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A molecular docking study of SARS-CoV-2 main protease against phytochemicals of Siddha Medicinal herb *Vilvam* (*Aegle marmelos*)

Research Article

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Abstract

Background: In December of 2019, mysterious pneumonia was reported. A novel coronavirus (nCoV) was identified as the causative agent for this pneumonia; it is now known as coronavirus 2. This pandemic has caused widespread alarm around the world. Now, countries around the world are preparing for the third and fourth waves of COVID-19. Objective: This research aims to conduct In Silico computational studies of phytoconstituents in leaf extracts of the Siddha medicinal herb *Aegle marmelos* (*Vilvam*), which are commonly used in the treatment of viral fever and respiratory infectious diseases and may be effective against the current pandemic novel coronavirus disease. Methodology: In Silico molecular docking analysis was performed for all the active compounds present in the herb *Aegle marmelos* (*Vilvam*) with potential targets SARS-CoV-2 Main Protease (PDB ID: 7JQ5). The ligand structures were prepared and optimized by AutoDockTools. The active sites docking study was performed using Autodock Vina for all the compounds. The inhibitor compound MPI8 bound inSARS-CoV-2 main protease Protein-Ligand complex (PDB ID: 7JQ5) is considered as the reference inhibitor molecule of this study. Results: Molecular docking of the 14 bioactive phytochemicals compounds from *Aegle marmelos leaves* carried out towards the active site of SARS-CoV-2 Main Protease protein (PDB ID: 7JQ5). The interactions of these compounds were comparatively analyzed with the reference inhibitor MPI8 bound inSARS-CoV-2 Main Protease protein-ligand complex (PDB ID: 7JQ5). These phytochemicals exhibited effective molecular interactions with the active residues enumerating their differential inhibition potency. Conclusion: Further research and clinical trials are needed whether this herb can be implemented to effectively treat and manage COVID-19.

Key Words: Molecular docking, Siddha medicine, Ayush Medicine, Network pharmacology, Indian Traditional Medicine.

Introduction

COVID-19 is also named SARS-CoV-2 which is a dreadful disease heading towards the world at this moment. This virus belongs to the family Coronaviridae. The family Coronaviridae has two genera-Coranavirus and Torovirus. They possess 12 and 2 species respectively. Only two species of genus coronavirus, human coronavirus 229E and the human coronavirus 0C43 cause respiratory and gastrointestinal infections (1). By infecting bronchial epithelial cells, pneumocytes and upper respiratory tract cells in humans, SARS-CoV, MERS-CoV and SARS-CoV-2 infections can develop into severe, life-threatening respiratory pathologies and lung injuries for which no specific prophylactic or therapeutic treatment has been approved to date. (2). On 31st December 2019, 27 cases of pneumonia of unknown aetiology were recognized in Wuhan City, in China. The causative organism was detected from the throat swab led by the Chinese Centre for Disease Control and Prevention (CCDC) on 7th January 2020 and was along these lines named Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) (3). The World Health Organization (WHO) named this entity COVID-19. On 30th January 2020, the WHO proclaimed the Chinese episode of COVID-19 to be a Public Health Emergency of International Concern representing a high hazard to nations with weak wellbeing frameworks (4). The first case of Covid-19 was reported on 27th Jan 2020 in India. The second wave resulted from approximately one lakh positive cases daily. The first case of the COVID-19 in the Indian state of Tamil Nadu was reported on 7 March 2020. As of July 29, 2021, exactly 4, 22,022 patients were died in India, with Maharashtra reported the highest rate of deaths of 1, 32,145. As of July 29, 2021, Tamilnadu had 2,55,3805 confirmed cases (5). Majority of the proposed COVID-19 vaccines requires a follow up dose with multiple shots. Additionally, SARS- CoV-2 has shown capacity to

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mutate and render certain vaccines ineffective. These challenges may be overcome by the discovery of a potent antiviral compound (6). To prevent, manage, and treat this devastating disease, it is a period to come back to our traditional Siddha system of medicine.

