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# 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 fevers, bacterial and viral infections, and Amrita (*Tinospora cordifolia* (Thunb.) Miers) is used for 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 6LU7) 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 6VXX interactions had 5 H bonds in each ligand Cordifolide A and Azadirachtin B. The prevention of virus 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.**

***Keywords*—SARS-CoV-2, *Azadirachta indica*, *Tinospora cordifolia*, Ayurveda Medicine, COVID-19**

## I. 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 [Wu, F et al (2020), Huang, C (2020), Zhu, N (2020), Zhou, P. et al.

(2020). Gorbalenya, A. E. et al. (2020), https://www.who.int.(2020)]. 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 [Zhang, L, Linet al (2020) Wang, M. et al. (2020). Devaux CA(2020)].

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) [Cui J, Li F, Shi ZL. (2019)]. The Spike glycoprotein acts as a viral antigen and binds to host cell receptors Angiotensin-Converting Enzyme 2 (ACE2) which is an initial entry point [Wu Aet al. (2020)]. The 3-Chymotrypsin like protease ($3CL^{pro}$) also called as Main protease ($M^{pro}$) and Papain Like protease ($PL^{pro}$) are important proteases to transcribe and replicate the viral genome encodes. Since $3CL^{pro}$ has a key role in replication, it is considered for studying as a drug target [Wang, M et al (2020)]. $M^{pro}$ and $PL^{pro}$ are cysteine proteases responsible for the segregation of viral polypeptides into functional proteins for replication and aggregation in host cells[Zhang L et al (2020)]. Since the surface Spike glycoprotein fusions with the cellular membrane through ACE2, drug candidates should prevent the protein-cell binding. Therefore, targeting ACE2, drug candidates shall block SARS-CoV-2 from entering into the host cells and prevent COVID-19 infection. Considering the activities of these proteases, 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 [Muhammad Tahir ul Qamar et al (2020)]. African medicinal plants were screened through *in silico* studies and bioactive alkaloids and terpenoids were docked to the $3CL^{pro}$ 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 [Gideon A (2020)]. 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 [Imane Abdellia B (2020)]. Digitoxigenin, b–Eudesmol and Crocin obtained from Moroccan plant showed inhibiting activity in SARS-CoV-2 [Aanouz I (2020)].

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 $M^{pro}$ 6LU7[Ashok Kumar Mishra 2020]. Molecules from *Rheum emodi, Thymus serpyllum* and *Artemisia annua* inhibited COVID-19 binding to ACE2 receptor [Rajan Rolta et al (2020)]. Piperolactam A, Schaftoside, Riboflavin, Absinthin, Anabsinthin, 3,4,5-tricaffeoylquinic acid are effectively docked with good affinities showing antiviral effect in SARS-COV-2 [Joshi, T et al (2020)]. Natural compounds Kaempferol, quercetin, demethoxycurcumin, curcumin, zingerol and gingerol revealed best docking affinities with $M^{pro}$ [Khaerunnisa S et al (2020)]. Berberine and Nimbin produced an inhibition effect in SARS-CoV-2 [Ambrish Kumar Srivastava et al (2020)].

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 [Pitchiah Kumar M et al (2020)].

An Ayurveda formulation- Samshanavati, containing one ingredient, i.e. *T. cordifolia* tablet is prescribed in COVID-19 [Advisory from Ministry of Ayush (2020)]. 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 [Guidelines for Ayurveda Practitioners (2020)]. Neem (*A. Indicia*) 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.*

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*indica* and *T. cordifolia* are included in the Ayurveda Formulary of India and Ayurveda Pharmacopeia of India [Ayurveda Formulary of India (2003,2000), Ayurveda Pharmacopeia of India (2005,2004)]

The present *in silico* study was aimed to find potential candidature of these two plants viz. Nimba-*Azadirachta indica* A. Juss and Amrita-*Tinospora cordifolia* (Thunb.) Miers as antiviral against SARS-CoV-2. Spike protease (PDB ID 6VXX) and $M^{pro}$ (PDB ID 6LU7) 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. Cycloeucalenone, 24-Methylenecycloartanol, Nimbolin, Nimocin, Cycloartanols Methylenecycloartanol were studied for SARS-CoV-2 inhibitory effects [Subhomoi Borkotoky, Manidipa Banerjee (2020)]. Azadirachtin, cardiofolioside, berberine, and kutkin were studied *in silico* in Alzheimer's disease and found with good affinities [Prashant Anthwal (2015)]. *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* [Singh S.S et al (2003)].

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 [Deepika Singh (2017)]. Hepatoprotective, antiulcer, antidiabetic, antioxidant, antipyretic, cytotoxic, immunomodulatory effects were found in *T. cordifolia*. Tinosporin, magnoflorine showed protection against aflatoxin-induced nephrotoxicity [Deepika Singh (2017), Atal C K et al (1986), Vedavathy S, Rao KN. (1991), Jagetia GC, Rao SK. (2006), Gupta SS (1967), Sinha, K. et al (2004)].

A diterpenoid, tinosporin showed an activity against HIV, HTLV and other viral diseases for its immunomodulatory and selective inhibition of the virus to target T helper cells [Chetan B, Nakum A. (2010)]. *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 [Raghu R, et al (2009)]. 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 [Gupta R, Sharma V. (2011)]. Stem and leaves extracts have shown a hepatoprotective effect in Swiss albino male mice against lead nitrate induced toxicity [Sharma V, Pandey D. (2010)]. The antibacterial activity was assayed against *Escherichia coli, Staphylococcus aureus* etc. [Narayanan A S (2011)]. This plant decreased the recurrent resistance of HIV to antiretroviral therapy (ART) and improve the outcome of the therapy [Gupta GD. (2010)].

