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

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still an ongoing global health emergency. Siddha Medicine is a valuable therapeutic choice which is classically used for treating both bacterial and viral respiratory infections. *Vishnu kiranthi Kudineer* is a drug indicated for all the 64 types of *suram* mentioned in Siddha sastric literature. An attempt is made to identify the possible inhibition of the phytocomponents of *Vishnu kiranthi Kudineer* in inhibiting ACE2 Receptor Spike protein in SARS-CoV-2 through molecular docking studies.

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

Results: Among the 11 active Phytocompounds present in the *Vishnu kiranthi Kudineer* formulation, Bisabolene, Linolenic acid and Corilagin possess 100% and Gallic acid , Gingerenone A, Squalene, Allose, Piperine, Maslinic acid and Amyrin possess 50% affinity by interacting with both the core target amino acids (31 LYS and 353 LYS) present on the target which shows the promising contrivance of ACE 2 receptor inhibition.

Conclusion: The phytocomponents showed possible affinity towards these targets and has the leads such as Bisabolene, Linolenic acid and Corilagin that may exerts promising inhibiting against ACE2 receptor.

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

INTRODUCTION

Coronaviruses have caused two large-scale pandemics in the past two decades, SARS and Middle East respiratory syndrome (MERS)[1,2]. The rapid transmission that occurs in COVID 19 is due to its person to person transmission and is effected through respiratory droplets. SARS-CoV-2 infects the host cells by latching on to the Human ACE 2 receptors through its enveloped spike glycoprotein. The coronavirus particles are spherical in shape having spike proteins around them. These proteins are responsible for virus replication in human host cells. Spike proteins after attaching with human cells, undergo structural changes, which results in a fusion of viral particle membrane with human host cell membrane. Thus, the viral RNA enters into the host cell and produces more viruses after copying its genome. SARS-CoV-2 spike proteins bind to the receptor proteins, on the host cell surface, known as angiotensin converting enzyme 2 (ACE2). The molecular level structure of SARS-CoV-2 spike protein has a Receptor Binding Domain (RBD) for binding to host human cells. Receptor Binding Domain (RBD) of spike glycoprotein interacts with ACE2 receptor in Protease Domain (PD) of the host human cell. Subsequently, it fuses with the viral and host membranes, causing viral infection[3].

Considering the preliminary data, it has been suggested that ACE2 is a membrane protein for SARS-CoV-2, that is identified to cause the respiratory disease outbreak in Wuhan in late 2019 [4,5]. Specifically, SARS-CoV-2 is a beta coronavirus, having similarity with SARS- CoV virus, in binding with human ACE2 receptor and spike glycoprotein for viral entry [6]. This spike protein fragment is responsible for the entry of both SARS-CoV-2 and SARS-CoV in human ACE2-expressing cells. Small molecules, which can affect the binding efficiency of spike protein with its receptor, may act as the viral attachment inhibitor for both infections[3]. 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[7,8].

ACE2 mediates SARS-CoV-2 infection

ACE2 is a membrane bound receptor for both coronaviruses such as SARS-CoV and SARS-CoV-2. Zhou et al.[9] has experimentally demonstrated that ACE2 is cellular entry receptor for SARS-CoV-2 in human host.

ACE2 is expressed in nearly all human organs in varying degrees. In the respiratory system, the traditional immuno histochemical method and recently introduced single-cell RNA-sequence analysis revealed that ACE2 is mainly expressed on type II alveolar epithelial cells, but weakly expressed on the surface of epithelial cells in the oral and nasal mucosa and nasopharynx, indicating that the lungs are the

