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Original Research Article

Molecular Docking Studies of Phytoconstituents Identified in Traditional Siddha Polyherbal Formulations Against Possible Targets of SARS-CoV-2

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Abstract

The Indian Traditional Medicines System has long used Siddha polyherbal formulations for different viral diseases. The ingredients of these formulas have been proven to be antiviral. The study focuses on *in silico* computational evaluation of phytoconstituents of the official Siddha formulation Kabasura, Thonthasura, and Vishasura Kudineer, which were widely used in treating viral fever and respiratory infections and may influence the current SARS-CoV-2 coronary virus pandemic. Maestro interface (Schrödinger Suite, LLC, NY) was used for molecular docking studies against M^{Pro} (PDB ID 5R82, 6Y2F, and 6LU7), Nsp15 endoribonuclease (6W01), RNA-dependent RNA polymerase (6M71), and spike protein (6VW1) of SARS-CoV-2. In addition, pharmacokinetics (ADME) and safety profile prediction studies were performed to identify the best drug candidates using Qikpro and Toxicity Estimation Software Tool (T.E.S.T). A total of 36 compounds were screened, of which nine displayed strong binding affinity and drug-likeness. Luteolin and chrysoeriol produced stronger results. These nine compounds were free of oral toxicity as evaluated by the Toxicity estimation software. Based on further *in vitro*, *in vivo*, and clinical effectiveness trials, these compounds may be used for the prevention or treatment as per the Indian system of traditional medicines.

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INTRODUCTION

Ever since the outbreak of COVID-19 caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, the world has witnessed the rapid spread of the pandemic across the world[1]. World Health Organization (WHO) reported approximately 82,579,768 COVID-19 cases and 1,818,849 deaths as of January 2nd, 2021, with cases reported in more than 222 countries/territories. This novel coronavirus outbreak has posed a severe burden to the global economic, medical, and public health infrastructure[2].

The COVID-19 is primarily a droplet-spread infection, and patients exhibit various symptoms of which fever, dry cough, and fatigue are predominant[3]. In some cases, the symptoms had rapidly developed to acute respiratory distress syndrome, metabolic acidosis, septic shock, coagulation dysfunction, eventually leading to multiple organ failure[4-6]. However, mild or asymptomatic COVID-19 patients can recover shortly after isolation and healthy lifestyle and food habits[7].

There is no particular treatment available for COVID-19 infection except for comprehensive support by the combination of broad-spectrum antibiotics, antiviral and anti-malarial drugs, corticosteroids, and convalescent plasma therapy[8]. Numerous clinical trials are in progress, including identifying vaccines against SARS-CoV-2. Researchers and health care professionals are in desperate search of an effective

cure for this pandemic. In the current scenario where the conventional drugs do not prove to be much efficacious, exploring the traditional system of medicine could be a feasible and hopeful strategy[9]. Traditional, complementary, and alternative medicine has a long history of providing primary beneficial health care to the population[10].

India has an unmatched alternative system of medicine in the form of Ayurveda, Yoga, and Naturopathy, Unani, Siddha, Homeopathy, which is now jointly referred to as Ayush, recognized by the Government of India[11]. Siddha Medicine is one of India's oldest (5000 years old) and well-documented medical systems and is practiced mainly in South India, especially in Tamil Nadu and Sri Lanka, Malaysia, Singapore, and Mauritius, where Tamils live[12]. In the current pandemic situation, many strategies would be highly critical to combat the rapid virus spread and treat the infection. Ministry of Ayush, Government of India has issued an "Advisory on Coronavirus" to manage this outbreak and broadly comprises of preventive and prophylactic symptom management of COVID-19 like illnesses and also insights to interventions based on Ayush systems of medicine through the evidence for immunity boosting as well as help in improving the respiratory symptoms[13].

Drug discovery and development involve a long time, a vast number of individuals, high prices. *In silico* screening approaches allow researchers to explore new and potentially active lead compounds in less time, expense, and humans[14]. Siddha polyherbal formulations are potent against several causative agents such as influenza, chikungunya, dengue, tuberculosis, and others[15-17]. Siddha medicines have been used successfully by Siddha practitioners and ordinary citizens for the treatment of many diseases for several years, such as Kabasura Kudineer during influenza outbreaks, Nilavembu Kashayam for dengue fever. Kabasura kudineer, Thonthasura kudineer, and Vishasura kudineer are polyherbal formulations that have long been used in Siddha medication for different health problems, including currently being developed for COVID-19 therapy[18-19]. These polyherbal formulas are made up of different medicinal plants.

