PalArch's Journal of Archaeology of Egypt / Egyptology

IN SILICO APPROACHES ON PHYTOCHEMICAL COMPONENTS OF *CITRUS LIMETTA* RISSO FOR THEIR SARS-COV-2 INHIBITORY ACTION

V. Arun Nivas[a1], R. Senthamarai[a2], A. M. Ismail[b], T Shri Vijaya Kirubha[a3], S. Shakila Banu[c]*

[a1,2,3] Department of Pharmacognosy, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamil Nadu, India.

[b] Department of Pharmacy Practice, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamil Nadu, India.

[c] Department of Pharmaceutical Chemistry, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamil Nadu, India.

*corresponding author: **V. Arun Nivas**,
Department of Pharmacognosy,
Periyar College of Pharmaceutical Sciences,
Tiruchirappalli, Tamil Nadu, India.

V. Arun Nivas[a1]*, R. Senthamarai[a2], A. M. Ismail[b], T Shri Vijaya Kirubha[a3], S. Shakila Banu[c], *in silico* approaches on phytochemical components of *citrus limetta* risso for their sars-cov-2 inhibitory action-- Palarch's Journal Of Archaeology Of Egypt/Egyptology 18(1). ISSN 1567-214x

Abstract:

The Novel Corona virus (Covid-19) belongs to a family of viruses called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that is currently challenging the global health. The first case of Covid-19 was reported and confirmed in Wuhan, China on December 31, 2019 and on January 30, 2020, the WHO (World Health Organisation) declared the outbreak as a public health emergency of international concern and on March 12 announced as pandemic due to its wide spread. Various treatment and management have been in progress on wide ranges. Molecular docking is a virtual screening method that cutback the expenses and time duration for identification of pharmacophore from natural products. This *in silico* study has been deployed to screen the phytochemical components D-limonene, α-pinene, β-pinene, Camphene, present in *Citrus limetta* Risso (Rutaceae) against the protein target of Corona virus, including SARS CoV-2 main protease, RNA-dependent RNA-polymerase and Spike receptor binding domain with the aid of AutoDock Vina software. The results obtained after docking showed a good binding affinity and implicated that the active phytoconstituents of the selected plant would be a supportive measure for the management of this pandemic disease upon further investigation.

Keywords: Covid-19*, In silico, Citrus limetta,* AutoDock Vina, Binding energy

1. Introduction:

In this modern era, the novel strain of corona virus (COVID-19) has ravaged the whole world. COVID-19 which belongs to a family of viruses called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is causing various symptoms such as pneumonia, fever, breathing difficulty and lung dysfunction. The first case of Covid-19 was reported and confirmed in Wuhan, China on December 31, 2019 and on January 30, 2020, the WHO (World Health Organisation) declared the outbreak as a public health emergency of international concern and in March 12 announced it as pandemic due to its wide spread. Various treatment and management have been given in wide regions by performing various clinical and preclinical testing.[1,2,3,4] One of such management has been focussed towards the *in silico* approach on phytochemical components present in the natural and traditional herbs.[5] The Citrus species are inherent source of valuable essential oil which might possess various pharmacological actions. One such species is *Citrus limetta* Risso of Rutaceae family. It is commonly known as Mosambi (sweet lime) that is rich in vitamin C. The essential oil from the leaves and fruits consists of various phytoconstituents including D-limonene, α-pinene, β-pinene, β-Myrcene, Neral, Citronellal, Camphene, etc.[6,7,8] These constituents are reported to possess Antioxidant, Anticancer, Antibacterial and Antiviral properties and also serve as Immune boosters.[9,10,11] The current study is aimed to explore the antiviral potential of components namely D-limonene, α-pinene, β-pinene and Camphene against SARS-CoV-2 main protease, Spike receptor binding domain and RNA-dependent RNA-polymerase (RdRp) by adopting computational (*in silico*) approaches. This study revealed the competence of *Citrus limetta* Risso for the treatment of SARS-CoV-2.

