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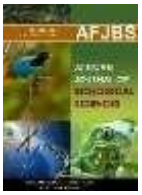

Computer Aided Identification Of Phytocompounds Of Nigella Sativa As Inhibitors Of Papain Like Protease(Plpro) Of SARS-COV2 And Their ADMET Analysis

Narayan Sarkar[1*], Barasha Ray[2], Pranjana Niyogi[3] Dr.Suresh Bharali[4], Dr.Gunajit kalita[5], Suranjana Das[6]

[1*]Phd. research scholar, Gauhati University (sarkarnarayn8@gmail.com)
[2]Msc Botany student, Gauhati University (Barasharoy6@gmail.com)
[3]Assistant professor, Dept. of Botany ,Birjhora Mahavidyalaya
[4]Associate Professor, Dept. of Botany , Tihu college
[5]Assistant Professor, Dept. of Botany, Nalbari college
[6]Dept of Botany Gauhati University (suranjanadas19@gmail.com)

*Corresponding Authors:- Narayan Sarkar
*Phd. research scholar, Gauhati University Email: sarkarnarayn8@gmail.com

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Abstract
The main causative agent of COVID 19 was SARS-COV 2(severe acute respiratory syndrome corona virus 2).This strain of virus uses an enzyme called papain like protease , which is required for processing of some polyproteins.This processing generate functional replicase complex and finally replication takes place. Therefore , to control the viral replication inside the host cell ,it is urgent to inhibit the function of papain like protease.For the discovery of drug against COVID 19 , identification of inhibitor of this protease is first step.In this study,molecular docking of 9 phytocompounds of Nigella sativa plant was done against this viral enzyme(pLpro). The result showed that .beta.-Amyrin has highest binding affinity. Later, ADMET analysis of this compound was done to establish it as a drug molecule. It has good drug-like properties and obeys Lipinski's Rule of 5.

Keywords Molecular docking, SARS-COV2, Replication, Lipinski's Rule of 5

INTRODUCTION

The novel coronavirus infection epidemic that started in China in late 2019 has been growing rapidly and cases have been reported worldwide. SARS -COV2 is a virus from coronaviridae family that has an envelope. The papain like protease pLpro is an essential coronavirus enzyme that is required for processing of viral polyproteins to generate a functional replicase complex and enable viral spread.[1,2]
Efforts to overcome the COVID-19 outbreak, such as the development of vaccines that are recognized as successful so far, then several drugs that are considered to have a good effect on the recovery of COVID-19 patients, as well as several metabolites from medicinal plants. which have been observed to give outstanding results in treatment. Commonly used antivirals often show limited efficacy and serious side effects, herbal extracts have been used for medicinal purposes

since ancient times and are known for their antiviral properties and more tolerable side effects (Ben-Shabat et al., 2020). Thus, nature-based pharmacotherapy can be an appropriate alternative to treat viral diseases.[3]

A molecular docking study of Turmeric active compounds against main protease of SARS-COV 2 , was done by Hasnaa et al.,(2022). The active compounds was identified from Turmeric plant which have antiviral activity by inhibiting the SARS-COV2 protein.

Nigella sativa is a popular plant species due to its culinary and medicinal properties.The seeds of this plant is rich in bioactive compounds and alkaloids , flavonoids etc. The seed oil from this plant can be considered as good candidates to formulate functional ingredients on the basis of scientific knowledge. The aim of this study was

1. Molecular docking of the phytocompounds of Nigella sativa plant against the Papain like protease (pLpro) of SARS-COV2.
2. ADMET analysis of the compounds which has highest binding affinity with viral protease pLpro, to establish it as a drug molecule.

METHODOLOGY

1.Preparation of the Target protein:

The PDB id: 6W9C of Papain like protease of SARS-COV2 was searched in Protein Data Bank(PDB)[www.rcsb.org] .The Three dimensional structure was visualized and the file was downloaded as PDB format.

