Ind. J. Pharm. Edu. Res., 2025; 59(2):682-694.
https://www.ijper.org

Original Article

Network Pharmacology-Based Modeling of Phytocompounds in a Traditional *Siddha* Formulation-*Kabasura kudineer* with Special Reference to SARS-CoV-2

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ABSTRACT

Background: *Kabasurak kudineer* (Kk), a traditional *Siddha* formulation containing 15 plant-based ingredients has been prescribed in *Siddha* medicine for the management and treatment of flu-like symptoms. Currently, the Ministry of AYUSH, Government of India has been prescribing Kk as a possible preventive and prophylactic formulation against COVID-19. **Objectives:** The present study focuses on computational methods and aims to identify host targets in different pathways where the phytocompounds of Kk possibly interact. **Materials and Methods:** Using the curated list of phytocompounds from 15 plants obtained from existing literature, each compound was searched against the Pubchem database and downloaded using which probable host targets were predicted in Swiss Target Prediction and Binding database. Probability scores of 0.5 and above was selected as high-confidence targets and a score of less than 0.5 as low-confidence targets. **Results:** We constructed a network of compounds and their possible interacting targets and identified three pathways, namely Phosphatidylinositol 3-Kinase (P13K/AKT), Repressor Activator Protein 1 (Rap1) and Mitogen Activated Protein Kinase (MAPK) pathways that the phytocompounds possibly modulate. Additionally, we performed molecular docking simulations for the phytocompounds with target's 3D structure to map the molecular interaction and ascertain their role in antiviral treatment, where we found ellagic acid as the best binder to the targets of these three pathways. **Conclusion:** Using a network pharmacology approach this study demonstrated that Kk can modulate three important molecular pathways (P13K/AKT, Rap1 and MAPK) associated with COVID-19 and this could be due to the effect of the phyto ingredient ellagic acid.

Keywords: COVID-19, Ellagic acid, *Kabasurak kudineer*, Molecular docking, Network Pharmacology.

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Received: 18-02-2024;
Revised: 05-08-2024;
Accepted: 29-01-2025.

INTRODUCTION

A sudden outbreak of pneumonia, initially observed in the Wuhan city of China's Hubei province in December 2019, gradually became a novel strain of coronavirus-SARS-CoV-2, a positive stranded enveloped RNA virus belonging to the Corona viridae family. What started as a relatively unsuspecting epidemic slowly started spreading throughout the globe across countries and territories and was subsequently declared by the World Health Organization (WHO) as a pandemic on 11 March (https://bestpractice.bmj.com/topics/en-gb/3000165; https://www.who.int/news-room/articles-detail/updated-who-advice-for-international-traffic-in-relation-to-the-outbreak-of-the-novel-coronavirus-2019-ncov-24-jan/; https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports).

While, it is known that the Angiotensin-Converting Enzyme-2 (ACE2) is the preferred host receptor for the virus to bind and gain entry, the role of host proteins in the cytoplasm and in various organelles towards the virus life cycle, specifically in the replication, production and maturation are explained in detail here.[1] Recently, the SARS-CoV-2 and host protein interactome and the X-ray and cryo-EM complex of ACE2 with the Spike Glycoprotein (S Protein) were published, that showed the

DOI: 10.5530/ijper.20256024

specificity of S protein for ACE2 was high with a binding affinity of 15 nM.[2,3] This implies that targeting the S protein with small molecule inhibitors is relatively difficult because the inhibitor has to competitively bind to ACE2 and its binding affinity should be relatively higher than the spike protein.

SARS-CoV-2 and Its Pathogenesis

SARS-CoV-2, a member of the Betacoronavirus genus, exhibits a single-stranded positive-sense RNA genome of approximately 30 kilobases.[4] This extensive genome is capable of encoding both structural and non-structural proteins critical to viral replication and infection. The virus utilizes its Spike (S) glycoproteins to attach to the Angiotensin-Converting Enzyme 2 (ACE2) receptors on host cells, a process further facilitated by the Host Transmembrane Protease Serine 2 (TMPRSS2).[5] Upon entry, the viral RNA is translated into polyproteins, which are subsequently processed by viral proteases such as 3CLpro and PLpro to yield functional non-structural proteins that drive the replication and transcription machinery. This process generates subgenomic RNA for structural protein synthesis, leading to the assembly of virions that bud from the host cell.[6-8]

The pathogenesis of COVID-19 is closely tied to both the direct cytopathic effects of the virus and the host's immune response. Viral replication in the respiratory epithelium initiates a cascade of inflammatory signaling, leading to the recruitment of immune cells and the release of cytokines. Severe cases are often characterized by dysregulated immune responses, including cytokine storms that contribute to widespread tissue damage, Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure.[9] This dual aspect of viral and host-mediated pathology underscores the need for therapeutic strategies that target both the virus and the inflammatory processes it induces.

Therapeutic Targets Involved in SARS-CoV-2 Infection

One hallmark of severe COVID-19 cases is the cytokine storm, an exaggerated immune response marked by the excessive release of pro-inflammatory cytokines such as Interleukin-6 (IL-6), Tumor Necrosis Factor-Alpha (TNF-α) and interferons. This hyperactivation of the immune system can lead to systemic inflammation, endothelial dysfunction and organ failure.[10-12] Therapeutic approaches targeting the cytokine storm include IL-6 inhibitors like tocilizumab and JAK-STAT pathway inhibitors, which aim to restore immune homeostasis.

Potential therapeutic targets also include viral proteins essential for replication, such as RNA-dependent RNA Polymerase (RdRp), the main Protease (3CLpro) and Papain-like protease (PLpro).[13] RdRp inhibitors like remdesivir mimic nucleotide substrates, halting viral RNA synthesis. Similarly, inhibitors targeting 3CLpro disrupt the processing of polyproteins necessary for viral assembly[14-16] These targets, alongside strategies to block the ACE2-S protein interaction and prevent viral entry, represent a multifaceted approach to mitigating SARS-CoV-2 infection. By combining antiviral agents with therapies modulating the immune response, the severity and progression of COVID-19 can be effectively managed.

The list of interacting viral-host proteins, called as interactome, has been performed for many corona viridae family of viruses, such as SARS, MERS and most recently with COVID-19.[17] From the viral entry inside the cell and up to its exit as mature virions, there are multiple host proteins that interact and aid in the production of viral particles. Specifically, it has been reported that apart from the structural proteins, the non-structural proteins have numerous interactions. An interactome data shows 27 discrete networks with intra-connectivity of some host proteins that are closely related.[18] Thus, it is possible to identify pathways and host targets that get regulated after the virus enters the cell.

