ADMET STUDIES OF THE PHYTOCONSTITUENT PRESENT IN THE LEAVES OF *AZADIRACHTA INDICA* AND ITS ROLE IN THE MANAGEMENT OF SARS-COV-2 VIRUS

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Abstract

Coronavirus (COVID-19) is a communicable disease that is engendered by the SARS-CoV-2 virus which causes very common to severe symptoms and recovery does not require any specialized treatment. In this study, we have evaluated the effect of the different phytoconstituent present in the leaf of *Azadirachta indica* as a role in the management and treatment of COVID-19. Different constituents such as Limocinin,

Rutin, Nimbinone, Nimbolin, Nimocin and Cycloartanols showed a satisfactory binding affinity towards SARS-CoV-2 protein molecular targets. These constituents were evaluated for various druggable properties like ADMET (Absorption, distribution, metabolism, excretion, and toxicity) profile along with its other physicochemical properties and medicinal chemistry that plays an important role in the draggability of these constituents.

Keywords: *Azadirachta indica*, Coronavirus, SARS-CoV-2

Introduction

Coronavirus disease 2019 also called Wuhan coronavirus/ Wuhan pneumonia [1] is a contagious disease that infects humans was initially identified in China, in December 2019[2]. It is characterized by fever [3], dry cough, tiredness, difficulty in breathing, sore throat, aches and pains, loss of taste or smell [4,5]. Severe symptoms of COVID-19 include hypoxia and dyspnea whereas shock, multiorgan failure and respiratory failure are the critical symptoms [6]. A novel coronavirus SARS-CoV-2 causes COVID-19 that communicates via airborne routes by inhalation of viral droplets formed by sneezing, coughing, and touching the contaminated surfaces [7]. Transmissibility sets about three days prior to the appearance of symptoms and continues for about 20 days [8]. SARS-CoV-2 belongs to the class of beta coronavirus which consists of a positive single-stranded RNA genome made up of ~30000 nucleotides encoding Spike (S) protein, Nucleocapsid (N) protein, Envelop (E) protein, Membrane (M) protein and several Non-structural proteins (nsp). The entry of virion into the host cell is initiated by the attachment of S-protein to the ACE2 (Angiotensin-converting enzyme 2) enzyme available on the exteriors of human lung cells. The S protein is later proteolyzed by host proteolytic enzymes like Furin at S1/S2 site [9]. Cleavage of the S2 domain leads to the release of viral RNA into the cytoplasm where the replication and transcription of the viral genome take place by the mediation of replication/transcription complex (RTC) which is made up of non-structural proteins and encoded in the genome of the viron[10]. Translation of viral RNA results in RNA replicase enzymes which use the viral RNA as a template to create negative sense RNAs. Generated negative sense RNAs act as a template to create further full-length genomes. Spike (S) protein, envelop (E) protein and Membrane (M) protein are produced in the host cytol and incorporated into the endoplasmic reticulum and later transferred into Endoplasmic Reticulum Golgi Intermediate Compartment (ERGIC) where it combines with nucleocapsid synthesized in the cytoplasm leading to the self-assembling of the virion. By the process called exocytosis, virions leave the cell to infect other host cells.

SARS-CoV-2 is found to have thousands of variants that are categorized as clades or lineages [11]. WHO recommends marking these variants using Greek Alphabets? To date, four potent variants are found transmitting across the globe namely Alpha, Beta, Gamma and Delta [12].

Diagnosis of covid-19 can be achieved by various methods like serology, viral cultures, and molecular methods. Important among them are RT-PCR which analyses nucleic acids in the sample to facilitate identification of the virus; POC tests to detect genes coding for N-protein present in the respiratory sample; immunoassay to quantify and identify antigen-antibody interaction. In recent times CRISPR based methods are used in the diagnosis of coronavirus disease 2019[13].

