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Anti-viral potential of traditional siddha formulation Nochi kudineer against 3-clpro main protease of sars-cov-2 virus: A computation approach

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Abstract
Novel corona virus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) characterized by acute respiratory illness potentially destabilize the health and wellness of the population around the globe. In search of several therapeutic preparations in management of COVID-19, herbs offer considerable better remedy as hypothesized by several research outcomes. Nochi kudineer is one such novel preparation of kudineer indexed in classic Siddha formularies indicated to counteract the infection and to enhance the immune makeup. The main aim of the present investigation is to evaluate the anti-viral potential of the phytotherapeutics such as Friedelin, β-pinen, Vitexin, Piperidine, Piperine, Ajoene, Allicin, Hydroxychavicol and Eugenol present in the formulation Nochi kudineer against the enzyme target 3-chymotrypsin-like protease ($3CL^{pro}$)by using Auto Dock prediction. Results of molecular docking analysis strongly suggested that out of nine phyto components leads such as Friedelin, Vitexin, Piperine, Ajoene and Allicin posse's maximum of 5 interactions with the core active amino acid residues present on the target. It was concluded from the results of the present investigation that phytochemicals present in the formulation Nochi kudineer reveals significant 3-CLpro enzyme inhibition property. Hence upon further clinical validation preparations like Nochi kudineer may be considered as a drug of choice for the management of COVID-19 in the near future.

Keywords: covid-19, sars-cov-2, $3cl^{pro}$, Nochi kudineer, phytocomponents, docking, siddha

Introduction

Emergence of novel corona virus threatens the social wellbeing of societies around the globe since Dec 2019. WHO and other health care authorities are constantly striving to get remedies for management of COVID 19. As the concern of WHO, novel corona virus has been permanently named as SARS-CoV-2 and the disease outcome of the infection named as Corona virus disease 2019 (COVID-2019) [1].

The peculiarity relies on SARS-CoV-2 infection and its pathogenicity over respiratory system calls for the need of acute emergency care. Effort of identifying therapeutic from the synthetic and natural source continuous to demand the versatile structural pharmacophore leads that should offer the entry and replication inhibition [2]. Among the several therapeutic targets for single stranded RNA viruses like SARS-CoV-2, some of the unique functional moieties like non-structural proteins (nsps), spike protein (S) gene, envelope shielded protein (E) gene, membrane protein (M), nucleocapsid protein (N) relatively attract the researchers.[3]

Even though drugs like Remdesivir, Ivermectin, Hydroxychloroquine reveals pronounced therapeutic efficacy in COVID management, their sustenance on long-term usage becomes questionable, due to number of hindering factors like unstable therapeutic profile, adverse events, resistance etc [4]. As the known source of synthetic molecules fail to offer the expected clinical recovery in COVID-19 cases it become essentially important to explore the alternate therapeutic leads from traditional medicines including herbal components [5].

For several centuries, herbs and its active therapeutics are contributing in treating human diseases. Unique feature of herbal medicine is that, they have a high degree of structural and functional specificity in comparison to synthetic molecules. Many herbal leads compassionately become enzyme inhibitors and may be in clinical utilization for many numbers of disease and disorders [6].

Siddha medicines provided a thoughtful clinical benefit during the pandemic spread of dengue cases in southern part of India, especially in Tamil Nadu. Studies that have been initiated on investigating the anti-viral potential of some Siddha medicines like Nilavembu Kudineer clearly justify the anti-viral potential in in-vitro. Decoction paves good attention for its compatibility with human biological system

as it mediates faster absorption, onset of action, extended pharmacological action, permeable for cell membranes etc. Kudineer preparations are known to setup steady state plasma concentrations which are highly required for declination of viral load in the infected host. The main aim of the present investigation is to evaluate the anti-viral potential of the phytotherapeutics such as Friedelin, β-pinen, Vitexin, Piperidine, Piperine, Ajoene, Allicin, Hydroxychavicol and Eugenol present in the formulation Nochi kudineer [7] against the enzyme target 3-chymotrypsin-like protease (3CL^{pro})by using Auto Dock prediction.

