

Some Compounds from Neem leaves extract exhibit binding affinity as high as -14.3 kcal/mol against COVID-19 Main Protease (M^{pro}): A Molecular Docking Study

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Abstract: Currently the new Coronavirus "COVID-19", also known as SARS-CoV-2, has infected nearly 3 million patients and nearly 200,000+ people have lost their lives due to this pandemic. There is an urgent need to find an antiviral agent that may slow down the spread of the virus. The aim of this study is to assess and evaluate compounds present in leaves of Neem tree (*Azadirachta Indica*) as potential inhibitors for COVID-19 Main Protease (M^{pro}) (PDB code: 6LU7). This will be done by blind molecular docking using PyRx and Auto Vina software. The compounds Hydroxychloroquine and Remdesivir were used for comparative study. The binding energies obtained from the docking of 6LU7 with meliacinanhdyride, nimocinol, isomeldenin, nimbolide, zafaral, nimbandiol, nimbin, nimbinene, desacetylnimbin were **-14.3**, -12.4, -12.3, -12.2, -11.9, -11.8, -11.7, -11.7, -11.4 kcal/mol respectively. Therefore Meliacinanhdyride (K_i=**33.36 pM**) and the compounds from Neem leaves may be a potential treatment option against COVID-19. In addition to that the leaves contain others compounds like Quercetin, Zinc, Vitamin A, Vitamin B1, B2, B6, Vitamin C, Vitamin E etc., which may boost immunity also (Garba, 2019). Further investigation is needed to evaluate the results of this study to consider Neem leaves as potential treatment option as it might inhibit the virus and boost immunity also.

Introduction:

In December 2019, health officials at Wuhan, China reported a cluster of pneumonia cases within the city. Shortly after that they determined that the pneumonia was caused by virus which is of Zoonotic origin and it's caused by novel coronavirus. It's now referred to as COVID-19 or SARS-CoV-2. Initially there was an assumption that the virus transmission is happening only between animals to humans because the initial cases were related to Huanan Sea Food market but it was soon found out that the virus could be also transmitted from human to human. Soon it spread from China to other parts of the world. Each and every country was affected by COVID-

19 . People around the world were affected directly and indirectly by it as the lockdowns imposed due to prevent the spread of COVID-19 made the economies come to a standstill which lead to loss of jobs for millions of people leading to worldwide recession. But till now no cure or drug has been found to prevent the spread of the virus and even some of the prominent drugs candidates also failed in clinical trials due to lot of reasons. And despite little evidence about their effectiveness against COVID-19, drugs like chloroquine and hydroxychloroquine, have been used for patients after it has been approved by FDA. And as of today Apr 26th, 2020 more than 200,000+ people have lost their lives to COVID-19 , so there is an urgent need to find a drug or cure to stop the virus spread worldwide as no drug or cure has been found till now.

Many scientists have started researching on the use of plant-based compounds as potential inhibitors against the COVID-19's main protease (Inderjeet, 2020), (Siti Khaerunnisa, 2020) .In this study, we have choosen to analyze compounds present in the leaves of “Neem” tree (Azadirachta Indica) as it is known to have very good antiviral properties and has been found to be an effective inhibitor against viruses like Herpes (Tiwari V, 2010), Smallpox, Chickenpox (Visweswaran, 2013) (Neem., 1992) and even against HIV (Udeinya IJ, 2004).

Neem: Neem also known as Azadirachta Indica (Nimtree or Indian lilac) is a tree in the mahogany family Meliaceae. It's one of the two species in the genus Azadiractha and is native to the Indian Sub-continent. It's mostly found in tropic and semi tropic regions around the world.. Neem leaves, seeds, flowers are well known for its medicinal properties in India for centuries. In Sanskrit, Neem is called as Universal healer (**Sarva Roga Nivarini**) which means one that cures all ailments and ills. In Africa it's called as “40 cures”. Neem leaves, fruits, flowers, oil from seeds has been used extensively in South East Asia to cure various diseases. And Neem is also known as Nimba which means 'bestower of good health'. The Neem leaf extracts have a powerful antiseptic, antifungal, antiviral and anti-bacterial effect. unlike synthetic chemicals that often produce side effects such as allergic reactions, rashes etc. And Neem when take in small doses doesn't have any side effects or create complications.