In the early days of the pandemic due to the lack of knowledge and understanding of SARS-CoV-2, the treatment options were limited creating a compulsion to initiate clinical research worldwide to mitigate the newly emerged illness. At present, several treatments are accessible which include anti-SARS-CoV-2 monoclonal antibodies, immunomodulators, anti-inflammatory agents, antiviral drugs [14] and a variety of novel vaccines in defence to the COVID-19 which activates the host immune system resulting in the production of antibodies against the virus. Currently, multiple kinds of vaccines are available globally including NVX-CoV2373 vaccine, mRNA-1273 vaccine, ChAdOx1 nCoV-19 vaccine, BNT162b2 vaccine and Ad26.COV2. S vaccine[15]. Preventive measures of COVID-19 transmission include frequent hand washing, use of face mask, social distancing, avoid touching face with unwashed hands, avoiding crowd, cleaning contaminated surfaces, quarantine, vaccination, etc [16].

Neem

Azadirachta indica, generally called Neem belongs to the family Meliaceae. It is also referred to as 'Indian Lilac' or 'Margosa'. The neem tree is a fast-growing evergreen tree commonly seen in India, Africa, and America [17,18]. It is effectively used in Ayurvedic medicine to treat a variety of human ailments. Neem leaf juice to treat skin problems, neem tea as a tonic, neem twigs to clean teeth and many more. The leaves of this plant yield different phytoconstituents mainly quercetin, nimbosterol, nimbin, nimbolide and nimbanene[19] listed in **(figure 1)**. These phytoconstituents possess various biological activities like antioxidant, anticancer, antibacterial, anti-inflammatory, antiviral, antifungal and antidiabetic properties. Although in adults no precise dose of neem has been fixed yet it appears to be risk-free in smaller quantities. Use of neem is associated with miscarriage, low blood sugar and infertility, and the extended use of which is found to be injurious to the liver and kidney [20]. Neem is found to be an excellent alternative to synthetic pesticides which also act as a fertilizer [21] and animal feed for rabbits and ruminants [22].

In an in vitro antiviral study of *Azadirachta indica* methanolic extract of leaf was found to hinder plaque formation of Coxsackievirus B mainly in six antigenic varieties at 96 hrs. In addition to inhibition of viral replication and yield, a reduction was observed. There exists evidence of polysaccharides of *Azadirachta indica* interfering in the replication of the PV-1 virus [23]. Azadirachtin and the aqueous extracts of neem

leaves were found to interfere in the replication of Dengue virus type 2[24]. Antiviral studies of neem extracts against bovine herpes type 1 and herpes simplex virus type 1 showed promising results. Aqueous extracts of stem and leaf of *Azadirachta indica* illustrated selective virucidal activity against BVH-1 whereas extracts of seeds and leaves show activity against HSV-1[25].

Neem against COVID-19

In a study conducted by Thirumalaisamy Rathinavel, et.al., to discover potential drug candidates for COVID-19, through different in silico virtual screening methods, three Indian traditional medicinal plants (ITMP) were selected in which *Azadirachta indica* was one among them. At the end of in silico virtual screening, ligands such as Baloxavir marboxil from the anti-viral drug group and Limocinin from *Azadirachta indica* were chosen as drug candidates for COVID-19. The outcome of the study was that these drug candidates were found to have a satisfactory binding affinity towards SARS-CoV-2 protein molecular targets, including excellent drug likeliness, pharmacokinetic, electrostatic potential profiles [26].

Titilayo Omolara Johnson, et.al, conducted similar research where the compounds revealed different levels of binding affinities towards the protein target SARS-CoV-2 3C-like protease. Rutin showed the highest binding affinity of -9.140 Kcal/mol and Nimbinone the lowest of -5.480 Kcal/mol among the ten top-scoring compounds [27].

Another study led by Subhomoi_Borkotoky, et.al, utilized various computational tools to pin down various small molecules that bind to the structural proteins, M and E of SARS-CoV-2. Molecular docking was used for the MD simulation and binding free energy calculations, where few feasible inhibitors of these proteins were identified which were the compounds derived from neem. Nimbolin A exhibited the strongest binding free energy with both E and M proteins. Other compounds i.e, Nimocin (24-Methylenecycloartanol) and Cycloartanols (24-Methylenecycloartan-3-one) were also common ligands that bind strongly to both proteins [28]. In addition, desacetylgedunin present in the seeds of Neem was found to inhibit PLpro (Papain like protease) of SARS-CoV-2[[29]].

