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Original Article

MOLECULAR DOCKING STUDY ON PHYTOCONSTITUENTS OF TRADITIONAL AYURVEDIC DRUG TULSI (OCIMUM SANCTUM LINN.) AGAINST COVID-19 M^{PRO} ENZYME: AN *IN SILICO* STUDY

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ABSTRACT

Objective: The aim of the present study was to assess bioactive compounds found in Tulsi as potential COVID-19 M^{pr} °inhibitor using molecular docking and to provide scientific justification in term of its active ingredient to target protein for prevention and symptomatic treatment of COVID-19.

Methods: COVID-19 M^{pr} °was docked with eight phytochemicals of Ocimum sanctum Linn. Using Autodock 4.2. Determination of active site and visualization of molecular interactions between ligands and target enzyme was done by Biovia Discovery Studio 4.5.

Results: Our result demonstrates that Vicenin, Caryophyllene, Cirsimaritin, Isothymusin and Isothymonin have a better binding affinity to target enzyme. However, only Vicenin exhibited better binding energy i.e.-7.02 kcal/mol to COVID-19 Main protease among other phytochemicals through some responsible interactions to inhibit the replication of SARS-CoV-2 in the human body, whereas Caryophyllene and Cirsimaritin exhibited similar binding affinity i.e.-6.46 kcal/mol but different interactions with target enzyme.

Conclusion: Tulsi (Ocimum sanctum Linn.) is a preeminent traditional drug of Ayurveda for prophylaxis and treatment of various ailments, including respiratory disorders like cough, cold and flu. With no specific therapies available, reevaluating and repurposing traditional drugs could be an effective approach for the prevention and treatment of SARS-CoV-2 infection. Therefore our study provides scientific evidence for the potential use of Tulsi as an adjunct therapy for the prevention and symptomatic treatment of COVID-19. However, further *in vitro* and *in vivo* studies should be conducted to validate use of proposed compounds in drug discovery and as therapeutics against COVID-19.

Keywords: Novel coronavirus, COVID-2019, SARS-CoV-2, Mpro, Medicinal plant compounds, Ocimum sanctum Linn., Tulsi, Holy basil, Molecular docking

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INTRODUCTION

Thepandemic COVID-19, which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has spread to 212 countries leading to global burden of 26,46,8031 laboratory-confirmed cases and 8, 71,166 casualties at the day of writing (September 5th, 2020) [1]. COVID-19 begin as acute respiratory distress syndrome with unknown etiology in China, later it crossed boundaries spreading to many other countries and clinically manifests as the disease of respiratory tracts with pneumonia-like symptoms and various other systemic disorders [2]. As of today, there are no specific anti-viral drugs or vaccines available to treat COVID-19 infected patients. Repurposing of FDA-approved drugs for other indications like antiviral agents, chloroquine, hydroxychloroquine etc., convalescent plasma, Immunological interventions are the few treatment modalities on which governments and clinicians are banking on to fight the pandemic [3, 4]. India has already entered into its third phase of lockdown in an effort to contain COVID-19 by breaking the chain of Human to human transmission. India stands quite low with laboratory-confirmed cases of 35043 and 1147 deaths [1, 4]. Like various anti-viral drugs and vaccines are under pipeline for treatment or prevention of SARS-CoV-2 infection, Traditional Chinese herbal medicines are also under investigation for treatment and/or prevention of COVID-19 infection in China [5, 6]. Similarly, Indian Ministry of AYUSH recommended Ayurvedic immunity promoting measures with reference to respiratory immunity supported by Ayurvedic literature and scientific publications. As per guideline, Drinking Tulsi (Ocimum sanctum Linn.) tea or decoction with other herbs can boost respiratory immunity [7]. Various clinical studies suggest that cytokine storm and over-activated Immune cell recruitment to the lungs characterize the severe lung damage and even deaths of COVID-19 patients. Moreover, old age and/or underlying disease conditions like hypertension, obesity, diabetes, cardiovascular disease and respiratory disease are the risk factors that determine the severity of outcomes [8]. Jagadeesh *et al.* suggested that a variety of immunotherapeutic approaches involving neutralization of inflammatory cytokines, immunomodulation and passive viral neutralization may be used as an adjunct therapy to treat COVID-19 patients [9]. In Ayurveda, Tulsi has a preeminent position known as 'The incomparable one' and 'Elixir of life' because of the diversity of pharmacological actions it possesses. There are mounting evidences which supports its multidimensional health benefits likeImmunomodulatory, antimicrobial, anti-inflammatory, chemopreventive, radioprotective, hepato-protective, neuro-protective, cardio-protective, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive, anti-carcinogenic, analgesic, anti-pyretic, anti-allergic, anti-asthmatic, anti-tussive, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritic, adaptogenic, anti-stressactivities [10]. Moreover, a set of preliminary studies have documented viral inhibitory potential of crude extract, polyphenol and terpenoid fractions of Tulsi plant partsagainst several viruses like Newcastle Disease virus, Vaccinia virus, Infectious Bursal Disease virus and H9N2 Influenza A virus [11]. However, there is paucity of data available on the selective chemical investigation of Ocimum sanctum Linn. phytoconstituents asanti-viral compound. Therefore, based on the above, as tulsiphytoconstituents may not only help in reducing the risk factors but also in decreasing viral load in COVID-19 patients because of its immunomodulatory, anti-inflammatory, and other pharmacological effects on all systems, Tulsi excels as suitable candidate to be repurposed as preventive and anti-viral therapy.