2. Materials and Methods:

2.1 Drug likeness Properties and ADME Screening of Phytoconstituents

Since most of the herbal medicines are taken orally, an *in silico* combining model of absorption, distribution, metabolism and excretion (ADME) was used to screen the phytoconstituents that are biologically active by oral administration. A free web tool SWISS ADME predictor was used to evaluate the pharmacokinetics, drug likeness and medicinal chemistry friendliness of phytoconstituents under investigation. The properties like molecular weight less than 500g/mol, less than 5 numbers of hydrogen bond donors, less than 10 numbers of hydrogen bond acceptors and less

than10 rotatable bonds were chosen as criteria to satisfy Drug likeness property. The search engine further gave a compiled result on lipophilicity and hydrophilicity of these molecules by integrating results obtained from various Log P and S prediction programmes called ILOGP, XLOGP3, WLOGP, ESOL and SILICOS-IT. Log P, a measure of lipophilicity of the molecule is the logarithm of the ratio of the concentration of drug substance between two solvents in an unionized form. Lipinski rule prescribes an upper limit of 5 for druggable compounds. The lower the log P values, the stronger the lipophilicity for the chemical substance. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. On the other side, low water solubility goes along with a bad absorption, and therefore, the general aim is to avoid poorly soluble compounds. 'Log S' is a unit of expressing solubility in itself, which is the 10-based logarithm of the solubility measured in mol/L. Distribution of LogS in traded drugs reveals a value somewhere between -1 to -4, will be optimum for better absorption and distribution of drugs in the body.

2.2 Retrieval and Preparation of Protein Structures:

The three-dimensional/crystal structures of protein targets from SARS-CoV-2 were obtained from Protein Data Bank. Targets chosen for this study included SARS-CoV–2 Main Protease (PDB ID: 1Q2W), RNA-dependent RNA-polymerase (RdRp) (PDB ID: 6M71) and Spike receptor binding domain (PDB ID: 6M0J). The water molecules, cofactors and other ligands were removed from SARS-CoV–2 Main Protease (PDB ID: 1Q2W), RNA-dependent RNA-polymerase (RdRp) (PDB ID: 6M71) and Spike receptor binding domain (PDB ID: 6M0J) through Molegro molecular viewer and were used for molecular docking studies.

2.3 Preparation of Ligands for Docking:

The 3D structures of chemical constituents from *Citrus limetta* viz., D-limonene, α-pinene, β-pinene, Camphene and standard drugs such as Hydroxychloroquine (HCQ), Remdesivir were retrieved from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and converted into PDB format using BOVIA Discovery studio Visualiser 2016. Energy minimization was done using Open babel version 2.4.1.

Figure 1 Fruits, leaves and Whole plant of *C. limetta*

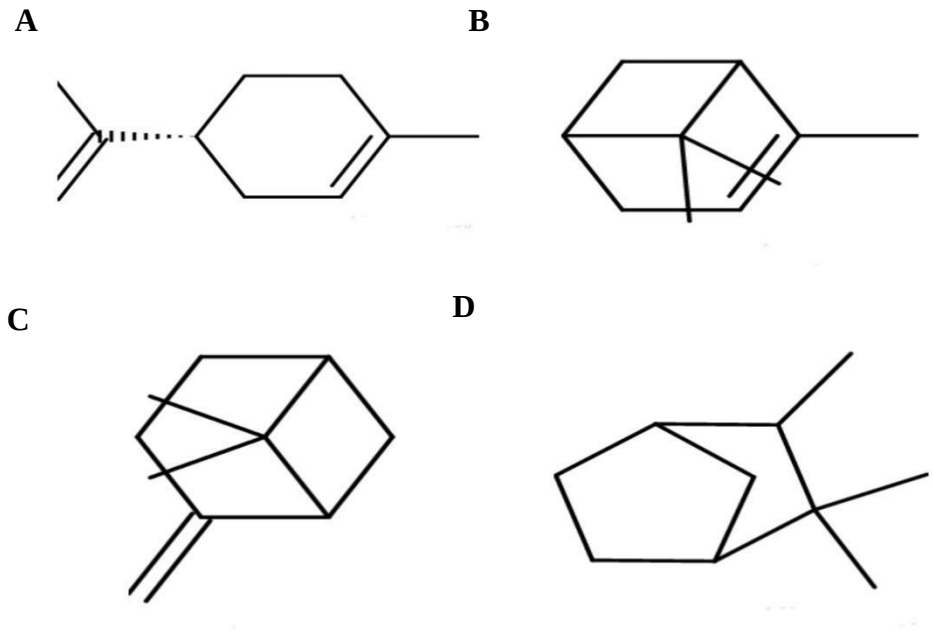

Figure 2 (A) D- limonene (B) α-pinene (C) β-pinene (D) Camphene

2.4 Detection of binding site and validation:

The interaction between the amino acids and ligands are considered as the active binding sites in the main protease. For the grid generation of spike protein, the interface residues of receptor binding domain with angiotensin converting enzyme 2 were selected, and for the RNA- dependent RNA-polymerase the binding sites were predicted using CASTp (Table 1). The size and location of binding sites were visualized and validated.

