Inhibitory Effect of Phytochemicals from *Azadirachta indica* A Juss. and *Tinospora cordifolia* (Thunb.) Miers against SARS-CoV-2 M^{pro} and Spike Protease- An *In Silico* Analysis

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Abstract

COVID 19 caused by SARS-CoV-2 is spreading worldwide and affected 10 million people with a mortality rate between 0.5 % to 5%. Medicinal plants from China, Morocco, Algeria, Africa and India were tested for antiviral efficacy in SARS-CoV-2. Ayurveda Medicine described many medicinal plants. The Nimba (*Azadirachta indica* A. Juss) is used in fever, bacterial and viral infections, and Amrita (*Tinospora cordifolia* (Thunb.) Miers) is used as antiviral, antipyretic, and anti-inflammatory purposes. The combination of both these plants is called Nimbamritam, and it is widely used in pyrexia, dermatitis, viral infections, etc. Spike protease (PDB ID 6VXX) and M^{pro} (PDB ID 6LU) were retrieved from RCSB and 16 ligands from *A. indica* and 6 ligands from *T. cordifolia* were obtained from IMPPAT and PubChem. AutoDock Vina embedded PyRx was used for docking. Remdesivir was taken as a reference drug. *In silico* study of Cordifolide A of *T. cordifolia* showed the highest scores with -8.2 Kcal/mol and -10.3Kcal/mol with M^{pro} protease and Spike protease respectively. Cordifolide A had 4 H bonds and Kaempferol had 7 non-conventional bonds, including van der Waal with M^{pro} (6LU7) protease. The interactions with 6VXX had 5 H bonds in each ligand Cordifolide A and Azadirachtin B. The prevention of virus entry by targeting spike protease host receptor ACE2 and restricting replication of the viral genome by targeting M^{pro} residues were identified in our study. *A. indica* and *T. cordifolia* are promising therapeutic agents in COVID 19.

Introduction

Ayurveda, a traditional medical system of India practiced worldwide is based on Tri-Humoral theory known as Tridosha which consists of Vata, Pitha, and Kapha. They have pathophysiological functions in the human body and diseases are caused due to the derangement of them. Drugs, herbs, or minerals make vitiated doshas into normalization to bring Health. The traditional Chinese medical system also has a similar strategy in health care. Medicinal plants are being used for health problems and many of these plant-derived drugs have been tested in vitro, in vivo, and clinical studies to prove their efficacy.

A virus, initially named as novel coronavirus 2019 (nCov209), now known as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) was identified at Wuhan, China. The disease caused by this virus is named as COVID 19, and the WHO has announced it as a pandemic [1–6]. Till the end of June 2020 nearly 10 million people were affected by COVID 19 disease with a mortality rate between 0.5 % and 5% in different countries. Despite in vitro, in vivo and clinical studies are being carried out internationally to combat the COVID-19 best cure is yet to be identified. Antiviral drugs Remdesivir, favipiravir, ribavirin; antimalarial chloroquine and hydroxychloroquine showed anti-SARS-CoV-1 and SARS-CoV-2. Vaccines and Plasma therapy-antibodies from COVID 19 recovered subjects are being tried in COVID 19 but awaited for satisfactory results [7–9].

The SARS-CoV-2 is a β coronavirus enveloped with RNA and consists of four structural proteins viz. Spike protein(S), Envelop protein(E), Membrane protein(M). and Nucleocapsid protein(N) [10]. Spike glycoprotein acts as a viral antigen and binds to host cell receptors Angiotensin-Converting Enzyme 2 (ACE2) which is an initial entry point [11]. The 3- Chymotrypsin like protease (3CLpro) also called as Main protease (Mpro) and Papain Like protease (PLpro) are important proteases to transcribe and replicate the viral genome encodes. Since 3CLpro has a key role in replication, it is considered for studying as a drug target [12].

Mpro and PLpro are cysteine proteases responsible for the segregation of viral polypeptides into functional proteins for replication and aggregation in host cells [7]. Since the surface Spike glycoprotein fusions with the cellular membrane through ACE2, drug candidates should prevent the protein-cell binding. Therefore, targeting ACE2 through drug candidates shall block SARS-CoV-2 from entering into the host cells and prevent COVID 19 infection. Considering the activities of these protease drugs, drugs having efficacy to prevent the binding and to inhibit virus replication are to be explored. Worldwide investigations on plant extracts are being undertaken through *in vitro*, *in vivo*, *in silico* or clinical trials.

