"IN SILICO ANALYSIS OF CARNOSIC ACID'S THERAPEUTIC POTENTIAL AS A MULTITARGET INHIBITOR AGAINST NIPAH VIRUS REPLICATION"

A Dissertation Submitted
In Partial Fulfillment of The Requirement for The
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in
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Submitted by:

AYUSHI GUPTA
2K22/MSCBIO/15

Under the supervision of:

Prof. YASHA HASIJA

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi college of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India

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Ayushi Gupta

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Thank you

Ayushi Gupta

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi college of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42.

CERTIFICATE'S DECLARATION

I hereby certify that the Project Dissertation titled "**In Silico Analysis of Carnosic Acid's Therapeutic Potential as A Multitarget Inhibitor Against Nipah Virus Replication**", which is submitted by Ayushi Gupta, 2K22/MSCBIO/15, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Ayushi

AYUSHI GUPTA

Place: Delhi

Date: 1/06/2024

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi college of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42.

CERTIFICATE BY THE SUPERVISOR

Certified that Ayushi Gupta (2K22/MSCBIO/15) has carried out their search work presented in this thesis entitled " **In Silico Analysis of Carnosic Acid's Therapeutic Potential as A Multitarget Inhibitor Against Nipah Virus Replication** " for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my/our (print only that is applicable) supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Place: Delhi

Date:

PROF. YASHA HASIJA

SUPERVISOR

Department of Biotechnology

Delhi Technological University

PROF. YASHA HASIJA

HEAD OF DEPARTMENT

Department of Biotechnology

Delhi Technological University

ABSTRACT

In 2023 the Kozhikode district of Kerala, a zoonotic virus with a high mortality rate (40%-70%) infected six people and took two lives. And the downside is that there is no approved medicine against this virus. This offender is a very small 40nm – 1900nm virus of paramyxoviridae family named as Nipah Virus, named for the Malaysian island of Sungai Nipah, where it was first discovered in 1999. It is endemic to Southeast-Asia and Western Pacific specially Bangladesh and India. Fruit bats, the natural reservoir of Nipah Virus are the ones who can transmit the infection to both humans and animals. In this paper we conducted an in-silico assessment of the ability of Carnosic Acid to inhibit Nipah Virus Proteins. The study made use of a number of computational tools, including BioVia Discovery Studio, Auto dock Tools, UCSF Chimera and PyRx Virtual Screening Tool. The results revealed that ligand was a potent inhibitor of Nucleoprotein (4CO6), Phosphoprotein (4N5B) and Large Protein (RNA dependent RNA Polymerase) (Modelled) with docking score of -7.6 kcal/mol, -7.4 kcal/mol and -7.7 kcal/mol, respectively, which is better than that of the standard control Ribavirin. However, the molecule was found to be class 3 toxic in toxicity testing and had a LD50 value of 287mg/kg. We have also explicated the roles of various viral proteins of Nipah Virus in its pathogenicity and infection process to try to determine the likelihood of preparing efficient inhibitors against them.

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi college of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42.

CANDIDATE'S DECLARATION

I, Ayushi Gupta, 2K22/MSCBIO/15 of MSc. Biotechnology, hereby declare that the project Dissertation titled "**In Silico Analysis of Carnosic Acid's Therapeutic Potential as A Multitarget Inhibitor Against Nipah Virus Replication**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science is an authentic record of my own carried out work under the supervision of professor Yasha Hasija. The matter presented in this report has not been submitted by me for the award of any other degree of this or any other Institute/University. The work has been accepted in IEEE conference with the following details:

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

The Nipah virus, a zoonotic virus from the genus Henipavirus within the Paramyxoviridae family, Nipah virus (NiV) has a negative-sense, single-stranded, enveloped RNA genome that is approximately 18.2 kb in length (Devnath et al., 2022).The virus particles range in diameter from 40 to 1900 nm. NiV can lead to fatal encephalitis and respiratory infections ranging from severe to fatal (Harcourt et al., 2000) NiV can lead to fatal encephalitis and respiratory infections ranging from severe to fatal. The symptoms at the very beginning of the infection are headache, fever, dizziness, and a sore throat. These symptoms can later develop into acute encephalitis, which leads to death in some cases (Hossain et al., 2008). Nipah virus (NiV) can be transmitted from infected pigs to humans, as seen in the Malaysia outbreak. It can also be transmitted directly from fruit bats, the natural reservoir of NiV, to humans and animals. This transmission occurs through the consumption of fruits or juices contaminated with the bats' body fluids, as observed in outbreaks in India and Bangladesh.(Rahman et al., 2012) However, incidences of person-to-person transmission were recorded in Bangladesh and India but not in Malaysia(Luby & Gurley, 2012)

The first Nipah virus (NiV) epidemic was documented in 1998 in Kampung Sungai Nipah, Malaysia and subsequently in Bangladesh. About 300 cases have been reported during the initial Nipah virus outbreak that occurred in Malaysia and Singapore in 1998–1999. The outbreak resulted in over 100 deaths due to encephalitis and respiratory illness caused by the NiV infection.(CDC & Nih, n.d.).

Recently, around September 12 and 15, 2023, six confirmed cases of the Nipah virus have been identified in the district of Kozhikode in Kerala, including two persons being declared dead by the Kerala government. (*Nipah Virus Infection - India*, n.d.). In South India, the NiV outbreak occurred in Kozhikode, Kerala, in May 2018, with 17 confirmed instances of mortality. This indicates high mortality and person-to-person transfer.(Thomas et al., 2019) The two outbreaks in India are in West Bengal, which saw the initial epidemic in 2001, and it happened there once again in 2007.(Thomas et al., 2019) . In Bangladesh, 161 individuals have died as a result of NiV outbreaks.

Since then several Nipah viral outbreaks have been reported in Bangladesh and IndiaNiV Malaysia (NiV-M) and the more virulent NiV Bangladesh (NiV-B) are the two strains of the Nipah virus (NiV) that have been reported. Due to its high mortality rate, the Centers for Disease Control and Prevention (CDC) in the USA has classified Nipah virus as a Bio-Safety Level-4 agent.(CDC & Nih, n.d.)

1.2 Objectives of this study:

1. To investigate natural active phytochemical as a substitutive therapeutic approach to ameliorate existing medication.
2. Pharmacokinetic analysis and prediction of drug likeliness properties of the lead
3. To conduct molecular docking studies to evaluate the affinity of Carnosic Acid active phytochemical from leaves of *Rosmarinus officinali* for the inhibition of in Nipah virus.