Materials and Methods

Protein-ligand docking

Computational biological investigation was performed using Auto Dock version 4 which evaluate the interaction between selected therapeutic leads (Friedelin, β-pinen, Vitexin, Piperidine, Piperine, Ajoene, Allicin, Hydroxychavicol and Eugenol) as shown in figure 2 and 3, with investigational target COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7.

Protein preparation

Three dimensional (3D) structure of COVID-19 main protease (3-chymotrypsin-like protease 3CL pro with protein data bank (PDB)-6LU7 (Figure 1) retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) [8].

Docking simulations

Molecular docking examination were performed using licensed version of Auto Dock 4, prediction between selected herbal lead compounds and their interactions with that of the selected protein target 3-chymotrypsin-like protease (3CL pro) were been carefully investigated. 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 programmed algorithm inbuilt with pre automation in the software. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking [9, 10].

Results

Binding affinity clearly distinguish the characteristic features of the functional moieties present in the drug molecules. In category of drugs like enzyme inhibitors, competent drug should binding on the active site of the enzymes. As per the earlier research it was clearly identified that the enzyme 3CL^{pro}reveals the presence of active including His 41, Phe 140, Gly 143, Cys 144, Cys 145, His 163, Glu 166, His 172 assist the polyprotein that necessitates the replication process.

It was evident from the outcome of the present investigation that the phytocomponents subjected for its inhibition efficacy hosting over the enzyme target provokes increasing order of affinity as follows Friedelin> Vitexin> Piperine> Ajoene> Allicin> Piperidine> Hydroxychavicol> Eugenol> β-pinen. As shown in Table 3 and in Figure 4 -5. Compounds such as Friedelin>Vitexin>Piperine>Ajoene> Allicin Ranks First with the maximum of five significant interaction on the active site of 3CL^{pro} followed by this compound Piperidine ranks second with four potential interactions. Similarly compounds Hydroxychavicol and Eugenol holds third rank with maximum of three interactions.

Discussion

COVID-19 attaining higher index in mean number of infections every hour, hence being need of the hour scientist depend more on the rapid screening techniques that yield a productive outcome with high reliability in lesser time. Globally, lakhs of library compounds from variety of source has been subjected to screening to hit the viable drug candidate [11].

Molecular docking is a powerful tool on the artificial intelligence platform, standard algorithm with optimized machine learning methodologies promptly identified the drug of choice against the specified receptor target. Docking works behind the principle of studying the binding behavior of the drug on the stable protein target. In majority of the cases enzyme could be the target of choice, 3D structure of the target under investigation subjected for possible docking with the promising leads molecules [12-17].

3CLpro structurally characterized by the existence of three active domains pooled by active molecular site. As a protein it has its own pattern of loops guarded by alpha and beta helices. While considering the amino acids His41, Cys144 and Cys 145 reveals efficient receipt of external functional groups preferably from the lead substrates. Hence it is well known fact that drug/ lead that host of these functional regions may emerge as better candidate for controlling viral multiplication in the host [18].

Binding of drugs with target enzyme has been controlled by varying factors includes, solvent exposure, specificity, nature of arrangement on substrate binding site, Concentration of dense amino acid sequence further make the aces ability of the pocket tougher for ideal drug target [19].

Promising lead molecule approached the target receptor in its active conformation state, further orientation of the amino acid chain also determines the potential binding affinity of the structurally versatile drugs [20]. Nochi kudineer is one such novel preparation of kudineer indexed in classic Siddha formularies indicated to counteract the infection and to enhance the immune makeup. It comprise of four core herbal ingredients including Nochi - *Vitex negunda*, Milagu - *Piper nigrum*, Poondu -*Allium sativum* and Kammaru vetrilai - *Piper betel.*

Firstly the herb *Vitex negunda* known for its anti-viral (Chikungunya virus) and anti-inflammatory potency [21]. *Piper nigrum* a potent plasma stabiliser, bio enhancer and anti-viral drug (herpes simplex and herpes zoster viruses) [22]. Extracts of *Allium sativum* traditionally used as anti-microbial agent and research evident that derivatives of *Allium sativum* may exert significant anti-viral property [23]. Floklore application of *Piper betel* leaf signifies the presence of anti-microbial, anti-inflammatory and anti-cancer activity [24].

