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IN-SILICO VALIDATION OF CLOVE AS A POTENTIAL NUTRACEUTICAL AGAINST SARS-COV-2

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Syzygium aromaticum, Clove, SARS-CoV-2, Spike protein, ACE2, M^{pro}, RdRp, Docking, Phytochemicals

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ABSTRACT: The pandemic COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has killed millions of human beings throughout the world and attaining natural immunity against this virus is the best remedy. Consumption of nutraceuticals having antiviral and immune-boosting ingredients is the natural way to fight against it. The main objective of the present study was to evaluate the anti-SARS-CoV-2 activity of phytochemicals from *Syzygium aromaticum* and identify lead molecules through an *in-silico* approach. To determine anti-SARS-CoV-2 activity, 249 phytochemicals from *Syzygium aromaticum* (Clove) were docked with each of the four therapeutic targets namely Spike protein (SP), Angiotensin-Converting Enzyme-2(ACE2), Main protease (M^{pro}) and RNA-dependent RNA polymerase (RdRp). Spike protein and ACE2 have a key role in viral entry into the human host and M^{pro} and RdRp have a key role in viral multiplication. The docked molecules with minimum binding energies ≤ -6 kcal/mol were considered active/hit molecules. The total number of hits obtained in the order of merit was 46, 51, 60, and 150 against RdRp, spike protein, $M^{pro,}$ and ACE2, respectively, and 46 phytochemicals showed inhibitory activity on all four targets. Further, top-ranked lead molecules' protein-ligand interaction, physiochemical and ADMET prediction indicated that the compounds bicornin, casuarictin and pedunculagin have good inhibitory activity against all the selected targets. However, bicornit was recommended as the best lead for further studies since it has comparatively good inhibitory and ADMET properties.

INTRODUCTION: The current pandemic COVID-19, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2 or 2019-nCoV), an enveloped positive-sense single-stranded RNA virus (+ssRNA) with a genomic size of ~30 kbp, belongs to the family Coronaviridae, subfamily Orthocoronovirinae and genus β-coronavirus [1].

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It induces severe respiratory diseases in humans and animals. Different strains of this virus have been reported earlier, including SARS (2002-2003), MERS (2012), and SARS-CoV-2 (2019-2020) [2]. The different variants of SARS-CoV-2 such as alpha, beta, gamma, delta and omicron, have been reported and these variants are considered as variants of concern by the World Health Organization [3].

The most important challenge in fighting against the current pandemic is the rapid mutating behaviour of the virus, creating a lot of uncertainties in the therapeutic strategies. The question arises whether the present vaccines and

drugs are effective against the new viral variants. Most importantly, this problem can be tackled by boosting the body's immune system to fight against these deadly viruses through natural products. Since, the beginning of human civilization, natural products particularly from plants, are being used as a potential remedy to fight against viruses and even recent investigations in this line revealed the role of plant-derived products in preventing and curing SARS-CoV-2 activity [4]. In the Indian traditional system of medicine, several plant species have been used against viral diseases. *Syzygium aromaticum* (L.) Merril & Perry commonly known as clove, belonging to the family Myrtaceae is one of the widely used spices against viral diseases and has a wide range of therapeutic activities in Indian traditional medicine. Clove is an indigenous species to Molucca Island, in eastern Indonesia also known as the 'Spice Islands'. It has been mainly grown in Indonesia, Madagascar, Tanzania, India, and Sri Lanka. It has anti-trypanosomal, anti-septic, anesthetic, anti-obesity, antiviral, anti-diabetic, cardio-protective, anti-bacterial, acaricidal, insecticidal, anti-cancer, anti-oxidant, anti-fungal, herbicidal, anti-spasmodic, anti-microbial, nematicidal, immune-stimulatory, anti-inflammatory, anti-thrombotic, anti-parasitic, anti-mutagenic, photo-cytotoxic and antipyretic activities [5].

Antiviral activity of clove against different types of viruses such as hepatitis C virus, herpes simplex virus, influenza virus, and dengue virus have been reported. Recently, clove has been used to prevent and treat COVID-19 due to its antiviral, anti-inflammatory, anti-thrombotic and immune-stimulatory properties [5]. However, the phytomolecules with antiviral activity and their molecular mechanism of drug activity are yet to be elucidated. In these backdrops, the present investigation aimed to determine the potential lead molecules with anti- SARS-CoV-2 activity in *S. aromaticum* through an *in-silico* approach.

