin, amentoflavone, silymarin, amoxycillin, curcumin* and ILs can be used as a potential antiviral agent against the main protease of SARS-CoV-2.

Author statement

Juhi Saraswat performed the research, analyzed the data, and wrote the paper.

Prashant Singh reviewed and edited the manuscript.

Rajan Patel is overall corresponding author and finalized/ designed the research.

All authors had read and approved the final manuscript.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2021.115298.

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