Journal of Phytology 2022, 14: 76-85
doi: 10.25081/jp.2022.v14.7842
https://updatepublishing.com/journal/index.php/jp

Research Article

ISSN: 2075-6240

Molecular docking analysis on 16 therapeutic ligands of *Ocimum tenuiflorum* L. (Tulasi) and their prospects in drug design for COVID-19

Guruprasad Anantharam, S. Geetha, P. Santhana Pandi, N. Krithika, C. V. Chittibabu*

Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous), Chennai – 600 005, Tamil Nadu, India

ABSTRACT

The PyRx software and Discovery studio were used in the present molecular docking studies of the 16 ligands of *Ocimum tenuiflorum* L., selected based on their high therapeutic potentials, viz., (E)-6-hydroxy-4,6-dimethylhept-3-en-2-one, Apigenin, Bieugenol, Cirsilineol, Cirsimaritin, β-Caryophyllene epoxide, Dehydrodieugenol B, Eugenol, Ferulaldehyde, Isothymonin, Isothymusin, Linalool, Luteolin, Ocimarin, Rosmarinic acid, and Thymol. Saquinavir was used as a positive control. The binding affinities of the 16 ligands to the main proteases of COVID-19 6LU7 and 6Y2E (critical for viral replication) and their ability to arrest the virus replication were recorded. The binding affinities of the ligands to 6LU7 and 6Y2E ranged from -4.3 and -4.7 kcal/mol (for (E)-6-hydroxy-4,6-dimethylhept-3-en-2-one) to -7.6 (for Rosmarinic acid to both target proteins). While the corresponding values for the control drug Saquinavir were -7.8 and -7.6 respectively. The Rosmarinic acid, in binding with both the proteases (-7.6 and -7.6 kcal/mol) showed six conventional hydrogen bonds, one carbon hydrogen bond (ASP 153 had one conventional hydrogen bond and one carbon hydrogen bond), one Pi-alkyl bond, one Pi-Pi stacked bond, eight van der waals bonds for 6LU7 protease; it formed three conventional hydrogen bonds, two Pi-alkyl bonds, one unfavourable donor – donor bond and 14 van der waals bonds with 6Y2E protease. The control drug – Saquinavir in binding with 6LU7 protease showed 12 van der waals, one alkyl, one Pi-alkyl, one Pi-cation, one Pi-stacked and four conventional hydrogen bonds, which indicates that it has less affinity when compared with Rosmarinic acid. Similarly, the control drug on binding with 6Y2E protease exhibited ten van der waals, four Pi-alkyl, one cation and three hydrogen bonds. The results are in conformity to similar other studies, and herald a promising scope for Rosmarinic acid as lead molecule in the drug discovery for COVID-19.

Received: September 11, 2021
Revised: July 13, 2022
Accepted: July 17, 2022
Published: July 27, 2022

***Corresponding author**
C. V. Chittibabu
E-mail: cvcgrab@gmail.com

KEYWORDS: COVID-19, Molecular docking, PyRx software, Rosmarinic acid, 6LU7 protease, 6Y2E protease

INTRODUCTION

The COVID-19, an infectious dreadful virus disease, has been pandemic since December 2019, and the unimaginable volume of human casualties witnessed so far has propelled the scientific community to explore the possibilities of developing safe herbal drugs (Chojnacka *et al.*, 2020), either as preventive or curative (Kiran *et al.*, 2020), against the COVID-19 (Sampangi-Ramaiah *et al.*, 2020). The present investigation on 16 therapeutic ligands of Ocimum tenuiflorum L., (Vernacular name, "Tulasi" in Tamil) (Singh & Chaudhuri, 2018) the latter being the key ingredient in Kandankathiri Legiyam-a Siddha polyherbal formulation used in respiratory diseases, comprise the binding affinities of them to the two main proteases of COVID-19, *viz.*, 6LU7 (Peele *et al.*, 2020) and 6Y2E, and their ability to arrest the virus replication (Sampangi-Ramaiah *et al.*, 2020). The PyRx software and Discovery studio were used in the study to discover lead molecules for the drug design and explore the possibility of repurposing the popular herbal drug in the treatment of COVID-19 (Trott & Olson, 2010). The COVID-19 proteases, 6LU7 (Sisakht *et al.*, 2021) and 6Y2E, are critical for viral replication and the effective binding of the viral proteases will arrest viral replication and the disease (Sampangi-Ramaiah *et al.*, 2020). Hence, the present investigation was taken up towards achieving this purpose.

Modern medicines of the 21st Century have their origin in traditional herbal medicinal practices. An array of compounds is reported as effective lead molecules in drug design and discovery (Wu *et al.*, 2020). *Ocimum tenuiflorum* L., is documented extensively in Siddha and Ayurveda literature to