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 $M^{pro}$ (PDB 6LU7) and exhibited good affinities [Vimal K. (2020)]. IL-6, TNF-a, 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 [Gupta R, Sharma V. (2011)].

## II. MATERIALS AND METHODS

**A. Proteins:**

Spike Protease PDB ID 6VXX and $M^{pro}$ PDB ID 6LU7 were retrieved from the RCSB protein data bank [www.rcsb.org/6VXX(2020),www.rcsb.org/6LU7 (2020)].

**B. Ligands:**

The following 22 ligands (*Azadirachta indica* A Juss -16, *Tinospora cordifolia* (Thunb.) Miers -6), were obtained from IMPPAT and PubChem [Karthikeyan Mohanraj et al (2018**),** Mohanraj, K. et al. (2018), https://pubchem(2020)].

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, Margosinolide 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

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CID_15215479, Tinosporinone CID_42607646 were obtained. For comparison with these ligands, antiviral molecule- Remdesivir CID_121304016 was selected for docking purpose.

**C. Molecular Docking:**

The AutoDock Vina embedded software PyRx was used for docking purposes [Dallakyan S, Olson (2015)]. 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 [Jin Z, et al (2020)]. The C chain N3 inhibitor in $M^{pro}$ protein PDB 6LU7 was deleted through AutoDock 4.2 and saved as .pdb file which used for docking purposes [Morris G. et al (2009)]. 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 interface 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 .pdqbt 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 [Dallakyan S, Olson (2015)]. 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.

**D. 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 [Dassault Systèmes (2017)].

Table 1. Molecules with Chemical formulae and application of 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 | $C_{20}H_{40}O_2$ | 312.000 | 1 | 2 | 7.112 | 96.415 |
| 2 | Azadirachtin | $C_{34}H_{44}O_9$ | 720.000 | 3 | 16 | -0.203 | 164.279 |
| 3 | Azadirachtin B | $C_{35}H_{44}O_{16}$ | 720.000 | 3 | 16 | -0.203 | 164.2796 |
| 4 | Azadirachtol | $C_{28}H_{36}O_{13}$ | 580.000 | 4 | 13 | -1.611 | 130.266 |
| 5 | Azadiradione | $C_{28}H_{34}O_5$ | 450.000 | 0 | 5 | 5.41 | 123.172 |
| 6 | Jatrorrhizine | $C_{20}H_{20}NO_4$ | 466.000 | 0 | 7 | 3.743 | 119.387 |
| 7 | Kaempferol | $C_{15}H_{10}O_6$ | 286.000 | 4 | 6 | 2.305 | 72.385 |
| 8 | Margosinolide | $C_{27}H_{32}O_8$ | 484.000 | 1 | 8 | 2.257 | 121.470 |
| 9 | Melianin | $C_{41}H_{58}O_9$ | 694.000 | 2 | 9 | 6.437 | 186.645 |
| 10 | Melianone | $C_{30}H_{46}O_4$ | 470.000 | 1 | 4 | 6.061 | 131.919 |
| 11 | Nimbidinin | $C_{26}H_{34}O_6$ | 442.000 | 3 | 6 | 2.489 | 115.907 |
| 12 | Nimbidiol | $C_{17}H_{22}O_3$ | 274.000 | 2 | 3 | 3.768 | 77.193 |
| 13 | Nimbinene | $C_{28}H_{34}O_7$ | 482.000 | 0 | 7 | 4.523 | 126.188 |
| 14 | Nimbiol | $C_{18}H_{24}O_2$ | 272.000 | 1 | 2 | 4.371 | 80.265 |
| 15 | Nimbolide | $C_{27}H_{30}O_7$ | 466.000 | 0 | 7 | 3.743 | 119.387 |
| 16 | Nimbolin A | $C_{39}H4_6O_8$ | 642.000 | 0 | 8 | 7.049 | 173.612 |
| 17 | Nimocin | $C_{33}H3_8O_4$ | 498.000 | 0 | 4 | 7.532 | 142.874 |
| 18 | Berberine | $C_{20}H_{18}NO_4$ | 336.000 | 0 | 5 | 2.307 | 93.548 |
| 19 | Columbin | $C_{20}H_{22}O_6$ | 358.000 | 1 | 6 | 2.532 | 88.555 |
| 20 | Cordifolide A | $C_{28}H_{38}O_{12}S$ | 336.000 | 0 | 5 | 2.307 | 93.548 |
| 21 | Tinosponone | $C_{19}H_{22}O_5$ | 330.000 | 1 | 5 | 2.836 | 84.739 |
| 22 | Tinosporinone | $C_{19}H_{18}O_6$ | 470.000 | 1 | 4 | 6.061 | 131.919 |
| 23 | Remdesivir | $C_{27}H_{35}N_6O_8P$ | 602.000 | 4 | 10 | 1.930 | 213.36 |

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**E. Drug-likeliness:**

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 [Lipinski CA (2004). Jayaram B (2012)].

## III. 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. Drug-likeness of these molecules is found favorable through Lipinski's Rule of Five. All 23 ligands qualify with no violation in Lipinski's Rule. Results are shown in Table 1. These ligands docked with SARS-CoV-2 proteases viz. Spike protease (S) and $M^{pro}$ 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 $M^{pro}$ (6LU7) protease and affinities were found between -4.4Kcal/mol and -8.2Kcal/mol.

