Research article

Molecular docking studies of *Ocimum americanum* secondary metabolites against SARS-Cov-2 spike protein

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ABSTRACT

Introduction and Aim: The outbreak of Covid-19 pandemic since December 2019 has raised serious global health concern. Because of rapid human to human transmission and non-availability of clinically proven drugs or vaccines, this Covid-19 pandemic has created a great threat to mankind. Many naturally derived molecules are being investigated for the treatment of Covid-19. *Ocimum americanum* is one such significant medicinal plant possessing a variety of biological activities.

Materials and Methods: In the present study, seven phytochemicals were selected from *O. americanum* and were docked against SARS-CoV-2 spike protein which is an important site for virus to enter the host cell. Docking was performed using Autodock Vina and the ADME properties of all these seven ligands were predicted using the Swiss-ADME tool. The bioactivity score was also predicted using the Molinspiration tool. Besides the secondary metabolites, all these analyses were also performed for well-known antiviral drugs namely lopinavir and ritonavir.

Results: The binding energy obtained from the docking studies of SARS-CoV-2 spike protein with Lopinavir, Ritonavir, Alpha-farnesene, Beta-farnesene, Eugenol, Linalool, Estragole, Limonene and 1,8-Cineole was found to be -5.2, -5.1, -4.7, -4.5, -4.3, -4.1, -4, -3.9 and -3.8 Kcal/Mol respectively. Swiss-ADME results also suggest that all the selected ligands follow the drug likeness properties and hence they could be taken for further drug discovery process.

Conclusion: From the present *in silico* study, it can be concluded that secondary metabolites of *O. americanum* have potential inhibiting activity against spike protein of SARS-CoV-2. Isolation and efficacy studies *in vitro* may provide an insight into the drug discovery to fight Covid-19.

Keywords: S protein; Coronavirus; Kanjankorai; ADME properties; Lopinavir; phytochemicals.

INTRODUCTION

SARS-CoV-2 (Severe acute respiratory syndrome CoronaVirus-2) is an infectious and pathogenic coronavirus of universal concern (1). It belongs to the class of Beta coronaviruses which includes SARS-CoV and MERS-CoV (2). The outbreak of coronavirus was announced as a health emergency and a pandemic on 30th Jan 2020 and 11th March 2020 by WHO. Since December 2019, majority of the world population is suffering from COVID-19 outbreak. The confirmed cases by 8th April 2022 reported 494,587, 638 cases across 210 countries and territories and a total death of 6,170,283 cases (3).

It is a single stranded ribonucleic acid (30kb) virus which causes a respiratory malady showing symptoms of cough, fever, and dyspnea (4). Covid-19 also exhibits symptoms like muscle pain, anoxia, fatigue, and abdominal discomfort. The spread of virus from the affected person is by means of close contact and through droplets spilled when in conversation or while coughing and sneezing (5). Lack of drugs and vaccines to treat Covid-19 along with rapid spreading is the major reason for the pandemic. Though certain drugs for the same kind of health complaints were described as potential drugs but have not yet been approved. Natural molecules from medicinal plants are being studied randomly to treat the covid-19 pandemic (6).

In search of drugs or molecules from a huge database or library, computational tools like molecular docking, screening, simulations etc., can help the drug discovery process for Covid-19 at a minimal expenditure (7). The present study was aimed at performing the molecular docking analysis of selected secondary metabolites of a traditionally significant medicinal plant, *Ocimum americanum.*

Ocimum americanum (synonym: *O. canum*) is commonly known as Hoary basil or Lime basil in English and *Kanjankorai* in Tamil. It belongs to the family *Lamiaceae*. This annual plant is native to Asia and Africa. It grows in broad daylight to partly shady conditions. It is a wild herb with a unique mint flavor,

hairy leaves and scented flowers which has gained significant attention to their potential medicinal properties. Different parts of this plant have been used to treat many ailments in various traditional medical systems around the globe. The leaf juice is rich in essential oils of medicinal value which have been used for the treatment of diabetes, constipation, diarrhea, piles, and dysentery in the traditional folklore medicine (8). As per traditional Siddha system of medicine, *O. americanum* was found to have digestive, expectorant, stimulant and diaphoretic activity. The whole plant decoction was given to treat cold, cough, fever, wheezing, dyspnea, body pain and loss of appetite (9). Many researchers have reported that *O. americanum* has antioxidant (10), antibacterial (11), antifungal (12), antiviral (13), immuno-modulatory (14), anesthetic (15), larvicidal, mosquito-repellent (16) and hepatoprotective activity (17). All these bioactivities are due to the different phytochemicals in abundance.

Seven such phytochemicals (secondary metabolites) were selected for the present docking studies based on their percent composition. They are Eugenol (Phenylpropanoid), Methyl chavicol or Estragole (Phenylpropane), Linalool (monoterpene alcohol), 1,8-cineole (monoterpenoid), Alpha and Beta farnesene (Sesquiterpene) and Limonene (monoterpene). Besides, well known standard antiviral drugs namely Lopinavir and Ritonavir were also used in the study.

The virus particle adheres to the host cell through its spikes which are projecting on its outer surface. The spike protein attaches to the host cell membrane through a receptor mediated interaction with ACE2 (Angiotensin Converting Enzyme- 2) and gets entry into the host cell (18). Hence, the selected secondary metabolites of *O. americanum* and the antiviral drugs were docked with the target SARS-CoV-2 spike protein to identify the potential lead molecules for further drug discovery process.