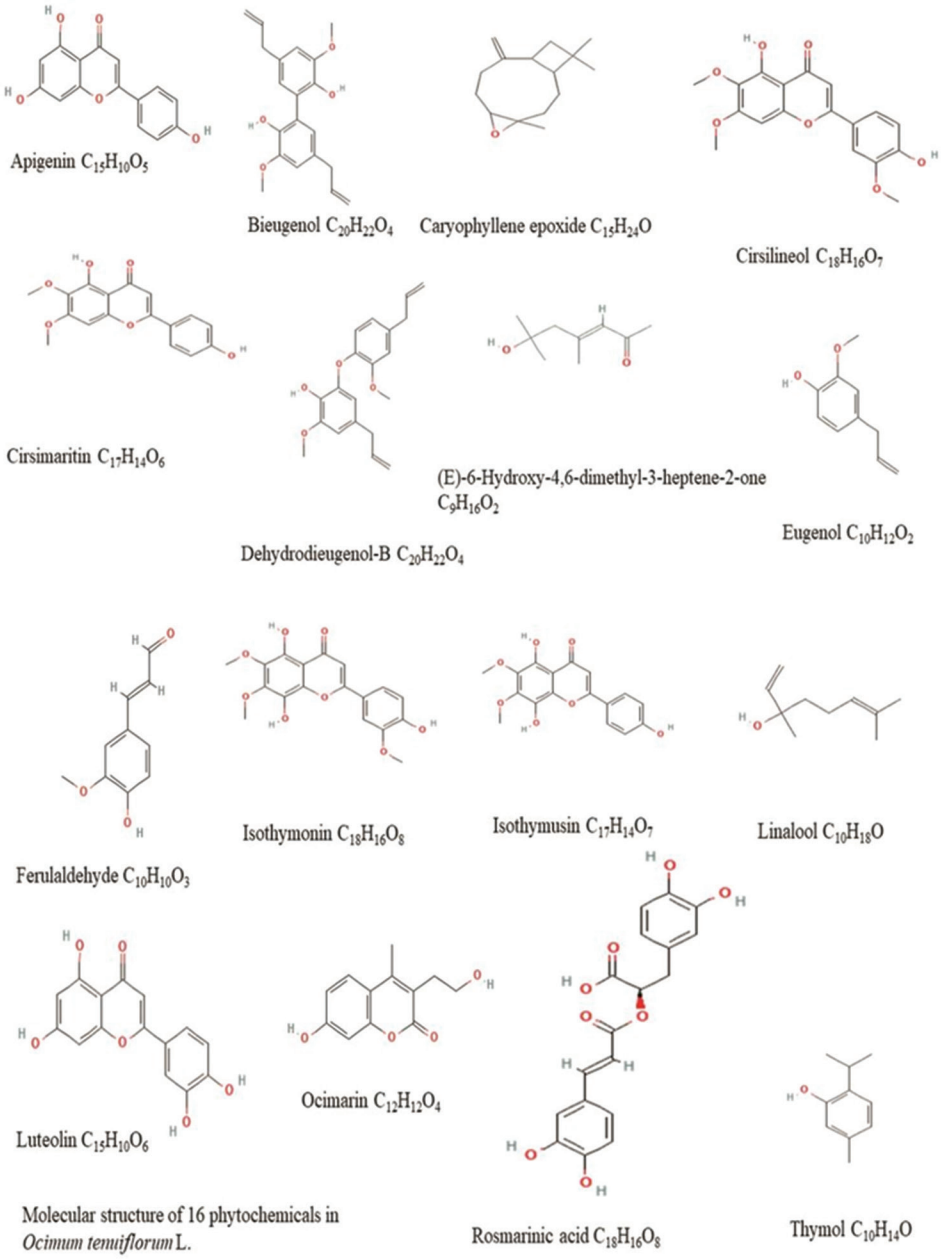

Figure 1: The Structural Formula of the 16 Selected Ligands of *Ocimum tenuiflorum* L

cure respiratory diseases (Singh & Chaudhuri, 2018). There are studies on *Ocimum tenuiflorum* L., to show the presence of several class of phytochemicals (Soni & Sosa, 2013) such as alkaloids, carbohydrates, phenolic compounds, tannins, flavonoids, steroids, and saponins (Siva *et al*., 2016) such phytochemicals possess antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anti-helminthic, antidiarrheal, analgesic, antipyretic, anti-inflammatory, antiallergic, antihypertensive activities (Pandey & Sharma, 2010). A total of 41 phytochemicals are reported in *Ocimum tenuiflorum* L., (Singh & Chaudhuri, 2018) and listed in Table – 1, out of these 41 reported phytochemicals, 23 phytochemicals are available in the PUBCHEM database (Kim *et al*., 2019) with canonical SMILES (Simplified Molecular Input Line Entry System), so these 23 phytochemicals are initially screened using SWISSADME software (Daina *et al*., 2017) for their drug-likeness (Bhadran *et al*., 2021).

MATERIALS AND METHODS

Selection of Ligands using SWISSADME Software

The SWISSADME software reports of 23 phytochemicals were analysed of as per Lipinski's rule of five for druglikeness (Lipinski *et al*., 2012), of these phytochemicals, Ocimumoside A, Ocimumoside B, Oleanolic acid, Orientin, Stigmasterol, Ursolic acid and Vicenin-2 are the violators of Lipinski's rule of five for drug design and these molecules were not taken for docking studies.

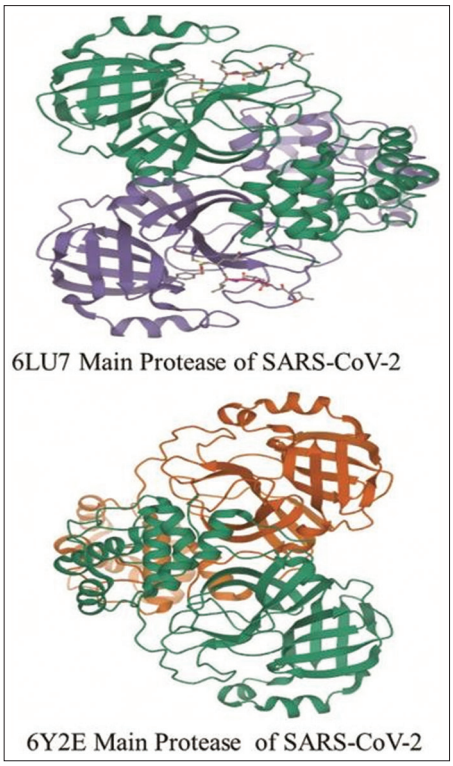

Figure 2: 3D View of 6LU7 and 6Y2E Protease of SARS-CoV-2

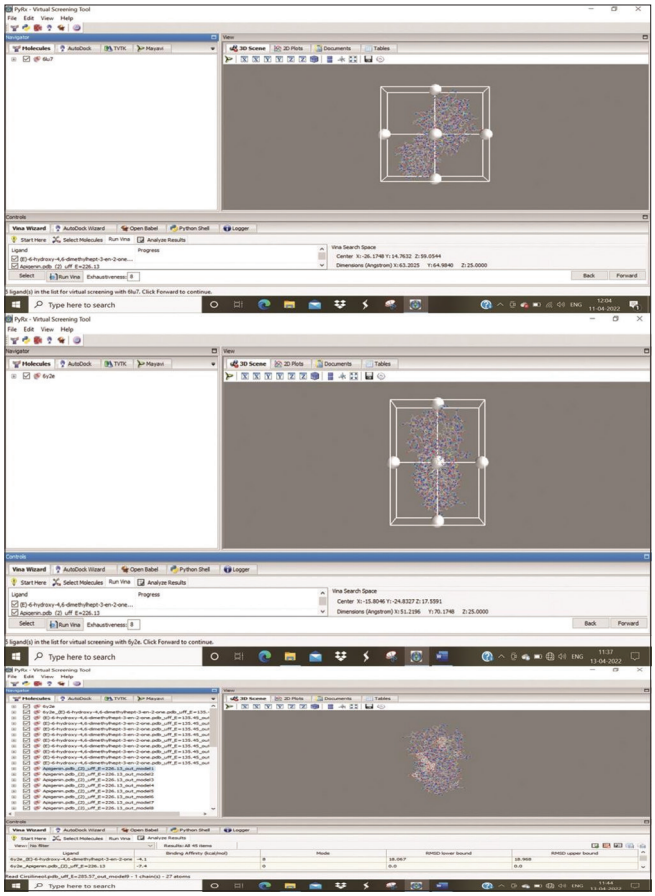

Figure 3: Grid Box Formed Around 6LU7 and 6Y2E Proteases of SARS CoV-2 and Output File with RMSD "0" Saved as PDB File

Whereas, (E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one, Apigenin, Bieugenol, Cirsilineol, Cirsimaritin, β-Caryophyllene epoxide, Dehydrodieugenol B, Eugenol, Feruladehyde, Isothymonin, Isothymusin, Linalool, Luteolin, Ocimarin, Rosmarinic acid, and Thymol are non-violaters of Lipinski's rule of five and hence these 16 molecules (Figure 1) were docked with 6LU7 and 6Y2E main proteases of SARS CoV2 (Figure 2).

Ligand Preparation

The input canonical SMILES from PUBCHEM database of these 16 phytochemicals (E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one, Apigenin, Bieugenol, Cirsilineol, Cirsimaritin, β-Caryophyllene epoxide, Dehydrodieugenol B, Eugenol, Feruladehyde, Isothymonin, Isothymusin, Linalool, Luteolin, Ocimarin, Rosmarinic acid, and Thymol are utilized to make PDB files through online source https://www.novoprolabs.com/tools/smiles2pdb the output was saved as PDB file for the molecules. The target proteins are 6LU7 and 6Y2E of SARS-CoV-2 (2019) were obtained from https://www.rcsb.org/website in PDB format (Sampangi-Ramaiah *et al*., 2020).

