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In-Silico approach of the Mangrove Triterpenoids against SARS-CoV-2 Main protease (Mpro)

Ramamoorthy Purushothaman [1*], Ganapathy Vishnuram[2] and Thirugnanasambandam Ramanathan[3]

[1, 2, 3] Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608502, Tamil Nadu, India.
* Correspondence: purushothaman.r1997@gmail.com

Abstract: Aim: In the present study, we performed an *in-silico* study of the Triterpenoid compounds from Mangrove plant as potential COVID-19 main protease (Mpro) inhibitors, which are used as a potential medicine target. **Methods:** In this study we used Molecular Docking using Auto Dock software. **Results:** The binding energies obtained from the docking of 6LU7 with Beta-amyrin, Betulin, germanicol, taraxerol, lupeol, lupane, simiarenol, Tirucallol, Ursolic acid, Oleanolic acid, and Alpha-amyrin were -8.37, -8.73, -8.06, -7.71, -8.32, -8.49, -8.16, -8.99, -9.24, -8.87 and -8.89 kcal/mol, respectively. Further, these results were also confirmed by drug-likeness properties by using Swiss ADME software. **Conclusion:** This study showed Triterpenoid compounds seemed to have the best potential to act as COVID-19 Mpro inhibitors and they have a potential lead compound for the development of drugs, which can be used against the SARS-CoV-2.

Keywords: SARS-CoV-2; Molecular docking; 6LU7; Mangrove; Triterpenoids; Drug likeness

1. Introduction:

Coronavirus disease (COVID-19) is a highly contagious disease caused by Severe Acute Respiratory Syndrome - Coronavirus 2 (SARS-Cov-2), which is responsible for a respiratory infection affecting different parts of the respiratory system, especially lungs [1, 2]. Presently, there is no specific therapy for COVID-19 treatment [3]. In recent times, Computational aided drug design (CADD) methods have exposed great implications and these techniques are faster and cost-efficient [4]. In the drug discovery process, *In-silico* methods are used in the early stages of Drug discovery to avoid the hazard of time and cost [5]. The lead compounds from natural sources are considered to be less in side effects and are low-cost nutraceuticals to control the SARS-CoV-2 infection [6]. This study mainly focused on Triterpenoid compounds from Mangroves. Mangroves are small tree or shrub that grows in coastal brackish or saline waters in muddy or rocky soils. Mangroves are facultative halophytes, being salt-tolerant, and they can quickly adapt themselves to coastal environmental conditions [7]. It is increasingly familiar that mangrove plants are rich in natural products and new chemical compounds. Mangroves have been given a considerable extent of scientific importance worldwide as they are known for their potent activity against many diseases. More than 16% of Mangrove phytoconstituents are considered to be terpenoids. Triterpenoids are the most representative group of Phytochemicals composed of 30 carbon atoms, polymerized to form six isoprene units. Triterpenoids are widely distributed in nature. The range of triterpenes is highly related with their wide range of pharmacological effects. In Asian countries, triterpenes are traditionally used as anti-inflammatory, analgesic, hepatoprotective, cardiotonic, and sedative agents [8]. **In the present study, we investigated Triterpenoid compounds that were already reported its presence in mangroves as potential inhibitors for COVID-19 main protease Mpro using molecular docking**. These findings will be helpful in the process of drug development against COVID-19.

2. Materials and methods:

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2.1. Protein preparation:

The main protease of SARS COVID-19 is Mpro and 3D structure was obtained from Research Collaboratory for Structural Bioinformatics Protein databank (RCSB PDB) (https://www.rcsb.org/), in PDB format. PDB is a library for the crystal structures of biological macromolecules [9]. PDB ID: 6LU7

2.2. Ligand preparation:

The 3D structures of triterpenoid compounds from Mangroves were obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/), in SDF format. Triterpenoids compounds like Beta-amyrin (CID_73145), Betulin (CID_72326), Germanicol (CID_122857), Taraxerol (CID_92097), Lupeol (CID_259846), Lupane (CID_9548715), Simiarenol (CID_12442794), Tirucallol (CID_101257), Ursolic acid (CID_64945), Oleanolic acid (CID_10494) and Alpha-amyrin (CID_73170) were used in this study.