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

| SL No | Ligand | 6LU7 | | | 6VXX | | |
|---|---|---|---|---|---|---|---|
| | | Bonding Affinity kcal/mol | RMSD hd | RMSD ld | Bonding Affinity kcal/mol | RMSD hd | RMSD 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 |

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Phytochemicals from *A. indica*, Kaempferol and Azadirachtin B showed high affinities with -7.8Kcal/ mol, and -7.7Kcal/mol respectively. 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. The Molecules from *T. cordifolia* showed good affinities as Cordifolide A -8.2kcal/mol, Tinosponone -7.4, Columbin -7.3, Berberin-6.8, Tinosporinone -6.5. Antiviral drug Remdesivir showed an affinity with -7Kcal/mol. 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 -10.3 Kcal/mol and -9.8 Kcal/mol respectively. Other molecules from *A. indica* produced good affinities as shown in Table 2. Nimocin -9.8 kcal/mol, 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. Conventional Hydrogen bonds, van der Waals, carbon bonds, Pi Alkyl, etc. with amino acids in 2D are depicted in Fig.2 and Fig 3.

Table 3. $M^{pro}$ 6LU7-Ligand 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 |

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| | | | | |
|---|---|---|---|---|
| 23. | Tinosporinone | -6.5 | GLY143, GLY166 | GUS41, MET49, CYS145, MET165 |

Ligands docked with $M^{pro}$ 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 TYR756, Melianin SER50, HIS49, THR761, GLN1036, ARG1107, Nimbinene ASN B1023, LYS304, Nimbidinin THR998, TYR756, Nimbiol ASN C 1023, Margosinolide TYR 756, ARG995, LEU517, PHE515, Azadirachtol ARG983, THR998, TYR C756, ASP994, Melianone THR430, ASP428, SER514,Jatrorrhizine GLY314, GLN1113, VAL1122, Azadiradione ARG995, ILE666, LYS733, Kaempferol TRP886,

Table 4. Spike protease 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 |

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Azadirachtin THR723, THR1027, Nimbidiol 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, and all results are depicted in Table 3, Table 4, Fig 4, Fig 5.

## IV. 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 $M^{pro}$ 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, which 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.