Phytochemicals from Chinese medicinal plants were screened for antiviral effects in SARS-CoV-2 [13]. African medicinal plants were screened through *in silico* studies and bioactive alkaloids and terpenoids were docked to the 3CLpro of the novel SARS-CoV-2. 10-Hydroxyusambarensine, Cryptoquindoline, 6-Oxoisoiguesterin, 22-Hydroxyhopan-3-one, Cryptospirolepine, Isoiguesterin and 20-Epibryonolic acid were found with good docking affinities [14]. Among Isothymol, Thymol, Limonene, P-cymene and c-terpinene from Algerian plant *Ammoides verticillata* (Desf.) Briq, Isothymol docked with best scores in SARS-

CoV-2 proteins [15]. Digitoxigenin, b-Eudesmol and Crocin obtained from Moroccan plant showed inhibiting activity in SARS-CoV-2 [16].

Biomolecules from Indian medicinal plants were reported to possess antiviral activity in SARS-CoV-2. Oleanic acid, Ursolic acid, Iso-Vallesiachotamine, Vallesiachotamine, Cadambine, Vincosamide-N-Oxide extracted from *Anthocephalus cadamba* showed binding with the spike protease of PDB 6VXX and Mpro 6LU7 [17]. Molecules from *Rheum emodi*, *Thymus serpyllum* and *Artemisia annua* inhibited COVID-19 binding to ACE2 receptor. [18]. Piperolactam A, Schaftoside, Riboflavin, Absinthin, Anabsinthin, 3,4,5-tricaffeoylquinic acid are effectively docked with good affinities showing antiviral effect in SARS-CoV-2 [19].

Natural compounds Kaempferol, quercetin, demethoxycurcumin, curcumin, zingerol and gingerol revealed best docking affinities with Mpro [20]. Berberine and Nimbin produced an inhibition effect in SARS-CoV-2 [21].

Plant derivatives mentioned in Siddha medicine, one of the traditional medicines practiced in the Southern part of India got the best docking result in a computer simulation.

Phytochemicals Cucurbitacin B and Cardiofoliolide B, Apiginin, Pyrethrin, Andrographolide, Vasicine, Carvacol, Eugenol and Zingiberene showed best binding affinities with Coronavirus spike glycoprotein trimer PDB 3JCL [22].

An Ayurveda formulation- Samshanavati, containing only one ingredient, i.e. *T. cordifolia* tablet is prescribed in COVID 19 [23]. The Government of India, Ministry of AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy) advised people to intake Samshanavati for improving immunity and in turn preventing SARS-CoV-2 infection [24]. Neem (*A. Indica*) a well-known plant in Ayurveda used for various disorders, including bacterial and viral infections.

In Ayurveda *A. indica* and *T. cordifolia* are known as Nimba and Amrita respectively, and some of the formulations e.g. Nimbamrtadi kashayam, Nimbamrithasavam, contain these both as main ingredients and are indicated in infectious diseases. *A. indica* and *T. cordifolia* are included in the Ayurveda Pharmacopeia of India and Ayurveda Formulary of India [25- 28].

The present *in silico* study was aimed to find potential candidature of these two plants viz. Nimba-*Azadirachta indica* A. Juss (Family *Meliaceae*) and Amrita-*Tinospora cordifolia* (Thunb.) Miers (Family Menispermaceae) as antiviral in SARS-CoV-2. Spike protease (PDB 6VXX) and Mpro (PDB 6LU) were selected for docking with the molecules from these plants. Remdesivir, an antiviral drug was docked as a reference molecule.

A. indica has 70 molecules, including Azadirachtin, Nimbidin, Azadirachtol, and Melionin. Cycloeucaenone, 24-Methylenecycloartanol, Nimbolin, Nimocin, Cycloartanols Methylenecycloartanol were studied for SARS-CoV-2 inhibitory effects [29]. Azadirachtin, cardiofolioside, berberine, and kutkin were studied *in silico* in Alzheimer's disease and found with good affinities [30]. *T. cordifolia* has 20 molecules including alkaloids, steroids, diterpenoid lactones, aliphatic and glycosides sterols. Tinosponone, Cordifolide A, Columbin, Berberine, etc. were found in *T. cordifolia* [31].

The Anti-inflammatory action of berberine, and the antiviral activity of tinosporin, jatrorrhizine cordifolioside A, magnoflorine and tinocordiside, and cordifolide were identified. Stems of *T. cordifolia* contain an appreciable quantity of zinc [32].

