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Indian herbal formulation Kaba Sura Kudineer possesses the most powerful ligands to block ACE2-RBD interaction of SARS-CoV-2 infection

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Medicinal herbs play an important role in the primary health care system of developing countries and always uphold the importance of ethnomedicinal studies in the drug discovery process. Indian Siddha practitioners urged people to consume a polyherbal formulation named 'Kaba Sura kudineer (KSK)' as a prophylactic measure against COVID-19. To validate the presence of anti-COVID 19 agents if any in KSK, virtual screening of 80 phytochemicals was done by blind docking, fixing the RBD-ACE2 complex as the target using PyRx software. The binding energy of the compounds was calculated using Autodock Vina. The SWISSADME server was used to identify the phytochemicals that obey the Lipinski rule and the blood-brain barrier permeability of the compounds. The outcome of the study revealed that phytochemicals such as diosgenin, diosgenone, coumaperine, bisdemethoxycurcumin, tinocordifolin, isovanillinand 1,8-Cineole displayed hydrogen bond interactions with the complex residues in the interacting site (−8.9 to −5.1 kcal/mol) and were found to obey the Lipinski rule as well as possess blood-brain barrier (BBB) permeability. Based on the highest docking score and the more number of interacting residues at the active site herein we suggest diosgenin and bisdemethoxycurcumin as potential inhibitors of SARS-Co-V-2.

Keywords: COVID-19, Herbal medicine, Kaba sura kudineer, Molecular docking, Phytochemicals

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The World Health Organization (WHO) dashboard revealed that 93,805,612 confirmed cases of COVID-19, including 2,026,093 deaths, have been reported worldwide as of 18th January, 2021. In India, from 3 to 18 January, 2021, there have been 10,571,773 confirmed cases of COVID-19 with 152,419 deaths as per the data available (https://covid19.who.int/region/searo/country/in). Medicinal herbs play an important role in the primary health care system of developing countries like India and always uphold the importance of ethnomedicinal studies in the drug discovery process. Antiviral drug discovery is always challenging as it poses a threat to the people who come in contact with the virus. For a virus that becomes pandemic, the challenges become multifold. Medicinal plants contain extractable biochemical and bioactive compounds, which can target certain viruses or can cure or prevent several viral diseases and infections[1]. Ayurveda and Siddha practices that originated in ancient India are still being widely used by both the rural and urban communities in various parts of the country. With the application of modern science and technology, it is plausible to effectively characterize bioactive and thereby the medicinal herbs as anti-infective agents. It will also uphold the value of traditional medicine which will be cost-effective with less or no adverse effects.

Kaba Sura Kudineer (KSK), is an Indian Siddha formulation that was highly valued by the Government of Tamil Nadu, India in their campaign as a prophylactic agent, as the government believed prevention is better than cure. Ministry of Ayush India, recommended KSK, as an add-on intervention to conventional care in the management of COVID-19[(2)]. In the name Kaba Sura Kudineer (KSK) the term kabam means 'cold/phlegm' and suram means 'a fever', People across Tamil Nadu were directed to

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consume this KSK as it was claimed to boost the immune system due to the presence of fifteen herbs with potent pharmacological properties. However, the efficacy of KSK against COVID-19 and whether the formulation possesses any anti-viral property or agents against SARS-CoV-2 is yet to be validated. The present study is aimed to discover whether any of the phytoconstituents that present in this herbal formulation could exhibit anti- SARS-CoV-2 properties by performing *in silico* molecular interactions between the phytochemical (ligand) and RBD-ACE2 complex (target). This is the first study that employed the whole RBD-ACE2 complex as a target to screen phytochemicals from KSK and uses the Lipinski rule to identify drug-like molecules.

Materials and Methods

Drug target preparation

To study the inhibitory effect of Kaba Sura Kudineer (KSK) against SARS-CoV-2 and ACE2 (Angiotensin-converting Enzyme-2) interaction, the complex structure of SARS-CoV-2 spike receptor-binding domain with ACE2 was considered. The crystal structure of the complex was taken from the RCSB (6M0J.pdb)[3]. The sequence length of ACE2 was 603 while that of spike RBD was 229. The ligands present in the crystal structure, namely NAG, ZN and CL, were removed. The missing residues in ACE2 (616-621) and RBD (319-332 and 527-547) were added using CHARMM-GUI[4]. The macromolecular complex structure was prepared for molecular docking in the PyRx software[5]. The interaction between the protein ACE2 and spike RBD was calculated using PDBSum[6] (Table 1).