CHAPTER 2

LITERATURE REVIEW

2.1 Nipah Virus Genome

The 18.2 kb long, negative-sense ssRNA genome of the Nipah Virus encodes for six main structural proteins (N, P, M, G, F and L) and three nonstructural proteins (W, V and C) (Debroy et al., 2023) (Table I). The non-structural proteins are also coded by the P gene, either by RNA editing (V and W proteins) or by an alternative open reading frame (C protein) (Kulkarni et al., 2009; Welch et al., 2023)

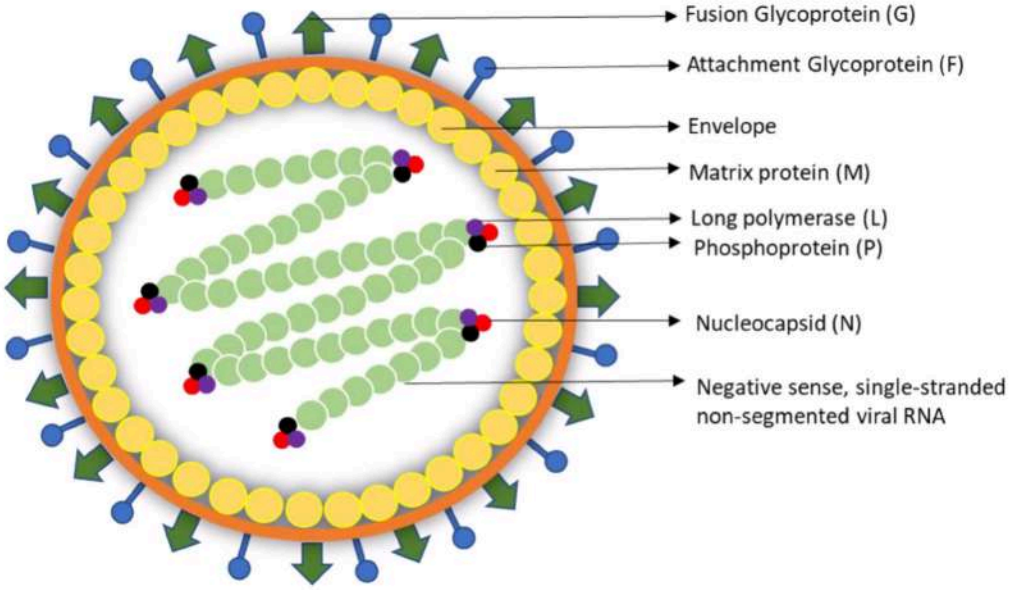

Fig 2.1 structural protein of Nipah Virus

The outer surface of the Nipah virus is characterized by a pleomorphic extracellular bilayer containing the attachment (G) and fusion (F) proteins. The inner surface is structured by matrix proteins (M) that form the viral capsid.(Kerry et al., 2019) The phosphoprotein (P), nucleoprotein (N), and RNA Dependent RNA-Polymerase (L) are all attached to the genomic RNA forming a ribonucleoprotein complex (Ker et al., 2021a). The matrix protein is found attached to the inner side of lipid membrane forming a protein shell and the G and F glycoproteins are present on the surface as spike proteins.(Talukdar et al., 2023)

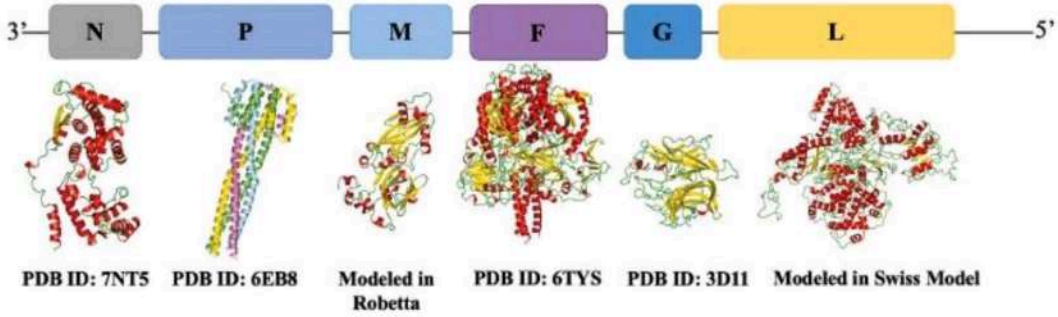

Fig 2.2 NiV proteins and the corresponding models and crystal structures.

TABLE 1: Nipah Virus Proteins and their Significance

Viral Protein	Significance	Reference
Structural Proteins		
Nucleoprotein (N)	Replication, regulation of transcription and encapsidation	(Sun et al., 2018a)
Phosphoprotein (P)	Polymerase cofactor, Chaperone and involved in host immunosuppresion	(Bruhn et al., 2014a)
Matrix protein (M)	Viral assembly and releaseing	(Dietzel et al., 2016)
Glycoprotein G	Attachment Glycoprotein- Mediates viral entry by binding to Ephrin B2 and B3 Receptors	(Priyadarsinee et al., 2022)
Glycoprotein F	Fusion Glycoprotein – Helps in fusion of virus and cell and also infected cell with non-infected one	(Morrison, 2003a)
Large protein (L)	RNA dependent RNA polymerase	(Sun et al., 2018b)
Non-Structural Proteins		
V protein	Inhibit host innate immune response by stopping interferon-β synthesis	(Childs et al., 2007)
W protein	Virulence factor causing disruption of host inflammatory response by downregulating cytokine production	(Enchéry et al., 2021)

C protein	Serves as a virulence factor and inhibits production of proinflammatory chemokines thereby affecting host immune response	(Mathieu et al., 2012)

2.2 Mechanism of Infection

The G and F surface glycoproteins are majorly responsible for the entry of virus in the host cell. The entry is mediated by the transmembrane attachment glycoprotein G which recognizes Ephrin B2 and Ephrin B3 receptors and the Fusion (F) glycoprotein promotes in the combining of the viral and target cell plasma membranes. On binding with Ephrin receptors, the G protein undergoes certain conformational modification which, in turn, causes the refolding of F protein. Initially expressed as a precursor (F0), the Fusion glycoprotein, is later cleaved into two subunits (named F1 and F2 attached by disulfide bond) by the endosomal protease cathepsin L(Morrison, 2003b). Following fusion viral RNA enters the host cell and is transcribed in the cell cytoplasm by viral polymerase enzyme L. The phosphoprotein P enhances polymerase processivity by acting as a cofactor. (Bruhn et al., 2014b)