MATERIALS AND METHODS:

Selection and Preparation of Macromolecule: SARS-CoV-2 genome encodes four main structural proteins *i.e.,* spike (S), nucleocapsid (N), envelope (E) and membrane (M) protein, along with 16 non-structural proteins (NSPs) and 5-8 accessory proteins [6]. SARS-CoV-2 enters the host cells by interaction of the envelope-anchored spike protein with human host cell receptor Angiotensin-Converting Enzyme-2 (ACE2), followed by membrane fusion and once the viral RNA is released into the host cell, genomic RNA undergoes translation. Processing of polyproteins pp1a and pp1ab into non-structural viral proteins (nsps) are mediated by proteases like main protease (M^{pro}) and papain-like protease (PL^{pro}). Non-structural proteins code for RNA-dependent RNA polymerase (RdRp/nsp12), a key enzyme involved in the replication and transcription of the virus[7]. Since these proteins play an important role in virulence, replication, and multiplication of virus, these are considered ideal targets for drug development against SARS-CoV-2.

Spike protein (PDB ID: 6M0J), ACE2 receptor from humans (PDB ID: 1R4L), M^{pro} (PDB ID: 7BUY), and RdRp (PDB ID: 7BV2) from SARS-CoV-2 were selected as targets. The 3D structures of the targets were downloaded from Protein Data Bank and the unwanted water molecules and hetero-atoms were excluded from the protein structure and finally converted into pdbqt format using the tool AutoDock 4.2 [8]. Then the active site residues of spike protein and ACE2 were found from the literature, and M^{pro} and RdRp were predicted based on the native ligands binding with these proteins using the tool PDB Sum.

Selection and Preparation of Ligands: 249 phytochemicals reported from *Syzygium aromaticum* were used as the ligands. The 3D structures of the ligands were obtained from the PubChem database in structural data format (sdf) and were then minimized using Universal Force Field (uff) with conjugate gradient as an optimization algorithm, followed by conversion of sdf to pdbqt format using Open Babel software integrated with PyRx 0.8 version virtual screening tool [9].

Molecular Docking: Docking was performed using AutoDock Vina 10 in the PyRx virtual screening tool based on the Lamarckian Genetic Algorithm (LGA) method. Before docking, the grids with X, Y, and Z coordinates were set around the active site of each target. After the execution of docking, the protein-ligand complex was viewed and saved in PDB format using PyMol 4.6.0 [11]. The

top-ranked five hits with the least binding energy were selected for further interaction analysis.

Post Docking Analysis: The protein-ligand interaction was analyzed and visualized using Discovery Studio Visualizer.

Physiochemical and Pharmacokinetic analysis: The pharmacokinetic properties such as absorption, distribution, metabolism, and excretion analysis of the top-ranked five hits from each target were performed using the SwissADME online program [12], where molecular descriptors such as molecular weight, hydrogen bond donor, hydrogen bond acceptor, logP and violation of Lipinski's rule of five were calculated and toxic properties were determined using pkCSM online program [13], as toxicity is the major concern in drug development.

RESULTS:

Structure of Target Proteins: Spike proteins are the protrusions on the surface of coronavirus that mediate receptor recognition and viral entry. Since spike protein is the first protein that interacts with the host cell, it can be considered an ideal target for drug development. Spike protein (PDB ID: 6M0J) consists of 832 amino acids with a molecular weight of 97.14 kDa.

Angiotensin-converting enzyme-2 (ACE2), a zinc carboxypeptidase, acts as the viral entry receptor, thus making it a potential therapeutic target [14]. ACE2 (PDB ID: 1R4L) consists of 673 amino acids with a molecular weight of 76.98 kDa. M^{pro} is a key enzyme involved in the proteolytic cleavage of polyproteins pp1a and pp1ab into functional non-structural proteins required for viral replication and multiplication [15]. M^{pro} (PDB ID: 7BUY) consists of 306 amino acids with a molecular weight of 34.36kDa. RNA-dependent RNA polymerase (RdRp) is a key enzyme involved in viral replication, thus making it an attractive target against SARS-CoV-2[7]. RdRp (PDB ID: 7BV2) consists of 1300 amino acids with a molecular weight of 159.19 kDa.

