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Original Research Article (Experimental)

In Silico computational screening of *Kabasura Kudineer* - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein

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ABSTRACT

Background: Siddha Medicine is a valuable therapeutic choice which is classically used for treating viral respiratory infections, this principle of medicine is proven to contain antiviral compounds.
Objective: The study is aimed to execute the *In Silico* computational studies of phytoconstituents of Siddha official formulation *Kabasura Kudineer* and novel herbal preparation - JACOM which are commonly used in treating viral fever and respiratory infectious diseases and could be affective against the ongoing pandemic novel corona virus disease SARS-CoV-2.
Method: Cresset Flare software was used for molecular docking studies against the spike protein SARS-CoV-2 (PDB ID: 6VSB). Further, we also conducted *in silico* prediction studies on the pharmacokinetics (ADME) properties and the safety profile in order to identify the best drug candidates by using online pkCSM and SwissADME web servers.
Results: Totally 37 compounds were screened, of these 9 compounds showed high binding affinity against SARS-CoV-2 spike protein. All the phytoconstituents were free from carcinogenic and tumorigenic properties. Based on these, we proposed the new formulation called as "SNACK–V"
Conclusion: Based on further experiments and clinical trials, these formulations could be used for effective treatment of COVID-19.

1. Introduction

The novel Coronavirus disease-2019 (COVID-19) is an ongoing pandemic caused by Severe Acute Respiratory Syndrome Corona-Virus 2 (SARS-CoV-2) [1]. COVID-19 has been declared a pandemic disease by WHO which has severely affected the livelihood of the population. SARS-CoV-2 has spread across the continents, as of April 11, 2020, has led to a total of 16,99,676 cases with a mortality of 1,02,734 among the registered cases. Presently, quarantine and symptomatic treatment protocol for disease management exists and there are no specific antiviral drugs available to combat this virus. As per Ministry of Health and Family Welfare, Govt. of India, in India there are 7447 Active cases and 239 deaths as on April 11, 2020; these data commensurate the impending risk facing the country. This pandemic is still ongoing, hence there is an urgent need to find new preventive and therapeutic agents as soon as possible [2].

Knowledge of Microbes and their Disease spread is clearly mentioned in Siddha which is evinced by "*Kirumiyal vandha thodam perugavundu lines mentioned in Guru naadi*"[3]. Siddha holistic approach will be helpful in combating COVID 19 using both therapeutic and non-therapeutic interventions. Siddhar's have advised evidence based treatment approach to understand a disease (*Noi naadi*), its etiology (*Mudhal Naadi*) based on those, fix a treatment (*Athu Thanikka Vainaadi*). As per basic Siddha Concept, Siddhar

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Theran has defined *Vatham* is responsible for creation, *Pittam* for prevention and *Aiyam* for destruction. Infections happen to a person when his immunity is challenged which could be related with reduction of *Pitham*. According to Siddha theory, in a COVID-19 infection there is initial increase of body temperature, cough and throat pain which may subside if there is good amount of immunity and these symptoms subside when *Pitta thathu* (Humor) come into action. If not, it escalates to a phase of *Kapha Dosham* (Disorder) which is said as "*Thanamulla sethumanthan ilagil veppu*". If not treated at this stage it slowly moves to a Stage of *Sanni* (Severe Pneumonia- Respiratory failure). It has been unanimously agreed to have equated diagnosis as *Kaphasuram* in Siddha in early stages moving towards *Sanni* and which is also reassured through Delphi or other sources of FGD (Focus group discussion).

The control and treatment of a viral infection depends mainly on the availability of antiviral drugs, which are few in numbers and usually are not directly acting on virus but prevent replication in the host. The Siddha herbal formulations having medicinal importance have proved to be potentially active against a wide range of causative agents as Influenza, Dengue, Chikungunya, Tuberculosis, etc [4–6]. Siddha medicines have been used effectively by human civilization over several centuries for treating various diseases and can be effectively employed to target the host response, like *Kabasura Kudineer* during influenza outbreaks [7]. Besides, during Dengue outbreak in India, a herbal formulation of Siddha medicine, *Nilavembu Kudineer* is used to prevent and control the morbidity level of public on contacting this viral fever [8].

Kabasura Kudineer, an official Siddha formulation described in Siddha manuscript '*Citta Vaittiyattirattu*' is used for *Aiyacuram* (phlegmatic fevers) and is a dependable Siddha prescription for fever with flu-like symptom [9]. Further, we choose another herbal formulation called "JACOM" a coded novel drug due to its Neuraminidase inhibition potential against inactivated influenza virus H1N1(Patent no.201741016901 A, dated 18.05.2018) [10].

Moreover, to screen out large number of herbs for compounds with antiviral activity against novel corona virus will be a challenge in very short period. Drug discovery is a time consuming, slow and challenging process [11,12], so it is necessary to depend on computational tools (Computer-aided drug design) to overcome these pitfalls to an extent. Of late, the impact on these tools for new drug development had made the drug discovery process very cost effective and time efficient [11]. For searching compounds, this ligand-based virtual screening tool is used to identify most probable molecule with pharmacological activity using molecular docking [13–15]. Similarly, for studies pharmacokinetics, toxicity, and drug-likeness prediction many algorithms exist which makes the job easier [16]. There are lots of evidence which prove the application of computational tools in the discovery of natural-derived drugs [17–20]. Hence, the aim of the current study is to apply this incredible *in-silico* screening methodology for the official Siddha formulation *Kabasura Kudineer* and the novel formulation JACOM against SARS- CoV-2 spike protein.