This study aims to evaluate the activity of phytoconstituents in Siddha polyherbal formulations against various potential SARS-CoV-2 targets using *in silico* methods. Thirty-six phytoconstituents were selected from these medicinal plants and docked against all potential SARS-CoV-2 targets, including M^{Pro}, Nsp15 endoribonuclease, RNA-dependent RNA polymerase (RdRp), and spike protein, utilizing Maestro 11.8 (Schrodinger 2018-4 package).

METHOD

Hardware and Software

Software used includes Maestro 11.8 from Schrödinger, Inc (https://www.schrodinger.com/products/maestro) and Toxicity Estimation Software Tool (T.E.S.T.) 4.2.1 from United States Environmental Protection Agency (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test).

Ligands

Hygrophila auriculata, Piper longum, Syzygium aromaticum, Tragia involucrata, Clerodendrum serratum, Anacyclus pyrethrum, Terminalia chebula, Adhatoda vasica, Coleus amboinicus, Saussurea lappa, Tinospora cordifolia, Andrographis paniculata, Sida acuta, Cyperus rotundus, and *Zingiber officinale* were the 15 ingredients of Kabasura Kudineer[20,21]. The Thonthasura Kudineer contains ten ingredients, including *Z. officinale, A. vasica, A. paniculata, T. cordifolia, Elettaria cardamomum, Solanum xanthocarpum, Trichosanthes cucumerina, Tephrosia purpurea, Mollugo cerviana*, and *Vitis vinifera*[22]. While the Vishasura Kudineer consists of nine ingredients, including *Azadirachta indica, Z. officinale, Hemidesmus indicus, Indigofera tinctoria, Aristolochia bracteolata, E. cardamomum, Vetiveria zizanioides, Santalum album*, and *Glycyrrhiza glabra*[23].

The major active phytoconstituents present in those plants were selected. The selected 36 phytoconstituents including β-sesquiphellandrene (PubChem ID 11106487), β-bisabolene (10104370), geranial/citral (638011), piperine (638024), piperlonguminine (5320621), eugenol (3314), β-caryophyllene (5281515), stigmasterol (5280794), squalene (638072), γ-sitosterol/clionasterol (457801), andrograpanin (11666871), moslosooflavone/5-hydroxy-7,8-dimethoxyflavone (188316), lupeol (259846), betulin (72326), chebulagic acid (442674), gallic acid (370), vasicinone (10242), carvacrol (10364), cirsimaritin (188323), chrysoeriol (5280666), luteolin (5280445), costunolide (5281437), elemol (92138), tinosponone (15215479), bharangin (194464),

scutellarein (5281697), magnoflorine (73337), cycleanine (121313), cyperene (99856), β-selinene (442393), zingiberene (92776), vasicine (442929), cucurbitacin B (5281316), andrographolide (5318517), apigenin (5280443), pyrethrin I (5281045), and the reference drugs (chloroquine, hydroxychloroquine, ivermectin, lopinavir, remdesivir, and ritonavir) were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Receptors

All potential SARS-CoV-2 targets, including M^{Pro}, Nsp15 endoribonuclease, RdRp, and spike protein, have been selected to evaluate the optimum ligand. The 3D structure of selected proteins has been downloaded from Protein Data Bank (https://www.rcsb.org). The PDB ID of the selected proteins was M^{Pro} (5R82, 6Y2F, 6LU7), Nsp15 endoribonuclease (6W01), RdRp (6M71), and spike protein (6VW1)[24-29].

Docking protocol

Preparation of ligands

The ligand minimization was carried out by the LigPrep module in Maestro 11.8. The 3D ligand structure was generated, and hydrogen atoms were introduced. Salt reduction and ionization (pH 7.0±2.0) were conducted, and the minimization was performed utilizing the OPLS-2005 force field[30,31].

Preparation of protein

Protein Preparation Wizard was used to prepare protein structures. Bond orders were assigned, and hydrogen atoms were inserted. Within 3 Å of the het groups, the water molecules were removed, and the missing side chains were filled with prime. As a result, hydrogen bonds (H-bond) were optimized and reduced using the OPLS 2005 force field. The co-crystallized ligand binding sites have been identified after elimination. The receptor grid was then created using the "Glide's Receptor Grid Generation" module with a 20 Å radius[30,31].