Active Site Determination: Active site residues of the selected proteins determined using PDBsum are as follows: Spike protein - Lys417, Gly446, Tyr449, Asn487, Tyr489, Gln493, Thr500, Asn501, Tyr505; ACE2 - Arg273, His345, Thr371, Glu375, His378, Glu402, Phe504, His505, Tyr515, Tyr510, Arg514; M^{pro} -Thr26, His41, Met49, Gly143, Ser144, Cys145, His164, Met165, Asp187 and RdRp - Arg555, Asp623, Ser 682, Thr687, Asp760.

Molecular Docking: A total of 249 phytochemicals derived from *Syzygium aromaticum* were docked with each of the four therapeutic targets, namely Spike protein, ACE2, M^{pro} and RdRp.

The docked molecules with binding energies ≤ -6 kcal/mol were considered active/hit molecules since many authors have reported that in AutoDock, the foregoing energy level can be treated as significant [16, 17].

The number of hits obtained in the order of merit was 46, 51, 60 and 150 against RdRp, spike protein, M^{pro} and ACE2, respectively.

Notably, out of the 249 phytochemicals screened, 46 of them showed inhibitory activity ($\Delta G \leq -6$ kcal/mol) on all four targets **Table 1**.

When compared to the reference drug remdesivir, 22, 22, 46, and 71 ligands showed better docking scores against M^{pro}, spike protein, RdRp, and ACE2, respectively. The top five hit molecules with the least binding energy and its' interactions obtained against the target spike protein, ACE2, M^{pro} and RdRp were depicted in **Tables 2, 3, 4** and **5,** respectively.

Among the selected leads casuarictin, bicornin and pedunculagin were common leads against all four targets, indicating the multi-target binding affinity of the phytomolecules. The compounds casuarictin and bicornin interact with both the catalytic dyads of M^{pro}, thus blocking His 41 and Cys145 catalytic residues, which can hinder the activity of M^{pro}.

The compound bicornin showed interactions with Lys 417, a critical residue of spike protein that is involved in the virulence of SARS-CoV-2. Except for three (casuariin with spike protein, pedunculagin with ACE2 and RdRp), all the top hit molecules formed H-bond interactions with the target proteins' catalytic residues, and seven of them showed unfavorable bonds.

TABLE 1: LIST OF PHYTOCHEMICALS FROM *SYZYGIUM AROMATICUM* WITH INHIBITORY ACTIVITY (BINDING ENERGY ≤ -6 KCAL/MOL) ON ALL THE SELECTED TARGETS

