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In Silico Docking Analysis of Poly Herbal Siddha Formulation *Notchi kudineer* in inhibiting ACE2 Receptor - PDB- 2AJF Spike protein SARS-CoV-2

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ABSTRACT

Background: COVID-19 is a deadly disease, where the infection is caused by SARS-CoV-2. The corona virus particles are spherical in shape having spike proteins around them. These proteins are responsible for virus replication in human host cells. Siddha system of medicine has diverse and extensive use of natural resources for the prevention and management of comorbid conditions, widespread epidemic or pandemic diseases.

Objective: The study is aimed to execute the In Silico computational studies of phytoconstituents of novel herbal preparation *Notchi kudineer*, mentioned in sastric Siddha text, which is commonly used in treating *Kulir suram* and could be effective against the ongoing pandemic novel corona virus disease SARS-CoV-2.

Methodology: In Silico molecular docking analysis was performed for phytocomponents present in the *Notchi Kudineer* formulation for targets ACE2 Receptor Spike protein, PDB - 2AJF using Autodock tool.

Result: Total of 9 bioactive lead compounds were retrieved from the herbs present in the formulations. From reported data of the herb, the leads such as Piper longuminine and Pellitorine possess 100% and Chavibetol, Piperic acid, Pyridoxine, Linalool and S-Allyl L-Cysteine possess 50% affinity by interacting with both the core target amino acids (31 LYS and 353 LYS) present on the target.

Conclusion: The phyto components showed possible affinity towards these targets and has the leads such as Piper longuminine and Pellitorine that may exerts promising inhibiting against ACE2 receptor.

Keywords: Covid-19, *Notchi Kudineer* Poly Herbal Siddha Formulation, Siddha Medicine.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by the recently emerged coronavirus, SARS-CoV-2, which was first reported in December 2019 in the city of Wuhan, Hubei province, PR China [1]. The outbreak has become a global pandemic.This virus seems to be much more contagious than severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV). Similar to other coronaviruses (SARS-CoV-1 and MERS-CoV), human-to-human transmission is well established for this virus, which has now spread globally [1,2]. Identically to SARS-CoV-1, which was responsible for the SARS outbreak in 2002–2004, the main target of SARS-CoV-2 is the respiratory tract, leading to typical clinical signs including fever, dry cough, fatigue,and dyspnoea [3].Full-length genome sequencing revealed that 2019-nCoV shares 79.5% sequence identity with SARS-CoV, and pairwise protein sequence analysis found that it belonged to the class of SARS-related coronaviruses [4]. Both 2019-nCoV and SARS-CoV enter host cell via thesame receptor, angiotensin-converting enzyme 2 (ACE2) [4]. Therefore, this virus was subsequently renamed SARS-CoV-2. Considering that the spike protein of SARS-CoV-2 interacts with ACE2, as does that of SARS-CoV, COVID-19 may have a pathogenic mechanism similar to that of SARS.

ACE2 mediates SARS-CoV-2 infection

Entry into host cells is the first step of viral infection. A spike glycoprotein on the viral envelope of the coronavirus can bind to specific receptors on the membrane of host cells. Previous studies have shown that ACE2 is a specific functional receptor for SARS-CoV [5]. Angiotensin-converting enzyme 2 (ACE2), the functional receptor of SARS-CoV-2, plays a crucial role in the pathogenesis of COVID-19, as it provides viral entry into human cells [6,7]. The viral spike (S) protein of SARS-CoV-2 binds to ACE2 as a cellular receptor in a similar way to SARS-CoV-1. Importantly, SARSCoV-2 is more pathogenic, at least in part because of its 10- to 20-fold increased binding affinity to ACE2 [7,8]. Membrane fusion of the virus and the host cell is activated after binding, and viral RNA is subsequently released into the cytoplasm, establishing infection.Evidently, SARS-CoV-2 cell entry and pathologic effects mainly occur in cells of the (upper) respiratory tract [9,10].