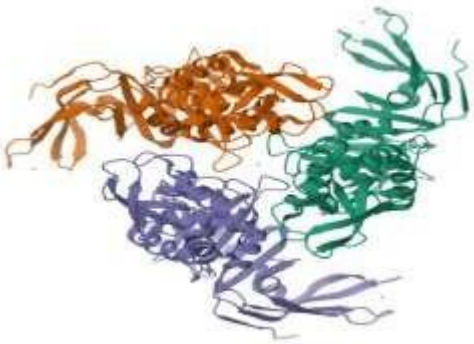
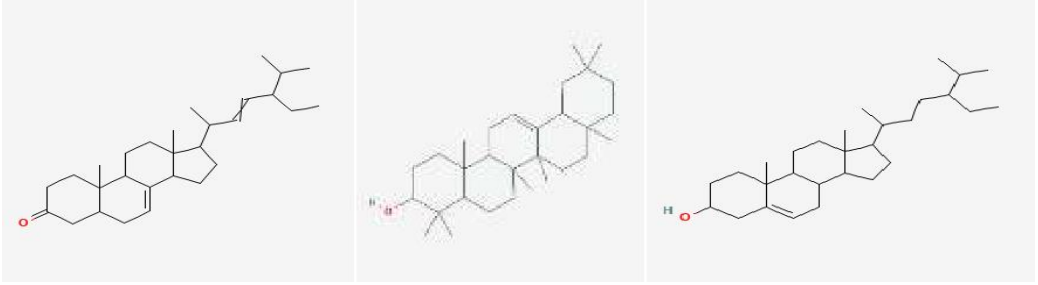


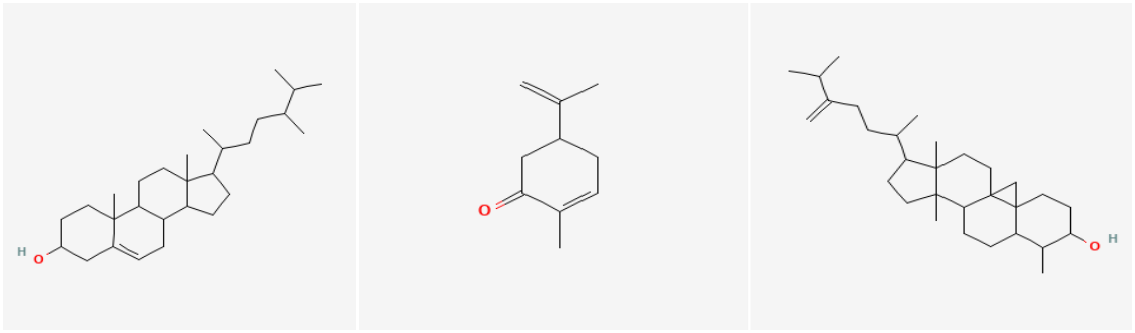
Fig:1. Crystal structure of Papain like protease of SARS-COV2

2.Preparation of the inhibitors:

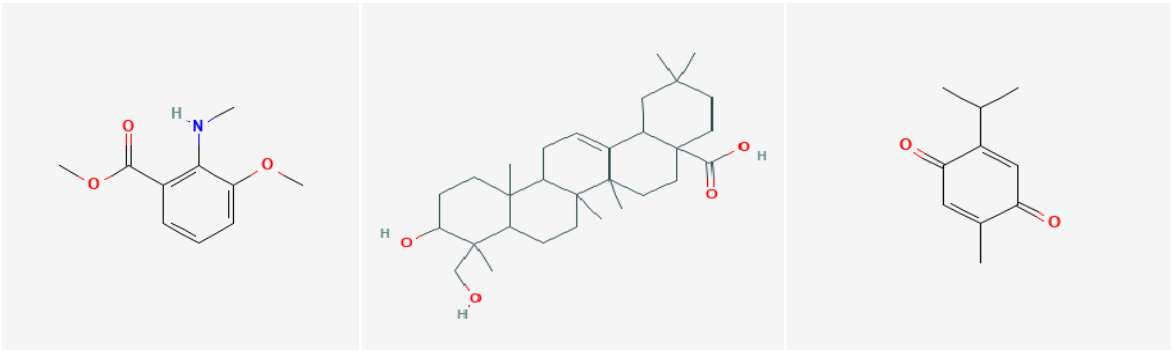
GC-MS analysis of the plant Nigella sativa resulted in 21 phytocompounds out of which 9 was selected for docking analysis. The Phytocompounds were searched in Pubchem and the structure of the compounds was searched and the file was downloaded as Sdf format.



