In silico computational evaluation of the components of the traditional siddha herbal formulation, *Vishasura kudineer (VSK)* for the inhibitory action against SARS-CoV-2 main protease (M^{pro}) or 3-chymotrypsin-like protease ($3CL^{pro}$)

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Abstract

Background: Outbreak of SARS-CoV-2 infection is a menace to public health. Among therapeutic targets of Corona virus, the main protease (M^{pro}), also known as 3-chymotrypsin-like protease ($3CL^{pro}$), essential for the replication of SARS-CoV-2, has emerged as the one best drug target.

Objective: The study is aimed at screening of phytocomponents of *Siddha* drug, *Vishasura Kudineer* (VSK) against against SARS-CoV-2 main protease (M^{pro}) or 3-chymotrypsin-like protease ($3CL^{pro}$).

Methods: Autodock program was used for the molecular docking studies against 3-chymotrypsin-like protease.

Results: Nimbin showed highest binding affinity against the target 3 CLpro. Indigotin, Paradol, Nimbin, Khusimol, Geraniol and Licochalcone A reveals maximum of 5 to 6 interactions with the core active amino acid residues present on the 3 CLpro followed by Salicylaldehyde, Piperonylic acid, β-Bisabolene, Genistein and Salannin with the maximum of 3 to 4 interactions.

Conclusion: It was concluded that these compounds may exerts promising inhibitory effect against 3 CL pro enzyme.

Keywords: *Siddha* medicine, *Vishasura Kudineer,* SARS-CoV-2 main protease (M^{pro}), 3-chymotrypsin-like protease ($3CL^{pro}$), Docking study

Introduction

Coronaviruses are positive-sense RNA viruses comes under the *Coronvirinae* subfamily, in the *Coronaviridae* family of the *Nidovirales* order. It was named after the spikes are resemble crowns on their surface. Based on their genetic structure, they may be divided into four primary subgroups: alpha, beta, gamma, and delta. Only mammals are infected with alpha and beta coronaviruses, which often cause respiratory problems in people and gastroenteritis in other animals.[1-3]

Only six different corona viruses were known to infect humans as of December 2019. In immunocompetent individuals, four of them (HCoV-NL63, HCoV-229E, HCoV-OC43, and HKU1) often caused mild symptoms like the common cold, whereas the other two have resulted in pandemics in the previous two decades. The severe acute respiratory syndrome

coronavirus (SARS-CoV) was the cause of the SARS outbreak in 2002–2003, which had a 10% mortality rate. The middle east respiratory syndrome coronavirus (MERS-CoV) also triggered a devasting pandemic in 2012, which had a 37% mortality rate.[4]

After H1N1 (2009), polio (2014), Ebola in West Africa (2014), Zika (2016), and Ebola in the Democratic Republic of the Congo (2019), the World Health Organization (WHO) declared the COVID-19 outbreak as a global public health emergency on January 30, 2020. On March 11, 2020, the WHO classified COVID-19 as a pandemic.[5] As of now, more than 200 countries are being affected by the corona virus infection outbreak that started in Wuhan, China in late 2019 and is now known as COVID-19.[6]

The coronavirus disease 2019 (COVID-19) is an acute respiratory illness caused by a novel coronavirus (SARS-CoV-2, formerly known as 2019-nCoV).[7] Coronavirus was named as 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) by the International Committee on Taxonomy of Viruses and the disease was called 'coronavirus disease 2019' (COVID-19) by the World Health Organization (WHO).[8]

The Siddha system of medicine has a long history in the management of infectious diseases. Herbal medicine consists of bioactive medicines with functional pharmacological effects. Siddha formulations are renowned for addressing infectious ailments without suffering from significant side effects or negative consequences because of its unique and trustworthy blend of herbal ingredients. *"Kirumiyal vandha thodam Perugavundu"* lines mentioned in Guru naadi is evidence that Siddha clearly mentions the knowledge of microbes and how diseases spread. Using both therapeutic and non-therapeutic interventions, the Siddha traditional approach will be beneficial in combating COVID 19. Siddhar's have recommended an evidence-based therapeutic method to comprehend a disease (*Noi Naadi*) and its aetiology (*Mudhal Naadi*), based on those, fix a treatment (*Athu Thanikka Vainaadi*). According to the fundamental Siddha concept described by Siddhar *theran, Vatham* is mainly responsible for creation, *Pittam* oversees prevention, and *kabham* oversees destruction. When a person's immune is weakened, infections might occur, which may be associated to a decrease in *Pitham*. According to Siddha theory, a COVID-19 infection causes an initial rise in body temperature, a cough, and throat discomfort. If there is a sufficient level of immunity, these symptoms may go away when Pitta thathu (Humor) takes effect. If not, it progresses to the *"Thanamulla sethumanthan ilagil veppu"* phase of the Kapha Dosham (Disorder). If left

untreated, it progresses gradually to the Stage of *Sanni* (Severe Pneumonia- Respiratory failure).[9]