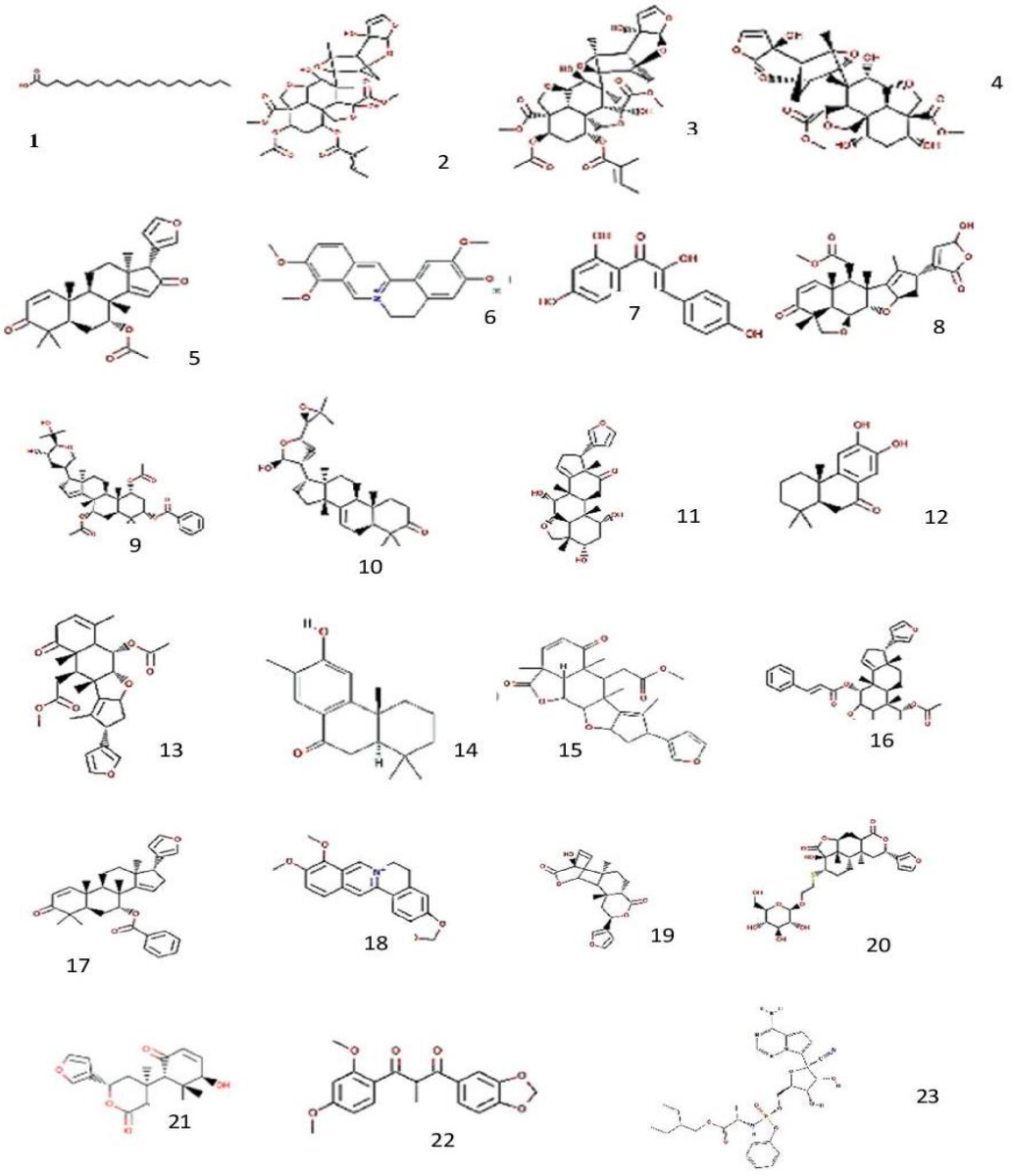

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Fig 1 Ligand 2D structures- 1 Arachidic acid 2 Azadirachtin 3. Azadirachtin B 4 Azadirachtol 5 Azadiradione 6 Jatrorrhizine 7 Kaempferol 8 Margosinolide 9 Melianin 10 Melianone 11 Nimbidinin 12 Nimbidiol 13 Nimbinene 14 Nimbiol 15 Nimbolide 16 Nimbolin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

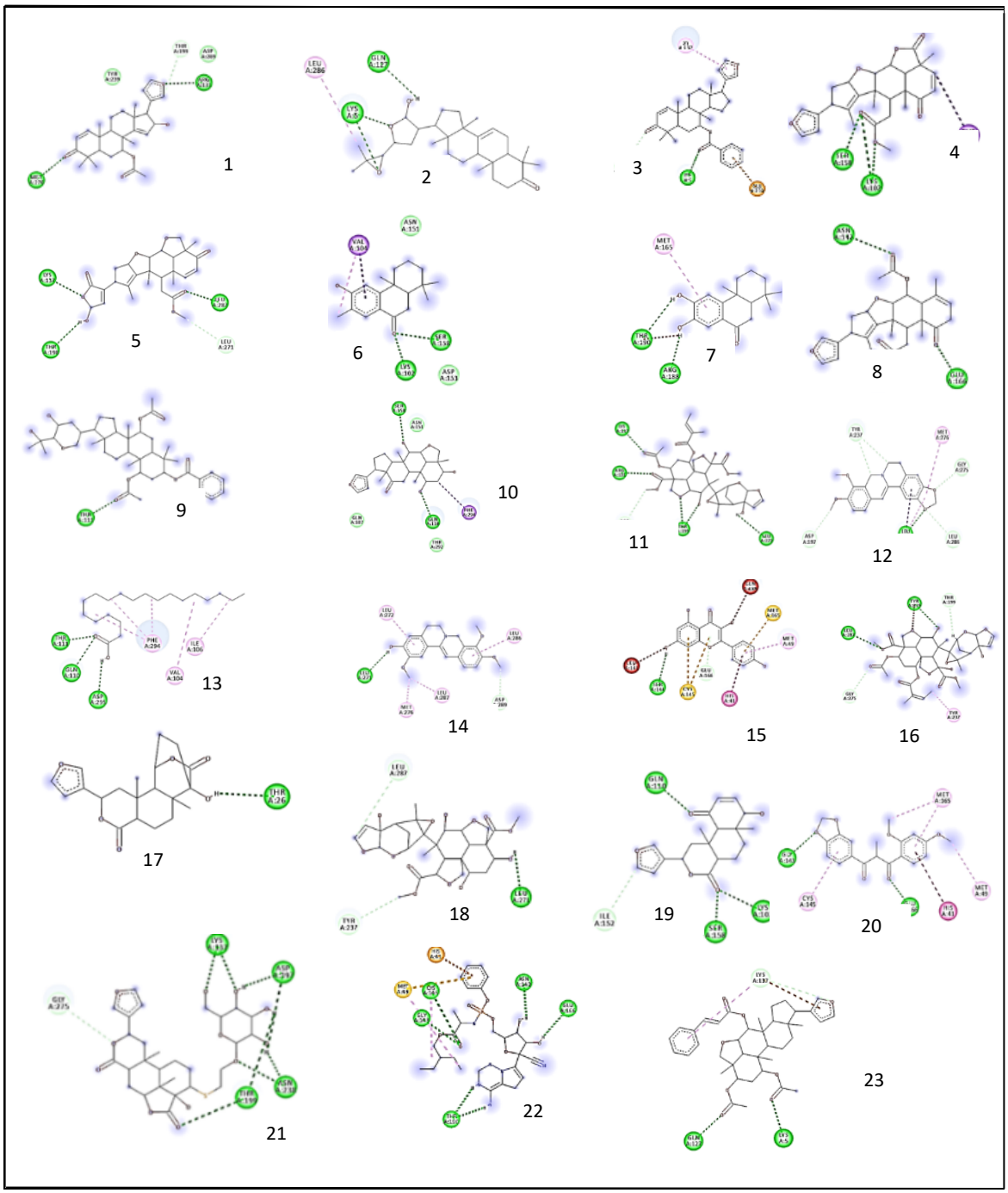


Fig.2 $M^{pro}$ 6LU7 2D Conformations; Legends: Green -Conventional Hydrogen Bond, Light Green- van Der Waal Carbon Hydrogen Bond, Light Red- Pi Alkyl, Dark Red- Stalk 1 Azadiradione 2 Melianone 3 Nimocin 4 Nimbolide 5 Margosinolide 6 Nimbiol 7 Nimbidiol 8 Nimbinene 9 Melianin 10 Nimbidinin 11 Azadirachtin 12 Berberine 13