Hepatoprotective, antiulcer, antidiabetic, antioxidant, antipyretic, cytotoxic, immunomodulatory effects were found in *T. cordifolia*. Tinosporin, magnoflorine showed protection against aflatoxin-induced nephrotoxicity [33–39].

A diterpenoid, tinosporin showed activity against HIV, HTLV and other viral diseases for its immunomodulatory and selective inhibition of the virus to target T helper cells [40]. *T. cordifolia* has been used as an excellent immune-stimulant and serves as a remedy against various microbial infections. With a polyclonal B cell mitogen, G1–4A on binding to macrophages have been reported to enhance the immune response in mice by inducing secretion of IL–1 together with activation of macrophages [41]. Its extract has shown to result in the up-regulation of IL–6 cytokine, resulting in acute reactions to injury, inflammation, activation of cytotoxic T cells, and B cell differentiation [42]. Stem and leaves extracts have shown a hepatoprotective effect in Swiss albino male mice against lead nitrate induced toxicity [43]. The anti-bacterial activity was assayed against *Escherichia*

coli, *Staphylococcus aureus* etc [44]. This plant decreased the recurrent resistance of HIV to antiretroviral therapy (ART) and improve the outcome of the therapy [45].

Molecules-Curcumin, nimbin, withaferin A, piperine, mangiferin, thebaine, berberine, and andrographolide showed significant binding affinity towards spike glycoprotein of SARS-CoV-2 and ACE2 receptor. Ligands berberine and nimbin from *T. cordifolia* and *A indica* respectively were docked with Mpro (PDB 6LU7) and exhibited good affinities [46]. IL-6, TNF- α , and IFNs were found to be elevated in patients with SARS-CoV-2 and drugs which are regulators of interleukins, chemokines, and cytokines are helpful in as adjuvant in COVID 19 [42].

Materials And Methods

Proteins:

Spike Protease PDB ID 6VXX and Mpro PDB ID 6LU7 were retrieved from the RCSB protein data bank [47, 48].

Ligands:

The following 22 ligands (*Azadirachta indica* A Juss –16, *Tinospora cordifolia* (Thunb.) Miers –6), were obtained from IMPPAT and PubChem [49–51]. The Molecules obtained from *A. indica* are Arachidic acid CID_10467, Azadirachtin CID_2263, Azadirachtin B CID_16126804, Azadirachtol CID_23256847, Azadiradione CID_12308714, Kaempferol CID_5280863, Margosinolid CASID_105404–75–9, Melianin A CID_101277363, Melianone [CID_44575793](#), Nimbidinin CID_101306757, Nimbidiol CID_11334829, Nimbinene CID_44715635, Nimbiol CID_11119228, Nimbolide CID_100017, Nimbolin A CID_101650373, Nimocin CASID_104522–76–1. *T. cordifolia* molecules Berberine CID 2353, Columbin CID_18502774, Cordifolide A CID_102451916, Jatrorrhizine CID_72323, Tinosponone CID_15215479, Tinosporinone CID_42607646 were obtained. For comparison with these ligands, antiviral molecule- Remdesivir CID_121304016 was selected for docking purpose.

Docking:

AutoDock Vina embedded software PyRx was used for docking purposes [52]. Two chains A and C were found in the PDB 6LU7 monomer retrieved from RCSB. The C chain N-[(5- Methylisoxazol-3-Yl)Carbonyl]Alanyl-L-Valyl-N~1~-(1r,2z)-4-(Benzyloxy)-4-Oxo-1-[(3r)-2-Oxopyrrolidin-3-Yl]Methyl}But-2-Enyl)-L-Leucinamide was the inhibitor ligand [53]. The C chain N3 inhibitor in Mpro protein PDB 6LU7 was deleted through AutoDock 4.2 and saved as.pdb file which used for docking purposes [54]. The pdb of Spike protein was directly docked with ligands in AutoDock vina in PyRx.

The protein in.pdb format was loaded in the PyRx and converted into Macromolecule and saved in.pdbqt format. Ligands in 2D SDF format were imported and modified with minimization and then converted into AutoDock ligand.pdqt format through Open Babel embedded in PyRx. Further, in the AutoDock Vina wizard docking was done with blind docking in the grid box. The molecular docking calculations have been performed as blind, i.e., covered the entire protein surface, not any specific region of the protein as the binding pocket to avoid sampling bias [52].