Table 1: Amino acid residues in the binding site

Target protein	Binding site residues
SARS-CoV-2 main protease	CYS145, THR25, THR26, HIS172, SER144, GLY143, ASN142, HIS163, GLU166, PHE140, HIS164, LEU141, ARG188, MET49, HIS141, GLN189, ASP187, PRO168, ALA191, THR190, GLN192, LEU167, MET165, GLY109, GLU240, PRO108, PRO132, ILE200, LEU202, HIS246
RNA-dependent RNA-polymerase	TRP509, LEU371, PHE368, ALA375, LEU372, TYR515, PHE506
Spike receptor binding domain	GLU435, GLU430, THR434, PHE428, ASN290, ILE291, PRO289, PRO415, THR414, LYS541, HIS540, PHE438

2.4 Molecular docking Analysis

Binding mode and interaction of SARS-CoV-2 Main Protease (PDB ID: 1Q2W), RNA-dependent RNA-polymerase (RdRp) (PDB ID: 6M71) and Spike receptor binding domain (PDB ID: 6M0J) with individual bioactive constituents of *Citrus limetta* and Standard drug (Hydroxychloroquine and Remdesivir) was performed using AutoDock Vina software. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. The protein was loaded in PyRx software, creating a PDBQT file that contains a protein structure with hydrogens in all polar residues. All bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The docking site on protein target was defined by establishing a grid box with the dimensions of X: 38.0729 Y: 33.3208 Z: 25.0000 Å, with a grid spacing of 0.375 Å, centered on X: 20.2892 Y: 10.3219 Z: 32.3218 Å. The best conformation was chosen with the lowest docked energy, after the docking search was completed. Eight runs with AutoDock Vina were performed in all cases per each ligand structure, and for each run the best

pose was saved. The binding affinity for best poses was taken as the final affinity value. The interactions of SARS-CoV–2 Main Protease (PDB ID: 1Q2W), RNA-dependent RNA-polymerase (RdRp) (PDB ID: 6M71) and Spike receptor binding domain (PDB ID: 6M0J with the ligand conformations, including hydrogen bonds and various other bonds were analyzed using BIOVIA Discovery Studio 2016.[13]

3. RESULTS AND DISCUSSION:

The results obtained from SWISS ADME predictor clearly indicated that the value of Log P, molar refractivity and the total polar surface area in these phytoconstituents were in excellent agreement with the Lipinski's rule of drug likeness. The Phytoconstituents also showed good hydrophilic-lipophilic balance, good bioavailability and a decent GI absorption.

Table 2: characteristic features of ligands

Compound	Molecular weight (g/mol)	No. of hydrogen bond donors	No. of hydrogen bond acceptors	No. of rotatable bonds	Total Polar Surface Area (A°)	Log P (iLOGP)	Log S (ESOL)
D-limonene	136.23	0	0	0	0.00	2.63	-3.51
α-pinene	136.23	0	0	0	0.00	2.59	-3.31
β-pinene	136.23	0	0	0	0.00	2.58	-3.34
Camphene	136.23	0	0	0	0.00	2.72	-3.50

Molecular docking of target proteins namely main protease, spike protein and RNA-dependent RNA polymerase was carried out with phytoconstituents using Autodock Vina software. Phytoconstituents from *Citrus limetta* Risso along with currently used drug like hydroxychloroquine and Remdesivir for the COVID-19 treatment were prepared for docking. Analysis of molecular docking revealed D-Limonene, a major phytoconstituents from *Citrus limetta* Risso to be the most promising inhibitor of targets in SAR-CoV-2. D-Limonene displayed strong binding with main protease having binding energy of -5.2 kcal/mol, -5.4 kcal/mol with RNA dependent RNA polymerase and -7.1 kcal/mol with Spike receptor binding domain. To obtain deeper insight into interaction pattern of D-Limonene with protein targets, protein – ligand interaction was plotted and key amino acid residues involved in interaction were identified. Analysis of interaction interface in the active site showed