1. Spinasterone 2..beta.-Amyrin 3..beta-sitosterol



4.24-alphamethylcholesterol 5.CARVONE 6.3-epicycloeucalenol



7.Damascenine 8.WilforolC 9.Thymoquinone

Fig2: 2D structures of the compounds

3. Drug likeness of the phytocompounds:

The drug like properties of the phytocompounds was predicted by Swiss.adme online server by pasting the SMILES of the compounds.The molecular weight, LogP, hydrogen bond donor, hydrogen bond acceptor, Topological polar surface area(TPSA) etc. were calculated to check if the compounds obey Lipinski's rule of 5.

4. Molecular docking:

Molecular docking was performed using CB-dock.CB-dock is a protein-ligand docking method which automatically identifies the binding sites , calculates the center and size, customises the docking box size according to query ligands and then perform the molecular docking with Autodock vina.Large scale benchmarks show that the cavity focused docking can enhance the hit ratio and accuracy of blind docking.Accordingly, CB-dock can fascilitate the docking procedure and improve the accuracy by predicting the binding sites of target proteins using curvature based cavity detection approach and the binding poses of query ligands using Autodock vina.

5. ADMET analysis:

The ADMET analysis (Absorption,Distribution, Metabolism, Excretion, and Toxicity) of the compounds was done by online server pkCSM(pharmacokinetics of small molecules) by copying and pasting canonical smiles on the pkCSM server.

RESULTS

1. Drug likeness of the compounds:

All the phytocompounds have Molecular weight <500, hydrogen bond donors <5, Hydrogen bond acceptor < 10, PSA<140. Therefore all of them have good drug like property and obeys lipinski's

rule of 5. However , 6 phytocompounds obey lipinski's rule of 5 with one violation as their MlogP>4.15. The drug likeness of the compounds is shown in table1.

Table1: Drug likeness of the compounds

Compound Name	MW	HBA	HBD	TPSA	MlogP	RO5	nVio
1.Spinasterone	410.67g/mol	1	0	17.07A^2	6.53	yes	1
2. .beta.-Amyrin	426.72	1	1	20.23	6.92	yes	1
3..beta.- Sitosterol	414.71	1	1	20.23	6.73	yes	1
4.24-alpha methylcholesterol	400.68	1	1	20.23	6.54	yes	1
5.CARVONE	150.22	1	0	17.07	2.10	yes	0
6. 3-Epicycloeucalenol	426.72	1	1	20.23	6.92	yes	1
7. Damascenine	195.22	3	1	47.56	1.36	yes	0
8.Wilforol C	472.70	4	3	77.76	4.97	yes	1
9.Thymoquinone	164.20	2	0	34.14	1.08	yes	0

MW- Molecular weight
HBA- Hydrogen bond acceptor
HBD-Hydrogen bond donor
TPSA- Topological polar surface area
RO5- Lipinski's Rule of 5
nVio- Number of violations

2.Molecular Docking:

The result of molecular docking showed different binding affinities of the compounds with the receptor papain like protease(PDB id:6W9C). .beta.-Amyrin has highest binding affinity with pLpro.