It was evident from the outcome of the present investigation that the phytocomponents subjected for its inhibition efficacy hosting over the enzyme target provokes increasing order of affinity as follows Friedelin> Vitexin> Piperine> Ajoene> Allicin> Piperidine> Hydroxychavicol> Eugenol>

β-pinen. Compounds such as Friedelin> Vitexin> Piperine>Ajoene> Allicin ranks first with the maximum of five significant interaction on the active site of $3CL^{pro}$ followed by this compound Piperidine ranks second with four potential interactions. Similarly compounds Hydroxychavicol and Eugenol holds third rank with maximum of three interactions.

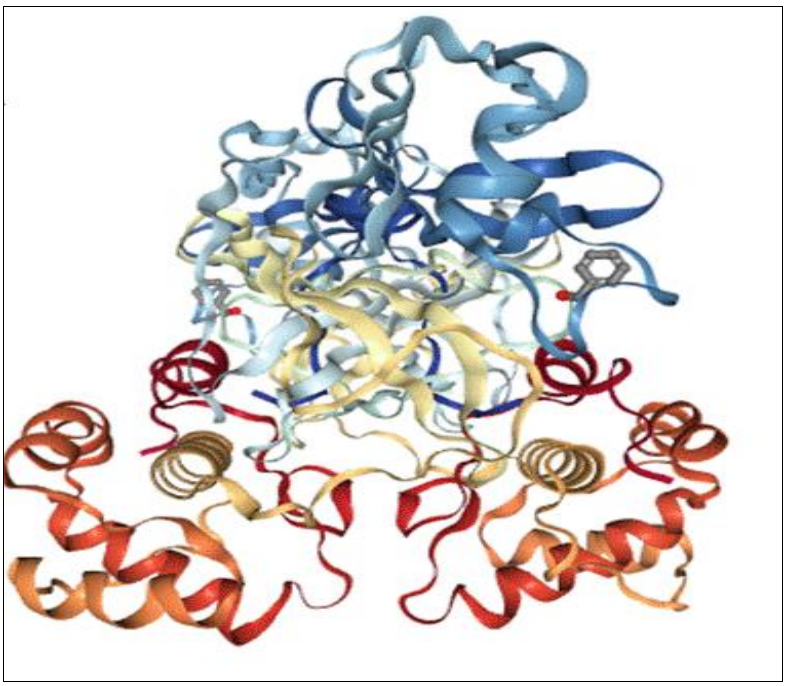

Fig 1: 3D crystalline structure of the target protein COVID-19 main protease (3-chymotrypsin-like protease (3CL pro) – PDB 6LU7