Molecular docking and free energy calculation

The molecular docking between receptor binding sites and ligands was conducted using the Glide Module of Maestro 11.8, and the lowest binding pose of each ligand was maintained. Glide docking scores were performed in three high-throughput virtual screening (HTVS), standard precision (SP), and extra precision (XP) modes. Firstly, docking was performed with reference molecules of respective proteins to validate the docking protocol. We used the XP mode for docking. After XP mode docking, compounds were sent to Prime MMGBSA from Maestro 11.8 for free energy calculations.

ADME and toxicity analysis

Out of the 36 compounds, ten compounds were chosen based on the docking performance. The chosen compounds were used in the ADME study using the QikProp module from Maestro 11.8, and the following parameters were determined.

1. The molecular weight of the molecule.
2. Predicted octanol/water partition coefficient.
3. Predicted brain/blood partition coefficient.
4. Percent human-oral absorption
5. Lipinski's rule of five.
   a. mol_MW <500
   b. QPlogPo/w <5
   c. donorHB ≤5
   d. accptHB ≤10
6. Jorgensen's rule of three
   a. QPlogS >-5.7
   b. QP PCaco >22 nm/s
   c. # Primary Metabolites <7

Toxicity was measured using T.E.S.T. 4.2.1. Oral rats LD_{50}, developmental toxicity, and Ames mutagenicity were conducted using four methods: Consensus system, Hierarchical clustering method, FDA method, and Nearest neighbor method[32].

1. Hierarchical method [HM]: Using the weighted average of estimates from several separate models, the toxicity of a specified question compound was determined. Using the Ward approach to fragment the training set into a sequence of structurally linked clusters, the separate models were obtained. A genetic algorithm-based approach was used to create models for each cluster. Models were created before runtime.
2. FDA Method [FM]: For and test product, the prediction was produced using a new model appropriate for chemicals closest to the test compound. Each model was generated at runtime.
3. Nearest neighbor method [NM]: The predicted toxicity was calculated by taking an average of the three chemicals most comparable to the research chemicals in the training kit.

4. Consensus Method [CM]: The predicted toxicity was calculated by taking an average of the predicted toxicity from the QSAR as mentioned earlier methods (provided the predictions were within the respective applicability domains).

RESULTS AND DISCUSSION

Molecular docking and free energy calculation

Compounds with a docking score of less than -6.0 were deemed possible candidates against SARS-CoV-2, as represented in **Table I** for a comparative study. Out of 36 molecules, luteolin, chrysoeriol, and cucurbitacin B have been associated with more than two receptor structures. Luteolin displays a docking score less than -6 with M^{Pro}, Nsp15 endoribonuclease, and RdRp, as seen in **Figure 1**.

Table I. Comparative docking analysis of ligands against M^{Pro}, Nsp15 endoribonuclease, RdRp, and spike protein

Compounds	Receptors (PDB ID)					
	5R82	6Y2F	6LU7	6W01	6M71	6VW1
Remdesivir	-5.478	-5.306	-7.189	-7.829	-8.643	-7.206
Hydroxychloroquine	-5.395	-2.741	-4.438	-4.814	-4.177	-8.748
Chloroquine	-4.203	-1.587	-3.98	-5.896	-2.191	N/A*
Lopinavir	-5.373	-3.5	-4.535	-5.953	-7.797	-6.702
Ritonavir	-3.927	-5.233	-6.79	-5.848	-1.198	-6.493
Ivermectin	-3.037	-3.427	-4.44	-4.187	-3.558	N/A
Luteolin	-7.408	-6.036	-7.47	-7.314	-6.304	N/A
Scutellarein	-6.807	-6.081	-7.587	-7.191	N/A	N/A
Chrysoeriol	-6.473	-6.394	-7.342	-6.43	-6.174	N/A
Cucurbitacin B	-6.267	N/A	-6.946	-7.021	-6.488	N/A
Apigenin	-6.065	N/A	-6.22	-6.41	N/A	N/A
Andrographolide	-6.042	N/A	N/A	N/A	N/A	N/A
Cirsimaritin	-6.031	N/A	-6.743	-6.461	N/A	N/A
Moslosooflavone	-6.003	N/A	-6.973	N/A	N/A	N/A
Gallic acid	N/A	N/A	N/A	-6.379	N/A	N/A
Pyrethrin	N/A	N/A	N/A	N/A	-6.704	N/A
Cycleanine	N/A	N/A	N/A	N/A	N/A	-6.907