Drug-like properties were calculated using Lipinski's rule of five [10, 11] Adherence with Lipinski's rule of five as calculated using SWISSADME prediction (http://www.swissadme.ch/).

2.3. Molecular Docking

The study was supported by tools like Autodock tools, MGL tools, and Rasmol. The docking analyses were performed by Autodock, Pymol, and Biovia discovery studio.

3. Results:

3.1. Selection of Phytochemicals:

Triterpenoid (Ligands) and standard (Nelfinavir) of total 12 compounds have been selected, based on adherence to Lipinski's rule of five. That can be used in molecular docking experiments with the target protein (6LU7). The drug scanning results (Table 1) shows that all tested compounds in this study were accepted by Lipinski's rule of five. 2D diagrammatic representations (Table 1) of selected Triterpenoid compounds interact with the target protein Mpro. 2D Visualization of Docking analysis results, including the H-bonds that interact with 6LU7 amino acids, were mentioned in Table 1.

3.2. Molecular docking of selected compounds:

Table 2 shows the molecular docking analysis results for standard drug and 11 Triterpenoid compounds against main protease of SARS COVID-19 (6LU7). The binding energies obtained from the docking of 6LU7 with Nelfinavir, Beta-amyrin, Betulin, germanicol, taraxerol, lupeol, lupane, simiarenol, Tirucallol, Ursolic acid, Oleanolic acid and Alpha-amyrin were -6.21, -8.37, -8.73, -8.06, -7.71, -8.32, -8.49, -8.16, -8.99, -9.24, -8.87 and -8.89 kcal/mol, respectively. The binding visualization of 6LU7 and selected Triterpenoid compounds from Mangrove like Beta-amyrin, Betulin, germanicol, taraxerol, lupeol, lupane, simiarenol, Tirucallol, Ursolic acid, Oleanolic acid and Alpha-amyrin and Nelfinavir (Standard Drug) was represented in Figure 1 (A to L), as potential inhibitors of COVID-19 Mpro.

4. Discussion:

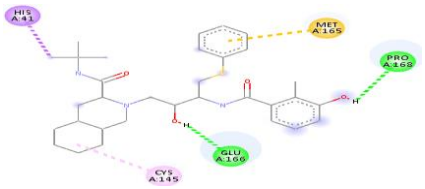
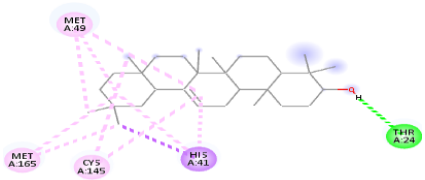
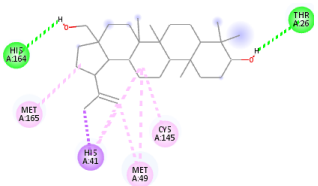
Nelfinavir forms many chemical bonds with 6LU7, including hydrogen bonds and hydrophobic bonds. **Several studies have recorded the abandunce of these triterpenoid compounds in Mangrove (Table 3)**. Selected Triterpenoid compounds from this study also forms many chemical bonds, similar to Nelfinavir. Therefore, the affinity of Ursolic acid bonds is higher compared with other compounds. The docking analysis in the present study showed the inhibition potential of several compounds, ranked by affinity (ΔG); Ursolic acid > Tirucallol > Alpha-amyrin > Oleanic acid >Lupane > Beta-amyrin > Germanicol > Simiarenol > Betulin> Taraxerol > Nelfinavir. Triterpenoid from Mangroves