2. Methods

2.1. Ligand preparation

Kabasura Kudineer Chooranam is a polyherbal formulation containing fifteen herbal drugs (Table 1) mixed in equal quantities and decoction is prepared. To prepare *Kabasura Kudineer Chooranam* all the fifteen ingredient drugs are coarsely powdered and mixed; 35 g of this powder is boiled with three liters of water and reduced to the volume of 1/12th. This has to be taken 30–60 mL twice or thrice daily [9]. The bioactive constituents used for docking were obtained from *Kabasura Kudineer Chooranam* are β-Sesquiphellandrene, β-Bisabolene, Geranial, Piperine, Piperlonguminine, Eugenol, β-Caryophyllene, Stigmosterol, 3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3- dihydrochromen-4-one, Squalene, γ-Sitosterol, Andrograpanin, 5-Hydroxy-7,8-dimethoxyflavanone, Lupeol, Betulin, Chebulagic acid, Gallic acid, Vasicinone, Carvacrol, Cirsimaritin, Chrysoeriol, 6-Methoxygenkwanin, Luteolin, Costunolide, Elemol, Tinosponone, Bharangin, Scutellarein, Magnoflorine, Cycleanine, Cyperene, β-Selinene [21–23] The bioactive constituents from JACOM are Vasicine, Andrographolide, Ursolic acid, Quercetin and Meliacine. The 2D structures of ligands are summarized in Supplementary Table S1. All the ligands were obtained from PubChem and prepared a single.sdf file, further optimization and minimization of all ligands were done in Cresset Flare software with default settings. The ligands file read in Autodetect under full protonation mode.

2.2. Protein preparation

To investigate the phytochemical analogs of Siddha formulation *Kabasura Kudineer Chooranam* and JACOM against SARS-CoV-2 virus, we have selected novel spike glycoprotein (PDB ID: 6VSB), a key target for therapeutics, vaccines and diagnostics in SARS-CoV-2. This spike glycoprotein 2019-nCOV S protein is a single receptor-binding domain (RBD) which binds to ACE2 (Angiotensin converting Enzyme-2) receptor on the host cell with high affinity, which makes it a key target for the novel coronavirus therapy development. The 3D structure of novel spike glycoprotein (PDB ID: 6VSB) were downloaded from Protein Data Bank (https://www.rcsb.org/structure/6VSB). The target protein was downloaded in PDB format and protein preparation was carried out in Cresset module Flare software with default settings. Missing residues, hydrogen's and 3D protonation were carried out on the target protein and minimized for the selected active residues [24].

2.3. Molecular docking studies

Molecular docking was carried for 32 phytochemical constituents of Siddha formulation *Kabaura Kudineer Chooranam* and 05 phytoconstituents of JACOM. The phytochemical analogs were docked with spike protein SARS-CoV-2 (PDB ID: 6VSB) by using Cresset Flare Docking software with default settings and the grid box was defined based on trial and error and carried out in normal mode [25,26]. The crystal structure of protein was obtained from protein data bank. The structures of phytochemical constituents were downloaded from the PubChem and the structures were converted into a single database file in sdf file format in Data warrior software. Best poses were generated and visualized in pose viewer and 3D images stored in storyboard. Analysis of docking results was done with Flare Software and the results are shown in Tables 1 and 2. Best score generating phytoconstituents in the largest cluster was analyzed for its interaction with the protein and 2D poses were obtained from LigPlus.

3. Results

3.1. Molecular docking studies

The molecular docking studies were carried out for the 32 phytochemical constituent's of Siddha formulation *Kabasura Kudineer Chooranam* and 05 phytochemical constituent's JACOM against coronavirus spike protein to identify the molecular interactions between target protein with ligands. All the phytochemical analogs were docked with spike protein SARS-CoV-2 (PDB ID: 6VSB) by using Cresset Flare Docking software.

Table 1
In silico docking studies of phytoconstituents of Siddha formulation *Kabasura Kudineer Chooranam* and JACOM against spike Protein SARS-CoV-2 (PDB ID: 6VSB) using docking software Cresset Flare.