S. no.	Phytochemical	Binding Energy(kcal/mol)			
		Spike Protein	ACE2	M^{pro}	RdRp
1	(-)-Epicatechin	-6.9	-8.8	-7.3	-6.6
2	3,3',4',5,5',7-Hexahydroxyflavylium	-7.8	-8.9	-7.7	-6.9
3	3,6,7-Trihydroxy-2-(4-hydroxy-3-methoxyphenyl)chromen-4-one	-6.6	-8.8	-7.9	-6.5
4	5,7-Dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	-6.9	-8.5	-7.3	-7
5	Apigenin	-7.3	-9.2	-8.2	-7
6	Astragalin	-6.5	-9.6	-7.6	-6.4
7	β-Carotene	-7.1	-10	-7.3	-6.7
8	β-Cryptoxanthin	-5.2	-10.3	-5.6	-7
9	β-Sitosterol	-6.7	-9.1	-6.5	-6.2
10	Bicornin	-9.2	-11.5	-9.5	-8.4
11	Biflorin	-6.3	-9	-7.1	-6.1
12	Campesterol	-7.5	-9.2	-6.3	-6.3
13	Campesterol glucoside	-6.9	-10	-7.7	-7.4
14	Casuarictin	-10.6	-11.7	-10	-8.2
15	Casuariin	-8.5	-6.4	-8.2	-7.4
16	Chlorogenic acid	-6.9	-8.7	-7.6	-6.6
17	Cianidanol	-7.1	-9.1	-7.4	-7.1
18	Curcumin	-7	-9.2	-6.6	-6
19	Daidzein	-7.2	-8.3	-6.6	-6.3
20	Ellagic acid	-6.7	-9.1	-8	-6.8
21	Epigallocatechin	-6.8	-8.7	-7.4	-6.7
22	Ethinylestradiol	-6.6	-9.4	-6.7	-6.6
23	Tellimagrandin II	-7.9	-8.3	-10.4	-7.8
24	Casuariin	-8.7	-10.8	-7.1	-7.1
25	Genistein	-6.7	-8.8	-6.8	-6.2
26	Glycitein	-6.4	-8.2	-6.6	-6.2
27	Hyperoside	-7.6	-9.9	-7.7	-7.2
28	Isobiflorin	-7.2	-8.2	-7.2	-6.3
29	Isoquercitrin	-7.4	-9.9	-8	-8.1
30	Kaempferide	-7.9	-8.5	-7.1	-6.3
31	Kaempferol	-7.6	-8.4	-7.1	-6.4
32	Luteolin	-7.8	-8.9	-7.7	-7.5
33	Malvidin	-7.4	-8.8	-8	-6.6
34	Maslinic acid	-6.7	-9.8	-6.2	-6.8
35	Maslinic acid methyl ester	-7.7	-9.4	-6.8	-6.7
36	Myricetin	-7.7	-9	-7.7	-7
37	Oleanolic acid	-7.7	-9.3	-6.9	-6.6
38	Pedunculagin	-9.3	-10.8	-9.3	-8.1
39	Quercetin	-7.7	-9.2	-7.5	-7.1
40	Rhamnetin	-7.5	-8.7	-7.6	-6.8
41	Rhamnocitrin	-7.2	-8.4	-7.2	-6.4
42	Rosmarinic acid	-7.3	-8.3	-7.2	-6.6
43	Gemin D	-8.4	-8.4	-7.5	-8.2
44	Rutin	-8.1	-10.5	-8.3	-7.5
45	Stigmasterol	-6.9	-9.6	-6.7	-6.5
46	Rugosin A	-8.3	-11.2	-9.1	-8.4
47*	Remdesivir	-7.3	-8	-7.4	-6.9

*Approved drug molecule

The compound casuarictin binds firmly with active site residues Tyr449, Gln493, Thr500, Tyr505 of spike protein, His378, Tyr510 of ACE2, His41, Gly143, Ser144, Cys145, Met165 of M^{pro} and Arg555 of RdRp. Similarly, compound bicornin forms H-bond with active site residues Lys417, Gln493, Tyr505 of spike protein, Arg273, His345 of ACE2, His41, Gly143, Ser144, Cys145, His164 of M^{pro} and Asp760 of RdRp. Compound pedunculagin blocks spike protein by forming H-

bond interactions with active site residues Gln493, Asn501, and Gly143 of M^{pro} but showed no H-bond interaction with both RdRp and ACE2. Strictinin interacts with catalytic residues His345, Thr371, Glu375, His378 of ACE2, Thr26, His41, Met49 of M^{pro} and Arg555 of RdRp. Similarly, gemin D blocks spike protein by forming H-bond interaction with Gln493, Asn501, and His345 of ACE2, and rugosin A only forms H-bond with Arg555 of RdRp.

TABLE 2: BINDING INTERACTION OF TOP-RANKED HITS WITH SPIKE PROTEIN

Hit Molecules	PubChem ID	BE (kcal/mol)	Active Site Residues		
			H-Bond	Bond Length (A^0)	Hydrophobic Interactions
Casuarictin	73644	-10.6	TYR449:HH-----O:Lig	2.74	
			GLN493:HE21--O:Lig	2.45	
			THR500:O------O:Lig	2.94	
			TYR505:C------O:Lig	3.45	
Pedunculagin	442688	-9.3	GLN493:OE1---O:Lig	3	
			GLN493:HE21--O:Lig	2.77	
			ASN501:OD1---C:Lig	3.79	
Bicornin	71308161	-9.2	LYS417:CE-----O:Lig	3.63	LYS417
			GLN493:HE21--O:Lig	2.49	
			TYR505:O------H:Lig	1.98	
Gemin D	471119	-8.7	GLN493:OE1----O:Lig	3.04	LYS417
			ASN501:HD22--O:Lig	2.89	
Casuariin	14035442	-8.5	Nil		

TABLE 3: BINDING INTERACTION OF TOP RANKED HITS WITH ANGIOTENSIN-CONVERTING ENZYME-2 (ACE2)