By above studies, it is promised that appropriate experimental study and optimization of neem, might add value to the development of specific therapeutics against COVID-19 if further elaborate research on clinical trials is made using these drugs candidates.

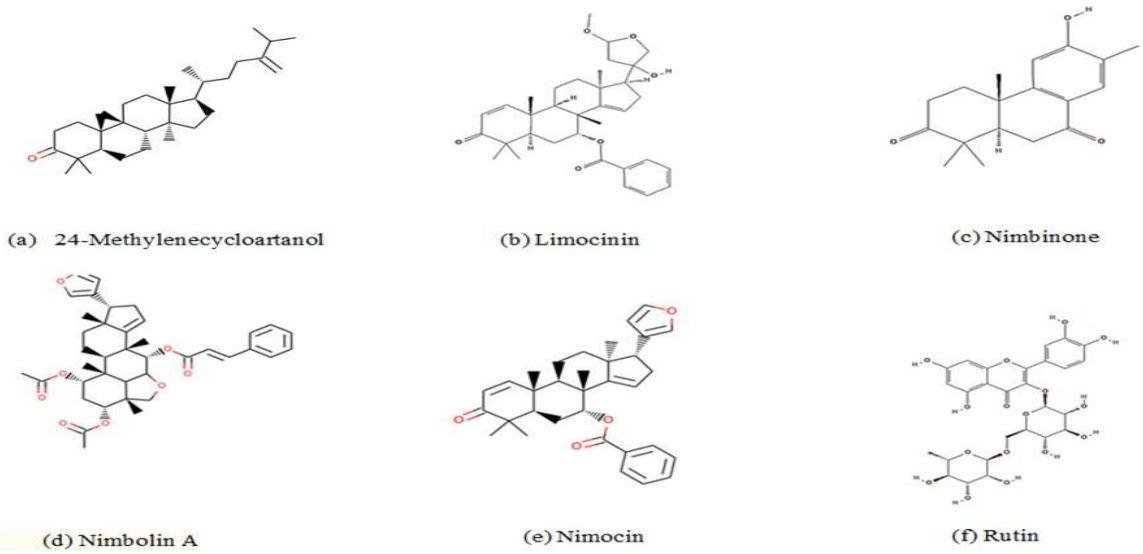

Figure 1: Structures of some phytoconstituents of *Azadirachta indica*

Table 1: Validation of Lipinski rule of selected phytochemicals

Chemical constituent	Properties				Lipinski Rule
	Molecular weight	nHA	nHD	LogP	
24-Methylenecycloartan-3-one	438.39	1	0	7.586	Accepted
Limocinin	548.31	6	1	4.42	Accepted
Nimbinone	286.16	3	1	2.848	Accepted
Nimbolin A	642.32	8	0	4.641	Accepted
Nimocin	440.4	1	1	7.548	Accepted
Rutin	610.15	16	10	-0.763	Rejected

nHA: Number of hydrogen bond acceptors (Optimal <10); **nHD:** Number of hydrogen bond donors (Optimal <5); **LogP:** Log of the octanol/water partition coefficient (Optimal <5); Molecular weight <500.

Table 2: Predicted physicochemical properties of selected phytochemicals

Chemical Constituent	Volume	n Rot	n Ring	nHet	f Char	nRig	SC	logS	logD
24-Methylenecycloartan-3-one	505.467	5	5	1	0	24	8	-6.742	5.848

Limocinin	579.567	5	6	6	0	33	9	-5.353	4.183
Nimbinone	307.403	0	3	3	0	18	2	-4.36	2.937
Nimbolin A	667.162	9	7	8	0	38	11	-5.53	3.514
Nimocin	508.103	5	5	1	0	23	9	-6.389	5.935
Rutin	552.318	6	5	16	0	30	10	-3.928	0.695

Volume: Van der Waals volume; **nRot:** Number of rotatable bonds (Optimal: 0~11); **n Ring:** Number of rings (Optimal: 0~6); **nHet:** Number of heteroatoms (Optimal:1~15); **f Char:** Formal charge (Optimal:-4 ~4); **nRig:** Number of rigid bonds (Optimal:0~30); **SC:** Sterio centre (Optimal: ≤ 2); **logS:** Log of the aqueous solubility (Optimal: -4~0.5 log mol/L); **logD:** logP at physiological pH 7.4 (Optimal: 1~3).

