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Research Article

COMBATING SARS-COVID-19 WITH LIGNANS OF JUSTICIA SPECIES: AN *IN SILICO* STUDY

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ABSTRACT

Coronavirus infection has spread worldwide and is posing a public health crisis on a global scale. Even though the World Health Organization first claimed to have complete control over the virus, the infection has taken the lives of more than 35 lakh people across the globe. The devastating effect of coronavirus is currently causing outrage in India. In the midst of the crisis, numerous research investigations are being conducted around the world. Various treatments, vaccines, clinical testing, and the synthesis of novel chemical entities are all being looked into. Current research is entirely focused on *in-silico* methods such as virtual screening, molecular docking, and calculating molecular properties. We have screened the lignans found in the Justicia species 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, Lignans, Molecular docking, SARS-CoV-2, *Justicia Procumbens*, AutoDock VINA.

1. INTRODUCTION

The Severe acute respiratory coronavirus, 2019 (SARS-CoV-2) contains four structural proteins, namely Spike (S) protein, membrane (M) protein,envelope (E) protein and nucleocapsid (N) protein [1]. The SARS-CoV-2 genome consists of more than 30000 nucleotides that encode about 29 proteins. Important proteins among them are spike protein, 3Clike protease ($3CL^{pro}$, M^{pro}), Papain-like protease (PL^{pro}), RNA-dependent RNA polymerase (RdRp), and nucleocapsid (N) protein [2]. The main protease and papain-like protease are involved in the viral replication process [3]. The main protease 6LU7 is a 3CLpro monomer and it consists of three domains [4]. Domains I and II are made up of β-barrels that form a chymotrypsin structure and contain the catalytic dyad histidine 41 (His41), and cysteine 145 (Cys145). Domain III consists of α-helices [5]. By binding antiviral drugs to the active site of the main protease M^{pro}, the protease activity can be inhibited and the synthesis of virions can be blocked. Therefore, the M^{pro} is our prime drug target to stop duplication process of the virus.

The study of interaction of the ligand with the protein molecule at the active site is known as molecular docking. The active site is a three-dimensional location on a protein molecule that has a direct impact on the protein's activity. Binding affinity is a measurement of how well ligands interact with receptor binding sites. The lower the binding affinity, the more efficiently it binds to the receptor at the desired location.

With over 600 species, Justicia is the largest genus in the Acanthaceae family. Justicia species contain a wide range of chemical classes, primarily alkaloids, lignans, flavonoids, and terpenoids. A wide range of lignans have been isolated from Justicia species. Lignans are a class of natural products with a wide range of biological effects. Lignans are polyphenols with low molecular weight. Lignans are phytoestrogen precursors and secondary plant metabolites which are formed by oxidative dimerization of two phenylpropanoids. Lignans are classified into eight classes and they are aryltetralin, arylnaphthalene, dibenzylbutane, dibenzyl-butyrolactone, dibenzocyclooctadiene, dibenzylbutyro-lactolfurofuran, and furan. Lignans could be used as lead compounds for the creation of novel cytotoxic therapeutics [6]. Lignans have antiangiogenic, anti-asthmatic [7], antileishmanial, antifungal, antiviral [8], antineoplastic [9], hypolipi-demic, antifeedant [10], antidepressant [11], insecticidal, cardiotonic, anti-inflammatory [12], analgesic, antiplatelet [13] properties.

Lignan Helioxanthin isolated from *Justicia flava* (J.flava) inhibits human hepatitis B viral replication and used in the treatment of HIV/AIDS in Uganda [14]. Lignans Diphyllin, Justicidin A, Justicidin B, Justicidinoside A, Justicidinoside B, Justicidinoside C, Diphylinapioside and Diphyllin apioside-5-acetate isolated from Justicia species shows antiviral activities [8]. The lignans Cilinaphthalide A, Diphyllin, Diphylinapioside, Diphyllin apioside-5-acetate, Justicidin A, Justicidin B, Justicidin E, Patentiflorin A, Patentiflorin B, Procumbenoside A, Tuberculatin and Taiwanin E, have antitumor activities [15]. Elenoside belonging to arylnaphthalene class of lignan, isolated from *Justicia hyssopifolia* (*J. hyssopifolia*) is the most pharmacologically probed lignan in the genus Justicia. Elenoside have muscle relaxant, sedative, cytotoxic, cardiotonic, antiviral, insecticidal, analgesic, lipid peroxidation inhibitory, anti-inflammatory, and stimulant activities [12]. As the lignans of Justicia species show widespread biological activities, we have preformed the molecular docking studies with these lignans as inhibitors of the main protease of the severe acute respiratory coronavirus 2019.