Neem as an Antiviral agent: Neem is known for its anti-viral properties and it is found to be effective against host of virus as shown below. Some pox virus have been traditionally treated with a paste of neem leaves by rubbing it directly into the affected skin. And research has shown crude neem extracts absorbed viruses and thereby preventing it from entering uninfected cells, thus Neem can be used for effective prevention against any viruses. Recent research has indicated there is lot more evidence for Neem's antiviral properties and neem leaves has been shown to be effective against smallpox, chickenpox, herpes virus, HIV, cancer, tumor etc., (Neem., 1992)

Since Neem is known to have more than 140+ compounds from its different parts like leaves, trunk, bark, root, flowers and seeds. Only selective compounds from Neem leaf has been considered for the present study as Neem leaves are available as powder

Compounds from Neem leaves selected for the study: Meliacin anhydride, Nimocinol, Isomeldenin, Nimbolide, Zafaral, Nimbandiol, Nimbin, Nimbinene and Desacetylnimbin. (Alzohairy, 2016) (Krishna, 2012) (Qiang Wu, 2014) (Siddiqui, 2004)

Experimental section:

Proteins/Macromolecule: For the experimental study COVID-10 Main proteases was obtained from PDB (<https://www.rcsb.org>) in pdb format. The 6LU7 protein's Chain A was used for macromolecule preparation.

Ligand Selection: Ligands were selected by Lipinski's rule of Five and almost all the ligands selected didn't have more than 1 violation and as per the rule they could be used in molecular docking experiments with the targeted protein as they didn't have more than 2 violations. Ligands calculation was done by using SwissADME(<http://www.swissadme.ch/>)

Here is the list of ligands selected

Table 1 shows the Lipinski's Rule of Five (RO5) of the docking compounds.

S.No	Compound	Molecular Formula	Lipinski's Rule of Five	
			Properties & Rule	Value
1	Meliacinanhydride	C ₃₁ H ₃₈ O ₁₀	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	570.63 2.67 1 10 1 Yes
2	Nimocinol	C ₂₈ H ₃₆ O ₅	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	452.58 4.26 5 1 0 Yes
3	Isomeldenin	C ₂₈ H ₃₈ O ₅	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	454.6 4.38 5 1 0 Yes
4	Nimbolide	C ₂₇ H ₃₀ O ₇	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5)	466.52 3.11

			Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	0 7 0 Yes
5	Zafaral	C ₂₉ H ₄₀ O ₆	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	484.62 3.93 0 6 0 Yes
6	Nimbandiol	C ₂₆ H ₃₂ O ₇	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	456.53 2.38 2 7 0 Yes
7	Nimbin	C ₃₀ H ₃₆ O ₉	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	540.6 3.2 0 9 1 Yes
8	Nimbinene	C ₂₈ H ₃₄ O ₇	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10)	482.57 3.41 0 7

			Violations Met Lipinski's Rule?	0 Yes
9	Desacetylnimbin	C ₂₈ H ₃₄ O ₈	Molecular weight (<500 g/mol) LogP (<5) Hydrogen bond donor (<5) Hydrogen bond acceptor (<10) Violations Met Lipinski's Rule?	498.57 2.77 1 8 0 Yes

All the above ligands met Lipinski's rule and only 2 of them had "1" violations where their mass was greater than 500 (Meliacinanhydride and Nimbin)

Molecular Docking:

Blind Molecular Docking was performed by PyRx-Auto Vina combination. SDF files of each of those above compounds were downloaded from PubChem and were imported in PyRx and then they were converted into Ligands. 6LU7 was also imported into PyRx. Since it's a blind molecular docking no ligand optimization was done and the grid co-ordinates were automatically chosen by the software itself.

Results:

Table 2 below shows the results of docking analysis between the different ligands from Neem and the receptor protein M^{pro} (6LU7). In the below table Ki Inhibition constant was calculated using the binding energy values at 25C (298K)

Compound Name	PUBCHE M ID	Binding Energy (ΔG) with 6LU7 (kcal/mol)	Ki (Inhibition Constant)
Meliacinanhydride	101355584	-14.3	33.36pM
Nimocinol	178770	-12.3	981.45pM
Isomeldenin	76316558	-12.2	1.16nM
Nimbolide	100017	-12	1.62nM
Zafaral	101355583	-11.9	1.92nM
Nimbandiol	157277	-11.8	2.28nM
Nimbin	108058	-11.7	2.69nM
Nimbinene	44715635	-11.7	2.69nM
Desacetylnimbin	176996	-11.4	4.47nM
Hydroxychloroquine	3652	-6.1	34.41 μ M
Remdesivir	121304016	-7.6	27.16 μ M

From the blind docking results with A Molecule of 6LU7 it's crystal clear that the compound Meliacinanhydride exhibited highest binding energy of -14.3 kcal/mol and an inhibition constant value of 33.36 pM (Picomolar) along with other compounds present in "Neem" leaves. The lowest binding energy among the selected compounds was "Desacetylnimbin" with -11.4 kcal/mol

And in comparison, the blind docking results for Remdesivir and HydroxyCholoroquine were -7.6 and -6.1 kcal/mol and their inhibition constants Ki at 25C (298K) were 27.16 and 34.41 μ M whereas Meliacinanhydride inhibition constant Ki at 25C was **33.36pM** (Picomolar)

