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Research Article

IN SILICO SCREENING AND MOLECULAR DOCKING OF PHYTOCHEMICALS OF *TERMINALIA ARJUNA* AND *TERMINALIA CHEBULA* AGAINST THE COVID-19 MAIN PROTEASE

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ABSTRACT

The exponential increase in coronavirus infection has created a catastrophe. India is witnessing a very difficult period because all ages, including young people and children, are affected by the second wave. The overburdened Medicare system in India, encounters acute shortages of medicines, oxygen and vaccines for health facilities. The main aim of this study was to develop new, highly stable SARS-CoV-2 main protease (M^{pro}) inhibitors. We have screened the phytochemical compounds present in the fruit of *Terminalia chebula* and bark of *Terminalia arjuna* as inhibitors of the main protease of the severe acute respiratory coronavirus 2019 (SARS-CoV-19). (6LU7). The research was carried out with the aid of AutoDock VINA. The results revealed that all-natural molecules examined were perceived in the active binding site with sizable binding energy.

Keywords: Corona virus, *Terminalia arjuna*, *Terminalia chebula*, Molecular docking, SARS-CoV-2, COVID-19 M^{pro}.

1. INTRODUCTION

SARS-CoV-2 is a positive-sense, single-stranded RNA beta-corona virus with a 30 kilo base genome that encodes viral proteins in up to 14 open reading frames [1, 2]. SARS CoV-2 includes 4 structural proteins, namely envelope (E), spike (S), membrane (M), and nucleocapsid (N). The S, M, and E structural proteins form the covering case of the corona virus. As M protein is more abundant, shape of the outer envelope is determined by M protein (M^{pro}). During replication process of the corona virus, the transmembrane proteins (S and M) play a dominant role. Using the spike protein, SARS-CoV-2 virus gets attached to angiotensin-converting enzyme 2 (ACE2) and hence ACE2 becomes a receptor for the COVID-19 virus [3]. In the replication process of the corona virus, the main protease plays a principal role [4]. Therefore, the M^{pro} is our prime drug target to stop duplication process of the virus.

Molecular docking is a structure-based drug design approach. It is commonly used due to its ability to project ligand binding configurations to the appropriate target binding site. Characterizing binding behaviour is critical for both judicious drug design and understanding basic biochemical processes [5]. The primary purpose of molecular docking is to achieve a ligand-receptor complex with enhanced conformation and lower binding free energy. The binding free energy is limited by a variety of factors, such as the hydrogen bond, electrostatic energy, torsional free energy, dispersion, repulsion, desolvation, total internal energy, and the energy of its unbound system. As a result, the expected binding free energy provides information on the existence of various types of interactions that are involved in molecular docking [6].

Natural phytochemicals extracted from plants have the ability to cure and prevent a variety of diseases. The rich biodiversity of medicinal plants makes them a gold mine for discovering novel compounds that can be used as medicines or as pilot molecules for the development of new drugs with various mechanisms of action. Terminalia is a genus of about 200-250 species in the Combretaceae family [7]. Terminalia genus plants are found primarily in tropical regions, with Southeast Asia having the highest genetic diversity. Different plant parts of Terminalia species, *T. arjuna* and *T. chebula*, among others, have been used in Ayurveda since ancient times. Due to the presence of a diverse range of phytochemical constituents in *T. arjuna* and *T. chebula,* these species have a variety of medicinal properties. Since antiquity, the fruit of *T. chebula* has been used as traditional medicine for household remedy against a

variety of human ailments. *T. chebula* has been widely used in Ayurvedic, Unani, and Homoeopathic medicines and has become a cynosure of modern medicine [8]. Main compounds among tannins found in *T. chebula* fruit are Terflavin A, Punicalagin, Chebulagic acid, Chebulinic acid, Corilagin, Casuarinin, Tercatain, Gemin, Tellimagrandin, Punicacortein C, Punicacortein D, Chebulic acid, Neochebulagic acid, Eschweilenol C, Phyllanemblinin E, Phyllanemblinin F [9-11]. The phenolic acid derivative Ellagic acid and the Flavonoids Rutin, Quercetin and Isoquercetin are also present in *T. chebula* fruit [12].