MATERIALS AND METHODS

Software and program

The main software program used for the study is AutoDock Vina (The Scripps Research Institute, USA). The visualization of structure of ligands and modifications were performed using Discovery Studio Biovia 2017(Dassault Systèmes, USA). AutoDock Tools version 1.5.4 (ADT; Scripps Research Institute, USA) was used for the grid box size determination and for the preparation of PDBQT file format of the SARS-CoV-3-human ACE2 complex.

Preparation of ligand structures

Structures of all the secondary metabolites of *O. americanum* and the antiviral drugs, lopinavir and ritonavir (Table 1) were downloaded in the Spatial Data File (.SDF) file format from the PubChem Compound Database (NCBI; https://pubchem.ncbi.nlm.nih.gov/). The structures were transformed to the.PDB format using Discovery Studio Biovia 2017. For studying the ligand structures w.r.t combinations of non-polar hydrogens, rotatable bonds and Gasteiger changes addition, ADT was used. Finally, the ligands were then converted to PDBQT format thereby enabling its usage in AutoDock4 (AD4) and AutoDock Vina (19).

Preparation of Target - Spike protein

The structure (Fig.1) of the Spike protein- Human ACE2 complex of SARS-CoV-2 (PDB code: 6LZG) was downloaded from the protein data bank of RCSB (http://www.rcsb.org). After removal of water molecules, hydrogen polarities were assigned and then calculated gasteiger charges to protein structures using ADT software. Finally, the protein structure was converted to. PDBQT format thereby enabling use in AutoDock Vina (20).

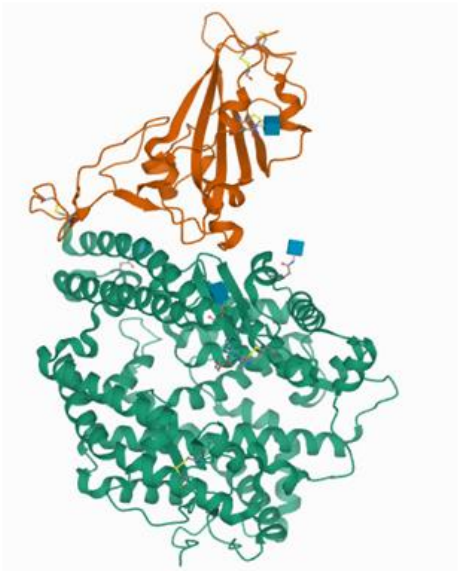
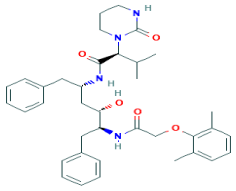
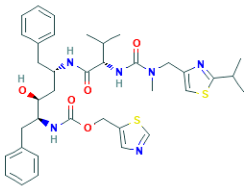
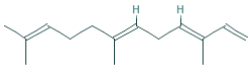
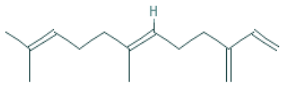
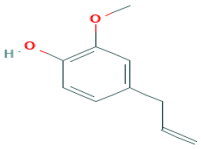
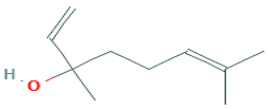
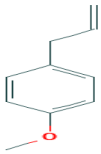
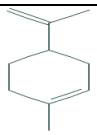
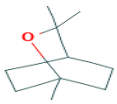


Fig. 1: SARS-CoV Spike Protein (PDB ID: 6LZG)

Docking Methodology

The AutoDock Vina program was used to study molecular docking. Ligands were docked individually to the receptor with grid coordinates and grid boxes of certain sizes. Polar hydrogen atoms and Kollman charges were added to the protein structure. The prepared file was saved in the .PDBQT format. Ligand-binding affinities were predicted as negative Gibbs free energy (ΔG) scores (kcal/mol) and this was calculated based on the AutoDock Vina scoring function (19). Post-docking analyses were visualized using Discovery Studio Biovia 2017, This may show the sizes and locations of ligand binding sites, hydrogen-bond interactions, hydrophobic interactions, and bonding distances. The binding poses of each ligand was observed and their interactions with the protein were characterized, and the best and most energetically favorable conformations of each ligand was selected.

Table 1: Structures and Pubchem CID of selected phytochemicals of *O.americanum*

Ligands	PubChem CID	Structure
Lopinavir	92727	
Ritonavir	392622	
Alpha Farnesene	5281516	
Beta Farnesene	5281517	
Eugenol	3314	
Linalool	6549	
Estragole	8815	
Limonene	22311	
1,8-cineole	2758	

RESULTS

In silico molecular docking studies

The results indicated strong interactions of the various secondary metabolites of *O. americanum* against Spike Protein-Human ACE2 complex of SARS-CoV-2. The result showed various modes of drug–protein interaction with docking score. The docking score is a measure of interaction of the target. The best mode of binding should be the most stable one for the ligand and it is the binding pose with least binding energy.

In the present study, various secondary metabolites of *O. americanum* namely Alpha- farnesene and Beta-farnesene, eugenol, linalool, estragole, limonene and 1,8-cineole were docked against the spike glycoprotein of SARS-CoV-2 and their docking energy was listed in Table 2. Besides the secondary metabolites, well known antiviral drugs like lopinavir and ritonavir were also docked with the protein of interest like a standard.