Availability of sufficient amounts of Nucleoprotein (N) is important for the switch between transcription to replication as it encapsidates the viral RNA when it is being synthesized to protect it from degradation. Here, phosphoprotein P acts as a chaperone for nucleoprotein and prevents it from binding to the host RNA. (Bruhn et al., 2014b)The glycoproteins G and F undergo co-translational translocation from where they reach the cell membrane via the secretory pathway whereas the other proteins are translated in the cytoplasm by free ribosomes. (Diederich & maisner, 2007) Eventually, the ribonucleoprotein complex (genomic RNA attached to N, P and L) as well as the other proteins reach the plasma membrane for virion assembly and budding. The matrix protein M aids in viral assembly and budding by forming dimers and then start associating as pseudo-tetramers causing curvature of the membrane. They maintain integrity of virion membrane by lining

at its inner side which is possible due to the presence of regions of positive charges and exposed hydrophobic residues at the membrane binding face. (Watkinson & Lee, 2016)

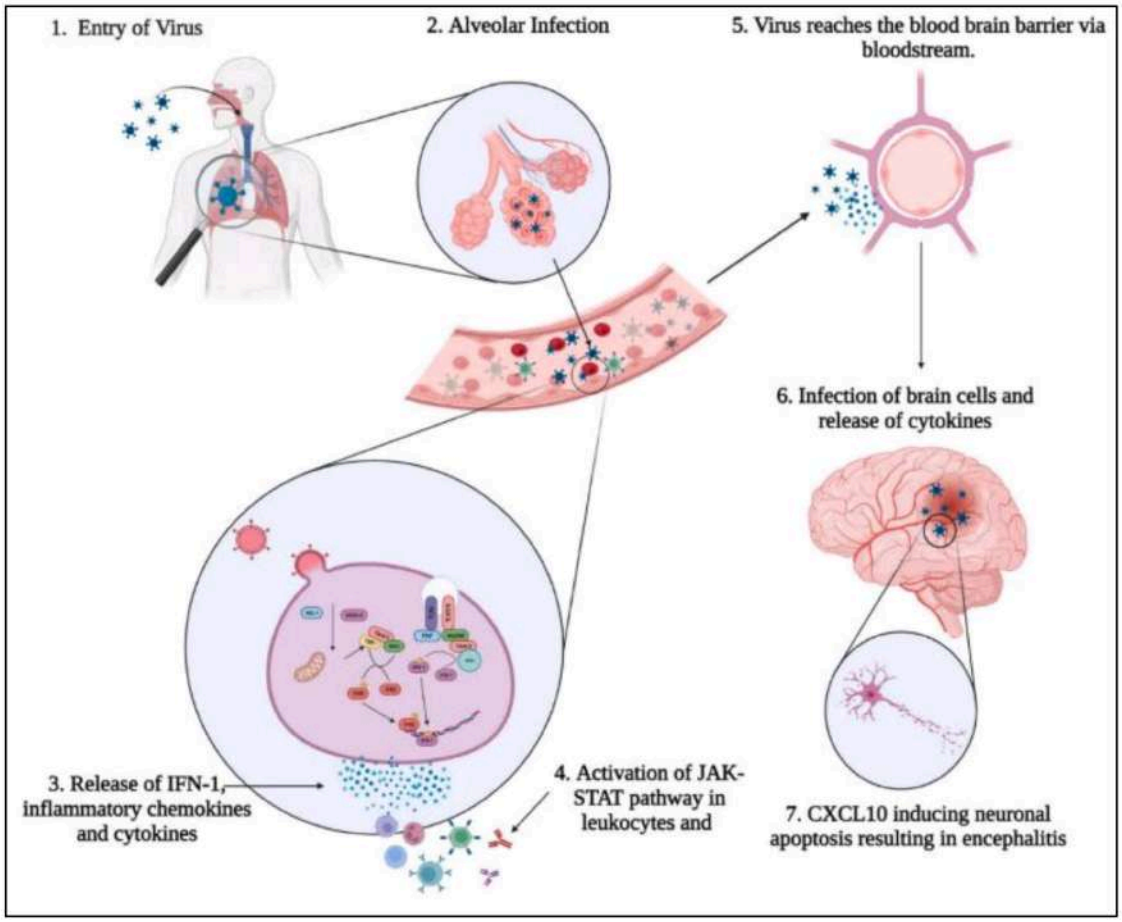

Fig2.3 Nipah virus disease progression and immunological response.

Moreover, the non-structural proteins aid the infection process by downregulating host immune system. The V and W proteins block IFN-induced signal transduction by binding to signal transducer and activator of transcription (STAT) proteins that are the necessary transcription factors in interferon (IFN) and cytokine signaling. (Keiffer et al., 2020)

2.3 Current therapeutics for Nipah Virus

The Nipah virus infection is a very contagious disease that affects animals as well as people.(White et al., 2020) . There isn't yet a particular medication or vaccination available to treat Nipah virus infection. Since no therapeutic FDA-approved drugs have been licensed for treatment, Supportive care can be provided to current patients to alleviate the symptoms and consequences associated with the diseases.(World Health Organization. National Guideline for Management, Prevention and Control of Nipah Virus Infection Including. 2011, n.d.)

2.3.1 Analogs of Nucleosides and Nucleic acids

Remdesivir: broad-spectrum antiviral prodrug that inhibits RNA polymerase. Both in vitro and in vivo research have shown that the Nipah virus inhibits replication. As a preventative measure against recurrence, it may serve as a therapeutic alternative and an adjuvant for viral clearance in survivors. The use of remdesivir in some individuals is supported by the strong viral clearance observed in experimental animals treated with the medication. High seropositive sustained titers are associated with partial viral clearance in people and an increased risk of infection recurrence.(Lo et al., 2019)

Favipiravir: a specific antiviral medication that inhibits RNA polymerase, has demonstrated a significant capacity to block transcription and replication in vitro, even at low doses. In experimental animal models utilizing Golden Hamsters, full resistance to lethal Nipah virus (NiV) doses has been demonstrated. This positions Favipiravir as a promising therapeutic agent for humans and a potential option for post-exposure prophylaxis.(Dawes et al., 2018)

Ribavirin: wide ranging antiviral, either by itself or in association with chloroquine, demonstrated a 36% relative mortality risk decrease during the Malaysia-Singapore epidemic. Nevertheless, in vivo research, including animal models using African Green Monkeys, did not indicate any decrease in mortality.(Dawes et al., 2018; Thakur & Bailey, 2019)