Every coronavirus comprises four structural proteins namely spike, envelope, nucleocapsid and membrane proteins. Among them, spike (S) protein is the most vital protein which controls the biological processes such as viral particle attachment, fusion and lastly entry in the host cell. As a result, it can be considered as a target for development of medicines in COVID-19, as well as SARS-CoV infection[11,12] . The S protein facilitates the entry of virus in human host cells. Initially it binds to ACE2 protein through its receptor-binding domain. Subsequently, it fuses with the viral and host membranes. Zhou et al.[13] has experimentally demonstrated that ACE2 is cellular entry receptor for SARS-CoV-2 in human host. ACE2, a type I membrane bound protein, is expressed in many tissues including heart, kidney, intestine except lungs. ACE2-expressing epithelial cells express several viral replication associated genes , signifying that these cells can facilitate coronavirus replication in the lung[14] . The presence of ACE2 receptor in other tissues, can explain the cause of kidney damage, heart failure and liver damage in COVID-19 infected patients.

Medicinal plants and herbs have shown promising anti-viral properties and multiple beneficial health applications as well as are being used as traditional practitioners to protect various health issues of humans and animals [15–17]. The medicinal plants and their derived phytoconstituents, herbs could provide the strong base for designing and developing the novel alternative and supplementary treatment for coronavirus with exploring phytotherapy approaches.*Notchi Kudineer* a Siddha formulation is indicated in the text Siddha Vaidhya Thirattu for treating various fevers and other conditions that cause moderate to severe respiratory symptoms.

Molecular docking analysis is an easier and effective way to identify the potential activities of bio-molecules against disease causing viral proteins.There are lots of evidence which prove the application of computational tools in the discovery of natural derived drugs [18-21]. Hence, the aim of the current study is to apply this incredible in-silico screening methodology for the sastric Siddha formulation Notchi Kudineer against SARS- CoV-2 spike protein.

Since no specifc medication is available to treat COVID-19, designing of new drug is important and essential. In this regard, in silico method plays an important role, as it is rapid and cost effective compared to the trial and error methods using experimental studies. An attempt is made to identify the possible inhibition of phytocomponents of Notchi Kudineer in inhibiting ACE2 Receptor Spike protein SARS-CoV-2 through molecular docking studies.

MATERIALS AND METHODS

Objective:

Binding of phytocomponents with the core amino acids (31 LYS and 353 LYS) of the target by forming hydrogen bond will hinder the function of the target Angiotensin-converting enzyme 2 (ACE2) receptors - PDB- 2AJF being recognized as binding site for novel corona virus for its pathogenesis essential for host-viral interaction. Thereby phytocomponents which inhibit the target ACE-2 may act as a potential therapeutic agent for management of COVID-19 and related symptoms.

Methodology

Docking calculations were carried out for retrieved phytocomponents against target protein ACE-2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (*Morris, Goodsell et al., 1998*). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.[22-25]

