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AN *IN-SILICO* INVESTIGATION ON ANTI-SARS-COV-2 ACTIVITY OF KABASURAKUDINEER AND THONTHASURAKUDINEER

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Abstract

Background: The novel severe acute respiratory syndrome coronavirus 2 (nSARS-CoV2), causing corona viral disease 2019, (COVID-19), is an important health concern to the entire globe. It started its journey from Wuhan, seafood market, spread to almost all the countries of the world, and has high contagious potential. Only precautionary measures such as social distancing, wearing masks, and regularly sanitizing the surfaces could prevent the disease, and no drugs or vaccines have been identified. In this situation, new drug invention is a challenging task, and the possibilities are repurposing strategies and exploration of phytochemical investigations, to identify the potent therapeutic molecules. At present, KabasuraKudineer and ThonthasuraKudineer are used by the Siddha medical practitioners as immunomodulators medications to treat viral infections and respiratory diseases. **Aim:**In this context, the current study aims to explore the antiviral potential of the chief phytoconstituents of these by *in-silico* approach.**Materials and methods**: In this study we docked the SARS-CoV-2 viral proteins with the main phytoconstituents of KabasuraKudineer and ThonthasuraKudineer, to assess their binding and inhibitory potential. For docking studies Grid-based Ligand and Docking with Energetics GLIDE module implemented inMaestro 12.5 version of Schrodinger LLC was used, and we also assessed the pharmacokinetic properties. **Key findings**: The docking results reveal most of the phytoconstituents of these formulations possess promising features of binding to COVID-19 proteins. Among these molecules, Luteolin, Vitexin, Chrysoeriol, Amentoflavone were found to possess higher binding affinities to COVID-19 proteins, by docking studies.

Keywords: SARS-CoV-2, KabasuraKudineer, ThondusaraKudineer, Molecular docking, COVID-19

INTRODUCTION

The Severe Acute Respiratory Syndrome corona virus-2 (SARS-CoV-2) originated from Wuhan, China, has become a worldwide pandemic [1]. On 30th January, World Health Organization (WHO) had declared it as novel Coronavirus disease 2019 (2019-nCoV, COVID-19) pandemic, with more than 35 million cases and 1 million deaths till date. COVID-19 is associated with fatigue, fever, muscle aches, dry cough, and shortness of breath, which leads to pneumonia or Acute Respiratory Distress Syndrome causing oxygen starvation and death. The incubation period of novel coronavirus is 1-14 days, and people with less immunity and comorbid conditions are more susceptible [2]. Coronavirus belongs to It is assumed that multiple mutations of a large family of Coronaviruses (CoV), altered the tissue tropism and leading to harboring in variety of hosts [3]. As on September 6th, 2020, the active cases are 14.11% (934427), 84.34% discharges (5586703), and 1.55% deaths (102685), in India [4]. Coronavirus belongs to Coronaviridae family having four genera (α, β, γ, δ), which is closely related to those found in bats. The virus is single-stranded RNA, positive-sense with 26-32 kb in length. The structural proteins are Spike (S), Envelope (E), Membrane (M), and Nucleocaspid (N) proteins [5, 6]. The identified proteases are main protease ($3CL^{pro}$), papain-like protease (PL^{pro}), RNA-Dependent-RNA-Polymerase (RdRp), and helicase [7]. The spike glycoprotein forms a trimer and receptor-binding with fusion and it also contains the antigenic determinants that neutralizes the antibodies formed by the host. The nonstructural proteins bind to single-stranded RNA and forms viral replication complex, which is a disulfide-linked homodimer, different from that of the previous SARS-CoV[8]. The treatment and control of COVID-19 depends mainly on extensive research and availability of antiviral drugs, which are very few and prevents only replication indirectly. It is a time consuming, costly search for new molecules and the adverse effect profiles could not be revealed within a short span of time. The wide spectrum of siddha formulations in Indian system of medicine, has been in use for several years for viral infections. The influenza outbreak was successfully controlled by KabasuraKudineer, and in dengue outbreaks Nilavembukudineer was proved to reduce morbidity and mortality [9]. The Ayurveda, Siddha, Unani and Homeopathy (AYUSH) department, under Ministry of AYUSH India, recommends KSKC for treating different

fevers [10, 11]. The KabasuraKudineer is a useful formulation for fever with flu-like symptoms, contains potential phytoconstituents to combat phlegmatic fevers. In addition to this Thonthasurakudineer is also used for treating viral infections by Siddha medical practitioners. With sound Indian medical system, scientific exploration of the potential of these formulations could be useful to encounter the COVID-19 with lesser adverse effects. With this background, the present study is designed to evaluate the potential of phytoconstituents of KabasuraKudineer against SARS-CoV-2 spike protein, non-structural protein (mention the name of nsp), and hydrolases.