2.4 Potential health benefits of Carnosic acid

Carnosic acid also possesses anti-bacterial, anti-inflammatory and neuroprotective activities. Carnosic acid demonstrate that it have inhibition activity against HIV , the antiviral effect of carnosic acid Results Carnosic acid from R. officinalis L. was identified as a potent candidate to prevent hRSV infection. carnosic acid was identified as the compound responsible for inhibiting hRSV replication, implying progression towards therapeutic use as a prophylactic for hRSV infection.(H. B. Shin et al., 2013)

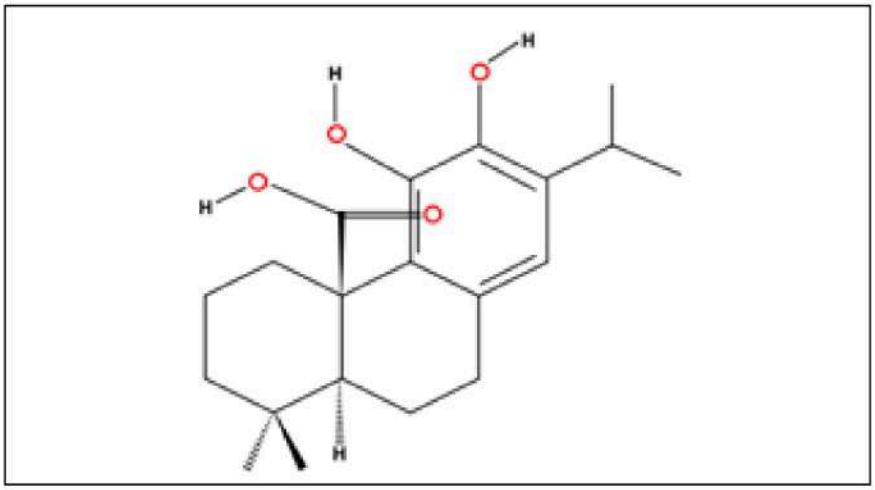

Fig 2.4 Carnosic Acid 2D structure

Antiviral Activity: Carnosic acid blocks both the A and B forms of the (hRSV) human respiratory syncytial virus, representing strong antiviral action against the virus. It is an option treatment for hRSV infections because it efficiently reduces viral gene expression without causing the generation of interferon or impacting cell viability. (Shuang-Cheng Ma, 2002; Wang et al., 2012)

Prophylactic and Therapeutic Agent: Research on carnosic acid have demonstrated its effectiveness against hRSV in both prophylactic and therapeutic capacities. Its capacity to treat hRSV infections is demonstrated by its inhibition of viral RNA synthesis, minimizing hRSV initial infection, and suppression of progeny virus generation. (Satoh et al., 2008)

Neuroprotective Activities: Apart from contributing to its antiviral characteristics, carnosic acid has been identified as having neuroprotective activities. These findings may have consequences for disorders involving degeneration of neurons or damage.(Satoh et al., 2008)

Anti-bacterial Activity: Rosmarinus officinalis, contains carnosic acid, which has antibacterial activity against a variety of microorganisms. Its antibacterial action has been shown in studies, making it a possible agent to fight bacterial infections. (Bernardes et al., 2010)(OLUWATUYI et al., 2004)

Anti-inflammatory Effects: Carnosic acid has anti-inflammatory properties and can help reduce inflammation in the body. This type of activity is helpful for disorders including arthritis, cardiac issues, and various cancers where inflammation is a significant factor. Carnosic acid may help alleviate the symptoms of inflammatory conditions by modulating inflammatory pathways. The biological structure and bioactive characteristics of carnosic acid are responsible for its antibacterial and anti-inflammatory activities. By modifying inflammatory signaling pathways, it may engage with certain targets in bacterial cells to suppress growth and provide anti-inflammatory effects.

2.5 Carnosic Acid as Viral Protein Inhibitor

Carnosic Acid (PubChem CID 65126), also called salvin, is a natural compound found in Lamiaceae family of plants such as rosemary (Rosmarinus officinalis) leaves and common sage (Salvia officinalis) (Birtić et al., 2015). It is a phenolic diterpene that belongs to the terpenoids class of plant secondary metabolites. It possesses antioxidative, anti-bacterial, anti-tumor, neuroprotective and anti-inflammatory activities (Bahri et al., 2016) .

Carnosic acid is mainly found in aerial parts of plants, especially in photosynthetic tissues like leaves. Trichomes in rosemary accumulate more carnosic acid than other leaf tissues. Studies have shown that English climate favors higher production of carnosic acid compared to Mediterranean conditions, and UV-B radiation can increase its yield. Its presence in plants is influenced by developmental stage, environmental conditions, and nutrient availability.

In their study, Shin et al. demonstrated the effect of carnosic acid on Respiratory Syncytial Virus (RSV), another virus from paramyxoviridae family. The study revealed that carnosic acid inhibited the replication and initial infection in RSV. Since the levels for viral RNAs were low upon treatment with carnosic acid, they suggested inhibition of viral proteins involved in viral RNA synthesis such as polymerase and cofactors as the possible mechanism (H.-B. Shin et al., 2013).

Nipah Virus Large protein (L) contains a RNA Dependent RNA Polymerase (RdRp) catalytic domain (residues VAL 715 – GLY 899), a polyribonucleotidyl transferase domain and a methyltransferase (MTase) domain (Gilman et al., 2019) (https://www.uniprot.org/uniprotkb/Q997F0/entry).

Nipah Virus Nucleoprotein (N) is a 532-residue long homomultimer that forms the nucleocapsid by binding to the viral RNA to protect it from the action of nucleases. Newly synthesized free nucleoprotein is also called as nascent nucleoprotein (N0). (Ker et al., 2021b). Nascent nucleoprotein binds to the phosphoprotein via its N terminus region to avoid getting polymerized before interacting with RNA that is done via its C-terminus.(Bruhn et al., 2014a; Yabukarski et al., 2014)

Nipah Virus Phosphoprotein (P) is a 709-residue long homo-tetramer. The residues 1 to 469 form the N-terminal domain where residues 1 to 50 contain the binding site for N0 (Nascent nucleoprotein) aiding its activity as a chaperone. The residues 470 to 578 of the C-terminal region of the protein contains a P multimerization domain critical for L-P complex formation during replication. It also contains a flexible linker and the X domain

(residues 660 to 709) which mediates binding of L-P complex to thenucleocapsid-RNA template complex (Bruhn et al., 2014a).

2.6 Factors Influencing Carnosic Acid Presence

- **Distribution in Plants**: Carnosic acid occurs specifically in aerial plant parts (leaves, sepals, and petals), and its concentration varies with the developmental stage and environmental factors.