Siddha medicines have been used effectively by human's traditional civilization over several centuries for treating different kinds of diseases and can be effectively recommended to target the host response, like *Kaba Sura Kudineer* during influenza outbreaks (7). The Siddha medical system is a well-known ancient Indian medicinal system. It plays a significant role in the treatment of Covid-19 disease, particularly in Tamil Nadu. The Siddha system of medicine considers the body as a conglomeration of three *dosham* known as *Vatham* (*Wind*), *Pitham* (*Fire*), and *Kabam* (*Water*), corresponding to the three elements of the universe. Equilibrium between the three *dosham* is necessary to maintain perfect health. Any derangement in the synergic action of these *dosham* transforms the body as a fertile ground to pop up any infection (8). Since Siddha system of medicine deals with the root cause of diseases, *Kaba Suram* is said to occur due to the aggravated humour of *Kabam* much more than its normalcy, facilitating a favourable environment for any respiratory infection (9). Many traditional herbs have been reported to possess potent anti-viral properties (10) and can aid in the reduction of *Kabam*.

As per the Siddha literature, *Aegle marmelos* (*Vilvam*) is used to treat various kinds of fever and viral infections (11). Some of the pharmacological studies reveal the Antipyretic and Analgesic properties of *Aegle marmelos* (*Vilvam*) (12). Some studies confer *Aegle marmelos* (*Vilvam*) numerous pharmacological actions associated to respiratory illnesses, including antiviral activity (13, 14), antipyretic activity (15, 16), anti-inflammatory activity (17), anti-histaminic effect, and anti-asthmatic activity (18).

Many analytical studies have been performed on these compositions and active compounds with their structure have been identified. The COVID-19 pathogen, SARS-CoV-2, requires its main protease (SC2M Pro) to digest two of its translated long polypeptides to form several mature proteins that are essential for viral replication and pathogenesis. Inhibition of this vital proteolytic process is effective in preventing the virus from replicating in infected cells and therefore provides a potential COVID-19 treatment option. Although we have yet to understand SARS-CoV-2 biology and COVID-19 pathogenesis, previous studies of SARS-CoV-1 have established that activity of both PL^{Pro} and M^{Pro} is essential to viral replication and pathogenesis. Of the two proteases, M^{Pro} processes 12 out of the total 15 Nsps; inhibition of this enzyme is anticipated to have more significant impacts on viral biology than that of PL^{Pro}. Therefore, small-molecule medicines that potently inhibit SARS-CoV-2 M^{Pro} ($SC2M^{Pro}$) (PDB ID: 7JQ5) (19), are potentially effective treatment options for COVID-19. Molecular docking studies can be performed to determine active natural compounds with inhibitory effects against both the protein involved in coronavirus disease pathogenesis.

Materials and methods

Protein-ligand docking

Protein preparation

The crystal structure of the SARS-CoV-2 Main Protease protein-ligand complex (PDB ID: 7JQ5) was obtained from RSCB Protein Data Bank. The 3D protein structures were optimized and prepared using AutoDockToolsv1.5.6. (20). The inhibitor MPI8 bound inSARS-CoV-2 Main Protease protein-ligand complex (PDB ID: 7JQ5) is considered as the reference inhibitor for comparison in this study.

Ligand preparation

The bioactive molecules constituents in the leaf extract of the medicinal herb *Aegle marmelos* (*Vilvam*) were reported as Methyldodecanol, Carene, Alpha-Amyrin, Alpha Cubebene, Cinnamamide, D-Limonene, Gamma- Sitosterol, Heneicosane, Humulene, Isoledene, Linalyl Acetate, Loliolide, O-Xylene, Tridecane. Hence, the 3D structural coordinates of these 14 bioactive phytochemicals obtained from PubChem were selected for docking studies. These ligand structures were further optimized and prepared using AutoDockToolsv1.5.6. (21).

Molecular Docking

Docking was performed with AutodockVina v112 (22), a prevalent molecular screening tool for predicting/identifying the approximate binding energy of each ligand with the target proteins. The docking parameters were optimized with the grid box centered on the MPI8 Inhibitor at the active site of the protein. Then, Docking analysis was carried out individually for each compound at the active ligand-binding site (MPI8 Inhibitor bound site) of SARS-CoV-2 Main Protease protein (PDB ID: 7JQ5) and the binding energies of each ligand found. All graphical presentations of the docked complexes were illustrated using Discovery studio visualizer v2.5 (BIOVIA, San Diego, CA, USA)