Materials and Methods

Platform for molecular modelling

The computational investigations were performed using the Schrodinger software (Maestro 12.5, Schrodinger 2020-3)

Ligand preparation

Atotal of thirty-seven major phytoconstituents reported from the KabasuraKudineer and ThonthasaraKudineer were selected to perform the molecular docking studies to screen and identify the potential of these ligands for their antiviral activity, specifically for COVID-19. PubChem database (https:// www.ncbi.nlm.nih.gov) was used to extract out the Structure Data Format (SDF) structures of the selected molecules. The phytochemicals generated were prepared using the Ligprep interface in Schrodinger with an Optimized Potential for Liquid Simulation (OPLS3e), at pH 7±1 using Epik. Desalt and generate tautomers were also selected on the ligprep interface and the stereoisomer computation was left at retain specific chiralities (vary other chiral centers) and to generate at most 32 per ligand. Energy minimization was performed using OPLS3e force field by using the standard energy function of molecular mechanics and RMSD cut off 0.01 Å to generate the lower-energy ligand conformer [12, 13, 14].

Preparation of protein structures

To combat the current situation of COVID-19 protein structure of COVID-19 main protease with co-crystallized structure (PDB IDs: 5R80, 5R81, 5R82, having resolution of < 2 Å, R-

Value Free <0.30, R-Value Work <0.25), CoV spike protein, corona spike glycoprotein trimer (PDB ID: 3JCL) and 2J97 Human coronavirus non-structural protein (nsp9) were obtained from the Protein Data Bank (http://www.rcsb.org) with good resolutions. Protein structures were prepared using protein preparation wizard in Maestro panel [15, 16, 17, 18, 19]. During preparation of protein bond orders were assigned and hydrogen atoms were added as well. Water molecules were removed within 3 Å of het groups. Finally, OPLS3e force field was applied to minimize the structure of protein (Schrodinger, LLC, NY, USA, 2020).

Receptor grid generation and Sitemap generation for identification of potential binding sites

Receptor grid was generated using receptor grid generation in the Glide application (GLIDE, version) of Maestro. Once the receptor grid was generated, ligands were docked to the protein using GLIDE docking protocol. The active site is generated with the computing cubic box of 10 Å x 10 Å x 10 Å, with a radius of 20 Å around the crystal structure [20, 21, 22]. In 3JCL and 2J97, having no ligands, active site was identified using sitemap tool in Schrodinger Maestro 2020-3

[23].

Molecular docking – Standard Precision mode

Molecular docking is a structure-based drug design approach to identify the essential amino acid interactions between selected protein and generated ligands with low energy conformation. Minimum interaction of the ligands characterized by the scoring function which used to foretell the binding affinity with the receptor. GLIDE Standard Precision (SP), docking protocol was applied without smearing any constraint. Flexible docking with SP protocol was performed to predict the binding affinity and ligand efficiency as inhibitor of COVID-19 target. Concluding energy assessment was done with the dock score. Visualization (Visualization tool has a name, pls mention the same here) of docked ligands was done by Maestro interface (Schrodinger Suite, LLC, NY)[24].

Docking in XP mode

Selected Compounds (mention the selection criteria here) were run in standard precision mode and based on the dock score, the top ligands with minimum energy (mention the cut-off value, also revise the sentence) were taken for extra precision (XP) mode, using the Maestro interface (Maestro 12.5 version, Schrodinger). In the XP mode, GScore, Dock Score, hydrophobic grid potential, hydrophobic enclosure reward, reward for hydrophobically packed H-bond, reward for hydrophilically packed correlated Hbonds, ChemScore H-bond pair term, electrostatic rewards, reward for pi-cation interactions, reward for Cl/Br in hydrophobic environment, reward for ligands with low molecular weight, reward for ligand atoms in a favorable electrostatic environment of the protein, and polar atom burial and desolvation penalties for intra-ligand contacts were calculated [25].