Ligand preparation

The key compounds present in each ingredient of KSK mentioned in (Supplementary material) Table S1 were considered for virtual screening. There were a total of 109 molecules and the available 3D structures for these molecules resulted in 80 being taken from PubChem[7] in the single SDF file format.

Molecular docking using Autodock Vina in PyRx and drug properties

After the preparation of macromolecule and ligand, blind docking was performed for 80 molecules against the complex using PyRx software. The parameters set are the conjugate gradient (CG) optimization of 15000 steps. The grid dimensions were set at a maximum of 61.7Å x 72.1Å x140.9Å with a grid center defined at −26.57,+17.94,−11.63 in X, Y, Z directions, respectively. The molecules which pose in the interacting site of ACE2-spike RBD were considered for further analysis. The binding energy of the compounds was calculated using AutodockVina[8], the available module in PyRx software. Figure 1 shows the molecular docking workflow to identify the effective phytochemicals in blocking the ACE2 and RBD interactions.

Lipinski rule and drug-likeness calculations

The SWISSADME server[9] was used to identify the phytocompounds that obey the Lipinski rule and have the blood-brain barrier permeability.

Results

Identification and optimization of the lead compounds present in KSK were performed through blind docking. Initially, the 3D structures of 80 compounds found in KSK were retrieved from the PubChem database and were blindly docked with the RBD-ACE2 complex as the target. Among these 80 compounds, only 18 compounds were found to be docked in the interacting site of RBD and ACE2 with binding affinity ranging from −12.5 to −3.3 kcal/mol (Table 2). It was observed that some molecules with good binding affinity were not having the perfect pose and those with good binding pose had a comparatively lower binding affinity.

Table 1 — The residues in human ACE2 interacting with the residues in RBD

S.No	ACE2	RBD
1	Glu35	Gln493
2	Lys31	Gln493
3	Asp30	Lys417
4	Gln24	Ala475, Asn487
5	Tyr83	Asn487, Phe486, Tyr489
6	Met82	Phe486
7	Leu79	Phe486
8	Phe28	Tyr489
9	Thr27	Tyr489, Phe456
10	His34	Tyr453, Leu455
11	Arg393	**Tyr505**
12	Glu37	**Tyr505**
13	Gly354	**Tyr505**, Gly502
14	Lys353	**Tyr505**, Gly502, Gly496, Asn501
15	Asn330	Thr500
16	Arg357	Thr500
17	Tyr41	Asn501, Thr500, Gln498
18	Asp355	Thr500
19	Gln42	Gln498, Gly446, Tyr449
20	Asp38	Tyr449

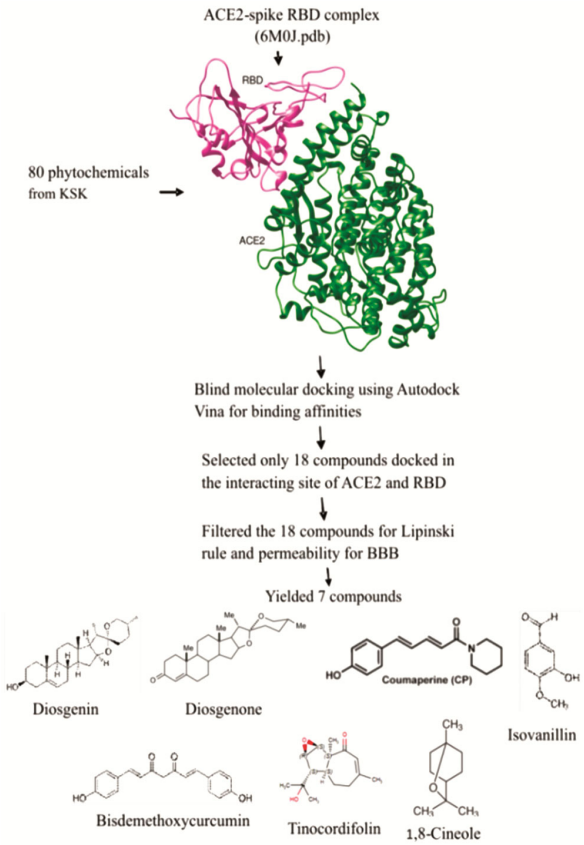

Fig. 1 — Shows the workflow of the molecular docking of phytochemicals to the ACE2-RBD complex.