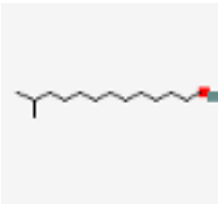
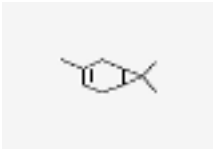
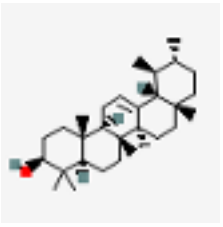
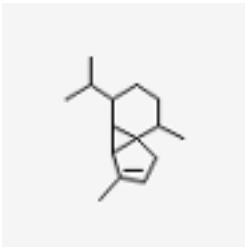
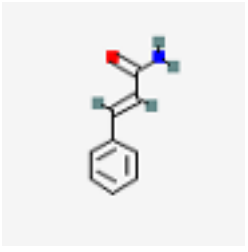
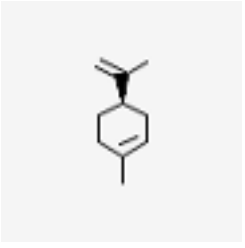
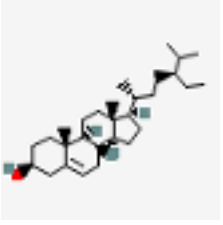
ADME properties prediction

The ADME (absorption, distribution, metabolism, excretion) properties of above mentioned natural compounds were predicted using SwissADME an online tool for ADME prediction (23). The bioactivity score of the best compound was predicted using mol inspiration (24).

Results

Molecular docking of the 14 bioactive phytochemical compounds from *Aegle marmelos* leaves was carried out towardsSARS-CoV-2 Main Protease protein (PDB ID: 7JQ5) to identify their molecular interactions mediating their inhibition potency. The inhibitor compound MPI8 bound in the SARS-CoV-2 Main Protease protein-ligand complex (PDB ID: 7JQ5) is considered as the reference inhibitor molecule in this study.

Table 1: Shows the bioactive compounds from *Aegle marmelos (Vilvam)* leaves

Sl.No	Pubchem ID	Compound Name	Molecular weight	Molecular formula	
1	33865	11-Methyldodecanol	200.36 g/mol	$C_{13}H_{28}O$	
2	26049	3-Carene	136.23 g/mol	$C_{10}H_{16}$	
3	73170	Alpha-Amyrin	426.7 g/mol	$C_{30}H_{50}O$	
4	86609	AlphaCubebene	204.35 g/mol	$C_{15}H_{24}$	
5	5273472	Cinnamamide,	147.17 g/mol	C_9H_9NO	
6	440917	D-Limonene	136.23 g/mol	$C_{10}H_{16}$	
7	457801	Gamma-Sitosterol	414.7 g/mol	$C_{29}H_{50}O$	

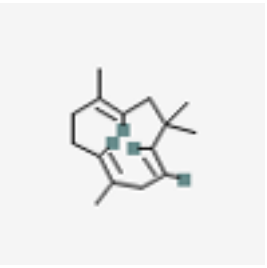
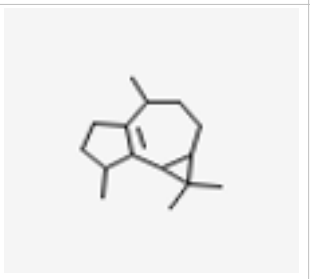
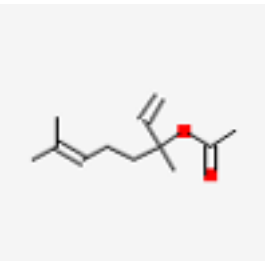
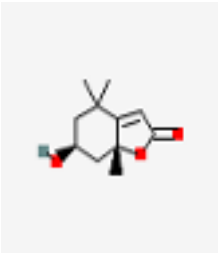
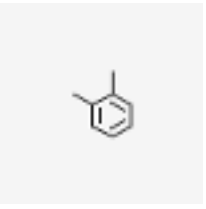
8	12403	Heneicosane	296.6 g/mol	$C_{21}H_{44}$	
9	5281520	Humulene	204.35 g/mol	$C_{15}H_{24}$	
10	530426	Isoledene	204.35 g/mol	$C_{15}H_{24}$	
11	8294	Linalyl Acetate	196.29 g/mol	$C_{12}H_{20}O_2$	
12	100332	Loliolide	196.24 g/mol	$C_{11}H_{16}O_3$	
13	7237	O-Xylene	106.16 g/mol	C_8H_{10}	
14	12388	Tridecane	184.36 g/mol	$C_{13}H_{28}$	

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Figure-I: The 3D Structure of SARS-CoV-2 Main Protease protein-ligand complex (PDB ID: 7JQ5) bound with the MPI8 Inhibitor