Table 2: Docking results of *O.americanum* secondary metabolites

Ligand Name	Pose energy (kcal/mol)	Interacting Amino acids
Lopinavir	-5.2	LYS 309, GLU 312, VAL 316, TRP 328, GLU 329
Ritonavir	-5.1	ARG 306, LYS 309, GLU 312, LYS 313, VAL 316, TRP328
Alpha-farnasene	-4.7	ARG 306, LYS 309, LYS 313
Beta-farnasene	-4.5	LYS 309, LYS 313, VAL 316
Eugenol	-4.3	GLU 312, ASN 322, MET 323
Linalool	-4.1	LYS 309, GLU 312, LYS 313
Estragole	-4	LYS 309, LYS 313, VAL 316
Limonene	-3.9	LYS 309, LYS 313
1,8- Cineole	-3.8	LYS 309, LYS 313

Lopinavir has docked with the spike glycoprotein with high negative binding energy of -5.2 Kcal/mol. Glu 312 of A chain of the spike protein formed a hydrogen bond with the ligand. Lys 309 and Glu 329 of A chain had pi-cation interactions with the ligand. Trp 328 was found to have Pi-Pi stacked interaction whereas Val 316 and Lys 309 were involved in Pi-alkyl interaction with the lopinavir (Fig. 2).

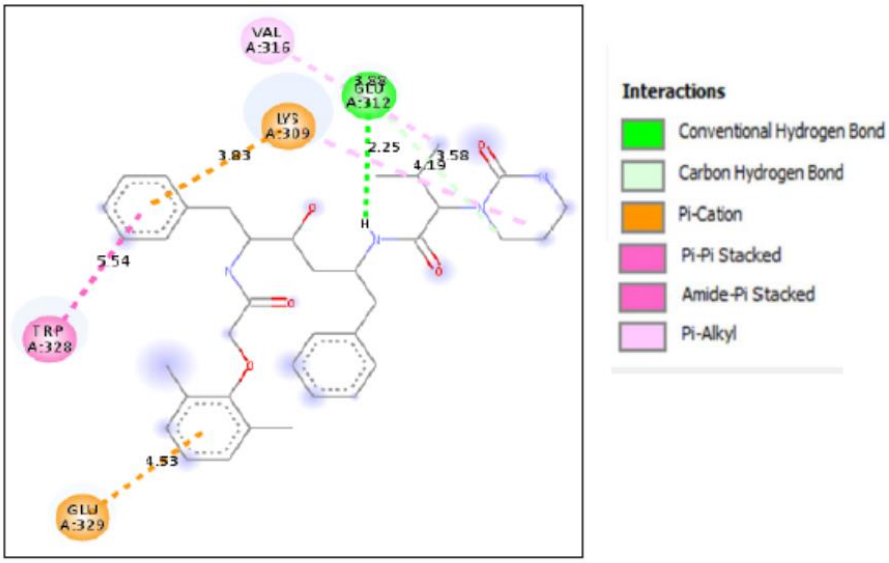


Fig. 2: Docking interaction of Spike protein (6LZG) with Lopinavir

The docking of ritonavir with the spike glycoprotein shows high affinity interaction with a binding energy of -5.1 Kcal/mol (Fig.3). The interaction involves two hydrogen bonding with Lys 309 and Arg 306 of A chain of Spike protein. The benzene ring shows pi-pi stacked interaction and amide–pi stacked interactions with Trp 328 & Glu 312 and Val 316 & Lys 313 respectively.

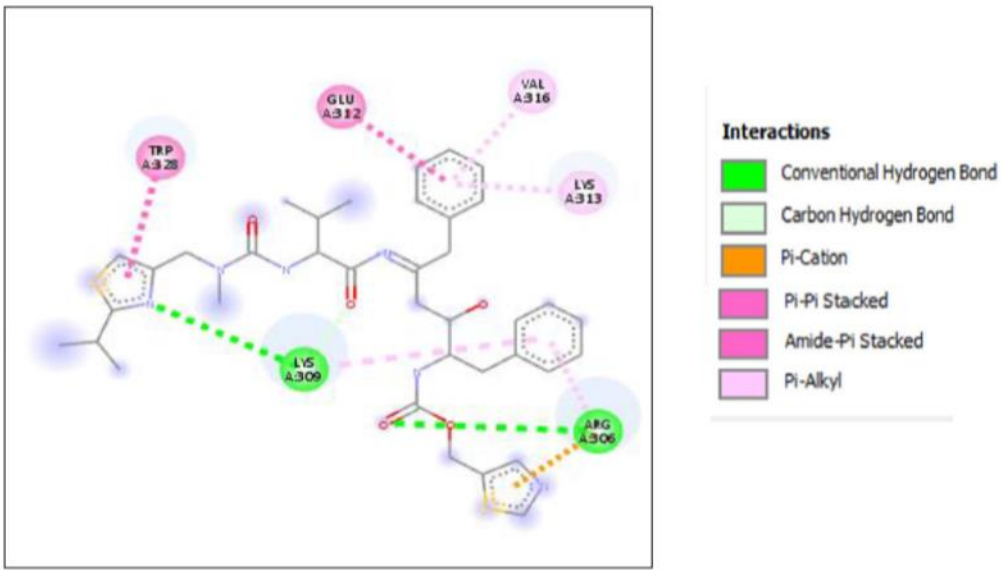


Fig. 3: Docking interaction of Spike protein (6LZG) with Ritonavir

Among seven secondary metabolites of *O. americanum*, alpha-farnesene docked with the target protein with a high binding affinity of -4.7 Kcal/mol. Though the docking energy was more negative, there was no hydrogen bonding involved in the docking interactions. Some of the alkyl interactions alone were found between the ligand and the Arg 306, Lys 309 and Lys 313 of A chain of the spike protein (Fig. 4).

