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ELUCIDATION OF PLAUSIBLE MECHANISMS OF KABASURAKUDINEER FOR COVID-19 BY MOLECULAR DOCKING AGAINST SARS COV-2

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Abstract:

The COVID-19 virus main protease (M^{pro})and papain like protease (PL^{pro}) which are the key CoV enzyme, in mediating viral replication and transcription, making it an attractive drug target for this virus. Also, the spike protein also the potential targets for the SARS Cov-2 from which viral entry inhibitors can be designed. The Kabasurakudineer is known to possess the immune modulating and some of the ingredients has anti-viral activity. The docking of phytoconstituents in Kabasurakudineer to the above mentioned three targets shows that the phytoconstituents binds near the hotspot-31 and hotspot-353 residues and key residues of the receptor binding domain of the spike protein. It also inhibits virus main protease (M^{pro}) and papain like protease (PL^{pro}) suggested by high binding affinity.

Keywords: *Kabasuraku dineer, COVID-19, SARS Cov-2,in-silico, molecular docking, Antiviral*

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INTRODUCTION:

In this world of fast and easy travel, emerging viruses possess major threat to world health. Coronaviruses are a notable example. Particularly virulent forms have emerged from their natural animal hosts and pose a threat to humans. In 2003, the SARS virus emerged in China from bat populations, moving to civets and finally to humans. Ten years later, the MERS virus also emerged from bats, transferring in the Middle East to dromedary camels and then to humans. Recently, another coronavirus SARS Cov-2 has emerged in China by way of animals in a live market.

COVID-19 is a virus belonging to the coronavirus family, which includes a large number of viruses that can cause a wide variety of diseases in humans. The SARS-CoV-2 virus that caused the current COVID-19 pandemic, which infected over 2,000,000 people causing over 150,000 deaths. The main clinical manifestation is an influenza-like illness associated with respiratory tract infection that can lead to severe hypoxemic pneumonia.

Coronaviruses contain a genome composed of a 30 kb—one of the largest of all RNA viruses. This genome acts just like a messenger RNA when it infects a cell, it directs the synthesis of two long polyproteins that include the machinery that the virus needs to replicate new viruses. These proteins include a replication/transcription complex that makes more RNA, several structural proteins that construct new virions, and two proteases. The proteases play essential roles in cutting the polyproteins into all of these functional pieces. Sequence and structural studies show that the proteases of these viruses can be very different, so drugs designed to fight one may not be effective against others. So, there is a need for broad-spectrum inhibitor that target against progenitor bat coronavirus.

A key host cellular protein required for the virus entry is angiotensin-converting enzyme 2 (ACE2) which express in many tissues including alveolar epithelial type II cells in lungs, oral mucosa and intestine, heart, kidney, endothelium and skin. ACE2-expressing cells can act as home cells and are prone to SARS-CoV-2 infection as ACE2 receptor facilitates cellular viral entry and replication. Patients with hypertension and diabetes mellitus may be at higher risk of SARS-CoV-2 infection, because they are treated with ACE inhibitors (ACEIs) or angiotensin II type-I receptor blockers (ARBs), which have been suggested to increase ACE2 expression[1].

Main Protease and Papain-like protease

Two types of cysteine proteases act on polyproteins (PPs) to release the NSPs (Non-Structural Proteins). The C-terminal end of these polyproteins is cleaved by chymotrypsin-like cysteine protease (main protease - M^{pro}) or 3C-like protease ($3CL^{pro}$) and the N-terminal end is processed by the papain-like protease (PL^{pro}). The M^{pro} ofSARS-CoV-2 is cleaving at 11 sites in the polyproteins. It is a dimer of two identical subunits that together form two active sites. The protein fold is similar to serine proteases like trypsin, but a cysteine amino acid and a nearby histidine perform the protein-cutting reaction and an extra domain stabilizes the dimer. This structure has a peptide-like inhibitor bound in the active site. PL^{pro}has single subunit and also uses a cysteine in the reaction. The PL^{pro} makes three specific cuts in the SARS polyproteins in the N-terminal region to generate three NSPs (NSP 1, 2, and 3). It makes three specific cuts in the SARS polyproteins, and also clips several proteins in the infected cell, and also causes deubiquitination which interferes with production of interferons which damage our defences against the virus. These cleavages results in release of 16 NSPs [2].Isotretinoin is a Potential papain like protease (PLpro) inhibitors which is a protein encoded by SARS-CoV-2 genes and considered one of the proteins that should be targeted in COVID-19 treatment by performing target-based virtual ligand screening.[3]

The rational strategies for destruction of SARS Cov-2are targeting spike protein and two the proteases which are main protease and papain like protease. The compounds bind with good and varying affinities to the target, so it may show the synergistic and potentiation action for each other.