The COVID-19 Main Protease (M^{pro}) participates in the assembly and multiplication of the SARS-CoV-2 virus is reported to be ideal target for disruption of viral self-replication without causing any harm to the host [12]. In our previous study, we reported M^{pro} inhibitory potential of Oxyacanthin against COVID-19 [13]. In another investigation kaempferol, quercetin, luteolin-7-glucoside,

demethoxycurcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin, and epicatechin-gallate have shown M^{pro}inhibitory potential against SARS-CoV-2 [14]. Prompted by this, in this study we analysed selected Ocimum sanctum Linn. phytoconstituents like Terpenoids viz. α-Thujene, Myrcene, Eugenol, Caryophyllenee and Phenolic compounds viz. Cirsimaritin, Isothymusin, Isothymonin, and Vicenin as potential inhibitory candidate against SARS-CoV-2 M^{pro}enzyme by *in silico* methods.

MATERIALS AND METHODS

Accession of target enzyme

The three-dimensional structure of protease enzyme (COVID-19 $3cl^{pro}$/M^{pro}(PDB ID: 6LU7), was downloaded from RCSB protein data bank (https://www.rcsb.org/) [15].

Ligand and macromolecule preparation

The chemical structures of ligands used in this study were designed and optimized using ChemSketch freeware 2015 and saved in. mol format, later was converted into. pdb format by Open Bable-2.3.2 software, required for execution in Autodock4.2 software. Macromolecule (target enzyme) was prepared before starting the molecular docking process, which involved removal of the water molecules and native ligand attached with target enzyme. After that hydrogen atoms were added into target the investigation ligand was loaded and their torsions along with rotatable bonds are assigned and the files are saved as ligand. PDBQT.

Molecular docking study

In the current study, binding modes of the chemical compounds from Ocimum sanctum Linn with target were identified using Autodock 4.2 software program. In this way, the 10 different conformers of the compounds were generated and blind docking was performed to know whether these molecules bind in the active site or anywhere in the target was demonstrated by Biovia Discovery studio 4.5 program. Binding site of herbal molecules on molecular target and the best conformers were represented with the lowest binding energy (-kcal/mol), which might pave the way to disclose the mode of actions of these ligands. The docking parameters were defined as coordinates of the center of binding site with x = 126, y = 126, z = 126 and binding radius = 0.375 Å. All Auto Dock output file (.dlg) were then analyzed through Analysis option provided in MGLTools-1.5.6 rc3. Further identifications of binding modes and preparation of 2D and 3D images were done through discovery studio 4.5.