PyRx Software and Discovery Studio

PyRx is software, it is used for molecular docking studies (Dallakyan & Olson, 2015). PyRx docks the compounds available in "Protein Data Base – (PDB)" format online in the data base against potential drug targets (Trott & Olson 2010). It enables pharmacologists to perform simulations for the drug design and discovery process (Raj, 2021). It comprises a docking wizard with easy-to-use user interface which makes it a valuable tool for computer-aided drug design (Chen, 2014). The rational drug design is made possible by this software because of its chemical spreadsheet-like functionality, and powerful visualization engine (Shaker *et al*., 2020). The software is unique in docking five ligands with the desired target protein (Figure 3). The latter feature of PyRx is explored in the present study (Chaudari *et al*., 2020). Discovery studio is allied software to PyRx for visualizing the protein database files of ligand and target before and after docking by PyRx (Shaker *et al*., 2020). It is mandatory that the input files must be in PDB format for both ligand and target protein (Yuliana *et al*., 2013).

Preparation of Target Protein and Molecular Docking

The water molecules and HET atoms of 6LU7 and 6Y2E were eliminated (Figure 2), and the polar hydrogens were added and saved in the system using Disovery studio software. Later, the saved PDB file of the target protein is given as an input file in PyRx software along with the PDB files of the 16 ligands (Figure 1). The files get converted to pdbqt format and then the docking site was confirmed by a grid box with the dimensions (Angstrom) of X: 63.20, Y: 64.98 and Z: 25.00 for 6LU7 protease (Ounthaisong & Tangyuenyongwatana, 2017) and for 6Y2E protease it is X: 51.21, Y:70.17, Z: 25.00 (Herowati & Widodo, 2014) (Figure 3).

The output of the docking score was obtained in CSV format was saved as MS-EXCEL spread sheet for tabulation. The docked

files with RMSD value with zero alone were saved as PDB file and visualized through discovery studio software to comprehend the ligand-protein interactions. It is pertinent to mention here that the RMSD < 2.0 Å provides a good solution, hence docked position with zero was prioritised and saved as PDB file (Figure 3) (Ramírez & Caballero, 2018) for visualization through Biovia discovery studio software. The output of PyRx software results were presented in figures 4-9 and Tables 1-3.

RESULTS AND DISCUSSION

The 16 ligands of *Ocimum tenuiflorum* L. (Figure 1) chosen based on their drug-likness as per Lipinski's rule of five (Lipinski *et al*., 2012) for drug design and presented in Table 1. The molecular docking studies revealed the values of their binding affinity to 6LU7 and 6Y2E proteases of COVID-19 with a score ranging from -4.3 kcal/mol and -4.7 kcal/mol respectively for (E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one of -7.6 kcal/mol for Rosmarinic acid (Table 2 and 3, Figure 1 -9).

The ascending order of binding affinity for the 16 ligands with 6LU7 protease was (E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one ($C_9H_{16}O_2$) < Linalool ($C_{10}H_{18}O$) < Thymol ($C_{10}H_{14}O$) < Ferulaldehyde ($C_{10}H_{10}O_3$) < Eugenol ($C_{10}H_{12}O_2$) < Bieugenol ($C_{20}H_{22}O_4$) < Ocimarin ($C_{12}H_{12}O_4$) < β-Caryophyllene epoxide ($C_{15}H_{24}O$) < Dehydrodieugenol B

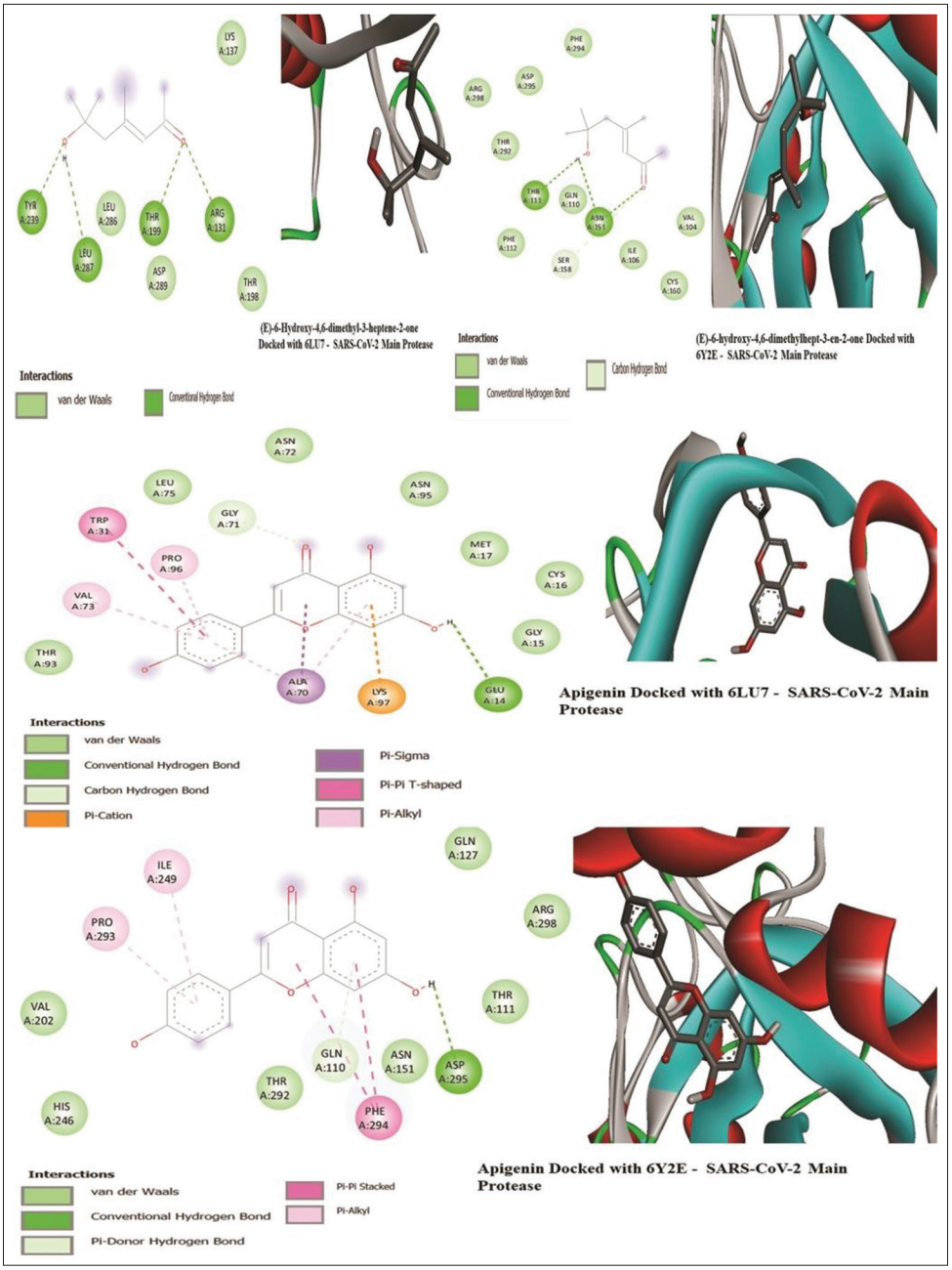

Figure 4: The 2D and 3D Visualization of Docking Analysis of Molecular Interaction of 6LU7 and 6Y2E Protease with (E)-6-hydroxy-4,6-dimethyl-3-heptene-2-one and Apigenin