primary target of SARS-CoV-2 [10, 11]. Moreover, ACE2 is highly expressed on myocardial cells, proximal tubule cells of the kidney, and bladder urothelial cells, and is abundantly expressed on the enterocytes of the small intestine, especially in the ileum [10–12]. About 67% of patients who developed diarrhea during the course of SARS and quite a number of patients with COVID-19 showed enteric symptoms [13–15]. Active viral replication in enterocytes of the small intestine has been reported, and SARS-CoV-2 has been successfully isolated from fecal specimens [16, 17] Traditional medicine is playing a key role in meeting global healthcare needs. Siddha is a unique system of medicine, originated in South India which incorporates the extensive use of herbs, inorganic substances and animal products for maintaining a healthy life. The control and treatment of a viral infection depends mainly on the availability of antiviral drugs, which are few in numbers and usually are not directly acting on virus but prevent replication in the host. Vishnukiranthi Kudineer is a Siddha formulation indicated for all the 64 types of suram in the text Balavagadam and is a dependable Siddha prescription for fever and associated symptoms [18].

Computational methods are playing increasingly larger and more important role in drug discovery and development [19-24] and are believed to offer means of improved efficiency for the industry. They are expected to limit and focus chemical synthesis and biological testing and thereby greatly decrease traditional resource requirements. Moreover, to screen out large number of herbs for compounds with antiviral activity against novel corona virus will be a challenge in very short period. Drug discovery is a time consuming, slow and challenging process [25,26] , so it is necessary to depend on computational tools (Computer-aided drug design) to overcome these pitfalls to an extent. Of late, the impact on these tools for new drug development had made the drug discovery process very cost effective and time efficient [25]. For searching compounds, this ligand-based virtual screening tool is used to identify most probable molecule with pharmacological activity using molecular docking [27-29]. There are lots of evidence which prove the application of computational tools in the discovery of natural derived drugs [30-33]. Hence, the aim of the current study is to apply this incredible in-silico screening methodology for the official Siddha formulation Vishnukiranthi Kudineer against SARS- CoV-2 spike protein.

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 phytocomponentsagainst 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 [34-37].

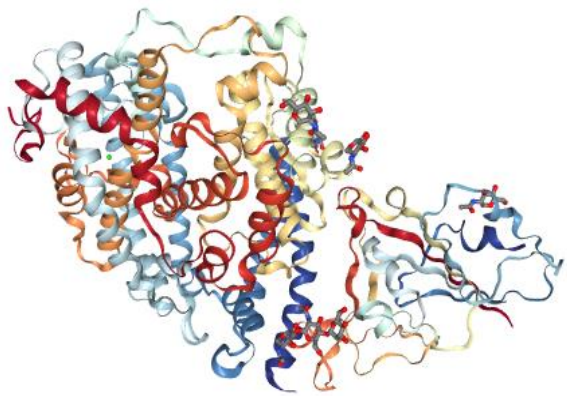
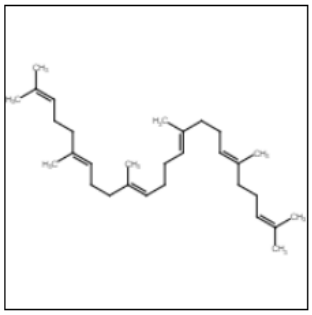


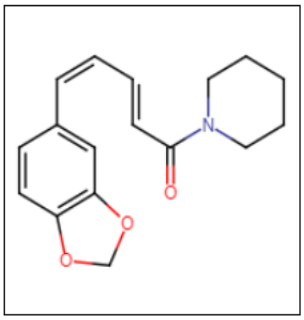
Fig .1 3D- Structure of Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Fig . 2 2D Structure of Selected Ligands