Plant Name	Compound name and Code	LF dG	LF VSscore	LF Rank Score	LF LE
Kabasura Kudineer Chooranam					
Zingiber officinale Rosc	β-sesquiphellandrene **(1)**	−6.638	−6.846	−2.658	−0.443
	β-bisabolene**(2)**	−6.562	−6.713	−2.8	−0.437
	Geranial**(3)**	−5.099	−5.319	−2.121	−0.464
Piper longum L	Piperine**(4)**	−6.768	−7.445	−4.143	−0.322
	Piperlonguminine**(5)**	−7.078	−7.7	−4.245	−0.354
Syzygium aromaticum	Eugenol**(6)**	−4.818	−5.559	−6.182	−0.402
	β-Caryophyllene**(7)**	−5.654	−5.918	−3.203	−0.377
Tragia involucrata L	Stigmosterol**(8)**	−9.724	−10.39	−7.466	−0.324
	3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3-dihydrochromen-4-one**(9)**	−6.433	−7.316	−9.011	−0.247
Anacyclus pyrethrum	Squalene**(10)**	−9.722	−10.187	−1.389	−0.324
	γ−Sitosterol**(11)**	−9.956	−10.521	−7.679	−0.332
Andrographis paniculata	Andrograpanin**(12)**	−6.819	−7.678	−7.854	−0.296
	5-Hydroxy-7,8-dimethoxyflavanone**(13)**	−7.356	−7.966	−9.035	−0.334
Hygrophilla auriculata (Schum.)Heine	Lupeol**(14)**	−8.337	−8.917	−6.41	−0.269
	Betulin**(15)**	−7.984	−9.117	−7.02	−0.249
Terminalia chebula Retz.	Chebulagic acid**(16)**	−10.769	−11.138	−9.723	−0.158
	Gallic acid**(17)**	−5.549	−6.602	−6.916	−0.462
Justicia adhatoda L.	Vasicinone**(18)**	−5.753	−6.272	−8.164	−0.384
Plectranthus amboinicus (Lour) Spreng	Carvacrol**(19)**	−5.322	−5.696	−6.923	−0.484
	Cirsimaritin**(20)**	−6.42	−7.227	−9.228	−0.279
	Chrysoeriol**(21)**	−7.954	−8.352	−11.392	−0.362
	6- Methoxygenkwanin**(22)**	−6.415	−7.527	−9.293	−0.279
Costus speciosus	Luteolin**(23)**	−8.149	−8.584	−11.159	−0.388
	Costunolide**(24)**	−6.081	−6.607	−3.799	−0.358
	Elemol**(25)**	−6.587	−6.696	−5.43	−0.412
Tinospora cordifolia (Willd.) Miers ex Hook.f&Thoms	Tinosponone**(26)**	−7.043	−7.434	−8.145	−0.293
Clerodendrum serratum L.	Bharangin**(27)**	−7.418	−7.744	−6.682	−0.309
	Scutellarein**(28)**	−7.805	−9.148	−10.277	−0.372
Sida acuta Burm. f.	Magnoflorine**(29)**	−7.635	−8.527	−9.762	−0.305
	Cycleanine**(30)**	−6.184	−8.214	−3.432	−0.134
Cypreus rotundus L.	Cyperene**(31)**	−6.024	−6.225	−3.558	−0.402
	β-selinene**(32)**	−6.33	−6.587	−3.412	−0.422
JACOM Formulation					
Justicia adathoda L.	Vasicine**(33)**	−5.19	−6.1	−7.67	−0.37
Carica Papaya	Quercetin**(34)**	−8.408	−8.59	−11.478	−0.382
Andrographis paniculata Burm.f.Nees	Andrographolide**(35)**	−7.74	−8.45	−7.85	−0.31
Ocimum tenuiflorum	Ursolic acid**(36)**	−7.08	−7.71	−5.1	−0.21
Melia azedarach	Meliacine**(37)**	−4.2	−8.76	−5.14	−0.88

The crystal structure of protein was obtained from pdb bank. The structures of phytochemical constituents were downloaded from the PubChem and the structures converted into a single database file in sdf file format in Data warrior software. To fight against this deadly virus, many X-ray crystal structures of proteins were reposited in pdb bank for Receptor-binding protein (RBD, trimer) with PBD ID 6CRV and 6VSB; Heptad repeat 2(HR2) with PBD ID 2FXP.

The SARS-CoV-2 virus binds to human cells through its spike glycoprotein, making this protein as key target to design potential therapeutics. In this regard, we have selected potential phyto constituents with previously reported antiviral activity for carrying out the docking studies with the viral spike glycoprotein.

Binding affinities of phytocompounds of siddha formulation *Kabasura Kudineer Chooranam* and JACOM towards active site of spike protein SARS-CoV-2 was studied in detail. Biological interaction analyses of phytoconstituents with spike protein SARS-CoV-2 were carried out to identify the compound having highest binding affinity with target proteinin the Flare software docking analysis.

The LF rank score is an indicator of the binding affinity of protein-ligand complex. The LF rank for each phytocompound is described in Tables 1 and 2 The binding orientation for each phytocompounds into the active site of SARS-CoV-2 spike protein is identified based on the molecule having the least LF rank score. The more the negative LF rank score represent the better affinity of the phytocompound against target SARS-CoV-2 spike protein.

Among the docking studies performed on phytocompound, all the analogs had effective binding interactions with SARS-CoV-2 spike protein (LF rank score range from −5.75 to −11.03). From the results it reveals that Phytoconstituents with highest docking LF rank score were seen for Chrysoeriol and Luteolin from *Kabasura Kudineer Chooranam* and Quercetin from JACOM with LF rank score values −11.478, −11.392 and −11.159, respectively. Whereas, 5-Hydroxy-7,8-dimethoxyflavanone, Cirsimaritin, Scutellarein with LF rank score of −9.035, −9.228, and −10.277, show moderate binding affinity against the target protein. Remaining analogs also show lower binding affinity towards SARS-CoV-2 spike protein. We further studied detailed binding orientation of top 11 phytocompounds in the active site of spike protein and best poses in 2D and 3D were generated.

The number of hydrogen bond and the number of amino acid residues of SARS-CoV-2 interacting with each phytocompounds are given in Table 2. From the detailed docking analysis, it is observed that Chrysoeriol, Luteolin, and Scutellarein show a high binding affinity with target protein SARS-CoV-2 spike protein. It is found that, these three compounds have formed H-bond contact with more than four amino acid residues in spike protein showing that it forms more number of H-bonds resulting in increased binding affinity with target protein Figs. 1–3.