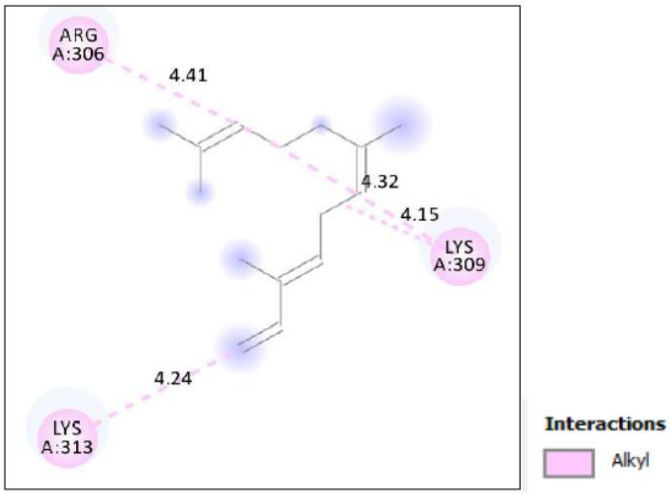


Fig. 4: Docking interaction of Spike protein (6LZG) with Alpha Farnesene

Fig. 5 shows the docking interaction of beta-farnesene with the target protein. The binding energy was found to be -4.5 Kcal/mol. Like alpha-farnesene, beta-farnesene also could not form any hydrogen bonding with the target protein. Only alkyl interactions were found with the Val 316, Lys 309 and Lys 313 of A chain of the spike protein.

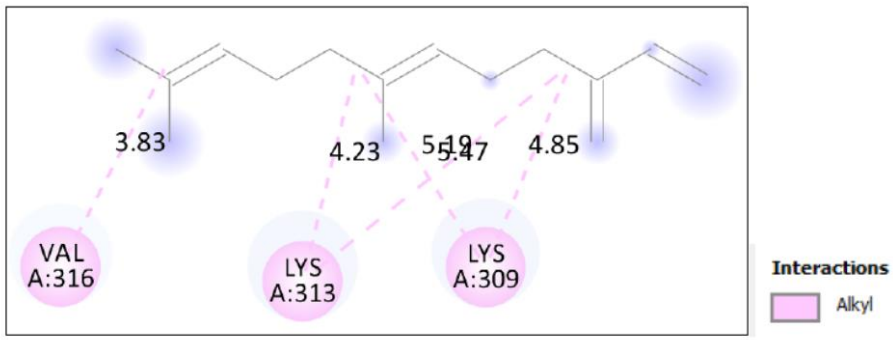


Fig. 5. Docking interaction of Spike protein (6LZG) with Beta Farnesene

Eugenol of *O. americanum* could dock with the A chain of SARS-CoV 2 spike protein with high affinity interactions and its energy was found to be -4.3 Kcal/mol. Significant binding was seen with three hydrogen bonds between hydrogen of hydroxyl group (attached to benzene ring) and Met 323 and Glu 312. The bond length was found to be 2.32 and 2.63 respectively. Eugenol also formed another hydrogen bonding with the oxygen of hydroxyl group (attached to benzene ring) and Asn 322 with a bond length of 3.18 (Fig. 6).