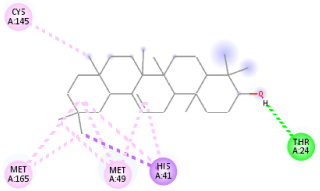
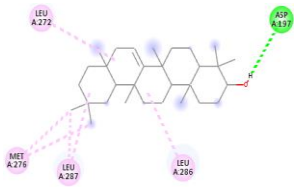
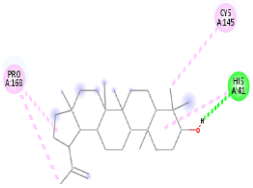
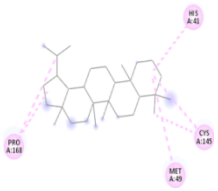
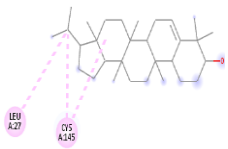
were the most recommended compounds as potential inhibitors of COVID-19 Mpro, which should be explored in future research.

5. Conclusions:

This study aimed to examine several Triterpenoid compounds from Mangroves that may be used to inhibit the COVID-19 infection pathway. Beta-amyrin, Betulin, germanicol, taraxerol, lupeol, lupane, simiarenol, Tirucallol, Ursolic acid, Oleanolic acid, and Alpha-amyrin have the good binding energies and inhibition constant. The affinity of Ursolic acid bonds is higher compared with other compounds. Therefore, Triterpenoids were the most recommended compounds found in Mangrove that may act as potential inhibitors of COVID-19 Mpro. However, further clinical trials must be investigated for the potential of the Triterpenoid compounds against viral infection for the development of drug from Mangroves followed by *in-vitro* and *in-vivo* studies.

Table 1. Properties of COVID-19 Mpro potential inhibitor candidates.

S. NO	Compound name	Molecular formula	Molecular Structure and interaction with 6LU7	Lipinski's rule of five	
				Properties	**Value**
1.	Nelfinavir	$C_{32}H_{45}N_3O_4S$		Molecular weight (<500 Da)	567.78 g/mol
				LogP (<5)	4.33
				H-Bond donor (5)	4
				H-bond acceptor (<10)	5
				Violation	1
2.	Beta amyrin	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	4.63
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
3.	Betulin	$C_{30}H_{50}O_2$		Molecular weight (<500 Da)	442.72 g/mol
				LogP (<5)	4.47
				H-Bond donor (5)	2
				H-bond acceptor (<10)	2
				Violation	1
4.	Germanicol	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	5.04
				H-Bond donor (5)	1

				H-bond acceptor (<10)	1
				Violation	1
5.	Taraxerol	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	4.73
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
6.	Lupeol	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	4.63
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
7.	Lupane	$C_{30}H_{52}$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	4.63
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
8.	Simiarenol	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	4.63
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
9.	Tirucallol	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	5.14

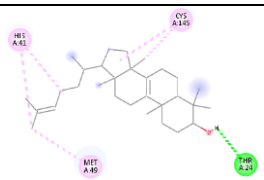
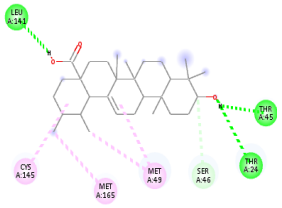
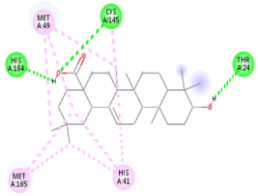
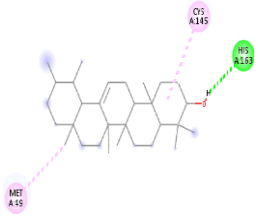
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
10.	Ursolic acid	$C_{30}H_{48}O_3$		Molecular weight (<500 Da)	456.70 g/mol
				LogP (<5)	5.82
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
11.	Oleanolic acid	$C_{30}H_{48}O_3$		Molecular weight (<500 Da)	456.70 g/mol
				LogP (<5)	5.82
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1
12.	Alpha-amyrin	$C_{30}H_{50}O$		Molecular weight (<500 Da)	426.72 g/mol
				LogP (<5)	6.92
				H-Bond donor (5)	1
				H-bond acceptor (<10)	1
				Violation	1

