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In Silico Docking Analysis of Poly Herbal Formulation *Aadathodai Kudineer* used in Siddha medicine in inhibiting Main Protease and ACE2 Receptor Spike protein SARS-CoV-2

Research Article

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Abstract

Corona virus disease (COVID-19) is an infectious pandemic disease caused by the newly discovered novel corona virus. World Health Organization has declared the global health emergency due to COVID19 outbreak. Currently, there is no specific treatment or vaccine for fighting against this infectious disease. *Aadathodai Kudineer* is a drug indicated for *Iya Erumal, Kozhai Kattu, Kabasuram.* Upon the mortality and severity of the disease COVID19, we tried to identify the possible inhibition of phytocomponents of *Aadathodai Kudineer* in inhibiting Main Protease and ACE2 Receptor Spike protein SARS-CoV-2 through molecular docking studies. Methodology: In Silico molecular docking analysis was performed for phytocomponents present in the *Aadathodai Kudineer* formulation for targets main protease and ACE2 Receptor Spike protein, PDB ID: 6LU7 and PDB ID: 2AJF using Autodock tool. ADME properties was also predicted for all the above compounds. Results: Among the 9 active Phytocompounds present in the *Aadathodai Kudineer* formulation, Lupeol showed high binding affinity with COVID19 main protease and ACE2 receptor which shows the promising contrivance of protease inhibition. The ADME suggested that the formulation is free from toxic. Conclusion: The phytocomponents showed possible affinity towards these targets and has the lead molecules that inhibits COVID19 main protease and ACE2 receptor.

Key Words: *Siddha formulation, Aadathodai Kudineer, SARS-CoV-2, COVID19, Molecular docking, ADME.*

Introduction

On 31st December 2019, 27 cases of pneumonia of unknown aetiology were recognized in Wuhan City, Hubei area in China. The causative organism was recognized from throat swab tests led by the Chinese Centre for Disease Control and Prevention (CCDC) on seventh January 2020, and was along these lines named Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). The World Health Organization (WHO) named this infection as 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 (1).

As of 27 July 2020, following data has been reported throughout the world, India & Tamil Nadu. More than 16.4 million cases of COVID-19 have been reported in 185 countries and territories, resulting in more than 6,46,641 deaths. More than 9.51 million people have recovered (2). The Ministry of Health and Family Welfare of India has confirmed a total of 14,35,453 cases, 9,17,567 recoveries (including 1 migration) and 32771 deaths in India. The Department of Health and Family Welfare of Tamil Nadu has confirmed a total of 2,13,723 cases, including 3493 deaths and 1,56,526 recoveries. Around 53703 active cases are reported (3).

The World Health Organization (WHO) welcomes innovations around the world including repurposing drugs, traditional medicines and developing new therapies in the search for potential treatments for COVID-19 (4). In China, traditional Chinese medicine is very useful to control and prevention and treatment for COVID 19 patients. In integrated approach is very success full treatment in COVID 19 patients in china (5).

Among six recognized streams of Indian Medicine System, Siddha medicine is one such traditional medicine originating in Tamil Nadu, India and practiced over centuries (6). Siddha system of medicine has played a major role in treating the diseases such as dengue, chikungunya. Both the TN Govt and union ministry of AYUSH has recommended an herbal siddha medicine called *Nilavembu Kudineer* as a treatment for dengue (7). This COVID 19 Pandemic Ministry of AYUSH publish the "Guidelines for Siddha Practitioners for COVID 19".

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Aadathodai Kudineer, a classical Siddha Formulation described in Siddha manuscript *Gunapadam Mooligai Vaguppu* and is used for *Iya Erumal, Kozhai Kattu, Kabasuram*. This poly herbal medicine contains three different ingredients such as *Aadathodai, Kandankathiri, Seenthil* (8). In Siddha treatment these three ingredients have been used extensively for the treatment of respiratory diseases such as cold, cough, whooping cough, chronic bronchitis, asthma, pneumonia and tuberculosis. *Aadathodai* has its own Anti-viral property (9), Anti-asthmatic, Bronchodilator activity, Anti-tubercular activity, Anti-bacterial activity (10 – 11), Antipyretic Activity (12). *Seenthil* has its own Antiviral property (13), Immunomodulatory activity (14), Anti-malarial, Anti-inflammatory, Antioxidant, Hepatoprotective, Immunomodulatory properties (15). *Kandankathiri* has it owns Anti-asthmatic activity, Anti-inflammatory activity, Cardio-protective activity (16), Antimicrobial activities, Anti-inflammatory activity, Anti-pyretic activity (17). Immunomodulatory activity, Anti-oxidant activity, Anti-pyretic activity, Hepatoprotective activity (18).

