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

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1. Introduction

The novel Coronavirus disease-2019 (COVID-19) is an ongoing pandemic caused by Severe Acute Respiratory Syndrome Coronavirus 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 Vainadi). As per basic Siddha Concept, Siddhar

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Theran has defined Vatham is responsible for creation, Pitam for prevention and Aiyam for destruction. Infections happen to a person when his immunity is challenged which could be related with reduction of Pitam. 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 sethamathan lagil lagpu 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 like 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 Vaittyiyattirattu’ is used for Ayurved (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 Neuroaminidase inhibition potential against inactivated influenza virus H1N1 (Patent no.20174106901 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 lack 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-Carophyllene, Stigmosterol, 3-(2,4- dimethoxyphenyl)-6,7- demethoxy-2,3-dihydrochromen-4-one, Squalene, γ-Sitosterol, Andrographanin, 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 Vascine, Andrographolide, Ursolic acid, Quercetin and Melicaine. 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 Kabasura 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.
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 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 phytoconstituents of siddha formula Kudarsha Kudineer Chooranam and JACOM towards active site of spike protein SARS-CoV-2 were carried out to identify the compound having highest binding affinity with target protein in 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 phytoconstituent is described in Tables 1 and 2. The binding orientation for each phytoconstituents 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 phytoconstituent against target SARS-CoV-2 spike protein. Among the docking studies performed on phytoconstituent, 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 Kudarsha 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 phytoconstituents 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 phytoconstituents 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.

| 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 |
| | Geraniol(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 | −7.78 | −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 |
| Tragacanthola L | Stigmasterol(8) | −9.724 | −10.39 | −7.466 | −0.324 |
| | 3-(2,4-dimethoxyphenyl)-6,7-dimethoxy-2,3-dihydrochroomen-4-one(9) | −6.433 | −7.316 | −9.011 | −0.247 |
| Anacyclus pyrethrum | Squalene(10) | −9.722 | −10.187 | −1.389 | −0.324 |
| | γ-Stotetol(11) | −9.956 | −10.521 | −7.679 | −0.332 |
| Androgaphis paniculata | Andrographan(12) | −6.819 | −7.678 | −7.854 | −0.296 |
| | 5-Hydroxy-7,8-dimethoxyflavanone(13) | −7.356 | −7.966 | −9.035 | −0.334 |
| Hygrophila 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 |
| | Cissampelos(20) | −6.42 | −7.227 | −9.228 | −0.279 |
| | Chrysosporum(21) | −7.954 | −8.352 | −11.392 | −0.362 |
| | 6-Methoxykumkwanin(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 |
| | Eromiel(25) | −6.587 | −6.696 | −5.43 | −0.412 |
| Tinospora cordifolia (Willd.) Miers ex Hook.f&Thoms | Tinosponnone(26) | −7.043 | −7.434 | −8.145 | −0.293 |
| Clorodendrum 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 |
| | Cycolamine(30) | −6.184 | −8.214 | −3.432 | −0.134 |
| Cypress rotundus L | Cyperenine(31) | −6.024 | −6.225 | −3.558 | −0.402 |
| | β-selinenine(32) | −6.33 | −6.587 | −3.412 | −0.422 |
| **JACOM Formulation** | | | | | |
| Justicia adhatoda L | Vasicinone(33) | −5.19 | −6.1 | −7.67 | −0.37 |
| Carica Papaya | Quercetin(34) | −8.408 | −8.59 | −11.478 | −0.382 |
| Androgaphis paniculata Burm.f.Lnees | 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 Docking studies of all the phytochemicals from two formulations, Kabasura Kudineer and JACOM into the active site of SARS-CoV-2 spike protein and corresponding hydrophobic interaction models, number of hydrogen bonds were 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.