Table 3: Predicted medicinal chemistry properties of selected phytochemicals

Constituents	Caco-2 Permeability	MDCK Permeability	Pgp-inhibitor	Pgp-substrate	HIA	F 20%	F 30%
24-Methylenecycloartan-3-one	-5.159	1e-05	0.12	0.0	0.006	0.986	0.991
Limocinin	-4.889	2.10E-05	1	0	0.003	0.048	0.016
Nimbinone	-4.845	2.20E-05	0.309	0.001	0.005	0.165	0.333
Nimbolin A	-5.087	2.70E-05	1	0.114	0.018	0.721	0.675
Nimocin	-5.11	1.20E-05	0.017	0	0.004	0.977	0.968
Rutin	-6.336	3.00E-05	0.002	0.978	0.925	0.234	0.999

Caco-2 Permeability: Optimal- higher than -5.15 Log unit; **MDCK Permeability:** low permeability: < 2 × 10−6 cm/s, medium permeability: 2–20 × 10−6 cm/s, high passive permeability: > 20 × 10−6 cm/s; **Pgp-inhibitor:** Category 1: Inhibitor; Category 0: Non-; **Pgp-substrate:** Category 1: substrate; Category 0: Non-substrate; **HIA:** Human Intestinal Absorption (Category 1: HIA+(HIA < 30%); Category 0: HIA-(HIA < 30%);); **F 20%:** 20% Bioavailability (Category 1: F20%+ (bioavailability < 20%), Category 0: F20%- (bioavailability ≥ 20%); **F 30%:** 30% Bioavailability (Category 1: F30%+ (bioavailability < 30%); Category 0: F30%- (bioavailability ≥ 30%).

Table 4: Predicted absorption properties of selected phytochemicals

Chemical constituent	Properties					
	QED	SA score	NP score	Pfizer Rule	GSK Rule	Golden Triangle
24-Methylenecycloartan-3-one	0.393	5.432	3.239	Rejected	Rejected	Rejected
Limocinin	0.373	5.149	2.77	Accepted	Rejected	Rejected
Nimbinone	0.793	3.552	2.666	Accepted	Accepted	Accepted
Nimbolin A	0.143	5.379	2.813	Accepted	Rejected	Rejected
Nimocin	0.425	5.447	3.267	Rejected	Rejected	Rejected
Rutin	0.14	4.783	2.015	Accepted	Rejected	Rejected

QED: A measure of drug-likeness based on the concept of desirability (Attractive: > 0.67; unattractive: 0.49~0.67; too complex: < 0.34); **SA score:** Synthetic accessibility score is designed to estimate ease of synthesis of drug-like molecules (SA score ≥ 6, difficult to synthesize; SA score<6, easy to synthesize); **NP score:** Natural product-likeness score (Range: −5 to 5. The higher the score is, the higher the probability is that the molecule is a NP); **Pfizer Rule:** logP> 3; TPSA < 75 (Compounds with a high log P (>3) and low TPSA (<75) are likely to be toxic); **GSK Rule:** MW ≤ 400; logP ≤ 4 (Compounds satisfying the GSK rule may have a more favourable ADMET profile); **Golden Triangle:** 200 ≤ MW ≤ 50; -2 ≤ logD ≤ 5 (Compounds satisfying the Golden Triangle rule may have a more favorable ADMET profile).

Table 5: Predicted distribution properties of selected phytochemicals

Constituent	Property			
	PPB	VD	BBB Penetration	Fu
24-Methylenecycloartan-3-one	96.15%	1.278	0.099	1.983%
Limocinin	98.17%	2.504	0.563	2.28%
Nimbinone	84.68%	0.739	0.326	14.80%
Nimbolin A	98.54%	2.169	0.758	7.10%
Nimocin	96.74%	1.291	0.089	1.99%
Rutin	83.81%	0.754	0.111	20.86%

PPB: Plasma Protein Binding (Optimal: < 90%); **VD:** Volume Distribution (Optimal: 0.04-20L/kg); **BBB Penetration:** Blood-Brain Barrier Penetration (Category 1: BBB+; Category 0: BBB-); **Fu:** The fraction unbound in plasms (Low: <5%; Middle: 5~20%; High: > 20%).