And also most of the ligands selected from Neem extracts other than the above compounds also exhibit high inhibition constant against COVID-19 Main Protease M^{Pro}(6LU7) (E.g Quercetin,Azadirachitin,Nimbolin etc.,)

Conclusion: Naturally occurring Neem tree has a host of compounds on its leaves, bark, trunk, seeds. Neem leaves has been already known for its antiviral and antibacterial properties around

the world. Since Meliacinanthrydride and other compounds are derived from Neem leaves, ingesting Neem leaves extract powder or crude Neem leaves contains might inhibit the COVID-19 virus by prevent it from replicating. Also Neem leaves are known to reduce blood sugar levels and also they act as ACE inhibitors. (Rotimi O. Arise, 2019) In addition to that Neem leaves has more than 140+ compounds like quercetin, zinc along with Vitamins like Vitamin C, E & K and ingesting them orally will also boost immunity. However, further in vitro and later in vivo tests are needed to evaluate compounds from Neem leaves as clinical drugs. Since the oral availability of these compounds are high further tests are needed to evaluate them against COVID-19 for clinical use.

References

- Alzohairy, M. A. (2016, March). *Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment*. Retrieved from <https://dx.doi.org/10.1155%2F2016%2F7382506>
- Derwand, M. S. (2020, April). Retrieved from Does Zinc Supplementation Enhance the Clinical Efficacy of Chloroquine/Hydroxychloroquine to Win Todays Battle Against COVID-19?: <https://www.preprints.org/manuscript/202004.0124/v1>
- Garba, M. (2019, July). *Quantitative Chemical Compositions of Neem Leaf Aqueous Extracts*. Retrieved from <https://www.rsisinternational.org/journals/ijrsi/digital-library/volume-6-issue-7/114-119.pdf>
- Inderjeet, A. (2020, March). *Eucalyptol (1,8 cineole) from Eucalyptus Essential Oil a Potential Inhibitor of COVID 19 Corona Virus Infection by Molecular Docking Studies*. Retrieved from <https://doi.org/10.20944/preprints202003.0455.v1>.
- Jalil, A. ,. (2014). *Screening and design of anti-diabetic compounds sourced from the leaves of neem (Azadirachta indica)*. Retrieved from <https://doi.org/10.6026/97320630091031>
- Krishna. (2012, February). *Antiangiogenic Effects and Therapeutic Targets of Azadirachta indica Leaf Extract in Endothelial Cells*. Retrieved from <https://doi.org/10.1155/2012/303019>
- Liu, X. Z. (2020). *The crystal structure of COVID-19 main protease in complex with an inhibitor N3*. Retrieved from <http://doi.org/10.2210/pdb6LU7/pdb>
- Mohammad. (2007). *Investigation of Compounds from Azadirachta indica (Neem)*. Retrieved from <http://dx.doi.org/10.3923/ajps.2007.444.445>

- Neem., N. R. (1992). *Neem: A Tree For Solving Global Problems*. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/25121266>
- Parida, U. P. (n.d.). Retrieved from Inhibitory potential of neem (*Azadirachta indica* Juss) leaves on Dengue virus type-2 replication: <https://www.ncbi.nlm.nih.gov/pubmed/11801392>
- Qiang Wu, M. K. (2014, May). *Preclinical Evaluation of the Supercritical Extract of Azadirachta Indica (Neem) Leaves In Vitro and In Vivo on Inhibition of Prostate Cancer Tumor Growth*. Retrieved from <https://doi.org/10.1158/1535-7163.MCT-13-0699>
- Rotimi O. Arise, a. M.-N. (2019, May). *Kinetics of angiotensin -1 converting enzyme inhibition and antioxidative properties of Azadirachta indica seed protein hydrolysates*. Retrieved from <https://dx.doi.org/10.1016%2Fj.heliyon.2019.e01747>
- Siddiqui. (2004, August). *Tetracyclic triterpenoids from the leaves of Azadirachta indica*. Retrieved from <https://doi.org/10.1016/j.phytochem.2004.04.031>
- Siti Khaerunnisa, H. K. (2020, March). *Potential Inhibitor of COVID-19 Main Protease (Mpro) From Several Medicinal Plant Compounds by Molecular Docking Study*. Retrieved from <https://doi.org/10.20944/preprints202003.0226.v1>
- Tiwari V, D. N. (2010, August). *In vitro antiviral activity of neem (Azadirachta indica L.) bark extract against herpes simplex virus type-1 infection*. Retrieved from <https://doi.org/10.1002/ptr.3085>
- Udeinya IJ, M. A. (2004, July). *An antimalarial extract from neem leaves is antiretroviral*. doi:<https://doi.org/10.1016/j.trstmh.2003.10.016>
- Visweswaran, V. (2013, July). *Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind*. Retrieved from [https://dx.doi.org/10.1016%2FS2221-1691\(13\)60105-7](https://dx.doi.org/10.1016%2FS2221-1691(13)60105-7)