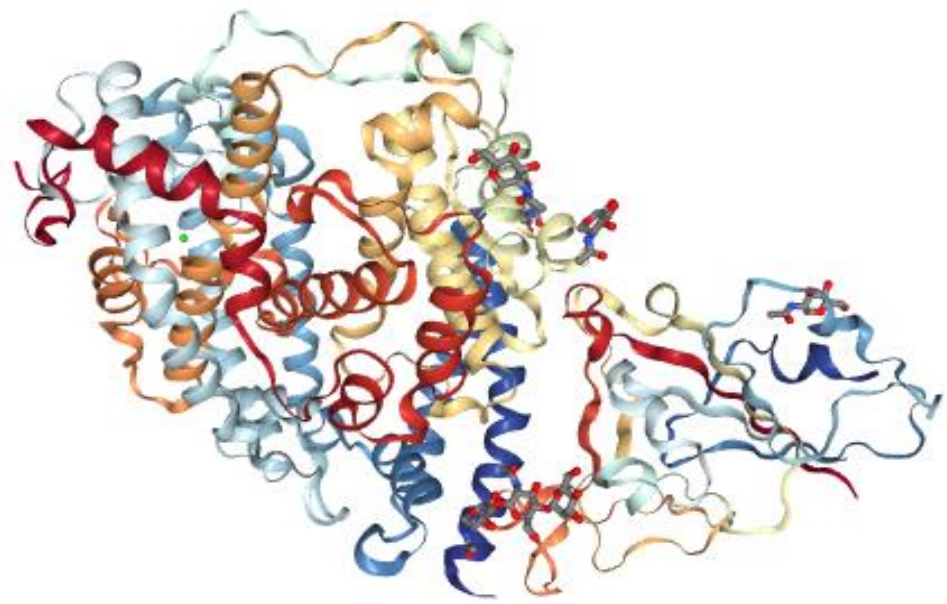
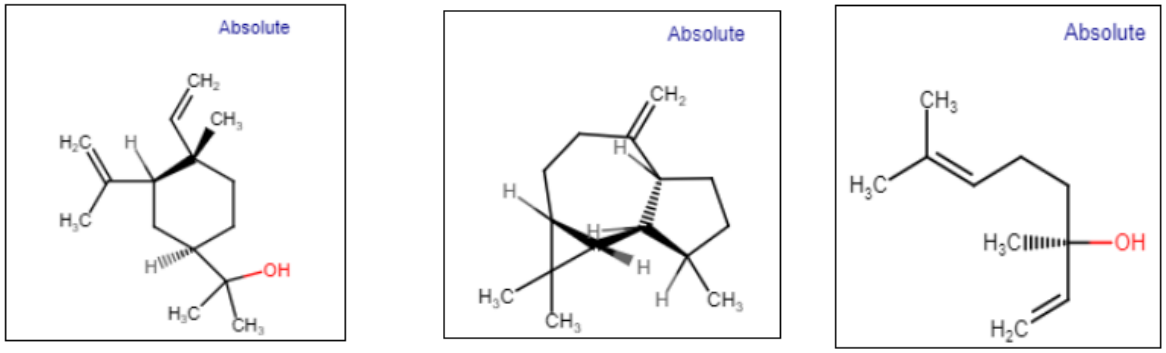
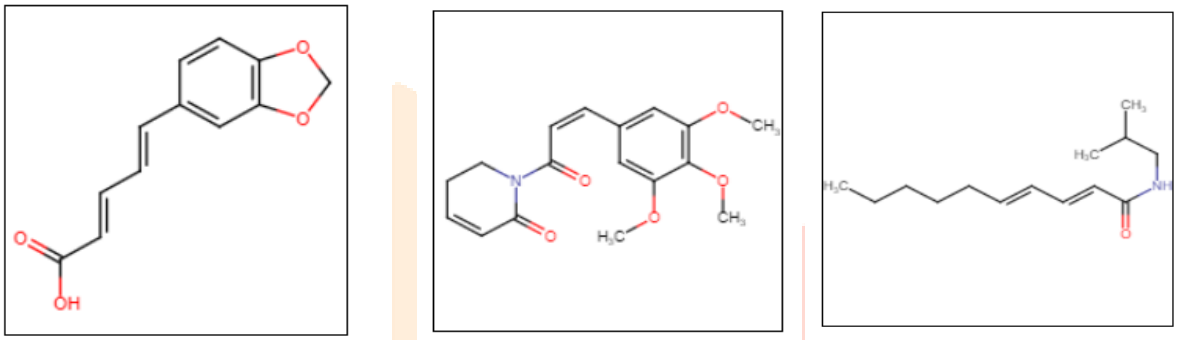


Fig 1: 3D- Structure of Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Elemol **Aromadendrene** **Linalool**



