Research Paper

Inhibitory Potentials of Phytoconstituents of *Phyllanthus amarus* against Severe Acute Respiratory Syndrome-Corona Virus-2 Main Protease: A Computational Aided Approach

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Sree *et al.*: Potential Inhibitors of Severe Acute Respiratory Syndrome-Corona Virus-2 Main Protease from the Phytoconstituents of *Phyllanthus amarus*

In 2019, a new strain of Coronavirus disease was identified in Wuhan, China. Specific therapies are unavailable and investigations regarding Coronavirus disease treatment are lacking. The present study aimed to assess bioactive compounds found in medicinal plant *Phyllanthus amarus* as potential Coronavirus disease main protease inhibitors, using a molecular docking study. Molecular docking was performed using Autodock 4.2. Coronavirus disease main protease was docked with several compounds, and docking was analysed by Autodock 4.2, Lopinavir was used as standard for comparison. The binding energies obtained from the docking of 6LU7 with native ligand, lopinavir, Phyllanthin, Hypophyllanthin, Hexanedioic acid, bis (2-ethylhexyl) ester, Benzeneethanamine,3,4-dimethoxy-n-((pentafluoro phenyl) methylene), Phenethylamine, 2-methoxy-alpha.-methyl-4,5-(methylenedioxy), Diisooctyl adipate, P-Tert-Octylresorcinol, 4-methyl-2,5-dimethoxy phenethylamine, 3-(2,4-dimethoxy-phenyl)-2-formylamino-propionic acid, ethyl ester, Rutin, Quercetin and Niranthin were -6.08, -4.44, -4.14, -2.32, -4.23, -4.07, -2.5, -4.66, -4.14, -3.38, -7.46, -6.28 and -4.62 kcal/mol, respectively. Therefore, lopinavir may represent potential treatment option and Rutin and Quercetin appeared to have the best potential to act as Coronavirus disease main protease inhibitors. Thus all the compounds have shown significant binding energy and potent inhibitory effect against Coronavirus disease main protease. However, further research is necessary to investigate their potential medicinal use.

Key words: Coronavirus disease, main protease, 6LU7, *Phyllanthus amarus*, phytoconstituents, molecular docking

Coronavirus Disease (COVID-19) is an ongoing pandemic of COVID-19 which is caused by Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2). The outbreak of COVID-19 was first identified in Wuhan, china, in December 2019[1]. It is easily transmissible and spread worldwide. Common symptoms are including fever, muscle and body aches, cough, sore throat, loss of smell and shortness of breath. Symptoms may appear 5-6 d after infection but it may range from 2-14 d. Table 1 shows COVID-19 outbreak updates.

At present, specific therapies such as vaccine, specific antiviral drugs for COVID-19 are not available and research regarding the treatment of COVID-19 is going on. Some preliminary studies have investigated potential combinations that include the protease inhibitor lopinavir/ritonavir, which is commonly used to treat Human Immunodeficiency Virus (HIV)/ acquired immunodeficiency syndrome patients, for the treatment of COVID-19-infected patients[2]. Other reported antiviral treatments form human pathogenic Corona Viruses (CoV) include nucleoside analogues, neuraminidase inhibitors, remdesivir, umifenovir (arbidol), Tenofovirdisoproxil (TDF) and Lamivudine (3TC)[2]. However, the measures that have been implemented remain limited to preventive and supportive therapies, designed to prevent further complications and organ damage. To treat COVID-19 infections, some preliminary studies have investigated potential combinations that include anti-malarial

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TABLE 1: COVID-19 OUTBREAK UPDATES ON MAY 20, 2020

Place	Total cases	Recovered	Total deaths
World wide	5036 815	1990 057	326 251
India	106 750	42 298	3303
America	1576 950	364 263	93 858
England	248 818	344	35 341
Italy	226 699	129 401	32 169
Spain	278 803	196 958	27 778
France	180 809	62 563	28 239
China	82 965	78 244	4634
Russia	308 705	85 392	2972
Pakistan	45 898	13 101	985
Tamilnadu	12 448	4895	84
Puducherry	18	-	1

drug chloroquinone and anti-HIV vaccines. The Main protease (M^{pro})/3 Chymotrypsin Like protease (3CLpro) from COVID-19 represent a potential target for the CoV replication inhibition[2]. It was observed that genome of CoV encodes two proteins ppla and pplb which are involved in spike, membrane, envelop nucleoprotein, replicase and polymerase activity of viruses. This function is performed by M^{pro}/3CLpro[3].

