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RESEARCH ARTICLE

A Molecular Docking Investigation of SARS- CoV-2's Main Protease against Phytocomponents of *Siddha* Formulation *Vishasura Kudineer*

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Abstract

Background: Covid-19 disease is a Wuhan, China originated acute viral respiratory illness. Even though the development of numerous vaccines, the threat of the Covid-19 virus endures nearly two years after the outbreak. The new mutant strain omicron has now spread to several countries. As a result, newer antiviral therapy is required to overcome this scenario. **Objective:** This study is aimed at phytocomponents in the *Siddha* formulation, *Vishasura Kudineer (VSK)*, it is widely used to manage fever and respiratory ailments and may be effective in combating the ongoing pandemic of novel coronavirus disease. **Methodology:** The principal protease 3CLpro of this new Coronavirus (SARS-CoV-2) is a possible focus for therapeutic development since it plays a major part in disease transmission. Molecular docking investigation for all active Phytocomponents found in the *Siddha* formulation *Vishasura Kudineer* with possible targets was carried in the major Protease of SARS-CoV-2 (PDB ID:6LU7). AutoDockTools was used to design and optimize the ligand structures. **Results:** Molecular docking of the 9 bioactive phytocomponents as Santalic acids (-6.39), Nimbolide (-6.38), Rutin, Aristolochic acid (-6.95), Glabridin (-7.53), Indirubin (-7.23), Sabinene (-5.36), β-vetivenene (-6.79), and Zingiberene (-6.47) from in the *Siddha sastric* formulation, *Vishasura Kudineer* performed on the SARS-CoV-2 Main Protease protein's active site (PDB ID: 6LU7). These phytochemicals had significant molecular interaction with the active residues, indicating their distinct inhibitory potencies. **Conclusion:** Whether this *Siddha* formulation could be used to effectively treat and manage COVID-19 and its variants, further research and clinical studies need to be done.

Keywords: Ayush, COVID-19, Molecular Docking, Network Pharmacology, *Siddha* Medicine

1. Introduction

Various infectious viral diseases have recently seemed to have a serious influence on millions of people's lives. Coronavirus has been known to cause human infections since the 1960s; it was only in the last two decades that the virus's potential to inflict severe epidemics became obvious. In the last couple of decades, COVID-19 has been the latest major coronavirus-related airway illness outbreak, and it has impacted negatively on the global socioeconomic balance. The coronavirus disease-19 (COVID-19) is a contagious disease and pathogenic virus transmitted by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that created a public health crisis[1]. It could be spread even during the asymptomatic incubation period (which appears in 50–60% of cases) and for up to two weeks following the symptom onset[2]. Each

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infected individual transmits an average of three other persons. The incubation period is usually 5–6 days (vary 1–14 days). The fatality rate among hospitalized patients is around 11 % and 15%[3]. COVID-19 has been detected in 276,436,619 people and reported to WHO as of December 23, 2021, with 5,374,744 deaths in 215 countries and territories around the world resulting from COVID-19[4]. In India, the Ministry of Health and Family Welfare reported 3, 47, 79,815 cases, and 4, 79,520 deaths. Tamil Nadu's Department of Health and Family Welfare has a sum of 27,42,821 cases have been confirmed, with 36714 deaths and 26,99,309 recoveries[5]. Rapid global spread in a short period has led to a huge number of people being affected, causing a major challenge to healthcare systems.

In the hunt for effective COVID-19 treatments, the World Health Organization (WHO) welcomes global innovations such as repurposing medicines, conventional medicines, and developing new therapeutics. *Siddha* medicine, one of the six authorized divisions of the conventional Indian Medical System, be a traditional medicine that originated in South India and has been used for hundreds of years[6]. It was also essential in the management of Covid-19 disease, especially in Tamil Nadu[7]. In a COVID-19 infection, as per *Siddha* theory, there is an early rise in the overall temperature, cough, and throat pain, which may reduce if there is a sufficient level of immunity, and these symptoms decline while *Pitta thathu* (Humor) refers to a process. Otherwise, it progresses to a stage of *Kapha Dosham* (Disorder), which is described as "*Thanamulla sethumanthan ilagilveppu*". If not treated at this stage it slowly moves to a Stage of *Sanni* (Severe Pneumonia- Respiratory failure)[8]. Many *Siddha* preparations for the treatment of *kapam*-related illnesses were formulated by *Siddhars*. In this way, during the covid pandemic in Tamil Nadu, many *Siddha* formulations were utilized. *Kabasura Kudineer* is a popular *Siddha* medicine that has shown to be successful in the treatment of mild and moderate Covid-19 positive cases. *Vishasura Kudineer* is another *Siddha* formulation that was effective during the global epidemic of covid-19. This is a necessary need toward scientifically proven *Siddha* formulations to fill the gap in novel antiviral therapy.