Table 6: Predicted metabolism properties of selected phytochemicals

Constituent	Property									
	CYP1A2 inhibitor	CYP1A2 substrate	CYP2C19 inhibitor	CYP2C19 substrate	CYP2C9 inhibitor	CYP2C9 substrate	CYP2D6 inhibitor	CYP2D6 substrate	CYP3A4 inhibitor	CYP3A4 substrate
24-Methylenecycloartan-3-one	0.048	0.54	0.139	0.968	0.188	0.649	0.258	0.773	0.87	0.859
Limocinin	0.007	0.924	0.152	0.885	0.243	0.067	0.293	0.075	0.695	0.714
Nimbinone	0.338	0.921	0.745	0.809	0.332	0.905	0.185	0.764	0.143	0.332
Nimbolin A	0.049	0.059	0.336	0.071	0.411	0.109	0.595	0.134	0.716	0.479
Nimocin	0.034	0.39	0.078	0.959	0.172	0.341	0.194	0.625	0.776	0.795
Rutin	0.013	0.026	0.011	0.05	0.002	0.246	0.007	0.155	0.013	0.003

CYP1A2/CYP2C19/CYP2C9/CYP2D6/ CYP3A4 inhibitor: Category 1: Inhibitor; Category 0: Non-inhibitor

CYP1A2/CYP2C19/CYP2C9/CYP2D6/ CYP3A4 substrate: Category 1: Substrate; Category 0: Non-substrate

Table 7: Predicted excretion properties of selected phytochemicals

Constituent	Property	
	CL	$T_{1/2}$

24-Methylenecycloartan-3-one	7.209	0.108
Limocinin	13.406	0.016
Nimbinone	16.851	0.546
Nimbolin A	4.516	0.168
Nimocin	7.676	0.044
Rutin	1.349	0.524

CL: Clearance (High: >15 mL/min/kg; moderate: 5-15 mL/min/kg; low: <5 mL/min/kg); **$T_{1/2}$:** Half-life (Category 1: long half-life; Category 0: short half-life; long half-life: >3h; short half-life: <3h).

Table 8: Predicted toxicity properties of selected phytochemicals

Constituents	Properties										
	hERG Blockers	H-HT	DILI	AMES Toxicity	Rat Oral Toxicity	FDA MDD	Skin Sensitization	Carcinogencity	Eyecorrosion	Eye Irritation	Respiratory Toxicity
24-Methylenecycloartan-3-one	0.103	0.305	0.4	0.007	0.273	0.61	0.447	0.216	0.105	0.334	0.953
Limocinin	0.013	0.185	0.026	0.011	0.054	0.974	0.075	0.111	0.003	0.155	0.958
Nimbinone	0.005	0.138	0.201	0.034	0.255	0.92	0.085	0.601	0.007	0.898	0.958
Nimbolin A	0.075	0.699	0.892	0.009	0.957	0.981	0.075	0.032	0.003	0.179	0.955
Nimocin	0.323	0.122	0.053	0.004	0.323	0.915	0.654	0.044	0.095	0.121	0.686
Rutin	0.017	0.092	0.982	0.805	0.05	0.014	0.036	0.064	0.003	0.01	0.015

hERGBlockers: Category 1: active, Category 0: inactive; **H-HT:** Human Hepatotoxicity (Category 1: H-HT positive(+); Category 0: H-HT negative(-)); **DILI:** Drug Induced Liver Injury (Category 1: drugs with a high risk of DILI; Category 0: drugs with no risk of DILI); **AMES Toxicity:** Category 1: Ames positive(+), Category 0: Ames negative(-); **Rat Oral Toxicity:** Category 0: low-toxicity, Category 1: high-toxicity; **FDAMDD:** Maximum Recommended Daily Dose (Category 1: FDAMDD (+); Category 0: FDAMDD(-)); **Skin Sensitization:** Category 1: Sensitizer, Category 0: Non-sensitizer; **Carcinogenicity:** Category 1: carcinogens, Category 0: non-carcinogens; **Eyecorrosion:** Category 1: corrosives, Category 0: noncorrosives; **Respiratory Toxicity:** Category 1: respiratory toxicants, Category 0:respiratory nontoxicants;**Eye Irritation:** Category 1: irritants, Category 0: nonirritants.