The bark of *T.arjuna* is used as an astringent, cardio tonic, demulcent, anti-dysenteric, expectorant, urinary astringent, styptic, and also used to cure fracture, leukorrhea, ulcers, diabetes, cardiopathy, anaemia, and cirrhosis [13]. The major Triterpenoids found in the bark of *T. arjuna* are Arjunin, Arjunic acid, Arjungenin, Terminic acid, Terminoltin, and Arjunolic acid. The bioactive tannins Punicalin, Castalagin, Casuariin, Casuarinin, Punicalagin, Terchebulin, and Terflavin C are found in the bark of *T. arjuna*. *T. arjuna's* bark also contains the glycosides Arjunetin, Arjunolone, Terminoside A, Termionic acid, and the flavonoids Arjunone, Baicalein, Pelargonidin, and Kempferol [14].

2. MATERIAL AND METHODS

From RCSB PDB repository [15] the 3D structure of the SARS-CoV-2 M^{pro} (6LU7) was downloaded and processed in AutoDock Tools [16]. The water, solvent molecules and the bound ligand were removed and then further processed with the addition of partial charges and polar hydrogens. The prepared structure was saved in AutoDock PDBQT format.

The ligand perception by any protein depends on 3-dimensional orientation and electrostatic interaction. Thus ligand preparation plays a vital role on the docking results. Molecules are in the ionized state in physiological conditions. But in databases molecules are stored in neutral forms. So before initiating docking process, it is essential to ionize the molecules by adding charges. The ligand molecules were downloaded from National Library of Medicine-PubChem [17] as sdf file. Using Open Bablel software, the ligand molecules were optimized by applying MM2 force field method and convert the sdf files into pdb file format.

AutoDockVina is used to identify the binding modes of phytochemical molecules with the target protein. Because of parallel computing performance and hybrid scoring function we have used AutodockVina for Molecular docking. Moreover, to confirm actual binding interaction with targets blind docking was performed and the best conformers were represented with lowest binding energy (-kcal/mol) which show way to disclose the mode of actions of these ligands. The docking parameters were defined as coordinates of the center of binding site with x=126, y=126, z=126 and binding radius = 0.531Å. Pymol and Discovery studio were used to investigate the docking poses and analyze the interactions of protein and ligand.

3. RESULTS AND DISCUSSION

All of the phytochemicals of *T. arjuna* and *T. chebula* studied, had a better interaction and a higher docking score. Table 1 lists the binding energies of the phytochemicals of *T. arjuna* and *T. chebula*. Most of the ligands formed several hydrogen bonds with the main protease M^{pro}. Hydrophobic, pi-cation, and pi-anion bonds were formed by these ligands when they interacted with M^{pro}. Re-docking was carried out to ensure precision and to find a better docking pose.

Sl. No	Ligands	Compound class	Plant and Part	Binding Affinity (kcal/mol)	Hydrogen-Binding Interaction
1	Arjunin	Triterpenoid	*T. arjuna* (Bark)	-9.9	ARG131, ASN238, LEU272, LEU287, TYR237
2	Punicallin	Tannin	*T. arjuna* (Bark)	-9.8	ASN142, CYS145, LEU141, SER144
3	Terflavin A	Tannin	*T. chebula* (Fruit)	-9.8	ASN151, ASP153, ASP245, LYS102, GLN110
4	Punicalagin	Tannin	*T. arjuna* (Bark), *T. chebula* (Fruit)	-9.7	LEU272, TYR237
5	Castalagin	Tannin	*T. arjuna* (Bark)	-9.5	ASN238, ASP197, LEU272, LEU287, MET276, THR199
6	Casuariin	Tannin	*T. arjuna* (Bark)	-9.2	ASP289, ASP197, THR199, TYR237, THR199