Hit Molecules	PubChem ID	BE (kcal/mol)	Active Site Residues		
			H-Bond	Bond Length (A^0)	Hydrophobic Interactions
Units	71308161	-11.7	ARG273:HH12--O:Lig	3.07	ARG273
			HIS345:HN------O:Lig	3.08	GLU375
Bicornin	73644	-11.7	HIS378:NE2-----H:Lig	3.03	
			TYR510:HH-----O:Lig	2.85	
			TYR510:HH-----O:Lig	2.91	
Strictinin	73330	-11.2	HIS345:HE2-----O:Lig	2.5	
			HIS345:HE2-----O:Lig	1.97	
			THR371:OG1----H:Lig	2.09	
			GLU375:OE1----H:Lig	2.4	
			HIS378:CD2-----O:Lig	3.14	
Gemin D	471119	-10.8	HIS345:HD1-----O:Lig	2.26	HIS345
Pedunculagin	442688	-10.8	Nil		

TABLE 4: BINDING INTERACTION OF TOP RANKED HITS WITH MAIN PROTEASE (M^{PRO})

Hit Molecules	PubChem ID	BE (kcal/mol)	Active Site Residues		
			H-Bond	Bond Length (A^0)	Hydrophobic Interactions
Strictinin	151590	-10.4	THR26:HN-----O:Lig	2.36	
			THR26:O-------H:Lig	2.59	HIS163
			GLY143:HN----O:Lig	2.44	MET165
Casuarictin	73644	-10	HIS41:CE1----O:Lig	3.77	MET49
			GLY143:HN---O:Lig	2.2	MET165
			SER144:OG---O:Lig	2.85	
			CYS145:HN---O:Lig	2.87	
			MET165:SD---O:Lig	2.01	
Bicornin	71308161	-9.5	HIS41:HE2----O:Lig	2.68	MET49
			HIS41:HD1---O:Lig	2.84	
			HIS41:H-------O:Lig	3.65	
			GLY143:HN---O:Lig	2.07	
			SER144:HG----O:Lig	2.42	

			SER144:HN----O:Lig	2.98	
			CYS145:HN---O:Lig	2.03	
			HIS164:O-----O:Lig	2.92	
			HIS164:O-----O:Lig	2.93	
Tellimagrandin II	442688	-9.3	GLY143:HN--O:Lig	2.11	MET49
Strictinin	73330	-9.1	THR26:O-----H:Lig	2.9	HIS41
			THR26:HN---O:Lig	2.34	MET49
			HIS41:CE1---O:Lig	3.42	MET165
			MET49:SD---H:Lig	3	

TABLE 5: BINDING INTERACTION OF TOP RANKED HITS WITH RNA-DEPENDENT RNA POLYMERASE (RdRp)

Hit Molecules	PubChem ID	BE (kcal/mol)	Active Site Residues		
			H-Bond	Bond Length (A^0)	Hydrophobic Interactions
Bicornin	71308161	-8.4	ASP760:OD1---O:Lig	3.09	ARG555
Strictinin	73330	-8.4	ARG555:HE----O:Lig	2.06	ASP623
			ARG555:HH21-O:Lig	2	
			ARG555:HH21-O:Lig	2.06	
Casuarictin	73644	-8.2	ARG555:HH21-O:Lig	2.77	ASP623
			ARG555:HH22-O:Lig	2.95	SER682
					ASP760
Rugosin A	16132354	-8.2	ARG555:HH21-O:Lig	2.4	
			ARG555:HH22-O:Lig	2.69	
			THR687:HN-----O:Lig	3.06	
Pedunculagin	442688	-8.1	Nil		AR555
					ASP760

Pharmacokinetics and Toxicity Prediction: Pharmacokinetic and ADMET properties were tested to optimize the ligands. Drug-likeness determined by Lipinski's 'Rule of 5' showed that all the top-rankedfive-hitmolecules showed three violations *i.e.,* MW > 500, H-bond donors > 5, and H-bond acceptors > 10. Generally, Lipinski's 'Rule of 5 is not applicable to natural compounds since several plant-derived drugs widely supplied today did not obey the above-said rule [18].