Pi-Alkyl formation of the D-Limonene with PRO 108, PRO 132, ILE 200, LEU 202 and HIS 246 with main protease. D-Limonene also showed Van der Waals interaction with GLU 240 and GLY 109 with main protease (Figure 3). In the RdRp-D-Limonene docked complex, strong Pi-Alkyl interaction were formed by residue PHE 368, LEU 371, LEU 372, ALA 375, PHE 506, TYR 515 and amino acid residue TRP 509 was observed to be involved in Pi-Sigma interaction with D-Limonene (Figure 4). Strong Pi-Alkyl interaction were seen in Spike protein-D-Limonene complex with amino acids ILE 291, PRO 415, PHE 438, HIS 540 and HIS 540 (Figure 5). The previous study reported that several phytocompounds of *Citrus limetta* Risso possess antiviral activities especially D-Limonene possess antiviral activities for influenza and herpes virus. [14, 15] The results also implicated that other phytoconstituents α-pinene, β-pinene and Camphene also showed significant interaction main protease (-4.6,-4.5 and -4.7 kcal/mol respectively), with RNA-dependent RNA-polymerase (-5.4,-5.4 and -5.5 kcal/mol respectively) and with Spike protein (-5.0,-4.8 and -4.8 kcal/mol). Also, the binding energies of bioactive compounds were nearly to that of standard drugs. The above results implicate the antiviral activity of medicinal plants that can aid in the treatment of COVID-19 infection.