Treatment regimen may be antiviral or immune booster. Currently, anti-viral treatments include Chloroquine (CQ) and Hydroxychloroquine (HCQ), Lopinavir/Ritonavir (Lop/r), Remdesivir, Favipavir, treatment of bacterial co-infection with Azithromycin, or modifying the inflammatory response of the host with Tocilizumab. But there may be still better ones remains unidentified.

Since discovery of novel drugs are time consuming even if designed and developed using CADD because many safety trials and regulatory approvals are needed. As it is pandemic, there is no time to develop novels. So drug repurposing is alternate source. Also, it should be readily available to everyone for treatment. Kabasurakudineer is also given to boost immunity and some ingredient in Kabasurakudineer also act as antiviral agents. From this background we try to work on molecular docking on COVID-19 targets.

Andrographolide, neo-andrographolide and 14-deoxy-11,12-didehydroandrographolide present in *Andrographis paniculata* has anti-viral and

immunostimulant activity[4]. Both costunolide and dehydrocostus lactone present in root of *Saussurealappa* supress HBsAg gene expression [5]. Gingerol is reported to possess anti-inflammatory, antioxidant and anti-microbial activity and Phyto some of gingerol reduces respiratory tract infection[6] which is the main pathogenesis of COVID-19. The eudesmane and patchoulane-type sesquiterpenoids in rhizomes of *Cyperusrotundus*, demonstrates anti-viral against HBV activity [7]. *Cissampelospareira*has anti-viral activity against all four strains of dengue viruses in and possess no toxic effects [8].

The above-mentioned literature survey confirms that the some of the phytocompounds present in Kabasurakudineer formulation has anti-viral activity and this gave us lead for anti-viral property for SARS CoV-2 proteins by molecular docking.

MATERIALS AND METHODS:

Target selection

Three targets6M0J - Crystal structure of SARS-CoV-2 spike receptor-binding domain bound with ACE2, 7BQY - **T**he crystal structure main protease SARS CoV-2**.** 6W9C - The crystal structure of papain-like protease of SARS CoV-2 are selected and downloaded from the RCSB Protein Data Bank, database for 3D structures of large biological molecules, including proteins and nucleic acids. Downloaded protein structures were prepared in Maestro. Fig1 shows main protease and papain-like protease downloaded from www.rcsb.org.

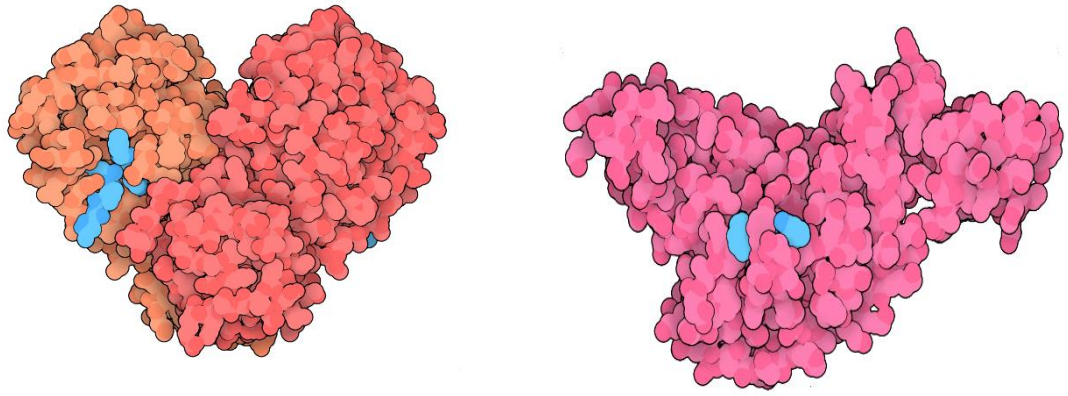

Fig 1 SARS main protease (Left) and papain-like protease (Right), with inhibitor (www.rcsb.org)

Ligand preparation

Thirty important phytoconstituents present in kabasurakudineer were selected and structures of all phytoconstituents were downloaded from PubChem database. All structures were subjected to energy minimization by Ligprep. This gave energetically stable conformations for the structures. The important markers of kabasurakudineer is given in table 1 and structures are represented in Fig2& Fig3.