The M^{pro} has 3 structural domains; domain I (residues 8-101) and domain II (residues 102-184) both have beta barrel motifs representing chymotrypsin catalytic domain and domain III (residues 185-200) with a helical structure participates in dimerization of protein and active enzyme production. M^{pro} is considered to be a suitable target for viral inhibitor development as an approach towards SARS. At present, natural compounds have served humans as cheaper and safer drug candidates against several diseases[4,5]. Most of the herbal drugs having antiviral properties which are used as supportive treatment for COVID-19 patients. *Phyllanthus amarus* (*P. amarus*) (Keezhkainelli) is one of the herbal drugs has got hepatoprotective properties, antioxidant and anti-inflammatory properties[6] and also antiviral properties against Hepatitis B virus and reverse transcriptase of retroviruses[7,8]. From the above light of information, it was thought worth to investigate the phytoconstituents of methanolic extract of *P. amarus* as potential inhibitor for COVID-19 M^{pro}.

MATERIALS AND METHODS

Ligand preparation:

The bioactive constituents used for docking were obtained from methanolic extract of *P. amarus* such as Phyllanthin, Hypophyllanthin, Hexanedioic acid, bis (2-ethylhexyl) ester, Benzeneethanamine,3,4-dimethoxy-n-(pentafluorophenyl) methylene), Phenethylamine, 2-methoxy-alpha.-methyl-4,5-(methylenedioxy), Diisooctyladipate, P-Tert-Octylresorcinol, 4-methyl-2,5-dimethoxy phenethylamine, 3-(2,4-dimethoxy-phenyl)-2-formylamino-propionic acid, ethyl ester, Rutin, Quercetin and Niranthin.

Ligands can be sketched using tools like Chemsketch or Marvinsketch. While selecting the ligand, the Lipinski rule of 5 should be applied (Table 2). The rule is very important for drug development where the pharmacologically active lead structure is optimized stepwise for increased activity and selectivity as well as drug like properties.

Protein preparation:

To investigate the phytochemical analogs of *P. amarus* against SARS-CoV-2 virus, we have selected COVID-19 3CLpro/M^{pro} (Protein Data Bank (PDB) ID: 6LU7)[9]. The structure of protein was obtained from PDB (https://www.rcsb.org/), in PDB format (fig. 1). PDB is an archive for the crystal structures of biological macromolecules, worldwide[10].

Active sites for 6LU7:

THR24, THR26, PHE140, ASN142, GLY143, CYS145, HIS163, HIS164, GLU166, HIS172.

Molecular docking studies:

Molecular docking was carried out for standard drug Lopinavir (anti-viral drug) and 12 phytochemical

constituents of methanolic extract of *P. amarus*. The phytochemical analogs were docked with COVID-19 3CLpro/M^{pro} (PDB ID: 6LU7) by using Autodock software with default settings and the grid box was defined based on trial and error and carried out in normal mode (fig. 2). The crystal structure of protein was obtained from protein data bank. The structures of phytochemical constituents were sketched by using Marvinsketch and the structures were converted into Three Dimensional (3D) images and saved as PDB format. Analysis of docking results was done with

TABLE 2: LIPINSKI RULE OF 5 FOR THE DOCKED PHYTOCONSTITUENTS

Compounds	Molecular weight	Log P	No. of atoms	H-bond donors	H-bond acceptors	Violations
Std	628.81	5.69	46	4	9	2
L1	418.53	3.92	30	0	6	0
L2	430.5	3.5	31	0	7	0
L3	370.57	7.36	26	0	4	1
L4	359.29	3.75	25	0	3	0
L5	209.25	1.2	15	2	4	0
L6	370.57	6.84	26	0	4	1
L7	222.33	4.5	16	2	2	0
L8	195.26	1.34	14	2	3	0
L9	281.31	1.55	20	1	6	0
L10	610.52	-1.06	43	10	16	3
L11	302.24	1.68	22	5	7	0
L12	432.51	3.95	31	0	7	0