Ligand	Docking score
1.Spinasterone	-9.2
2. .beta.-Amyrin	-9.9
3..beta.- Sitosterol	-8.2
4. 24-alpha methylcholesterol	-8.3
5.CARVONE	-6.2
6. 3-Epicycloeucalenol	-8.3
7. Damascenine	-5.4
8.Wilforol C	-9.3
9.Thymoquinone	-6.4

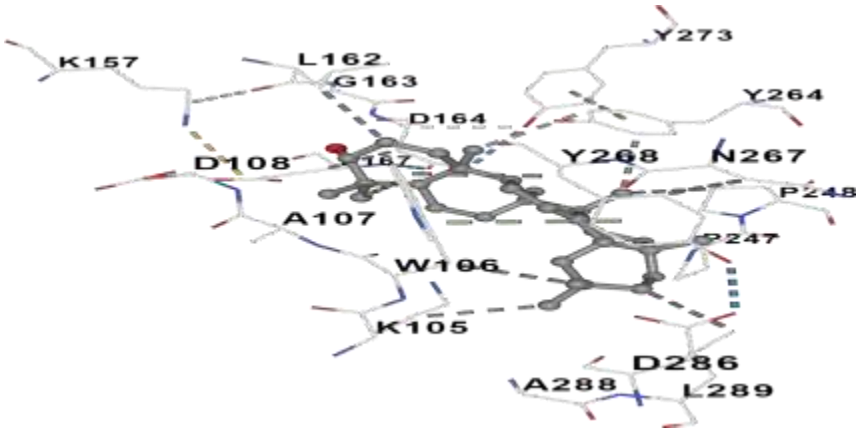


Fig3: Interaction of .beta.-Amyrin with papain like protease of SARS-COV2.

3. ADMET analysis of .beta.-Amyrin:

Absorption	Water solubility	-6.531	Numeric (log mol/L)
Absorption	Caco2 permeability	1.226	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	93.733	Numeric (% Absorbed)
Absorption	Skin Permeability	-2.811	Numeric (log Kp)
Absorption	P-glycoprotein substrate	No	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	Yes	Categorical (Yes/No)
Distribution	VDss (human)	0.268	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0	Numeric (Fu)
Distribution	BBB permeability	0.667	Numeric (log BB)
Distribution	CNS permeability	-1.773	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	Yes	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	No	Categorical (Yes/No)
Excretion	Total Clearance	-0.044	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	-0.56	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	Yes	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.478	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	0.873	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)

Toxicity	T.Pyriformis toxicity	0.383	Numeric (log ug/L)
Toxicity	Minnow toxicity	-1.345	Numeric (log mM)

DISCUSSION

The druglikeness of phytocompounds of Nigella sativa was studied and checked for Lipinski's rule of 5. Lipinski's Rule of Five also known as Rule of five is a rule of thumb for evaluating a drug or determining whether a chemical compound with a particular pharmacological or biological activity has properties that make it an orally administered drug in humans. Based on these rules, 9 active compounds of the Nigella sativa plant were investigated to determine whether or not they matched the requirements of Rule Five. From the screening results, the test ligands used for molecular binding showed that all the compounds used met the five criteria, so they tended to be clinically active if given orally because of their good absorption.

From the molecular docking analysis it was found that the compound .beta.-Amyrin has highest binding affinity with papain like protease.Later, ADMET analysis of this compound was done. According to Chander et al., (2017), a compound is said to have good absorption if its absorption value is >80%, and its absorption is bad if it is <30%.The compound has good absorption intensity. The compound is predicted to be able to penetrate the drug through the bloodstream in the brain. CNS or Central Nervous System Permeability is the ability of a drug to penetrate the central nervous system. Polymorphisms in the CYP family may have the greatest impact on the fate of therapeutic drugs. The CYP2D6, 2C19, and 2C9 polymorphisms are the most frequent variations in phase I drug metabolism, as nearly 80% of currently used drugs are metabolized by these enzymes.

CONCLUSION

The compound .beta.-Amyrin with docking score -9.9 has good interaction with papain like protease of SARS -COV2. So,This compound can inhibit the function of this viral enzyme and replication of the virus inside host cell will not take place.Again this compound obeys Lipinski's rule of 5, has drug like properties. It has good absorption intensity, less toxicity.So, this compound may be used for treatment of COVID19. For further validation of this compound it is suggested for pre-clinical and clinical trials to make them successful and eventually marketed.

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