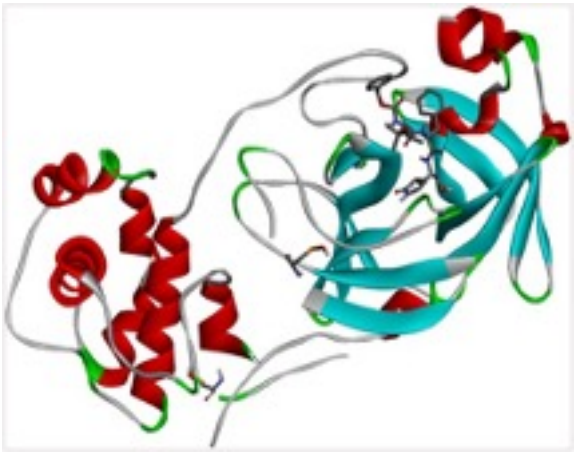
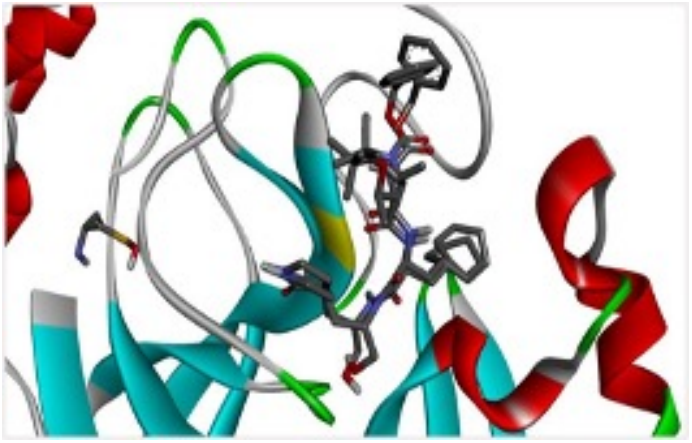


Figure-II: shows the docked conformation of MPI8 inhibitor with B.E of -7.7 Kcal/mol superimposed with the original protein-ligand complex crystal conformation (7JQ5)



This demonstrates that the docking prediction is quite close to the actual bound complex, demonstrating the reference compound MPI8 inhibitor's interactions with the SARS-CoV-2 Main Protease.

The bonded interactions were found with Phe140, Glu166, Gly143, Ser144, Cys145 through conventional H-bonds and the non-bonded interactions with Tyr54, Asp187, Arg188, Leu167, Gln192, His164, His172, Leu141, Asn142, Met165 through Van der Waals interactions and His41, Met49 through Alkyl interactions. The inhibition potency of the compounds was analyzed based on their Binding energy profile and they are bonded and non-bonded interactions with the active site residues. The predicted docking score is represented as B.E which depicts the approximate binding property of these ligands with the protein is given in **Table- II.**

Table- II: Shows binding property of these ligands with the protein

Ligands	ID	Binding Energy (Kcal/mol)
MPI8 – inhibitor	MPI8	-7.7
Alpha-Amyrin	73170	-7.6
Gamma-Sitosterol	457801	-6.7
AlphaCubebene	86609	-5.7
Loliolide	100332	-5.7
Isoledene	530426	-5.4
3-Carene	26049	-5.1
Humulene	5281520	-5.7
Cinnamamide	5273472	-5.1
Linalyl Acetate	8294	-4.8
D-Limonene	440917	-4.7
Heneicosane	12403	-4.5
11-Methyldodecanol	33865	-4.4
O-Xylene	7237	-4.1
Tridecane	12388	-3.7

Based on their Binding profile, the top 5 phytochemical molecules Alpha-Amyrin, Gamma Sitosterol, Alpha Cubebene, Loliolide and Humulene were revealed to have a comparatively better B.E. than the MPI8 inhibitor. As a result, their molecular interactions were evaluated to see how effective they were at inhibiting MPI8. Hence, their molecular interactions were analyzed to evaluate their inhibition efficacy in comparison to MPI8.

Figure-III - The bonded and non-bonded interactions of the MPI8 inhibitor are represented in the 2D ligand interactions profile

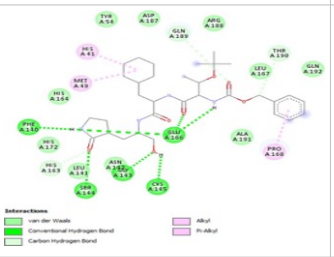


Figure-IV: The bonded and non-bonded interactions of Alpha-Amyrin are represented in the 2D ligand interactions profile

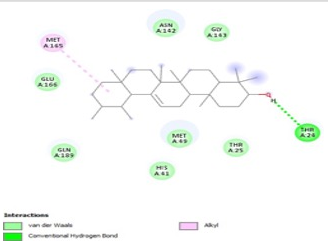


Figure-V: The bonded and non-bonded interactions of Gamma-Sitosterol are represented in the 2D ligand interactions profile