7	Terchebulin	Tannin	*T. arjuna* (Bark), *T. chebula* (Fruit)	-9.2	GLU288, GLY170, LYS5, PHE140
8	Eschweilenol C	Tannin	*T. chebula* (Fruit)	-9.1	LEU287, LYS137, THR199
9	Tercatain	Tannin	*T. chebula* (Fruit)	-9.1	ASN238, GLU290, LEU271, LYS137, LYS236, TYR239
10	Terflavin C	Tannin	*T. arjuna* (Bark)	-9	GLN189, GLU166, HIS41, LEU141, MET49, THR24, THR190
11	Casuarinin	Tannin	*T. arjuna* (Bark), *T. chebula* (Fruit)	-8.9	ARG105, ASN203, GLY109, GLN110, HIS246, PRO108
12	Neochebulagic acid	Tannin	*T. chebula* (Fruit)	-8.8	ASN238, ASP289, LEU287, LYS137, LYS236, THR199, TYR239
13	Phyllanemblinin E	Tannin	*T. chebula* (Fruit)	-8.8	GLY143, GLU166, HIS163, HIS172, PHE140, SER46, SER144, THR26, THR45
14	Punicacortein C	Tannin	*T. chebula* (Fruit)	-8.8	ALA285, LEU272, LEU287, TYR237, TYR239
15	Gemin	Tannin	*T. chebula* (Fruit)	-8.7	ARG188, CYS145, GLU166, HIS163, HIS164, MET165, PHE140, THR190
16	Ellagic acid	Phenolic derivative	*T. chebula* (Fruit)	-8.6	ASP295, GLN110, THR111, THR292
17	Isoquercetin	Flavonoid	*T. chebula* (Fruit)	-8.6	ASN142, GLU166, HIS41, HIS164, MET165, SER144
18	Rutin	Flavonoid	*T. chebula* (Fruit)	-8.6	CYS145, THR26
19	Chebulagic acid	Tannin	*T. chebula* (Fruit)	-8.5	LEU271, LEU 287, THR199
20	Corilagin	Tannin	*T. chebula* (Fruit)	-8.5	ARG131, ASP197, ASP289, LEU287
21	Arjunetin	Glycosides	*T. arjuna* (Bark)	-8.4	LYS137
22	Chebulinic acid	Tannin	*T. chebula* (Fruit)	-8.1	ARG131, ASN238, LEU271, LEU287, LYS137
23	Tellimagrandin I	Tannin	*T. chebula* (Fruit)	-8	GLN110, PHE294, SER158, THR111
24	Arjunic acid	Triterpenoid	*T. arjuna* (Bark)	-7.9	ASP289
25	Baicalein	Flavonoid	*T. arjuna* (Bark)	-7.9	GLU166, GLY143, LEU141
26	Terminic acid	Triterpenoid	*T. arjuna* (Bark)	-7.9	ARG131
27	Arjungenin	Triterpenoid	*T. arjuna* (Bark)	-7.7	ARG131, THR199
28	Arjunolic acid	Triterpenoid	*T. arjuna* (Bark)	-7.6	ARG131

The molecular docking results revealed that ligand Arjunin top ranked among the phytochemicals of *T. Arjuna* and *T. Chebula* investigated. Arjunin showed a binding energy of -9.9 kcal/mol and formed five hydrogen bond with the amino acid residues TYR237, LEU287, LEU272, ARG131 and ASN238. It formed a Pi-Cation bond with residue LYS137 and a Pi-Cation bond with residue LYS137. The interaction is given in Fig. 1.

The tannin, Punicalin interacted strongly with the main protease M^{pro} with a binding energy of -9.8 kcal/mol by the formation of hydrogen bonds with residuesLEU141, SER144, ASN142 and CYS145. It also interacted with the residues GLN189, GLU166, PRO168, ASN142 through the formation of carbon hydrogen bond, Pi-Donor Hydrogen Bond, Pi-Alkyl and Pi-Sigma respectively. Fig. 2 represents the interaction between M^{pro} and Punicalin.

The tannin, Terflavin A also interacted with a binding energy of -9.8 kcal/mol with residues ASP153, ASP245, LYS102, GLN110 and ASN151 of M^{pro} through hydrogen bonds. It has formed Carbon Hydrogen, Pi-Sigma, Pi-Pi Stacked and Pi-Alkyl bonds with residues PRO293, ILE249, PHE294, and VAL104 respectively. The interaction is shown in Fig.3.