The interaction analysis of Chrysoeriol, Cirsimaritin, and Magnoflorine - SARS-CoV-2 spike protein complex reveals that amino acids Cys336, Asp364, Ser373, Asn343, Cys336, Gly339, Asp364,

Table 2
Amino acid residues of SARS-CoV-2 spike protein participated in H-Bond and hydrophobic interactions with ligands.

Compound Code	LF Rank Score	Interactions	
		H-Bonding	Hydrophobic
β-sesquiphellandrene **(1)**	−2.65	NHB	Ser373, Phe374
β-bisabolene**(2)**	−2.8	Phe342, Ser373,	Phe338, Gly339
Geranial**(3)**	−2.12	NHB	Ser373, Phe374,
Piperine**(4)**	−4.14	Phe374, Trp436	Phe338, Ser373,
Piperlonguminine**(5)**	−4.24	Phe338	Ser373, Phe342, Cys336, Leu335, Val367
Eugenol**(6)**	−6.18	Asn343, Phe342,	Ser373
β-Caryophyllene**(7)**	−3.20		Phe338, Gly337
Stigmosterol**(8)**	−7.46	Cys336, Gly336,	Phe342, Asn343, Ser373,
3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3- dihydrochromen-4-one**(9)**	−9.01	Arg509, Trp436,	Phe374, Phe342, Asn343, Thr345, Ala344, Leu441
Squalene**(10)**	−1.38	NHB	Thr345, Asn643, Phe342, Asn343, Phe338, Leu335
γ−Sitosterol**(11)**	−7.67	Cys336, Gly339	Ser373, Phe374, Val510
Andrograpanin**(12)**	−7.85	Asn343	Phe342, Leu335, Asp364
5-Hydroxy-7,8-dimethoxyflavanone**(13)**	−9.03	Asp 364, Gly339	Cys336, Phe337, Leu335, Phe342, Phe338, Leu368
Lupeol**(14)**	−6.41	Thr345	Asn343, Ser373, Thr345, Arg509
Betulin**(15)**	−7.02	Thr345, Ser373	Asn422, Val341, Arg509, Phe373, Thr345
Chebulagic acid**(16)**	−9.72	Tyr369, Asn370, Tyr369, Phe377, Cys379, Lys378	Lys378, Phe337, Phe342, Cys336
Gallic acid**(17)**	−6.91	Lys356, Val341	Ala397, Val341, Lys356
Vasicinone**(18)**	−8.16	Cys336, Gly339	Val397, Cys336, Phe338, Leu335, Asp364
Carvacrol**(19)**	−6.92	Asp364	Cys336, Leu335, Asp364
Cirsimaritin**(20)**	−9.22	Cys336, Asp364, Ser373, Asn343	Phe338, Phe342, Phe374, Ser373
Chrysoeriol**(21)**	−11.39	Cys336, Gly339, Asp364,	Phe338, Phe342, Phe374, Leu335, Val367, Ser373
6- Methoxygenkwanin**(22)**	−9.29	Cys336, Phe342	Ser373, Phe342, Leu368, Phe338, Leu335
Luteolin**(23)**	−11.15	Asp364, Val367, Ser371, Ser373, Cys336, Val362	Phe338, Gly339, Phe374, Phe342
Costunolide**(24)**	−3.79	Phe515, Gly431	Val511, Phe515, Gly431
Elemol**(25)**	−5.43	Asp364, Asp364	Phe374, Phe342, Asn343,
Tinosponone**(26)**	−8.14	Phe342, Gly339	Trp436, Asn343, Leu368, Val367
Bharangin**(27)**	−6.68	Phe338, Gly339,	Phe337, Phe342, Ser373
Scutellarein**(28)**	−10.27	Cys336, Phe338, Gly339, Asp364, Val362	Ser373, Phe374, Leu335, Asn343
Magnoflorine**(29)**	−9.76	Arg346, Val341, Thr345	Ala344, Lys356, Ala397
Cycleanine**(30)**	−3.43	Ser373	Phe374, Trp436,
Cyperene**(31)**	−3.55	NHB	Ser373
β-selinene**(32)**	−3.41	NHB	Phe342, Ser373
JACOM Formulation			
Vasicine**(33)**	−7.67	Phe 338, Asn343	Gly339
Quercetin**(34)**	−11.47	Asp364	Phe338, Leu335, Gly339, Leu368, cys336, he374
Andrographolide**(35)**	−7.85	Asp364, Phe368, Gly339, Asn343	Cys336, Phe342, Leu368, Phe374
Ursolic acid**(36)**	−5.1	Val367	Leu368
Meliacine**(37)**	−5.14	Phe338	Val367, Ser371, Leu368, Phe338
Hydroxychloroquine**(38)**	−8.35	Phe342, Asn343	Gly339, Phe338, Leu368, Trp436, Ser373, Phe374

NHB: No Hydrogen Bond Interactions.