Table 2 — Binding energy of the ligand to the interacting site of ACE2-RBD complex after blind docking

Name	Pubchem ID	Binding Energy (kcal/mol)
Serratagenic Acid	21594175	−12.5
Isovanillin	12127	−5.2
Coumaperine*	10131321	−6.7, −6.5, −6.4
Pyrrolidine	31268	−3.3
Bisdemethoxycurcumin	5315472	−6.6
Sitosterol-D-Glucoside*	71628	−7.7, −7.6
Iridin*	5281777	−8.0, −7.5
Quercetin-3-O-Rutinoside	5280805	−8.2
Tinocordifolin	100926540	−6.4
α-c*is*-Bergamotene	6429303	−6.2
1,8-Cineole	2758	−5.1, −4.6
Diosgenin*	99474	−8.9, −8.4
Diosgenone	10251134	−8.7
Lupeol	259846	−8.6
Chebulinic Acid	72284	−9.7
Casuarinin	157395	−10.6
Terchebulin*	16175789	−10.1,−9.8
Terflavin A	16175788	−10.2

*Ligand showing more than one pose interacts with the complex site and their energy for each site is given.

After filtering the compounds in the SWISSADME server, only 7 phytochemicals were found to obey the Lipinski rule and BBB permeability. Table 3 gives Lipinski's rule of five attributes for the selected 7 phytochemicals. The docked pose of the seven compounds was further analyzed and their binding affinity ranged from −8.9 to −5.1 kcal/mol. Each of these seven compounds has 1 to 5 hydrogen bond interactions and from 27 to 41 close-contacts with the complex in the interacting site, substantiating the inhibitory property of these ligands on RBD and ACE2 complex formation a vital initial step in SARS-COV2 infection. The compounds inhibiting the interaction of ACE2 and RBD were tabulated in Table 4. Further analysis revealed that these seven molecules displayed hydrogen bond interactions with the complex residues in the interacting site. Though the findings provide concrete information on the inhibitory property of these seven compounds in the formation of the RBD-ACE complex, the investigations on the dynamics of the phytochemicals in the complex structure will provide an in-depth understanding of this interaction mechanism between ligand and RBD-ACE2 complex and the dynamics study will be done in near future.

Tracing of those compounds based on their binding affinity, using their PubChem ID led to the identification of seven ligands viz., diosgenin (−8.9 kcal/mol), diosgenone (−8.7 kcal/mol), coumaperine (−6.7 kcal/mol), bisdemethoxycurcumin (−6.6 kcal/mol), tinocordifolin (−6.4 kcal/mol), isovanillin (−5.2 kcal/mol) and 1,8-Cineole (−5.1 kcal/mol). Figure 2 shows the binding pose of the ligands with the target complex. From Table 1, it was clear that the residues Y41 and K353 present in ACE2 were found to have strong interactions with the residues in RBD. The phytochemicals were found to have 27 to 38 strong interactions with the complex. Further ranking of phytochemicals revealed diosgenin and

Table 3 — The Lipinski's rule of five for the 7 phytochemicals

Phytochemicals	miLogP	TPSA	natoms	MW	nON	nOHNH
Diosgenin	4.52	38.69	30	414.62	3	1
Diosgenone	4.38	35.53	30	412.6	3	0
Coumaperine	2.84	40.54	19	257.33	2	1
Bisdemethoxycurcumin	1.75	74.6	23	308.33	4	2
Tinocordifolin	2.68	49.83	18	250.33	3	1
Isovanillin	1.44	46.53	11	152.15	3	1
1,8-Cineole	2.58	9.23	11	154.25	1	0

Table 4 — Binding affinity of the phytochemicals to the interacting site of ACE2-RBD complex

Name	Pubchem ID	Binding affinity (kcal/mol)	Rank	Total number of interactions with the complex residues
Diosgenin	99474	−8.9	1	38 (E: Gly485, Cys488)*
Diosgenone	10251134	−8.7	2	36 (A: Asn49, Lys68)*
Coumaperine	10131321	−6.7	3	33 (A: Asn33, Lys353; E:Gly496)*
Bisdemethoxycurcumin	5315472	−6.6	4	33 (A: Arg393; E: Gln409, Gly496, Tyr505)*
Tinocordifolin	100926540	−6.4	5	33 (A: Gln76)*
Isovanillin	12127	−5.2	6	32 (A: Gln76; E: Asn487, Tyr489)*
1,8-Cineole	2758	−5.1	7	27 (A: Gln76)*

*Intermolecular hydrogen bond interaction of lead compounds with chain A (ACE2) and chain E (RBD) is given.