Even though coronaviruses have multiple known therapeutic targets, the main protease (M^{pro}), also known as 3-chymotrypsin-like protease ($3CL^{pro}$), has emerged as the one best drug target. The large polypeptide, which is translated from the viral RNA, is specially processed by the M^{pro} at 11 splicing sites, predominantly Leu to Gln (Ser, Ala and Gly). These important splicing areas are not human-homologous. For SARS-CoV-2, inhibiting enzyme activity may impede viral pathogenesis, making it a desirable therapeutic target. Also, using computer-aided drug design, a mechanism-based peptide-like inhibitor (N3) was found, and its crystal structure in association with the M^{pro} of SARS-CoV-2 was subsequently established.[6]

Until now, there are several vaccines have been granted authorization, but only three antiviral drugs have received approval or emergency use authorization from the United States Food and Drug Administration (FDA) such as Remdesivir, Casirivimab + Imdevimab, and Sotrovimab. This indicates an urgent need for more antiviral drugs.[10]

The benefits of decoction Pay close attention to how well it works with the human biological system, as it facilitates quicker absorption, an earlier commencement of effect, a longer duration of pharmacological activity, permeability of cell membranes, etc. It is well known that Kudineer preparations provide steady state plasma concentrations, which are crucial for the decline of viral load in the infected host.[11]

Vishasura kudineer is a traditional Siddha herbal formulation comprising nine unique blend of herbal ingredients such as *Azadirachta indica A Juss (Vembu), Indigofera tinctoria Linn (Avuru/Neeli), Zingiber officinale Roscoe (Chukku), Hemidesmus indicus Linn. R. Br. (Nannari), Aristolochia bracteolata Lam. (Aadutheendapaalai), Vetiveria zizanioides Linn. Nash (Vettiver), Glycyrrhiza glabra Linn. (Adhimadhuram), Elettaria cardamomum (Elam), and Santalum album (Sandanam).*[12]

As suggested in the siddha literature *"Kaaviya sura nool",* this versatile formulation claims significant anti-viral potential, but as on date there is no proper documentary evidence advocating the desired mechanism of action in managing SARS-COV-2 by the way of 3CL pro inhibition. Hence, the main aim of current In-silico study is to investigate and explore the anti-viral Potential of the phytochemicals such as *Salannin, Nimbin, Genistein, Indigotin,*

Paradol, β-bisabolene, Salicylaldehyde, Piperonylic acid, Khusimone, Khusimol, Geraniol, Licochalcone A in the traditional herbal formulation *Visasura kudineer Chooranam* complex against the enzyme target 3-Chymotrypsin-like protease (3CLpro) by using Auto Dock Prediction, which may be used in anti-SARS-COV-2 drug development.

Materials and methods

Target protein preparation

Crystalline structure of the target protein COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7 **(Figure 1)** was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added.

Ligand preparation

We selected different orientation of the lead molecules (phyto-constituents) from *Vishasura kudineer* complex **(Table 1)** with respect to the target protein was evaluated by Auto dock program and the best dock pose was selected based on the interaction study analysis. Selected 12 phyto-constituents of *Vishasura kudineer* complex were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)

Protein and ligand docking analysis

Docking calculations were carried out for retrieved phytocomponents against target protein 3CL pro. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Auto grid program. Auto Dock 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. 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.[13-14]

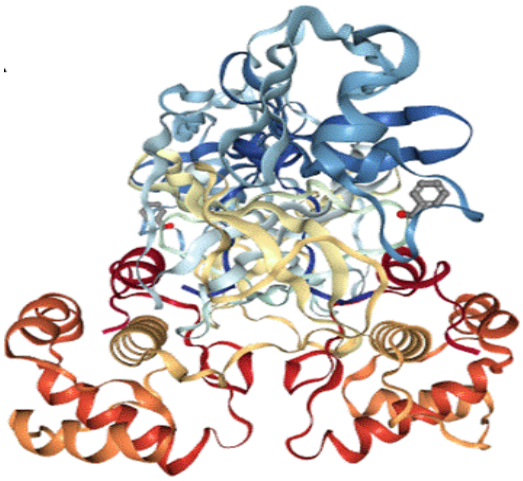


Figure 1: 3D-crystalline structure of the target protein COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Table 1: Selected different orientation of the lead molecules (Phyto-constituents) from *Vishasura kudineer* (VSK) complex