*N/A: Not available

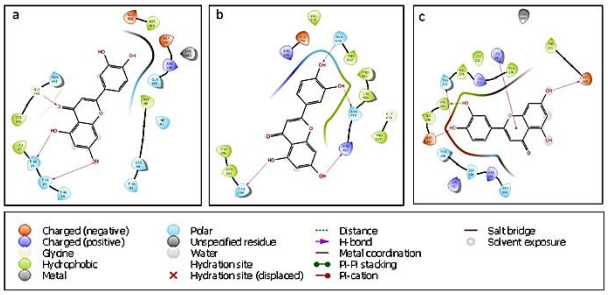

Figure 1. Binding-interaction analysis of luteolin with (**a**) M^{Pro}, with (**b**) RdRp, and with (**c**) Nsp15 endoribonuclease.

Chrysoeriol also displays a docking score less than -6.0 with M^{Pro}, Nsp15 endoribonuclease, and RdRp, as seen in **Figure 2**. The associations of luteolin and chrysoeriol with various SARS-CoV-2 target forms were comparatively analyzed, in which H-bond and hydrophobic pockets were presented in **Tables II** and **III**. Luteolin shows hydrogen bonding with nearly four amino acids of most of the targets. This finding shows its high binding potency towards the SARS-CoV-2.

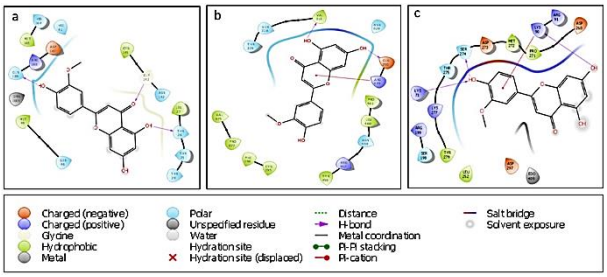

Figure 2. Binding-interaction analysis of chrysoeriol with (**a**) M^{Pro}, with (**b**) RdRp, and with (**c**) Nsp15 endoribonuclease.

Table II. Binding interactions of luteolin with the active sites of different targets in SARS-CoV-2

Target	PDB ID	H-Bond	Hydrophobic pocket
M^{Pro}	5R82	GLY 143, THR 26, THR 25	CYS 145, MET 165, MET 49, LEU 27
	6LU7	THR 26	LEU 27, CYS 145, CYS 44, MET 49, PRO 52, TYR 54, MET 165
	6Y2F	GLU 166, LEU 141, HIE 163, HIE 41	LEU 27, VAL 42, CYS 44, TYR 54, MET 49, PHE 140, LEU 141, LYS 145, MET 165
RdRp	6M71	THR 394, ARG 457, ASN 628, ASN 459	PHE 396, CYS 395, VAL 315, PRO 627, PRO 461, LEU 460, PRO 677
Nsp15 endoribo-nuclease	6W01	ASP 268, ASP 297, THR 275, VAL 295	PRO 271, LEU 252, VAL 295, ILE 296, VAL 276, TYR 279

Table III. Binding interactions of chrysoeriol with the active sites of different targets in SARS-CoV-2

Target	PDB ID	H-Bond	Hydrophobic pocket
M^{Pro}	5R82	GLN 189, GLY 143, THR 26	CYS 145, LEU 27, MET 49, MET 165
	6LU7	THR 26	CYS 44, PRO 52, MET 49, TYR 54, MET 165, CYS 145, LEU 27
	6Y2F	ASP 187, GLU 166, LEU 141	CYS 44 , LEU 141, CYS 145, MET 165, TYR 54, MET 49
RdRp	6M71	VAL 315, GLU 350	VAL 315, PRO 461, LEU 460, PHE 396, CYS 395, TYR 456, PRO 677, VAL 675
Nsp15 endoribo-nuclease	6W01	LYS 71, SER 275, LYS 90	TYR 279, MET 272, PRO 271, LEU 252

In the molecular docking of phytoconstituents with M^{Pro} (5R82), luteolin had a higher affinity with a docking score of -7.408, followed by scutellarein and chrysoeriol with docking scores of -6.807 and -6.473, respectively. These phytoconstituents had a higher affinity to M^{Pro} (5R82) than remdesivir, displaying a docking score of -5.478. Chrysoeriol had a higher affinity with a docking score of -6.394, followed by scutellarein and luteolin with docking scores of -6.081 and -6.036, respectively with the target M^{Pro} (6Y2F). These phytoconstituents had a higher affinity to M^{Pro} (6Y2F) than remdesivir, with a docking score of -5.306. Scutellarein had a greater affinity with a docking score of -7.587, followed by luteolin and chrysoeriol with -7.470 and -7.342, respectively, for molecular docking of phytoconstituents with M^{Pro} (6LU7). These phytoconstituents had a higher affinity than remdesivir, which had a docking score of -7.189.