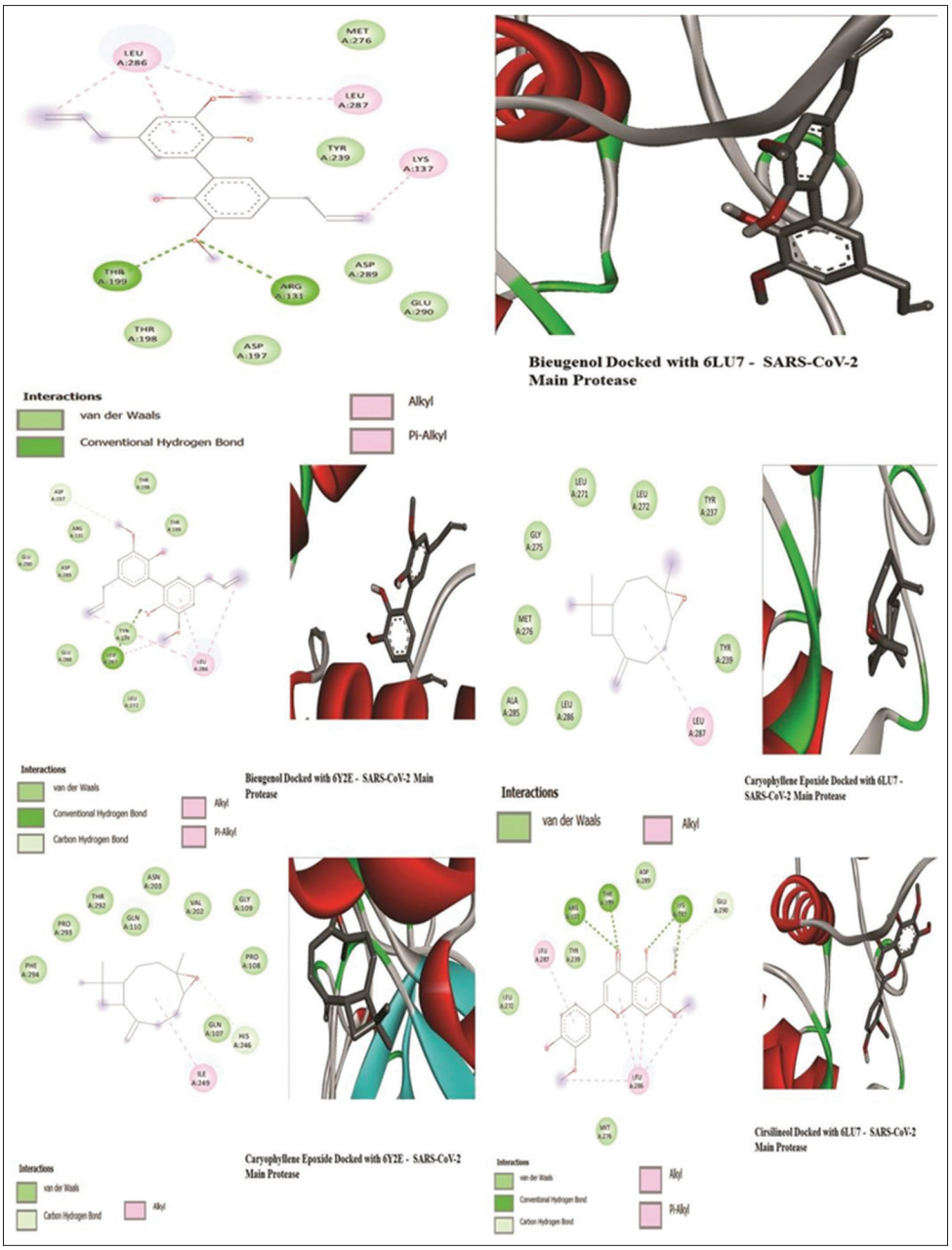

Figure 5: The 2D and 3D Visualization of Docking Analysis of Molecular Interaction of 6LU7 with Bieugenol, Caryophyllene Epoxide, Cirsilineol and 6Y2E Protease with Bieugenol and Caryophyllene Epoxide

($C_{20}H_{22}O_4$) < Cirsimaritin ($C_{17}H_{14}O_6$) < Isothymusin ($C_{17}H_{14}O_7$) < Apigenin ($C_{15}H_{10}O_5$) < Cirsilineol ($C_{18}H_{16}O_7$) < Isothymonin ($C_{18}H_{16}O_8$) < Luteolin ($C_{15}H_{10}O_6$) < Rosmarinic_acid ($C_{18}H_{16}O_8$) (Table 1).

The 13 different types of bonding of the 16 ligands with the target protein 6LU7 were recorded. Single C-H bond was evident in the case of Apigenin, Cirsilineol, Cirsimaritin, Dehydroeugenol B, Isothymonin, Isothymusin, Ocimarin and Rosmarinic acid (Table 2). While that of conventional hydrogen bond, Rosmarinic acid displayed six followed by Cirsimaritin with four, cirsilineol with three, and Dehydrodieugenol B, Isothymonin, Isothymusin, and Ocimarin formed two conventional hydrogen bonds each. With regard to the Apigenin it revealed a single conventional hydrogen bond with 6LU7 protease (Table 2). The rest of the ligands did not exhibit such C-H bond and conventional hydrogen bond. It is pertinent to mention here that different types of pi-bonds and van der waals electrostatic attractions are weaker than C-H bond and conventional hydrogen bonds. Thus, it is evident that Rosmarinic acid has a strong binding affinity with 6LU7 than the other 15 ligands (Figure 4-9).