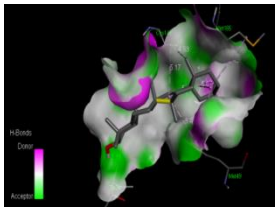
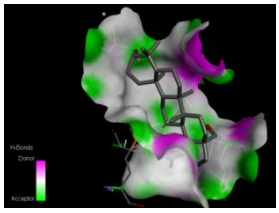
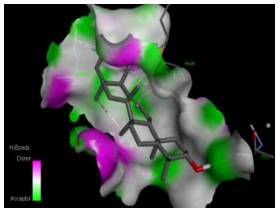
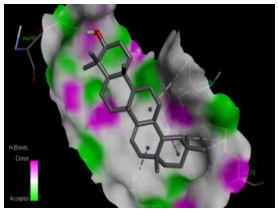
Table 2. Molecular docking analyses of Triterpenoid compounds against 6LU7.

Protein	**Ligand**	Lowest Binding Energy (Kcal/mol)	Ligand Efficiency	Inhibition Constant	Intermolecular Energy (kcal/mol)	VDW-H Bond Desolvation Energy (kcal/mol)
6lu7	**Nelfinavir**	**-6.21**	-9.709	27.83 uM	-9.79	-9.77
	Beta – amyrin	**-8.08**	-4.445	1.20 µM	-8.37	-8.33
	Betulin	**-7.54**	-6.161	2.96 µM	-8.73	-8.70
	Germanicol	**-7.76**	-4.229	2.04 µM	-8.06	-8.02
	Taraxerol	**-7.41**	-25.601	3.68 µM	-7.71	-7.43

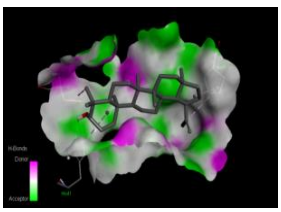
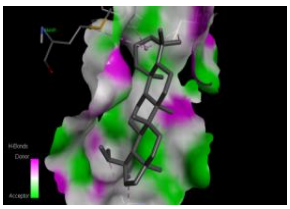
	Lupeol	**-7.73**	-10.786	2.17 µM	-8.32	-8.31
	Lupane	**-8.19**	-9.698	996.23 nM	-8.49	-8.48
	Simiarenol	**-7.56**	-10.006	2.87 µM	-8.16	-8.15
	Tirucallol	**-8.99**	-4.998	255.21 nM	-10.49	-10.46
	Ursolic acid	**-9.24**	-3.784	168.90 nM	-10.13	-10.05
	Oleanic acid	**-8.87**	-5.134	314.36 nM	-9.77	-9.69
	Alpha –amyrin	**-8.89**	-11.721	306.52 nM	-9.18	-9.14

Table 3. Triterpenoids compounds from Mangrove.

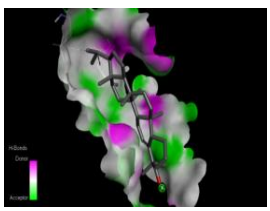
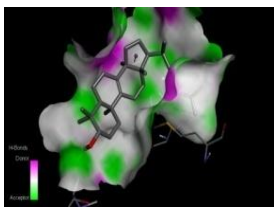
Compounds	**Species name**	**Parts**	**References**
Beta amyrin	*Rhizophora mucronata*	Bark	Rohini, R.M *et al*., 2009
Betulin	*Rhizophora mucronata*	Leaf	Ghosh A *et al.*, 1985
Germanicol	*Rhizophora sp*	Leaf	Koch, B.P *et al.*, 2003
Taraxerol	*Avicennia marina*	Root	Mahera, S.A *et al.*, 2011
Lupeol	*Rhizophora mucronata*	Bark	Rohini, R.M *et al*., 2009
Lupane	*Ceriops decandra*	Leaf	Ponglimanont, C. and Thongdeeying, P., 2005
Simiarenol	*Rhizophora mucronata*	Bark	Rohini, R.M *et al*., 2009
Tirucallol	*Excoecaria agallocha*	Leaf	Zou, J.H *et al.*, 2006
Ursolic acid	*Brugurera gymnorhiza*	Leaf	Ghosh, A *et al.*, 1985
Oleanic acid	*Acanthus ilicifolius*	Leaf	Ghosh, A *et al.*, 1985
Alpha-amyrin	*Ceriops decandra*	Leaf	Ghosh, A *et al.*, 1985