For managing and treating diseases like SARS-CoV-2, a multi-drug formulation that could bind with multiple targets associated with the disease and could exhibit a synergistic effect is the need of the hour. Here, the formulations from *Ayurveda* and *Siddha* become relevant as the formulations, in general, are multi-drug in nature working in a synergistic way. Hence, in the present study we have selected a natural *Siddha* formulation *Kabasurak kudineer* (Kk).

The clinical usage and dosage of Kk is prescribed in ancient Siddha texts. Specifically, Kk is used for treating *Kabasuram* (Swine flu), as mentioned in *Siddha* texts like *Theran karisal, Suravagadam, Yugi chinthamani* etc., with major symptoms that include body ache, fever, cough, fatigue, diarrhea, sore throat, shortness of breath and chest pain.[19-21] The *Siddha* manuscript *Citta vaittiyattirattu* (CVT) also mentions the usage of this formulation in treating *Aiyacuram* (Phlegmatic fevers). Previously Kk was effectively used to target host response during the outbreak of influenza virus (https://www.nhp.gov.in/swine-flu_mtl). 5 g of Kk powder can be mixed and boiled with 300 mL of water, reducing it to 30 mL and can be given with honey as vehicle, to the patients twice a day or as directed by the physician.[22] This formulation is made up of 15 botanical ingredients (Table 1).

Network pharmacology or Poly-pharmacology is a technique that brings systems biology and computer-based virtual high-throughput screening together to study, understand, illustrate and visualize the action of multi-component drugs and formulations and the concerted interactions of their ingredients with multiple targets.[66,67] This strategy could be used for better prediction of impending diseases and their subsequent preventive measures, to search and identify novel therapeutic targets, to re-purpose existing drugs and improve their safety and efficacy and to design new personalized drugs.[68,69] Exploration of traditional poly-herbal formulations and their synergistic action against multiple targets associated with diseases is a

well-documented approach in Chinese medicine[70-81] and is recently gaining momentum with regard to Indian traditional system of medicine (*Ayurveda* and *Siddha*) and medicinal plants too.[82]

With respect to Kk, recent reports talk about the *in silico* docking of phyto ingredients of Kk with the COVID-19 spike glycoprotein.[83] The studies report appreciable binding activity of the Kk active ingredients against the viral glycoprotein and both the studies have proposed that the effective action of the phytocompounds of Kk against the viral protein may be due to its immunomodulatory potential. However, there have been no reports on the molecular mechanistic action of Kk, its interaction, modulation or regulation of pathways and genes associated with SARS-CoV-2 till date to the best of our knowledge.

Hence, the present study is an attempt to carry out a network pharmacological approach to investigate the interactions between the phytoconstituents of Kk and multiple targets associated with SARS-CoV-2 and to comprehend the mechanistic action of the formulation.

MATERIALS AND METHODS

Materials

A network pharmacology model using the ingredients of *Kabasurak kudineer* and their bioactive compounds was developed using Cytoscape 3.2.1, a java based open-source software. The reported active compounds were created in 3D and saved in .sdf format. CORINA structure generator (https://www.mn-am.com/products/corina) was used to obtain the 3D structure of the phytocompounds. These were then queried in Binding DB (https://www.bindingdb.org/) database to get high ranked hits. These hits were then mapped to other target databases such as Uniprot, therapeutic target database and others.

Compound Screening

After the data collection from CVT and identifying the phytocompounds of the plants, they were searched against the PubChem database[84] to obtain the structure information in SMILES format. For those compounds having no structure in PubChem, they were not used in constructing the network construction and were marked separately. The next task was to identify the potential targets in the human body which would interact with the phytocompounds. For this purpose, Swiss target prediction server (https://www.swisstargetprediction.ch/) was used.[85] The potential targets were extracted along with the probability score of the compound that might bind with a particular receptor. An arbitrarily selected value of 0.5 was chosen as the threshold and any target below this threshold were classified as low confidence targets. Targets with a score of 0.5 and above were classified as high confidence targets.

Network Construction

To visualize the interactions between phytocompounds and their respective target proteins, a network was constructed using Cytoscape, a widely used network visualization platform.[86] In this network, the phytocompounds and their targets were represented as nodes, while the interactions between them were depicted as edges. This approach facilitated a comprehensive view of the molecular relationships.

Following the network creation, the structures of the host target proteins were retrieved for further analysis. The UniProt database served as the primary resource for obtaining structural information. For targets with listed structures in UniProt, several filtering criteria were applied to ensure the selection of high-quality data. These criteria included:

Method of structure determination: Preference was given to structures determined using X-ray crystallography or cryo-electron microscopy due to their higher resolution.

Resolution: Only structures with resolution values within an acceptable range (typically <2.5 Å) were selected to ensure structural accuracy.

Sequence length: Targets with complete sequences matching the experimental data were prioritized to avoid partial or truncated structures.

Domain information: The presence of well-annotated domains relevant to the target's function was considered essential.

Table 1: Ingredients of *Kabasurak kudineer* and their pharmacological activity.

Sl. No.	Ingredient Name	Botanical name	Part(s) used	Quantity	No. of Phytocompounds	Pharmacological activity
1	Dry ginger/*Chukku*	*Zingiber officinale* Roscoe	Rhizome	1	41 compounds.[23]	Dissolve phlegm associated with colds or chronic bronchitis, treat asthma and cough due to cold and coldness associated with shock.[24]
2	Long pepper/*Thippili*	*Piper longum* L.	Fruit	1	Fruit and Root-23 compounds.[25] Fruit-16 compounds.[25]	Decoction of immature fruits and roots is used in chronic bronchitis, cough and cold.[25]