Materials and methods

Aadathodai Kudineer is a polyherbal Siddha formulation and active compounds in the formulation is indicated for *Iya Erumal, Kozhai Kattu, Kabasuram*. This formulation is having the following Siddha medicinal plants (Table 1).

Table 1: *Aadathodai Kudineer* their botanical name and its source.

S. No	Common Name	Botanical Name
1	Aadathodai	*Justicia adhatoda* L
2	Kandankathiri	*Solanum surattense* BURM. F.
3	Seenthil	*Tinospora cordifolia* (WILLD.) HOOK.F. & THOMS

Protein ligand docking

Protein preparation

The three-dimensional crystal structure of SARS-CoV-2 main protease (3-chymotrypsin-like protease (3CL pro) [PDB ID - 6LU7] and Angiotensin-converting enzyme 2 (ACE2) receptors – [PDB ID - 2AJF] was retrieved from the Protein Data Bank (PDB) and energy minimized and then converted into their corresponding PDBQT formats.

Ligand preparation

The phytocomponents identified in *Aadathodai Kudineer* formulation such as Adhatodine, Anisotine, Berberine, Tinosporide, Apigenin, Diosgenin, Lupeol, Vasicinone, Vasicoline were selected for docking from the *Aadathodai Kudineer* formulation. Ligand molecules were downloaded from Pubchem (19) in sdf format. Optimization was done with the force field type MMFF94 using Openbable softwares and saved as pdbqt format and used in docking studies.

Molecular Docking

Docking was performed with Autodock tool (20) a prevalent molecular screening tool for identifying binding energy between the 3D structures of each ligand and target proteins. Two target proteins PDB ID - 6LU7 and PDB ID - 2AJF were +9 selected, a Grid-free docking performed, and the binding energies of each ligand found.

ADME properties prediction

The ADME (absorption, distribution, metabolism, excretion and toxicity) properties of above mentioned phytocomponents predicted using SwissADME an online tool for ADME prediction (21).

Results and Discussion

Aadathodai Kudineer Chooranam is one among the poly herbal formulation used for the treatment of COVID-19, Molecular docking studies carried out for the nine phytocomponents present in *Aadathodai Kudineer.* SARS-CoV-2 use ACE2 receptors to gain entry to the target cells causing Corona Viral disease 19 in humans (22) and the main protease 3CLpro is highly essential for cleavage of polyprotein to get16 non-structural proteins (called nsp1-nsp16). These non-structural proteins are highly essential for viral replication. For this purpose, AutoDock4 docking program was employed, which uses Lamarckian genetic algorithm. The results of the binding affinity of these compound with their respective targets are given in (Table 2).

Table 2: Binding energy of various compounds involved in *Aadathodai Kudineer*

S. No.	Compounds	Binding Free energy Kcal/ mol 3CLPRO	Binding Free energy Kcal/ mol ACE2
1	Adhatodine	-7.47	-5.13
2	Anisotine	-7.42	-4.18
3	Berberine	-5.99	-5.344
4	Tinosporide	-5.77	-3.86
5	Apigenin	-4.55	-2.23
6	Diosgenin	-7.96	-6.27
7	Lupeol	-8.38	-6.18
8	Vasicinone	-5.38	-3.59
9	Vasicoline	-7.69	-5.32

Among all the docked compounds that showed a range of binding affinity Lupeol showed 8.38 kcal/ mol for 3CLPRO and 6.27 kcal/mol for ACE2 receptor respectively. Adhatodine, Anisotine, Tinosporide, Apigenin and Vasicoline possess 100% binding efficacy by interacting with both the core target amino acids (31 LYS and 353 LYS) present on the target. Followed by this other phytocompounds such as Berberine, Diosgenin, Lupeol and Vasicinone possess 50% affinity by binding with one of the target amino acid either with

31 LYS or with 353 LYS present on the target receptor ACE-2. Anisotine has maximum of 6 interactions with the core active amino acid residues present on the target. Followed by this the compounds such as Adhatodine, Tinosporide and Lupeol ranked second with the maximum of 5 interactions. Similarly, the compounds Berberine, Apigenin and Vasicinone ranks third with the maximum of 4 interactions with the active site of the target enzyme 3CLpro. The ADME properties predicted using swissADME suggested that did not show any hepatocellular toxicity. The compound did not also show any blood-brain barrier crossing suggesting low toxicity induced upon intake. The compound is shows 90% solubility concentration of this phytocompound.