2. MATERIAL AND METHODS

From RCSB PDB repository [16] the 3D structure of the SARS-CoV-2 M^{pro} (6LU7) was downloaded and processed in AutoDock Tools [17]. 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 Nation Library of Medicine-PubChem [18] as sdf file. Using Open Bablel software, the ligand molecules were optimized by applying MM2 force field method and converted the sdf files into pdb file format.

AutoDock Vina 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 Autodock Vina 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. During the docking process, the ligands were assumed to be flexible while the protein was considered to be rigid. The Auto Grid engine in Pyrex was used to generate the grid parameters. Pymol and Discovery studio were used to investigate the docking poses and analyze the interactions of protein and ligand.

3. RESULTS AND DISCUSSION

The top 20 lignans with the best docking score are presented in Table 1. On the basis of their score values, these molecules were ranked. All the lignans formed several hydrogen bonds with the main protease M^{pro}. Hydrogen bond interaction includes the formation of Conventional Hydrogen Bond, Pi-Donor Hydrogen Bond and Carbon Hydrogen Bond. Hydrophobic interaction includes Alkyl, Pi-Alkyl, Amide-Pi Stacked, Pi-Pi T-shaped and Pi-Sigma bonds formation. Re-docking was carried out to ensure precision and to find a better docking pose.

Table 1: Lignans with the best docking score

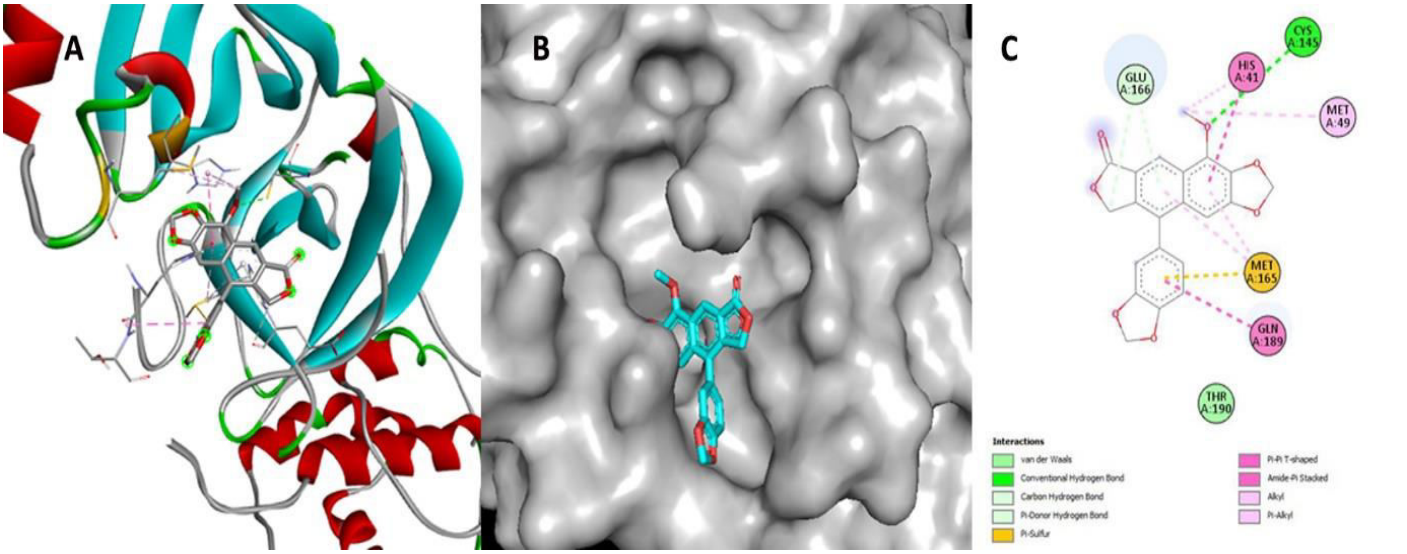
Sl. No.	Name of compound	Binding Energy	Hydrogen Bond	Hydrophonic Bond	Justicia species
1	Justicidin G	-9.1	CYS145, GLU16	HIS41, GLN189, THR190, MET49, MET165,	*J. neesii* [19]
2	Elenoside	-9	ASN142, HIS164, CYS145	HIS41, MET165	*J. hyssopifolia* [20]
3	Justalakonin	-9	THR26, GLY143, GLU166, ASN142	HIS41, CYS145, MET149	*J. purpurea* [21]
4	Neojusticin A	-8.9	GLU166	HIS41, GLN189, THR190, MET 165	*J. procumbens* [6]
5	Taiwanin C	-8.9	GLY143, HIS41, CYS145	MET49, HIS41	*J. procumbens* [22]