Remdesivir shows greater affinity with a docking score of -7.829, followed by scutellarein and cucurbitacin B with a score of -7.314 and -7.191, respectively, in the docking analysis with Nsp15 endoribonuclease (6W01). With RdRp (6M71), remdesivir had a higher affinity with a docking score of -8.643, followed by pyrethrin and cucurbitacin B with docking scores -6.704 and -6.488, respectively. Hydroxychloroquine had a higher affinity with a docking score of -8.748, followed by remdesivir and cycleanine, which had a docking score of -7.206 and -6.907, respectively, with the target spike protein (6VW1). Most phytoconstituents exhibited similar reference drugs in binding energies and binding pockets, except gallic acid, pyrethrin, chebulagic acid, and cycleanine.

Chrysoeriol shows less hydrogen bonding than the luteolin but better than other phytoconstituents. The hydrogen bonding of both luteolin and chrysoeriol could be increased by substitute better chemical groups. The prime MM-GBSA was generally accepted for the re-scoring of docked complexes. Both of the chosen complexes were subjected to prime MM-GBSA measurements after XP Docking[33]. MM-GBSA DG-bind scores for all chosen compounds were displayed in **Table IV**. The negative DG-bind values indicate that the selected compounds associate favorably with the receptor. Ligand binding energies for both substances vary from -40.0 to -100.0 kcal/mol. The binding energies of several of the substances were relatively close to those of the reference drug binding energy. These findings indicate that the selected compounds would inhibit SARS-CoV-2.

Table IV. MM-GBSA DG-bind values of selected compounds

Compounds	Receptors (PDB ID) (kcal/mol)					
	5R82	6Y2F	6LU7	6W01	6M71	6VW1
Remdesivir	-63.6	-74.01	-79.74	-61.48	-73.53	-47.55
Hydroxychloroquine	-77.77	-94.09	-64.02	-46.21	N/A*	-64.72
Chloroquine	-62.03	-87.32	-78.62	-36.65	N/A	-66.13
Lopinavir	-59.92	-52.27	-48.39	-47.87	-93.51	-70.62
Ritonavir	-93.22	-88.95	-96.23	-69.29	N/A	-31.95
Ivermectin	-62.33	-59.89	-55.15	-66.39	N/A	-54.7
Luteolin	-45.23	-26.87	-54.84	-41.3	-48.75	N/A
Scutellarein	-43.21	-41.37	-50.83	-44.21	N/A	N/A
Chrysoeriol	-56.63	-23.2	-56.63	-39.5	-54.83	N/A
Cucurbitacin B	-82.11	N/A	-63.88	-58.96	-79.78	N/A
Apigenin	-45.79	N/A	-52.44	-43.85	N/A	N/A
Andrographolide	-69.79	N/A	-51.5	N/A	N/A	N/A
Cirsimaritin	-53.92	N/A	-55.73	-50.21	N/A	N/A
Moslosooflavone	-51.89	N/A	-56.77	N/A	N/A	N/A
Gallic acid	N/A	N/A	N/A	-18.15	N/A	N/A
Pyrethrin	N/A	N/A	N/A	N/A	-79.94	N/A
Cycleanine	-63.6	-74.01	-79.74	-61.48	-73.53	-47.55

*N/A: Not available

ADME analysis

The absorption, distribution, metabolism, and elimination of substances play an essential role in the drug development phase. *In silico* ADME analysis would save thousands of dollars spent in the drug development phase by producing fewer new compounds[34]. The ADME parameters, such as mol MW, QPlogPo/w, QPlogBB, percent human oral absorption, Rule of Five, and Rule of Three using QikProp showed a better score for the docked compounds[35]. Both of the chosen nine compounds have enhanced ADME properties and drug-likeness according to the spectrum as shown in **Table V**. All of the nine phytoconstituents have enhanced ADME properties. Cucurbitacin B violates a rule of 1 of 5, which was appropriate. Gallic acid and pyrethrin were in breach of a law of three that was fitting. Luteolin and chrysoeriol display improved drug-likeness and high binding capacity, all of which were essential to the drug candidate.