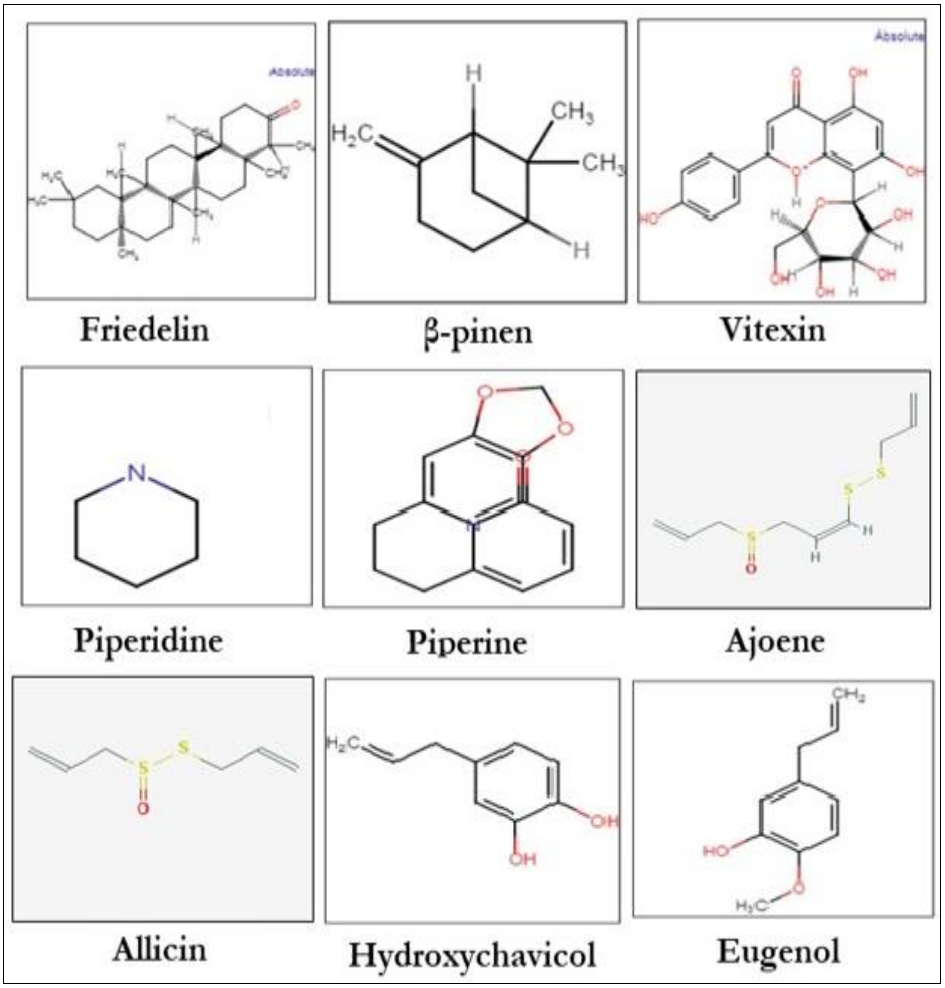

Fig 2: 2D Structure of Selected Ligands

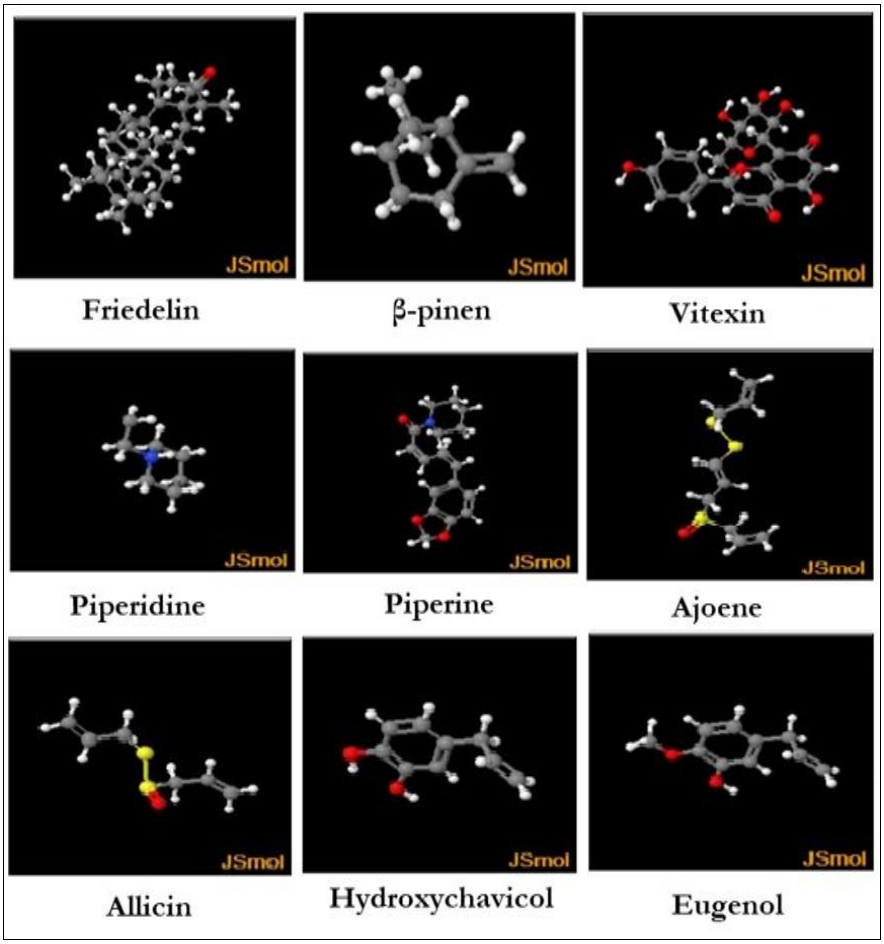

Fig 3: 3D Structure of Selected Ligands