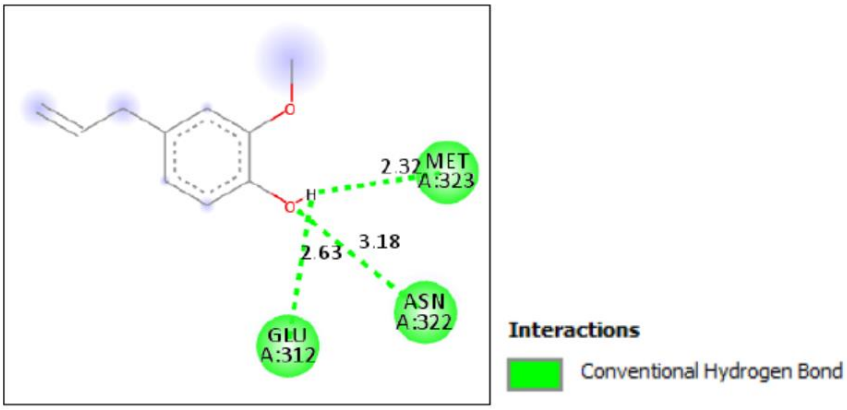


Fig. 6. Docking interaction of Spike protein (6LZG) with Eugenol

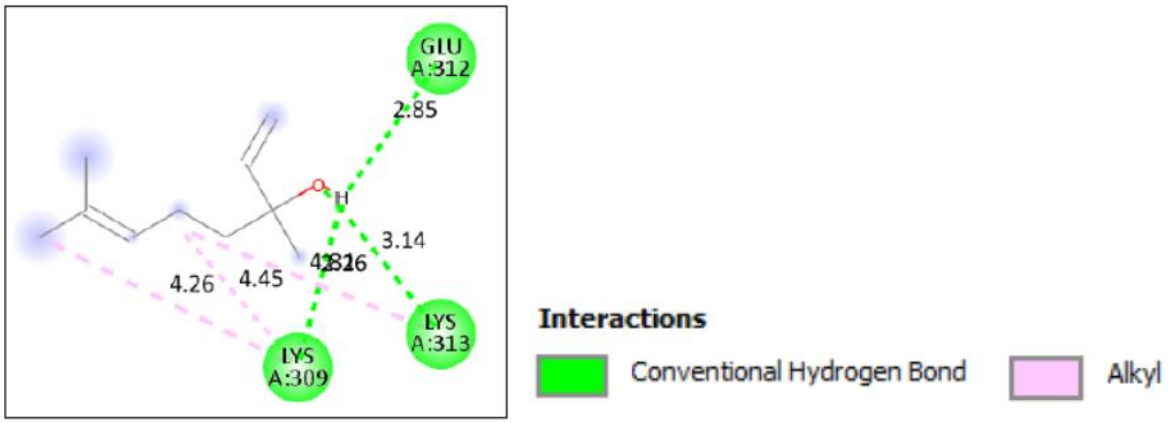


Fig. 7: Docking interaction of Spike protein (6LZG) with Linalool

Fig. 7 depicts the docking interaction of linalool with the A chain of SARS-CoV-2 spike protein. With three hydrogen bonding, linalool forms a high affinity interaction with a binding energy of -4.1 Kcal/mol. Two hydrogen bonds were formed between the hydrogen of hydroxyl group and Lys 309 & Glu 312. Another hydrogen bond was found between the oxygen of hydroxyl group and Lys 313. Few alkyl interactions were also observed between the ligand and Lys 309 and Lys 313.

Estragole binds to the A chain of the spike protein with a binding energy of -4 Kcal/mol. There was no hydrogen bonding observed between the benzene ring and the Val 316 of estragole. In addition, alkyl interactions were also found between Lys 309 and Lys 313 of spike protein and the ligand (Fig. 8). Figs. 9 and 10 show the docking interactions of limonene and 1,8-cineole respectively. Limonene binds with the A chain of the target protein with a binding affinity of –3.9 Kcal/mol. It binds by alkyl interactions with the Lys 309 and Lys 313 of the target protein. 1,8-cineole docks with the A chain of the spike protein through four alkyl interactions with Lys 309 and Lys 313 with a binding affinity of -3.8 Kcal/mol.