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Arachidic acid 14 Jatrorrhizine 15 Kaempferol 16 Azadirachtin B 17 Columbin 18 Azadirachtol 19 Tinosponone 20 Tinosporinone 21 Cordifolide A 22 Remdesivir 23 Nimbolin

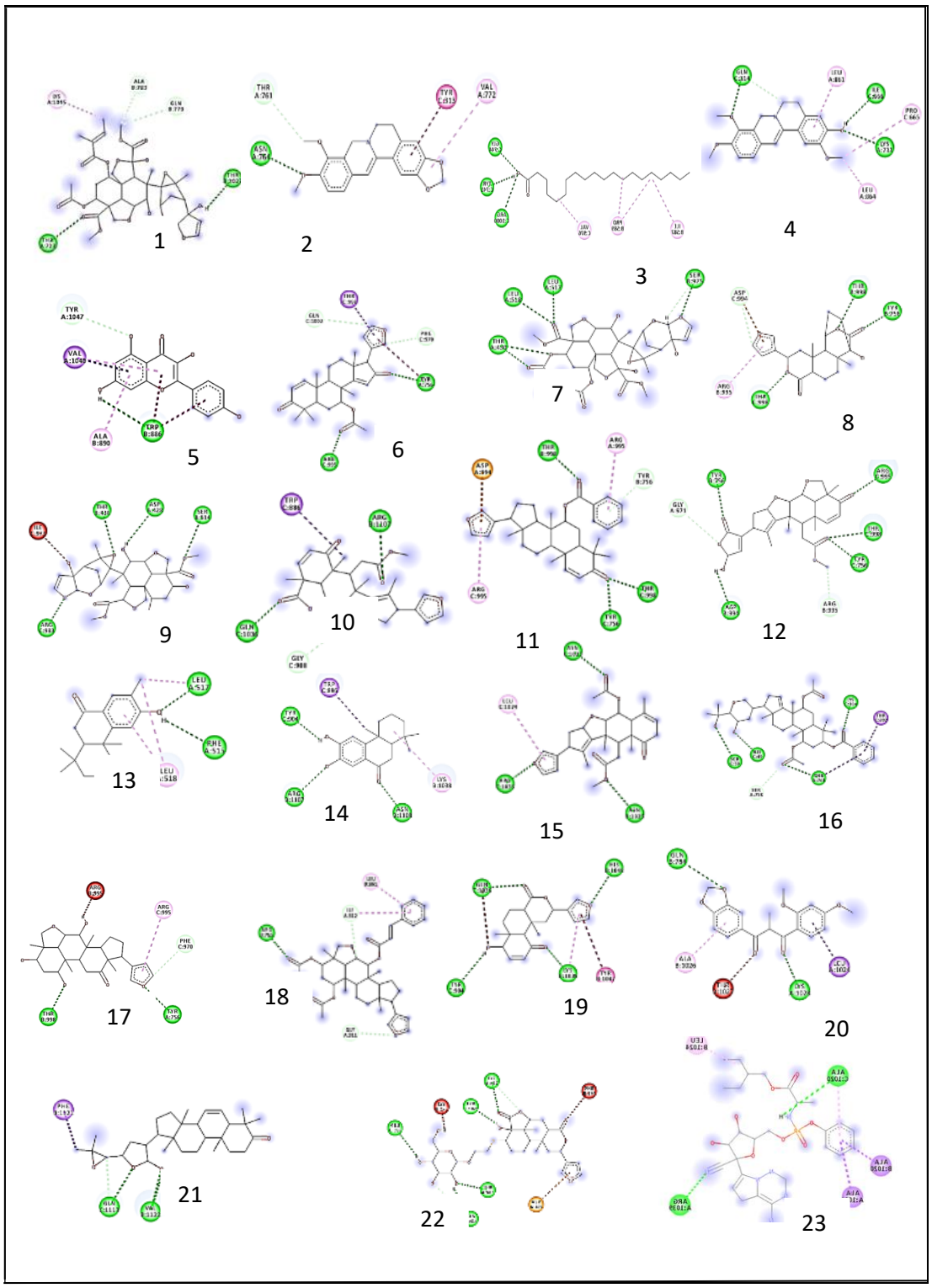


Fig. 3 Spike protease 6VXX 2D Ligand Conformations- Legends: Green -Conventional Hydrogen Bond, Light Green- Carbon Hydrogen Bond, Light Red- Pi Alkyl, Dark Red- Pi Stak;1 Azadirachtin 2 Berberine 3 Arachidic acid 4 Jatrorrhizine 5 Kaempferol 6 Azadiradione 7 Azadirachtin B 8 Columbin 9 Azadirachtol 10 Nimbolide 11

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Nimocin 12 Margosinolide 13 Nimbiol 14 Nimbidiol 15 Nimbinene 16 Melianin 17 Nimbidinin 18 Nimbolin 19 Tinosponone 20 Tinosporinone 21 Melianone 22 Cordifolide A 23 Remdesivir

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.

These active molecules might inhibit the viral pathogenesis with a more efficient inhibitory effect against viral replication. IL-6, TNF-a, 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 [Gupta R, Sharma V. (2011)].