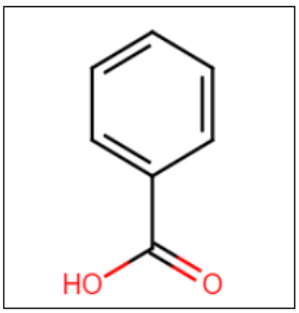
Squalene



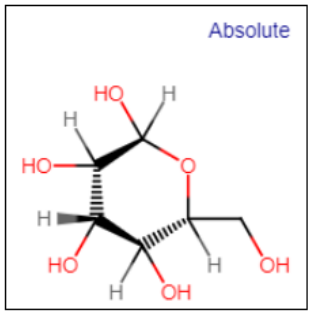
Piperine



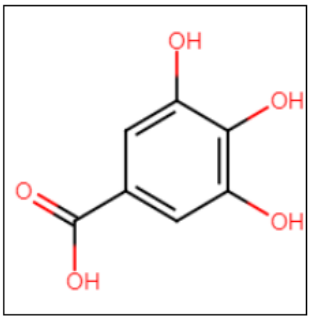
Benzoic Acid



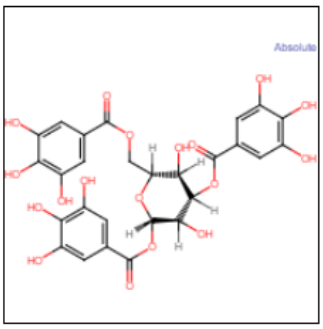
D-Allose



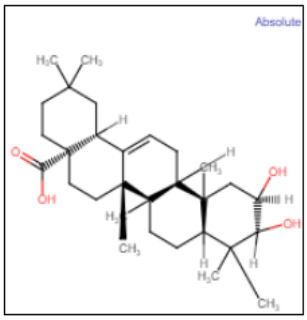
Gallic acid



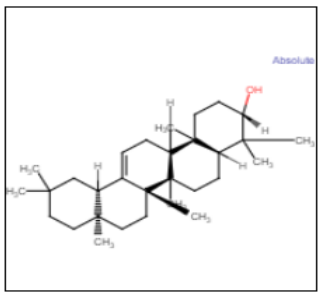
Corilagin



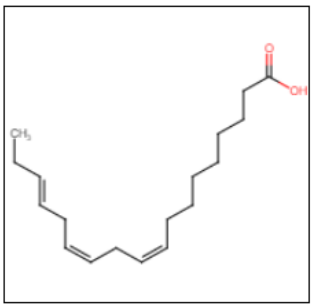
Maslinic acid



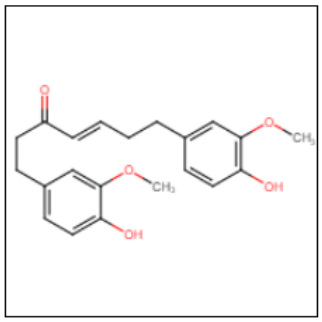