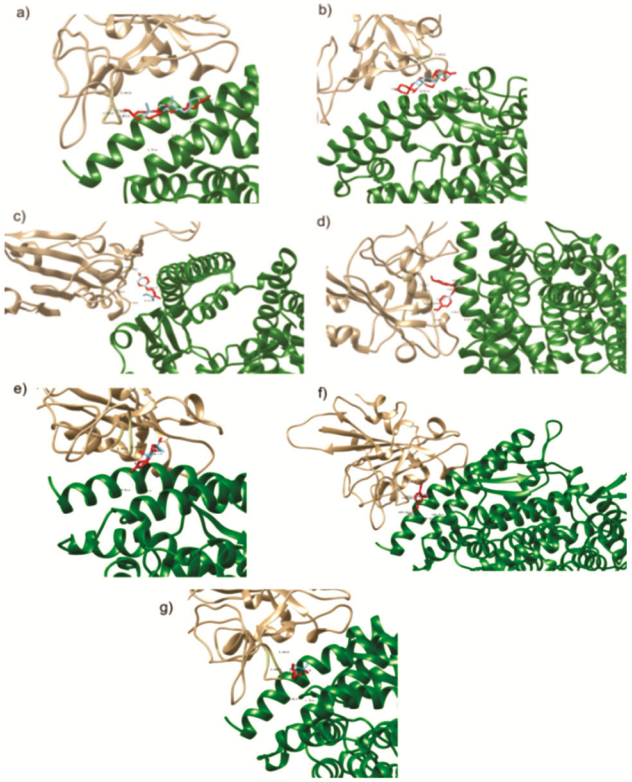

Fig. 2 — Depicts the interaction of the ligands in the interacting site of the ACE2-RBD complex a) Diosgenin b) Diosgenone c) Coumaperine d) Bisdemethoxycurcumin e) Tinocordifolin f) Isovanillin and g) 1,8-Cineole respectively. The interacting residues in ACE2 (chain A: Green) and RBD (chain E: Light Brown) are shown.

bisdemethoxycurcumin as potential anti-SARS Co-V-2 agents based on the docking score and number of interacting residues.

Discussion

The novel coronavirus disease (COVID 19) caused by the SARS-Co-V-2 remains in upsurge day by day increasing the morbidity and mortality mostly due to lung pathology[10]. In humans, transmission occurs via droplets as with other respiratory viruses and the virus is reported to survive on different surfaces for days and remains alive in aerosols for hours[11]. WHO insists to follow frequent personal hygiene and social distancing to minimize viral exposure. The infection rate of nSARS-COV2 outstripped the previous outbreak caused by the SARS-COV and MERS-COV though COVID-19 has many clinical features similar to SARS. It has been established that SARS-COV2 shares sequence homology with the SARS-COV and a bat coronavirus[12]. Despite its analogy to SARS-COV, the transmission efficiency and diagnostic methods of SARS-COV2 are rather contrasting which is probably due to the nucleotide changes in the spike (S) protein and its receptor-binding domain (RBD) which makes the drug development strategy an arduous one.

Like the other members of the coronavirus family that caused SARS and MERS, SARS-Co-V2 is also shown to use the angiotensin-converting enzyme 2 (ACE2) a member of the ACE family of dipeptidyl-carboxydipeptidase and is highly homologous to ACE1 as its receptor for infecting humans[13]. The report showed that the ACE2-binding affinity of the receptor-binding domain (RBD) in the S1 subunit of S protein of SARS-CoV-2 is 10 to 20 fold higher than that of SARS-CoV[14]. The signature spike protein named S protein comprising two subunits S1 and S2 of the coronavirus is the key for the viral entry into human cells via ACE2. This is initiated by the RBD of the S1 subunit in S protein, followed by the self-folding of the S2 subunit that brings the interaction of the heptad repeat 1 (HR1) and 2 (HR2) domains of the S2 subunit forming a six-helix bundle (6-HB) fusion core that facilitates the proximity of the viral and cellular membranes for fusion and infection[15]. Infection of SARS-CoV-2 is reported to down-regulate ACE2 expression in lung cells causing dysfunction of the renin-angiotensin system that

results in the disturbances of blood pressure, fluid/electrolyte balance associated with increased inflammation and vascular permeability in the airways[16]. Drug development strategies targeting the RBD-ACE2 complex will be of great potential as the mechanism of cell entry reveals that the formation of the RBD-ACE2 complex is the initial step of infection.