- **Environmental Conditions**: Temperature, rate of precipitation, and relative water content in the plant may affect the carnosic acid levels. In the presence of elevated temperatures and low precipitation, water concentrations in rosemary are reduced, comes with reduced concentrations of carnosic acid.(Birtić et al., 2015)

- **Nutrient Availability**: The amount of carnosic acid very much depends on its nutrient availability or limitation. For example, salt stress induced by high sodium levels decreases the content of carnosic acid, while potassium and calcium increase the accumulation of carnosic acid in leaves.

- **Trichome Contribution**: Research indicates that trichomes may have an impact on carnosic acid levels. In particular, there are signs that trichomes are the location of a sizable amount of carnosic acid in rosemary leaves. Trichome-specific gene expression suggests a function for them in the carnosic acid biosynthesis pathway.(Birtić et al., 2015)

CHAPTER - 3

MATERIALS AND METHODOLOGY

3.1 TOOLS & SOFTWARES

This is a brief overview of the software and databases used in in-silico molecular docking and pharmacological study.

3.1.1. Blastp (protein-protein BLAST)

The Basic Local Alignment Search Tool (BLAST) can identify regions of local similarity between sequences. This software evaluates the statistical significance of matches between nucleotide or protein sequences and sequence databases.

3.1.2. SWISS-MODEL

SWISS-MODEL, through its web interface, provides various levels of user engagement. In the "first approach mode," users only need to provide a protein's amino acid sequence to generate a 3D model. The server then automatically manages template selection, alignment, and model construction(Schwede et al., 2003).

3.1.3 UNIPROT

The Universal Protein database (UniProt) is a dependable, extensive, and openly accessible primary database for protein sequences and functional annotation. It is managed by the UniProt Consortium, a collaboration between the Swiss Institute of Bioinformatics (SIB), the Protein Information Resource (PIR), and the European Bioinformatics Institute

(EBI). The main efforts include creating an intuitive UniProt website, archiving sequences, manually curating protein sequences with computational analysis assistance, and cross-referencing to other databases for additional value-added information. UniProt comprises four main components, each serving a specific purpose: the UniProt Knowledgebase, UniProt Reference Clusters, UniProt Archive, and UniProt Metagenomic and Environmental Sequences database. Every three weeks, UniProt is updated and released. It is available for online searches and download at (http://www.uniprot.org).

3.1.4. RCSB PDB (Protein Data Bank)

The Protein Data Bank (PDB) is a crucial resource for structural biology researchers. It offers access to three-dimensional structures of biological macromolecules, such as proteins, nucleic acids, and complex assemblies, determined through experimental methods. The PDB is managed by the Worldwide Protein Data Bank (wwPDB), a global cooperation of organizations.(Berman et al., 2000)

Important aspects of the PDB

- **Repository of 3D structures**: since 1971, PDB has served as a comprehensive database store of 3D structural data for biological macromolecules. It includes information and the 3D structure of proteins and nucleic acids.
- **Open accessible data**: RSCB PDB is freely available; it provides open access to its database. Researchers can retrieve data from this database and use this information for their work**.**
- **Experimental determination of structure:** these methods X-ray crystallography, NMR spectroscopy, and cryo-electron microscopy are used for the determination of PDB structure of the proteins and nucleic acids
- **Structural Annotations and Metadata**: PDB data include detailed structural annotations and metadata. This covers details on biological function, ligand-

binding sites, post-translational changes, and other important features. It also offers information about the protein's or nucleic acid sequence. Annotations may include data on biological assembly, crystal packing, and experimental settings.

- **Unique identification number:** for easy retrieval and referencing of the molecules.
- **Visualization:** PDB provides visualizations of the protein structure for analyzing the molecular structure.
- **Data Deposition & Community Contribution:** The PDB permits researchers worldwide to deposit data and contribute to the community. Sharing empirically discovered structures among academics broadens knowledge and improves research quality. Placing the data in the PDB allows for future research to build on previous discoveries.

3.1.5 PubChem

PubChem (https://pubchem.ncbi.nlm.nih.gov/)(Kim et al., 2023) The National Centre for Biotechnology Information (NCBI), a part of the National Library of Medicine (NLM) in the US, manages the PubChem database. It is a premium resource for scientists and researchers who are working in chemistry, biology, drug discovery, and bioinformatics. PubChem covers the biological roles, chemical compositions, and properties of small organic molecules.

Substances, compounds, and bioassays are the three main components of the database. Under substance, you find information on unique chemical compounds, including their chemical structures, synonyms, and links to related sites. In-depth details on specific chemical compounds are provided in the compounds section, including links to academic publications, descriptions of their physical and chemical properties, and anticipated and

experimental features. Biological activities information about chemicals are provided under bioassays sections.

3.1.6 UCSF Chimera 1.17.3

Chimera is a software used for the visualization and analysis of molecular structures. including conformational ensembles, sequence alignments, density maps, supramolecular assemblies, docking results, and pictures of excellent quality can be produced. Chimera is available for free download.(Pettersen et al., 2004)

3.1.7 AutoDock Tools 1.5.6

AutoDock is a suite a docking tools. Its purpose is to dock small molecules, such as drug candidates or substrates, would attach to a known three-dimensional receptor. For academics working in high-performance computing facilities and handling large-scale computational workloads, Autodock is an indispensable tool for molecular modeling. It is particularly helpful for research on biomolecular interactions and drugs development.(Morris et al., 2009)

3.1.8 PyRx 0.8

PyRx is a program designed for virtual screening in computational drug discovery. It is useful for screening chemical libraries against potential targets for drugs(Dallakyan & Olson, 2015).

3.1.9 BIOVIA Discovery Studio Visualizer

Drug development and research require the analysis and interpretation of enormous biological data. A comprehensive tool known as BIOVIA Discovery permits bioinformatics processes. It offers researchers a variety of data management, analysis, visualization, and predictive modeling functions. BIOVIA Discovery. By combining many data formats, enabling cutting-edge analytic techniques, and encouraging

collaboration, BIOVIA Discovery promotes the development of bioinformatics research and the discovery of new treatments.

3.1.10 Pharmacological study

Pharmacological study assesses if test compounds have drug-like characteristics, determining their potential as drug molecules. To achieve this, the following assessments were completed.

RO5 analysis: Lipinski's rule of five

The compounds' bioavailability may be evaluated based on the extent to which they adhere to Lipinski's rule of five, or RO5. An open-access SwissADME server is used for predication, the canonical smiles for each molecule were obtained and were used as inputs for this tool and radars for bioavailability were deployed.

Toxicity analysis

Toxicity analysis were done on the basis of toxicity end points (Carcinogenicity, Immunogenicity, Mutagenicity and Cytotoxicity). The drug likeness is shown in the bioavailability radar where the pink area represents the optimal range.