A **B**

C **D**

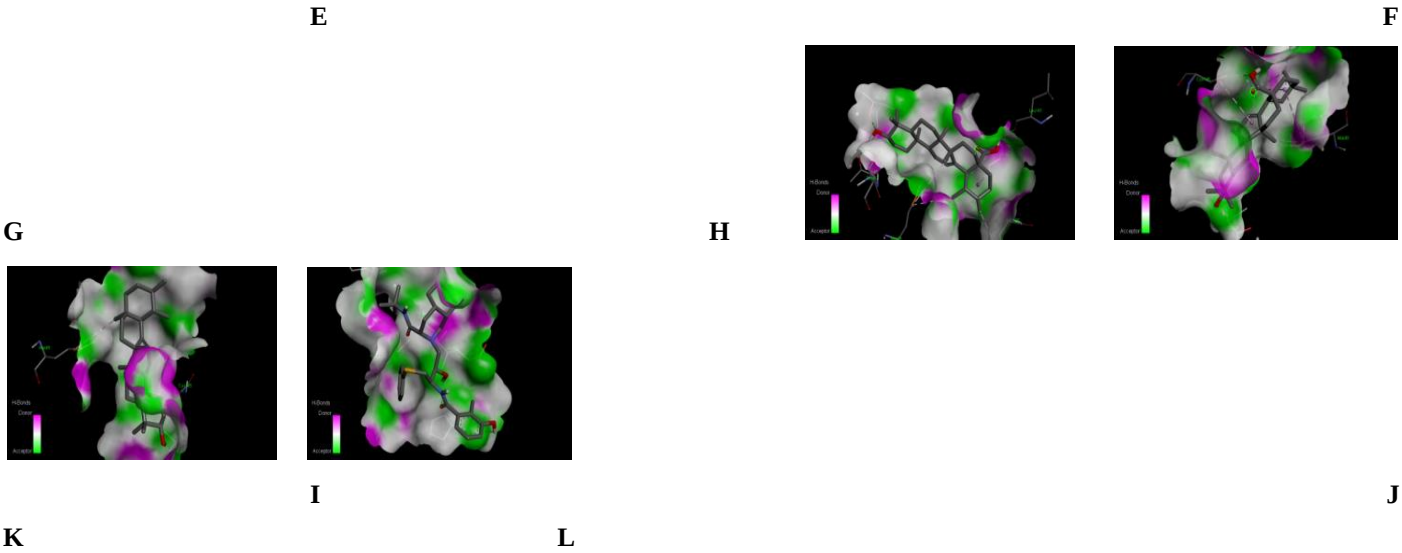


Figure 1. 3D visualisation of Docking analysis in 6LU7 binding with Beta-amyrin (A), Betulin (B), germanicol (C), taraxerol (D), lupeol (E), lupine (F), simiarenol (G), Tirucallol (H), Ursolic acid (I), Oleanolic acid (J), Alpha-amyrin (K) and Nelfinavir (L) using Biovia Discover Studio. The green and pink colour represents H-bonds Acceptor and Donor region respectively.

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Conflicts of Interest: The authors have no conflict of interest.

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