Table V. ADME prediction for the selected compound

Compounds	Mol MW	QPlogBB	QPlogPo/w	Percent human oral absorption	Rule of Five	Rule of Three
Andrographolide	350.454	-1.222	1.437	79.068	0	0
Apigenin	270.241	-1.411	1.624	73.955	0	0
Chrysoeriol	300.267	-1.409	1.81	76.672	0	0
Cucurbitacin B	558.711	-1.964	2.92	67.293	1	0
Gallic acid	170.121	-1.659	-0.585	41.441	0	1
Luteolin	286.24	-1.91	0.941	62.05	0	0
Pyrethrin	372.46	-1.157	4.385	100	0	1
Scutellarein	286.24	-1.819	1.001	63.924	0	0
Moslosooflavone	298.295	-0.43	3.165	100	0	0

In silico toxicity study

The oral rat LD_{50}

The endpoint of the oral rat LD_{50} was the amount of the chemical (chemical mass per rat body weight) that destroys half of the rats when administered orally[36]. The oral rat LD_{50} was conducted in four methods for all of the chosen compounds, and the findings were comparatively evaluated in **Table VI**. All substances have been shown to have an acceptable toxicity limit for drug production and preclinical and clinical assessment.

Developmental toxicity

Developmental toxicity includes embryonic and fetal mortality, miscarriage, and other abnormal developmental symptoms such as liver toxicity, lowered body weight, growth, developmental retardation, and physical abnormalities (teratogenic effects)[37]. Developmental toxicity was performed in four approaches with all of the chosen compounds, and the findings were comparatively analyzed in **Table VI**. A predicted value greater than 0.5 indicates toxicity. Except gallic acid, all other compounds show developmental toxicity.

Ames mutagenicity

In Ames assay, frame-shift mutations or base-pair substitutions could be identified by exposure of histidine-dependent strains of *Salmonella typhimurium* to the test compound. When these strains were exposed to a mutagen, reversing mutations that restore the functional capacity of the bacteria to synthesize histidine would cause the bacterial colony to develop on a medium histidine deficiency (revertant)[38].

A compound was labeled Ames positive if it significantly induces the development of the reverting colony in at least one of the five strains. If a compound was positive for the Ames test, it could be a possible mutagen[39]. Ames mutagenicity was conducted in four methods for all of the chosen compounds, and the findings were comparatively analyzed in **Table VI**. A predicted value greater than 0.5 indicates mutagenicity. All the nine phytoconstituents except pyrethrin were not mutagens based on the results on the Ames mutagenicity as predicted by T.E.S.T software.

Table VI. Predicted value for oral rat LD_{50} – Log^{10} (mol/kg), developmental toxicity, and Ames mutagenicity

Compounds	Oral rat LD_{50} - Log^{10} (mol/kg)				Developmental toxicity				Ames mutagenicity			
	HM	FM	NM	CM	HM	FM	NM	CM	HM	FM	NM	CM
Andrographolide	2.82	3.87	5.32	4.00	0.76	0.81	1.00	0.82	0.32	0.49	0.33	0.38
Apigenin	2.17	2.28	2.14	2.20	0.94	0.35	N/A	0.65	0.06	0.47	0.33	0.29
Chrysoeriol	2.30	3.11	3.05	2.82	1.04	1.13	N/A	0.95	0.04	0.18	0.33	0.18
Cucurbitacin B	3.87	4.18	3.37	3.81	0.77	0.69	1.00	0.83	0.02	0.00	0.00	0.01
Gallic acid	1.88	1.35	1.69	1.64	0.34	0.39	0.33	0.34	0.29	0.64	0.00	0.31
Luteolin	2.37	1.85	2.14	2.12	0.89	1.20	N/A	0.88	0.31	0.62	0.67	0.53
Pyrethrin	3.28	3.36	2.77	3.14	0.70	0.98	N/A	0.84	0.92	1.12	0.67	0.90
Scutellarein	2.38	2.05	2.14	2.19	0.89	1.30	N/A	0.92	0.28	0.17	0.67	0.37
Moslosooflavone	3.35	3.20	3.37	3.31	0.99	1.09	0.67	0.89	0.65	0.07	0.00	0.24

HM: Hierarchical method; FM: FDA method; NM: Nearest neighbor method; CM: Consensus method; N/A: Not available

Our current research has chosen three Official Siddha Formulation Kabasura, Thonthasura, and Vishasura Kudineer to test its potential against SARS-CoV-2 targets. Siddha medicine is one of the oldest Indian systems of medicine. The methods of Siddha emerged in India, and it was most commonly practiced in India, especially in southern regions. Siddha medicinal plants were a promising area for the treatment of a wide variety of diseases. Siddha medicinal plants might also be considered a new choice for their role in overcoming viral transmission[40,41].