3.2 METHODOLOGY

3.2.1 Identification and selection of natural compound

A literature review was conducted to identify a natural compound that inhibits the effect of the Nipah virus. Rosemary (Rosmarinus officinalis), a Lamiaceae family of plants, is selected, and its components are studied. It was found that Carnosic acid (PubChem CID 65126), also called salvin, is a natural compound present in rosemary that provides an inhibitory effect on the replication machinery of the Nipah virus.

3.2.2 In silico toxicology and ADME properties prediction

Swiss ADME server was used to predict the Pharmacokinetic (ADME) properties of the molecule and the toxicological analysis was performed using the ProTox-II online tool. SDF file of carnosic acid 2-D structure was provided as input on both the webserver tools.

3.2.3 Retrieval of protein structure

RCSB-PDB was used to retrieve the crystal structure of the protein. The PDB file of the Nipah Virus Phosphoprotein multimerization domain has a PDB ID of 4N5B. The resolution of this structure is 2.2 Å. Ligand IMD is complexed with protein structure. So, the protein structure requires preparation for molecular docking.

The crystal 3D structure of Nucleoprotein of PDB:ID 4CO6 was taken from RCSB-PDB. It is the crystal structure of the nucleoprotein-phosphoprotein complex. It had a resolution of 2.50 Å. Bromide ion and Chlorine ion are present as ligands in this complex. Nucleoproteins are made from chains A, B, and C. phosphoprotein is needed to be removed from this complex for docking, as we require nucleoprotein as a target protein. So, need to be prepared before docking.

3.2.4 Protein structure modeling

Large protein or RNA dependent RNA Polymerase 3D structure was modelled using SWISS-MODEL and the FASTA format protein sequence was taken from NCBI Protein.

3.2.5 Preparation of protein

The proteins were prepared for docking using UCSF Chimera (v1.17.3) software and Autodock tools (v4.2.6). All redundant non-standard residues and water molecules were removed and then saved in .pdb format using UCSF Chimera (v1.17.3) software. Polar H-atoms as well as Kollman charges were added using Autodock tools (v4.2.6) from The Scripps Research Institute, USA and the protein thus prepared was saved in PDBQT format. While the NiV-N (4CO6) structure is a NiV nucleoprotein–phosphoprotein (N-P) complex thus the phosphoprotein chains were removed using the UCSF Chimera (v1.17.3) software.

3.2.6 Retrieval and preparation of ligand

The data on ligands was obtained from published literature. The ligand (Carnosic acid) is one of the bioactive phytochemical component of R. officinalis leaf extract. The 3-D crystal structure of carnosic acid (test ligand) and Ribavrin (positive control) were retrieved from PubChem database and the files were saved in 3D.sdf format.

UCSF Chimera v1.17.3 software was for preparation of the ligand and positive control. The file is converted from .sdf file to .pdb format. Theses ligands were prepared one by one using UCSF Chimera. This was followed by converting the .pdb file into .pdbqt format.

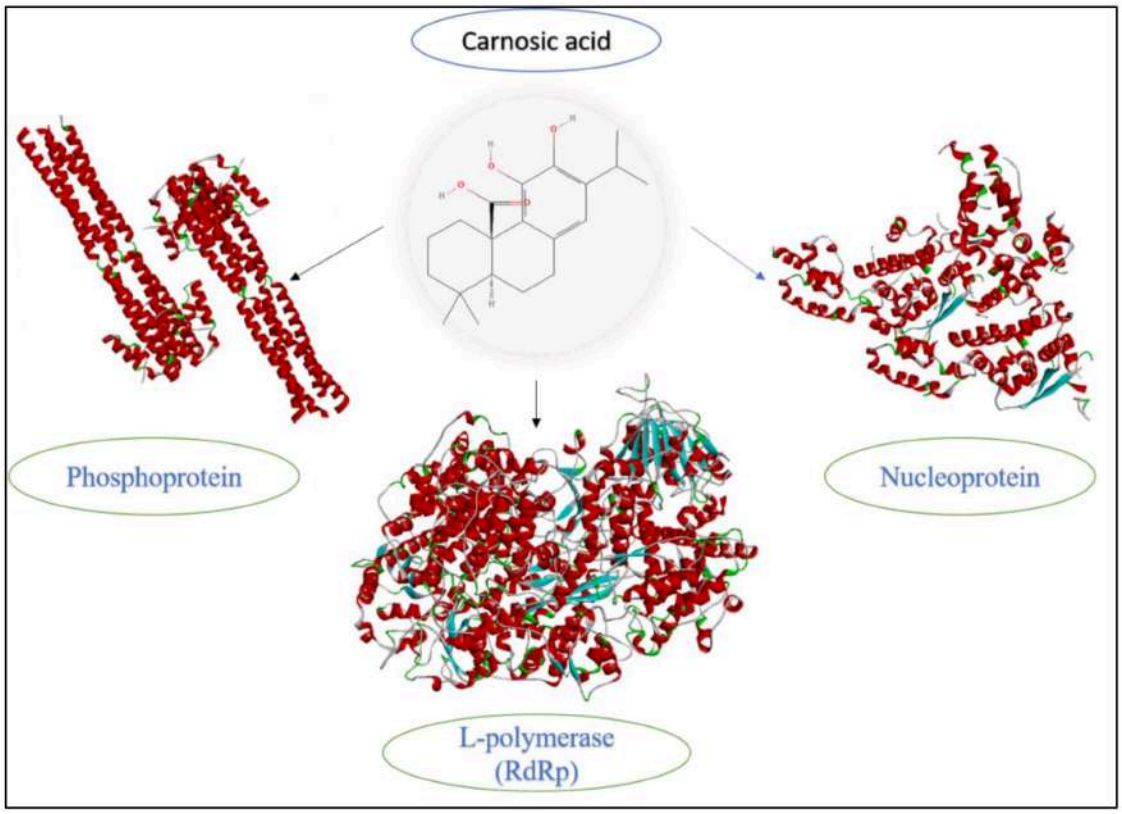

Fig3.1 The structure of the target phosphoprotein (PDB ID: 4N5B), nucleoprotein (PDB ID: 4CO6), and L-polymerase (RdRp) (Modeled) and test ligands.

3.2.7 Grid box formation and Molecular Docking

Molecular docking is a computational method utilized to anticipate the ligand pose best fit to the active site of protein. The ligand carnosic acid was docked to the prepared Phosphoprotein (P), Nucleoprotein (N) and Polymerase protein (L) of Nipah Virus using PyRx Virtual Screening Tool. Both proteins and ligands were uploaded into the software and the favorable pose was predicted using molecular docking.