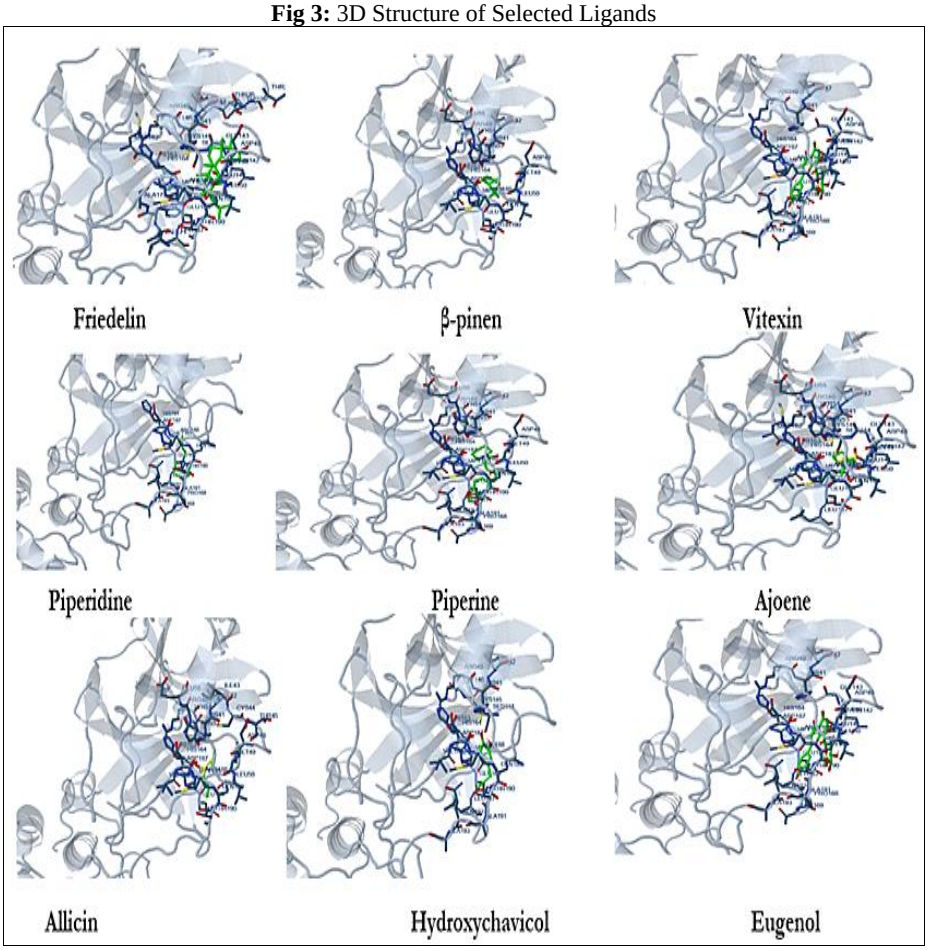

Fig 4: Representing best docking pose of phytotherapeutic lead molecules against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7