S. No	Name of the Herbs	Family	Phytochemicals selected for Docking
1	*Azadirachta indica A Juss (Vembu)*	Meliaceae	Salannin, Nimbin[15]
2	*Indigofera tinctoria Linn (Avuru/Neeli)*	Fabaceae	Genistein, Indigotin[16]
3	*Zingiber officinale Roscoe (Chukku)*	Zingiberaceae	Paradol, β-bisabolene[17-18]
4	*Hemidesmus indicus Linn. R. Br. (Nannari)*	Apocynaceae	Salicylaldehyde[19]
5	*Aristolochia bracteolata Lam. (Aadutheendapaalai)*	Asclepiadaceae	Piperonylic acid[20]
6	*Vetiveria zizanioides Linn. Nash (Vettiver)*	Poaceae	Khusimone, Khusimol[21]
7	*Glycyrrhiza glabra Linn. (Adhimadhuram)*	Fabaceae	Geraniol, Licochalcone A[22]

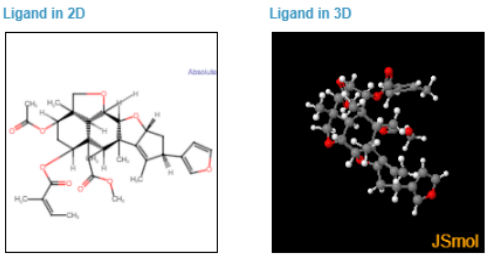
Results and discussion

The molecular docking studies were carried out for the 12 Phytochemical constituents of traditional Siddha herbal formulation *Visasura kudineer Chooranam* complex against COVID-19 main protease (3-chymotrypsin-like protease ($3CL^{pro}$) – PDB 6LU7 to identify the molecular interactions between target protein with ligands. All the phytochemical analogs were docked with enzyme target 3-Chymotrypsin-like protease ($3CL^{pro}$) by using Auto Dock Prediction. Binding affinities of phytocompounds of siddha herbal formulation VSK towards target protein SARS-CoV-2 was studied in detail. Binding free energy is frequently used to determine the affinity of drug's biomolecular interactions and the efficacy. Complete characterization of binding-competent protein conformations, ligand binding poses, and binding/unbinding kinetics is therefore needed for a thorough understanding of protein/ligand binding.[23]

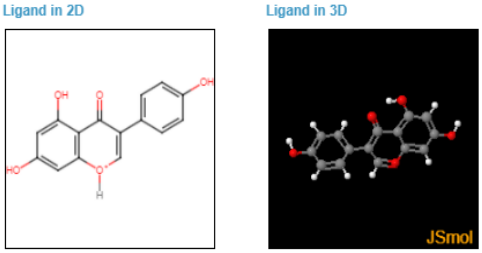
Total of 12 bioactive lead compounds were retrieved from the list of herbal ingredients provided for In-silico investigation. Out of twelve compounds' the leads such as Indigotin, Paradol, Nimbin, Khusimol, Geraniol and Licochalcone A reveals maximum of 5 to 6 interactions with the core active amino acid residues present on the target 3 CLpro. Followed by this, the compounds such as Salicylaldehyde, Piperonylic acid, β-Bisabolene, Genistein and Salannin ranked second with the maximum of 3 to 4 interactions with the active site of the target enzyme 3CLpro.

In the present investigation, Nimbin ranks top with highest binding free energy -8.94 kcal/mol followed by Licochalcone A (-7.73 kcal/mol), Salannin (-7.58 kcal/mol), Indigotin (-7.16 kcal/mol), β-bisabolene (-6.68 kcal/mol), Khusimone (-6.23 kcal/mol), Genistein (-6.13 kcal/mol), Khusimol (-6.01 kcal/mol), Paradol (-5.72 kcal/mol), Geraniol (-5.03 kcal/mol), Piperonylic acid (-3.89 kcal/mol), and Salicylaldehyde (-3.56 kcal/mol). Total of 12 bioactive lead compounds were retrieved from the ingredients of Vishasura kudineer complex. Out of twelve compounds' the leads such as Nimbin, Indigotin, Paradol, Khusimol, Geraniol and Licochalcone A reveals maximum of 5 to 6 interactions with the core active amino acid residues present on the target 3 CLpro. Followed by this the compounds such as Salicylaldehyde, Piperonylic acid, β-Bisabolene, Genistein and Salannin ranked second with the maximum of 3 to 4 interactions with the active site of the target enzyme 3CLpro.