Sl. No.	Ingredient Name	Botanical name	Part(s) used	Quantity	No. of Phytocompounds	Pharmacological activity
3	Cloves/Lavangam	*Syzygium aromaticum* (L.)	Flower bud	1	18 compounds[26]	Expectorant; Soothe certain respiratory conditions like cold, cough, asthma, bronchitis and sinusitis; helps in clearing the nasal tract.[27]
4	Climbing nettle roots/*Sirukanjori*	*Tragia involucrata* L.	Root	1	8 compounds.[28]	Leaf-effective in treating pain and bronchitis; Root-treatment of high fever.[29]
5	Pellitory/ *Akkarakaram*	*Anacyclus pyrethrum* (L.) Lag.	Root	1	10 compounds.[30]	Akarkara root exhibits anticatarrhal properties, i.e. it expels old catarrh.[30]
6	*Barleria* roots/*Neermulli ver*	*Hygrophila auriculata* (Schumach.) Heine	Root	1	2 compounds.[31]	Tonic for asthma.[31]
7	*Chebulic myrobalan*/*Kadukkai thole*	*Terminalia chebula* Retz.	Pericarp	1	13 compounds.[32,33]	Antitussive;[34] Used in treatment of asthma, cough, dyspnea.[35]
8	*Adhatoda* root/*Adhatoda* ver	*Justicia adhatoda* L.	Root	1	4 compounds.[36-38]	Bronchodilatory activity;[39] Used in treating chest and respiratory track infection;[40] treatment of whooping cough, chronic bronchitis, asthma and excessive phlegm.[41]
9	Indian borage/ *Karpooravalli*	*Coleus amboinicus* Lour.	Leaf	1	10 compounds.[42-45]	Used in treatment of chronic coughs, asthma, bronchitis and sore throat;[46] has bronchodilatory activity;[47] Used to treat catarrhal infections;[48] juice/decoction can be a worthy treatment for influenza, cough, bronchitis and throat problems.[49]
10	*Costus* root/*Kottam*	*Saussurea costus* (Falc.) Lipsch.	Root	1	26 compounds.[50]	Roots are used mainly as an antispasmodic in asthma, cough.[51]
11	*Tinospora* stem/*Seenthil*	*Tinospora cordifolia* (Willd.) Hook.f. and Thomson.	Stem	1	39 compounds.[52]	Can be given as adjuvant therapy in chronic bronchitis patients in addition to standard treatment.[53]
12	Beetle killer root/*Siruthekku*	*Rotheca serrata* (L.) Steane and Mabb.	Root	1	27 compounds.[54]	Root used in treatment of asthma.[55]
13	Nilavembu samoolam	*Andrographis paniculata* (Burm.f.) Nees	Whole plant	1	11 compounds.[56]	Good remedy as treatment for common respiratory infections such as colds and flu.[57]
14	Vattathiruppi Ver	*Cissampelos pareira* L.	Root	1	6 compounds.[58-63]	Used in treatment of fever.[64]
15	Nut grass tubers/*Koraikizhangu*	*Cyperus rotundus* L.	Rhizome	1	28 compounds.[65]	Tubers are used to treat cough, fever; Considered as an antispasmodic, antitussive; prescribed to treat bronchitis.[65]

This systematic process ensured the incorporation of reliable and high-quality structural data into the analysis, thereby enhancing the robustness of subsequent modeling and interpretation steps.

Molecular Modeling

For targets that did not have published structures, the Rosetta algorithm implemented in Robetta server[87] and Swissmodel server[88] was used for structure prediction, using a comparative modeling approach.

Assessing the quality of 3D modelled structures

The three-dimensional structures of the target proteins were generated using the Robetta and SwissModel servers.[89,90] To ensure the reliability and accuracy of the predicted structures, a systematic quality assessment was conducted.

For models generated by the Robetta server, only those with a confidence score of 0.8 or higher were retained for further analysis. This threshold ensured the inclusion of high-quality models with robust structural predictions. Similarly, the SwissModel-generated structures were evaluated using their associated quality scores, including the Global Model Quality Estimation (GMQE) and QMEAN scores. Models with GMQE values above 0.7 and favorable QMEAN Z-scores, indicating agreement with high-resolution experimental structures, were selected.

Additionally, structural integrity and stereochemical quality were validated using tools such as PROCHECK and MolProbity. These tools assessed parameters like Ramachandran plot distributions, bond angles and overall structural geometry, ensuring that the models adhered to standard protein structure quality benchmarks. This multi-step approach ensured that only the most reliable models were used for subsequent analysis.

Molecular Docking

To find out the interactions between the receptor and the ligand we did not specify the binding site but we used an unbiased approach of blind docking.[91,92] In this we, defined the 3D grid map of the AutoGrid step such that 95% of the target is defined as the binding site. This unbiased approach has been shown to give better results. An exhaustive molecular docking approach-with 100 dockings for each phytocompound was performed using the lamarkian genetic algorithm in AutoDock 4.2. The compiled data obtained from molecular docking is listed in Supplementary Table S1. The overall lowest energy confirmation of the ligand, from all 100 docked confirmations was extracted and the coordinates were converted into a pdb file. This lowest energy confirmation-ligand was visualized along with the macromolecule in pymol and