Results of docking affinities were saved in.csv format and bonding with residues were saved in.dsv format for further analysis. Binding energies with the highest negative scores were considered for good docking between protein and ligand. Each protein-ligand docking emerged with 9 poses and the highest score from these poses was noted for its efficacy.

Analysis:

Bonding analysis of ligand-residue conformations was done with Discovery Studio Visualizer 2020 of BIOVIA software and their target residues were recorded. The receptor-ligand interactions were documented along with 2D diagrams. The conventional Hydrogen bonds, non -conventional van der Waal, Pi Alkyl, etc., were recorded [55].

Drug-likeness:

Lipinski's Rule of Five is adopted for all ligands for their drug-likeness. More than two violations among five rules disqualify for drug utility. Lipinski's rule of Five includes Molecular mass less than 500 Dalton, high lipophilicity (expressed as LogP less than 5), less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, Molar refractivity should be between 40–130 [56, 57].

Results

The 2D structures of *A. indica* molecules -Arachidic acid, Azadirachtin, Azadirachtin B, Azadirachtol, Azadiradione, Margosinolide, Melianin, Melianone, Nimbidinin, Nimbidiol, Nimbinene, Nimbiol, Nimbolide, Nimbolin A, Nimocin; and *T. cordifolia* molecules Berberine, Columbin, Cordifolide A, Jatrorrhizine, Kaempferol, Tinosponone, Tinosporinone; and antiviral molecule- Remdesivir were shown in Fig.1. Druggability of these molecules is found favorable through Lipinski's Rule of Five. All 23 ligands qualify with no violation in Lipinski's Rule. Table 1. These ligands docked with SARS-CoV-2

proteases viz. Spike protease (S) and Mpro protease and generated negative values for energies in Kcal/mol. Binding energy – 5.0 Kcal/mol and above was considered as drug potential for preventing spike protease attaching cellular membrane and ACE2 interaction.

Best affinities with root mean square deviation (RMSD) obtained in the PyRx AutoDock Vina were recorded. Among the 9 poses from each ligand best affinity of docking with RMSD was taken for analysis. All ligands were docked successfully with Mpro (6LU7) protease and affinities were found between –4.4 Kcal/mol and –8.2 Kcal/mol. Phytochemicals from *A. indica*, Kaempferol and Azadirachtin B showed high affinities with –7.8Kcal/mol, and –7.7Kcal/mol respectively.

Table 1. Molecules with Lipinski Rule of Five

Sl No.	Ligand	Molecular Formula	Mass (<500 Dalton)	Hydrogen Bond Donor (<5)	Hydrogen Bond Acceptors (<10)	LogP (<5)	Molar Refractivity (Between 40-130)
1.	Arachidic acid	C20H40O2	312.000	1	2	7.112	96.415
2.	Azadirachtin	C34H44O9	720.000	3	16	-0.203	164.279
3.	Azadirachtin B	C35H44O16	720.000	3	16	-0.203	164.2796
4.	Azadirachtol	C28H36O13	580.000	4	13	-1.611	130.266
5.	Azadiradione	C28H34O5	450.000	0	5	5.41	123.172
6.	Jatrorrhizine	C20H20NO4	466.000	0	7	3.743	119.387
7.	Kaempferol	C15H10O6	286.000	4	6	2.305	72.385
8.	Margosinolide	C27H32O8	484.000	1	8	2.257	121.470
9.	Melianin	C41H58O9	694.000	2	9	6.437	186.645
10.	Melianone	C30H46O4	470.000	1	4	6.061	131.919
11.	Nimbidinin	C26H34O6	442.000	3	6	2.489	115.907
12.	Nimbidiol	C17H22O3	274.000	2	3	3.768	77.193
13.	Nimbinene	C28H34O7	482.000	0	7	4.523	126.188
14.	Nimbiol	C18H24O2	272.000	1	2	4.371	80.265
15.	Nimbolide	C27H30O7	466.000	0	7	3.743	119.387
16.	Nimbolin A	C39H46O8	642.000	0	8	7.049	173.612
17.	Nimocin	C33H38O4	498.000	0	4	7.532	142.874
18.	Berberine	C20H18NO4	336.000	0	5	2.307	93.548
19.	Columbin	C20H22O6	358.000	1	6	2.532	88.555
20.	Cordifolide A	C28H38O12S	336.000	0	5	2.307	93.548
21.	Tinosponone	C19H22O5	330.000	1	5	2.836	84.739
22.	Tinosporinone	C19H18O6	470.000	1	4	6.061	131.919
23.	Remdesivir	C27H35N6O8P	602.000	4	10	1.930	213.36

Other molecules docked with 6LU7 protease showed good affinities; Azadiradione –7.6, Margosinolide –7.6, Melianin–7.5, Nimbolide –7.4, Azadirachtol –7.2, Nimbidin–7.2, Jatrorrhizine–7.1, Nimbidiol –7.1, Azadirachtin–7, Melianone–6.8, Nimocin–6.6, Nimbinene- 6.4, Nimbiol –6.4, Nimbolin –5.1kcal/mol.