Amyrin



Linolenic Acid



Gingerenone-A



β-bisabolene

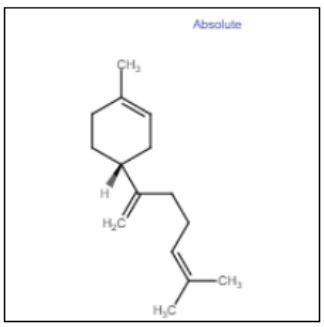


Fig .3 3D Structure of Selected Ligands

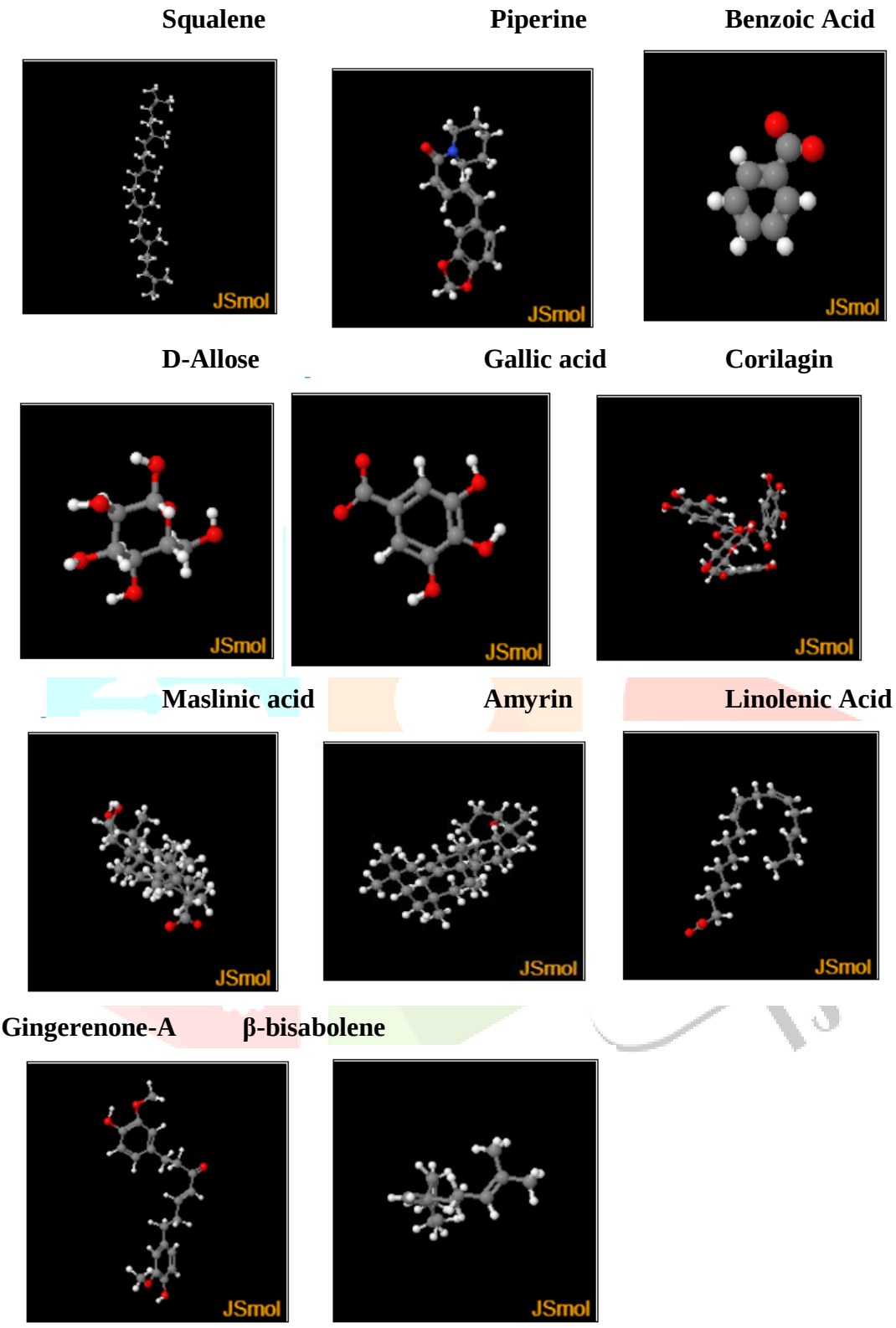
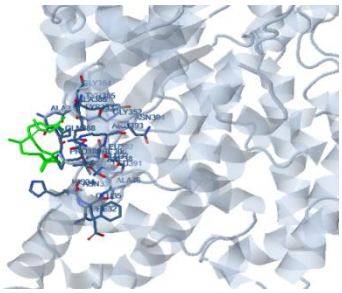
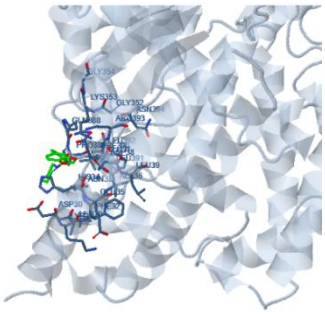
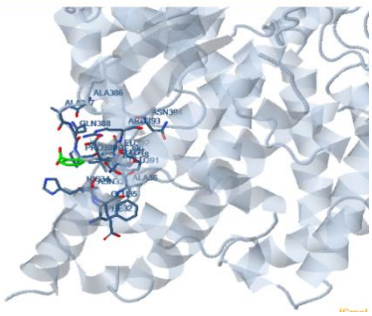
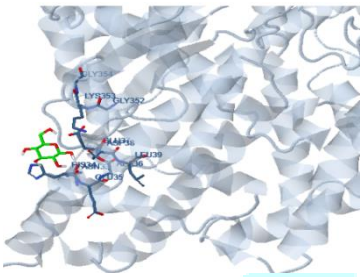
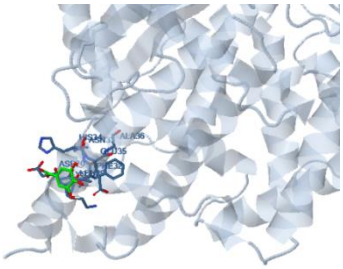
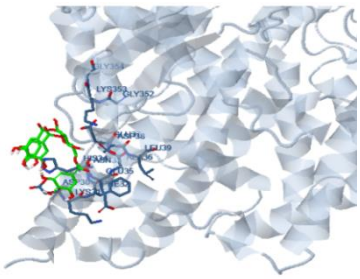