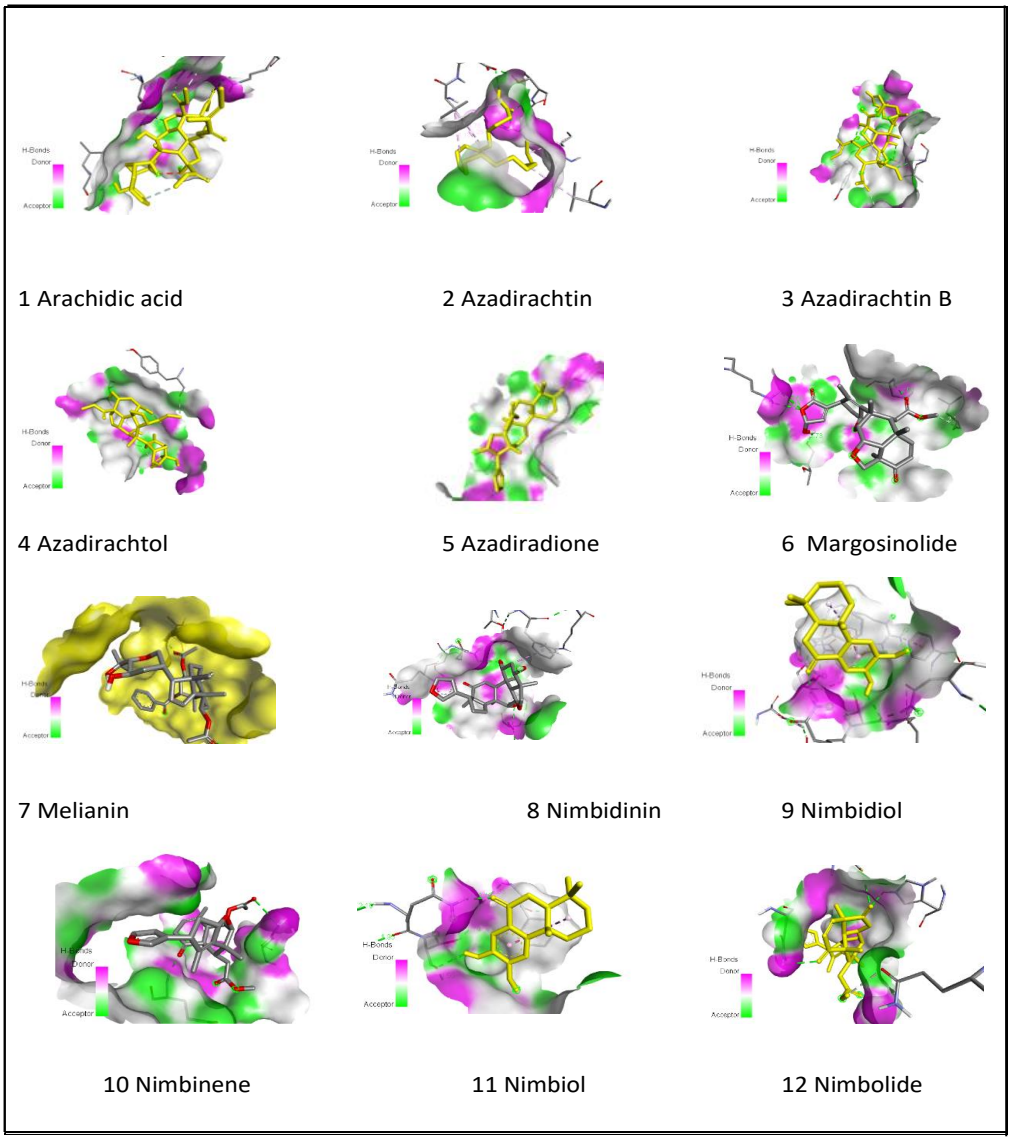


Fig. 4 M$^{pro}$ 6LU7 Ligand Docking conformations with Hydrogen bonds

*T cordifolia* immune-regulatory effect may intervene with the IL 6. TNF-a etc. and regulate cytokines to produce a therapeutic effect in COVID-19. 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 (PDBID: 2AJF) hence, in turn, prevent COVID-19 virus

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binding to the host receptor ACE2 [Rajan Rolta et al(2020)].