Arg346, Val341, and Thr345 have played important role in the formation of H-bond network. The possible binding orientation of phytocompounds from Siddha formulation *Kabasura Kudineer Chooranam* and JACOM into the active site of SARS-CoV-2 spike protein and corresponding hydrophobic interaction models, number of hydrogen bonds are shown in Tables 1 and 2 and Figs. 1 and 3. The Docking studies of all the phytochemicals from two formulations were compared with positive control Hydroxychloroquine and found that all docked ligands were interacting with the same amino acid residues. The validation docking and Hydroxychloroquine has LF rank score −8.35 and forms two H-bond interactions with Phe342 and Asn343 Fig. 4.

Flare was used to perform *in silico* computational studies, prediction of cavity, assigning bond orders, structure refinement, defining the active sites of the SARS-CoV-2 and structure preparation. The protein preparation was carried out with Flare and the chain was treated to add missing hydrogen, assign proper bond orders. The structure output format was set to pose viewer file so as to view the output of resulting docking studies and hydrogen bond interactions of different poses with the protein. The 2D and 3D interactions were generated with Ligplus and storyboard in Cresset. All the studied Phytoconstituents have showed excellent free energy of binding interactions with SARS-CoV-2 Figs. S1−S8.

3.2. In Silico *prediction of drug likeliness, and synthetic accessibility*

Rule of 5 by Lipinski is a significant criterion to evaluate drug likeliness and if a specific chemical compound with a certain biological activity has physio-chemical properties that would make it a likely orally active drug in humans. Lipinski's rule evaluates the different descriptors which are important for a drug design. Lipinski's rule of five states that (i) molecular mass less than 500 Da, (ii) no more than 5 H-bond donors, (iii) no more than 10 H-bond

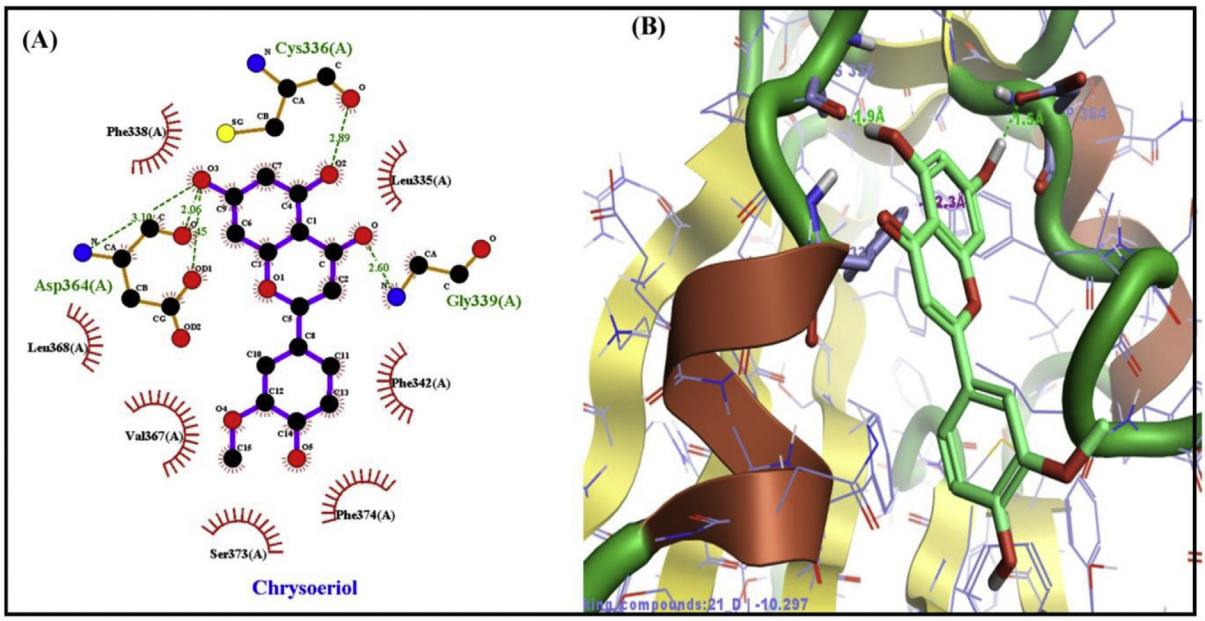

Fig. 1. Molecular docking results of Chrysoeriol into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Chrysoeriol with SARS-CoV-2 Spike protein (B) Binding mode of Chrysoeriol in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

acceptors, (iv) O/W partition coefficient log P not greater than 5. If the molecule violates more than 3 descriptor parameters, it will not fit into the criteria of drug likeliness and it is not considered in order to proceed with drug discovery.

Supplementary Tables S2 and S3 depicts the drug likeliness and various rules like Lipinski rule of five, Veber Ghose, Muegge and Egan rules were applied to all phytochemical constituents. From the data, most of the Phytoconstituents obeyed the rules only few analogs violated. The low value of synthetic accessibility indicates that all the phytoconstituents could be synthesized. These results indicate the active ingredients of two Siddha Formulations of *Kabasura Kudineer Chooranam* and JACOM have drug like properties.

3.3. In Silico *simulation of Pharmacokinetic Properties*

In silico pharmacokinetics properties of phytochemical constituents of Siddha formulation *Kabasura Kudineer Chooranam* and JACOM were carried out with online pkCSM webserver.