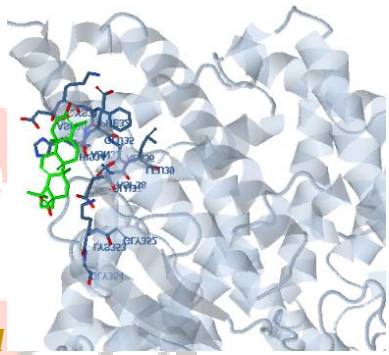
Fig .4 ***Docking Pose***

Squalene *Piperine* *Benzoic* *Acid*

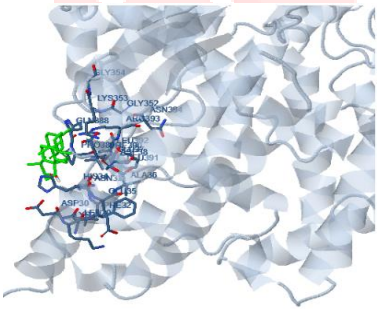
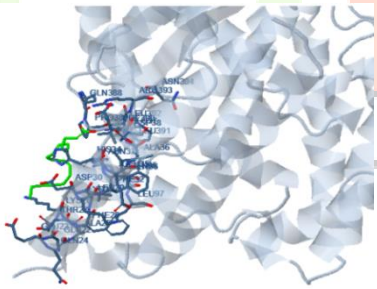




Allose *Gallic acid* *Corilagin*



Maslinic acid *Amyrin* *Linolenic* *acid*

Gingerenone A *Bisabolene*

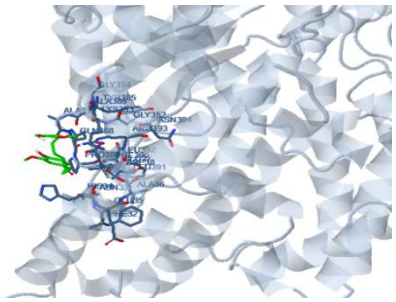
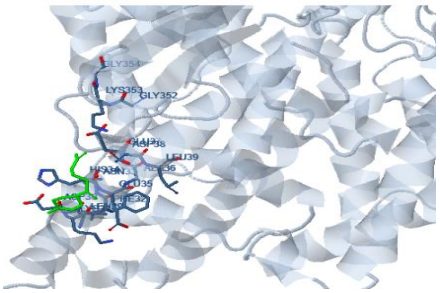
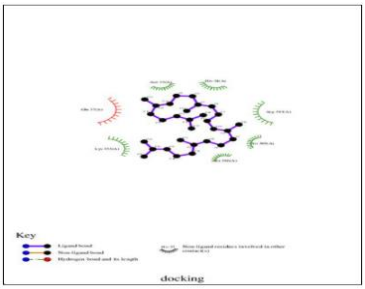
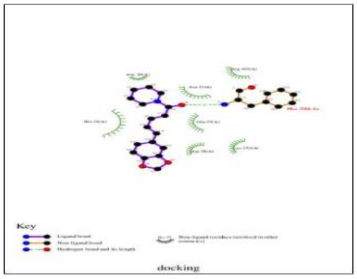