Table 3: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Adhatodine	337.416 g/mol	$C_{20}H_{21}N_3O_2$	1	2	4
Anisotine	349.4 g/mol	$C_{20}H_{19}N_3O_3$	1	5	4
Berberine	336.4 g/mol	$C_{20}H_{18}NO_4$	3	0	4
Tinosporide	374.4 g/mol	$C_{20}H_{22}O_7$	1	7	1
Apigenin	270.24 g/mol	$C_{15}H_{10}O_5$	3	5	1
Diosgenin	414.6 g/mol	$C_{27}H_{42}O_3$	1	3	0
Lupeol	426.7 g/mol	$C_{30}H_{50}O$	1	1	1
Vasicinone	202.21 g/mol	$C_{11}H_{10}N_2O_2$	1	3	0
Vasicoline	291.4 g/mol	$C_{19}H_{21}N_3$	0	2	2

Table 4: Summary of the molecular docking studies of compounds against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM) (**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Adhatodine	-5.13	172.73	-0.82	-5.04	530.48
Anisotine	-4.18	867.69	-0.06	-5.03	503.55
Berberine	-5.344	103.4	-1.14	-4.88	551.99
Tinosporide	-3.86	1.47*	-0.16	-4.62	444.65
Apigenin	-2.23	23.08	-0.12	-4.05	430.24
Diosgenin	-6.27	25.36	-0.02	-6.57	582.39
Lupeol	-6.18	29.59	-0.16	-6.77	655.34
Vasicinone	-3.59	2.34*	-0.17	-3.89	344.17
Vasicoline	-5.32	126.28	-0.60	-5.23	463.48

Table 5: Amino acid Residue Interaction of Lead against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Molecule	Interactions	Amino Acid Residue- Binding						
Adhatodine	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-
Anisotine	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-
Berberine	1	33 ASN	34 HIS	37 GLU	38 ASP	353 LYS	389 PRO	393 ARG
Tinosporide	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-
Apigenin	2	31 LYS	34 HIS	38 ASP	353 LYS	-	-	-
Diosgenin	1	33 ASN	34 HIS	37 GLU	38 ASP	353 LYS	389 PRO	393 ARG
Lupeol	1	30 ASP	33 ASN	34 HIS	37 GLU	353 LYS	389 PRO	393 ARG
Vasicinone	1	31 LYS	34 HIS	35 GLU	38 ASP	-	-	-
Vasicoline	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS	-

Table 6: Summary of the molecular docking studies of compounds against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM) (**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Adhatodine	-7.47	3.36	-0.36	-8.29	766.16
Anisotine	-7.42	3.66	-0.03	-8.11	790.62
Berberine	-5.99	40.33	-0.17	-6.71	726.25
Tinosporide	-5.77	59.28	-0.07	-6.59	758.09
Apigenin	-4.55	45.50	-0.21	-6.39	650.78
Diosgenin	-7.96	1.46	-0.18	-8.29	832.88
Lupeol	-8.38	716.67**	-0.03	-8.98	911.38
Vasicinone	-5.38	113.14	-0.10	-5.68	493.23
Vasicoline	-7.69	2.31	-0.32	-7.61	687.35

Table 7: Amino acid Residue Interaction of Lead against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Molecule	Interactions	Amino Acid Residue- Binding									
Adhatodine	5	41 HIS	49 MET	54 TYR	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN
Anisotine	6	27 LEU	41 HIS	144 SER	145 CYS	163 HIS	165 MET	166 GLU	-	-	-
Berberine	4	41 HIS	49 MET	145 CYS	165 MET	166 GLU	189 GLN	-	-	-	-
Tinosporide	5	41 HIS	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	189 GLN	-	-
Apigenin	4	41 HIS	49 MET	52 PRO	54 TYR	145 CYS	163 HIS	166 GLU	189 GLN	-	-
Diosgenin	3	25 THR	41 HIS	49 MET	143 GLY	145 CYS	166 GLU	189 GLN	-	-	-
Lupeol	5	41 HIS	142 ASN	145 CYS	165 MET	166 GLU	168 PRO	189 GLN	-	-	-
Vasicinone	4	27 LEU	41 HIS	144 SER	145 CYS	163 HIS		-	-	-	-
Vasicoline	3	165 MET	166 GLU	167 LEU	168 PRO	189 GLN	192 GLN	-	-	-	-