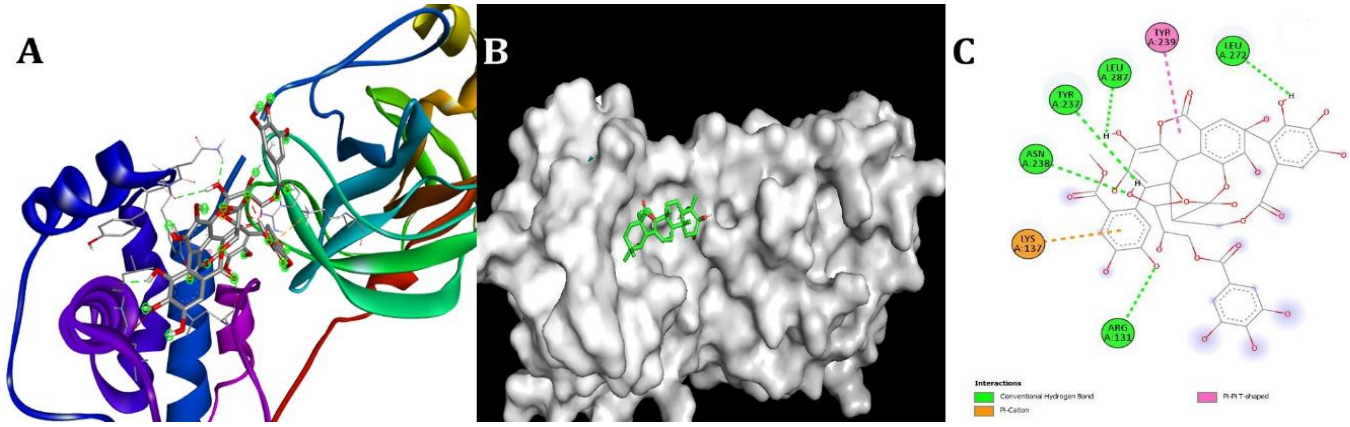

Fig. 1: Arjunin docked in Covid-19 main protease M^{pro} (PDB ID 6LU7) with (a) Amino acid residues involved in interaction (with ligand as grey sticks), (b) Best binding mode in the cavity of protein (with ligand as green color sticks) and (c) Binding interaction of Arjunin with amino acid with hydrogen bond (green dash line).

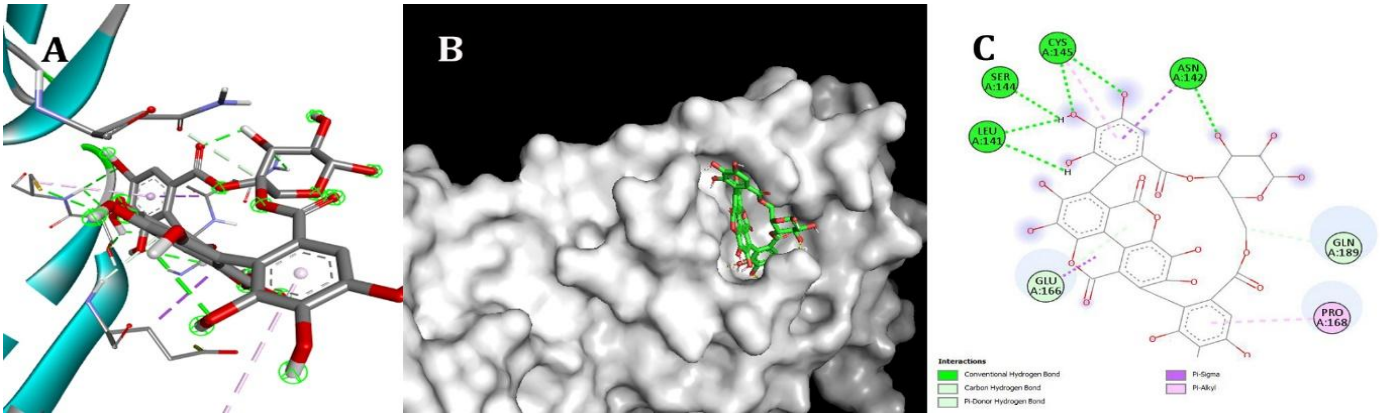

Fig. 2: Punicalin docked in Covid-19 main protease M^{pro} (PDB ID 6LU7) with (a) Amino acid residues involved in interaction (with ligand as grey sticks), (b) Best binding mode in the pocket of protein (with ligand as green color sticks) and (c) Binding interaction of Punicalin with amino acid with hydrogen bond (green dash line).