Fig .5 *Docking Pose 2D Interaction Plot*

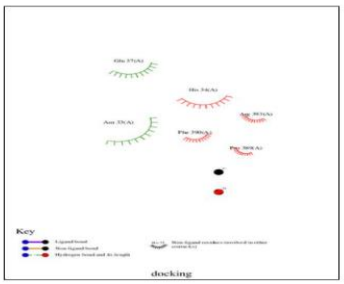
Squalene



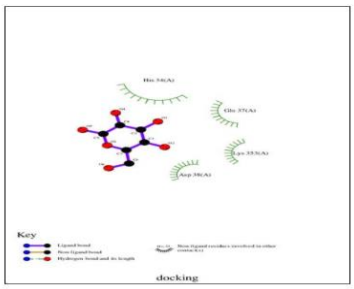
Piperine



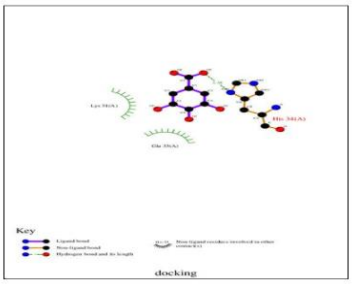
Benzoic Acid



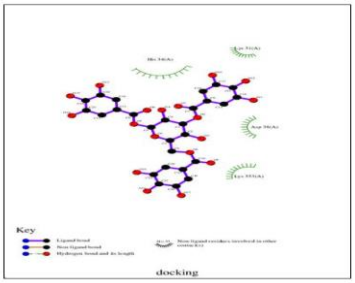
Allose



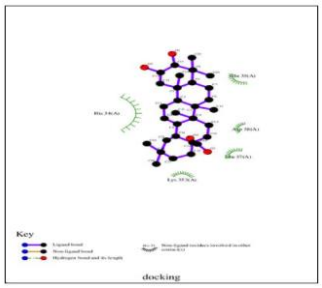
Gallic acid



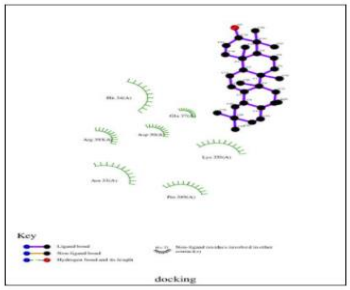
Corilagin



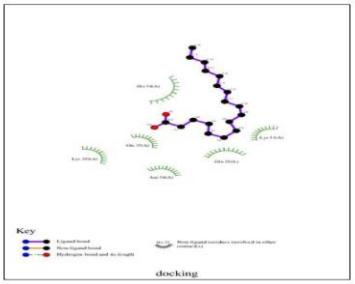
Maslinic acid



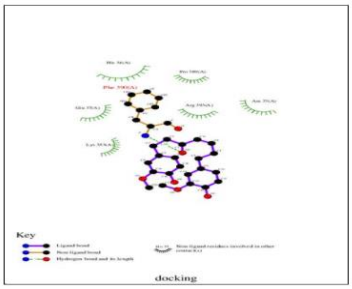
Amyrin



Linolenic acid



Gingerenone A



Bisabolene

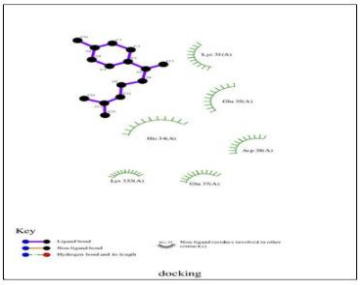


Table. 1 Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Squalene	410.7 g/mol	$C_{30}H_{50}$	0	0	15
Piperine	285.34 g/mol	$C_{17}H_{19}NO_3$	0	3	3
Benzoic Acid	122.12 g/mol	$C_7H_6O_2$	1	2	1
Allose	260.14 g/mol	$C_6H_{13}O_9P$	6	9	7
Corilagin	634.5 g/mol	$C_{27}H_{22}O_{18}$	11	18	3
Gallic acid	170.12 g/mol	$C_7H_6O_5$	4	5	1
Maslinic acid	472.7 g/mol	$C_{30}H_{48}O_4$	3	4	1
Amyrin	426.7 g/mol	$C_{30}H_{50}O$	1	1	0
Linolenic acid	278.4 g/mol	$C_{18}H_{30}O_2$	1	2	13
Gingerenone A	356.4 g/mol	$C_{21}H_{24}O_5$	2	5	9
Bisabolene	204.35 g/mol	$C_{15}H_{24}$	0	0	4

Table. 2 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
Squalene	-4.58	442.77	-0.01	-5.95	685.03
Piperine	-4.70	358.39	-0.18	-5.41	556.36
Benzoic Acid	-2.19	24.91*	-0.60	-2.49	306.15
Allose	-3.47	2.87*	-0.13	-2.70	286.63
Corilagin	-8.08	1.20	-0.14	-3.71	475.84
Gallic acid	-3.24	4.19*	-0.16	-2.83	279.72
Maslinic acid	-5.63	74.73	-0.31	-5.34	559.09
Amyrin	-6.13	32.16	-0.09	-6.43	639.20
Linolenic acid	-2.15	26.45*	-0.35	-5.71	603.08