Table 1 important markers of kabasurakudineer

Name of the plant	Marker	Known Properties
Andrographis paniculata	Andrographolide	Anti-influenza
*Saussureacostus*or*Saussurealappa*	Costunolide	Anti-inflammatory
Cyperusrotundus	Mustakone Isocyperol Acyperone kaempferol luteolin Quercetin patchoulenone	fevers, digestive system disorders, dysmenorrhea
Zingiber officinale	Gingerol Linalool oxide	Antibacterial
Piper longum	Piperine	Anti-inflammatory
Syzygiumaromaticum	Eugenol	Antimicrobial
Tragia involucrate		Given in fever when the extremities are cold; also, for pain in arms and legs
Anacyclus pyrethrum	Gallic acid Daidzein Icariin	Antimicrobial
Hygrophilliaauriculata	Lupeol (LP) Stigmasterol (ST)	Anti-inflammatory

Terminalia chebula	Ellagic acid (EA)	Antimicrobial
Justicia adhatodaorAdathodavasika	Vasicine Vasicinone Embelin	Antimicrobial
Coleus amboinicus	Carvacrol Thymol α-terpineol	Essential oils used as mosquito repellant
Tinosporacordifolia	Tinosporaside	
Clerodendronserratum	Lupeol	
Cissampelospareira	Warifteine Berberine Hayatininmethochloride	Antimalarial Antiviral especially against Dengue virus

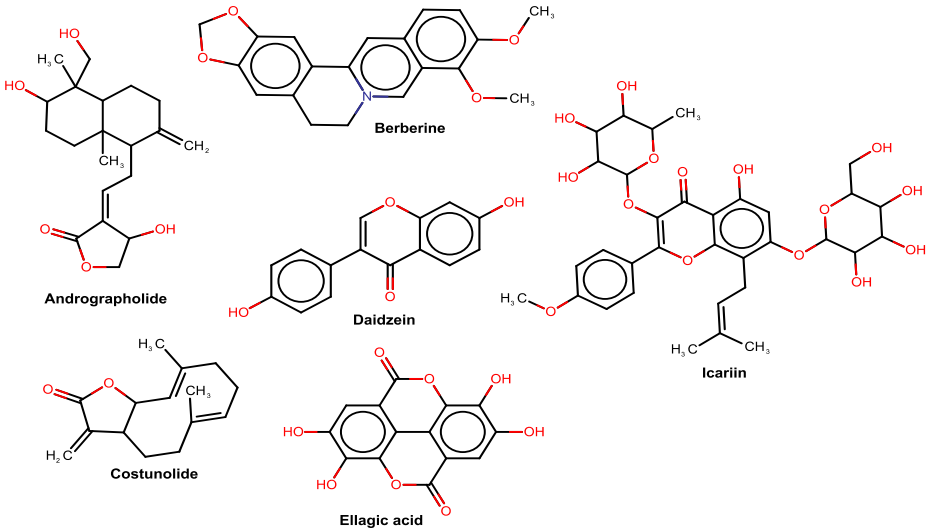

Fig2 Structures of some important Phytoconstituents from KabasuraKudineer

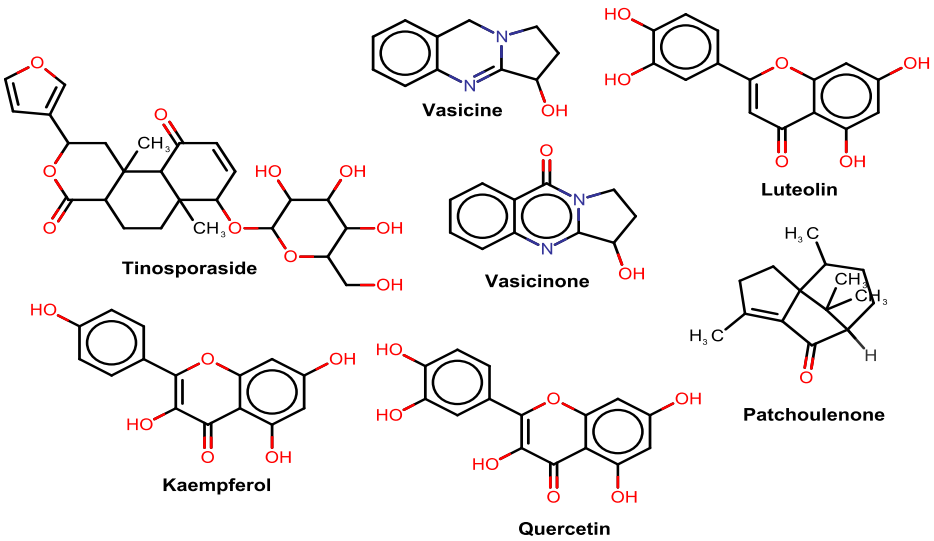

Fig3 Structures of some important Phytoconstituents from KabasuraKudineer

Molecular docking Calculations

In order to understand how the phytoconstituents bind to the target, docking analysis were performed using Schrodinger maestro v11.8 software. The validation of docking is done and proceeded to docking of ligands. The receptor-ligand interactions, interacting amino acid residue, bond type and bond distance were noted.