The ascending order of binding affinity of the 16 ligands with 6Y2E protease was (E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one ($C_9H_{16}O_2$) < Linalool ($C_{10}H_{18}O$) < Eugenol ($C_{10}H_{12}O_2$) < Thymol ($C_{10}H_{14}O$) < Ferulaldehyde ($C_{10}H_{10}O_3$) < Bieugenol ($C_{20}H_{22}O_4$) < β-Caryophyllene epoxide

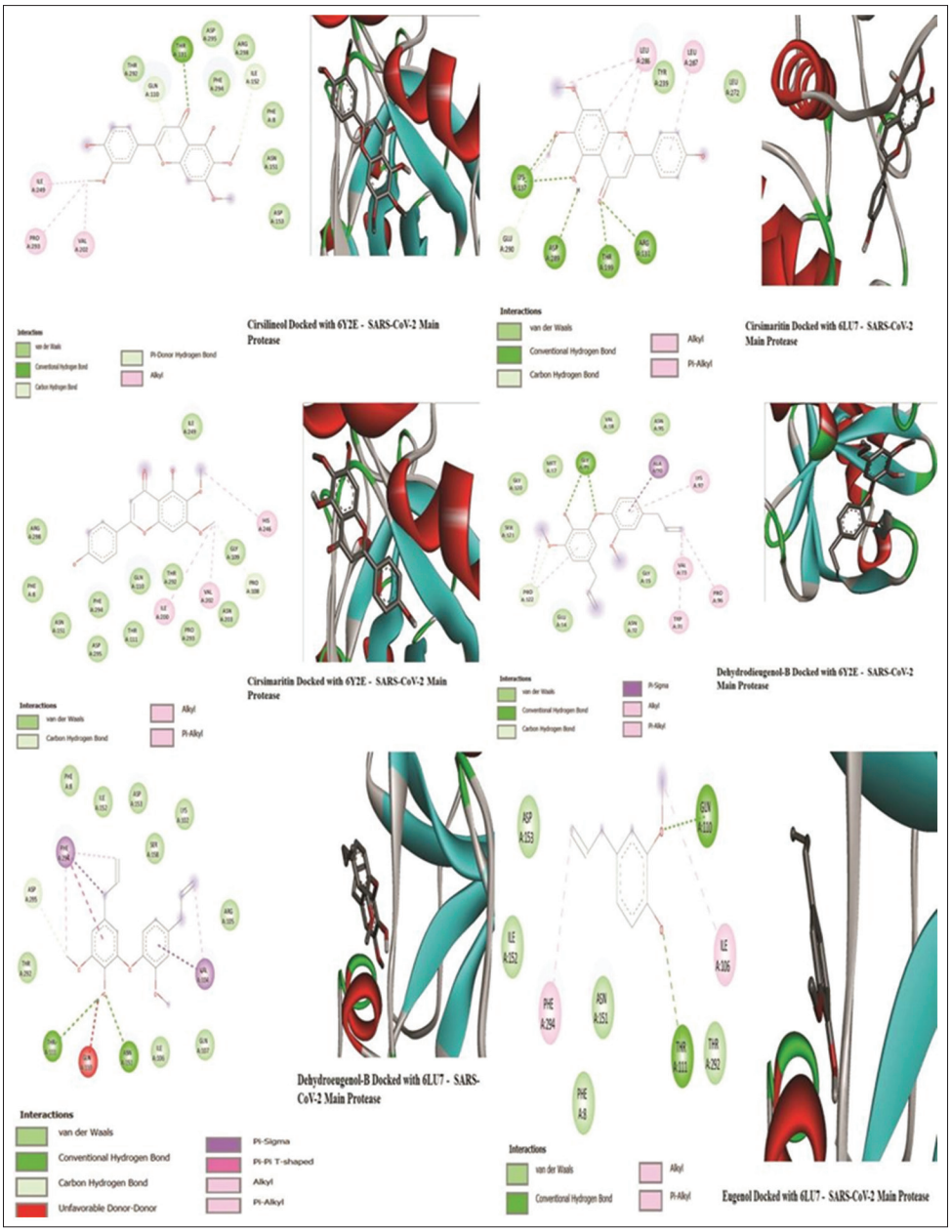

Figure 6: The 2D and 3D Visualization of Docking Analysis of Molecular Interaction of 6LU7 with Cirsimaritin, Dehydrodieugenol-B, Eugenol and 6Y2E Protease with Cirsilineol, Cirsimaritin, Dehydrodieugenol-B

($C_{15}H_{24}O$) < Dehydrodieugenol B ($C_{20}H_{22}O_4$) < Ocimarin ($C_{12}H_{12}O_4$) < Isothymusin ($C_{17}H_{14}O_7$) < Cirsimaritin ($C_{17}H_{14}O_6$) < Cirsilineol ($C_{18}H_{16}O_7$) < Isothymonin ($C_{18}H_{16}O_8$) < Apigenin ($C_{15}H_{10}O_5$) < Luteolin ($C_{15}H_{10}O_6$) < Rosmarinic_acid ($C_{18}H_{16}O_8$) (Table 1).

The molecular docking results of 16 ligands with 6Y2E protease exhibited that (E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one ($C_9H_{16}O_2$), Bieugenol, Cirsilineol, Dehydrodieugenol B, Ferulaldehyde and Isothymonin have one C-H bond each. Whereas regarding the formation of conventional hydrogen bonds, Ocimarin has formed four, followed Rosmarinic acid and Ferulaldehyde three each, (E)-6-hydroxy-4,6-dimethylhept-3-en-2-one ($C_9H_{16}O_2$), Eugenol and Luteolin formed two each. The Apigenin, Bieugenol, Cirsilineol, Dehydroeugenol B, Isothymonin, Isothymusin, Linalool and Thymol contributed one conventional bond each (Table 3). Although Rosmarinic acid did not formed a C-H bond with 6Y2E protease, but it has three conventional hydrogen bonds, 14 van der Waals electrostatic attractions, two pi-alkyl bonds and one unfavourable donor bond with 6Y2E protease. So, Rosmarinic acid shall be considered as a promising drug candidate to combat SARS CoV2 (Table 3).

The binding affinity of the control drug Saquinavir were -7.8 and -7.6 for 6LU7 and 6Y2E respectively (Table 1). Whereas, that of Rosmarinic acid, in binding with both the proteases the binding affinity were -7.6 and -7.6 kcal/mol. The Rosmarinic acid showed six conventional hydrogen bonds, one carbon hydrogen