Discussion

The current study was carried out to determine the pharmacokinetic properties and drug-likeness of the constituents of *Azadirachta indica* which in virtual molecular docking showed promising binding affinity towards SARS-CoV-2 viral protein molecules causing respiratory tract infections in humans with mild to critical symptoms. The phytoconstituents of neem were investigated for their binding pattern with SARS-CoV-2 protein molecular targets by Thirumalaisamy Rathinavel, et.al., Titilayo Omolara Johnson, et.al, Subhomoi_Borkotoky, et.al, and Baildya N, et al. which revealed that 24-Methylenecycloartan-3-one, Limocinin, Nimbinone, Nimbolin A, Nimocin and Rutin present in Azadirachta indica exhibit acceptable binding affinity towards the target protein under investigation. This motivates further investigations to explore the pharmacokinetics and drug-likeness of these compounds in the hope of the discovery of a new drug molecule for the treatment of COVID-19.

The physicochemical properties and medicinal chemistry of the selected compounds were obtained from the ADMETlab 2.0 online tool **(Table:1-3)** to validate various rules and parameters to determine the drug-likeness of the molecules. By analyzing the molecular weight, logP, logD, number of hydrogen bond acceptors and donors Lipinski's rule, Pfizer rule, GSK rule and Golden triangle for the selected compounds were validated. Out of six molecules, Nimbinone was found to accept all the four rules and possess good oral bioavailability, less toxic and have a more favourable ADMET profile. Limocinin and Nimbolin A accepted Lipinski's and Pfizer's rule which indicates good oral bioavailability and less toxic profile of these compounds. 24-Methylenecycloartan-3-one and Nimocin accepted Lipinski's rule whereas Rutin accepted Pfizer's rule alone.

Absorption plays a very important role in the bioavailability and therapeutic efficacy of any drug molecule. Seven absorption properties of the chosen molecules were inspected **(Table:4)** and it was learned that Nimbinone and Limocinin satisfy all the seven properties and possess good absorption profile followed by Nimocin with five, 24-Methylenecycloartan-3-one and Nimbolin A with four and Rutin with only three satisfying properties.

The distribution of a drug in the body determines the extent of transfer of the therapeutic agent into various body fluids and compartments to exert its action. Protein binding, Volume of distribution (VD), Blood-Brain Barrier (BBB) penetration and Unbound fraction of drug in the plasma (Fu) were investigated **(Table: 5)** which uncovered that Nimbinone and Rutin fulfill all the criteria followed by Limocinin, Nimocin and 24-Methylenecycloartan-3-one with an optimum volume of distribution and BBB penetrability and Nimbolin A possesses optimal VD and satisfied value of Fu. Any xenobiotic that enters the body must be ejected out. This process is facilitated by metabolism which involves changing

the chemical structure of the molecule via different reactions like reduction, oxidation, conjugation, hydrolysis, etc. facilitated by various enzymes that ease the excretion of ingested xenobiotics. The current study examined the probability of the selected compounds for their substrate and inhibitory activity towards five specific enzymes namely CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 **(Table: 6).** The compounds Nimbinone and Nimocin were found to have a higher probability of being a substrate to all the five enzymes; 24-Methylenecycloartan-3-one and Rutin have a greater probability of inhibiting the enzyme CYP3A4 whereas Limocinin exhibit a higher chance of inhibiting CYP2C9 and CYP2D6 enzymes and Nimbolin A appears to be a substrate for only CYP1A2.