TABLE 6: ADMET ANALYSIS OF SELECTED HIT MOLECULES USING PKCSM SERVER

Property	Model name	Units	Hits							
			Pedunculagin	Casuarictin	Casuarictin	Gemin D	Rugosin A	Strictinin	Tellimagrandin II	Pedunculagin	Bicornin
Absorption	Water solubility	log mol/L	-2.89	-2.89	-2.89	-2.89	-2.89	-2.89	-2.89	-2.89	-2.89
	Intestinal absorption	% Absorbed	31.56	100	100	64.69	21.8	67.51	41.54	100	
	Skin Permeability	log Kp	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	
	P-glycoprotein substrate	Yes/No	Y	Y	Y	Y	Y	Y	Y	Y	
	P-glycoprotein I inhibitor	Yes/No	Y	Y	Y	Y	N	Y	Y	Y	
	P-glycoprotein II inhibitor	Yes/No	Y	Y	Y	Y	N	Y	Y	Y	
Distribution	VDss	log L/kg	-0.03	-0.01	-0.006	0.64	-0.01	0.53	-0.006	0.028	
	BBB permeability	log BB	-5.13	-3.86	-2.78	-2.73	-5.7	-2.61	-4.49	-3.06	
Metabolism	CNS permeability	log PS	-6.38	-5.87	-5.34	-5.14	-6.73	-5.07	-6.00	-5.41	
	CYP2D6 substrate	Yes/No	N	N	N	N	N	N	N	N	
	CYP3A4 substrate	Yes/No	N	N	N	N	N	N	N	N	
	CYP3A4 inhibitior	Yes/No	N	N	N	N	N	N	N	N	
Excretion	Total Clearance	log ml/min/kg	0.02	-0.1	-0.13	0.38	0.003	0.26	0.11	-0.03	
	Renal OCT2 substrate	Yes/No	Y	Y	Y	Y	N	Y	Y	Y	
Toxicity	AMES toxicity	Yes/No	N	N	N	N	N	N	N	N	
	Max. tolerated	log mg/kg/day	0.43	0.43	0.43	0.438	0.438	0.438	0.438	0.438	

dose									
hERG I inhibitor	Yes/No	N	N	N	N	N	N	N	N
hERG II inhibitor	Yes/No	Y	Y	Y	Y	N	Y	Y	N
Hepatotoxicity	Yes/No	N	N	N	N	N	N	N	N
Skin Sensitisation	Yes/No	N	N	N	N	N	N	N	N
T. Pyriformis toxicity	log ug/L	0.28	0.28	0.28	0.285	0.285	0.285	0.285	0.285
Minnow toxicity	log mM	14.85	16.81	18.77	13.31	16.1	11.79	15.85	14.14

(VDss=Volume of Distribution, BBB=Blood Brain Barrier, CNS=Central Nervous System, CYP=Cytochrome P, OCT2=Organic Cation Transporter 2, hERG=human ether-a-go-go-gene).

The pkCSM analysis results were depicted in **Table 6**. The compounds casuariin, casuarictin and pedunculagin showed 100% intestinal absorption but rugosin A showed a lower absorption rate. Similarly, except rugosin, all the compounds were both substrates and inhibitors of P-glycoprotein ABC transporters. The distribution profile indicated that the compounds have a moderate blood-brain barrier (BBB) and central nervous system (CNS) permeability, and compounds gemin D and strictinin showed a high volume of distribution (VDss) compared to other molecules. The metabolism revealed that neither of the compounds inhibits most CYP 450 isoforms. Further, the excretion profile revealed that compounds casuarictin and pedunculagin have a negative rate of total clearance from the body. The toxicity analysis indicated that the compounds were not mutagenic, hepatotoxic, did not induce any skin sensation, and were not an inhibitor of hERG I.