From the data of pharmacokinetic properties shows that Lupeol, Betulin, Cycleanine, β-selinene, Quercetin, Andrographanin and Tinosponone have the highest gastrointestinal absorption, tissue distribution (Vd), and respectable total clearance

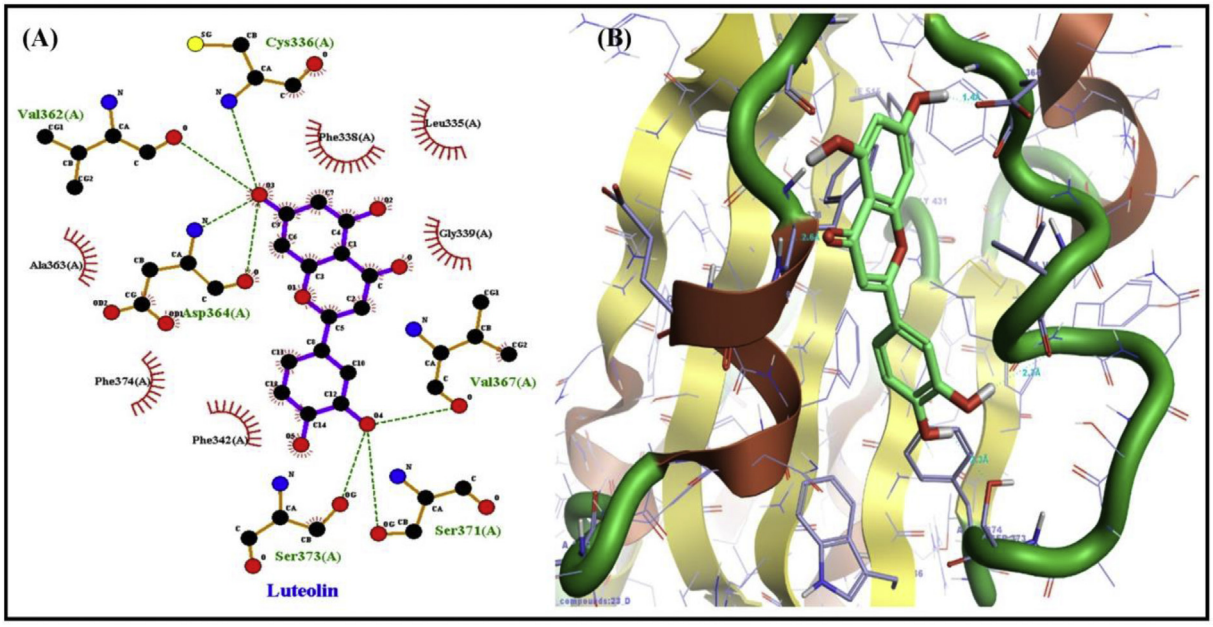

Fig. 2. Molecular docking results of Luteolin into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Luteolin with SARS-CoV-2 Spike protein (B) Binding mode of Luteolin in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

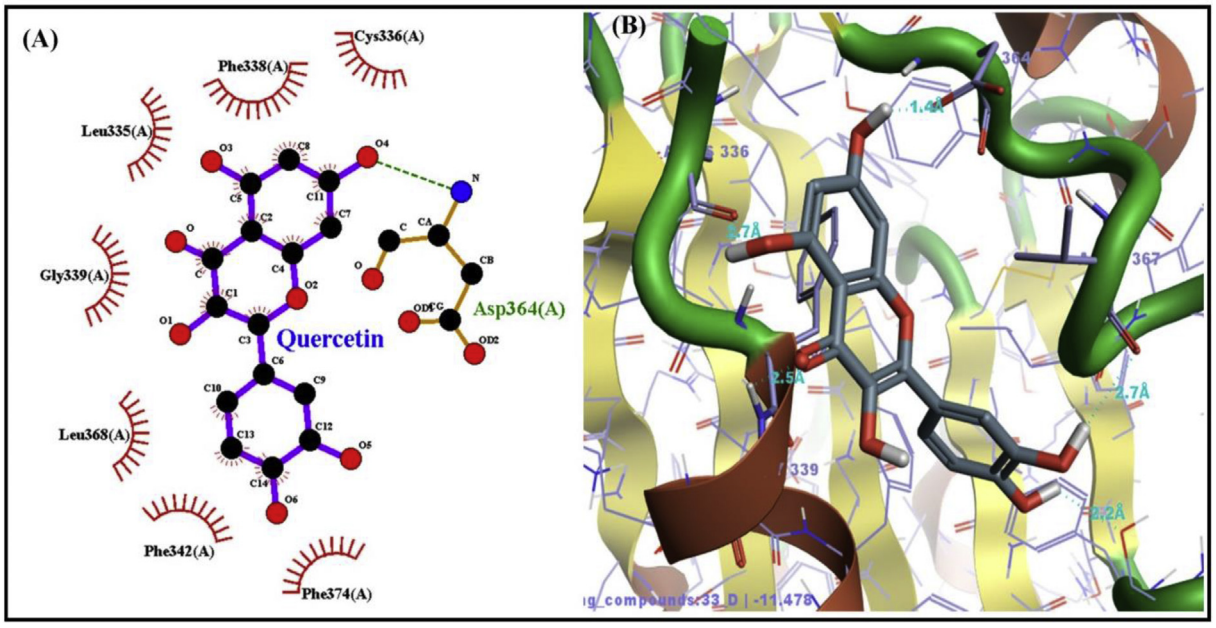

Fig. 3. Molecular docking results of Quercetin into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Quercetin with SARS-CoV-2 Spike protein (B) Binding mode of Quercetin in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

Supplementary Table S4. The Lupeol and Betulin ingredients of *Kabasura Kudineer Chooranam* formulation have 100% bioavailability and other ingredients also having oral bioavailability >80%. For JACOM formulation Ursolic acid has 100% bioavailability and other ingredients also having >80% bioavailability.