ADMET Properties

ADMET properties were calculated using Qikprop v 3.5 tool of Schrodinger [26, 27]. It predicts both physicochemically significant descriptors and pharmacokinetic relevant properties. Qikprop provides ranges for comparing a particular molecule's properties with those of 95% of drugs. Qikprop evaluates the acceptability of analogs based on Lipinski's rule of five, which is essential to ensure the drug-likeness pharmacokinetic profile using rational drug design. All the analogs were neutralized before used by Qikprop.

RESULTS

The Siddha formulation KabasuraKudineer and ThonthasuraKudineercontains twenty-two medicinal plants. For in silico docking studies, the thirty-seven reported phytoconstituents were taken as potential ligands (Table 1). The molecular docking studies were performed for the 37 ligands on the binding pockets of the COVID-19 targets (3JCL, 2J97, 5R80, 5R81, and5R82). All the ligands were docked against the targets and ranked based on their dock score (Table 2).

Table 1: Phytoconstituents from Kaba Sura KudineerChoornam and ThondusaraKudineer

	Botanical name	Tamil name	Major Phytoconstituents
1	*Zingiber officinale*	Chukku	Zingiberne, β-sesquiphellandrene, β-bisabolene, Geranial
2	*Piper longum*	Thippili	Piperine, Piperlongumine
3	*Syzygiumaromaticum*	Kirambu	Eugenol
4	*Tragia involucrate*	Sirukanchori	Costunolide, β-caryophyllene
5	*Anacyclus pyrethrum*	Akkrrakkaram	Pyrethrin, γ-sitosterol
6	*Hydrophilia auriculata*	Mulliver	Apigenin, lupeol
7	*Terminalia chebula*	Kadukkaithol	Chebulic acid, Chebulagic acid, Gallic acid
8	*Adathodavasica / Justicia adathoda*	Adathodai	Vasicine
9	*Coleus amboinicus / Plecanthrusamboinicus*	Karpuravalli	Myrtenol, Carvacrol, Crisimaritin, Chrysoeriol,
10	*Saussurealappa*	Kostam	Costunolide
11	*Tinospora cordifolia*	Seendhil	Cordiofolioside A
12	*Clerodendrumserratum*	Siruthekku	Carvacol, Bharangin, Scutallerien
13	*Andrographis paniculata*	Nilavembu	Andrographolide, Andrograpanin, 5-hydroxy-7,8-dimethoxyflavone
14	*Sida acuta*	Vattathiruppi	Carvacol, Magnoflorine, Cycleanine
15	*Cyperus rotundus*	Korai kizhangu	Amentoflavone, Cyperene, β-selinene
16	*Elettaria cardamomum*	Elam	Eugenol
17	*Solanum xanthocarpum*	Kandangathri	Stigmasterol
18	*Trichosanthescurcumerina*	Peipudal	Cucurbitacin B
19	*Tephrosia purpuria*	Mutkavelai	Stigmasterol
20	*Mollugocerviana*	Parpadgam	Vitexin
21	*Vitis vinifera*	Thrakshai	Vitexin
22	*Costusspeciosus*	Ven Kottam	Luteolin, Costunolide

Sitemap was identified for 3JCL and the phytoconstituents were docked with the identified best site. Site map1 was selected for the study (Table 3). The docking results ligands with 3JCL in SP mode reveal Crisimaritin has lowest dock score of -7.072 and Scutallerein showed a docking score of -6.69 when compared to other ligands. Remaining ligands showed docking score of -6.58 to -2.11. The docking score may be accredited to the hydrogen bonding interactions with at the Ser 309, Gln 314 and Cys 580 at the active site. Out of 37 ligands, 10 were taken for further studies, docking in XP mode, and vitexin exhibited lowest docking score of -8.8 and followed by luteolin with -8.19 (Fig 1, Fig 2). The inhibition constant Ki was found to be lowest for luteolin -39.631, whereas it was -23.417 for Crisimaritin. Amentoflavone exhibited lowest binding energy Ki of -52.734. In the XP docking mode only luteolin exhibited activity profile. All other ligands revealed GLIDE scores of -7.96 to -3.72. The docking score may be of luteolin in XP mode may be accredited to the hydrogen bonding interactions with ILE666 at the active site. The docking score may be of vitexin in XP mode may be accredited to the hydrogen bonding interactions with Gln 314, Cys 633, and Asn 668 at the active site. Sitemap was identified for 2J97 and the phytoconstituents were docked with the identified best site (Table 4).