Grid box is created to establish a 3D confined space in which the docking computational methods can be conducted. Generating a grid box has several advantages over blind

docking, including lower energy use, lower processing costs, and faster analysis of specific regions. Protein grids were prepared according to x: 69.35, y: 0.10, z: 43.12 for NiV-N, x: -4.58, y: 22.21, z -2.81 for NiV-P and x: 109.29, y: 107.39, z: 120.73 centers for NiV-L. The binding affinity was enumerated for the ligand posture that best suited the protein. The optimal fit is achieved during molecular docking when the maximum negative binding energy and RMSD value is 0.

3.2.8 Molecular docking analysis and visualisation

Biovia Discovery Studio v21.1.0.20298 (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/) was used for analysis of interactions and visualization of the protein-ligand docked complexes. Molecular docking studies were also performed with Ribavirin as a positive control. Ribavirin is a broad-spectrum antiviral that is one of the first drugs to be used to treat patients infected with Nipah Virus in Malaysia 1998/1999 outbreak and also recently during Kerala, India 2018 outbreak. It has also been licensed for the treatment of respiratory syncytial virus (RSV) infection.

CHAPTER 4

RESULTS

4.1 Pharmacological Analysis

Pharmacological properties of carnosic acid such as solubility (Log *S*), lipophilicity (Log $P_{o/w}$); Pharmacokinetic properties (absorption in the GI tract, Blood Brain Barrier penetration) and Drug likeness (Lipinski's rule of violation and Bioavailability Score) was examined. (Lipin et al., 2021)The results are depicted in Table II. Carnosic acid was less water soluble as compared to ribavirin. The *n*-octanol/water partition coefficient (log $P_{o/w}$) value is a measure of lipophilicity which is positive for lipophilic molecules and negative for water soluble molecules. The results suggest that carnosic acid has a log $P_{o/w}$ value of 3.82 and has high absorption in the GI tract however the control Ribavirin has a log $P_{o/w}$ value of -2.05 and has low GI absorption.

Table 2 Pharmacological ADME predication Result

ADME prediction		
Parameters	**Carnosic acid**	**Ribavirin**
Log S	-5.03, Moderately soluble	-0.21, Very soluble
Lipinski	Yes; 0 violation	Yes; 0 violation
Log Po/w	3.82	-2.05
GI absorption	High	Low
BBB permeant	No	No

Toxicity studies (Table IV) were done on the basis of toxicity end points (Carcinogenicity, Immunogenicity, Mutagenicity and Cytotoxicity). The drug likeness is shown in the bioavailability radar (Fig.5.) where the pink area represents the optimal range

Table 3. toxicity predication result analysis

Toxicity prediction		
Toxicity Target	**Carnosic acid**	**Ribavirin**
LD 50	287mg/kg	2700mg/kg
Carcinogenicity	Inactive (P:0.60)	Active (P:0.56)
Immunotoxicity	Active (P:0.68)	Inactive (P:0.99)
Mutagenicity	Inactive (P:0.84)	Inactive (:0.85)
Cytotoxicity	Inactive (P:0.94)	Inactive (P:0.72)

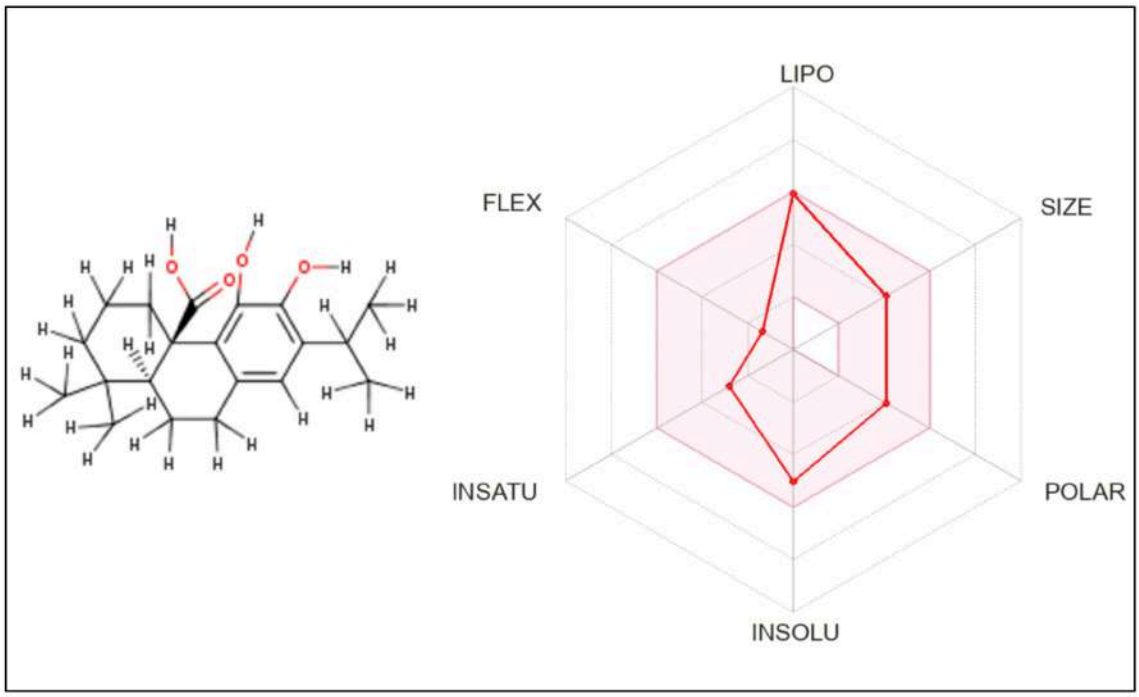

Fig 4.1: SwissADME bioavailability radar of Carnosic Acid depicting the Drug Likeness

4.2 Molecular docking analysis

PyRx Virtual screening tool was run to measure the binding affinity of carnosic acid against Nipah Virus proteins. All three-test ligand-protein complexes showed a stronger binding affinity as compared to the control Ribavirin. Binding affinity of carnosic acid with Nucleoprotein was –7.6 kcal/mol, better than Carnosic acid - Phosphoprotein complex which has binding affinity of – 7.4 kcal/mol. Carnosic acid – large protein complex had the most effective binding with affinity of –7.7 kcal/mol. Table 2 shows the binding site analysis of ligand-protein docking. Carnosic acid interacted with the N-terminal domain of Nucleoprotein and the binding residues were VAL 58, ALA 64, ALA 65, LYS 69, ILE 131 out of which 2 were conventional hydrogen bonds (ALA 65 and LYS 69) (Fig.4.2.). In Phosphoprotein, Carnosic acid interacted with the C terminal domain at residues LYS 525, ILE 527, ASN 528, ASP 530 and formed 2 conventional hydrogen bonds (LYS 522 and ASN 528) (Fig.4.3.). Binding residues in large protein were PHE 1556, ILE 1685, LEU 1688, ASN 1689. (Fig.4.4).