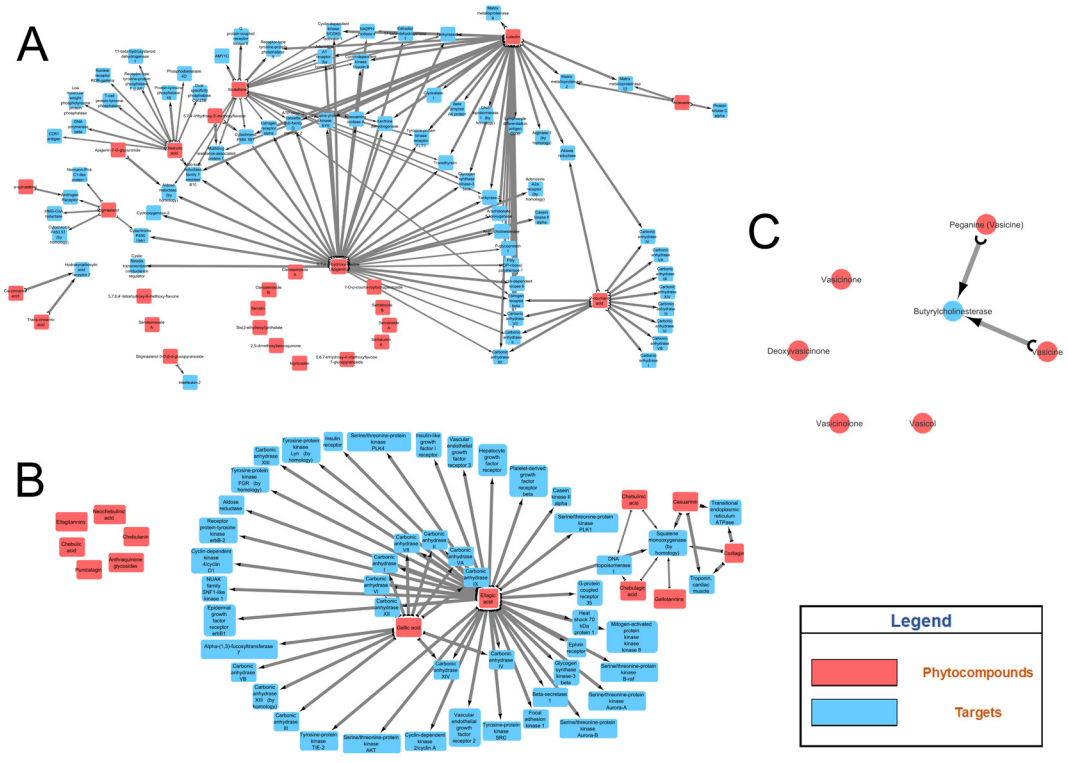

Figure 1: The networks of A) Beetle Killer root, B) *Chebulic myrobalan* and C) Adhatoda root the edges, represented in grey shows the interaction between a target and a phytocompound. The width of the edges depends on the potential binding probability score of the target with its phytocompound, the width increases as the probability score increases. The probability scores of potential targets for all the plants range from 0 to 1 classically, but after filtering them out, the high confidence targets range from 0.5 to 1. The plants have probability scores with their respective targets as following: A) Beetle Killer root-0.509 to 0.971, B) *Chebulic myrobalan*-0.874 to 1.000 and C) Adatoda root-1.000.

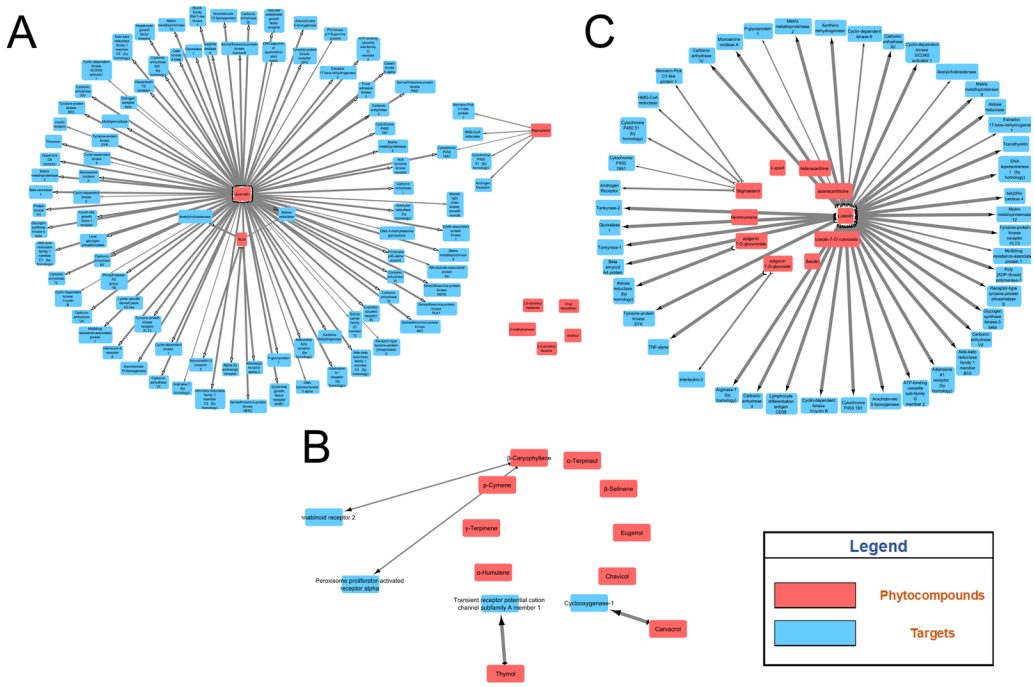

Figure 2: The networks of A) Climbing Nettle roots, B) Indian Borage and C) *Hygrophilla auriculata* (Schumach.) Heine The edges, represented in grey shows the interaction between a target and a phytocompound. The width of the edges depends on the potential binding probability score of the target with its phytocompound, the width increases as the probability score increases. The probability scores of potential targets for all the plants range from 0 to 1 classically, but after filtering them out, the high confidence targets range from 0.5 to 1. The plants have probability scores with their respective targets as following: A) Climbing Nettle roots-0.514 to 1.000, B) Indian Borage-0.699 to 1.000 and C) *Hygrophilla auriculata* (Schumach.) Heine-0.514 to 1.000.

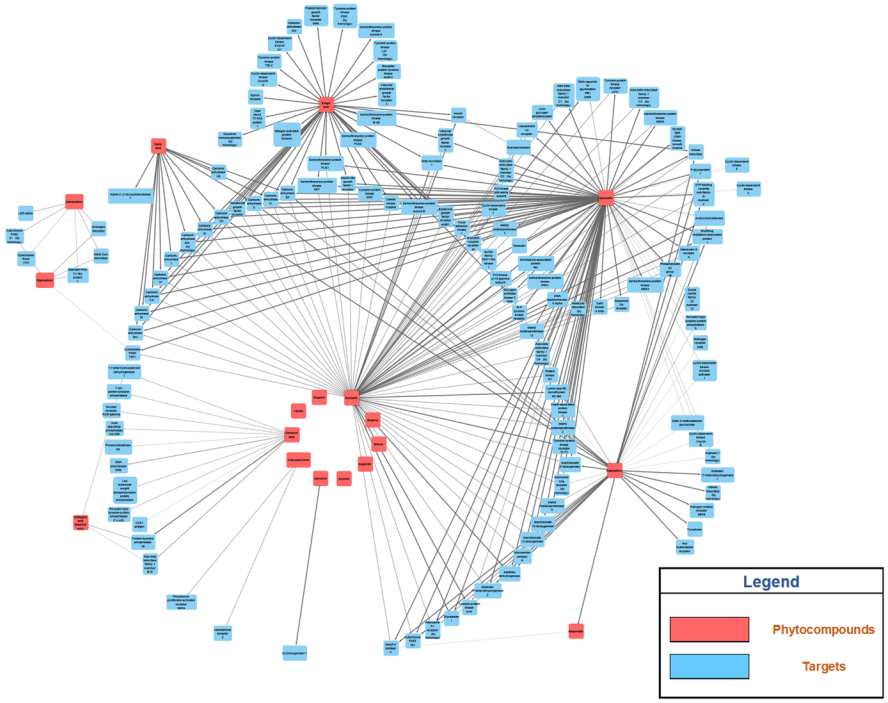

Figure 3: The network of Cloves The edges, represented in grey shows the interaction between a target and a phytocompound. The width of the edges depends on the potential binding probability score of the target with its phytocompound, the width increases as the probability score increases. The probability scores of potential targets for all the plants range from 0 to 1 classically, but after filtering them out, the high confidence targets range from 0.5 to 1. The probability scores for this network ranged from 0.501 to 1.000.

the interactions mapped. We mapped pdb ids into uniprot ids by using tool, Retrieve/ID mapping (https://www.uniprot.org/uploadlists/).