RESULTS AND DISCUSSION

Data obtained by molecular docking study is arranged as tabulated form (table 1). Table 1 shows the 2d interactions of ligands with target enzymes and necessary hydrogen bond formation by ligands with targets required for inhibition of target enzyme of covid-19. Whatever investigational ligands have been included in the study are found in the different extracts of Ocimum sanctum Linn. plant. Fig. 1 indicates the active site of target to confirm the binding site of investigational ligands if they were interacted with the amino acids in the active site of target or found to be attached any other site inside the target. The active site of this target enzyme comprises of HIS41, MET 49, PHE140, LUE141, ASN142, GLY143, HIS163, HIS164, MET165, GLU166, LEU167, PRO168, HIS172, GLN189, THR190, ALA191 (fig. 1). As well superimposition is also performed using discovery studio to check the actual position of compounds after molecular docking study onto target enzyme (fig. 2 and 3). The best poses of natural ligands and enzyme complexes are described [fig. 4(A-I)]. Being a chymotrypsin-like cysteine protease in nature, main protease enzyme Mpro executes a vital role in provoking replication and transcription of virus [16]. Therefore we opted for this enzyme as a prime target for molecular docking study to identify promising inhibitors of this deadly virus. Results indicated that Vicenin might be promising in case of inhibition of the main protease enzyme. Rationales for being an inhibitor, are that chemical compounds have to show some specific binding interactions with amino acids in active sites (active pocket) of target enzyme. Also these interactions should be compared with native ligand attached with enzyme as downloaded (pdb). Interactions of the native ligand with enzyme revealed that proper inhibition of main protease enzyme requires the formation of hydrogen bonds with specific amino acids residues such as Phe140, Gly143, His163, His164, Glu166, Gln189, Thr190, If any investigational compound able to interact by forming Hydrogen bond with all or some of above-mentioned amino acid residues then possibly that particular compound or some of the compounds included in this study might be potent inhibitor of target enzyme. After the superimposition, it was also found that all investigational ligands can bind to the target enzyme at the same position (fig. 2). In current study, three compounds such as α-Thujene, Myrcene could not afford requisite hydrogen bond formations with target enzyme as well as exhibited very low binding energy i.e.-4.820,-4.28kcal/mol respectively. Also, Caryophyllenee could not afford the hydrogen bond formation but exhibited better binding affinity (-6.46kcal/mol) compared to α-Thujene, and Myrcene. Cirsimaritin showed moderate binding energy (-6.61kcal/mol) and was able to form three hydrogen bonds with amino acid residues Leu141, Ser144, and Glu166 (with hydrogen bonds distance 2.01Å, 2.22Å, and 1.83Å, respectively) but only interaction with Glu166 might be required for inhibition of enzyme, remaining other two H-bonds formation were not mandatory for enzyme inhibition. Eugenol demonstrated very less binding energy (-4.5 kal/mol) and made two hydrogen bond with Glu166 (with hydrogen bonds distance 2.11Å and 2.31Å). Isothymonin and Isothymusin both exhibited moderate binding energies (-5.92 and-6.23 kcal/mol, respectively) and could afford only one mandatory h-bond formation with Thr190 andGlu166 respectively (with hydrogen bonds distance 2.12Å and 3.08Å respectively), whereas other h-bonds were common in both. For comparative purposes, Remdesivir was included in the study but it showed very least binding affinity (-3.7 6kcal/mol), although it could interact with target enzyme in the active pocket. Vicenin proved itself most efficacious compound among other investigational compounds because of having the highest binding affinity (-7.02) and by forming hydrogen bonds with amino acid residues i.e. Thr26, Phe140, His163, Glu166, and Thr190, most of them were supposed to be mandatory of inhibition of target enzyme. For the symptomatic treatment of covid-19 and its different variants, some allopathic medicines were employed, such as hydroxychloroquine (Antimalarial drug), Remdesivir (Antiviral), Dexamethasone (Steroid) and other antioxidants. Shree *et al.*, 2020 also emphasized the importance of herbal medicines to combat against covid-19 and concluded that Vicenin exhibited significant binding affinity as compared to the native ligand and could interrupt the interactions of covid-19 with host cell without producing any toxicity [17].