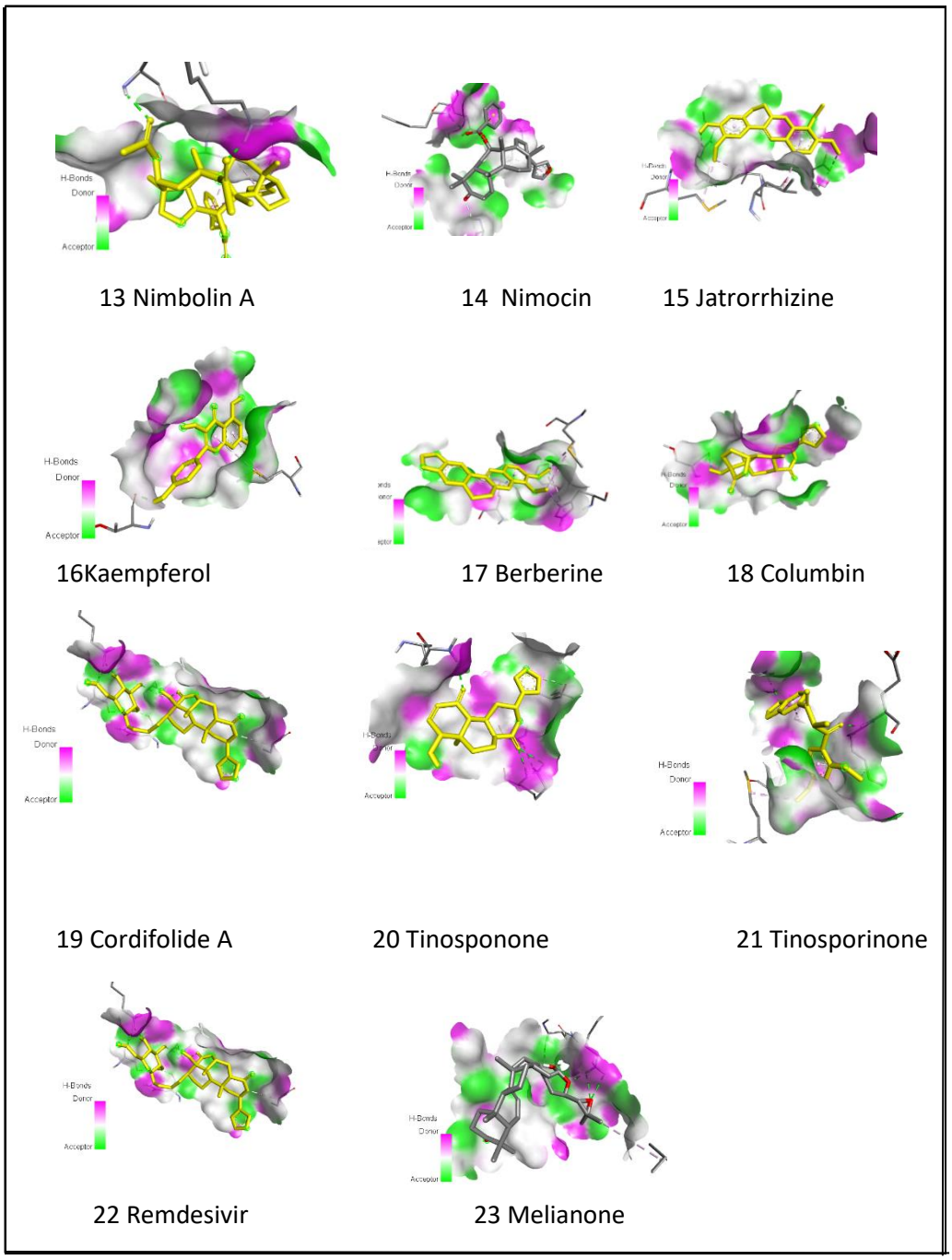


Fig 4. Contd. M$^{pro}$ 6LU7 Ligand conformations with Hydrogen bonds

Nimbin, berberine, mangiferin of *A indica* showed significant binding affinity towards spike protein of SARS-CoV-2 and ACE2 receptor [Vimal K et al (2020)]. 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

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activity against HIV, HTLV and other viral diseases for its immunomodulatory and selective inhibition of the virus to target T helper cells [Chetan B, Nakum A. (2010)].