The process of ousting the unchanged or metabolized drug molecule from the body is referred to as excretion. Clearance (rate of drug elimination) and Half-life (time taken for the plasma concentration of drug to reduce to half of its initial value) determines the excretion profile of a molecule. The compound Nimbinone was found to have high clearance, 24-Methylenecycloartan-3-one, Limocinin and Nimocin present moderate clearance and Nimbolin A and Rutin exhibit low clearance. Nimbinone and Rutin have a moderate probability of having a long half-life whereas Nimbolin A, 24-Methylenecycloartan-3-one, Nimocin and Limocinin have a low probability of having a long half-life **(Table: 7).**

The chosen compounds were analyzed for their probability of inducing toxicity to various animal and human organs. A total of eleven toxicity properties were evaluated computationally **(Table: 8)**. All the compounds except Nimocin and Rutin have higher chances of inducing Respiratory toxicity, molecules excluding 24-Methylenecycloartan-3-one and Rutin stands a greater probability of being FDAMDD (FDA Maximum recommended Daily Dose) positive, Nimbolin A depicts high chances of Drug-induced liver injury (DILI) and rat oral acute toxicity whereas Nimbinone could exhibit high Human Hepatotoxicity (H-HT) and Rutin was found to have a high risk of DILI and AMES toxicity.

Conclusion

The phytoconstituents like Limocinin, Rutin, Nimbinone, Nimbolin, Nimocin and Cycloartanols of *Azadirachta indica* have shown a satisfactory binding affinity towards the SARS-CoV-2 protein molecular target. Virtual pharmacokinetics and toxicity profile prediction has indicated that Nimbinone possesses excellent drug-likeness and a satisfactory ADMET profile. Apart from Nimbinone Limocinin reflects good absorption and excretion data, Rutin was found to exhibit favourable distribution and metabolism, Nimocin holds a better metabolism profile. As evidence for these studies, a further clinical investigation is needed. It can be concluded that as for the ongoing pandemic the need for the discovery of new drugs in treatment is a major concern. Hence, neem becomes an ideal starting point to identify a new therapeutic compound.

Reference

1. Jiang S, Xia S, Ying T, Lu L. A novel coronavirus (2019-nCoV) causing pneumonia-associated respiratory syndrome. Cellular & Molecular Immunology. 2020 May; 17 (5): 554. doi:10.1038/s41423-020-0372-4.
2. Page J, Hinshaw D, McKay B. In Hunt for Covid-19 Origin, Patient Zero Points to Second Wuhan Market – The man with the first confirmed infection of the new coronavirus told the WHO team that his parents had shopped there. The Wall Street Journal. 2021 February 26; Retrieved 27 February 2021.
3. Islam MA. Prevalence and characteristics of fever in adult and paediatric patients with coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of 17515 patients. PLOS ONE. 2021 April; 16 (4): e0249788. doi:10.1371/journal.pone.0249788.
4. Saniasiaya J, Islam MA. Prevalence of Olfactory Dysfunction in Coronavirus Disease 2019 (COVID-19): A Meta-analysis of 27,492 Patients. The Laryngoscope. 2021 April; 131 (4): 865–878. doi:10.1002/lary.29286.
5. Agyeman AA, et al. Smell and Taste Dysfunction in Patients With COVID-19: A Systematic Review and Meta-analysis. Mayo Clin Proc. 2020 August; 95(8). 95 (8): 1621–1631. doi:10.1016/j.mayocp.2020.05.030.
6. Interim Clinical Guidance for Management of Patients with Confirmed Coronavirus Disease (COVID-19. U.S. Centers for Disease Control and Prevention (CDC). 2020 April 6. Archived from the original on 2 March 2020. Retrieved 2020 September 21.
7. Wang CC, et al. Airborne transmission of respiratory viruses. Science. 2021 August; 373 (6558). doi:10.1126/science.abd9149.
8. Communicable Diseases Network Australia. "Coronavirus Disease 2019 (COVID-19) - CDNA Guidelines for Public Health Units". Version 4.4. Australian Government Department of Health. Retrieved 17 May 2021.
9. Boopathi, et al. Novel 2019 Coronavirus Structure, Mechanism of Action, Antiviral drug promises and rule out against its treatment. Journal of Biomolecular Structure and Dynamics. 2020; 1-14. 10.1080/07391102.2020.1758788.
10. Hasan, et al. A review on the cleavage priming of the spike protein on coronavirus by angiotensin-converting enzyme-2 and furin. 2020.
11. Koyama T, Platt D, Parida L. Variant analysis of SARS-CoV-2 genomes. Bulletin of the World Health Organization.2020 July; 98 (7): 495–504. doi:10.2471/BLT.20.253591.