RESULT AND DISCUSSION:

By comparing SARS-CoV RBD and SARS-CoV-2 RBD from the literature, the key interactions are obtained. The structure (PDB ID: 2AJF) SARS-CoV RBD contains a core and a receptor-binding motif (RBM); the RBM mediates contacts with ACE2. The surface of human ACE2 (hACE2) contains two virus-binding hotspots that are critical for SARS-CoV binding. At the SARS-CoV/hACE2 interface, salt bridge in hotspot-31 (Lys31 and Glu35) and hotspot-353 (Lys353 and Asp38) occurs. But both salt bridges are weak, because of relatively long distance between these interactions. Burial of these weak salt bridges in hydrophobic environments upon virus binding would enhance their energy. This process is facilitated through the hotspots' interactions with nearby RBD residues. Hotspot-31 and Hotspot-353 requires support from Leu455 and Asn501 respectively from SARS-CoV-2 RBM/hACE2 interface (Tyr442 and Thr487 in SARS-CoV RBM). Due to these changes, the support from nearby RBD residues decreases, still become well stabilized at the SARS-CoV-2 RBM/hACE2 interface [9].

The docking score is given in the table. The good score compounds are Andrographolide, Berberine, Costunolide, Tinosporaside, Vasicine, Vasicinone, Ellagic acid, kaempferol, luteolin, quercetin, Icariin, Piperine, Stigmasterol. These bind with spike protein, Main Protease and Papain-like proteasewith good binding affinity. The interface residues in spike protein are TYR41, GLU42, GLU37, ASP38, GLU35, ASP30, TYR83.Lipinski's rule used to evaluate drug-likeness for orally active drug in humans. It states that not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, molecular mass less than 500 daltons, log P that does not exceed 5. The Lipinski's rule violations are only one for the constituents Berberine, Hayatininmethochloride, Lupeol, Stigmasterol. Two violations include Warifteine and three violation in Icariin.