However, Arachidic acid showed –4.4kcal/mol affinity. Molecules from *T. cordifolia* showed good affinities as Cordifolide A –8.2, Tinosponone –7.4, Columbin –7.3, Berberin–6.8, Tinosporinone –6.5. Antiviral drug Remdesivir showed an affinity with –7. Spike protein 6VXX was docked with all ligands and showed good binding energies between –5.6 Kcal/mol and –10.3Kcal/mol. Among these, Cordifolide A and Nimocin showed high affinities with –10.3Kcal/mol and –9.8 Kcal/mol respectively. Other molecules from *A. indica* produced good affinities; Nimocin –9.8, Azadirachtin B –9.6, Nimbolin–9.4, Nimbolide –9.3, Nimbinene–9.2, Margosinolide –9.1, Melianone–9, Azadiradione–8.9, Melianin –8.9, Nimbidinin –8.7, Nimbiol –8.7, Azadirachtol –8.6, Jatrorrhizine –8.5, Kaempferol –8.4, Azadirachtin –8.2, Nimbidiol–7.7, Arachidic acid –5.6, Molecules

from *T. cordifolia* showed affinities; Tinosponone –8.9, Columbin –8.3, Berberine –8, Tinosporinone –7.6 Antiviral drug Remdesivir showed an affinity with –7.6Kcal/mol. Table 2

Table 2. Receptors with Ligand Affinities and Root Mean Square Deviation (RMSD)

SL No	Ligand	6LU7	6VXX				
		Bonding Affinity kcal/mol	RMSD	RMSD	Bonding Affinity kcal/mol	RMSD	RMSD
			hd	ld		hd	ld
1.	Arachidic acid	-4.4	0	0	-5.6	0	0
2.	Azadirachtin	-7	0	0	-8.2	0	0
3.	Azadirachtin B	-7.7	0	0	-9.6	0	0
4.	Azadirachtol	-7.2	0	0	-8.6	0	0
5.	Azadiradione	-7.6	0	0	-8.9	0	0
6.	Berberine	-6.8	0	0	-8	0	0
7.	Columbin	-7.3	0	0	-8.3	0	0
8.	Cordifolide A	-8.2	0	0	-10.3	0	0
9.	Jatrorrhizine	-7.1	0	0	-8.5	0	0
10.	Kaempferol	-7.8	0	0	-8.4	0	0
11.	Margosinolide	-7.6	0	0	-9.1	0	0
12.	Melianin	-7.5	0	0	-8.9	0	0
13.	Melianone	-6.8	0	0	-9	0	0
14.	Nimbidinin	-7.2	0	0	-8.7	0	0
15.	Nimbidiol	-7.1	0	0	-7.7	0	0
16.	Nimbinene	-6.4	0	0	-9.2	0	0
17.	Nimbiol	-6.4	0	0	-8.7	0	0
18.	Nimbolide	-7.4	0	0	-9.3	0	0
19.	Nimbolin	-5.1	0	0	-9.4	0	0
20.	Nimocin	-6.6	0	0	-9.8	0	0
21.	Remdesivir	-7	0	0	-7.6	0	0
22.	Tinosponone	-7.4	0	0	-8.9	0	0
23.	Tinosporinone	-6.5	0	0	-7.6	0	0

Conventional Hydrogen bonds, van der Waals, carbon bonds, Pi Alkyl, etc. with amino acids in 2D are depicted in Fig.2 and Fig 3. Ligands docked with Mpro protein residues having H bonds are noted as Kaempferol SER144, Azadirachtin B LEU287, TYR239, Azadiradione MET276, ARG131, Margosinolide LYS137, LEU287, Melianin THR111, Nimbolide, SER158, LYS102, Azadirachtol LEU271, Nimbidinin GLN110, SER158, Jatrorrhizine

LEU271, Nimbidiol THR190, ARG188, Azadirachtin ARG131, THR199, LEU272, Remdesivir THR190, GLU166, ASN142, CYS145, GLY143, Melianone LYS5, GLN127, Nimocin LYS5, Nimbinene ASN142, GLU166, Nimbiol LYS102, SER158, Nimbolin GLN127,

LYS5, Arachidic acid THR111, GLN110, ASP295, Cordifolide A LYS137, ASP197, ASN238, THR199, Tinosponone GLN110, ER158, LYS102, Columbin THR26,

Berberine LEU287, Tinosporinone GLY143, GLY166.