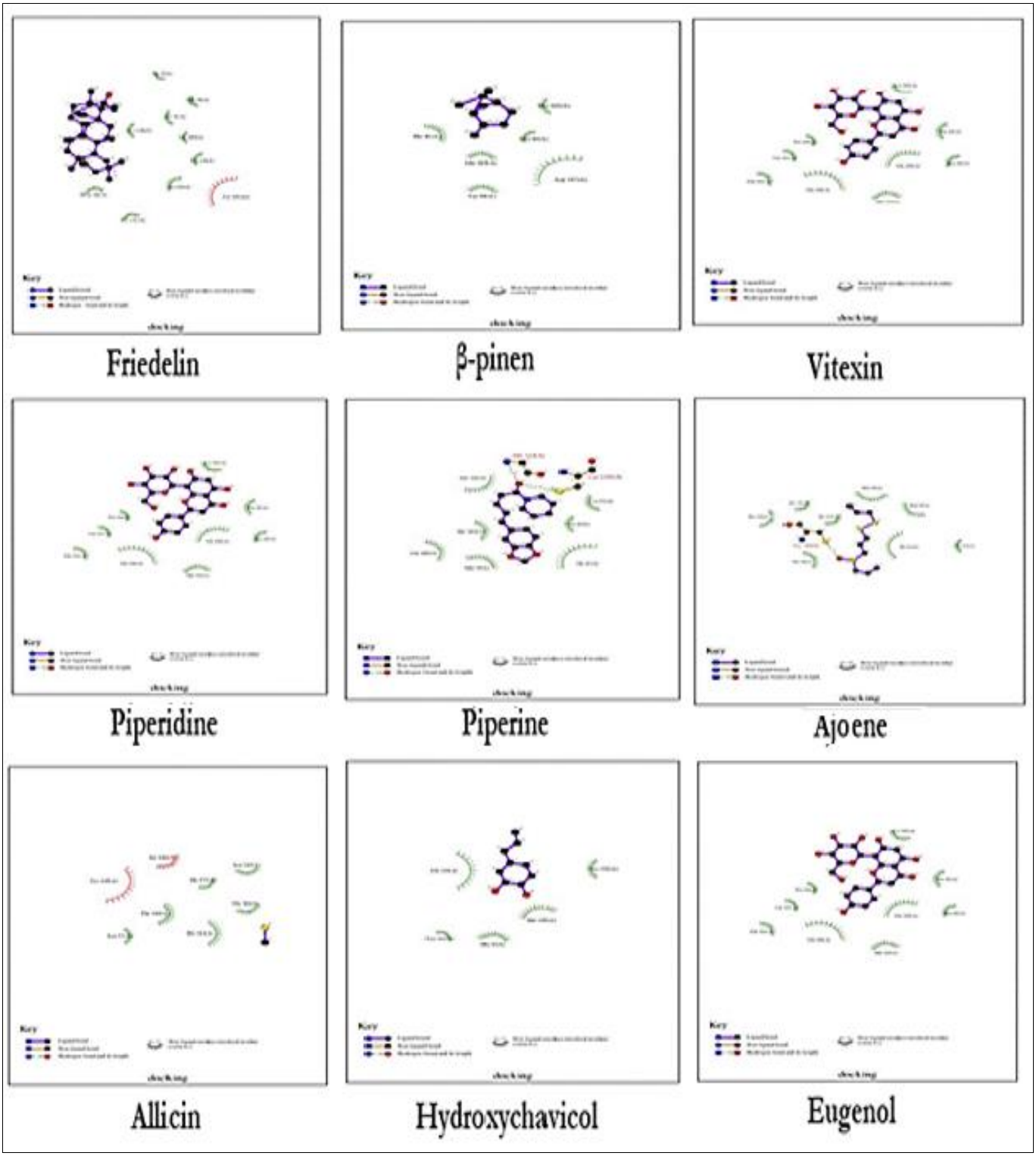

Fig 5: Representing interaction analysis plot of phytotherapeutic lead molecules against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7