6	Taiwanin E	-8.8	GLY143, GLU166, ASN142	HIS41, CYS145, MET49, CYS145	*J. procumbens* [23]
7	Taiwanin E methyl ether	-8.8	GLY143, GLU166, ASN142	HIS41, CYS145, MET49	*J.betonica* [24], *J.procumbens* [23], *J.purpurea* [21]
8	Tuberculatin	-8.8	THR190, HIS41, GLU166	MET49, CYS145, MET165	*J. ciliate, J.betonica* [24]
9	Justicidin E	-8.7	HIS41, GLY143, CYS145	CYS145, HIS41	*J. procumbens* [6]
10	Justicinol	-8.7	ASN142, LEU141	HIS41, MET165	*J. patentiflora* [25]
11	juspurpurin	-8.6	HIS41, GLY143, CYS145, THR45		*J. purpurea* [21]
12	Justicidone	-8.6	HIS163, PHE140, GLN189	MET165	*J. hyssopifolia* [20]
13	Patentiflorin B	-8.6	THR199, LEU287, LEU287	TRY237	*J. patentiflora* [25]
14	diphyllin apioside-5-acetate	-8.5	GLY143, PRO168,THR190 ,GLN189, GLU166	MET165	*J. procumbens* [8]
15	Jusmicranthin	-8.4	HIS163, MET165	MET49, CYS145, MET49, MET165, HIS41, HIS163	*J. neesii* [26]
16	Patentiflorin A	-8.4	CYS145, HIS163, THR190, GLU166	GLU166	*J. patentiflora* [25]
17	Prostalidin A	-8.4	CYS145, GLU166	HIS41, GLN189, THR190, MET165, MET149, CYS145	*J. prostrate* [11]
18	Justisolin	-8.3	LEU141, SER144, HIS41, CYS145, THR45		*J. simplex* [27]
19	Diphyllinapioside	-8.2	CYS145,PHE140, GLU166	GLN189,MET165	*J. procumbens* [8]
20	Helioxanthin	-8.1	HIS41, ASN142, THR26, GLU166	HIS41, MET49, CYS145	*J. flava* [14]

The main protease of SARS-CoV-2 M^{pro}, has an active binding site that is predominantly positioned in a hydrophobic cleft and incorporates Cys145 and His41 [28]. In these active sites of the protease, CYS145 and HIS41 amino acid residues interact to form a catalytic (base-nucleophilic) dyad. It is reported that the inhibition of the SARS-COV M^{pro} is mainly due to an irreversible covalent bond by Cys145, or by reversible interaction with Cys145 and His41. His41 polarises and deprotonates the nucleophile (Cys145) to increase its reactivity [29]. The inhibitor attacks Cys145 by building an intermediate complex before release the enzyme or forming directly an irreversible complex.