Piperic acid **Piperlonguminine** **Pellitorine**



S- allyl cysteine **Pyridoxine** **Chavibetol**

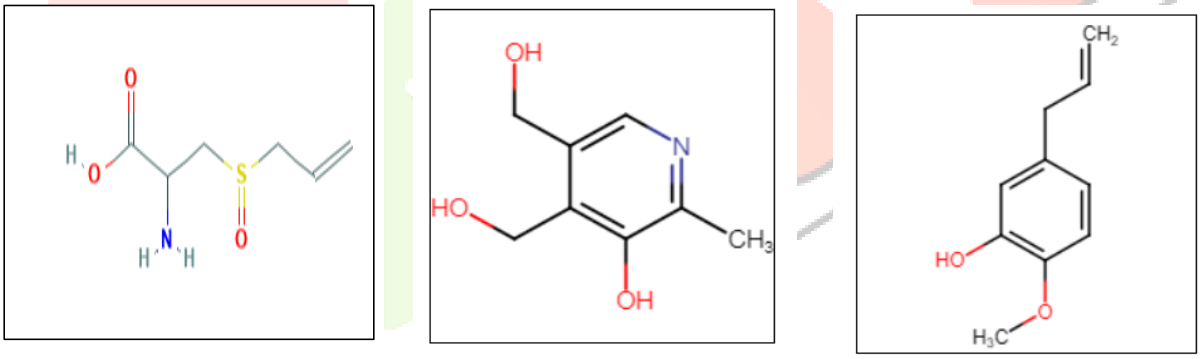
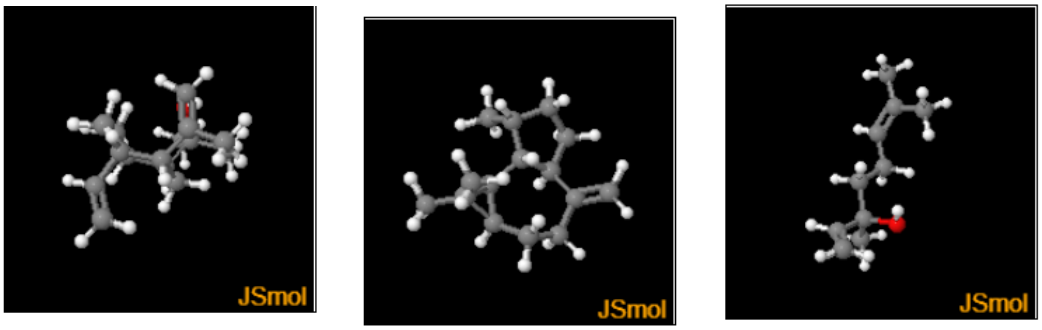
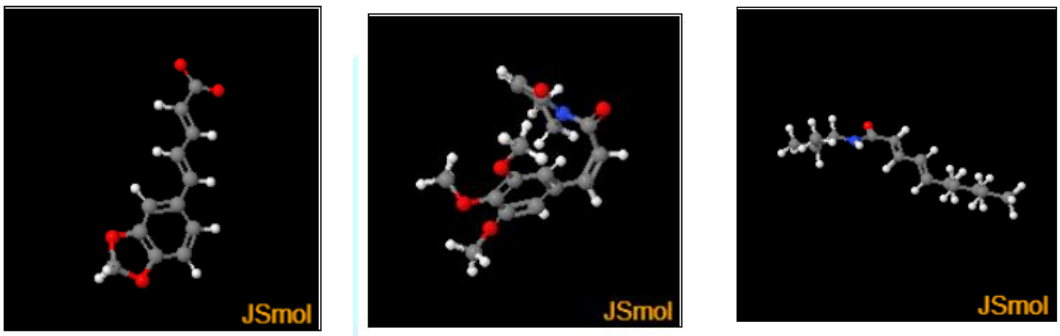