Mekala and Krishnamurthy[42] performed the phytochemical screening and pharmacological update on Kabasura Kudineer Choornam and Nilavembu Kudineer Choornam. Kabasura Kudineer was found to have alkaloids, carbohydrates, glycosides, heart-glycosides, flavonoids, phenols, saponins, and hydrolyzable present in Kabasura Kudineer Choornam. In addition to the broad range of other pharmacological operations, the ingredients in Kabasura Kudineer show that most of the components were antipyretic, anti-inflammatory, antimicrobial, and immunostimulant[43]. Therefore, it was scientifically rational to use it in respiratory viral infection.

The molecular docking study of Thonthasura Kudineer ingredients demonstrated affinity with the Coronavirus Spike (S) glycoprotein, carried out by Kumar *et al*[22]. Vishasura Kudineer was a polyherbal formulation from the Siddha literature 'Kaaviya Sura Nool'. Vishasura Kudineer was traditionally used for symptoms associated with viral fever. Its portion demonstrates antiviral activity against a wide variety of viruses. It might also be antipyretic, antiasthmatic, anti-inflammatory, antioxidant, hepatoprotective, and immunostimulant[18].

Various research studies have been performed on different formulations of Siddha and its phytoconstituents against selective targets for SARS-CoV-2[19,22,44]. The main protease (M^{Pro}, $3CL^{Pro}$, Nsp5) proteolytically cleaves the overlapping pp1a and pp1ab polyproteins to functional proteins, crucial in viral replication. In the viral replication cycle, the M^{Pro} acts as the primary enzyme. Its inhibition could thus interfere with the production of infectious virus particles and reduce disease symptoms[45].

The SARS-CoV-2 spike protein mediates the binding of the virus to its receptor angiotensin-converting enzyme 2 (ACE2) and facilitates the integration of viral and host cell membranes and the entrance of the virus into the host cell. Thus, the Spike protein was vital in neutralizing and T-cell reactions and maintaining immunity during SARS-CoV-2 infection. Given the essential role of the S-protein in viral infection and adaptive immunity, most methods and therapies were based on the S-protein[46]. RNA-dependent RNA Polymerase was an enzyme that replicates RNA from an RNA template. RNA-dependent RNA Polymerase was one of the Nsp (Nsp12) that plays a key role in the coronavirus life cycle[47].

Nsp15 was responsible for protein interaction with the innate immune response, although other studies suggest that the mechanism was independent of endonuclease activity. In order to conceal it from the host's immune system, there were also reports that Nsp15 degrades viral RNA[48]. Nevertheless, in coronavirus biology, Nsp15 was important. The active site, located in a shallow groove between the two β-sheets, carries six key residues conserved among SARS-CoV-2, SARS-CoV, and MERS-CoV proteins: His235, His250, Lys290, Thr341, Tyr343, and Ser294[27].

CONCLUSION

The present research was planned to classify potential drug candidates exhibiting potential binding affinity to all possible SARS-CoV-2 targets (M^{Pro}, Nsp15 endoribonuclease, RdRp, and spike protein). Based on the findings obtained from molecular docking, free energy measurement, ADME analysis, as well as toxicity analysis, luteolin and chrysoeriol exhibit stronger docking score, binding energy, ADME properties, and lower toxicity than all other compounds.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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None.

DATA AVAILABILITY

All data are available from the authors.

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AUTHORS' CONTRIBUTIONS

Logesh Kumar Selvaraj: conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, visualization, writing – original draft. **Geethanjali Thayumanavan**: data curation, investigation, writing – original draft. **Srikanth Jeyabalan**: conceptualization, investigation,

project administration, software, supervision, validation, writing – review & editing. **Sugin Lal Jabaris**: supervision, validation, writing – review & editing.

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