Drug repurposing is an effective strategy in discovering or developing drug molecules with new pharmacological targets or therapeutic indications. As drug repurposing helps to overcome the issues associated with preclinical development and optimization, it saves time and reduces expenses as well as failures typically associated with the drug discovery process[17]. The application of computer-aided drug design also potentiates drug repurposing as it gives cues about the functional probability of the drugs. In drug repurposing, structure-based approaches have been extensively used to analyze and predict the activity of ligands using proteins as targets. In the current COVID-19 scenario, where there is an accelerated drug discovery process as no proper drug is identified yet to fight against SARS-Co-V-2, this computer-aided drug discovery becomes indispensable due to time constraints. Molecular docking, a versatile tool used to predict the geometry and to score the interaction of a protein in complex with a small-molecule ligand[18] is employed in the present study to identify the anti-SARS-Co-V-2 phytochemical from the herbs of KSK targeting RBD-ACE2 complex genesis a key process involved in the mechanism of cell entry by SARS-Co-V-2.

Among these phytochemicals, the first two ranked compounds diosgenin and diosgenone were from the herb *Costus speciosus* (Koen Ex. Retz) Sm. (Family-Costaceae*)* (Venkottam/ Crepe Ginger) and the next two compounds Coumaperine and Bisdemethoxycurcumin were from the herb *Piper longum L*. (Thippili/ Long pepper). While the other compounds tinocordifolin, isovanillin, and 1, 8-Cineole was from three different herbs *Tinospora cordifolia* (Willd.) Miers*, Zingiber officinale* Roscoe *and Cyperus rotundus* Lrespectively. *Diosgenin* ($C_{27}H_{42}O_3$) a C27 spiroketal steroid sapogenin belonging to a family of spirostanol steroidal compounds has the highest docking score. Other than *Costus speciosus* used in the polyherbal formulation KSK, diosgenin is also present in several plants, namely *Dioscorea* spp., *Trigonella* spp., and *Smilax* spp. A series of preclinical and mechanistic studies have been performed to understand the real importance and benefits of diosgenin against a variety of pathologies including metabolic diseases (diabetes, obesity and dyslipidemia, including hypercholesterolemia), inflammatory diseases, and cancer as reviewed by Jesus[19]. Diosgenin resembles the steroidal structure of glucocorticoids like dexamethasone, a synthetic glucocorticoid with anti-inflammatory and immunosuppressant activities. Junchao *et al*.[20], reported that diosgenin, by its ability to induce the expression of glucocorticoid receptors (GRs), downregulated the pro-inflammatory cytokines TNF-a, IL-1b and IL-6 and exhibited anti-inflammatory effects in the trachea of asthmatic mice. Though diosgenin displayed the highest score and active site interaction with RBD-ACE amino acid residues Gly485, Cys488) followed by its derivative diosgenone, the phytochemical bisdemethoxycurcumin also presents as a potential anti-COVID-19 agent because of its ability to interact with four amino acid residues (Arg393; Gln409, Gly496, Tyr505 in RBD). Bisdemethoxycurcumin, from *Piper longum* used in the polyherbal formulation KSK is well known as an active ingredient of *Curcuma longa,* had already been reported to suppress TNF-induced NF-κB activation a key inflammatory signaling pathway[21]. Bisdemethoxycurcumin interacts strongly with Tyr505, a key residue in RBD that interacts strongly with the residues Glu37, Lys353, Gly354 and Arg393 in ACE2. These findings substantiate that this polyherbal formulation might act against SARS-Co-V2 infection. The majority of the Indian population relies on medicinal herbs as they are a good alternate for curing many diseases[22]. However considerable knowledge accumulated by the villagers and tribals on herbal medicine remains unknown to the scientists[23] and hence these herbal remedies must undergo preclinical and clinical investigations to establish their immense therapeutic value. In this context, our preliminary findings open a novel perspective to test diosgenin and bisdemethoxycurcumin either as an individual or as synergistic phytochemicals against the novel coronavirus SARS-Co-V-2.

Conclusion

In silico screening of key phytochemicals from the medicinal herbs of a polyherbal formulation "Kaba Sura Kudineer" identified seven phytochemicals as drug-like candidates. Out of seven, based on the high molecular docking score and the more number of

interaction residues, the two phytochemicals diosgenin from *Costus speciosus* and bisdemethoxycurcumin from *Piper longum* are suggested as potential inhibitors and the findings warrant appropriate preclinical and clinical investigations to develop these phytochemicals as anti-COVID-19 agents.

Supplementary Data

Supplementary data associated with this article is available in electronic form at http://nopr.niscair.res.in/jinfo/ijtk/IJTK_20(04)(2021)885-890_SupplData.pdf

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Conflict of Interest

The authors declare that there is no competing or conflict of interest.

Authors' Contributions

SN and RL designed the concept and wrote the manuscript with significant inputs from SM. RL performed the *in silico* analysis and edited the final draft of the manuscript. All contributing authors have agreed to submission of this manuscript for publication.

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