Fig 2: 2D Structure of Selected Ligands

Elemol **Aromadendrene** **Linalool**



Piperic acid **Piperlonguminine** **Pellitorine**



S- allyl cysteine **Pyridoxine** **Chavibetol**

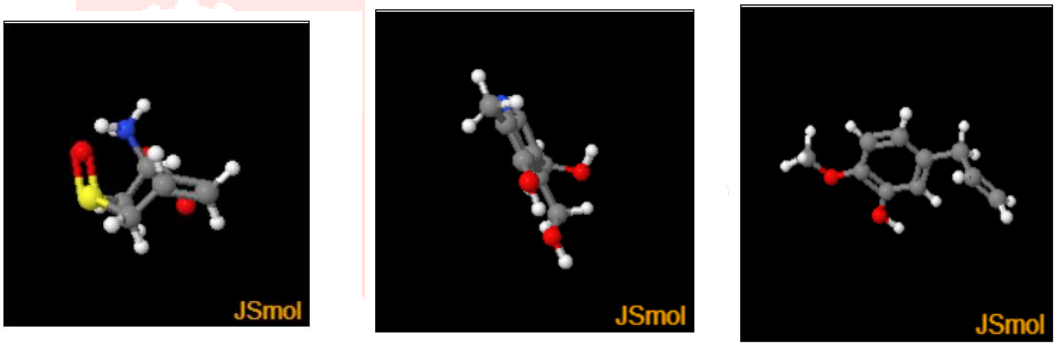
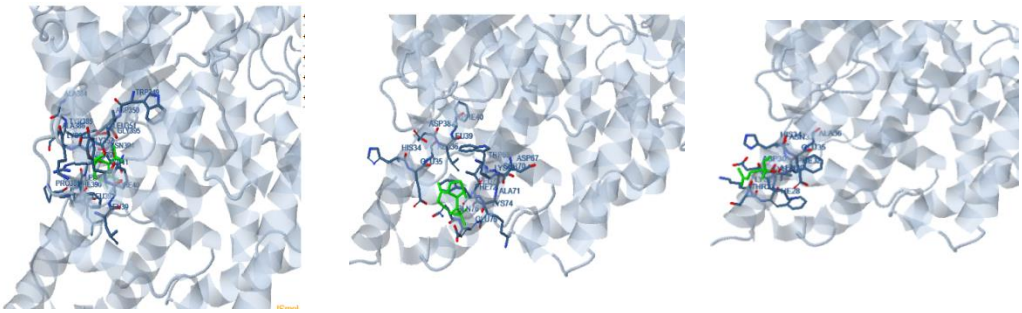