KEGG Enrichment Analysis

We mapped pdb ids into uniprot ids by using tool, Retrieve/ID mapping (https://www.uniprot.org/uploadlists/). These target proteins were considered for pathway analysis in StringDB[93] and KEGG Mapper.[94] In StringDB, the confidence score for interactions were 0.4, 0.7 and 0.9. The proteins interactions as evidenced by gene fusion and experimental data were selected. The pathways of these proteins were analyzed further. The proteins were taken as input in KEGG Mapper, a tool to map pathways. In KEGG Mapper, we used the search pathway option and we selected "hsa". We the proteins exported from StringDB were given as input, the important pathways were identified. The targets were selected for pathway reconstruction using Pajek software.[95]

RESULTS

The current study involved a network pharmacological approach to understand the plausible molecular mechanistic action of Kk in the treatment of SARS-CoV-2 by studying the interactions between phytoconstituents of Kk and multiple targets associated with SARS-CoV-2. A network interaction was constructed between the active ingredients of Kk, their multiple targets and the plausible pathways.

Network Pharmacology Scores

Adathoda root has about 6 phytocompounds of which only two of them share the only possible high confidence target for this plant (Figure 1A), *Chebulic myrobalan* has 13 phytocompounds and 48 targets (Figure 1B) and *Beetle killer* root has 27 phytocompounds and 70 targets (Figure 1C). The plants have probability scores with their respective targets as following: *Beetle killer*root-0.509 to 0.971, *Chebulic myrobalan*-0.874 to 1.000 and *Adathoda* root-1.000.

Climbing nettle roots have about 8 phytocompounds and 96 high confidence targets (Figure 2A), *Indian borage* has 10 phytocompounds and 4 targets (Figure 2B) and *Hygrophilla auriculata* has 10 phytocompounds and 44 targets (Figure 2C). The plants have probability scores with their respective targets as following: A) *Climbing nettle* roots-0.514 to 1.000, *Indian borage*-0.699 to 1.000 and *Hygrophilla auriculata*-0.514 to 1.000.

Clove (Figure 3) is the ingredient in this herbal concoction which has the greatest number of phytocompounds with most interacting targets and hence the largest network with 18 phytocompounds and 285 potential high confidence targets. The probability scores of potential targets for all the plants range from 0 to 1 classically,

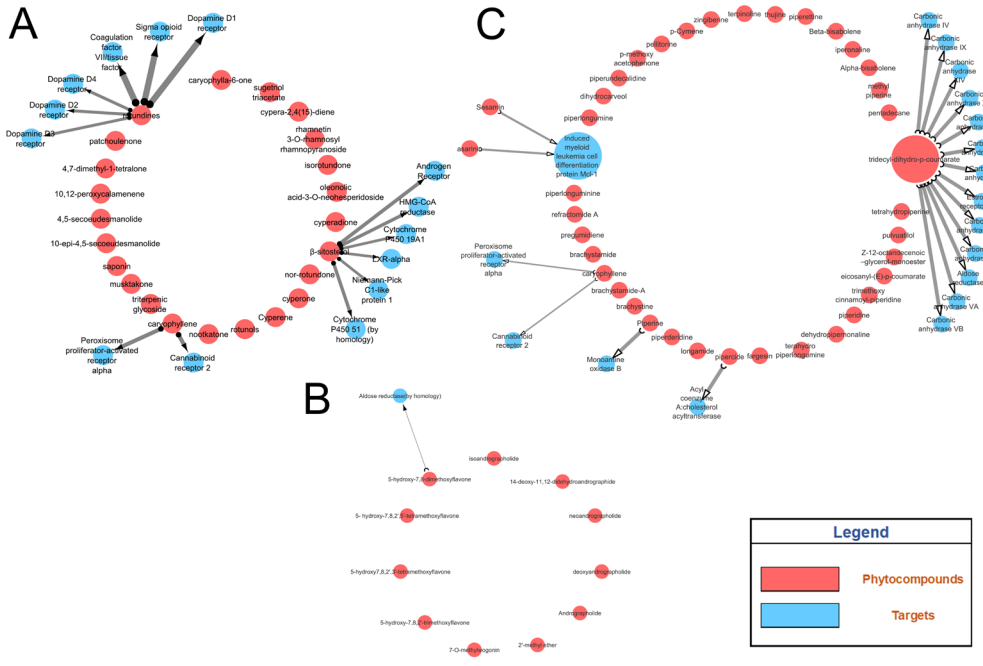

Figure 4: The networks of A) Nut Grass Tubers, B) *Nilavembu samoolam* and C) Long Pepper The edges, represented in grey shows the interaction between a target and a phytocompound. The width of the edges depends on the potential binding probability score of the target with its phytocompound, the width increases as the probability score increases. The probability scores of potential targets for all the plants range from 0 to 1 classically, but after filtering them out, the high confidence targets range from 0.5 to 1. The plants have probability scores with their respective targets as following: A) Nut Grass Tubers-0.555 to 0.967, B) *Nilavembu samoolam*-0.522 and C) Long Pepper-0.699 to 1.000.

but after filtering them out, the high confidence targets range from 0.5 to 1. The probability scores for this network ranged from 0.501 to 1.000.

Nut grass tubers have about 22 phytocompounds and 14 high confidence targets (Figure 4A), *Nilavembu samoolam* has 11 phytocompounds and only one potential high confidence target (Figure 4B) and *Long pepper* has 38 phytocompounds and 18 targets (Figure 4C). The plants have probability scores with their respective targets as following: *Nut grass* tubers-0.555 to 0.967, *Nilavembu samoolam*-0.522 and *Long pepper*-0.699 to 1.000.

Pellitory has about 10 phytocompounds and 2 high confidence targets (Figure 5A), *Vattathiruppi ver* has 6 phytocompounds and 8 targets (Figure 5B) and *Tinospora* stem has 46 phytocompounds and 58 targets (Figure 5C). The plants have probability scores with their respective targets as following: *Pellitory*-0.791 to 1.000, *Vattathiruppi ver*-0.504 to 0.879 and *Tinospora* stem-0.531 to 0.647.