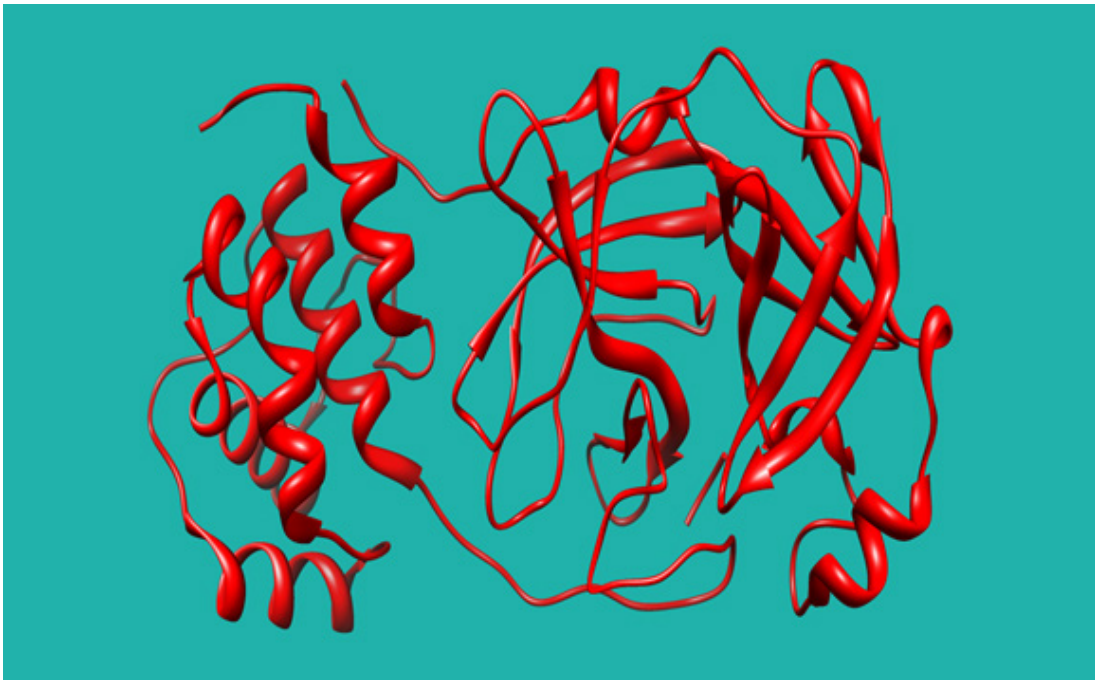

Fig. 1: Target protein (6LU7) for molecular docking

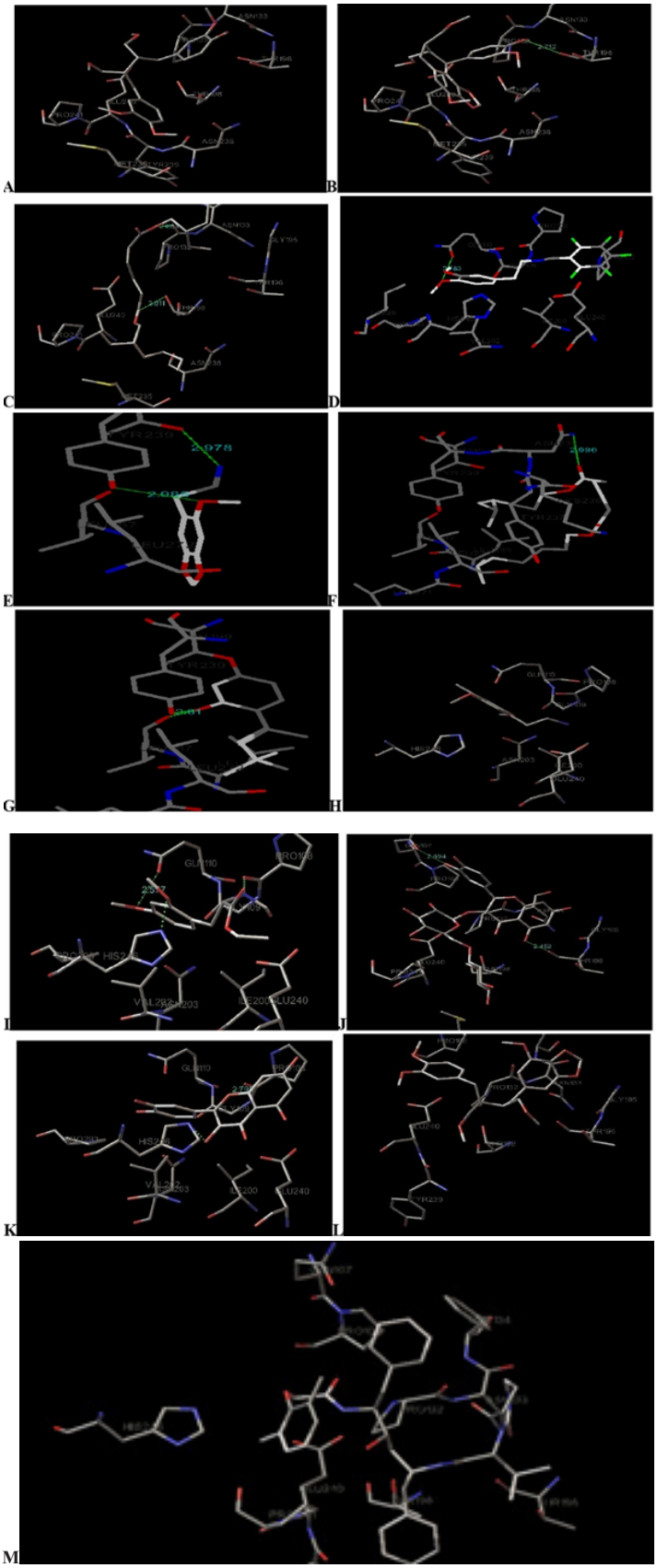

Fig. 2: Interaction of compounds with amino acid residues of M^{pro} COVID-19; (A): Phyllanthin; (B): Hypophyllanthin, (C): Hexanedioic acid, bis (2-ethylhexyl) ester, (D): Benzeneethanamine, 3, 4-dimethoxy-n-[(pentafluoro phenyl) methylene], (E): Phenethylamine, 2-methoxy-alpha.-methyl-4,5-(methylenedioxy), (F): Diisooctyladipate, (G): P-Tert-Octylresorcinol, (H): 4-methyl-2,5-dimethoxy phenethylamine, (I): 3-(2,4-dimethoxy-phenyl)-2-formylamino-propionic acid, ethyl ester, (J): Rutin, (K): Quercetin, (L): Niranthin, (M): Lopinavir

TABLE 3: MOLECULAR DOCKING ANALYSIS OF SELECTED PHYTOCONSTITUENTS AGAINST 6LU7

Compounds	Binding energy (kcal/mol)	Ligand efficiency	Inhibitory constant (μM)	Vanderwaals desolvation energy	Intermolecular energy
Std	-6.08	-0.13	34.74	-10.38	-10.56
L1	-4.44	-0.15	559.12	-8.2	-8.32
L2	-4.14	-0.13	919.66	-6.62	-6.53
L3	-2.32	-0.09	19.87	-8.01	-7.99
L4	-4.23	-0.17	794.62	-5.84	-6.02
L5	-4.07	-0.27	1.03	-4.62	-4.97
L6	-2.5	-0.1	14.82	-8.04	-8.16
L7	-4.66	-0.27	384.34	-5.91	-5.85
L8	-4.14	-0.3	920.17	-3.69	-5.34
L9	-3.38	-0.17	3.34	-6.03	-6.06
L10	-7.46	-0.17	3.4	-9.15	-9.25
L11	-6.28	-0.29	24.87	-6.48	-6.58
L12	-4.62	-0.15	407.56	-8.1	-8.2

Autodock Software and the results are compared with the standard drug and shown in Table 3.