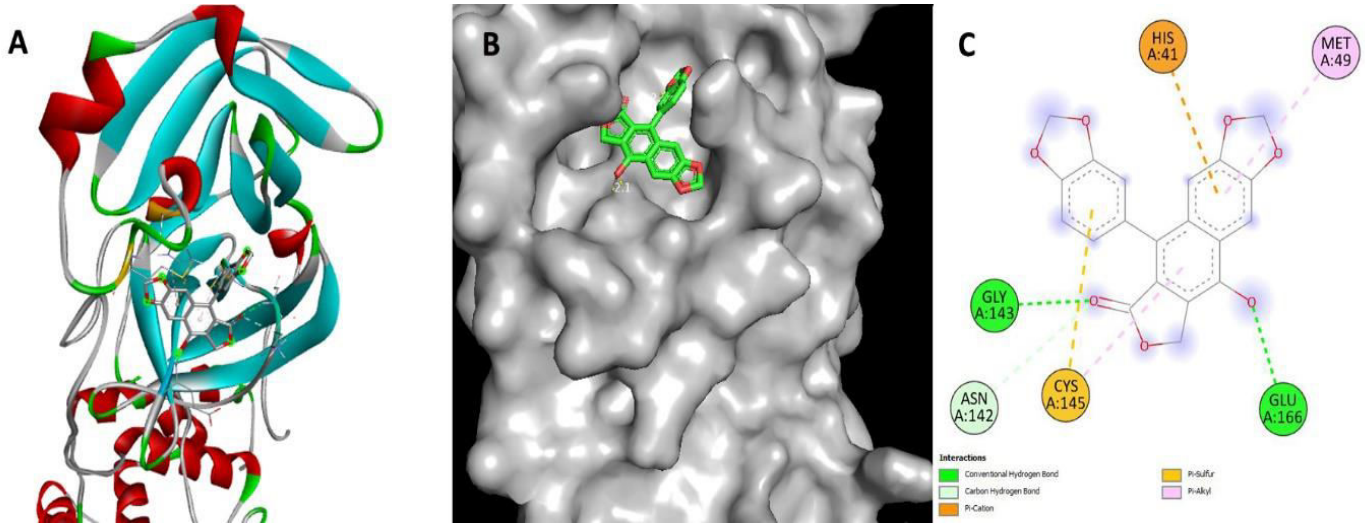
The molecular docking results revealed that lignan Justicidin G top ranked among the investigated lignans of Justicia species. Lignan Justicidin G interacted with a binding energy of -9.1kcal/mol. It formed a Conventional Hydrogen Bond with amino acid residue CYS145 and also interacted with amino acid residue HIS41 through Pi-Pi T-shaped hydrophobic bond. It interacted with the amino acid residues GLU166, GLN189, THR190, MET49 and MET165 by the formation of Carbon Hydrogen Bond, Amide-Pi Stacked, Alkyl and Pi-Alkyl bonds. The interaction is given in fig. 1.

The lignan Elenoside strongly interacted with a binding energy of -9 kcal/mol by forming bonds with ASN142, HIS164, CYS145, HIS41 and MET165 amino acid residues. Lignan Taiwanin E interacted with main protease of SARS-CoV-2 M^{pro} by forming 3 hydrogen bonds and 4 hydroponic bonds. It formed a Pi-Sulfur bond with residue CYS145 and a Pi-Cation eletrostatic interaction with HIS41. Fig. 2 represents the interaction between M^{pro} and Lignan Taiwanin E.



(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 colour sticks) and (c) Binding interaction of Lignan Justicidin G with amino acid with hydrogen bond (green dash line).

Fig. 1: Lignan Justicidin G docked in Covid-19 main protease Mpro (PDB ID 6LU7)



(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 colour sticks) and (c) Binding interaction of Lignan Taiwanin E with amino acid with hydrogen bond (green dash line).

Fig. 2: Lignan Taiwanin E docked in Covid-19 main protease Mpro (PDB ID 6LU7)