In the name of *Vishasura Kudineer*, there are many formulations available. The ingredients vary from one to another. In our docking research, we studied *Vishasura Kudineer,* a *Siddha* formulation that has nine ingredients listed in the *Siddha* manuscript '*Agathiyar kaaviya suranool*'. These ingredients of *Vishasura Kudineer* have been used extensively in *Siddha* treatment for the treatment of viral fever, fevers of unknown origin, and respiratory diseases like common cold and cough. The antiviral activity of ingredients in *Vishasura Kudineer* is revealed by the various pharmacological activities of the compounds. In animal studies, they showed anti-inflammatory, antipyretic, and immunomodulatory action[9]. As a result, the present study aims to use a computational method to evaluate the Preparation of *Siddha*, *Vishasura Kudineer* against SARS-CoV-2 main protease.

2. Methodology

Vishasura Kudineer is a polyherbal preparation of *Siddha* with principal components that are used to treat all kinds of fever, Cough, particularly fevers of unknown origin. The following *Siddha* medicinal herbs are included in this *Vishasura Kudineer* preparation (Table 1).

Table 1. The components of *Vishasura Kudineer* and its botanical name, phytoconstituents selected for docking

S. NO.	Vernacular Name	Botanical Name	Phyto components
1	*Vembu*	*Azadirachta indica*	Nimbolide[14]
2	*Avuri*	*Indigofera tinctoria*	Indirubin[15]
3	*Sukku*	*Zingiber officinalae*	Zingiberene[16]
4	*Nannari*	*Hemidesmus indicus*	Rutin[17]
5	*Aadutheendapalai*	*Aristolochia bracteata*	Aristolochic acid[18]
6	*Vettiver*	*Vettivera zizanoids*	β-vetivenene[19]
7	*Adhimathuram*	*Glycyrrhiza glabra*	Glabridin[20]
8	*Ealam*	*Elettaria cardomum*	Sabinene[21]
9	*Santhanam*	*Santalum album*	Santalic acids[22]

3. Protein-Ligand Docking

3.1 Protein Preparation

Binding of Phyto components with the core amino acids (Leu 27, His 41, Gly 143, Cys 145, His 163, His 164, Met 165, Glu 166, Pro 168, His 172) of the target by forming hydrogen bond will hinder the function of the target COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) - PDB- 6LU7. It is thought to be a possible target since it is required for polyprotein cleavage to get16 non-structural proteins (called nsp1-nsp16). These non-structural proteins are very necessary for viral replication and survival. Thereby phytocomponents that inhibit the target 3CL proenzyme could be used as a possible treatment agent for COVID-19 and its symptoms.

3.2 Ligand Preparation

The bioactive Phyto components in the *Siddha* formulation *Vishasura Kudineer* were reported as Santalic acids, Nimbolide, Rutin, Aristolochic acid, Glabridin, Indirubin, Sabinene, β-vetivenene, and Zingiberene. As a result, the PubChem 3D structural components of these 9 bioactive substances were chosen for docking investigations. Using AutoDockToolsv1.5.6, these ligand structures have been further optimized and synthesized[10].

3.3 Docking of Proteins and Ligands

The RSCB Protein Data Bank provided the crystal structure of the SARS-CoV-2 Main Protease protein-ligand complex. AutoDockToolsv1.5.6 has been used to optimize and generate the 3D protein structures (Table 2)[11].

3.4 Molecular Docking

For the obtained phytocomponents, docking computations were done against the target protein 3CL pro. With the assistance of AutoDock tools, essential hydrogen atoms, Kollman unified atom type charges, and solvation parameters were incorporated[12]. The Autogrid application was used to create affinity (grid) maps with a grid point spacing of 0.375. The van der Waals and electrostatic components were calculated using AutoDock parameter set- and distance-dependent dielectric functions, correspondingly. The Lamarckian Genetic Algorithm (LGA) and the Solis and Wets local search approach were used to simulate docking[13]. The ligand molecules' positions, orientations, and torsions were randomly chosen. During docking, all rotatable torsions were relieved. Each docking experiment was made up of two separate runs, each of which was programmed to end after a maximum of 250000 energy evaluations. The population was limited to 150. During the search, a 0.2 translational step was used, as well as 5 quaternion and torsion steps.