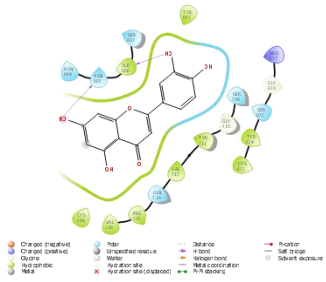

Figure 1: Docking interactions of Luteolin with 3JCL

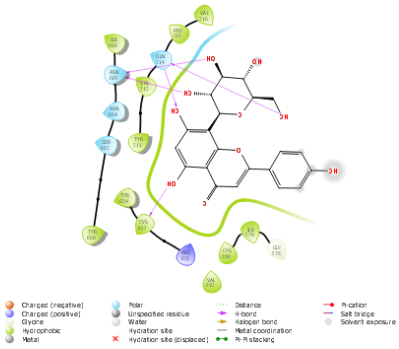

Figure 2: Docking interactions of Vitexin with 3JCL

SNO	LIGAND	3JCL			2J97			5R80			5R81		
		Dscore	Gscore	eModel	Dscore	Gscore	eModel	Dscore	Gscore	eModel	Dscore	Gscore	eModel	Dscore
	Amentoflavone	-5.925	-6.049	-52.734	-4.509	-4.633	-51.165	-3.701	-3.825	-48.847	-5.834	-5.958	-63.318	-4.113
	Andrographanin	-4.218	-4.218	-23.738	-3.587	-3.587	-26.986	-3.211	-3.211	-31.12	-4.192	-4.192	-19.267	-3.402
	Andrographolide	-3.16	-3.161	-16.292	-3.34	-3.34	-27.334	-3.394	-3.394	-29.996	-3.763	-3.763	-30.567	-4.065
	Apigenin	-5.72	-5.76	-36.081	-4.491	-4.531	-32.79	-5.519	-5.559	-38.666	**-6.41**	**-6.45**	**-52.389**	-5.527
	Betabisabolene	-3.665	-3.665	-20.511	-3.702	-3.702	-17.089	-4.822	-4.822	-30.97	-5.207	-5.207	-32.724	-4.98
	Betacaryophyllene	-3.985	-3.985	-17.523	-4.698	-4.698	-19.101	-3.385	-3.385	-13.813	-4.034	-4.034	-17.75	-4.264
	Betaselinene	-4.291	-4.291	-17.468	-4.486	-4.486	-18.61	-4.127	-4.127	-20.51	-4.847	-4.847	-21.027	-3.985
	Betasesquiphellandrene	-3.22	-3.22	-15.317	-3.215	-3.215	-17.105	-4.601	-4.601	-29.631	-4.525	-4.525	-31.29	-3.949
	Betulin	-2.514	-2.514	-12.635	-3.043	-3.043	-26.143	0	0	0	-3.715	-3.715	-15.998	-1.963
	Bharangin	-4.831	-4.88	-30.526	-3.562	-3.611	-26.143	-4.273	-4.322	-32.489	-5.441	-5.49	-37.546	-3.735
	Carvacrol	-5.599	-5.599	-29.417	**-5.263**	**-5.263**	**-20.169**	**-6.595**	**-6.595**	**-35.281**	**-7.109**	**-7.109**	**-37.483**	-5.627
	Chebulagic acid	-4.514	-5.115	-48.946	0	0	0	0	0	0	0	0	0	0
	Chebulic acid	-3.729	-3.77	-30.054	-3.371	-3.413	-25.596	-3.373	-3.414	-32.143	-3.934	-3.976	-33.838	-4
	Chrysoeriol	**-6.485**	**-6.524**	**-39.877**	-4.281	-4.321	-33.824	-5.825	-5.865	-40.741	**-6.557**	**-6.597**	**-53.71**	-4.744
	Costunolide	-4.051	-4.051	-20.292	-4.488	-4.488	-20.206	-5.399	-5.399	-27.962	**-6.014**	**-6.014**	**-29.612**	-4.58
	Crisamarit in	**-7.072**	**-7.072**	**-23.417**	-4.117	-4.117	-20.206	-5.252	-5.252	-43.558	**-7.043**	**-7.043**	**-60.798**	-5.169
	Cucurbitacin B	-3.753	-3.753	-38.835	-3.846	-3.846	-37.575	-1.741	-1.741	-25.327	-2.955	-2.955	-32.243	-2.993
	Cycleanine	-2.093	-2.145	-22.113	0	0	0	0	0	0	0	0	0	0
	Cyperene	-4.353	-4.353	-14.726	-4.353	-4.353	-14.726	-5.854	-5.854	-29.297	-4.456	-4.456	-19.01	0