RESULTS AND DISCUSSION

Computational biology means *in silico* investigation which is an essential arm of biotechnology focused at enhancing a deeper insight of bimolecular interactions in order to address cellular disease pathogenesis while having immense contribution towards design and development of possible therapeutic candidates[11]. Indeed, this technique has assisted in identifying lead compounds for various diseases[12].

The exponential increase in cases and mortality of COVID-19 has called for a need to develop drugs to treat this infection. Using *in silico* and molecular docking approaches, this study investigated the inhibitory effects of Pradimicin A, Lamivudine, Plerixafor and Lopinavir against SARS-CoV-2 M^{pro}[13]. Recent study on *Spondias mombim* L molecular docking of the studied enzymes with chlorogenic acid, lutein and zeaxanthin were carried out using PatchDock. SMEAF had remarkable enzyme inhibitory effects against Phosphodiesterase-5 (PDE-5), arginase, Angiotensin I-Converting Enzyme (ACE), cholinesterase, Monoamine Oxidase A (MAO), Ecto-5' Nucleotidase (E-NTDase), tyrosinase and stimulated Sodium (N^+)-Potassium (K^+) Adenosine-Triphosphatase (ATPase) activities[14].

On the other hand, the emergence and sporadic spread of COVID-19 has called for the urgent identification of novel drug and repurposing of existing available chemotherapeutic agents against this pandemic. Corona viruses have a long history of infecting animals and humans and causing respiratory, digestive, liver and central nervous system diseases in them[15]. Currently, COVID-19 has emerged in the human population and is a potential threat to global health, worldwide[15]. The virus polyprotein encodes two proteases, the 3CLpro (M^{pro}) and a papain-like protease; both are vital targets for drug discovery platforms against coronaviruses. Accordingly, the M^{pro} is the most probable antiviral candidate due to its crucial role in self-maturation and ensuing development of polyproteins[16].

However, no approved drug currently exists to treat the disease. The currently available drugs for COVID-19 treatment primarily act on the M^{pro}. The aim of this study was to examine the phytoconstituents of medicinal plant *P. amarus* that may be used to inhibit the COVID-19 infection pathway. Table 2 shows that Lipinski's rule of five for tested compounds. As per docking score obtained in energy kcal/mol as shown in Table 3. The highest docking score was obtained for the derivative L_{10} with score of -7.46 kcal/mol followed by compound L_{11} with score of -6.28 kcal/mol. Our study revealed that all the phytoconstituents present in

P. amarus had satisfactory binding affinity and potent inhibitory effect for the SARS-CoV-2 M^{pro} (fig. 3). The docking analysis in the present study showed the inhibition potential of several compounds, ranked by affinity (ΔG); Rutin (L10)>Quercetin (L11)>Lopinavir (Std)>P-Tert-Octylresorcinol (L7)>Niranthin (L12)>Phyllanthin (L1)>Benzeneethanamine, 3,4-dimethoxy-n-((pentafluoro phenyl) methylene) (L4)>Hypophyllanthin (L2)>4-methyl-2,5-dimethoxy phenethylamine (L8)>Phenethylamine, 2-methoxy-alpha.-methyl-4,5-(methylenedioxy) (L5)>3-(2,4-dimethoxy-phenyl)-2-formylamino-propionic acid, ethyl ester (L9)>Diisooctyladipate (L6)>Hexanedioic acid, bis (2-ethylhexyl) ester (L3). Table 4 shows that hydrogen bond formation and its distance and amino acid involved in H-bond interactions. However, further