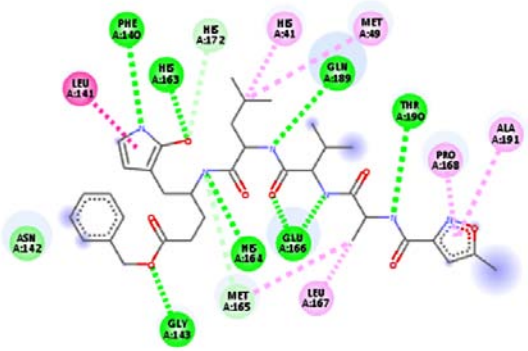


Fig. 1: Active site of target enzyme

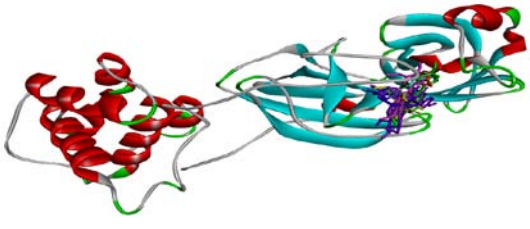
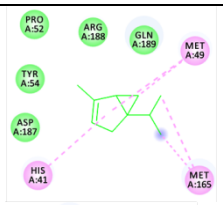
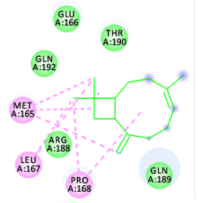
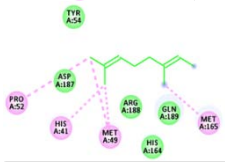
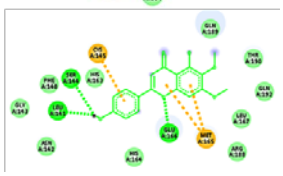
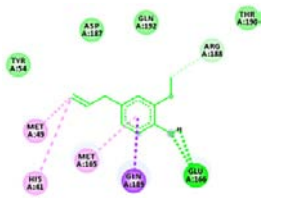
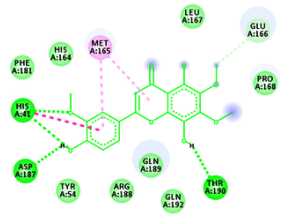
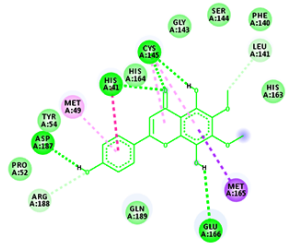
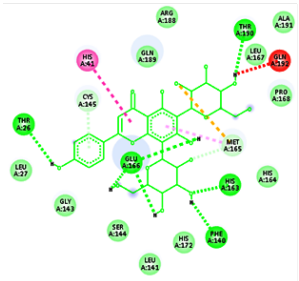
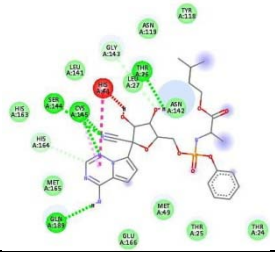


Fig. 2: Superimposition of investigational ligands without ramdesivir

Table 1: Structures of ligands and hydrogen bond formation between ligands and target