Similarly, ligands docked with spike protein and their target residues noted as Nimocin TRY756, THR C998, THR B998, Azadirachtin B THR430, LEU518, LEU517, SER975,

Nimbolin ARG765, Nimbolide GLN1036, ARG1107, Nimbinene ASN B1023, ASN C 1023, Margosinolide TYR 756, ARG995, THR998, TYR C756, ASP994, Melianone GLN1113, VAL1122, Azadiradione ARG995, TYR756, Melianin SER50, HIS49, THR761, LYS304, Nimbidinin THR998, TYR756, Nimbiol LEU517, PHE515, Azadirachtol ARG983, THR430, ASP428, SER514, Jatrorrhizine GLY314, ILE666, LYS733, Kaempferol TRP886, Azadirachtin THR723, THR1027, Nimbiolol ARG1107, ASN1108, TYR904, Remdesivir ARG139, ALA1020, Arachidic acid GLY744, TYR741, Cordifolide A GLN414, THR415, GLU988, TYR369, LYS417, Tinosponone TYR904, LYS1038, HIS1048,

GLY1036, Columbin THR998, TYR756, Berberine ASN764, Tinosporinone LYS1028, GLN784 Table 3, Table 4, Fig 4, Fig 5

Discussion

Ayurveda medicine described many plants having the pharmacological actions, including antiviral, immune-modulatory, etc. Ligands from Nimba and Amrita (*A. indica* and *T. cordifolia*) were screened *in silico* to establish anti-SARS-CoV-2 activity. Ligand interactions by binding with residues of Spike protease or Mpro protease showed good binding affinities.

The pharmacophore of a molecule includes the Hydrogen bond acceptor, H bond donor, negative and positive functional features with hydrophobic, aromatic groups. The present molecules were studied with a reference ligand- Remdesivir and showed similar essential features of the reference drug. The lesser energy showed the greater possibility of the drug candidate for prevention as well as to cure. In the present study, with computational docking tools AutoDock Vina, it is established that all tested ligands successfully docked against the inhibitory region of the main protease of the SARS-CoV-2 virus with docking scores between -4 Kcal/mol and -10 Kcal/mol. Further ligands were also had the best affinities with Spike protease which are responsible for viral entry at host ACE2. In a multidrug therapy, molecules contemporize and produce synchronized synergetic action. It is evidenced from our *in silico* study that ligands had common H bond residue interactions. A combination of molecules from *A. indica* and *T. cordifolia* may synchronize and prognosticate to establish an effective therapy in COVID 19.

Table 3. 6LU7 Affinities with Amino acid interactions

Sl. No	Ligand	Affinities kcal/mol	Conventional H Bonds	Van der Waals and Other Bonds Carbon H, Pi Alkyl etc
1.	Kaempferol	-7.8	SER144	LEU141, CYS145, GLU166, GUS41, MET49, MET165, GLN189
2.	Azadirachtin B	-7.7	LEU287, TYR239	GLY275, THR199, TYR237
3.	Azadiradione	-7.6	MET276, ARG131	TYR239
4.	Margosinolide	-7.6	LYS137, LEU287	LEU271
5.	Melianin	-7.5	THR111	
6.	Nimbolide	-7.4	SER158, LYS102	PHE294
7.	Azadirachtol	-7.2	LEU271	TYR237, LEU287
8.	Nimbidinin	-7.2	GLN110, SER158	PHE294
9.	Jatrorrhizine	-7.1	LEU271	ASP289, LEU287, MET276, LEU286, LEU272
10.	Nimbidiol	-7.1	THR190, ARG188	MET165
11.	Azadirachtin	-7	ARG131, THR199, LEU272	
12.	Remdesivir	-7	THR190, GLU166, ASN142, CYS145, GLY143	MET49, HIS41
13.	Melianone	-6.8	LYS5, GLN127	LEU286
14.	Nimocin	-6.6	LYS5	SER139, GLU290, LYS137
15.	Nimbinene	-6.4	ASN142, GLU166	
16.	Nimbiol	-6.4	LYS102, SER158	VAL104
17.	Nimbolin	-5.1	GLN127, LYS5	LYS137
18.	Arachidic acid	-4.4	THR111, GLN110, ASP295	PHE294, ILE106, VAL104
19.	Cordifolide A	-8.2	LYS137, ASP197, ASN238, THR199	
20.	Tinosponone	-7.4	GLN110, SER158, LYS102	ILE152
21.	Columbin	-7.3	THR26	
22.	Berberine	-6.8	LEU287	LEU287, TYR237, LEU286, ASP197, GLY275, MET276
23.	Tinosporinone	-6.5	GLY143, GLY166	GUS41, MET49, CYS145, MET165