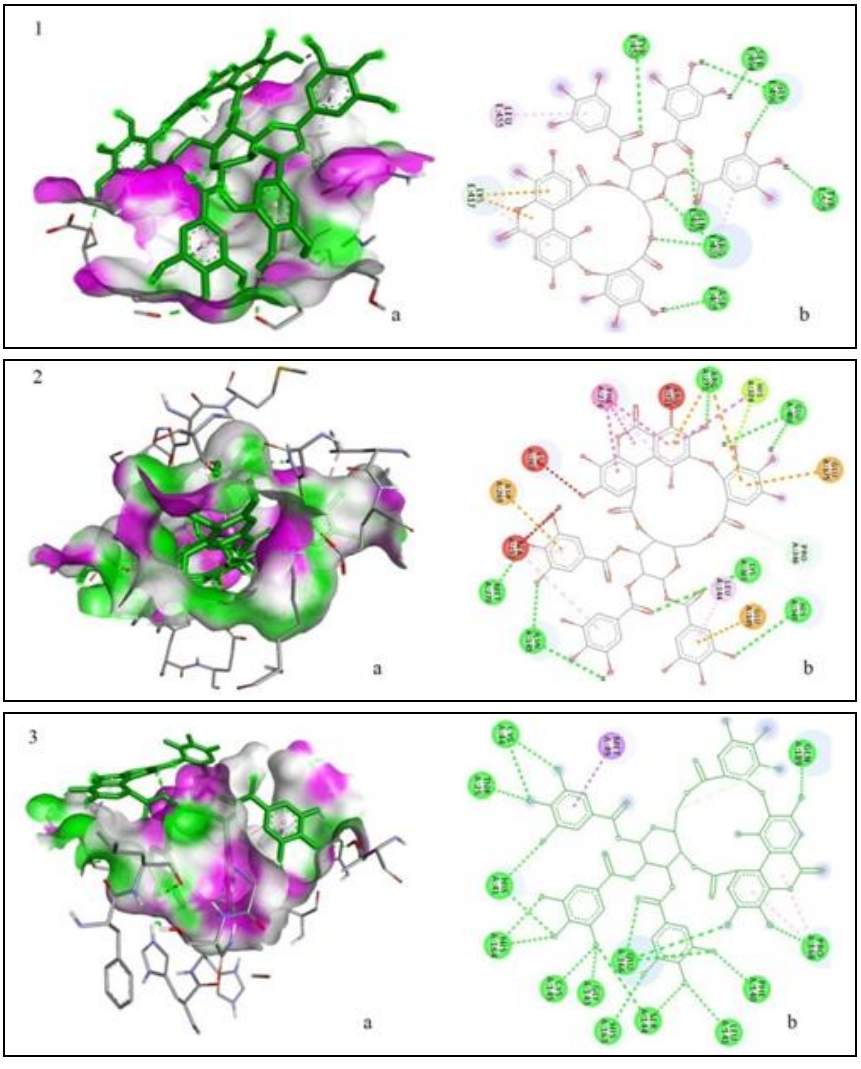
DISCUSSION: Plants are a rich source of chemical molecules with diverse therapeutic activities and the application of *in-silico* method is the best option for primary screening of a large number of chemical molecules quickly and cost-effectively for validating their therapeutic activity. *In-silico* approach offers an in-depth theoretical analysis of molecular interactions, drug-likeness, and ADMET properties and provides a clear insight into the therapeutic activity of chemical molecules on its target without the utilization of chemicals and live materials.

The selection of the right target and identification of ligands with inhibitory activity on multiple targets involved in pathogenesis /disease network pathways is recently accepted as a better approach than the earlier single-target approach, particularly in diseases caused by rapidly mutating pathogens that attain drug resistance[19]. SARS-CoV-2 is a rapidly mutating virus, and determining leads with inhibitory activity on multiple targets involved in different phases of the disease-causing pathway is the better way to control and prevent the disease. Several authors have demonstrated that many natural compounds have an inhibitory effect on several target proteins. For example, the compounds such as stigmasterol, β-sitosterol and campesterol have an inhibitory effect on nine Russell's viper venom proteins[20]. Generally, plant extract contains a plethora of chemical molecules with diverse therapeutic activities at various levels. Those molecules' individual, synergistic and collective activity can effectively control the disease with multi-factorial causation like snake envenomation. Recently several authors have chosen a multi-target approach to screen natural compounds against SARS-CoV-2 [21]. Based on the foregoing reports, four target proteins, which have a key role in viral infection and multiplication, were selected in the current investigation. The targets spike protein and ACE2 have a key role in receptor recognition and entry into the host cell, and M^{pro} and RdRp have a critical role in viral replication [7, 14, 15].