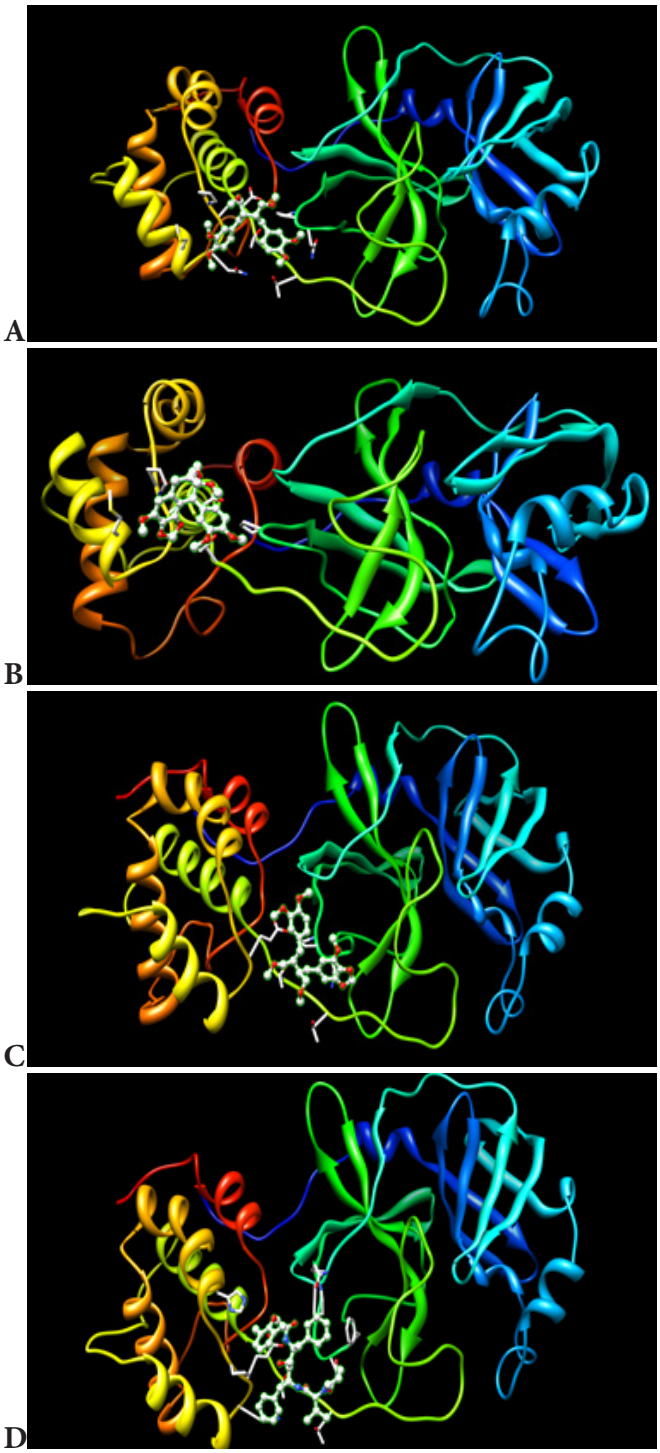

Fig. 3: Binding mode of compounds in SARS-COV-2 mainprotease; (A): Phyllanthin, (B): Hypophyllanthinin, (C): Niranthin, (D): Lopinavir

TABLE 4: HYDROGEN BOND FORMATION AND ITS DISTANCE AND AMINO ACID INVOLVED IN H-BOND INTERACTIONS

Compounds	No. of hydrogen bonds formed	Amino acid involved in hydrogen bond interactions	Distance between donor and acceptor (A˚)
Std	0	-	-
L1	0	-	-
L2	0	-	-
L3	2	THR198	2.811
L4	1	GLN110	2.783
L5	2	THR199;TYR239	2.985; 2.978
L6	1	ASN238	2.996
L7	1	TYR239	2.61
L8	0	-	-
L9	1	GLN110	2.577
L10	1	GLN107	2.694
L11	0	-	-
L12	0	-	-

research is necessary to investigate the potential uses of the phytoconstituents of *P. amarus* plant.

Conflict of interest:

The authors declared no conflict of interests.

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