S. No.	Ligand	Binding energy (kcal/mol)	2D structures of interactions	Types of interactions
1	α-Thujene	-4.820		Conventional H-bond: NIL Van der vaals: Pro52, Tyr54, Asp187, Arg188, Gln189 Pi-alkyl: His41, Met49, Met165
2	Caryophyllenee	-6.46		Conventional H-bond: NIL Van der vaals: Glu166, Arg188, Gln189, Thr190, Gln192 Pi-alkyl: Met165, Leu167, Pro168
3	Myrcene	-4.28		Conventional H-bond: NIL Van der vaals: Tyr54, His164, Asp187, Arg188, Gln189 Pi-alkyl: His41, Met49, Met165
4	Cirsimaritin	-6.46		Conventional H-bonds: Leu141:O: Acceptor-LIG1:H: Donor, H-bond distance-2.01Å Ser144:OG: Acceptor-LIG1:H: Donor, H-bond distance-2.22Å Glu166:HN: Donor-LIG1:O: Acceptor, H-bond distance-1.83Å Van der vaals: Phe140, Asn142, Gly143, His163, His164, Leu167, Arg188, Gln189, The190, Gln192 Pi-sulfur: Cys145, Met165
5	Eugenol	-4.5		Conventional H-bond: Glu166:HN: Donor-LIG1:O: Acceptor, H-bond distance-2.11Å Glu166:O: Acceptor-LIG1:H: Donor, H-bond distance-2.31Å Van der vaals: Tyr54, Asp187, Gln189, The190 Pi-alkyl: His41, Met49, Met165 Pi-SigmaGln189 Carbon-Hydrogen bond: Arg188
6	Isothymonin	-5.92		Conventional H-bond: His41:HD1: Donor-LIG1:O: Acceptor, H-bond Distance-2.69Å His41:HD1: Donor-LIG1:O: Acceptor, H-bond Distance-2.81Å Asp187:O: Acceptor-LIG1:H: Donor, H-bond Distance-1.90Å Thr190:O: Acceptor-LIG1:H: Donor, H-bond Distance-2.12Å Van der vaals: Tyr54, His164, Leu167, Pro168, Phe181, Arg188, Gln192 Carbon-Hydrogen bond: Glu166, Gln189 Pi-Pi stacked: His41 Pi-alkyl: Met165
7	Isothymusin	-6.23		Conventional H-bond: Asp187:O: Acceptor-LIG1:H: Donor, H-bond Distance-2.34Å His41:HE2: Donor-LIG1:O: Acceptor, H-bond Distance-2.72Å Cys145:SG: Donor-LIG1:O: Acceptor, H-bond Distance-2.85Å Cys145:SG: Acceptor-LIG1:H: Donor, H-bond Distance-2.98Å Van der vaals: Pro52, Tyr54, Phe140, Gly143, Ser144, His163, His164, Gln189 Carbon-Hydrogen bond: Leu141, Arg188 Pi-Pi stacked: His41 Pi-alkyl: Met49 Pi-sigma: Met165
8	Vicenin	-7.02		Conventional H-bond: Thr26:O: Acceptor-LIG1:H: Donor, H-bond Distance-2.51Å Phe140:O: Acceptor-LIG1:H: Donor, H-bond Distance-2.46Å His163:HE2:Donor-LIG1:O: Acceptor, H-bond Distance-1.97Å Glu166: O: Acceptor-LIG1:H: Donor, H-bond Distance-2.41Å Glu166: OE1:Acceptor-LIG1:H: Donor, H-bond Distance-2.03Å Glu166: OE2:Acceptor-LIG1:H: Donor, H-bond Distance-2.65Å Thr190:O: Acceptor-LIG1:H: Donor, H-bond Distance-2.08Å Van der vaals: Leu27, Leu141, Gly143, Ser144, His164, Leu167, Pro168, His172, Arg188, gln189, Ala191 Carbon-Hydrogen bond: Cys145, Met165 Pi-Pi T shaped: His41

S. No.	Ligand	Binding energy (kcal/mol)	2D structures of interactions	Types of interactions
9	Remdesivir	-3.76		Conventional H-bond: Thr26:O: Acceptor-LIG1:H: Donor, H-bond Distance-2.12Å Cys145:HN: Donor-LIG1:N: Acceptor, H-bond Distance-2.25 Å Cys145:SG: Donor-LIG1:N: Acceptor, H-bond Distance-3.45 Å Ser144:HN: Donor-LIG1:H: Acceptor, H-bond distance-2.45Å Van der vaals: THr24, Thr25, Met49, Thr118, Asn119, Leu141, Asn142, His163, Met165, Glu166 Carbon-Hydrogen bond: Gly143, His164