Dimethoxy flavone	**-6.493**	**-6.495**	**-42.192**	-4.371	-4.372	-31.263	-5.751	-5.753	-39.628	-5.746	-5.747	-47.979	-5.785
Eugenol	-4.205	-4.205	-26.238	-3.896	-3.896	-22.494	-5.313	-5.313	-36.97	-5.583	-5.583	-38.473	-4.114
Gallic acid	-5.83	-5.837	-34.63	-4.281	-4.288	-23.669	-5.911	-5.918	-36.421	**-6.68**	**-6.687**	**-41.633**	**-6.05**
Gammasit osterol	-2.97	-2.97	-17.622	-3.05	-3.05	-26.058	-2.221	-2.221	-7.821	-4.044	-4.044	-24.211	0
Geranial	-2.884	-2.884	-20.511	-2.728	-2.728	-17.657	-4.122	-4.122	-28.124	0	0	0	-3.486
Lupeol	-2.111	-2.111	-12.635	-2.226	-2.226	-20.415	0	0	0	0	0	0	-2.361
Luteolin	**-6.58**	**-6.62**	**-39.631**	-4.93	-4.97	-37.336	-5.51	-5.55	-39.041	**-6.362**	**-6.402**	**-57.512**	-5.445
Magnoflori ne	-4.72	-5.151	-38.018	-3.357	-3.788	-27.338	-4.533	-5.124	-18.86	-4.763	-5.195	-27.788	-3.295
Myrtenol	-4.833	-4.833	-21.576	-4.683	-4.683	-21.39	**-6.76**	**-6.76**	**-32.026**	-5.41	-5.41	-24.103	-3.56
Piperine	-5.678	-5.678	-35.825	-4.109	-4.109	-29.825	-5.688	-5.688	-46.977	**-6.527**	**-6.527**	**-58.538**	-5.253
Piperlongu minine	-5.85	-5.85	-35.14	-3.798	-3.798	-28.905	-4.442	-4.442	-37.489	-5.631	-5.631	-46.379	-4.433
Pyrethrin	-3.862	-4.273	-26.156	-3.002	-3.412	-27.077	-5.332	-5.743	-41.671	-5.689	-6.1	-50.805	-3.798
Scutallerei n	**-6.69**	**-6.738**	**-41.915**	**-5.013**	**-5.061**	**-38.741**	-5.452	-5.5	-39.958	**-6.591**	**-6.639**	**-59.273**	-5.571
Stigmaster ol	-3.497	-3.497	-18.214	-3.002	-3.412	-27.077	-3.003	-3.003	-9.338	0	0	0	0
Tinosporin one	-5.182	-5.482	-37.44	-3.476	3.776	-31.496	-5.205	-5.205	-44.68	-5.807	-6.107	-49.715	-4.732
Vasicine	**-6.26**	**-6.271**	**-30.269**	**-5.032**	**-5.044**	**-24.217**	**-7.048**	**-7.059**	**-39.791**	**-8.89**	**-8.901**	**-54.109**	**-7.073**
Vitexin	-5.814	-5.849	-47.935	-4.385	-4.421	-47.531	-4.785	-4.785	-45.257	-5.387	-5.423	-48.097	-5.033
Zingiberen e	-4.25	-3.88	-34.66	-3.418	-3.418	-17.23	-4.31	-4.31	-27.161	-4.938	-4.938	-31.501	-4.168