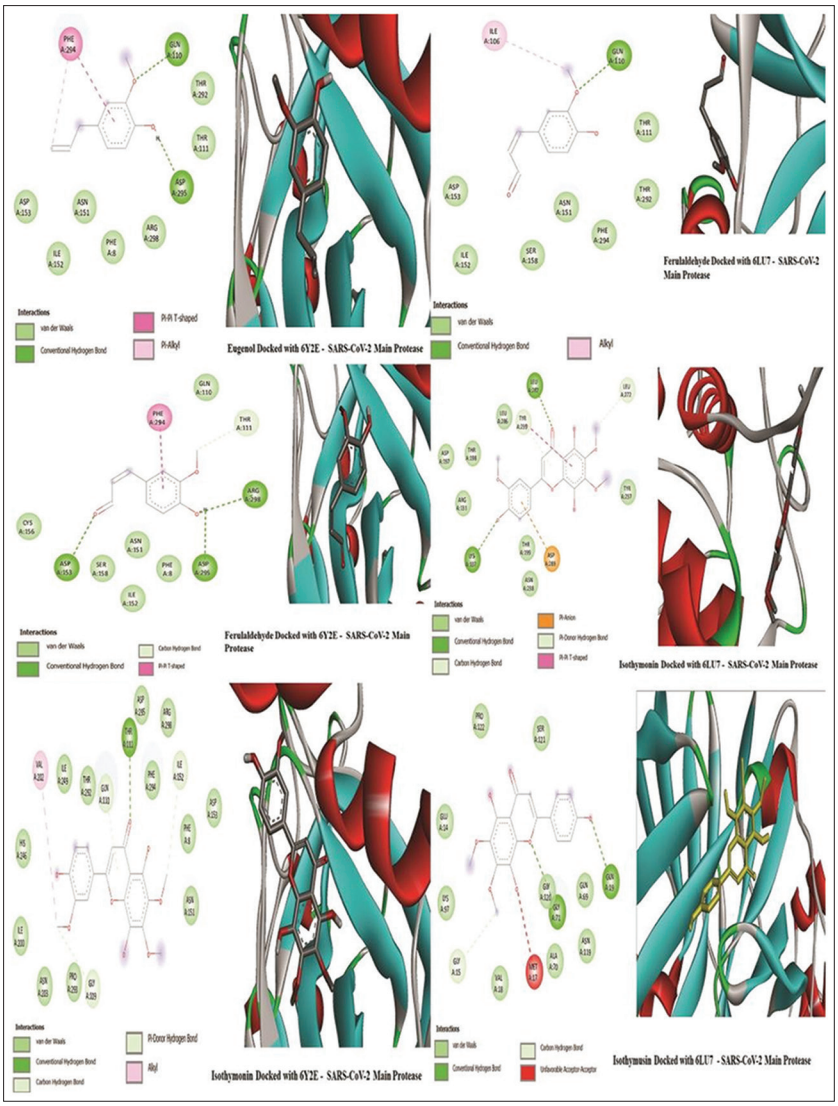

Figure 7: The 2D and 3D Visualization of Docking Analysis of Molecular Interaction of 6LU7 with Feruladehyde, Isothymonin, Isothymusin and 6Y2E with Eugenol, Ferulaldehyde, Isothymonin.

Table 1: The 16 ligands of *Ocimum tenuiflorum* and their binding affinity to 6LU7 and 6Y2E proteases of SARS – CoV2

S.No	Ligand (Molecular formula)	Molecular weight	Binding affinity to 6LU7 (kcal/mol)	Binding affinity to 6Y2E (kcal/mol)
1	(E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one	156.22	-4.3	-4.7
2	Apigenin ($C_{15}H_{10}O_5$)	270.24	-6.7	-7.4
3	Bieugenol ($C_{20}H_{22}O_4$)	326.39	-5.9	-5.6
4	Cirsilineol ($C_{18}H_{16}O_7$)	344.32	-6.8	-6.7
5	Cirsimaritin ($C_{17}H_{14}O_6$)	314.29	-6.5	-6.6
6	β-Caryophyllene epoxide ($C_{15}H_{24}O$)	220.35	-6	-6
7	Dehydrodieugenol B ($C_{20}H_{22}O_4$)	326.39	-6.1	-6.3
8	Eugenol ($C_{10}H_{12}O_2$)	164.20	-5.5	-5
9	Ferulaldehyde ($C_{10}H_{10}O_3$)	178.18	-5.4	-5.5
10	Isothymonin ($C_{18}H_{16}O_8$)	360.0	-6.8	-6.7
11	Isothymusin ($C_{17}H_{14}O_7$)	330.29	-6.6	-6.5
12	Linalool ($C_{10}H_{18}O$)	154.25	-4.5	-4.7
13	Luteolin ($C_{15}H_{10}O_6$)	286.24	-7.1	-7.5
14	Ocimarin ($C_{12}H_{12}O_4$)	220.22	-5.9	-6.5
15	Rosmarinic_acid ($C_{18}H_{16}O_8$)	360.3	-7.6	-7.6
16	Thymol ($C_{10}H_{14}O$)	150.22	-4.8	-5.4
17	Saquinavir ($C_{38}H_{50}N_6O_5$)*	670.84	-7.8	-7.6

*Positive Control - Synthetic HIV drug (Megha Hastantram Sampangi-Ramaiah *et al.*, 2020)

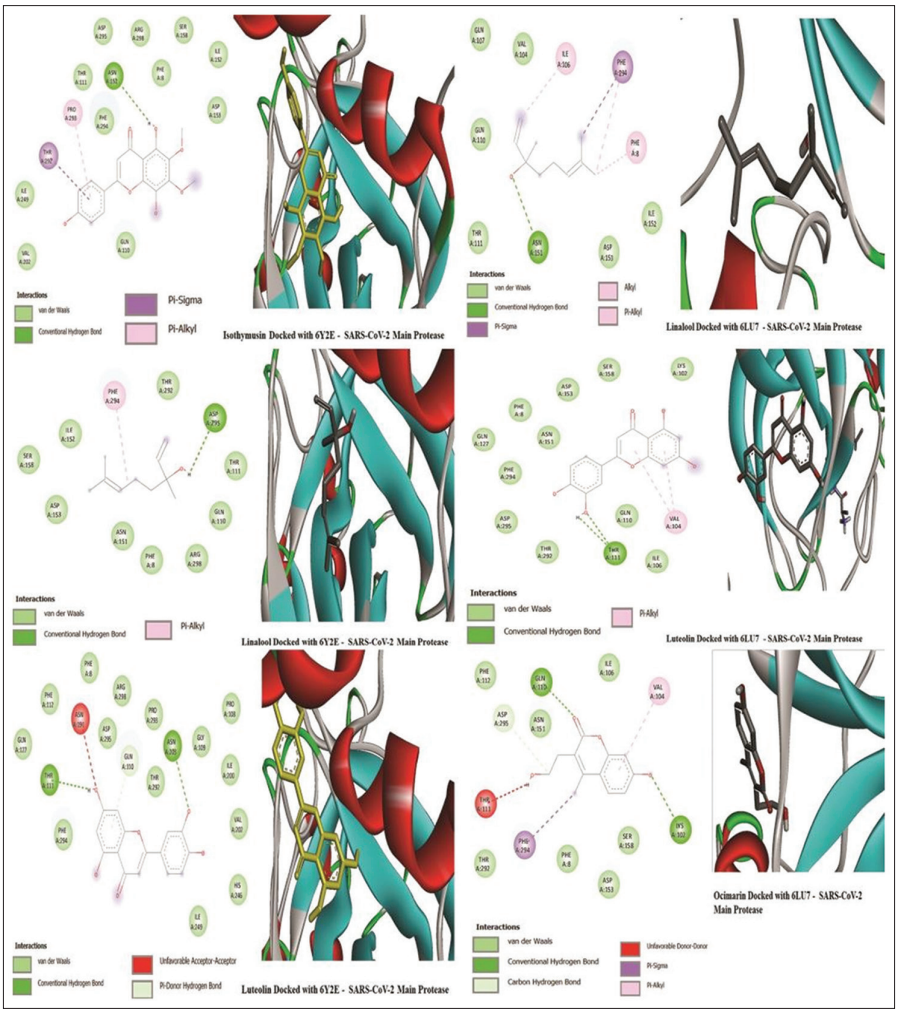

Figure 8: The 2D and 3D Visualization of Docking Analysis of Molecular Interaction of 6LU7 with Linalool, Luteolin, Ocimarin and 6Y2E with Isothymusin, Linalool, Luteolin

Table 2: The 16 ligands of *Ocimum tenuiflorum* and the details of different bonds formed with 6LU7 protease of SARS-CoV2