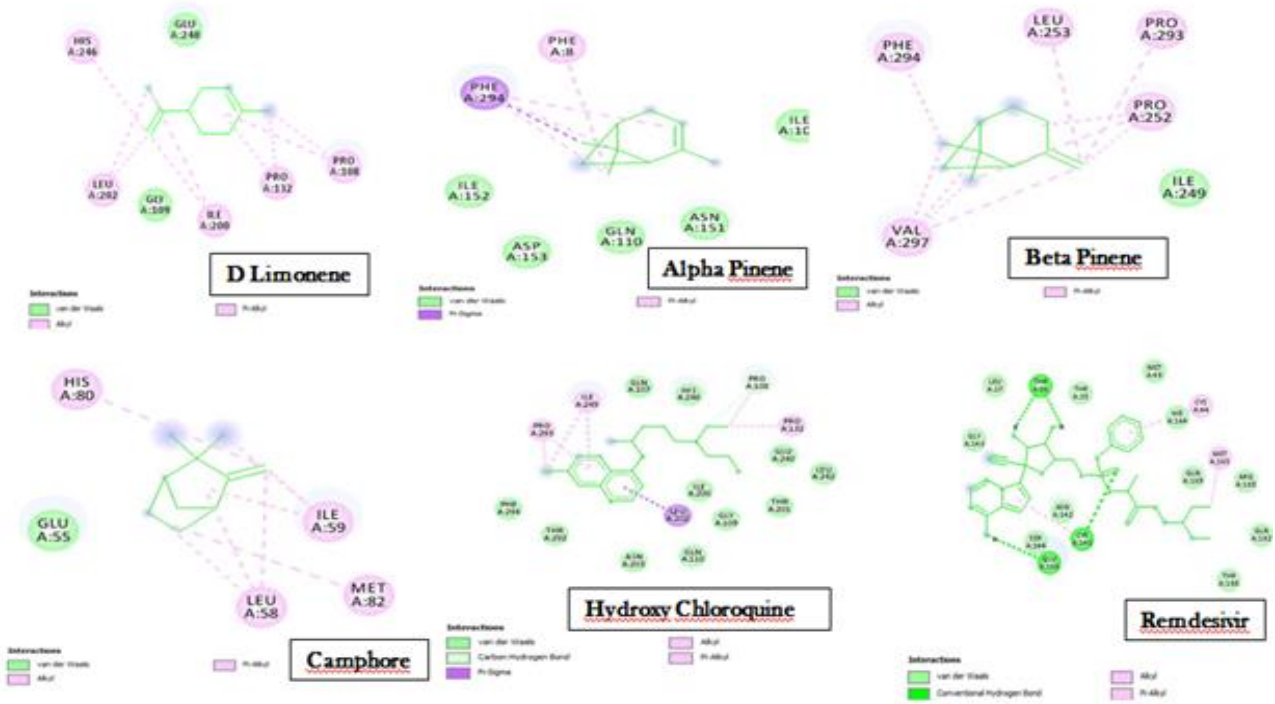

Figure 3 ligand interactions with 1Q2W

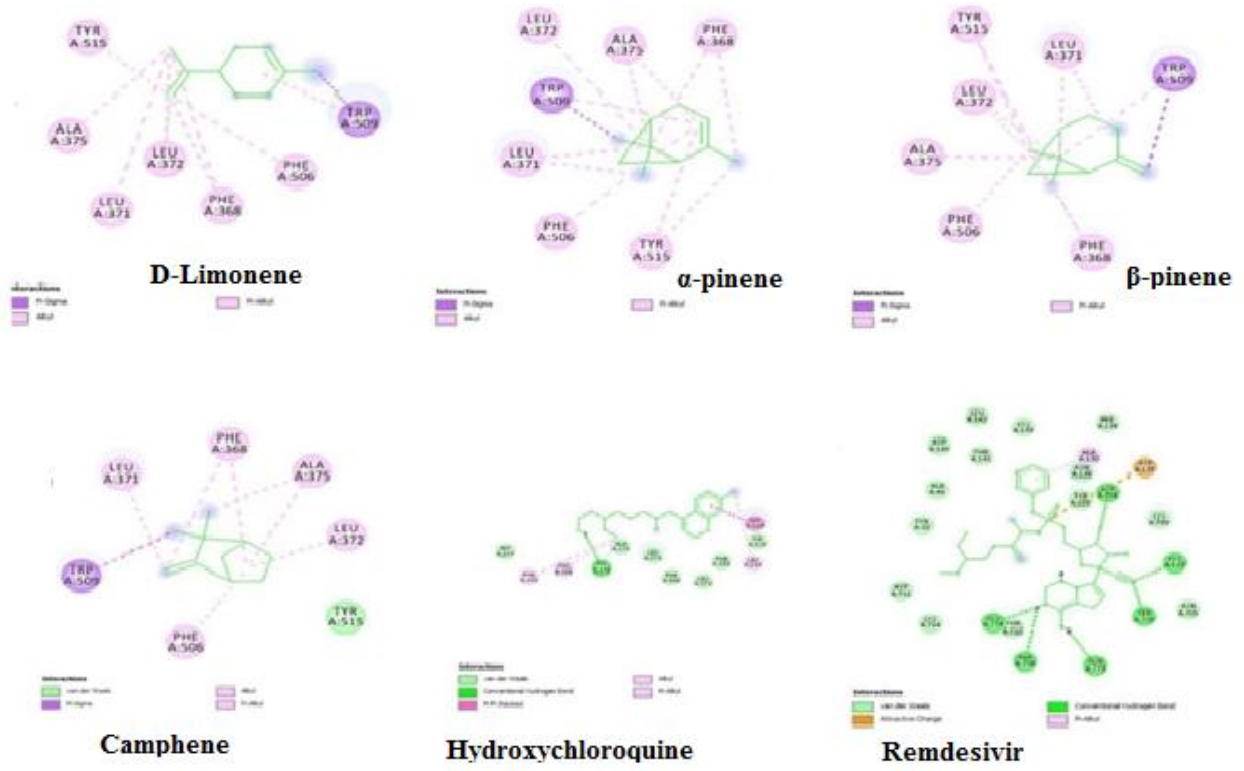

Figure 4 ligand interactions with 6M71

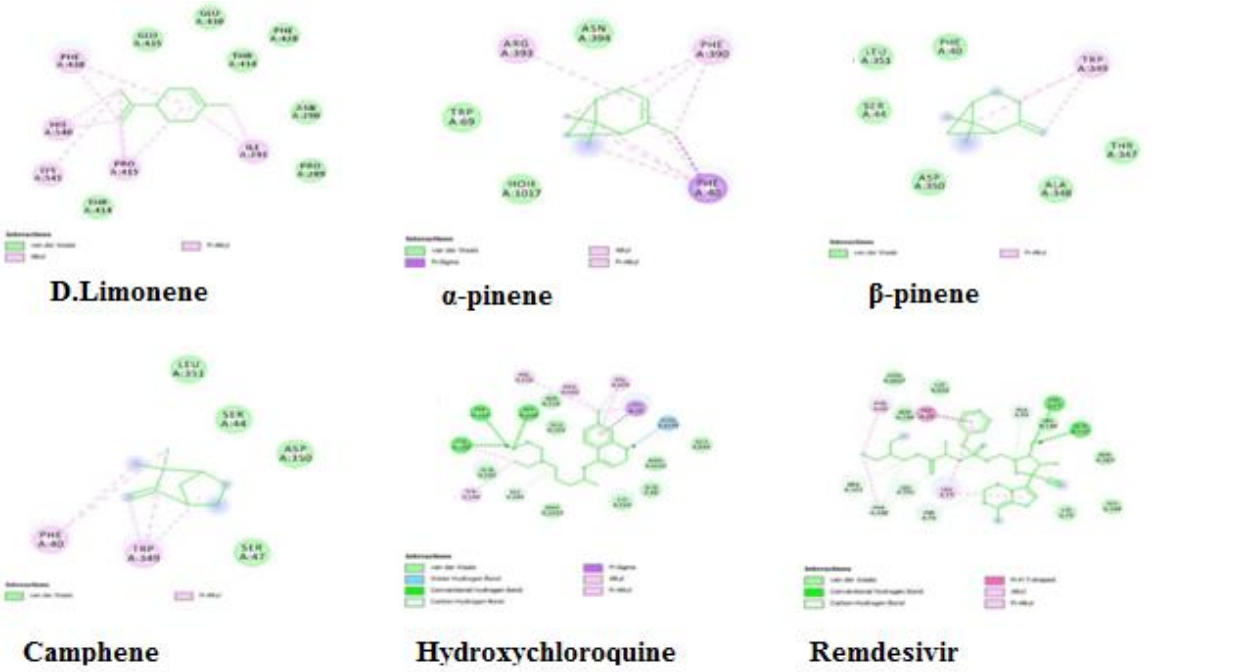

Figure 5 Ligand interactions with 6M0J

Table 3: Binding energy and RMSD values of ligands with targeted protein

S.No.	Name of the ligand	Binding energy target protein 1Q2W	Binding energy with target protein 6M71	Binding energy with target protein 6MOJ	RMSD
1	D-limonene	-5.2	-5.4	-7.1	0
2	α-pinene	-4.6	-5.4	-5.0	0
3	β-pinene	-4.5	-5.4	-4.8	0
4	Camphene	-4.7	-5.5	-4.8	0
5	Hydroxychloroquine	-5.9	-6.1	-6.5	0
6	Remdesivir	-6.4	-8.2	-7.7	0

4. Conclusion:

The COVID-19 outbreak is an unprecedented global public health challenge and needs immediate intervention. Various preventive measures, treatments have been implemented for the management of this disease. Natural resources play an indispensable role in the management of COVID-19. Traditional Indian medicinal plants have long been used for treatment of several diseases including viral diseases. *Citrus limetta* Risso, is a significant aromatic medicinal plant reported to possess broad spectrum of medicinal uses including antitumor, antioxidant, antibacterial, antiviral activities etc. The antiviral property of *C. limetta* had been reported in many articles. The preset study was attempted to prove that phytocompound D-Limonene from *Citrus limetta* Risso acts as a promising adjunct in the treatment of COVID-19. D-Limonene possesses excellent drug likeness parameters with zero violations of Lipinski's Rule and very good ADME pharmacokinetic properties. Finally, D-Limonene proves anti-viral efficiency against SAR CoV-2 by showing the highest binding affinity and interaction with multiple targets of COVID-19 including Viral proteases, RNA binding Protein and Spike protein. This implies that the active phytoconstituents especially D- Limonene of *Citrus limetta* Risso would serve as a supportive measure for the management of this pandemic disease upon further investigation.

5. Acknowledgement:

We are grateful to the Management and Principal of Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamil Nadu, India for their valuable support and guidance throughout this work.