Table 2: Comparative docking study results of COVID-19 enzymes

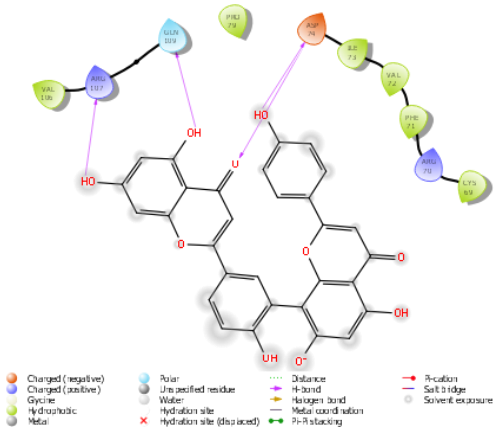

Figure 3: Docking interactions of Amentoflavone with 2J97

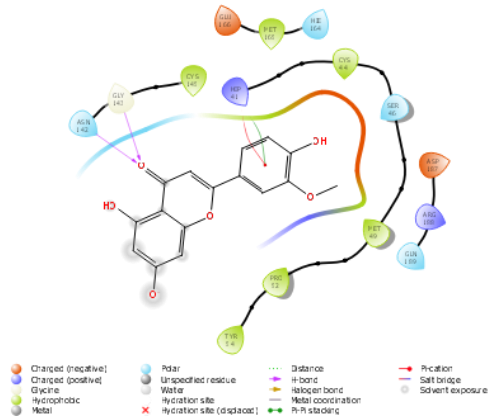

Figure 4: Docking interactions of Chrysoeriol with 5R80

Table 3: Site map identification for 3JCL

3JCL	HBD	HBA	Phil	Phob	Surf
Sitemap 1	8701.847	14147.6	22539.5	2434.896	33813.5
Sitemap 2	7108.991	11635.4	18729.3	1977.601	27488.2
Sitemap 3	5573.060	8648.993	14013.1	1700.946	20812.6
Sitemap 5	4344.364	6047.387	10307.9	1196.843	14977.8
Sitemap 4	2517.537	3046.419	5700.850	799.472	8933.637

Table 4: Site map identification for 2J97

2J97	HBD	HBA	Phil	Phob	Surf
Sitemap 1	179.191	120.166	303.797	11.530	454.911

The docking results ligands with 2J97 in SP mode reveal Carvacrol has lowest dock score of -5.263 and Scutallerein showed a docking score of –5.061 when compared to other ligands. Remaining ligands showed docking score of -5.032 to 3.776. The docking score may be accredited to the hydrogen bonding interactions with at the Val 72, Asp 74, and Gln 109 at the active site (Fig 3). Then, top 10 ligands were taken for further studies, docking in XP mode, only Amentoflavone exhibited lowest docking score of -6.69. The inhibition constant Ki was found to be lowest for Amentoflavone as -51.165, whereas it was -47.531 for Vitexin. The docking results ligands with 5R80 in SP mode reveal Vasicine has lowest dock score of -7.048 and Myrtenol showed a docking score of

-6.76 when compared to other ligands. Remaining ligands showed docking score of -6.595 to -1.741. Even though both the ligands had good contact with the active site, no bonding interactions were revealed. In further docking in XP mode, and Chrysoeriol exhibited lowest docking score of -6.02 and followed by luteolin with -5.93. The inhibition constant Ki was found to be lowest for Amentoflavone -48.847, whereas it was -46.977 for Pyrethrin. The docking score may be of Chrysoeriol in XP mode may be accredited to the hydrogen bonding interactions with Asn 142, Gly 143, and Pi-cation interactions with Histidine Protonated 41 at the active site (Fig 4). The docking score may be of Luteolin in XP mode had no interactions, in spite of good contact with the active site. The docking results ligands with 5R81 in SP mode reveal Vasicine has lowest dock score of -8.89 and Carvacrol showed a docking score of -7.109 when compared to other ligands. Remaining ligands showed docking score of -7.043 to -2.955. The docking score may be accredited to the hydrogen bonding interactions with at the His 41 and Glu 166 at the active site. Out of 37 ligands, 10 were taken for further studies, docking in XP mode, and Luteolin exhibited lowest docking score of -7.82 and followed by Scutallerein with -7.37. The inhibition constant Ki was found to be lowest for Amentoflavone -63.318 followed by Crisimaritin with -60.798. The docking score may be of luteolin in XP mode may be accredited to the hydrogen bonding interactions with Thr 190 and His 41 Pi-Pi stacking at the active site (Figure 5, Figure 6).