S. No	Ligand	Van der waals	Alkyl C – H	Pi-Alkyl	Pi-Hydrogen	Pi-Anion	Pi-cation	Pi-Sigma	Pi-Stacked	Pi-Pi T-shaped	Conventional Hydrogen	Unfavourable-Acceptor	Unfavourable-Donor	Total
1	(E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one	4	-	-	-	-	-	-	-	-	4	-	-	8
2	Apigenin	7	-	1	2	-	-	1	1	-	1	1	-	-	14
3	Bieugenol	6	2	-	1	-	-	-	-	-	-	2	-	-	11
4	Cirsilineol	4	1	1	1	-	-	-	-	-	-	3	-	-	10
5	Cirsimaritin	2	1	1	1	-	-	-	-	-	-	4	-	-	9
6	β-Caryophyllene epoxide	8	1	-	-	-	-	-	-	-	-	-	-	-	9
7	Dehydrodieugenol B	9	1	1	1	-	-	-	2	-	1	2	-	1	18
8	Eugenol	5	1	-	1	-	-	-	-	-		2	-	-	9
9	Ferulaldehyde	7	1	-	-	-	-	-	-	-	-	1	-	-	9
10	Isothymonin	7	-	1	-	1	1	-	-	-	1	2	-	-	13
11	Isothymusin	9	-	1	-	-	-	-	-	-	-	2	1	-	13
12	Linalool	6	1	-	1	-	-	-	1	-	-	1	-	-	10
13	Luteolin	11	-	-	1	-	-	-	-	-	-	1	-	-	13
14	Ocimarin	7	-	1	1	-	-	-	1	-	-	2	-	1	13
15	Rosmarinic acid	8	-	1	1	-	-	-	-	1	-	6	-	-	17
16	Thymol	4	3	-	1	-	-	-	1	-	-	1	-	-	10
17	Saquinavir*	12	1	-	1	-	-	1	-	1	-	4	-	-	20
Total		116	13	8	13	1	1	2	6	2	3	38	1	2	206

*Positive Control - Synthetic HIV drug (Megha Hastantram Sampangi-Ramaiah *et al.*, 2020)

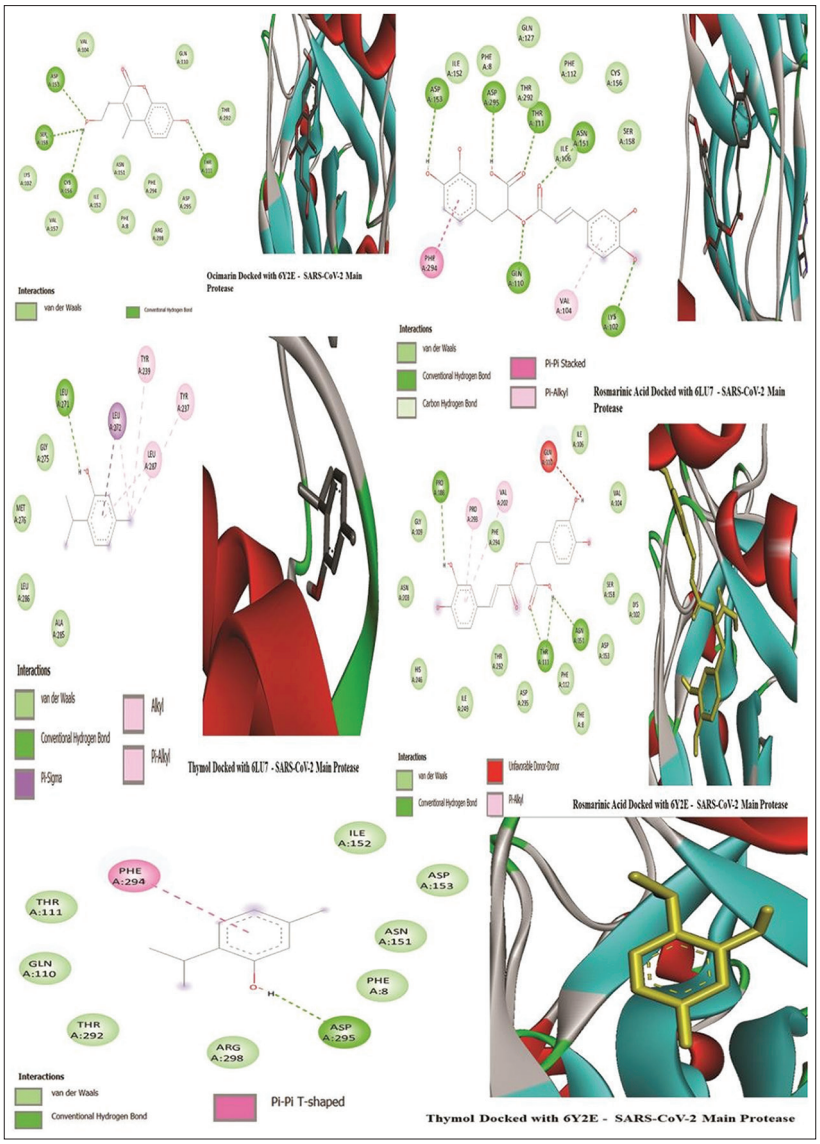

Figure 9: The 2D and 3D Visualization of Docking Analysis of Molecular Interaction of 6LU7 with Rosmarinic Acid, Thymol and 6Y2E with Ocimarin, Rosmarinic Acid, Thymol

Table 3: The 16 ligands of *Ocimum tenuiflorum* and the details of different bonds formed with 6Y2E protease of SARS-CoV2

S. No	Ligand	Van der waals	Alkyl	C – H	Pi-Alkyl	Pi-Hydrogen	Pi-Cation	Pi-Sigma	Pi-Stacked	Pi-Pi T shaped	Conventional Hydrogen	U-Ac	U-Do	Total
1	(E)-6-hydroxy-4,6-dimethylhept-3-en-2-one	9	-	1	-	-	-	-	-	-	2	-	-	12
2	Apigenin	7	-	-	2	1	-	-	1	-	1	-	-	12
3	Bieugenol	8	1	1	2	-	-	-	-	-	1	-	-	13
4	Cirsilineol	7	3	1	-	1	-	-	-	-	1	-	-	13
5	Cirsimaritin	12	1	1	2	-	-	-	-	-	-	-	-	16
6	β-Caryophyllene epoxide	9	1	1	-	-	-	-	-	-	-	-	-	11
7	Dehydrodieugenol B	8	1	1	3	-	-	1	-	-	1	-	-	15
8	Eugenol	7	-	-	1	-	-	-	-	1	2	-	-	11
9	Ferulaldehyde	6	-	1	-	-	-	-	-	1	3	-	-	11
10	Isothymonin	12	1	1	-	1	-	-	-	-	1	-	-	16
11	Isothymusin	11	-	-	1	-	-	1	-	-	1	-	-	14
12	Linalool	9	-	-	1	-	-	-	-	-	1	-	-	11
13	Luteolin	14	-	-	-	1	-	-	-	-	2	1	-	18
14	Ocimarin	11	-	-	-	-	-	-	-	-	4	-	-	15
15	Rosmarinic acid	14	-	-	2	-	-	-	-	-	3	-	1	20
16	Thymol	8	-	-	-	-	-	-	-	1	1	-	-	10
17	Saquinavir*	10	-	-	4	-	1	-	-	-	3	-	-	18
Total		162	8	8	18	4	1	2	1	3	27	1	1	236

*Positive Control - Synthetic HIV drug (Megha Hastantram Sampangi-Ramaiah *et al.*, 2020)

bond (ASP 153 had one conventional hydrogen bond and one carbon hydrogen bond), one Pi-alkyl bond, one Pi-Pi stacked bond, eight van der Waals bonds for 6LU7 protease; it formed three conventional hydrogen bonds, two Pi-alkyl bonds, one unfavourable donor – donor bond and 14 van der Waals bonds (Figure 9).