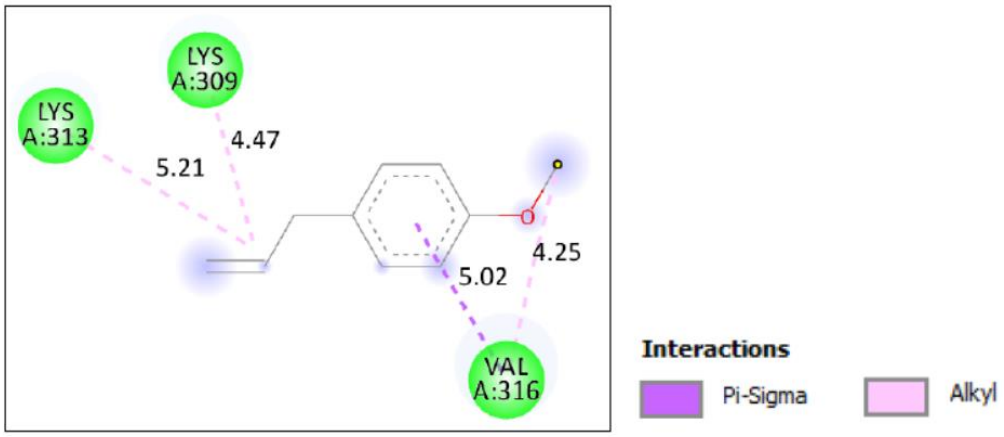


Fig. 8: Docking interaction of Spike protein (6LZG) with Estragole

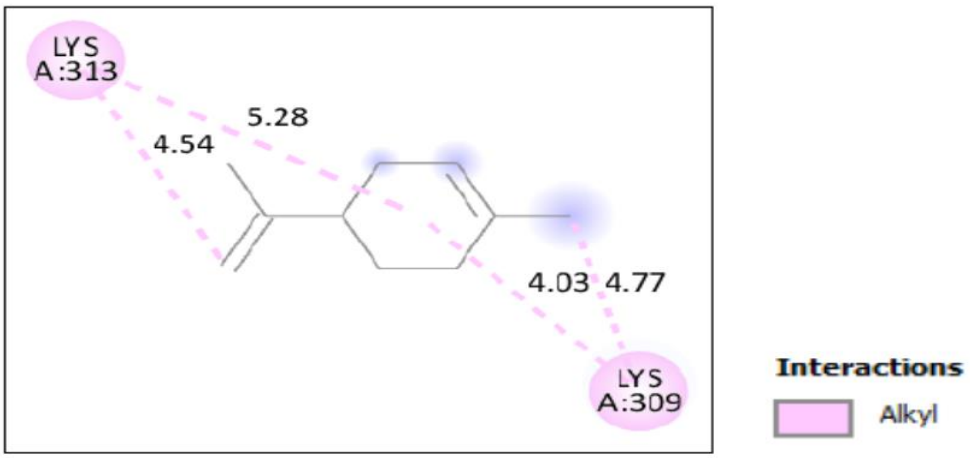


Fig. 9: Docking interaction of Spike protein (6LZG) with Limonene

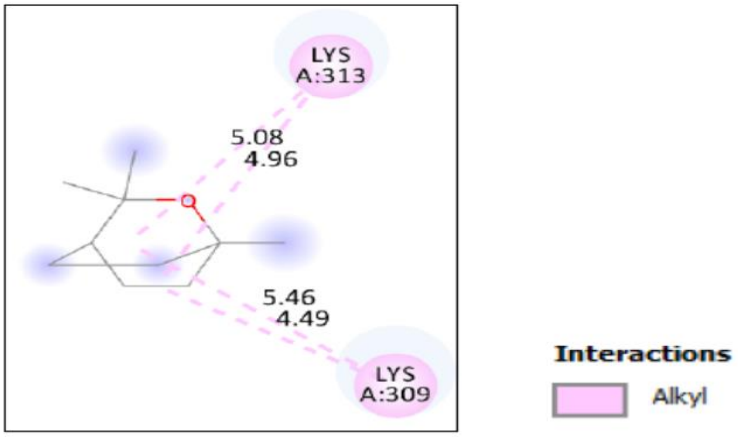


Fig.10: Docking interaction of Spike protein (6LZG) with 1,8-cineole

In silico prediction of ADME properties

Many *in silico* tools are available online to predict the ADME parameters of the lead molecules from their molecular structure (21). Swiss ADME tool is one of the freely accessible online tools for ADME predictions. Lipinski's Rule of 5 (RO5) plays a very important criterion in the prediction of drug likeness of any lead molecule. RO5 states that i) Molecular weight of a ligand should be less than or equal to 500 (MW< 500) ii) the compound's lipophilicity, C log P should be lesser than or equal to (C log P< 5) iii) the number of hydrogen bond donors should be less than (HBD<5) iv) Number of hydrogen bond acceptors should be less than 10 (HBA< 10) and v) Polar surface area (PSA)<140 (22). If any of the lead molecule violates more than 3 descriptors that molecule will not obey the criteria of drug likeness and hence it is not considered for further drug discovery process.

Table 3 depicts the predicted physicochemical properties of both the antiviral drugs (lopinavir and ritonavir) and the secondary metabolites of *O. americanum*. Almost all the compounds were in the acceptable range of Lipinski's R05 indicating their potential for use as drug-like molecules.

Table 4 depicts the drug likeliness of all the ligands based on the rules of Lipinski, Ghose, Veber, Egan and Muegge. Almost all the ligands obeyed all the rules, and few violations were also observed for certain ligands. Appreciable bioavailability score and synthetic accessibility score of all the ligands also makes them considerable for further drug discovery procedure.

Table 3: Physicochemical properties of selected ligands of *O.americanum* using Swiss ADME tool

Ligands	Formula	MW	# H-bond acceptors	# H-bond donors	MR	TPSA	Consensus Log P	Water Solubility
Lopinavir	C37H48N4O5	628.8	5	4	187.92	120	4.37	Poorly soluble
Ritonavir	C37H48N6O5S2	720.94	7	4	197.82	202.26	5.04	Insoluble
Alpha Farnesene	C15H24	204.35	0	0	72.32	0	4.96	Moderately soluble
Beta Farnesene	C15H24	204.35	0	0	72.32	0	4.97	Moderately soluble
Eugenol	C10H12O2	164.2	2	1	49.06	29.46	2.25	Soluble
Linalool	C10H18O	154.25	1	1	50.44	20.23	2.66	Soluble
Estragole	C10H12O	148.2	1	0	47.04	9.23	2.78	Soluble
Limonene	C10H16	136.23	0	0	47.12	0	3.37	Soluble
1,8-cineole	C10H18O	154.25	1	0	47.12	9.23	2.67	Soluble

Table 4: Predicted drug-likeliness of selected ligands of *O.americanum* using Swiss ADME tool

Ligands	Lipinski #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	Bioavail ability Score	Lead likeness #violations	Synthetic Accessibility
Lopinavir	1	3	1	0	3	0.55	3	5.67
Ritonavir	2	4	2	1	4	0.17	3	6.45
Alpha Farnesene	1	0	0	0	2	0.55	2	3.72
Beta Farnesene	1	0	0	0	2	0.55	2	3.42
Eugenol	0	0	0	0	1	0.55	1	1.58
Linalool	0	1	0	0	2	0.55	1	2.74
Estragole	0	1	0	0	2	0.55	1	1.28
Limonene	0	1	0	0	2	0.55	2	3.46
1,8-cineole	0	1	0	0	2	0.55	1	3.65

Table 5: Predicted pharmacokinetics of selected ligands of *O.americanum* using Swiss ADME tool

Ligands	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s) Skin permeability
Lopinavir	High	No	Yes	No	Yes	No	No	Yes	-5.93
Ritonavir	Low	No	Yes	No	No	No	No	Yes	-6.4
Alpha Farnesene	Low	No	No	Yes	No	Yes	No	No	-3.2
Beta Farnesene	Low	No	No	Yes	No	Yes	No	No	-3.27
Eugenol	High	Yes	No	Yes	No	No	No	No	-5.69
Linalool	High	Yes	No	No	No	No	No	No	-5.13
Estragole	High	Yes	No	Yes	No	No	No	No	-4.81
Limonene	Low	Yes	No	No	No	Yes	No	No	-3.89
1,8-cineole	High	Yes	No	No	No	No	No	No	-5.3

Table 5 shows the predicted pharmacokinetic properties of secondary metabolites of *O. americanum* and of the antiviral drugs, lopinavir and ritonavir. Molecules like eugenol, linalool, estragole, 1,8-cineole and lopinavir have high GI absorption. All the selected ligands are non-substrates of Permeability glycoprotein (P-gp) and hence all the ligands possess BBB permeability. Most of the *O. americanum* ligands are not the inhibitors of CYP isoenzymes and hence they may be cleared properly through various metabolic biotransformation. Only a few ligands were inhibitors of CYP isoenzymes but not for all the types of isoforms.

The bioavailability score of the selected ligands were predicted using the Molinspiration tool and the scores were depicted in table 6. From the table it was observed that the target of lopinavir and ritonavir were GPCR ligands and protease inhibitor respectively. The target of alpha-farnesene would be an ion channel modulator whereas beta farnesene would be a nuclear receptor ligand.

Linalool and 1,8-cineole targets might be an active ion channel modulator wherein estragole could be an enzyme inhibitor and the limonene could be an enzyme inhibitor or ion channel modulator else nuclear receptor ligand. The bioactivity of eugenol might be an ion channel modulator or enzyme inhibitor.