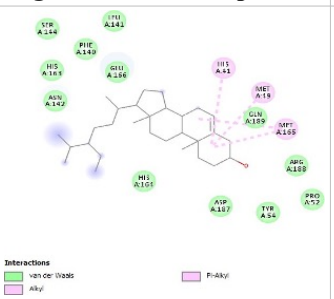


Figure-VI: The bonded and non-bonded interactions of Alpha Cubebene are represented in the 2D ligand interactions profile

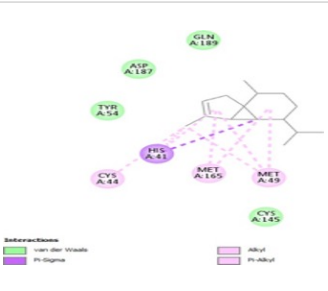


Figure-VII: The bonded and non-bonded interactions of Loliolide are represented in the 2D ligand interactions profile

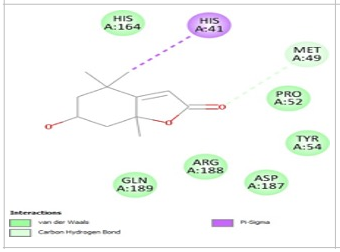


Figure-VIII: The bonded and non-bonded interactions of Humulene are represented in the 2D ligand interactions profile

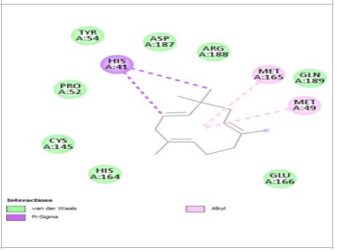


Table – III: The top 5 phytochemicals of the herb with better binding energy and bonded, non-bonded interactions are summarized

Ligands	Binding Energy (Kcal/ mol)	Bonded Interactions H-Bond	Non bonded interactions Vander Waals, and Alkyl, Pi-Alkyl
MPI8 - inhibitor	-7.7	Phe140, Glu166, Gly143, Ser144, Cys145, Gln189, His163, Thr190	Tyr54, Asp187,Arg188, Leu167, Gln192,His164, His172, Leu141, Asn142, Met165 and His41, Met49
Alpha-Amyrin	-7.6	Thr24	Asn142, Gly143, Glu166, Gln189, His41, Met49, Thr25 and Met165
Gamma-Sitosterol	-6.7	-	Ser144, His163, Asn142, Phe140, Leu141, Glu166, His164, Asp187, Tyr54, Arg188, Gln189, Pro52 and Met49, Met165, His41
Alpha Cubebene	-5.7	-	Tyr54, Asp187, Gln189, Cys145 and Cys44, Met165, Met49, His41
Loliolide	-5.7	Met49	His164, Pro52, Tyr54, Gln189, Arg188, Asp187
Humulene	-5.7		Tyr54, Pro52, Cys145, His164, Glu166, Asp187, Arg188, Gln189 and Met165, Met49, His41

Although the bonded and non-bonded interactions of these compounds vary, the crucial interaction residues mediated by the inhibitor MPI8 were maintained by these phytochemicals, indicating that these compounds could act asSARS-CoV-2 Main protease inhibitors with similar efficacy to MPI8. This could elucidate that why extracts that constitute these phytochemicals are active. This could explain that's why the extracts that constitute these compounds are active.

Conclusion

Various Phytoconstituents of the medicinal herb of *Aegle marmelos* (*Vilvam*) such as Methyldodecanol, Carene, Alpha-Amyrin, AlphaCubebene, Cinnamamide, D-Limonene, Gamma- Sitosterol, Heneicosane, Humulene, Isoledene, Linalyl Acetate, Loliolide, O-Xylene, Tridecane were selected for docking from the leaf extract of the medicinal herb *Aegle marmelos* (*Vilvam*). 3D structures of 14 bioactive were taken from PubChem structures revels significant binding against receptor Spike protein of COVID-19. If in silico investigations are confirmed with antiviral activity trials, it could be a future treatment against this coronavirus infection. In addition, preclinical and clinical research must be carried out to evaluate the exact mechanism. India is a developing country with a moderate rate of economic growth. This simple herb is usually always available at Shiva temples. This simple herb will save many poor individuals from this pandemic illness if its usefulness is proven.

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Conflict of Interest

We announce that there is no conflict of interest in this study.

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