These active molecules might inhibit the viral pathogenesis with a more efficient inhibitory effect against viral replication. IL-6, TNF- α , and IFNs were found to be elevated in patients with SARS-CoV-2 and drugs which are regulators of interleukins,

chemokines, and cytokines are helpful in as adjuvant in COVID 19 [42]. *T. cordifolia* immune-regulatory effect may intervene with the IL 6. TNF- α etc and regulate cytokines to produce a therapeutic effect in COVID 19.

Table 4. 6VXX- Ligands affinities with Amino acid interactions

Sl. No.	Ligand	Affinity kcal/mol	Conventional Hydrogen Bonds	Other Bonds van der Waals, Pi Alkyl etc
1.	Nimocin	-9.8	TRY756, THR C998, THR B998	ASP994, TYR756, ARG A995, ARG C995
2.	Azadirachtin B	-9.6	THR430, LEU518, LEU517, SER975	
3.	Nimbolin	-9.4	ARG765	ILE312, LEU861, GLY311
4.	Nimbolide	-9.3	GLN1036, ARG1107	TRP886, GLY908
5.	Nimbinene	-9.2	ASN B1023, ASN C 1023	LUE1024
6.	Margosinolide	-9.1	TYR B756, ARG995, THR998, TYR C756, ASP994	GLY971, ARG995
7.	Melianone	-9	GLN1113, VAL1122	PHE1121, GLN1113
8.	Azadiradione	-8.9	ARG995, TYR756	PHE970, TYR756, GLN1002, THR998
9.	Melianin	-8.9	SER50, HIS49, THR761, LYS304	THR302
10.	Nimbidinin	-8.7	THR998, TYR756	ARG995, ARG C995, PHE970
11.	Nimbiol	-8.7	LEU517, PHE515	LEU518
12.	Azadirachtol	-8.6	ARG983, THR430, ASP428, SER514	ILE973
13.	Jatrorrhizine	-8.5	GLY314, ILE666, LYS733	LEU864, PRO665, LEU861
14.	Kaempferol	-8.4	TRP886	TYR1048, VAL1040, ALA890
15.	Azadirachtin	-8.2	THR723, THR1027	GLN779, ALA783, LYS1045
16.	Nimbidiol	-7.7	ARG1107, ASN1108, TYR904	LYS1038, TRP886
17.	Remdesivir	-7.6	ARG139, ALA1020	ALA A1020, ALA B1020, ALA C1020, LEU1024
18.	Arachidic acid	-5.6	GLY744, TYR741	ILE587, PRO589, VAL976
19.	Cordifolide A	-10.3	GLN414, THR415, GLU988, TYR369, LYS417	ASP405, GLN414, LYS378, PHE374
20.	Tinosponone	-8.9	TYR904, LYS1038, HIS1048, GLY1036	TYR1047, GLN1036, LYS1038
21.	Columbin	-8.3	THR998, TYR756	ASP994, ARG995
22.	Berberine	-8	ASN764	THR761, TYR313, VAL772
23.	Tinosporinone	-7.6	LYS1028, GLN784	ALA1026, LEU1024, THR1027

Indian medicinal plants are being used to treat viral infections, and could physically bind COVID–19 target proteins such as SARS-CoV–2 spike glycoprotein (PDB ID: 6VXX), SARS-CoV–2 spike ectodomain structure (PDB ID: 6VYB), and SARS

coronavirus spike receptor-binding domain (PDB ID: 2AJF) hence, in turn, prevent COVID–19 virus binding to the host receptor ACE2 [18]. Nimbin, berberine, mangiferin of *A. indica* showed significant binding affinity towards spike protein of SARS-CoV–2 and ACE2 receptor [46]. Molecules from *A. indica* and *T. cordifolia* may be useful as a prophylactic as well as therapeutic agents due to restricting viral attachment to the host cells and prevent replication of viral RNA.