Table 8: *In silico* pharmacokinetics properties of phytochemical constituents of *Aadathodai Kudineer*

Phytochemical constituent	Intestinal absorption	BBB permeability	Human Vd (L/kg)	Total clearance (mg/kg/ day)	Renal OCT2 substrate
Adhatodine	86.22	-0.127	0.08	0.58	No
Anisotine	94.97	-0.381	-0.092	0.675	No
Berberine	97.147	0.198	0.58	1.27	No
Tinosporide	100	-0.482	0.15	0.414	No
Apigenin	93.25	-0.734	0.822	0.566	No
Diosgenin	96.565	0.2	0.426	0.328	Yes
Lupeol	95.782	0.726	0	0.153	No
Vasicinone	92.532	-0.206	0.142	0.568	No
Vasicoline	93.064	0.614	0.76	0.609	Yes

Table 9: The predicted pharmacokinetics properties of phytochemical constituents for Cytochrome Inhibition and P-glycoprotein studies for *Aadathodai Kudineer*

Phytochemical constituent	CYP2D6 and CYP3A4 substrate	CYP enzymes inhibition	P-gp substrate	P-gp I or II inhibition
Adhatodine	No	CYP1A2	No	No
Anisotine	CYP3A4	CYP1A2 CYP2C19 CYP2C9 CYP3A4	substrate	P-gp I and II
Berberine	CYP3A4	CYP1A2 CYP2D6 CYP3A4	substrate	P-gp II
Tinosporide	CYP3A4	No	No	No
Apigenin	No	CYP1A2 CYP2C19	substrate	No
Diosgenin	CYP3A4	No	No	P-gp I and II
Lupeol	CYP3A4	No	No	P-gp I and II
Vasicinone	No	CYP1A2	substrate	No
Vasicoline	CYP3A4 CYP2D6	CYP1A2 CYP2C19 CYP2C9 CYP2D6	No	No

Table 10: The predicted toxicity of phytochemical constituents of *Aadathodai Kudineer*

Phytochemical constituent	AMES tox.	hERG I or II inhibition	Hepatotoxicity	Skin sensitization	Carcinogenicity	Human maximum tolerated dose (mg/kg/day)	Oral rat acute toxicity (mol/kg)	Oral rat chronic tox. (mg/kg_bw/day)
Adhatodine	Yes	No	No	No	No	0.15	1.956	2.564
Anisotine	NO	NO	Yes	No	No	0.158	2.268	1.758
Berberine	Yes	No	Yes	No	No	0.144	2.571	1.89
Tinosporide	No	No	Yes	No	No	-0.373	3.165	0.967
Apigenin	No	No	No	No	No	**0.328**	2.45	2.2983
Diosgenin	No	hERG II	No	No	No	-0.559	1.921	1.452
Lupeol	No	hERG II	No	No	No	-0.502	2.563	0.89
Vasicinone	No	No	No	No	No	0.332	1.91	1.708
Vasicoline	Yes	hERG II	Yes	No	No	0.088	2.621	0.728

Figure 1a: 3D- Structure of Angiotensin-converting enzyme 2 (ACE2) receptor- PDB ID 2AJF

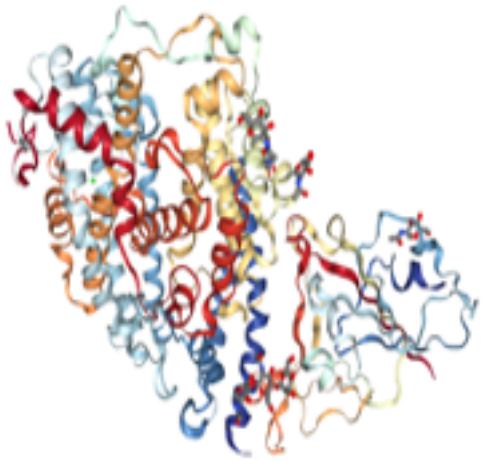

Figure 1b: 3D crystalline structure of the target protein COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB ID 6LU7