| Compound Code | LF Rank Score | Interactions | Hydrophobic |
|---------------|---------------|--------------|-------------|
| β-sesquistereol(1) | -2.65 | NHB | Ser373, Phe374 |
| β-sisibolene(2) | -2.8 | Phe342, Ser373, Asp364, Val367 | Gly339 |
| Geranial(3) | -2.12 | NHB | Ser373, Phe374 |
| Piperine(4) | -4.14 | Phe374, Trp346 | Ser373, Phe342, Asn343, Gly339, Leu335, Val367 |
| Piperlonguminine(5) | -4.24 | Phe338 | Ser373, Phe342, Asn343, Gly339, Leu335, Val367 |
| Eugenol(6) | -6.18 | Asn343, Phe342, Asn343, Gly339, Leu335, Val367 | Ser373 |
| β-Caryophyllene(7) | -3.20 | NHB | Phe338, Gly339 |
| Stigmasteryl(8) | -7.46 | Cys336, Gly339 | Phe342, Asn343, Gly339, Leu335, Val367 |
| 3-(2,4- dimethoxyphenyl)-6,7- dimethoxy-2,3-dihydrochromen-4-one(9) | -9.01 | Arg509, Trp346 | Phe342, Asn343, Thr345, Ala344, Leu441 |
| Squalene(10) | -1.38 | NHB | Thr345, Asn343, Gly339, Leu335, Val367 |
| γ-Sitosterol(11) | -7.67 | Cys336, Gly339 | Ser373, Phe374, Val510 |
| Andrographalin(12) | -7.85 | Asn343 | Phe342, Leu335, Asp364 |
| 5-Hydroxy-7,8-dimethoxyflavanone(13) | -9.03 | Asp364, Gly339 | Cys336, Phe337, Leu335, Asp342, Gly339, Leu368 |
| Lupeol(14) | -6.41 | Thr345 | Asn343, Thr345, Phe374, Gly339, Leu368 |
| Betulin(15) | -7.02 | Thr345, Ser373 | Asn422, Arg509, Phe373, Thr345 |
| Chebulagic acid(16) | -9.72 | Tyr369, Asn370, Phe338, Gly339, Cys379, Lys378 | Val397, Asn334, Gly339, Ala397, Val367 |
| Gallic acid(17) | -6.91 | Lys356, Val341 | Ala397, Val341, Gly339, Leu335 |
| Vasicine(18) | -8.16 | Cys336, Gly339 | Val397, Asn334, Gly339, Ala397, Val367 |
| Carvacrol(19) | -6.92 | Asp364 | Cys336, Leu335, Asp364 |
| Cirsimaritin(20) | -9.22 | Cys336, Asp346, Ser373, Asn343 | Phe338, Phe342, Phe374, Ser373 |
| Chrysoeriol(21) | -11.39 | Cys336, Gly339, Asp364, | Phe338, Phe342, Phe374, Leu335, Val367, Ser373 |
| 6-Methoxyenokwainon(22) | -9.29 | Cys336, Phe342 | Ser373, Phe374, Leu335, Val367, Gly339, Leu335 |
| Luteolin(23) | -11.15 | Asp364, Val367, Val367, Ser373 | Cys336, Gly339, Leu335, Val367, Ser373 |
| Costunolide(24) | -3.79 | Phe515, Gly431 | Val511, Phe515, Gly431 |
| Elemol(25) | -5.43 | Asp364, Gly339, Asp364 | Val431, Asp342, Gly339, Gly339 |
| Tinosponone(26) | -8.14 | Phe342, Gly339 | Val342, Gly339, Gly339, Leu335, Val367 |
| Bharangol(27) | -6.88 | Phe338, Gly339 | Val338, Gly339, Gly339, Gly339, Leu335, Val367 |
| Scutellarein(28) | -10.27 | Cys336, Phe338, Gly339, Asp364, Val362 | Val338, Gly339, Gly339, Gly339, Leu335, Val367 |
| Magnololmine(29) | -9.76 | Arg346, Val341, Thr345 | Ala344, Gly339, Leu335, Ala397 |
| Cyclicarne(30) | -3.43 | Ser373 | Phe342, Trp346, Leu335 |
| Cyperene(31) | -3.55 | NHB | Ser373 |
| β-selinene(32) | -3.41 | NHB | Phe342, Ser373 |
| JACOM Formulation | | | |
| Viscadin(33) | -7.67 | Phe338, Asn343 | Gly339 |
| Quercetin(34) | -11.47 | Asp364 | Phe338, Leu335, Gly339, Gly339, Gly339, Leu335, Gly339, Leu335, Gly339, Leu335, Val367 |
| Andrographolide(35) | -7.85 | Asp364, Phe368, Gly339, Asn343 | Cys336, Phe342, Leu335, Val367 |
| Ursolic acid(36) | -5.1 | Val367 | Leu368 |
| Melacin(37) | -5.14 | Phe338 | Val367, Ser371, Leu368, Phe338 |
| Hydroxychloroquine(38) | -8.35 | Phe342, Asn343 | Gly339, Phe338, Leu368, Trp346, Ser373, Phe374 |

**Table 2**

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

Arg346, Val341, and Thr345 have played important role in the formation of H-bond network. The possible binding orientation of phyto compounds 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 were 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.

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
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, Andrograparin and Tinosponone have the highest gastrointestinal absorption, tissue distribution (Vd), and respectable total clearance.
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.
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 reinitate 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-dimethoxyflavone(-9.03), Tinosponone(-8.14), Cirsimaritin(-9.22), Chrysoeriol(-11.39), 6-Methoxykwanin(-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 amboinicus) 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. Based on these results, we proposed a novel herbal formulation called “SNACK-V” (Sida acuta, Andrographis paniculata, Tinospora Cordifolia, Costus speciosus, Plectranthus amboinicus) 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. Based on these results, we proposed a novel herbal formulation called “SNACK-V” (Sida acuta, Andrographis paniculata, Tinospora Cordifolia, Costus speciosus, Plectranthus amboinicus) 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. Based on these results, we proposed a novel herbal formulation called “SNACK-V” (Sida acuta, Andrographis paniculata, Tinospora Cordifolia, Costus speciosus, Plectranthus amboinicus) 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.

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. amboinicus 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

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

Table 3: Proposed SNACK –V formulation containing plants and their phytoconstituents with Dock score.
“SNACK-V” which contains nine phytoconstituents from the six plant herbs.

Conflict of interest
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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