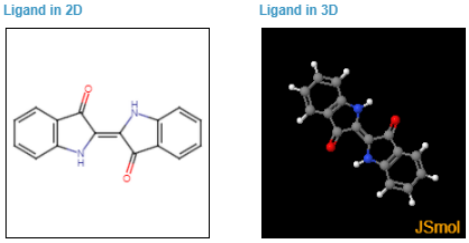
Salannin



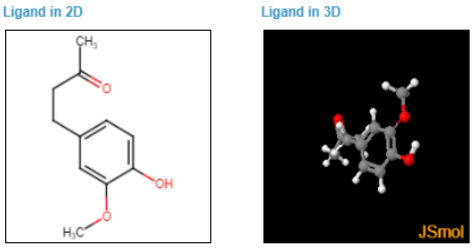
Genistein



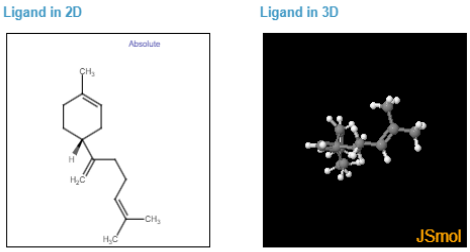
Indigotin



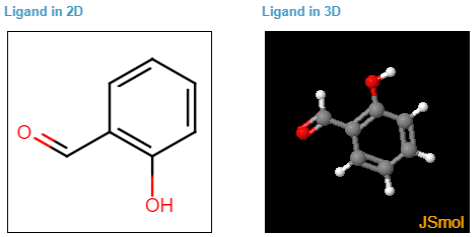
Paradol



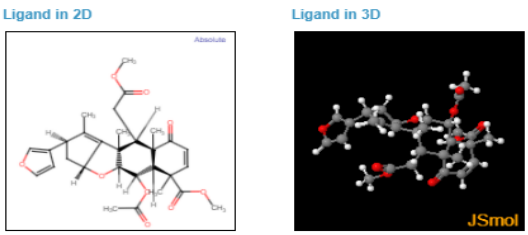
β-bisabolene



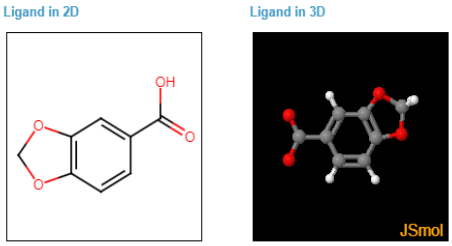
Salicylaldehyde



Nimbin



Piperonylic acid



Khusimone

Khusimol

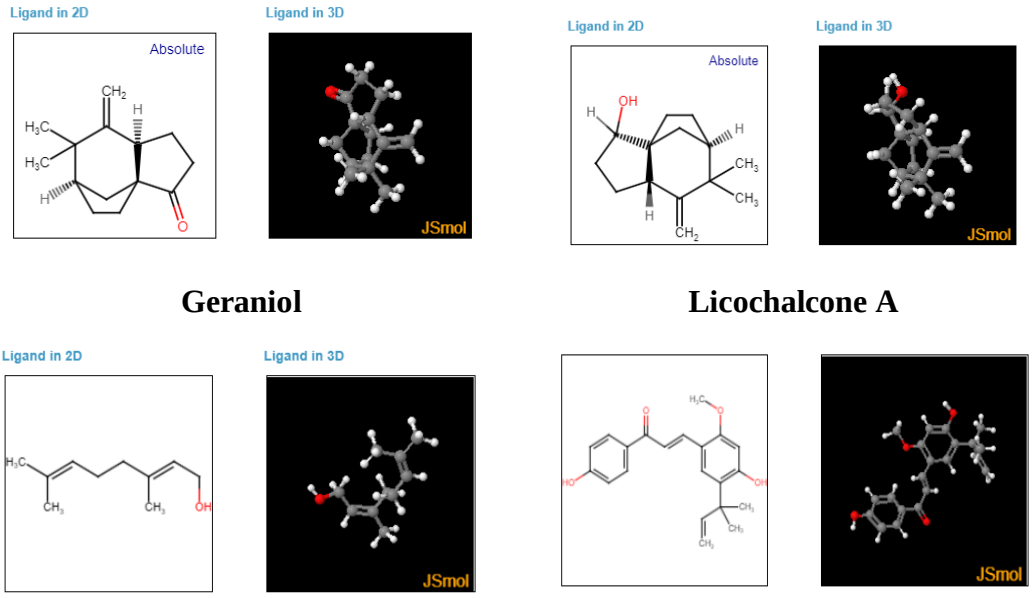


Figure 2: 2D and 3D illustration of the selected Phyto-therapeutic ligand compounds from Vishasura kudineer (VSK) complex

Table 2: Phytochemical properties of the Phyto-therapeutic ligand compounds selected for docking analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Salannin	596.7	$C_{34}H_{44}O_9$	0	9	9
Genistein	270.24	$C_{15}H_{10}O_5$	3	5	1
Indigotin	262.26	$C_{16}H_{10}N_2O_2$	2	3	1
Paradol	278.4	$C_{17}H_{26}O_3$	1	3	10
β-bisabolene	204.35	$C_{15}H_{24}$	0	0	4
Salicylaldehyde	122.12	$C_7H_6O_2$	1	2	1
Piperonylic acid	166.13	$C_8H_6O_4$	1	4	1
Khusimone	204.31	$C_{14}H_{20}O$	0	1	0
Khusimol	220.35	$C_{15}H_{24}O$	1	1	1
Geraniol	154.25	$C_{10}H_{18}O$	1	1	4
Licochalcone A	338.4	$C_{21}H_{22}O_4$	2	4	6
Nimbin	540.6	$C_{30}H_{36}O_9$	0	9	8