6. References:

1. Tripathi, *et al.* (2020). Identification of bioactive molecule from *Withania somnifera* (Ashwagandha) as SARS-CoV-2 main protease inhibitor. *Journal of Biomolecular Structure & Dynamics*, 1–14. Advance online publication. https://doi.org/10.1080/07391102.2020.1790425
2. McKee, D. L., *et al.* (2020). Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacological Research*, *157*, 104859. https://doi.org/10.1016/j.phrs.2020.104859
3. Hui, D. S., *et al*. (2020). The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. *International Journal of Infectious Diseases : IJID : official publication of the International Society for Infectious Diseases*, *91*, 264–266. https://doi.org/10.1016/j.ijid.2020.01.009
4. Mora, J. R., Iwata, M., & von Andrian, U. H. (2008). Vitamin effects on the immune system: vitamins A and D take centre stage. *Nature Reviews. Immunology*, *8*(9), 685–698. https://doi.org/10.1038/nri2378
5. Chikhale, R. V., *et al.* (2020). SARS -Cov-2 host entry and replication inhibitors from Indian ginseng: an *in-silico* approach. *Journal of Biomolecular Structure & Dynamics*, 1–12. Advance online publication. https://doi.org/10.1080/07391102.2020.1778539
6. Devgan, Kirandeep & Kumar, Nitin &Alam, Md & Singh, Baljit. (2018). Development of Sweet Lime (*Citrus limetta* Risso) Pomace Integrated Rice-based Extruded Product: Process Optimization. 55.
7. Ayangla, N. W., N. E. E. T. U. Singh, and A. J. A. Y. Kumar. "Phytochemical analysis of plant species of genus Zanthoxylum." *International Journal of Medicine and Pharmaceutical Science* 6.1 (2016): 1-8.
8. Shree Dhar Gautam. GC-MS analysis of *Citrus limetta* Risso (Sweet lime) peel extract. The Pharma Innovation Journal. 2018; 7(6): 01-04.
9. Koula, D. O. U. K. A. N. I., G. A. C. E. M. Nacera, and B. E. N. L. A. R. B. I. Hayat. "Physicochemical and phytochemical characterization of some Algerian honeys types." *International Journal of Applied, Physical and Bio-Chemistry Research* 4.6 (2014): 1-16.
10. Mansi Pandit, N. Latha. *In silico* studies reveal potential antiviral activity of phytochemicals from medicinal plants for the treatment of COVID-19 infection, 14 April 2020, PREPRINT (Version 1)

available at Research Square https://doi.org/10.21203/rs.3.rs-22687/v1

11. Parivuguna, V., A. Doss, and B. Abhitha. "Anticandidal activity of the leaf extracts of Fragaria virginiana Duchense. and Lantana camara Linn." *International Journal of Bio-Technology and Research* 3.2 (2013): 21-26.
12. Khan, Ahmed & Mahmood, *et al*. . (2016). Phytochemical and pharmacological properties on *Citrus limetta* (Mosambi). Journal of Chemical and Pharmaceutical Research. 2016. 555-563.
13. Colecio-Juárez, M.C.*et al.* (2012). Characterization of volatile compounds in the essential oil of sweet lime (*Citrus limetta* Risso.) Chilean Journal of Agricultural Research. 72. 275-280.
14. Othayoth, R. A. J. A. T. H., S. R. A. V. Y. A. Kalivarapu, and M. A. H. E. N. D. R. A. N. Botlagunta. "Nanophytomedicine and drug formulations." *Int J Nanotechnol Appl* 4 (2014): 1-8.
15. Li, Y., Liu, X., *et al*. 2020). Traditional Chinese herbal medicine for treating novel coronavirus (COVID-19) pneumonia: protocol for a systematic review and meta-analysis. *Systematic Reviews, 9*(1), 75. https://doi.org/10.1186/s13643-020-01343-4
16. Herowati, Rina & Widodo, *et al* (2014). Molecular Docking Studies of Chemical Constituents of *Tinospora cordifolia* on Glycogen Phosphorylase. Procedia Chemistry. 13. 10.1016/j.proche.2014.12.007.
17. Krupanidhi, S., *et al*. (2020). Screening of phytochemical compounds of *Tinospora cordifolia* for their inhibitory activity on SARS-CoV-2: an *in silico* study. *Journal of Biomolecular Structure & Dynamics*, 1–5. Advance online publication. https://doi.org/10.1080/07391102.2020.1787226
18. Astani, A., & Schnitzler, P. (2014). Antiviral activity of monoterpenes beta-pinene anD-Limonene against herpes simplex virus in vitro. *Iranian Journal of Microbiology, 6*(3), 149–155.
19. Maii M. Nagy, Dalia A. Al-Mahdy, Omnia M. Abd El Aziz, Ahmed M. Kandil, Mona A. Tantawy & Taha S.M. El Alfy (2018) Chemical Composition and Antiviral Activity of Essential Oils from *Citrus reshni* Hort. ex Tanaka (*Cleopatra mandarin*) Cultivated in Egypt, Journal of Essential Oil Bearing Plants, 21:1, 264-272, DOI: 10.1080/0972060X.2018.1436986