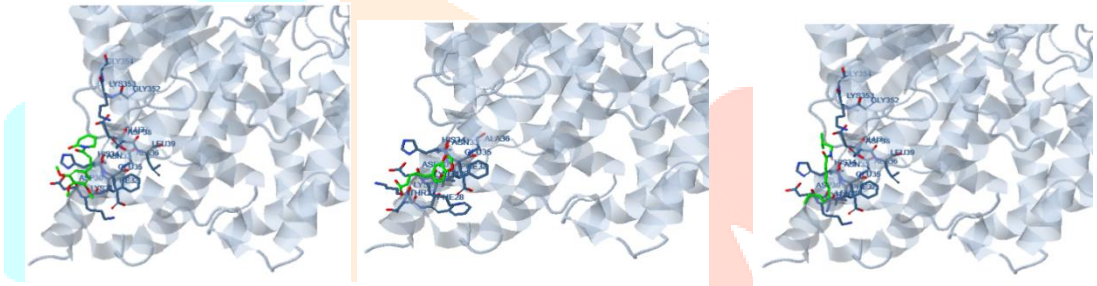
Fig 2: 3D Structure of Selected Ligands

Docking Pose

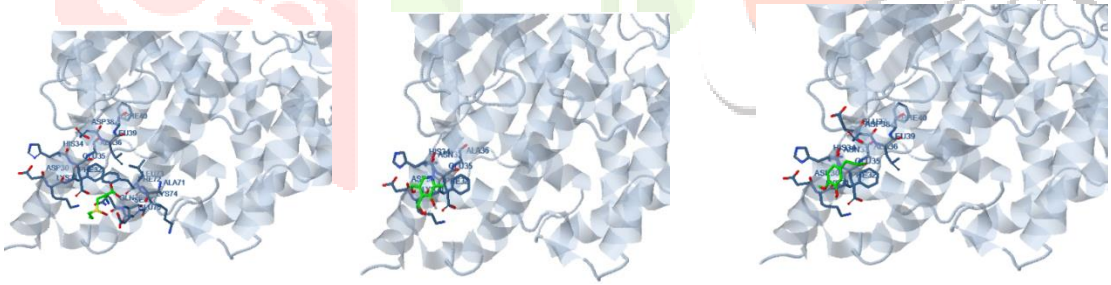
Elemol ***Aromadendrene*** ***Linalool***



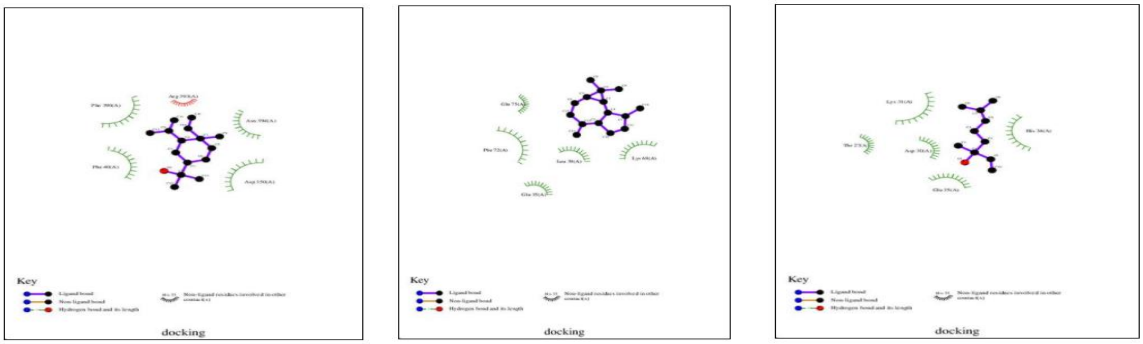
Piperic acid ***Piperlonguminine*** ***Pellitorine***



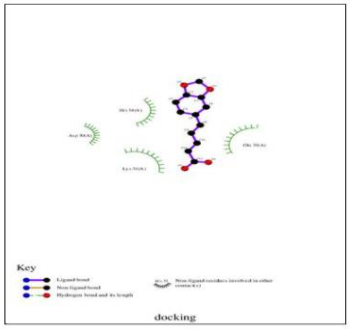
S-Allyl L-Cysteine ***Pyridoxine*** ***Chavibetol***



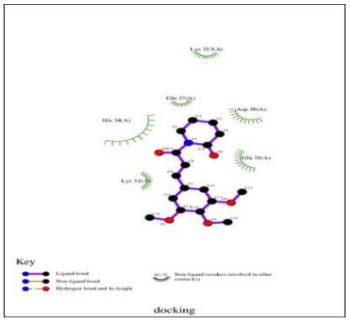
Elemol ***Aromadendrene*** ***Linalool***