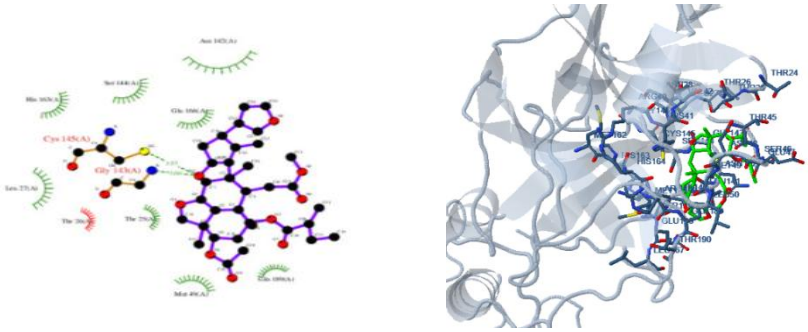
Table 3: Summary of the molecular docking studies of Phyto-therapeutic ligand compounds selected from VSK complex against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM/Mm/nM	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Salannin	-7.58 kcal/mol	2.77 µM	-0.20 kcal/mol	-9.73 kcal/mol	955.331
Genistein	-6.13 kcal/mol	31.96 µM	-0.10 kcal/mol	-6.61 kcal/mol	594.345
Indigotin	-7.16 kcal/mol	5.64 µM	-0.01 kcal/mol	-7.16 kcal/mol	621.64
Paradol	-5.72 kcal/mol	64.26 µM	-0.02 kcal/mol	-6.39 kcal/mol	531.035
β-bisabolene	-6.68 kcal/mol	12.59 µM	-0.00 kcal/mol	-7.80 kcal/mol	604.722
Salicylaldehyde	-3.56 kcal/mol	2.44 mM	-0.04 kcal/mol	-4.17 kcal/mol	333.524
Piperonylic acid	-3.89 kcal/mol	1.42 mM	-0.19 kcal/mol	-4.18 kcal/mol	439.359
Khusimone	-6.23 kcal/mol	26.91 µM	-0.01 kcal/mol	-6.23 kcal/mol	485.672
Khusimol	-6.01 kcal/mol	39.01 µM	-0.16 kcal/mol	-6.31 kcal/mol	520.839
Geraniol	-5.03 kcal/mol	207.12 µM	-0.05 kcal/mol	-6.51 kcal/mol	517.288
Licochalcone A	-7.73 kcal/mol	2.15 µM	-0.08 kcal/mol	-10.03 kcal/mol	728.848
Nimbin	-8.94 kcal/mol	280.13 nM	-0.12 kcal/mol	-9.49 kcal/mol	945.058