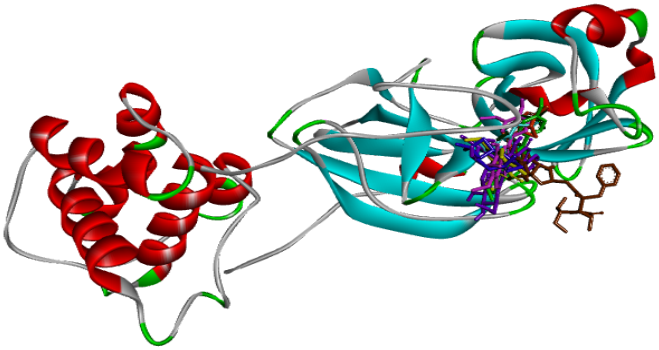


Fig. 3: Superimposition of investigational ligands with ramdesivir

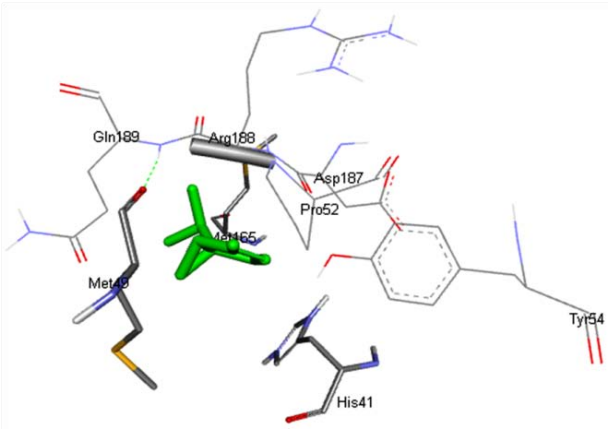


Fig. 4(A): 3D image of interactions of α-thujene with the target enzyme

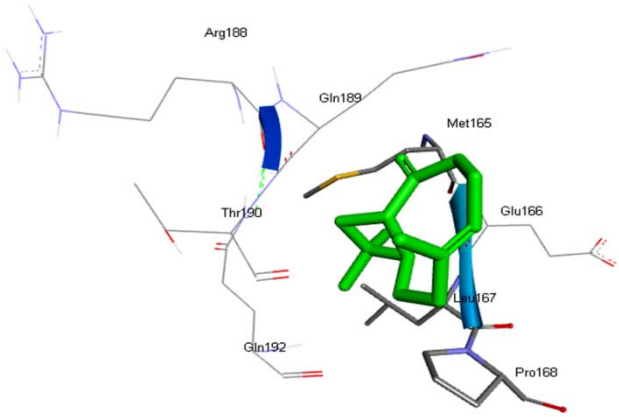


Fig. 4(B): 3D image of interactions of caryophyllenee with target enzyme

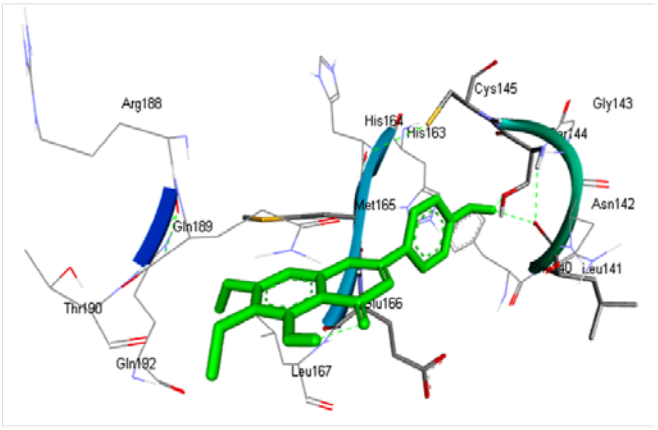


Fig. 4(C): 3D image of interactions of cirsimaritin with target enzyme

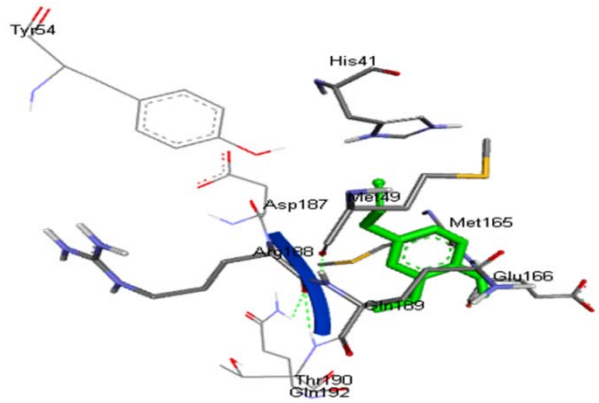


Fig. 4(D): 3D image of interactions of eugenol with target enzyme

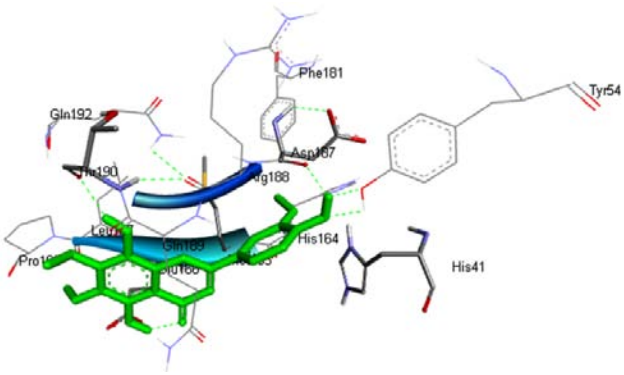


Fig. 4(E): 3D Image of interactions of isothymonin with target enzyme

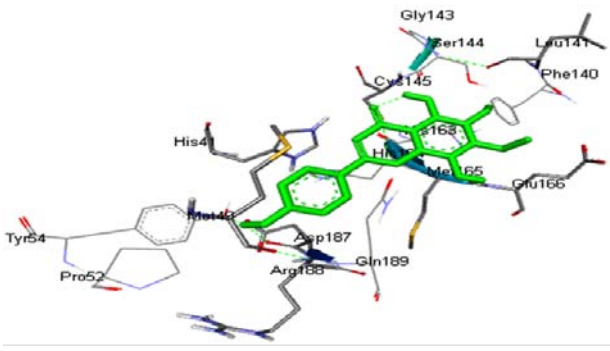


Fig. 4(F): 3D image of interactions of isothymusin with target enzyme

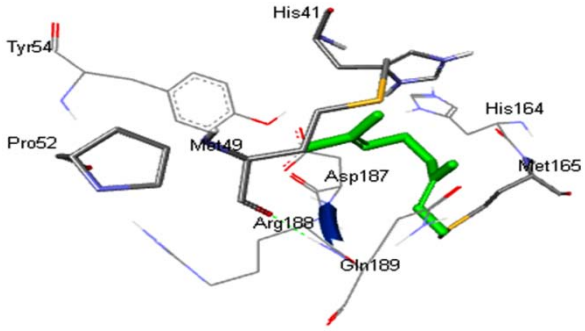


Fig. 4(G): 3D image of interactions of myrcene with target enzyme

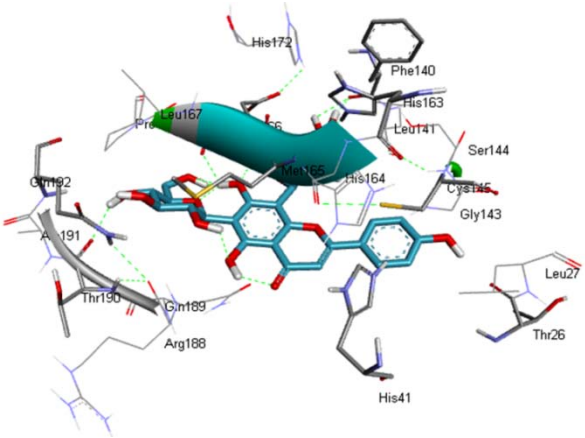


Fig. 4(H): 3D image of interactions of vicenin with target enzyme

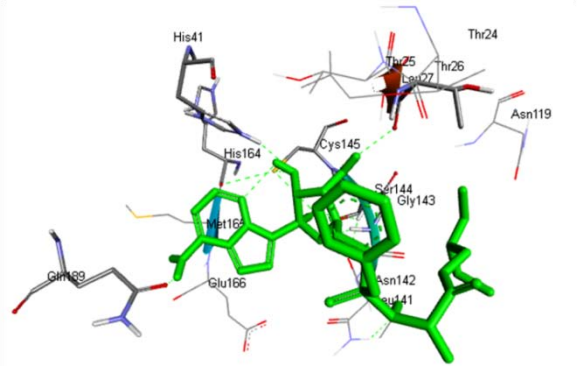


Fig. 4(I): 3D image of interactions of remdesivir with target enzyme

CONCLUSION

Amid COVID-19 crisis there is an urgent unmet need of effective therapy. Conventional drug development approaches are costly and time-consuming. Therefore army of the researcher is using alternative techniques like computer-assisted structure-based drug design (SBDD) or the computer added drug design (CADD) to reduce the cost and time. In the present study, we demonstrate the bioactive constituent of traditional ayurvedic drug Tulsi have significant binding affinity to COVID-19 Main protease (M^{pro}), indicating that they have the potential to control viral multiplication in addition to their reported immunomodulatory activity in host cells. In conclusion, Tulsi may be used as an adjunct therapy to boost individual's immunity and preventing the risk of infection with SARS-CoV-2, with symptomatic treatment of other respiratory symptoms like cough, cold and flu. However, further *in vitro* and *in vivo* studies should be conducted to validate use of proposed compounds in drug discovery and as therapeutics against COVID-19.

Human and animal rights

No Animals/Humans were used for studies that are base of this research.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

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FUNDING

Nil

AUTHORS CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: AA and NKJ; data collection: AA; analysis and interpretation of results: GTK and MT; draft manuscript preparation: NKJ and AA. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The author declares no conflict of interest, financial or otherwise.

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