Costus root and *Dry ginger* had no potential targets (Figure 6). Specifically, *Costus* root has 24 phytocompounds (Figure 6A) and the same is about 41 in the case of *Dry ginger* (Figure 6B).

Molecular Pathway Identification

The KEGG mapper analysis revealed that Kk could be involved in modulating 3 major pathways namely the Phosphatidylinositol 3-Kinase (P13K/AKT), Mitogen Activated Protein Kinase (MAPK) and Repressor Activator Protein 1 (Rap1) signaling pathways (Figure 7).

The proteins were mapped into three important pathways A) MAPK pathway B) PI3K AKT Pathway and Rap1 pathway. MAPK pathway, we have 18 proteins, colored in grey color. 8 proteins from our data have been highlighted. Similarly, PI3K-Akt pathway, we have 26 proteins, colored in grey color. 12 proteins from our data have been highlighted. PI3K-Akt pathway, we have 16 proteins, colored in grey color. 8 proteins from our data have been highlighted.

DISCUSSION

Over the centuries, *Siddha* medicines and formulations have been found to be effective in the treatment of various infectious diseases, the recent example being the use of the *Siddha* preparation *Nilavembu kudineer* against dengue.[96] Now, against the SARS-CoV-2, *Siddha* medicines are at the forefront again.

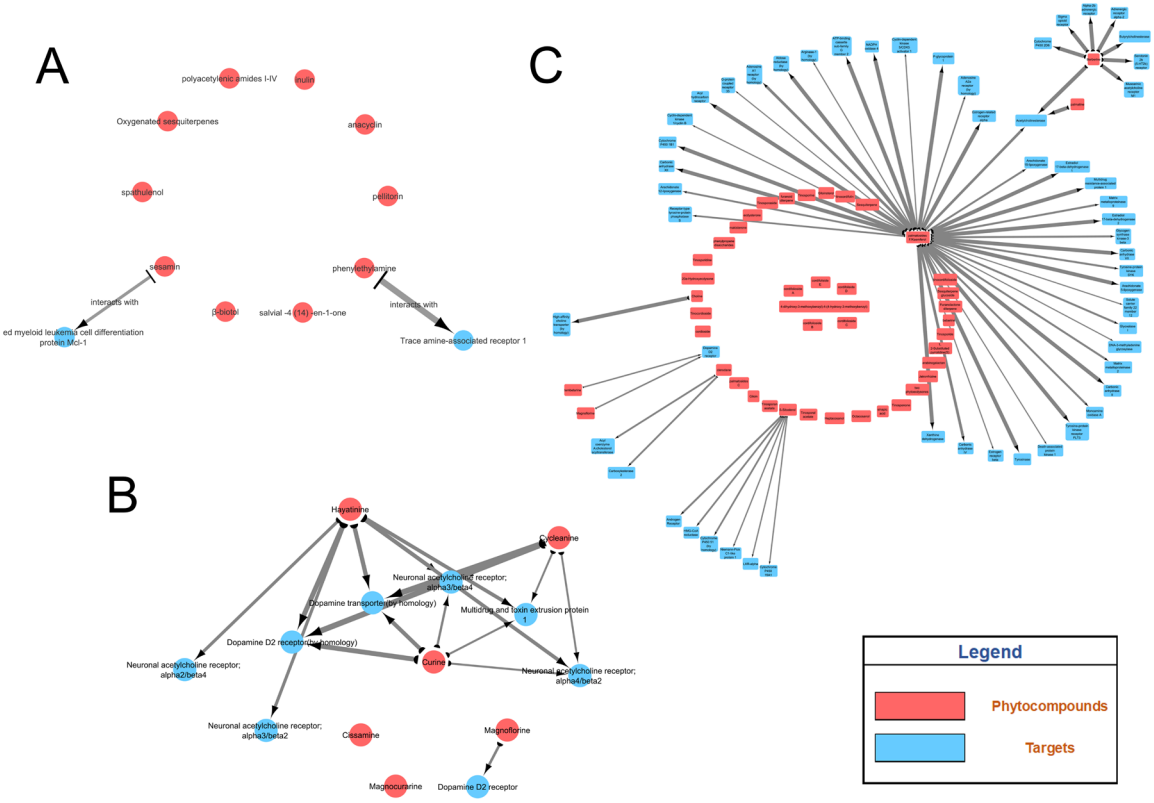

Figure 5: The networks of A) Pellitory, B) *Vattathiruppi Ver* and C) *Tinospora* stem the edges, represented in grey shows the interaction between a target and a phytocompound. The width of the edges depends on the potential binding probability score of the target with its phytocompound, the width increases as the probability score increases. The probability scores of potential targets for all the plants range from 0 to 1 classically, but after filtering them out, the high confidence targets range from 0.5 to 1. The plants have probability scores with their respective targets as following: A) Pellitory-0.791 to 1.000, B) *Vattathiruppi Ver*-0.504 to 0.879 and C) *Tinospora* stem-0.531 to 0.647.