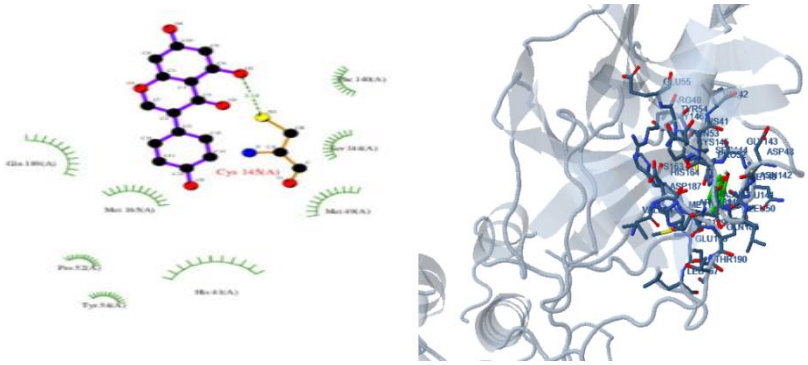
Table 4: Interaction of Phyto-therapeutic ligand compounds with biologically significant Amino acid residues of COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Molecule	Interactions	Amino Acid Residue- Binding										
Salannin	4	25 THR	26 THR	27 LEU	49 MET	142 ASN	143 GLY	144 SER	145 CYS	163 HIS	166 GLU	189 GLN
Genistein	3	41 HIS	49 MET	52 PRO	54 TYR	140 PHE	144 SER	145 CYS	165 MET	189 GLN		
Indigotin	5	41	140	142	144	145	163	164	165	189		

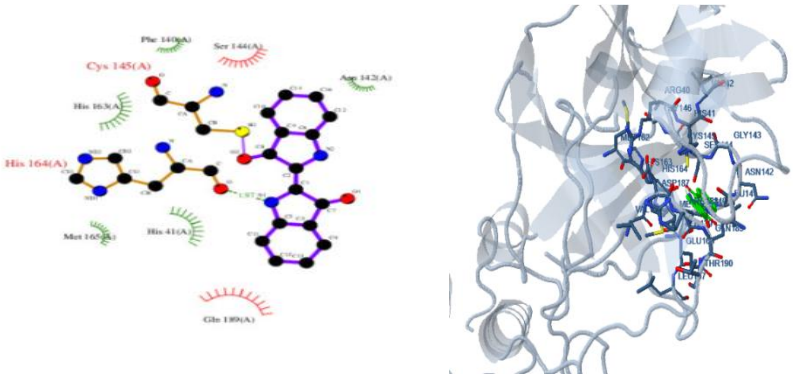
		HIS	PHE	ASN	SER	CYS	HIS	HIS	MET	GLN		
Paradol	5	41 HIS	143 GLY	144 SER	145 CYS	163 HIS	165 MET	166 GLU	187 ASP	189 GLN		
Nimbin	6	41 HIS	49 MET	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	167 LEU	168 PRO	189 GLN
β-Bisabolene	4	41 HIS	49 MET	54 TYR	140 PHE	144 SER	145 CYS	163 HIS	165 MET	189 GLN		
Salicylaldehyde	3	41 HIS	49 MET	54 TYR	164 HIS	165 MET	187 ASP	189 GLN				
Piperonylic acid	3	140 PHE	142 ASN	144 SER	145 CYS	163 HIS	166 GLU					
Khusimone	2	41 HIS	49 MET	54 TYR	165 MET	187 ASP	189 GLN					
Khusimol	5	140 PHE	142 ASN	144 SER	145 CYS	163 HIS	165 MET	166 GLU	172 HIS			
Geraniol	5	27 LEU	140 PHE	144 SER	145 CYS	163 HIS	166 GLU	172 HIS				
Licochalcone A	5	41 HIS	49 MET	142 ASN	144 SER	163 HIS	165 MET	166 GLU	168 PRO	189 GLN	192 GLN	