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
Friedelin	426.7 g/mol	$C_{30}H_{50}O$	0	1	0
β-pinen	136.23 g/mol	$C_{10}H_{16}$	0	0	0
Vitexin	432.4 g/mol	$C_{21}H_{20}O_{10}$	7	10	3
Piperidine	85.15 g/mol	$C_5H_{11}N$	1	1	0
Piperine	285.34 g/mol	$C_{17}H_{19}NO_3$	0	3	3
Ajoene	234.4 g/mol	$C_9H_{14}OS_3$	0	4	8
Allicin	162.3 g/mol	$C_6H_{10}OS_2$	0	3	5
Hydroxychavicol	312.31 g/mol	$C_{15}H_{20}O_7$	5	7	5
Eugenol	164.2 g/mol	$C_{10}H_{12}O_2$	1	2	3

Table 2: 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
Friedelin	-8.84	333.79**	-0.06	-8.11	777.49
β-pinen	-5.72	64.21	-0.01	-5.72	419.64
Vitexin	-9.07	224.89**	-1.21	-7.69	792.47
Piperidine	-4.86	273.83	-0.39	-5.43	417.67
Piperine	-7.46	3.39	-0.03	-7.81	626.97
Ajoene	-4.83	287.80	-0.07	-7.08	625.31
Allicin	-3.34	3.55*	-0.05	-4.78	409.46
Hydroxychavicol	-5.37	115.78	-0.10	-5.31	453.86
Eugenol	-4.97	226.32	-0.03	-5.37	493.64

Table 3: 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									
Friedelin	5	25 THR	41 HIS	49 MET	142 ASN	144 SER	145 CYS	163 HIS	166 GLU	172 HIS	189 GLN
β-pinen	2	41 HIS	49 MET	54 TYR	165 MET	187 ASP	189 GLN				
Vitexin	5	41 HIS	142 ASN	145 CYS	165 MET	166 GLU	168 PRO	189 GLN	192 GLN		
Piperidine	4	41 HIS	54 TYR	145 CYS	164 HIS	165 MET	187 ASP				
Piperine	5	25 THR	27 LEU	41 HIS	49 MET	142 ASN	143 GLY	145 CYS	165 MET	189 GLN	
Ajoene	5	41 HIS	49 MET	54 TYR	140 PHE	144 SER	145 CYS	163 HIS	165 MET	166 GLU	
Allicin	5	27 LEU	140 PHE	142 ASN	144 SER	145 CYS	163 HIS	166 GLU	172 HIS		
Hydroxychavicol	3	41 HIS	145 CYS	165 MET	189 GLN	192 GLN					
Eugenol	3	41 HIS	44 CYS	49 MET	52 PRO	54 TYR	145 CYS	165 MET	187 ASP	189 GLN	

Conclusion

India becomes rich in herbal heritage that claim the traditional therapies like Siddha and Ayurveda on hosting the measure for several infectious disease. Documentary evidence claim the rich historic culture of availing herbs as remedy for detoxification, rejuvenation and reformation of body and soul. Siddha system of medicine hypothecates that fever as an indication of increased kabam happens to be the outcome of the microbial infection. Balancing three humours like vatham, pitham and kabam boost the immune system to combat the viral infections. Polyherbal preparation of composite blend of multiple phyto components, Siddha system often relies on polyherbal preparations for its efficacious therapy. In our present investigation it was clearly evident that phyto components leads such as Friedelin, Vitexin, Piperine, Ajoene and Allicin present in the formulation Nochi kudineer posses maximum interactions with the core active amino acid residues present on the target $3CL^{pro}$. Hence upon further clinical validation preparations like Nochi kudineer may be considered as a drug of choice for the management of COVID-19 in the near future.

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