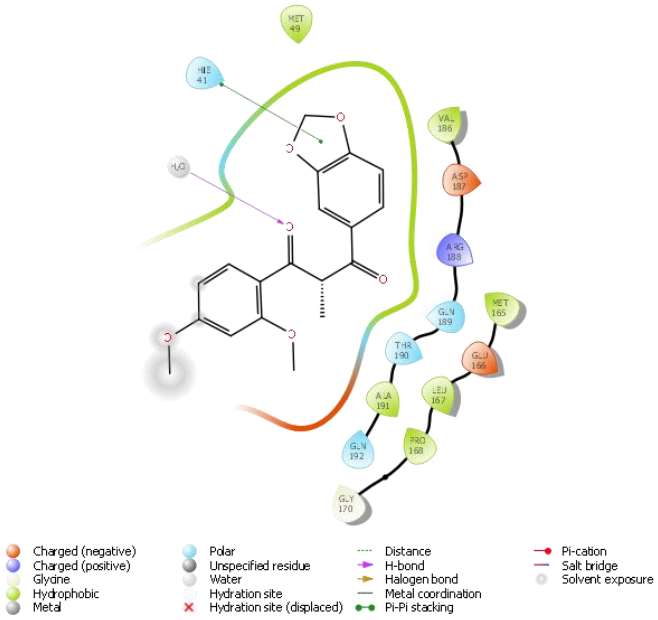

Fig 5: Docking interactions of Luteolin

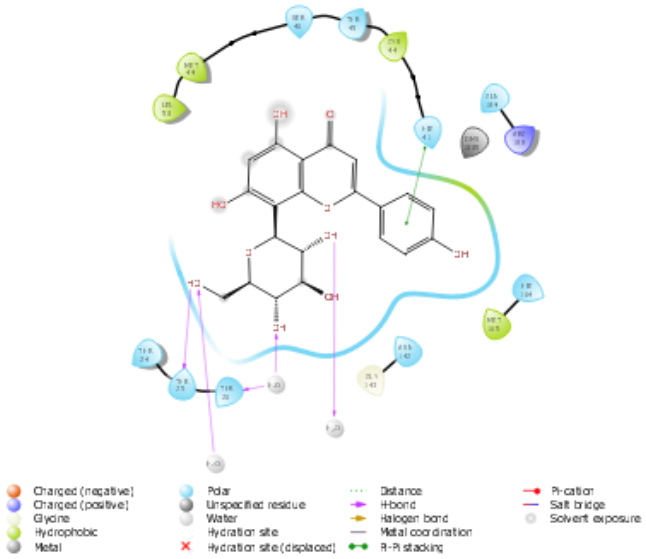

Fig 6: 5R81 and Vitexin with 5R82

The docking results ligands with 5R82 in SP mode reveal Vasicine has lowest dock score of -7.073 and Gallic acid showed a docking score of -6.05 when compared to other ligands. Remaining ligands showed docking score of -5.571 to -1.963. The docking score may be accredited to the hydrogen bonding interactions with at the Gln 189, and His 41 Pi-cation with Pi-Pi stacking at the active site. Top 10 ligands were taken for further studies, docking in XP mode, and vitexin exhibited lowest docking score of -8.67 and followed by luteolin with -6.92. The inhibition constant Ki was found to be lowest for Luteolin -46.046, whereas it was -45.711 for Amentoflavone. The docking score of luteolin in XP mode may be accredited to the hydrogen bonding interactions with Thr 25 and His 41 Pi-stacking at the active site. The ADMET descriptors contribute to the development of successful ligands. So, the pharmacokinetic properties should be added in addition to the virtual screening. In the current study 51 descriptors and pharmaceutically pertinent properties were investigated using Qikprop (Qikprop, version, Schrodinger) in comparison with those of 95% of known drugs. Most of the compounds have followed the Lipinski's rules, molecular weight <500 Da, octanol/water partition coefficient (QlogPo/w) <5, hydrogen bond acceptor <10 and donor <5. The % oral absorption was found in the range of 80-100% in most of the derivatives. Out of the 37 ligands, Betabisabolene, Betacaryophyllene, Betaselinene, Betasesquiphellandrene, and Cyperpene has lesser drug likeness properties. The ligands having the probability of false positives in High Throughput Screening (HTS) assays were identified in Chebulic acid, Chrysoeriol, and Pyrethrin. Blockade of HERG

K+ channels were predicted in Pyrethrin, Crisimaritin, Vitexin, Piperlongumine, Scutallerein, and Chebulic acid.