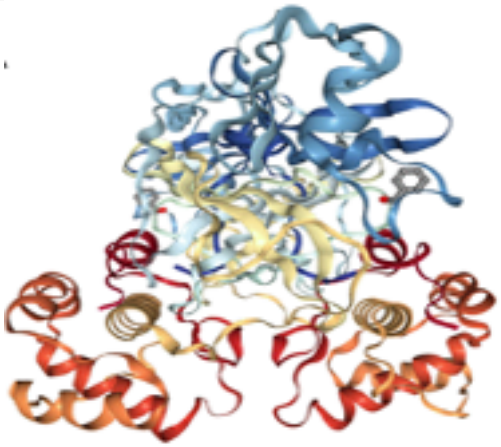

Figure 2: 2D and 3D Structure of Selected Ligands

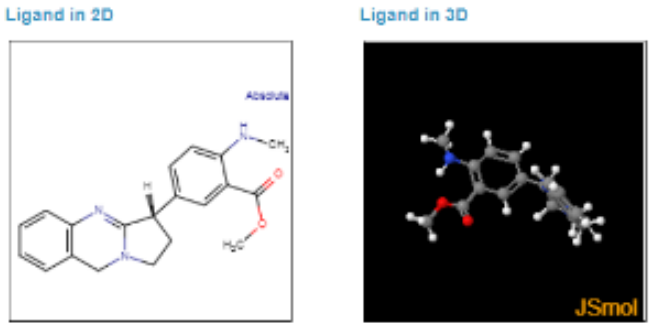

Figure 2a : Adhatodine

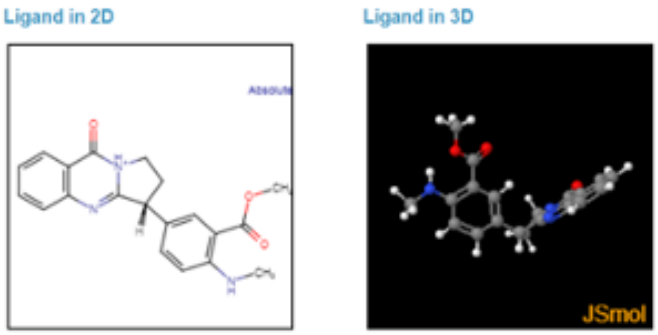

Figure 2b : Anisotine

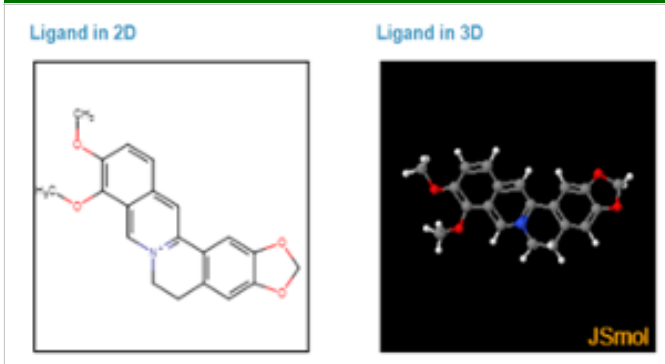

Figure 2c : Berberine

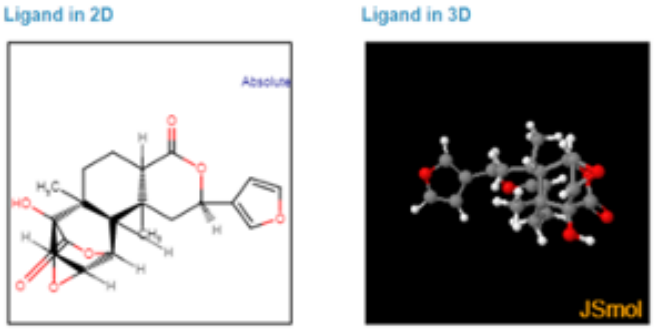

Figure 2d : Tinosporide

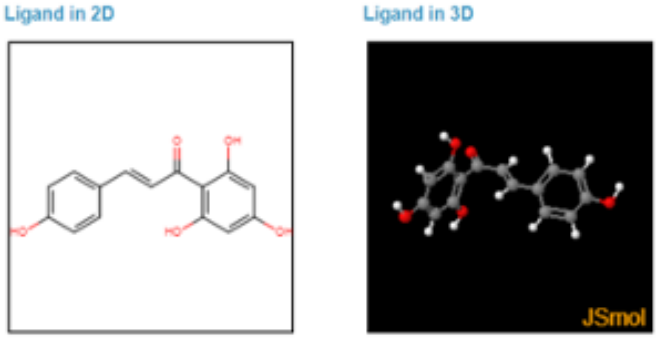

Figure 2e : Apigenin

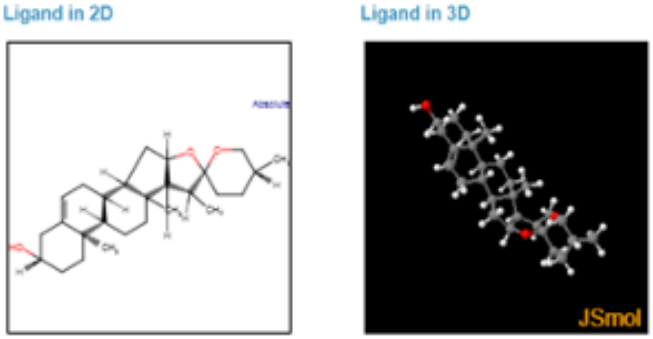

Figure 2f : Diosgenin

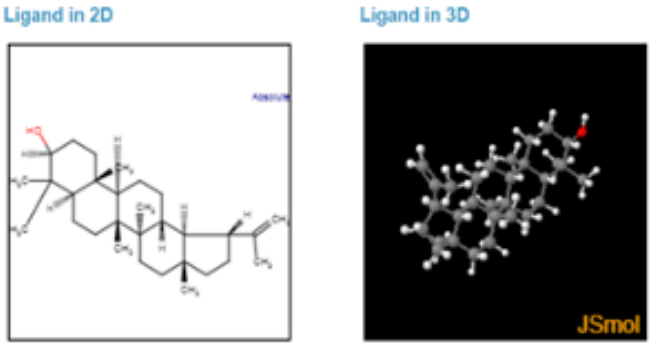

Figure 2g : Lupeol

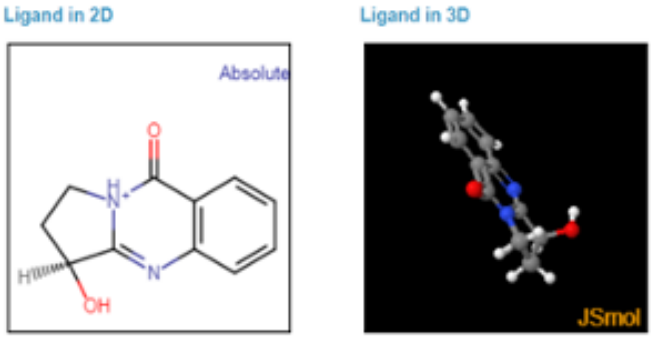

Figure 2h : Vasicinone

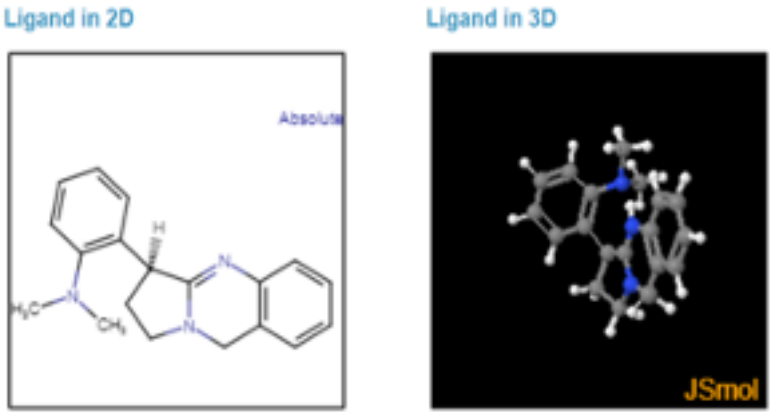

Figure 2i : Vasicoline

Conclusion

Various Phytoconstituents of the *Aadathodai Kudineer* Chooranam such as Adhatodine, Anisotine, Tinosporide, Apigenin, Vasicoline, Berberine, Diosgenin, Lupeol and Vasicinone present in the herbs of the formulation *Aadathodai Kudineer* revels significant binding against the target protein thereby it was concluded that these compounds may exerts promising inhibiting against ACE-2 receptor and hereby halt the host-viral interface and exerts promising inhibiting against 3 CL pro enzyme and hereby halt the formation of 16 non-structural proteins (nsp1-nsp16) that are highly essential for viral replication and there by prevents the viral survival in the host environment. This formulation have high binding affinities to the C3-like protease and ACE2 Receptor Spike protein of COVID-19 and can possibly be future therapeutics against this coronavirus if in silico studies are confirmed with antiviral activity studies. It remains a possibility that this formulation on treatment may contribute to the lower incidences of COVID-19 cases in India. Further, preclinical and clinical trials have to be conducted in order to know the exact mechanism and efficacy of *Aadathodai Kudineer* in SARS-CoV-2.

Conflict of Interest

Nil.

Funding Information

Nil.

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