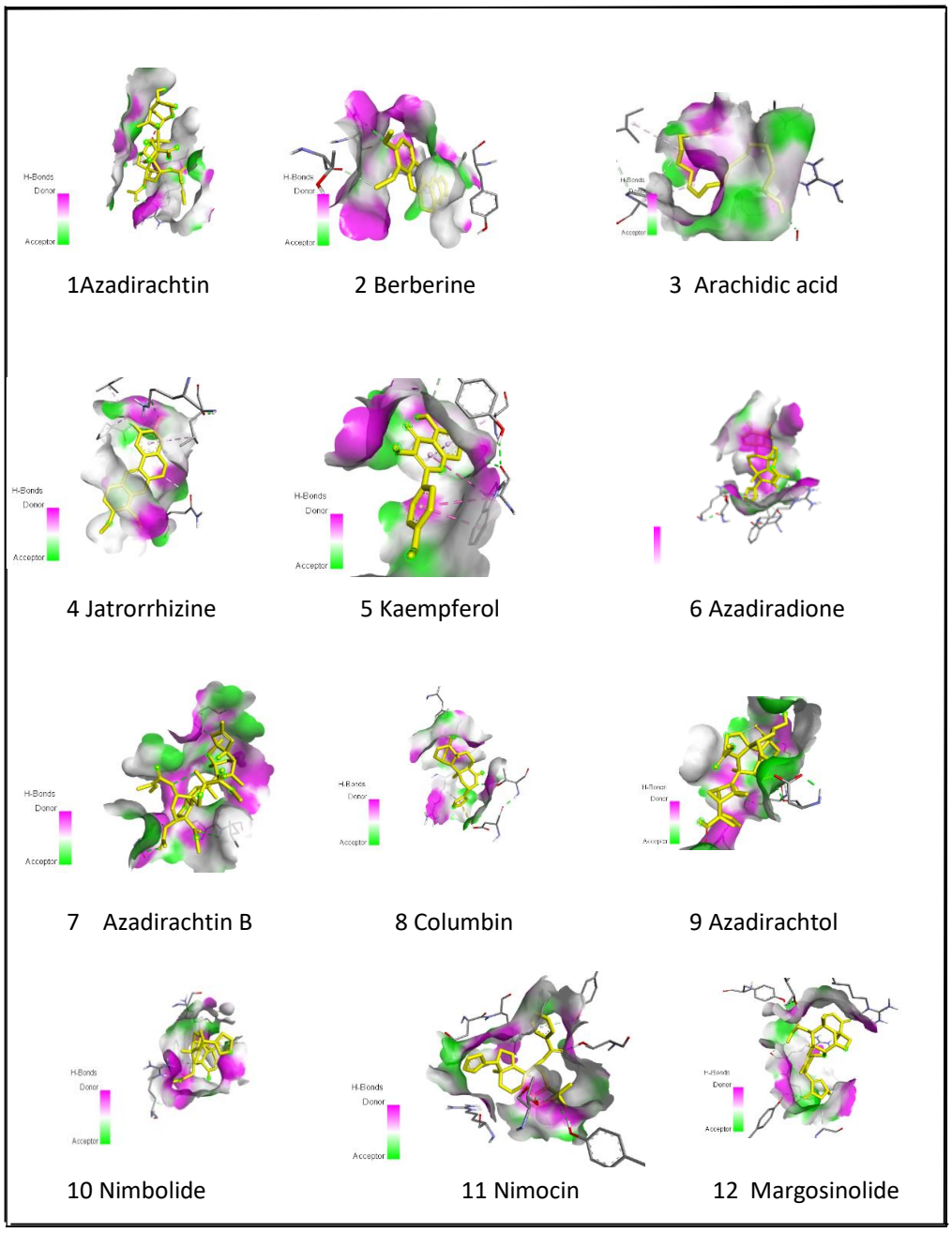


Fig. 5 Spike protease 6VXX with ligand conformations with Hydrogen bonds

Dry cough, fever, dyspnea 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 [Marius Ueffing et al (2020)]. In the low immune older subjects COVID-19 produces severe symptoms and comorbidities may increase the mortality rate in senior citizens.

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The immune-enhance effect of the *T cordifolia* may help in reducing the severity of symptoms.

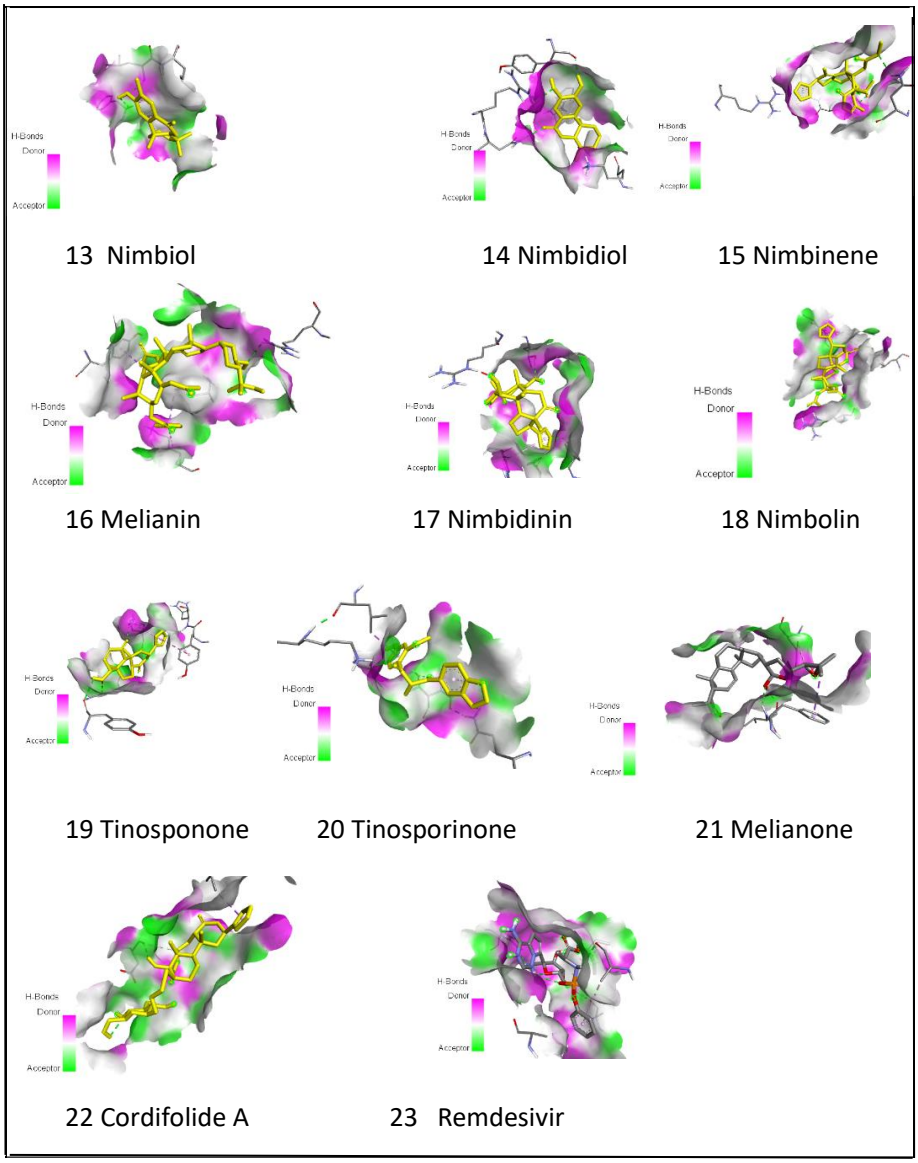


Fig. 5 contd. Spike protease 6VXX with ligand conformations with Hydrogen bonds

## V. CONCLUSION

The Nimba- *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 $M^{pro}$ protease, and substantiate the claim for anti-SARS-CoV-2 by preventing the spike protease-ACE2 target.

Further docking with $M^{pro}$ 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.

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