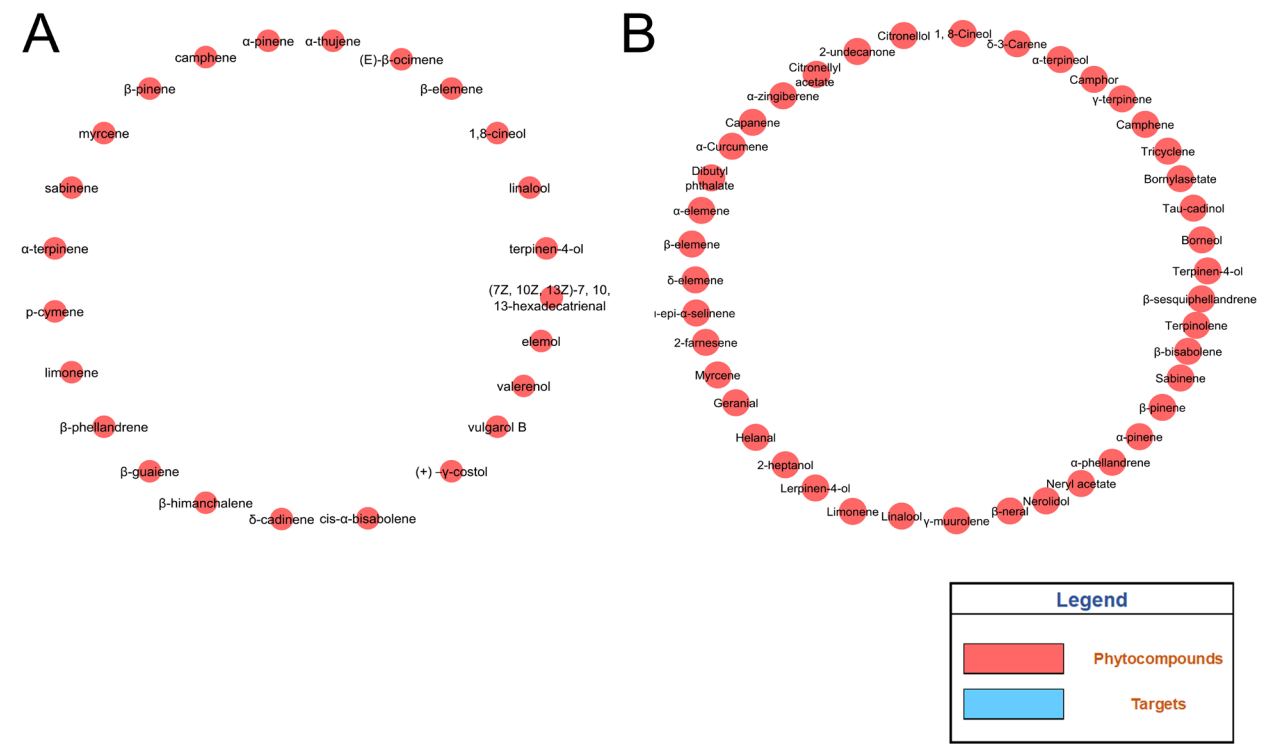

Figure 6: The networks of A) *Costus* root and B) Dry Ginger The ingredients of the concoction put in this image have no potential targets so the networks without any edges representing only the phytocompounds. *Costus* root has 24 phytocompounds and the same is about 41 in the case of Dry Ginger.

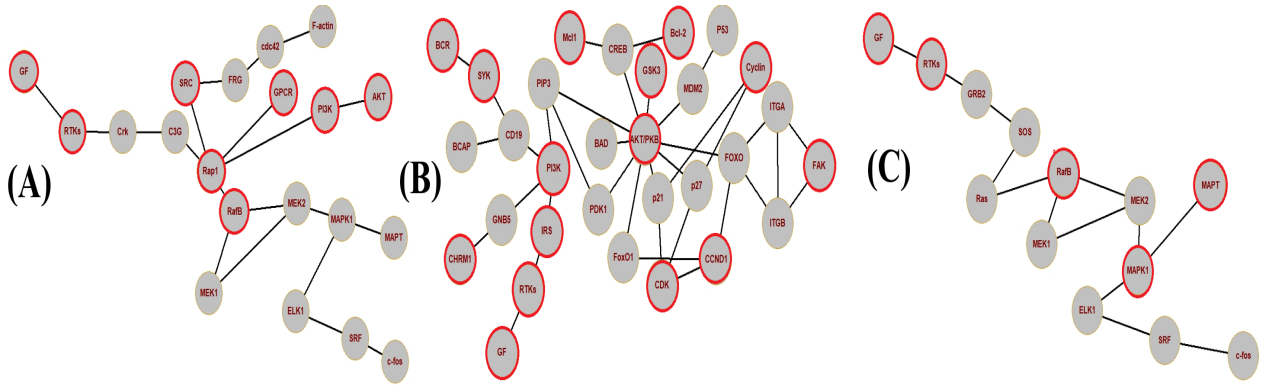

Figure 7: Pathways identified with KEGG Mapper. The proteins were mapped into three important pathways A) MAPK pathway B) PI3K AKT Pathway and Rap1 pathway. MAPK pathway, we have 18 proteins, colored in grey color. 8 proteins from our data have been highlighted. Similarly, PI3K-Akt pathway, we have 26 proteins, colored in grey color. 12 proteins from our data have been highlighted. PI3K-Akt pathway, we have 16 proteins, colored in grey color. 8 proteins from our data have been highlighted.

The coactive effects of *Siddha* medications with the conventional treatment have resulted in a promising improvement in the condition of COVID-19 patients.[97] Initial inflammatory stage of COVID-19 infection involved the administration of *Poorna chandirodayam* and *Gorojanai mathirai* along with other herbal and herbomineral *Siddha* formulations was performed to minimize hypoxia.[98] One research group have drawn the conclusion that *Nagaradi Kashaya* may be effective in battling SARS-CoV-2 and for performing additional clinical trials, *Nagaradi Kashaya* may be a lead candidate.[99] To strengthen immunity against the COVID-19 infection, the AYUSH ministry suggested the herbal combination of *Ocimum tenuiflorum, Cinnamomum verum, Piper nigrum, Zingiber officinale* and *Vitis vinifera* as they were found to modulating the signalling of HIF-1, p53, PI3K-Akt, MAPK, cAMP, Ras, Wnt, NF-kappa B, IL-17, TNF and cGMP-PKG among other diverse pathways.[100] Recently, quite a few studies have been centered on *Kk*, a *Siddha* formulation made up of 15 botanical ingredients and a wide range

of pharmacological activities.[23-65] Nine major active ingredients of the *Siddha* official formulation *Kk* were observed to be a potent inhibitor of SARS-CoV-2 spike protein and a new formulation called SNACK-V was proposed.[83] In one report, Kk used in the strategy against COVID-19 considerably decreased SARS-CoV-2 viral load among asymptomatic COVID-19 cases and did not record any negative effects but the pathways were not enriched and promising compound from Kk was not reported.[101]

In the present study, we constructed a network of compounds from Kk and their possible interacting targets and identified three pathways, namely P13K/AKT, Rap1 and MAPK pathways that the phytocompounds possibly modulate. Interestingly, all these 3 pathways are being looked on as possible therapeutic targets against COVID-19. All these 3 pathways when activated, result in the increased expression and release of pro-inflammatory mediators such as IL-6, TNF-α, IL-1β and activation of factors like Activated Protein-1 (AP-1) and Nuclear Factor Kappa B (NFκB)[102,103] and there have been recent literature evidences that indicate an increased level of inflammatory mediators like cytokines and chemokines in COVID-19 patients.[104-106]