Many authors have selected these proteins as targets for screening phytomolecules and identified lead molecules [22]. Out of 249 phytochemicals screened against the foregoing target proteins, 46 have an inhibitory effect on all the targets. These molecules showed binding energy ≤ -6 kcal/mol, which has been considered the minimum standard binding energy level for considering the molecule as active [16, 17]. When compared to the reference drug remdesivir several phytochemicals have a better docking score against all the selected targets here. Further, top-ranked lead molecules' protein-ligand interaction, physiochemical, and ADMET prediction indicated that the compounds bicornin, casuarictin and pedunculagin have good inhibitory

activity against all the selected targets. However, in ADMET profile studies, casuarictin and pedunculagin showed a negative rate of clearance from the body. Since bicornin showed good inhibitory activity on all the selected four target proteins of SARS-CoV-2 and also possessed good ADMET properties, it can be recommended as a lead for further *in-vitro* and *in-vivo* studies.

The protein-ligand interaction studies revealed that bicornin blocks M^{pro} by interacting with the catalytic dyads His41 and Cys145 and interacting with Lys 417 of the spike protein, which is a major factor for the virulence of SARS-CoV-2 **Fig. 1**. Also, the binding energies obtained from docking the compound bicornin with the target proteins *viz.*, spike protein, human ACE2, M^{pro} and RdRp were -9.2, -11.7,-9.5 and -8.4kcal/mol, respectively which was better than the reference drug remdesivir. Thus, bicornin, a naturally occurring ellagitannin can be recommended as a common lead against spike protein, human ACE2 receptor, $M^{pro,}$ and RdRp of SARS-CoV-2. Although bicornin showed three violations in Lipinski's rule of five, it has good drug-likeness properties and is non-toxic, non-mutagenic and non-carcinogenic. According to Veith and Sutherland *et al.* hypothesis, oral drugs for difficult targets like proteases and GPCR-pep families have a significantly higher molecular weight, thus bicornin may be a promising drug candidate against SARS-CoV-2 [23]. Molecular docking studies have also reported the inhibitory role of bicornin from *Syzygium aromaticum* against the main protease of SARS-CoV-2 [24]. The American Food and Drug Administration agency has approved that the intake of *S. aromaticum* ingredients is safe and WHO has recommended the daily intake as 2.5mg/kg body weight [25]. However, further *in-vitro* and *in-vivo* studies are required to establish bicornin as a potential drug candidate against SARS-CoV-2.



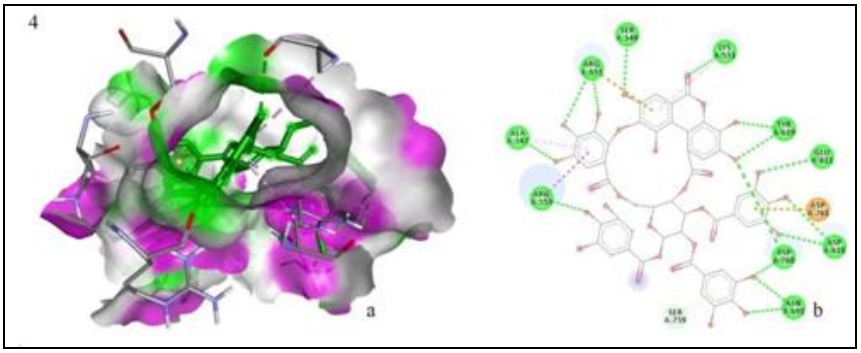


FIG. 1: DOCKING BETWEEN TARGET PROTEINS AND THE BEST LEAD MOLECULE BICORNIN (A) 3D VIEW AND (B) 2D VIEW. 1 (A & B) SPIKE PROTEIN AND BICORNIN, 2 (A & B) ACE2 AND BICORNIN, 3 (A & B) M^{PRO} AND BICORNIN, 4 (A & B) RDRP, AND BICORNIN

CONCLUSION: The *in-silico* analysis unequivocally demonstrated that the clove is a potential nutraceutical to check out SARS-CoV-2 infection, since it contains several phytomolecules having significant inhibitory effects on major targets with key roles during the infection and multiplication phases of the virus. Out of 249 phytochemicals screened, 46 of them have reasonably good inhibitory activity on all the selected targets when compared to the approved drug 'remdesivir'. Based on the overall results, bicornin can be recommended as the best lead for further study. However, as suggested by WHO and based on the present investigation, daily intake of clove can be recommended as the natural remedy against SARS- CoV-2.

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