The Cytochrome P450 and P-glycoprotein simulation studies for substrate and inhibition were performed for all selected Phytoconstituents of two Siddha formulations by using online webserver. The results show that most of the Phytoconstituents has less CYP inducing and P-gp compatibility property Supplementary Table S5. Piperine, piperlonguminine, Stigmosterol, 3-(2,4-dimethoxyphenyl)-6,7-dimethoxy-2,3- dihydrochromen-4-one, Squalene, γ-sitosterol, Andrograpanin, 5-Hydroxy-7,8-dimethoxyflavanone, Lupeol, Betulin could undergoes metabolism via CYP3A4 enzyme Supplementary Table S5 Moreover, β-Sesquiphellandrene, β-Bisabolene, Geranial, Gallic acid, Carvacrol, Costunolide, and Elemol were free from drug–drug interaction via the inhibition of cytochrome-P (CYP) or P-glycoprotein (P-gp) I and II enzymes Supplementary Table S5.

3.4. In Silico *toxicity prediction*

Toxicity assessment was performed for the selected phytoconstituents of Siddha formulations and results show that very few analogs have deviated toxicity prediction. Overall the study indicates, the ingredients of these two formulations are free from carcinogenic, teratogenic, and tumorigenic properties Supplementary Table S6.

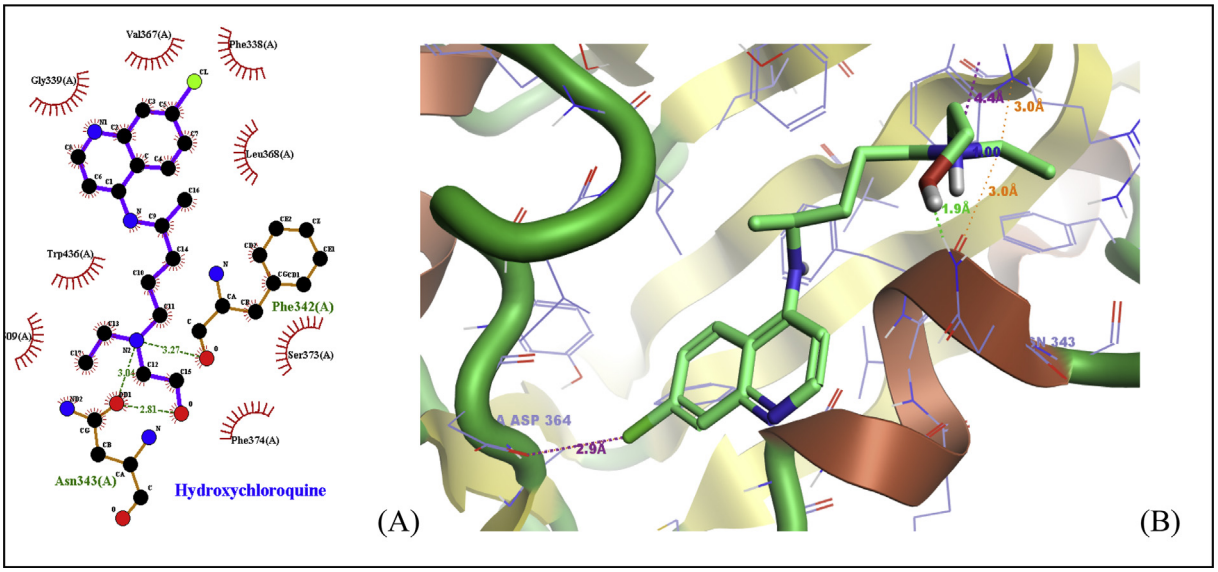

Fig. 4. Molecular docking results of Hydroxychloroquine into SARS-CoV-2 spike protein. (A) Hydrophobic interaction of Hydroxychloroquine with SARS-CoV-2 Spike protein (B) Binding mode of Hydroxychloroquine in SARS-CoV-2 Spike protein. Amino acid residues involved in H-bond formation and H-bond networks are shown.

Table 3
Proposed SNACK –V formulation containing plants and their phytoconstituents with Dock score.

S.No	Plant Name	Phytoconstituents	LF Rank Score
1	*Sida acuta* Burm. f.	Magnoflorine	−9.76
2	*Andrographis paniculata*	5-Hydroxy-7,8-dimethoxyflavanone	−9.03
3	*Tinospora cordifolia*	Tinosponone	−8.14
4	*Plectranthus amboinicus*	Cirsimaritin	−9.22
		Chrysoeriol	−11.39
		6- Methoxygenkwanin	−9.293
5	*Justicia adhatoda* L	Vasicinone	−8.16
		Quercetin	−11.47
6	*Costus speciosus*	Luteolin	−11.15

4. Discussion

In this work, we have chosen Official Siddha Formulation *Kabasura Kudineer Chooranam* and JACOM (patented formulation). Modern medicines focus on killing the virus but not on increasing the host immunity. In case of Siddha medicine, herbs like *Amukkara*, *Nilavembu* are immuno-modulator and having the capacity to inhibit the virus by enhancing and restoring immunity of human. So, we are utilizing this strength of Siddha medicine to arrive upon a potent formulation that is both anti-viral and Immuno-modulatory with minimum side effects on patients who are immuno compromised as well as those who have co-morbid conditions.