Gingerenone A	-5.38	113.99	-0.17	-4.14	502.68
Bisabolene	-3.88	1.43*	-0.01	-4.85	457.45

Table. 3 Amino acid Residue Interaction of Lead against Angiotensin-converting enzyme 2 (ACE2) receptor- PDB 2AJF

Bisabolene	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS		
Corilagin	2	31 LYS	34 HIS	38 ASP	353 LYS				
Gallic acid	1	31 LYS	34 HIS	35 GLU					
Gingerenone A	1	33 ASN	34 HIS	37 GLU	353 LYS	389 PRO	390 PHE	393 ARG	
Linolenic acid	2	31 LYS	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS		
Squalene	1	33 ASN	34 HIS	37 GLU	353 LYS	386 ALA	389 PRO	393 ARG	
Allose	1	34 HIS	37 GLU	38 ASP	353 LYS				
Piperine	1	30 ASP	33 ASN	34 HIS	37 GLU	38 ASP	353 LYS	389 PRO	393 ARG
Amyrin	1	30 ASP	33 ASN	34 HIS	37 GLU	353 LYS	389 PRO	393 ARG	
Benzoic Acid	0	33 ASN	34 HIS	37 GLU	389 PRO	393 ARG			
Maslinic acid	1	34 HIS	35 GLU	37 GLU	38 ASP	353 LYS			

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 Bisabolene,Linolenic acid and Corilaginpossess 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 Gallic acid ,Gingerenone A, Squalene, Allose, Piperine,Maslinic acid and Amyrinpossess 50% affinity by binding with target amino acid 31 LYS present on the target receptor ACE

CONCLUSION

Based on the results of the computational analysis it was concluded that the bio-active compound's like Bisabolene,Linolenic acid, Corilagin,Gallic acid, Gingerenone A, Squalene, Allose, Piperine,Maslinic acid and Amyrinpresent 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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