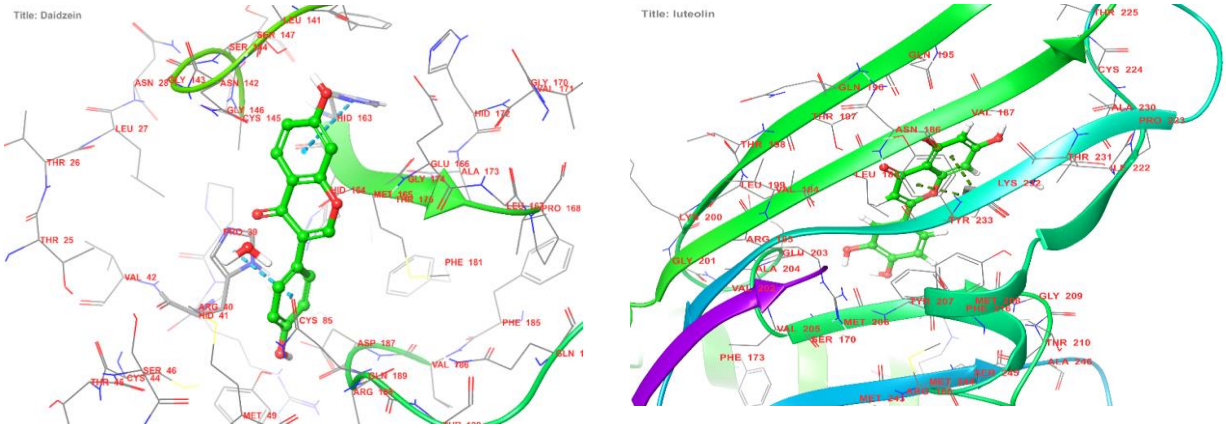

Fig. 4 Binding of Andrographolide and Quercetin with spike protein

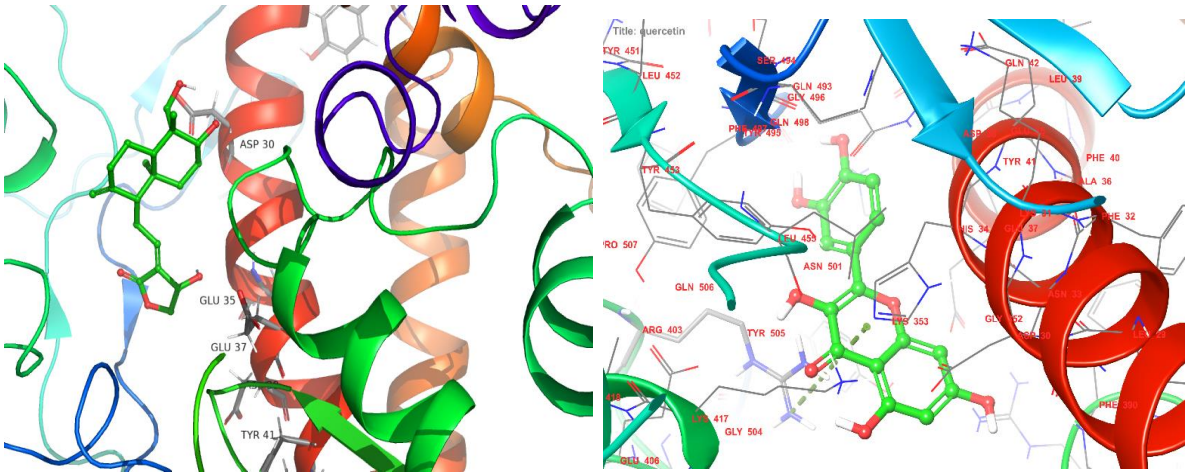

Fig. 5Binding of Daidzein and Quercetin with main protease and papain like protease respectively.

The phytoconstituents in kabasurakudineer binds ininterface residues in spike protein. The distance between the ligands and the key residues are given in table. The ligands are close to the interface residues. From this it can be concluded that that these ligands prevent the binding of SARS-Cov-2 to ACE2 receptor and prevent the viral entry into the cells. The ligands slide in the interface residuesof spike protein/ACE2 complex making it effective inhibition.The flavonoids luteolin and quercetin have potential anti-viral activity against enterovirus 71 and coxsackievirus A16, which causes hand, foot and mouth disease (HFMD) in paediatric [10]. Several structural analogues of Quercetin-7-Rhamnoside like quercetin, apigenin, luteolin and catechin, also showed moderate anti-Porcine epidemic diarrhoea virus [11]. By corroborating with above references it reveals that flavonoids are the important component in the kabasurakudineer which is responsible for inhibition of SARS-CoV-2 virus.

Table 2 Dock score of KabasuraKudineer Phytoconstituents with Spike protein, Main Protease and Papain-like protease