DISCUSSION

The COVID-19 pandemic has shuddered the entire globe by human-human transmission, with significant morbidity and mortality, with all the healthcare and research professionals are in the search of remedies surmounting the viral episode. Many new medical strategies with modern medicines like Remdesvir, Favipravir, and Itolizumab have emerged, which addresses the control of viral replication process, and do not have an effect on the host immunity [28, 29]. All the herbal medications suggested by AYUSH, such as KabasuraKudineer and ThonthasuraKudineer, focusses both on antiviral property along with boosting the host immunity. Apart from this these medications could be a valuable add-on therapies for allopathic medications too. The immune modulating effects of KSKC has been already reported, which reduces the TNF production, signaling pathway. The phlegm will be expelled by the formulation, which improves the respiratory health. It has also been mentioned that the metabolites of the phytoconstituents also contribute to antitussive effects. With this background, the *in-silico* approach was taken to investigate the inhibitory activities of phytoconstituents from KSKC and TSK, to address the SARS-CoV-2 virus, which, the world is struggling to recover from its deadly health and economic damages. For this study, five COVID-19 proteins, 3JCL, 2J97, 5R80, 5R81, and 5R82 were taken from the protein data bank. The major phytoconstituents of KSKC and TSK were docked with such proteins and studied. The deadly pneumonia caused by coronaviruses is due to transmembrane spike protein S, which is a transmembrane protein carrying fusion functions and carries receptor-binding domains. The fusion proteins play an important role in entry of the virus and one such protein was taken for the current study, 3JCL [30]. Previous reports on this protein reported the potential of KSKC and Zingiberene, Andrographolide, Vasicine, Cordiofolioside B, Apigenin were found to having docking interactions, but the active site was not mentioned. In this study, the active site was identified, then the phytoconstituents were docked, and the interactions were studied. In our study, Luteolin and Vitexin had good docking interactions.Luteolin, structurally related to quercetin, has been recently investigated for its antiviral property through inhibiting the entry of SARS-CoV-2, which has EC50 of 10.6μM, LD50 of 456mg/kg, CC50 of

0.155mM, and selective index of 240 [31]. As an ingredient in KSKC it could be a valuable antiviral molecule to inhibit the viral entry in COVID-19. Vitexin has been recently explored to interact with human entry receptor ACE2, which is an important entry regulatory for SARS-CoV-2. Vitexin is an apigenin flavone glycoside has been found to have good docking score with stabilization of viral spike proteins [32]. The 2J97 protein is a non-structural protein 9 (Nsp9), which regulates viral replication through binding through disulfide-bonds. The nutgrass, *Cyperus rotundus*, has been widely used in Ayurveda, for several ailments. The important constituent amentoflavone has been proven for its antiviral property against influenza A, influenza B, and anti-herpes properties [33]. In the current study, Amentoflavone has shown good docking interactions and could successfully inhibit viral replication by binding in the active site of viral replication proteins. Many proteases are involved in processing of the SARS-CoV-2 viral proteins, and targeting such proteases could be useful targets to find successful drug candidates. Many investigations are in process for main proteases such as $3CL^{pro}$, and in the current study, three proteases are selected for docking analysis. In this investigation, we found Chrysoeriol, Luteolin, and Vitexin are the best drug candidates, through good docking score with these viral proteases. Chrysoeriol is an important flavonoid, found in many herbs, used in different ailments. Recently the bronchodilatory effect of chrysoeriol was proved [34], and its antiviral property in SARS-CoV-2 was explored in the present study. Previous reports suggest many allopathic medications have such good interactions with these proteases, and they are lopinavir, asuprenavir, indinavir, ritonavir, remdesvir, methisazone, and paritaprevir[35, 36]. It is well known that all antiviral drugs allopathic medications possess numerous adverse effect profiles, and in such cases, herbal medications such as KabasuraKudineer and ThonthasuraKudineer, having a long history of medical practice, could be useful in the current pandemic scenario.

CONCLUSION

The SARS-CoV-2 viral spike proteins and proteases are important targets of the viral entry into host cells, and identification of molecules to inhibit it and prevent entry could be useful drug candidates to combat the viral infection. Herbal medications could be useful formulations with less adverse effect profile, when compared to allopathic medications. The most widely used antiviral herbal medications are subjected to in-

silico investigations, and our findings prove good binding interactions of most ingredients, and Luteolin, Vitexin, Chrysoeriol, and Amentoflavone have higher binding affinities and interactions with the spike proteins and proteases of COVID-19. Further in the ADME analysis, it revealed most of the phytoconstituents has good oral bioavailability and drug-likeness properties. Based on these findings, we propose that KabasuraKudineerChoornam and ThondusaraKudineer may provide better drug therapy by interacting with the key viral proteins of SARS-CoV2 novel corona virus COVID-19 infections.

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