Table 6: Calculated bioactivity scores of selected ligands of *O.americanum* using Molinspiration tool

Ligands	GPCR ligand	Ion channel modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
Lopinavir	0.04	-0.78	-0.55	-0.66	0.42	-0.37
Ritonavir	-0.33	-1.41	-1.02	-1.41	0.35	-0.74
Alpha Farnesene	-0.30	0.14	-0.62	0.17	-0.63	0.45
Beta Farnesene	-0.44	-0.05	-0.77	0.07	-0.68	0.27
Eugenol	-0.86	-0.36	-1.14	-0.78	-1.29	-0.41
Linalool	-0.73	0.07	-1.26	-0.06	-0.94	0.07
Estragole	-1.06	-0.51	-1.40	-1.03	-1.39	-0.62
Limonene	-0.91	-0.27	-2.01	-0.34	-1.38	-0.21
1,8-cineole	-0.93	0.01	-1.60	-1.07	-0.90	-0.15

DISCUSSION

In silico Molecular docking studies

Molecular docking is a virtual screening approach which has become the most preferable tool for the drug discovery process. The reason behind this is time and cost for *silico* drug studies in comparison to laboratory experiments. Ligand-Protein binding is possible only when the free energy change is negative, that is when the ΔG is low in the system. A negative docking score specifies the stability of the resulting complexes that is a characteristic for any efficacious drugs. Besides binding energy, molecular interactions like hydrogen bond, hydrophobic and electrostatic interactions are also suggestive of ligand docking under favorable conformations (23).

Hydrophobic interactions are key to the stability of the proteins. Though protein stability is also determined by hydrogen bonding, even in the smallest globular proteins the stability is determined by hydrophobic interactions (23). Hence, in many native proteins, folding configuration is mainly determined by hydrophobic binding. The result of the present study shows the involvement of hydrogen bonds, hydrophobic (pi-pi stacked, amide-pi stacked and pi-alkyl) and electrostatic (pi-cation) bonds that are mediated by different amino acids in each ligand and protein interaction. Most of the secondary metabolites namely alpha-farnesene, beta-farnesene, linalool, estragole, limonene and 1,8-cineole have formed hydrophobic interactions with Lys 309 and Lys 313. The same amino acids were also involved in the hydrogen bonding with Linalool. No electrostatic

interactions were observed between the protein and the secondary metabolites of *O. americanum*. Many researchers have reported the antiviral activity of *O. americanum*. In the traditional Siddha system of medicine also the whole plant decoction of *O. americanum* is prescribed for various respiratory disorders. Thus, in keeping with these findings, the present study shows that the secondary metabolites of *O. americanum* have potential bioactivity against spike protein of SARS-CoV-2.

In silico prediction of ADME properties

In silico tools like Swiss- ADME and Molinspiration were used to predict the ADME properties and bioactivity of *O. americanum* ligands respectively. Almost all the ligands possess the drug likeness properties demonstrating their potential for use as drug like molecules. Though few violations were observed, that is also under acceptable terms only. A compound being a substrate or non-substrate of the permeability glycoprotein (P-gp) determines the active outflow through biological membranes. The most vital role of P-gp is to protect the central nervous system from xenobiotics. If any drug is a substrate for P-gp, then it does not exhibit BBB permeability (24). Interestingly, all the selected ligands were non-substrate of P-gp and thus they exhibit BBB permeability.

The Cytochrome P450 (CYP) family of isoenzymes is a significant player in any drug elimination process through metabolic biotransformation. About 50 to 90% of drug molecules are substrates of 5 major isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2DC and CYP3A4). Inhibition of these isoenzyme forms is one of the major causes of pharmacokinetic related drug-drug interactions (25), which leads to toxic and adverse effects due to minimal clearance and accumulation of the drug or its metabolites at physiological conditions. Most of the selected ligands are not the CYP isoforms inhibitor and thus they might be easily cleared off from the system through appropriate metabolic reactions. Though few ligands are predicted to be an inhibitor of some CYP isoforms, it might be eliminated by other isoenzymes of CYP leaving no toxicity inside the biological system. The bioactivity towards GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets were predicted by the Molinspiration tool. The larger the value of the score, the greater the probability that the ligand will be active. Almost all the ligands have potential bioactivity and thus it can be confirmed that the secondary metabolites of *O. americanum* could be effective towards binding to SARS-CoV-2 spike protein to exhibit its activity.

Linalool and 1,8-cineole targets might be an active ion channel modulator wherein estragole could be an enzyme inhibitor and the limonene could be an enzyme inhibitor or ion channel modulator else nuclear receptor ligand. The bioactivity of eugenol might be an ion channel modulator or enzyme inhibitor.