In addition, activated P13K/AKT and MAPK pathways have been found to be associated with viral endocytosis[107] and lung tissue fibrosis[108,109] especially in COVID-19 patients. A clathrin-mediated pathway regulated by activated P13K/AKT seems to facilitate the endocytosis of SARS-CoV-2, leading to reduction of ACE2 and a subsequent increase in the levels of inflammatory cytokines finally causing lung fibrosis. Similarly, it has been shown that vital thrombotic events such as platelet aggregation, arterial thrombosis and endothelial dysfunction observed in COVID-19 patients may be due to disproportionately activated p38 MAPK pathway that may in turn lead to apoptosis, impaired contraction and fibrosis.[110]

The Rap1 signaling pathway has been identified as one of the prominent pathways involved in the regulation of immune system. The Rap1 pathway activates 3 different secondary messengers namely 3′,5′ Cyclic Adenosine Monophosphate (cAMP), Calcium Ion (Ca^{2+}) and Diacylglycerol (DAG) that are required for cell position signaling in viral infections.[82,111] The activation of cAMP triggers a cascade reaction activating cAMP dependent Protein Kinase-A (PKA) and an Exchange Protein Activated by cAMP (EPAC). It is also involved in the opening of ion channels that help in regulation of Ca^{2+} levels and thereby controls T cell proliferation and cytokine production.[112,113] The cAMP/PKA pathway has been found to modulate the proliferation and transcription of cytokine genes like TNF-α, IFN-γ and Interleukins.[114] cAMP is found to have dual and contrasting roles with regard to viral infection where on one hand it could decrease viral entry and replication and on the other hand it could suppress antiviral immune responses. Hence therapeutic medications that activate cAMP could act as immunosuppressants and those which repress cAMP signaling could act as immunostimulators.[113]

Based on the results obtained in this study and the available information on the 3 pathways, it could be safely hypothesized that the plausible molecular mechanistic action of Kk in the management and treatment of SARS-CoV-2 could be through inhibition of P13K/AKT, MAPK and Rap1 signaling pathways, that would in turn result in reduction of expression and release of pro-inflammatory cytokines, viral endocytosis and lung fibrosis all of which are associated with COVID-19. In the present study, among all the phytoconstituents that interacted with multiple protein targets, Ellagic acid was found to have the best binding affinity [Supplementary Table S1]. Ellagic acid is a polyphenol that is found mainly in fruits. The ability of Polyphenols' potential to treat cancer by blocking the PI3K/Akt/mTOR signaling pathway has been reported,[115] and the compound ellagic acid has been reported to show anti-inflammatory and anti-cancer activity.[116-118] Interestingly, Ellagic acid is currently being put forth as a possible therapeutic agent against COVID-19 based on its proven therapeutic ability. The antiviral effect of Ellagic acid against Influenza A (H3N2), Rhinoviruses (HRV-2,3 and 4), Ebola, HIV-1, HSV-1 and noroviruses have been well documented.[119-121]

Ellagic acid's protective effect against lung damage could be attributed to its excellent anti-oxidant potential and it was also found to possess anti-inflammatory activity and could bring down the levels of IL-6.[122] In addition, Ellagic acid was also found to exhibit synergistic action with antimalarial drugs that are currently being prescribed as a treatment strategy against COVID-19.[123] In another recent study carried out by Usama Ramadan *et al.* 2020,[124] when more than 496 phenolic compounds were virtually screened against the active site of SARS-CoV Main protease, it was found that only Ellagic acid along with acetylglucopetunidin and isoxanthohumol obeyed the Lipinski's and Veber's rules of drug-likeness and possessed higher degree of bioavailability.

CONCLUSION

There have been multiple reports about the *Siddha* formulation Kk being effective in preventing and curing the viral disease. However, there is a paucity of studies performed to identify the molecular level mechanism of Kk and its effectiveness with respect to COVID-19. We hope that this study fills that gap and also shows that using computational techniques such as network pharmacology and molecular docking, potential phytocompounds and their most probable host targets can be identified. Specifically, we identified the role of Kk phytocompounds and their interaction with 3 major pathways namely the Phosphatidylinositol 3-Kinase (P13K/AKT), Mitogen Activated Protein Kinase (MAPK) and Repressor Activator Protein 1 (Rap1) signaling pathways. It has to be noted that these three pathways are potential therapeutic targets against COVID-19 as reported in literature. Also, that the high specific binding of Ellagic acid, using a blind molecular docking approach, is statistically significant. This study clearly

illustrates that, traditional formulations like Kk with specific active ingredients could prove to be beneficial as an alternative source of therapeutic intervention both in the treatment and prevention of COVID-19. Further wet lab experiments and clinical trials could confirm and establish the effectiveness of this formulation against COVID-19.

ACKNOWLEDGEMENT

We acknowledge SASTRA (Deemed to be University) for the Infrastructure support provided to carry out this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Kk: *Kabasura kudineer*; **SMILES:** Simplified Molecular Input Line Entry System; **DB:** Database; **P13K/AKT:** Phosphatidylinositol 3-kinase; **MAPK:** Mitogen activated protein kinase; **Rap1:** Repressor activator protein 1; **ACE2:** Angiotensin-converting enzyme-2; **SARS:** Severe acute respiratory syndrome; **MERS:** Middle East respiratory syndrome; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **EPAC:** Exchange protein activated by cAMP.

CREDIT AUTHOR STATEMENT

Brindha Pemaiah, Ragothaman M. Yennamalli, Sriram Sridharan and Davidraj Chellappan conceived the idea and designed the experiments; Yatindrapravanan Narasimhan and Shashank Ravichandran performed the molecular docking and draft of the figures, Krishna Kant Gupta performed the network analysis and wrote the first manuscript draft; All authors were involved in editing and finalizing the manuscript.

SUMMARY

Kabasura kudineer (Kk) is a classical *Siddha* medicine used for the management of many types of fevers and flu with respiratory complications. In this study, a network pharmacological approach has been employed to investigate the interactions between the phytoconstituents of Kk and multiple targets associated with SARS-CoV-2 and also obtain valuable inputs on the mechanism of action of the formulation. The present study indicates that Kk could modulate three important molecular Pathways (P13K/AKT, Rap1 and MAPK) associated with COVID-19 and this could be due to the effect of one of its phytoingredient, Ellagic acid.

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Cite this article: Gupta KK, Narasimhan Y, Ravichandran S, Pemaiah B, Chellappan D, *et al*. Network Pharmacology-Based Modeling of Phytocompounds in a Traditional *Siddha* Formulation-*Kabasura kudineer* with Special Reference to SARS-CoV-2. Indian J of Pharmaceutical Education and Research. 2025;59(2):682-94.