The *Kabasura Kudineer* increases the immunity and could act as immuno modulator as this virus is adversely affecting the immune response by effecting signaling pathway of TNF production as recent findings shows [27]. The formulation chosen are aimed at increasing immunity and also to expel out the *kapham* and reinstate respiratory health. Drugs in these formulations majorly possess Bitter taste or pungent taste. These drugs on post digestive transformation get converted to hot potency which increases and normalizes *pitham* and expel out excessive *kapham* out of lungs, which is the rationale behind selecting these formulations.

Based on these results, nine phytoconstituents (6 plants) were found to be the best lead and drug candidates with good synthetic accessibility. The nine phytoconstituents with the LF rank Score viz., Magnoflorine (−9.76), 5-Hydroxy-7,8-dimethoxyflavanone(-9.03), Tinosponone(-8.14), Cirsimaritin(-9.22), Chrysoeriol(-11.39), 6-Methoxygenkwanin(-9.293), Vasicinone(-8.16), Quercetin(-11.47) and Luteolin(-11.15) are having highest binding affinity with spike protein and the plants associated with the Phytoconstituents were chosen for novel "SNACK-V" formulation. These 6 plants containing 9 phytochemicals have interaction score higher than the positive control Hydroxychloroquine. Based on these results, we proposed a novel herbal formulation called "SNACK -V" (*Sida acuta*, *Adhatoda vasica*, *Andrographis paniculata*, *Tinospora Cordifolia, Costus speciosus*, *Plectranthus ambonicus*) it may have high probability of directly inhibiting the novel corona virus (2019-nCoV), possibly providing instant help in the prevention and treatment of the pneumonia that it can cause Table 3. This formulation having herbs that possess bitter taste increases *pittam* and expels out *kapham* for their properties of immunomodulation, expectorant and antipyretic. These effects reinstate Gaseous exchange normalizing *trithodam* and *Sanni* Symptoms are wiped away thereby restoring normal health.

Tinospora cordifolia is a one of the drug of choice in conditions wherever pitta is diminished and *kapha* dominates [28]. Due to its bitter taste in post digestive transformation it turns into hot potency as a pungent active molecule and helps in reinstating *pitta* to normalcy and eliminates *kapha* slowly out of the body. It is useful also in settling fever. Later studies had proved its efficacy as an antiviral and an immunomodulator. Its effect against HIV has been documented via clinical evaluation [29].

A. paniculata by its bitter taste and hot potency helps in all fevers by precipitating diaphoresis [28], in dengue out break and during other disaster mitigation interventions it was the drug of choice even by public health authorities [5]. By possessing anti-inflammatory, analgesic, anti pyretic and immuno - modulatory activity [30] this has also proven to inhibit dengue virus [5].

Adhatoda vasica is bitter in taste and turns into hot potency. It is also an expectorant and very useful in *kapha* disorders [28]. Studies suggest that extracts have strong anti-influenza virus activity that can inhibit viral attachment and/or viral replication, and may be used as viral prophylaxis [31].

P. ambonicus is a plant having pungent taste and gets converted to hot potency post transformation, possess diaphoretic and expectorant property [28]. Many antimicrobial studies have established its effectiveness in lower respiratory symptoms like pneumonia [32].

C. speciosus is bitter in taste and turns into hot potency [28], indicated in fever and used as an expectorant. Studies have proved that it inhibits *Herpes simplex* and Varicella virus [33].

S. acuta is bitter in taste and turns into hot potency. It is also an expectorant and very much useful in *kapha* disorders [28]. Studies show this herb inhibits the replication of dengue viruses in cell cultures and protected mice against dengue infection. It also showed antipyretic and anti-inflammatory effects [34]. To summarize, these above mentioned 6 plants possess both anti viral and immuno-modulatory property, also all the bioactive compounds are non-toxic and non-carcinogenic. However, further experimental studies and clinical studies are required to validate the results.

Siddha medicine is one of best way to control the COVID-19. The docking studies of bioactive compounds from *Kabasura Kudineer* and JACOM showed that stronger binding affinity with good ADMET properties. Further we propose a new formulation as SNACK-V. Given their binding affinity towards SARS-CoV-2 spike protein and *in silico* safety studies, these two formulations qualify as a potential therapeutic for further *in vitro, in vivo* and clinical studies.

5. Conclusion

Spike protein is an important target for binding with the ACE2 of the host cell, and the inhibitors of this protein could be a potential target for COVID-19 infection. In this study, we have done the *in silico* molecular docking studies for the 37 phytoconstituents against the spike protein of SARS-CoV-2 (PDB ID: 6VSB). The results shown that Chrysoeriol and Luteolin from *Kabasura Kudineer Chooranam* and Quercetin from JACOM have high binding affinity and good binding interactions with spike protein. Further, *In silico* pharmacokinetic and toxicity prediction shown that all the phytoconstituents have good oral bioavailability and free from toxicity. Based on these, we proposed the new formulation called as

"SNACK−V" which contains nine phytoconstituents from the six plant herbs.

Conflict of interest

None.

Source of funding

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2020.05.009.

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