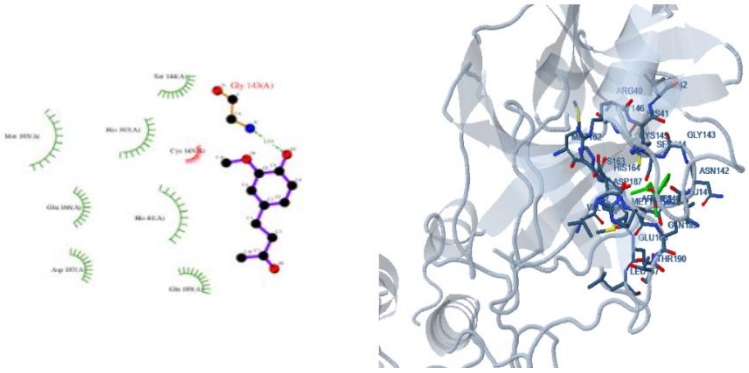
(A. Salannin)



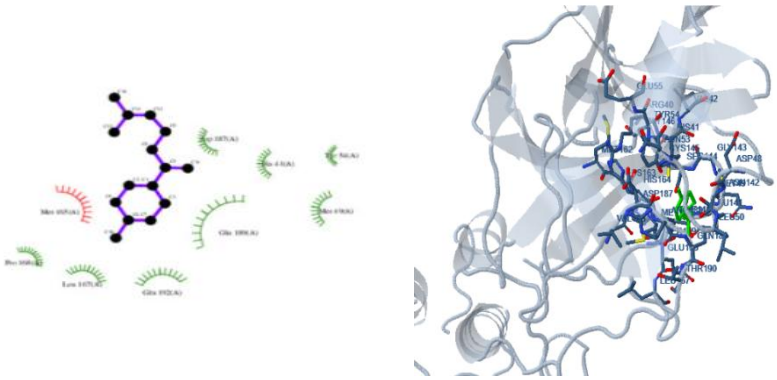
(B. Genistein)



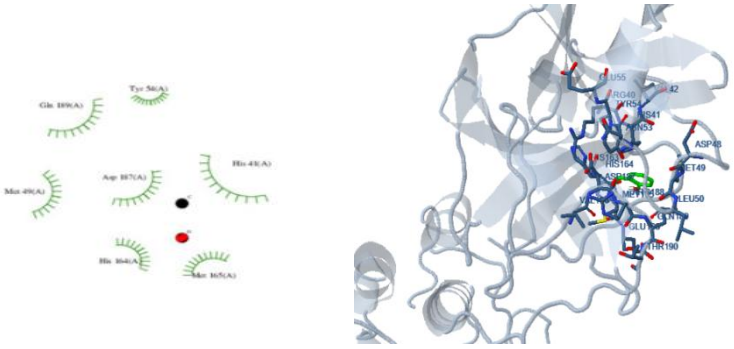
(C. Indigotin)



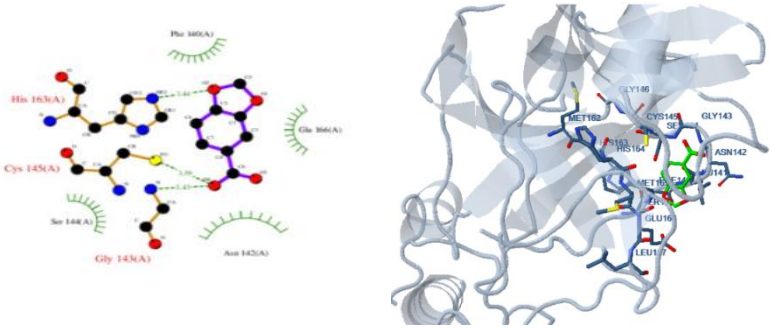
(D. Paradol)



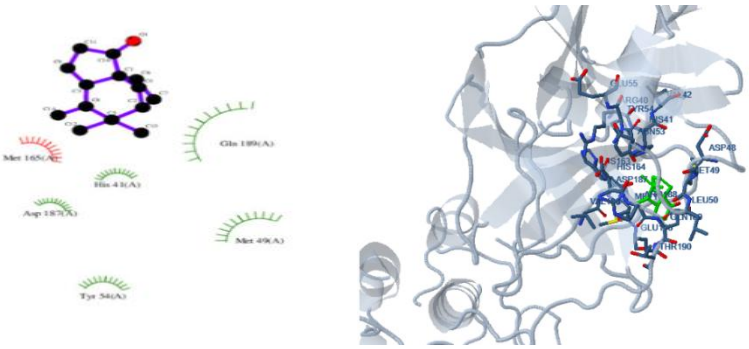
(E. β-bisabolene)