4. Discussion of the Results

Vishasura Kudineer Chooranam is one of the poly herb *Siddha* preparations used among *Siddha* professionals to treat COVID-19. Based on molecular docking investigations utilizing AutoDock tools, the inhibitory possibility and efficiency of phytocomponents from *Vishasura Kudineer* towards the novel coronavirus 3CLpro were studied. The key proteases 3CLpro PDB ID

Table 2. Properties of the ligands in the compounds selected for docking analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Zingiberene	204.35 g/mol	$C_{15}H_{24}$	0	0	4
Santalic acids	234.33 g/mol	$C_{15}H_{22}O_2$	1	2	4
Nimbolide	466.5 g/mol	$C_{27}H_{30}O_7$	0	7	4
Indirubin	262.26 g/mol	$C_{16}H_{10}N_2O_2$	2	3	1
Rutin	610.5 g/mol	$C_{27}H_{30}O_{16}$	10	16	6
Aristolochic acid	341.27 g/mol	$C_{17}H_{11}NO_7$	1	7	2
Glabridin	324.4 g/mol	$C_{20}H_{20}O_4$	2	4	1
β-vetivenene	202.33 g/mol	$C_{15}H_{22}$	0	0	0
Sabinene	136.23 g/mol	$C_{10}H_{16}$	0	0	1

(6LU7) were investigated as potential SARS - CoV-2 target proteins. The 9 bioactive phytochemical components from the *Siddha sastric* formulation *Vishasura Kudineer* were docked against the SARS-CoV-2 Main Protease protein (PDB ID: 6LU7) to analyze the chemical connections that cause it to be effective at inhibiting. The major protease 3CLpro is required for polyprotein breakdown into 16 non-structural proteins (called nsp1-nsp16). These non-structural proteins play a crucial role in the replication of viruses. The results of these bioactive compounds' binding affinity with their respective targets are shown in (Table 3).

The herbs provided for In-silico analysis resulted in a set of 9 bioactive phytocompounds. Out of nine compounds,' the leads such as Santalic acids, Nimbolide, Rutin, Aristolochic acid, and Glabridin reveal an

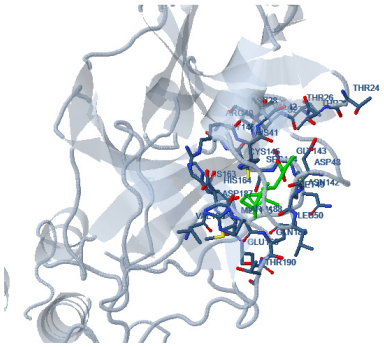
Table 3. Compounds in molecular docking investigations against COVID-19's main protease (3-chymotrypsin-like protease (3CL pro) - (PDB ID: 6LU7)

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM) (**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Zingiberene	-6.47	11.54	-0.01	-7.84	605.01
Santalic acids	-6.39	20.55	-0.22	-7.82	581.04
Nimbolide	-6.38	21.02	-0.14	-7.40	777.90
Indirubin	-7.23	5.00	-0.03	-7.23	585.55
Rutin	-4.76	324.63	-0.78	-5.11	690.29
Aristolochic acid	-6.95	8.11	-0.25	-7.91	672.53
Glabridin	-7.53	3.00	-0.18	-8.44	742.65
β-vetivenene	-6.79	10.50	-0.02	-7.09	536.69
Sabinene	-5.36	117.70	-0.01	-5.66	420.49

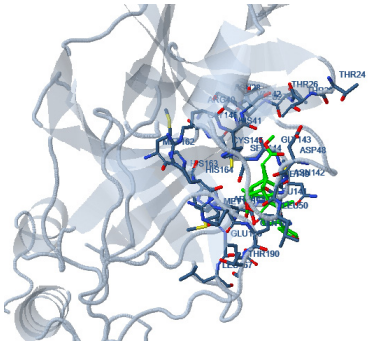
Table 4. Lead's amino acid residue interactions with COVID-19's key protease 3-chymotrypsin-like protease (3CL pro) - (PDB: 6LU7)

Molecule	Interactions	Amino Acid Residue- Binding										
Zingiberene	3	41 HIS	44 CYS	49 MET	52 PRO	54 TYR	167 LEU	189 GLN				
Santalic acids	7	25 THR	27 LEU	41 HIS	49 MET	145 CYS	165 MET	187 ASP	189 GLN			
Nimbolide	9	25 THR	27 LEU	41 HIS	49 MET	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN
Indirubin	5	41 HIS	49 MET	165 MET	166 GLU	168 PRO	189 GLN					
Rutin	6	41 HIS	49 MET	54 TYR	142 ASN	143 GLY	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN
Aristolochic acid	6	25 THR	27 LEU	41 HIS	49 MET	54 TYR	145 CYS	165 MET				
Glabridin	7	41 HIS	54 TYR	141 LEU	142 ASN	165 MET	166 GLU	187 ASP	189 GLN			
β-vetivenene	3	27 LEU	144 SER	145 CYS	163 HIS	166 GLU						
Sabinene	4	41 HIS	49 MET	54 TYR	164 HIS	165 MET	187 ASP	189 GLN				