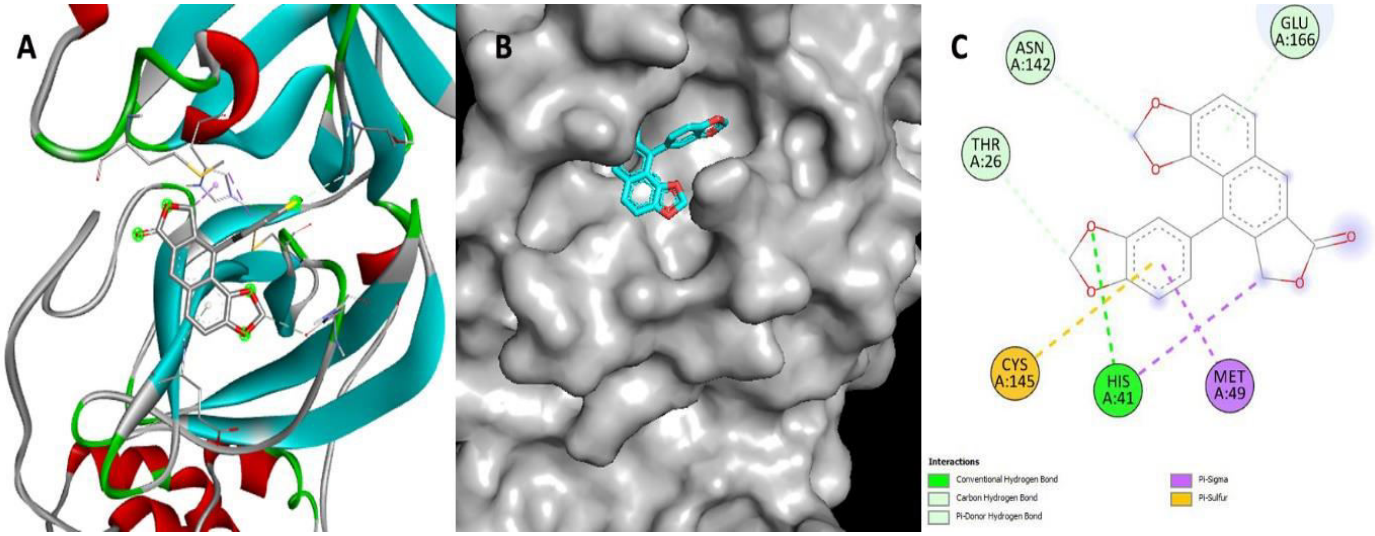
Lignan Helioxanthin which inhibits human hepatitis B viral replication, interacted with Covid-19 main protease M^{pro} through 4 hydrogen bonds, 2 hydrophobic bonds and a Pi-Sulfur bond. Lignan Helioxanthin formed a Pi-Sulphur bond with active residue CYS145 and formed a conventional hydrogen bond with the residue HIS41.The interaction is shown in fig.3.

DongWha Pharm, a leading Korean pharmaceutical company, invented a new drug named DW2008 [30] from the extract of *Justicia procumbens* to treat COVID-19. The *in vitro* antiviral experiments performed at the Institute Pasteur Korea against COVID-19 demonstrated that DW2008 had 1.7, 3.8, and 4.7 times higher antiviral activity compared to chloroquine, remdesivir, and Kaletra respectively [31]. In our studies we found that the active biological phytochemicals present in *J. procumbens* are lignans. The lignans present in *J. procumbens* are Neojusticin A, Taiwanin E, Taiwanin E methyl ether, Tuberculatin, Justicidin E, Taiwanin C, diphyllinapioside-acetate, Diphyllinapioside, Justicidinoside C, Justicidinoside A, Justicidinoside B, Pinoresinol, Neojusticin B, Justicidin B, Diphyllin, Justicidin A,

Procumbenoside A, Justin B, Chinensinaphthol methyl ether, Ciliatoside A, Cilinaphthalide B.

These lignans had a high affinity for the target protein and formed several hydrogen bonds with it.Due to the presence of these lignans *J. procumbens* is able to acts as antiviral against COVID-19.

All of the investigated lignans of Justicia species interacted with the catalyst residues (Cys145 and His41) in the SARS-Cov-2 M^{pro} main protease. Our findings show that these compounds may inhibit M^{pro}, the main protease of SARS-COV-2, by forming transient complexes.



(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 colour sticks) and (c) Binding interaction of Lignan Helioxanthin with amino acid with hydrogen bond (green dash line).

Fig. 3: Lignan Helioxanthin docked in Covid-19 main protease Mpro (PDB ID 6LU7)

4. CONCLUSION

In this study we have screened the lignans of Justicia species against SARS-COV-2 main protease. Docking results show that all the lignans interacted with Cys145 and His 41 amino acid residues which are responsible for inhibitory activity, thus indicating that the screened lignans have inhibitory effect on SARS-COV-2 main protease. As the Justicia species plants have multiple lignans, the whole plant can be used as medicine. Most of the plants of Justicia species are invasive herbs and hence they are economically viable. Experimental studies are necessary to confirm that these lignans to be used as drug against COVID-19.

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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