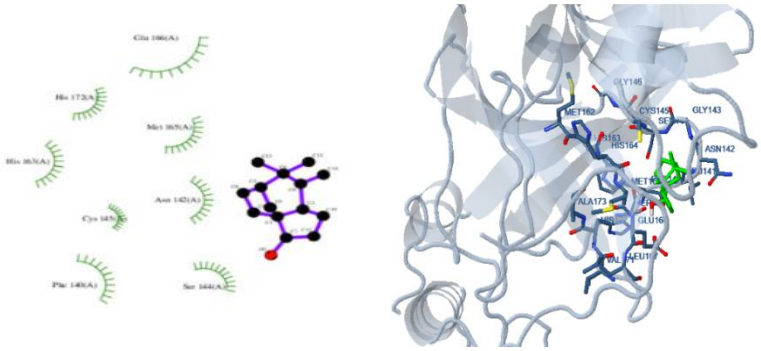
(F. Salicylaldehyde)



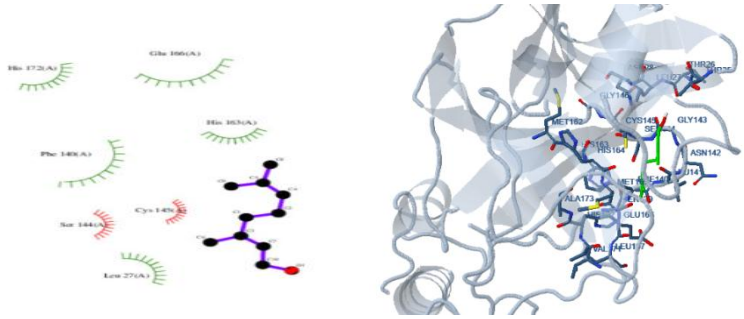
(G. Piperonylic acid)



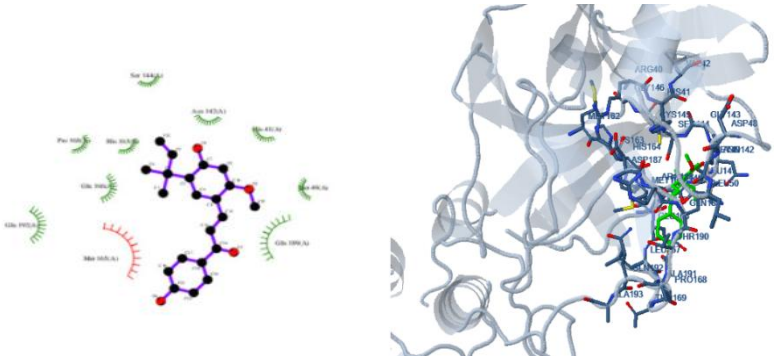
(H. Khusimone)



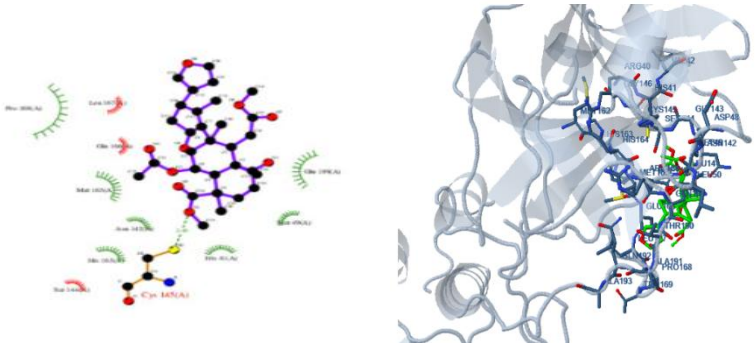
(I. Khusimol)



(J. Geraniol)



(K. Licochalcone A)



(L. Nimbin)

Figure 3: The binding affinity of Phyto-therapeutic ligand compounds (A) Salannin, (B) Genistein, (C) Indigotin, (D) Paradol, (E) β-bisabolene, (F) Salicylaldehyde, (G) Piperonylic acid, (H) Khusimone, (I) Khusimol, (J) Geraniol, (K) Licochalcone A, (L) Nimbin present in the *Vishasura kudineer* (VSK) with COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

Conclusion

Based on the results of the computational analysis it was concluded that the bio-active compound's such as Indigotin, Paradol, Nimbin, Khusimol, Geraniol , Licochalcone A, Salicylaldehyde, Piperonylic acid, β-Bisabolene , Genistein and Salannin present in the herbs revels significant binding against the target protein 3CL pro thereby it was concluded that these compounds may 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.

Conflicts of interest

Authors declare that they have no conflicts of interest

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