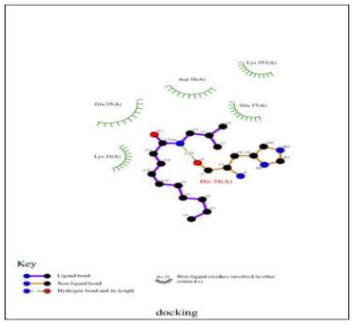
Piperic acid



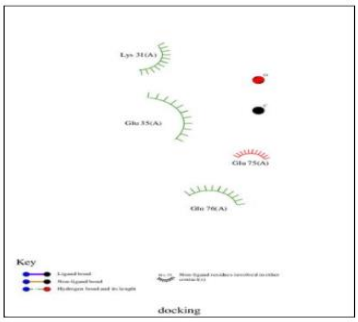
Piperlonguminine



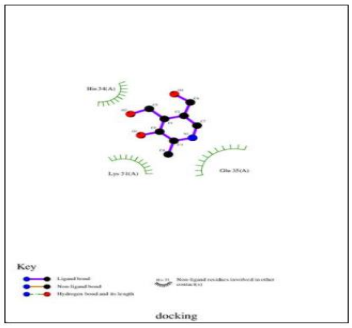
Pellitorine



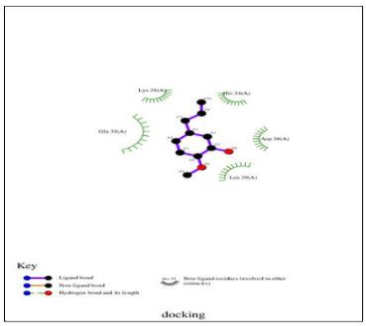
S-Allyl L-Cysteine



Pyridoxine



Chavibetol



Ligand Properties of the Compounds Selected for Docking Analysis

Compound	**Molar weight g/mol**	**Molecular Formula**	**H Bond Donor**	**H Bond Acceptor**	**Rotatable bonds**
Chavibetol	164.2 g/mol	$C_{10}H_{12}O_2$	1	2	3
Pellitorine	223.35 g/mol	$C_{14}H_{25}NO$	1	1	8
Piperic acid	218.2 g/mol	$C_{12}H_{10}O_4$	1	4	3
Piperlonguminine	273.33 g/mol	$C_{16}H_{19}NO_3$	1	3	5
Pyridoxine	169.18 g/mol	$C_8H_{11}NO_3$	3	4	2
Linalool	154.25 g/mol	$C_{10}H_{18}O$	1	1	4
Aromadendrene	204.35 g/mol	$C_{15}H_{24}$	0	0	0
Elemol	222.37 g/mol	$C_{15}H_{26}O$	1	1	3
S-Allyl L-Cysteine	161.22 g/mol	$C_6H_{11}NO_2S$	2	4	5

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
Chavibetol	-2.95	6.89*	-0.05	-3.45	365.94
Pellitorine	-3.44	3.03*	-0.04	-5.55	514.87
Piperic acid	-2.76	9.52	-0.55	-3.62	404.82
Piperlonguminine	-3.37	3.38*	-0.08	-4.47	475.04
Pyridoxine	-2.47	15.46*	-0.38	-4.10	296.92
Linalool	-2.62	12.05*	-0.01	-4.08	372.08
Aromadendrene	-4.37	631.17	-0.01	-4.37	426.33
Elemol	-5.32	126.23	-0.26	-7.03	546.5
S-Allyl L-Cysteine	-4.33	673.86	-1.29	-4.18	312.11

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

Chavibetol	1	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	39 LEU
Pellitorine	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS
Piperic acid	1	30 ASP	31 LYS	34 HIS	35 GLU		
Piperlonguminine	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS
Pyridoxine	1	31 LYS	34 HIS	35 GLU			
Linalool	1	27 THR	30 ASP	31 LYS	34 HIS	35 GLU	
Aromadendrene	0	35 GLU	39 LEU	68 LYS	72 PHE	75 GLU	
Elemol	0	40 PHE	350 ASP	390 PHE	393 ARG	394 ASN	
S-Allyl L-Cysteine	1	31 LYS	35 GLU	72 PHE	75 GLU	76 GLN	

OBSERVATION AND INFERENCE

Total of 9 bioactive lead compounds were retrieved from the herbs present in the formulations. From reported data of the herb, the leads such as Piperlonguminine and Pellitorine 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 phyto-compounds such as Chavibetol, Piperic acid, Pyridoxine, Linalool and S-Allyl

L-Cysteine possess 50% affinity by binding with target amino acid 31 LYS present on the target receptor ACE-2.

CONCLUSION

Based on the results of the computational analysis it was concluded that the bio-active compound's like Piperlonguminine, Pellitorine ,Chavibetol, Piperic acid, Pyridoxine, Linalool and S-Allyl L-Cysteine present in the formulation 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.

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