S.No	KabasuraKudineer Phytoconstituents	6M0J - Spike Protein		7BQY – MPro		6W9C - PLPro		Lipinski rule violation
		Dock score	Glide energy	Dock score	Glide energy	Dock score	Glide energy	
1.	Alpha-cyperone	-5.15	-21.2	-5.724	-30.37	-3.67	-19.67	0
2.	Alpha-terpineol	-4.689	-21.15	-4.721	-18.95	-3.41	-16.6	0
3.	Andrographolide	-5.524	-27.13	-7.872	-29.8	-5.63	-23.19	0
4.	Berberine	-6.862	-31.48	-7.496	-37.05	-5.09	-23.65	1
5.	Carvacrol	-5.52	-19.37	-5.098	-18.03	-3.45	-14.05	0
6.	Costunolide	-4.118	-22.73	-5.877	-32.5	-4.23	-20.33	0
7.	Daidzein	-7.406	-24.86	-7.371	-34.14	-4.05	-20.26	0
8.	Ellagic acid	-6.858	-28.63	-9.144	-27.7	-5.49	-29.04	0
9.	Embelin	-5.645	-35.85	-6.213	-38.67	-4.35	-26.27	0
10.	Eugenol	-5.891	-22.69	-4.852	-25.26	-3.65	-15.93	0
11.	Gallic acid	-6.548	-24.79	-5.764	-24.39	-4.81	-18.42	0
12.	Gingerol	-6.208	-38.34	-6.671	-35.18	-3.65	-24.05	0
13.	Hayatininmethochloride	-	-	-	-	-7.4	-36.36	1
14.	Icariin	-10.19	-52.27	-11.48	-52.51	-3.65	-18.7	3
15.	Linalool oxide A	-5.004	-17.51	-4.869	-24.22	-4.38	-24.41	0
16.	Lupeol	-4.923	-26.33	-7.344	-29.43	-4.53	-16.92	1
17.	Mustakone	-4.073	-19.13	-6.361	-27.18	-2.75	-24.58	0
18.	Piperine	-8.819	-34.92	-6.92	-31.52	-4.58	-19.5	0
19.	Rotundone	-4.158	-21.58	-6.226	-28.46	-5.5	-27.13	0
20.	Stigmasterol	-7.007	-28.69	-6.973	-24.6	-5.42	-25.22	1
21.	Tinosporaside	-6.575	-33.31	-7.564	-39.88	-4.15	-22.04	0
22.	Vasicine	-5.436	-18.17	-5.268	-25.81	-4.22	-20.25	0
23.	Vasicinone	-5.298	-24.42	-5.621	-24.17	-5.54	-30.74	0
24.	Warifteine	-8.254	-37.44	-5.53	-30.63	-4.43	-22.29	2
25.	isocyperol	-4.757	-18.72	-5.893	-28.29	-5.72	-24.17	0
26.	kaempferol	-7.594	-25.6	-8.88	-35.44	-5.86	-23.35	0
27.	luteolin	-7.93	-30.42	-9.19	-33.32	-3.32	-15.77	0
28.	patchoulenone	-	-	-4.912	-24.98	-5.82	-26.66	0
29.	quercetin	-8.263	-30.04	-8.936	-41.52	-4.22	-17.66	0
30.	thymol	-4.573	-19.63	-4.788	-18.8	-	-	0

Table 3 Distance between interface residues of Spike protein with KabasuraKudineer Phytoconstituents

S.No	Title	TYR41	ASP38	GLU37	GLU35	ASP30
1.	Alpha-cyperone	6.434	2.464	1.87	4.605	5.132
2.	Alpha-terpineol	5.686	2.496	2.062	4.68	5.69
3.	Andrographolide	7.608	4.748	3.424	5.28	3.005
4.	Berberine	5.917	2.063	2.226	4.482	4.421
5.	Carvacrol	6.009	2.283	2.572	4.145	5.745
6.	Costunolide	7.527	4.646	2.449	5.363	4.343
7.	Daidzein	6.273	4.058	2.365	6.936	5.485
8.	Ellagic acid	13.283	10.022	6.927	7.578	2.252
9.	Embelin	6.411	3.517	2.327	5.136	2.777
10.	Eugenol	6.586	2.675	2.749	4.212	6.475
11.	Gallic acid	6.824	2.853	3.423	3.587	6.428
12.	Gingerol	6.831	2.881	2.513	4.158	5.135
13.	Icariin	9.06	7.121	2.332	7.289	3.339
14.	Linalool oxide A	6.016	1.974	2.178	3.327	6.561
15.	Lupeol	11.57	9.131	6.152	7.867	1.928
16.	Mustakone	8.512	5.984	2.45	5.951	4.155
17.	Piperine	6.96	2.569	2.272	4.28	3.751
18.	Rotundone	16.19	12.999	9.618	9.649	2.387
19.	Stigmasterol	6.543	2.904	2.236	5.189	3.819
20.	Tinosporaside	10.857	8.232	6.134	8.307	2.829
21.	Vasicine	6.661	2.151	3.896	2.297	6.7
22.	Vasicinone	6.312	2.84	1.902	4.6	6.194
23.	Warifteine	8.056	5.17	2.414	6.251	4.029
24.	isocyperol	10.976	8.311	5.294	7.683	5.767
25.	kaempferol	6.612	2.412	2.235	4.45	5.909
26.	luteolin	7.068	3.107	2.363	3.73	6.267
27.	quercetin	6.684	2.539	2.386	4.078	6.11
28.	thymol	6.462	2.707	2.336	5.277	5.573

CONCLUSION:

The presence of many compounds in the formulation will provide greater probability in neutralising the threat of virus than with the single molecule. The main proteases from PDB ID: 6M2N in protein data bank in complex with a novel inhibitor - 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one in which the basic nucleus is flavone, many of the phytoconstituents present in Kabasurakudineer has this flavone nucleus.Molecular docking studies shows that one or many of the phytoconstituent can be taken as lead forantiviral activity. Further research in *in-vitro* and *in-vivo* studies should be carried out.The development of a vaccine is another important aspect to tackle the problems immediately.

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