12. SARS-CoV-2 Variant Classifications and Definitions. CDC.gov. Centers for Disease Control and Prevention. 11 February 2020. Archived from the original on 29 June 2021. Retrieved 18 June 2021.
13. Giri, B, et al. Review of analytical performance of COVID-19 detection methods. Anal Bioanal Chem 2021. 413, 35–48. https://doi.org/10.1007/s00216-020-02889-x.
14. Coopersmith CM et al. The Surviving Sepsis Campaign: Research Priorities for Coronavirus Disease 2019 in Critical Illness. Crit Care Med. 2021 Apr 01;49(4):598-622.
15. Titilayo Omolara Johnson, et.al. Computer-Aided Identification of Bioactive compounds of Azadirachta indica (Neem) with Potential activity against SARS-CoV-2 main protease. https://doi.org/10.21203/rs.3.rs-841180/v1. 2021;5-6.
16. Scientific Brief: SARS-CoV-2 and Potential Airborne Transmission. COVID-19 Published Science and Research. U.S. Centers for Disease Control and Prevention (CDC). 11 February 2020. Retrieved 30 October 2020.
17. R. K. Bijauliya, et al. An updated review of pharmacological studies on Azadirachta indica (neem). International Journal of Pharmaceutical Sciences and Research. 2018; 9(7); 2645-2646.
18. Sharma Pankaj, et al. Review on neem (Azadirachta indica): Thousand problems one solution. 2012; 2(12); 97.
19. Alzohairy MA. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evid Based Complement Alternat Med [Internet]. 2016 [cited 2021 Jun 9];2016.
20. "Neem". Drugs.com. 2020 August 13. Retrieved 21 September 2020.
21. Heinrich W. Scherer, et al. "Fertilizers", Ullmann's Encyclopedia of Industrial Chemistry (7th ed.), 2017.
22. Badam L, Joshi SP, Bedekar SS. 'In vitro' antiviral activity of neem (Azadirachta indica. A. Juss) leaf extract against group B coxsackieviruses. The Journal of Communicable Diseases. 1999 Jun;31(2):79-90.
23. Ligia Carla Faccin-Galhardi, et al. The in vitro antiviral property of Azadirachta indica polysaccharides for poliovirus. Journal of Ethnopharmacology. 2012; 142 (1): 86-90.
24. M.M Parida, et al. Inhibitory potential of neem (Azadirachta indica Juss) leaves on Dengue virus type-2 replication. Journal of Ethnopharmacology. 2002; 79(2): 273-278.
25. Hassan Amer, Wafaa A. Heimy, Hanan A.A. Taie. In Vitro Antitumour and Antiviral Activities of Seeds and Leaves Neem (Azadirachta indica) Extracts. International Journal of Academic Research. 2010 March.

26. Thirumalaisamy Rathinavel, et al. Virtual screening of COVID-19 drug from three Indian traditional medicinal plants through in silico approach. Research Journal of Biotechnology. 2020;15 (10):139.
27. Titilayo Omolara Johnson, et.al. Computer-Aided Identification of Bioactive compounds of Azadirachta indica (Neem) with Potential activity against SARS-CoV-2 main protease. https://doi.org/10.21203/rs.3.rs-841180/v1. 2021;5-6.
28. Subhomoi Borkotoky and Manidipa Banerjee. A computational prediction of SARS-CoV-2 structural protein inhibitors from Azadirachta indica (Neem). Journal of Biomolecular Structure and Dynamics. 2021;1(39).
29. Baildya N, et al. Screening of potential drug from Azadirachta Indica (Neem) extracts for SARS-CoV-2: An insight from molecular docking and MD-simulation studies. Journal of Molecular Structure. 2021 Mar 5;1227:129390.