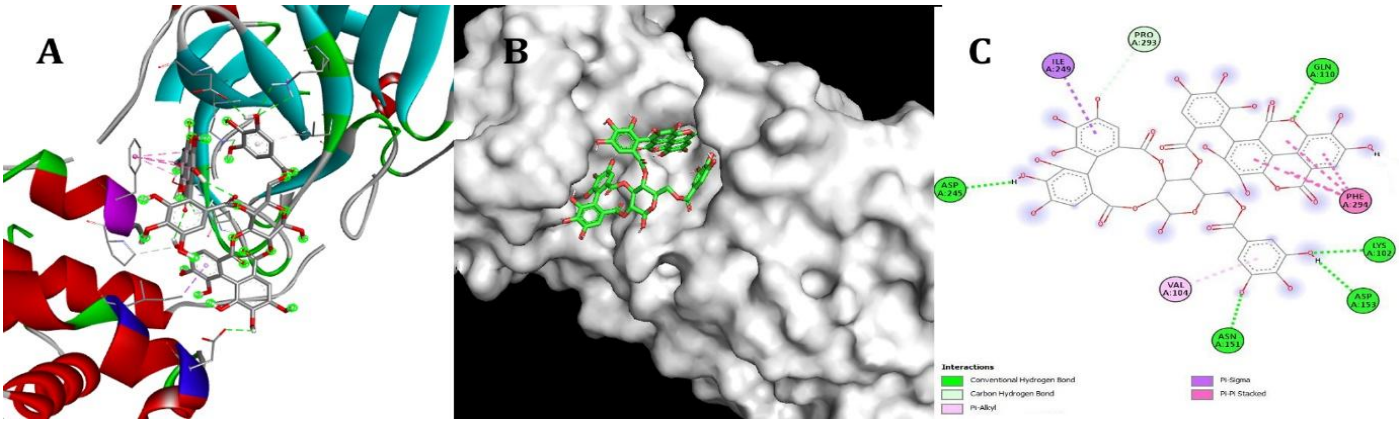

Fig. 3: Terflavin A docked in Covid-19 main protease M^{pro} (PDB ID 6LU7) with (a)Amino acid residues involved in interaction (with ligand as grey sticks), (b) Best binding mode in the cavity of protein (with ligand as green color sticks) and (c) Binding interaction of Terflavin A with amino acid with hydrogen bond (green dash line).

Arjunetin, Castalagin, Casuariin, Casuarinin, Chebulagic acid, Chebulinic acid, Corilagin, Ellagic acid, Eschweilenol C, Gemin, Isoquercetin, Methyl Neo chebulagate, Neochebulagic acid, Phyllanemblinin E, Punicacortein C, Punicalagin, Rutin, Tellimagrandin I, Tercatain, Terchebulin and Terflavin C strongly interacted with M^{pro}. The abundance of the above bioactive phytochemicals in the bark and fruit of *T. arjuna* and *T. chebula* trees, as well as their bioavailability in human bodies, will determine their efficacy in combating covid-19.

4. CONCLUSION

The main protein protease M^{pro} is responsible for the COVID-19 virus's duplication phenomenon. All of the ligands tested had a high affinity for the target protein and formed several hydrogen bonds with it. The binding poses of the phytochemicals of *T. arjuna* tree's bark and *T. chebula* tree's fruit have been analyzed by MD simulations. These natural compounds may work in tandem with pharmacological treatments to combat the new corona virus. These phytochemicals have the ability to treat Covid-19 and its real efficiency can be ascertained by its in-vitro, in-vivo and clinical studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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