CONCLUSION

SARS-COV-2 is an infectious and communicable disease that is threatening humankind globally. The impact of this pandemic situation has compelled the researchers' worldwide to find a cure at the shortest possible time. Docking studies carried out with natural products will allow the planning of *in vitro* studies with the cell lines in an effective way. This study is one such attempt to understand the interaction of the phytochemicals present in *O. americanum* against the spike protein of SARS-COV-2. The results indicated have shown that the phytochemicals show a strong binding to the spike protein. However, further studies are required at the pre-clinical and clinical level to confirm the result and to identify the exact mechanism of action of the secondary metabolites of *O. americanum* against SARS-COV-2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Cai, G., Cui, X., Zhu, X., Zhou, J. A Hint on the COVID-19 Risk: Population Disparities in Gene Expression of Three Receptors of SARS-CoV. Preprints 2020 doi:10.20944/ preprints202002. 0408.v1.
2. Chen, Y., Liu, Q., Guo, D. Emerging Coronaviruses: genome structure, replication, and pathogenesis. J Med Virol. 2020; 92(4):418-423.
3. World Health Organization (WHO), "Coronavirus disease 2019 (COVID-19) Situation Report – 58, 18 March. pp. 1–9, 2020. Available at https://www.who.int/emergencies/ diseases/novel-coronavirus-2019/situation-reports.
4. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., *et al*. China Novel Coronavirus I., Research T. A Novel Coronavirus from patients with pneumonia in China 2019. N Engl J Med. 2020; 382: 727-733.
5. Chan, J.F., Yuan, S., Kok, K.H., To, K.K., Chu, H., Yang, J., *et al*. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 2020; 395: 514-523.
6. Vardhan, S., Suban K. Sahoo. Searching inhibitors for three important proteins of COVID-19 through molecular docking studies. arXiv preprint arXiv:2004.08095. 2020 Apr 17.
7. Hall, D.C. Jr, Ji, H.F. A search for medications to treat COVID-19 via *in silico* molecular docking models of the SARS-CoV-2 spike glycoprotein and 3CL protease. Travel Med Infect Dis. 2020 May-Jun; 35: 101646. doi: 10.1016/j.tmaid.2020.101646.
8. Reena Parida, I. Sriram Sandeep, Bijay Kumar Sethy, Santilata Sahoo, Sujata Mohanty. Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oil from Lime Basil (*Ocimum americanum*): A Potent Source for Natural Antioxidant. Int J Pharm Pharm Sci. 2014; 6(7): 487-490.
9. Murugesa K.S. Mudaliyar. Gunapadam Mooligai Vaguppu. 2nd ed., Part I. Chennai: Department of Indian Medicine and Homeopathy; 2002. p. 421-423.

10. Karau, G.M., Njagi, E.N.M., Machocho, A.K., Wangai, L.N., Thinga, M.J. Chemical composition and *in vitro* antioxidant activities of *Ocimum americanum.* Advances in Analytical Chemistry. 2015; 5(2): 42-49.
11. Oyedemi, S.O., Oyedemi, B.O., Coopoosamy, R.M., Prieto, J.M., Stapleton, P., Gibbons, S. Antibacterial and norfloxacin potentiation activities of *Ocimum americanum* L. against methicillin resistant *Staphylococcus aureus*. South African Journal of Botany. 2017; 109: 308-314.
12. Sroisiri Thaweboon, Boonyanit Thaweboon. *In vitro* Antimicrobial activity of *Ocimum americanum* L. essential oil against oral microorganisms. Southeast Asian J Trop Med Public Health. 2009; 40(5): 1025-1033.
13. Yucharoen,R., Anuchapreeda,S., Tragoolpua, Y. Anti-herpes simplex virus activity of extracts from the culinary herbs *Ocimum sanctum* L., *Ocimum basilicum* L. and *Ocimum americanum* L. African Journal of Biotechnology. 2011; 10(5): 860-866.
14. Sunitha, K., Nasreen Begum. Immunomodulatory activity of methanolic extract of *Ocimum Americanum* seeds. IJRPC. 2013; 3(1):95-98.
15. de Lima Silva,L, Garlet,Q.L., Koakoski, G., de Abreu, S., Mallmann,C. A., Baldisserotto, B. *et al*. Anesthetic activity of the essential oil of *Ocimum americanum* in Rhamdia quelen (Quoy & Gaimard, 1824) and its effects on stress parameters. Neotropical Ichthyology. 2015;13(4): 715-722.
16. Kazembe, T.C., Chaibva, M. Mosquito repellency of whole extracts and volatile oils of *Ocimum americanum*, *Jatropha curcas* and *Citrus limon*. Bulletin of Environment Pharmacology and Life sciences. 2012; 1(8): 64-71.
17. Aluko, B.T, Oloyede, O.I., Afolayan, A.J. Hepatoprotective activity of *Ocimum americanum* L leaves against paracetamol – induced liver damage in rats. American Journal of Life Sciences. 2013; 1(2): 37-42.
18. Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., *et al*. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020; 63(3): 457- 460.
19. Trott, O., Olson, A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading J Comput Chem. 2010; 31: 455-461.
20. Morris, G., Huey, R. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem. 2009; 30: 2785-2791.
21. Tian, S., Wang, J., Li, Y., Li, D., Xu, L., Hou, T. The application of *in silico* drug-likeness predictions in pharmaceutical research. Adv Drug Deliv Rev 2015; 86: 2-10.
22. Veber, D.F., Johnson, S.R., Cheng, H.Y., Smith, B.R., Ward, K.W., Kopple, K.D. Molecular properties that influence the oral bioavailability of drugs. J Med Chem. 2002; 45: 2615-2623.
23. Afriza, D., Suriyah, W.H., Ichwan, S.J.A. *In silico* analysis of molecular interactions between the anti-apoptotic protein survivin and dentatin, nordentatin, and quercetin. J Phys Conf Ser 2018 1073 032001. doi:10.1088/1742-6596/1073/3/032001.
24. Van Waterschoot, R.A.B., Schinkel, A.H.A. Critical analysis of the interplay between cytochrome P450 3A and P-glycoprotein: recent insights from knockout and transgenic mice. Pharmacological Reviews. 2011; 63: 390–410.
25. Huang, S.M., Strong, J.M., Zhang, L., Reynolds, K.S., Nallani, S., Temple. R., *et al*. New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. J Clin Pharmacol. 2008; 48(6): 662-670.