CONCLUSION

As the binding affinity of Saquinavir (Sampangi-Ramaiah *et al*., 2020), the synthetic control drug, with 6LU7 protease showed 12 van der Waals, one alkyl, one Pi-alkyl, on Pi-cation, one Pi-stacked, four conventional hydrogen bonds, which indicates that it has less affinity when compared with Rosmarinic acid. Similarly, the synthetic control drug on binding with 6Y2E protease exhibited 10 van der Waals, four Pi-alkyl, one cation, three hydrogen bonds. The results are in conformity to similar other studies and herald a promising scope for Rosmarinic acid as lead molecule in the drug discovery for COVID-19.

REFERENCES

Bhadran, S., George, S. A., Malla, S., & Puttaraju, H. B. (2021). *In silico* prediction and molecular docking study on the interaction of bioactive compounds of *Adenanthera pavonina* exploring the potential antifungal activity against *Candida glabrata* cell wall proteins. *International Journal of Pharmaceutical Sciences and Drug Research*, *13*(1), 51–59.

Chaudari, R. N., Khan, S. L., Chaudhary, R. S., Jain, S. P., & Sidduqui, F. A. (2020). B-sitosterol: isolation from *Muntingia calabura* Linn bark extract, structural elucidation and molecular docking studies as potential inhibitor of sars-cov-2 mpro (COVID-19). *Asian Journal of Pharmaceutical and Clinical Research*, *13*(5), 204–209. https://doi.org/10.22159/ajpcr.2020.v13i5.37909

Chen, S. J. (2014). A potential target of tanshinone IIA for acute promyelocytic leukemia revealed by inverse docking and drug repurposing. *Asian Pacific Journal of Cancer Prevention*, *15*(10), 4301–4305. https://doi.org/10.7314/apjcp.2014.15.10.4301

Chojnacka, K., Witek-Krowiak, A., Skrzypczak, D., Mikula, K., & Młynarz, P. (2020). Phytochemicals containing biologically active polyphenols as an effective agent against Covid-19-inducing coronavirus. *Journal of Functional Foods, 73,* 104146. https://doi.org/10.1016/j.jff.2020.104146

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports, 7,* 42717. https://doi.org/10.1038/srep42717

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. In J. E. Hempel, C. H. Williams & C. C. Hong (Eds.), *Methods in Molecular Biology: Vol. 1263. Chemical Biology: Methods and Protocols*. New York: Humana Press. https://doi.org/10.1007/978-1-4939-2269-7_19

Herowati, R., & Widodo, G. P. (2014). Molecular docking studies of chemical constituents of *Tinospora cordifolia* on glycogen phosphorylase. *Procedia Chemistry, 13,* 63–68. https://doi.org/10.1016/j.proche.2014.12.007

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2019). PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Research, 47*(D1), D1102–D1109. https://doi.org/10.1093/nar/gky1033

Kiran, G., Karthik, L., Shree Devi, M. S., Sathiyarajeswaran, P., Kanakavalli, K., Kumar, K. M., & Ramesh Kumar, D. (2020). *In Silico* computational screening of Kabasura Kudineer - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein. *Journal of Ayurveda and Integrative Medicine, 13*(1), 100324. https://doi.org/10.1016/j.jaim.2020.05.009

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews, 64,* 4-17. https://doi.org/10.1016/j.addr.2012.09.019

Ounthaisong, U., & Tangyuenyongwatana, P. (2017). Cross-docking study of flavonoids against tyrosinase enzymes using PyRx 0.8 virtual screening tool. *Thai Journal of Pharmaceutical Sciences, 41,* 189–192.

Pandey, G., & Sharma, M. (2010). Pharmacological activities of *Ocimum sanctum* (Tulsi): A review. *International Journal of Pharmaceutical Sciences Review and Research, 5*(1), 61–66.

Peele, K. A., Durthi, C. P., Srihansa, T., Krupanidhi, S., Ayyagari, V. S., Babu, D. J., Indira, M., Reddy, A. R., & Venkateswarulu, T. C. (2020). Molecular docking and dynamic simulations for antiviral compounds against SARS-CoV-2: A computational study. *Informatics in Medicine Unlocked, 19,* 100345. https://doi.org/10.1016/j.imu.2020.100345

Raj, R. (2021). Analysis of non-structural proteins, NSPs of SARS-CoV-2 as targets for computational drug designing. *Biochemistry and Biophysics Reports, 25,* 100847. https://doi.org/10.1016/j.bbrep.2020.100847

Ramírez, D., & Caballero, J. (2018). Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? *Molecules, 23*(5), 1038. https://doi.org/10.3390/molecules23051038

Sampangi-Ramaiah, M. H., Vishwakarma, R., & Shaanker, R. U. (2020). Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease. *Current Science, 118*(7), 1087–1092. https://doi.org/10.18520/cs/v118/i7/1087-1092

Shaker, B., Yu, M. S., Lee, J., Lee, Y., Jung, C., & Na, D. (2020). User guide for the discovery of potential drugs via protein structure prediction and ligand docking simulation. *Journal of Microbiology, 58*(3), 235–244. https://doi.org/10.1007/s12275-020-9563-z

Singh, D., & Chaudhuri, P. K. (2018). A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). *Industrial Crops and Products, 118,* 367–382. https://doi.org/10.1016/j.indcrop.2018.03.048

Sisakht, M., Mahmoodzadeh, A., & Darabian, M. (2021). Plant-derived chemicals as potential inhibitors of SARS-CoV-2 main protease (6LU7), a virtual screening study. *Phytotherapy Research, 35*(6), 3262–3274. https://doi.org/10.1002/ptr.7041

Siva, M., Shanmugam, K., Shanmugam, B., Venkata Subbaiah, G., Ravi., S., Sathyavelu., K., & Mallikarjuna., K. (2016). *Ocimum sanctum*: a review on the pharmacological properties. *Int. J. Basic Clin. Pharmacol, 5,* 558–565. https://doi.org/10.18203/2319-2003.ijbcp20161491

Soni, A., & Sosa, S. E. (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *Journal of Pharmacognosy Phytochemistry, 2*, 22–29.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry, 31*(2), 455-461. https://doi.org/10.1002/jcc21334

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M., Li, X., Zheng, M., Chen, L., & Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica. B, 10*(5), 766–788. https://doi.org/10.1016/j.apsb.2020.02.008

Yuliana, D., Bahtiar, F. I., & Najib, A. (2013). *In Silico* screening of chemical compounds from roselle (*Hibiscus Sabdariffa*) as angiotensin-i converting enzyme inhibitor used PyRx Program. *ARPN Journal of Science and Technology, 3*(12), 1158–1160.