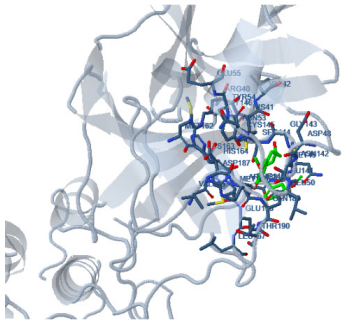
optimum of 6 to 7 linkages with the target's core active amino acid residues 3CLpro. Compounds like Indirubin, Sabinene, β-vetivenene, and Zingiberene placed second, with a maximum of 3 to 5 connections with the active position of the objective enzyme 3CLpro. According to findings, certain VSK compounds may have antiviral properties against the evolving coronavirus SARS-CoV-2 by inhibiting the host cell receptor or suppressing the main viral protease needed for host cell replication. In the future, this research is intended for therapeutic development for viral diseases like COVID-19 shown in (Table 4 and Figure 1).



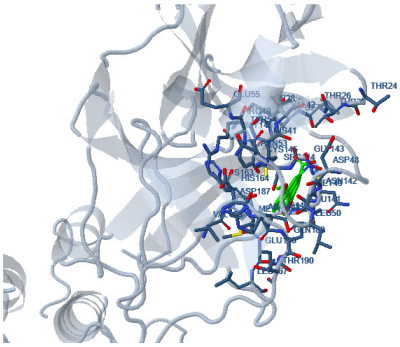
Santalic acids with the major protease of COVID-19 (3-chymotrypsin-like protease (3CL pro) -PDB- 26LU7



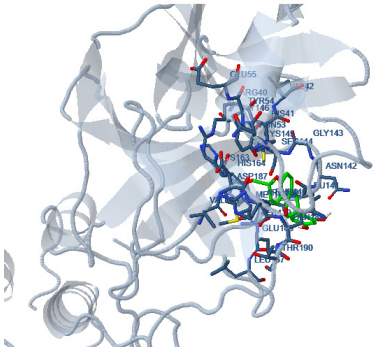
Nimbolide with the major protease of COVID-19 (3-chymotrypsin-like protease (3CL pro) -PDB- 26LU7



Rutin with the major protease of COVID-19 (3-chymotrypsin-like protease (3CL pro) -PDB- 26LU7



Aristolochic acid with the major protease of COVID-19 (3-chymotrypsin-like protease (3CL pro) -PDB- 26LU7



Glabridin with the major protease of COVID-19 (3-chymotrypsin-like protease (3CL pro) -PDB- 26LU7

Figure 1. Main compounds in molecular docking investigations against COVID-19's main protease (3-chymotrypsin-like protease (3CL pro).

5. Conclusion

The *Siddha* herbal infusion *Vishasura Kudineer* has many medicinal plant ingredients. The biomolecules which are present in those herbals which are formulated in this *Siddha* medicine *Vishasura Kudineer* shows inhibition while docking against the major protease of COVID-19 (3-chymotrypsin-like protease (3CL pro) -PDB-26LU7. The Phytochemicals present in the ingredients of *Vishasura Kudineer*, such as Santalic acids, Nimbolide, Rutin, Aristolochic acid, Glabridin, Indirubin, Sabinene, β-vetivenene, and Zingiberene, show significant binding energy against the target molecule 3CL pro, according to the results of the computational analysis. Especially, the Compound Glabridin and Indirubin shows high binding affinity such as -7.53 Kcal/mol and -7.23 Kcal/mol respectively. The biocompound Glabridin present in the herb *Glycyrrhiza glabra* showed high therapeutic potency against SARS-CoV2 in previous research works[23]. The Phytocompounds such as Aristolochic acid, *β*-vetivenene, Zingiberene, Santalic acids, Nimbolide, Sabinene, Rutin shows more binding potential against the target protein molecule which are -6.95 Kcal/mol, -6.79 Kcal/mol, -6.47 Kcal/mol, -6.39 Kcal/mol, -6.38 Kcal/mol, -5.36 Kcal/mol, -4.76 Kcal/mol respectively. The plant *Azadirachta indica*, *Zingiber officinale* is referred to treat COVID19 due to its medicinal value[24,25]. As an outcome, it was concluded that these molecules could have a potential inhibitory effect on the 3 CL proenzyme, blocking the synthesis of 16 non-structural proteins (nsp1-nsp16), which are necessary for replication of virus and thereby restricting viral survival in the host atmosphere. The Molecular Docking studies of these Phyto compounds concludes that this drug may be beneficial to treat SARS-Cov-2.

6. References

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