The tinosporin of *T. cordifolia* showed activity against HIV, HTLV and other viral diseases for its immunomodulatory and selective inhibition of the virus to target T helper cells [40]. Dry cough, Fever, Dyspnoea and Myalgia are the main symptoms, and in severely affected COVID 19 subjects, Acute Respiratory Distress is observed. Apart from antiviral efficacy, *A. indica* and *T. cordifolia* have anti-inflammatory, analgesic and antipyretic actions. Therefore, a combination of these plants may improve clinical conditions such as fever, myalgia and cough in COVID 19 subjects.

The role of the immune system was explained in SARS-CoV–2 infection [58]. In the immune depleted older subjects COVID 19 produces severe symptoms, and comorbidities may increase the mortality rate in senior citizens. The immune-enhance effect of the *T. cordifolia* may help in reducing the severity of symptoms.

Conclusion

Neem *A. indica* and Amrita *T. cordifolia* have antiviral, antipyretic and anti-inflammatory actions, and a combination of these two plants is promising drug therapy for prevention and intervention in SARS-CoV–2 infection.

Our *in silico* study revealed that 22 molecules of these plants had good binding affinities with Spike protease and Mpro protease, and substantiate the claim for anti-SARS-CoV–2 by preventing the spike protease-ACE2 target. Further docking with Mpro protease by the ligands producing affinities reiterate that replication of the viral genome will be prevented. Protein- ligand docking of these phytochemicals, on comparison with reference synthetic antiviral Remdesivir, showed equivalent to Remdesivir. In addition to the antiviral effect, this combination has a role in symptomatic relief from fever, cough, myalgia. Therefore, with the multidrug therapy containing *A. indica* and *T. cordifolia* promises an effective alternate solution in COVID 19. However, since the present study conducted *in silico* we need to establish antiviral activity *in vitro*, *in vivo*, and clinical studies in COVID 19.

Declarations

Conflict of Interest: There is no conflict of interest with this work.

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Figures

Figure 1

Ligand 2D structures 1 Arachidic acid 2 Azadirachtin 3. Azadirachtin B 4 Azadirachtol 5 Azadiradione 6 Jatrorrhizine 7 Kaempferol 8 Margosinolide 9 Melianin 10 Melianone 11 Nimbidinin 12 Nimbiol 13 Nimbinene 14 Nimbiol 15 Nimbolide 16 Nimbolin 17 Nimocin 18 Berberine 19 Columbin 20 Cordifolide A 21 Tinosponone 22 Tinosporinone 23 Remdesivir

Figure 2

6LU7 2D Conformations; Legends: Green -Conventional Hydrogen Bond, Light Green- van Der Waal Carbon Hydrogen Bond, Light Red- Pi Alkyl, Dark Red- Stalk 1
 1 Azadiradione 2 Melianone 3 Nimocin 4 Nimbolide 5 Margosinolide 6 Nimbiol 7 Nimbidol 8 Nimbinene 9 Melianin 10 Nimbidinin 11 Azadirachtin 12 Berberine 13 Arachidic acid 14 Jatrorrhizine 15 Kaempferol 16 Azadirachtin B 17 Columbin 18 Azadirachtol 19 Tinosponone 20 Tinosporinone 21 Cordifolide A 22 Remdesivir 23 Nimbolin

Figure 3

6VXXX 2D Ligand Conformations- Legends: Green -Conventional Hydrogen Bond, Light Green- Carbon Hydrogen Bond, Light Red- Pi Alkyl, Dark Red- Pi Stak;1 Azadirachtin 2 Berberine 3 Arachidic acid 4 Jatrorrhizine 5 Kaempferol 6 Azadiradione 7 Azadirachtin B 8 Columbin 9 Azadirachtol 10 Nimbolide 11 Nimocin 12 Margosinolide 13 Nimbiol 14 Nimbiol 15 Nimbinene 16 Melianin 17 Nimbidinin 18 Nimbolin 19 Tinosponone 20 Tinosporinone 21 Melianone 22 Cordifolide A 23 Remdesivir

Figure 4

6LU7 Ligand Docking conformations with Hydrogen bonds

Figure 5

6VXX with ligand conformations with Hydrogen bonds