The binding residues for the positive control Ribavirin in Nucleoprotein were PHE 38, VAL 58, ALA 65, SER 67, GLY 71, ALA 72, THR 75 with binding affinity of -7.1 kcal/mol. In Phosphoprotein it docks at THR 487, LYS 525, ILE 527 ASN 528, ASP 530 ARG 532 with binding affinity of -6.1 kcal/mol and in large protein at ASP 1052, SER 1059, GLY 1060, ASN 1061, ASP 1064 SER 1065, GLN 1066 with binding affinity of -6.8 kcal/mol**.** (Fig

Table 4 – Docking Results

Proteins	Binding Analysis			
	Carnosic Acid		*Ribavirin*	
	Binding residues	**Binding Affinity (kcal/mol)**	**Binding residues**	**Binding Affinity (kcal/mol)**
Nucleoprotein	VAL 58, ALA 64, ALA 65, LYS 69, ILE 131	-7.6	PHE 38, VAL 58, ALA 65, SER 67, GLY 71, ALA 72, THR 75	-7.1
Phosphoprotein	LYS 525, ILE 527, ASN 528, ASP 530	-7.4	THR 487, LYS 525, ILE 527 ASN 528, ASP 530 ARG 532	-6.1
Large Protein (RdRp)	PHE 1556, ILE 1685, LEU 1688, ASN 1689	-7.7	ASP 1052, SER 1059, GLY 1060, ASP 1064 SER 1065, GLN 1066	-6.8

The results suggest that carnosic acid is a potent inhibitor of the Nipah Virus replication as it binds to the multimerization domain of the phosphoprotein which is essential for genome replication. The multimerization region is essential for L-P complex formation and is also required for stable binding of P to the N-RNA template. This association of proteins is essential for both transcription and replication processes to carry through (Curran, 1998; De et al., 2000). It can also bind to the N- terminal domain in nucleoprotein that is essential for its interaction with phosphoprotein to form N-P complex prior to encapsidation. However, carnosic acid does not bind to large protein in the RdRp domain. Thereby we can say that it does not directly inhibit its catalytic activity.

Our findings coincide with Shin et al. where they demonstrated carnosic acid as an inhibitor of Respiratory syncytial virus replication(H.-B. Shin et al., 2013). Through this study we describe how carnosic acid can also inhibit another paramyxovirus, that is, Nipah virus replication. We suggest that this ligand might exhibit its anti-viral activity by interfering with Phosphoprotein and Nucleoprotein functions thereby impairing viral replication as well as transcription.

4.3 Visualize 2D structure

The 2D structural of the best pose between ligands and proteins was obtained using BIOVIA Discovery Studio 2021 to analyze the interactions.

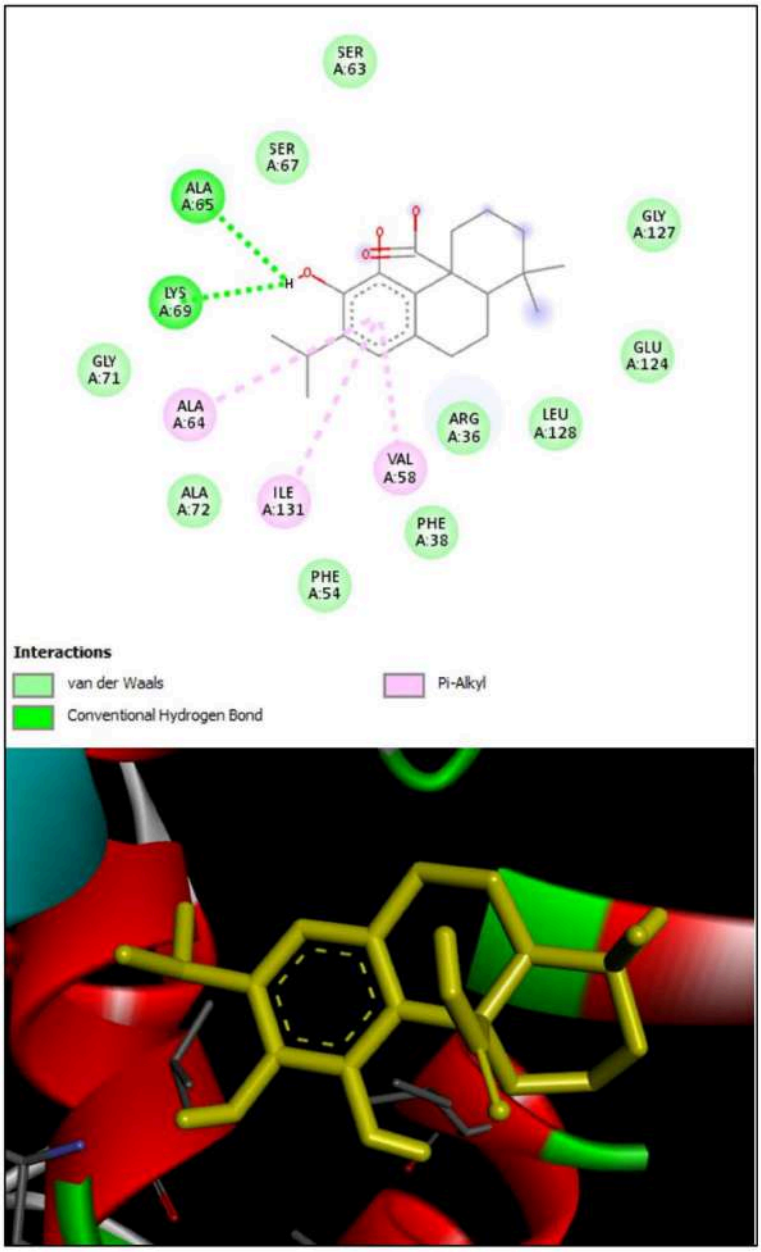

Fig 4.2- 3D and 2D interaction between Carnosic Acid (PubChem CID 65126) extracted from rosemary (Rosmarinus officinalis) leaves and to be used as a potential inhibitor of proteins of Nipah Virus NiV-P (phosphoprotein)

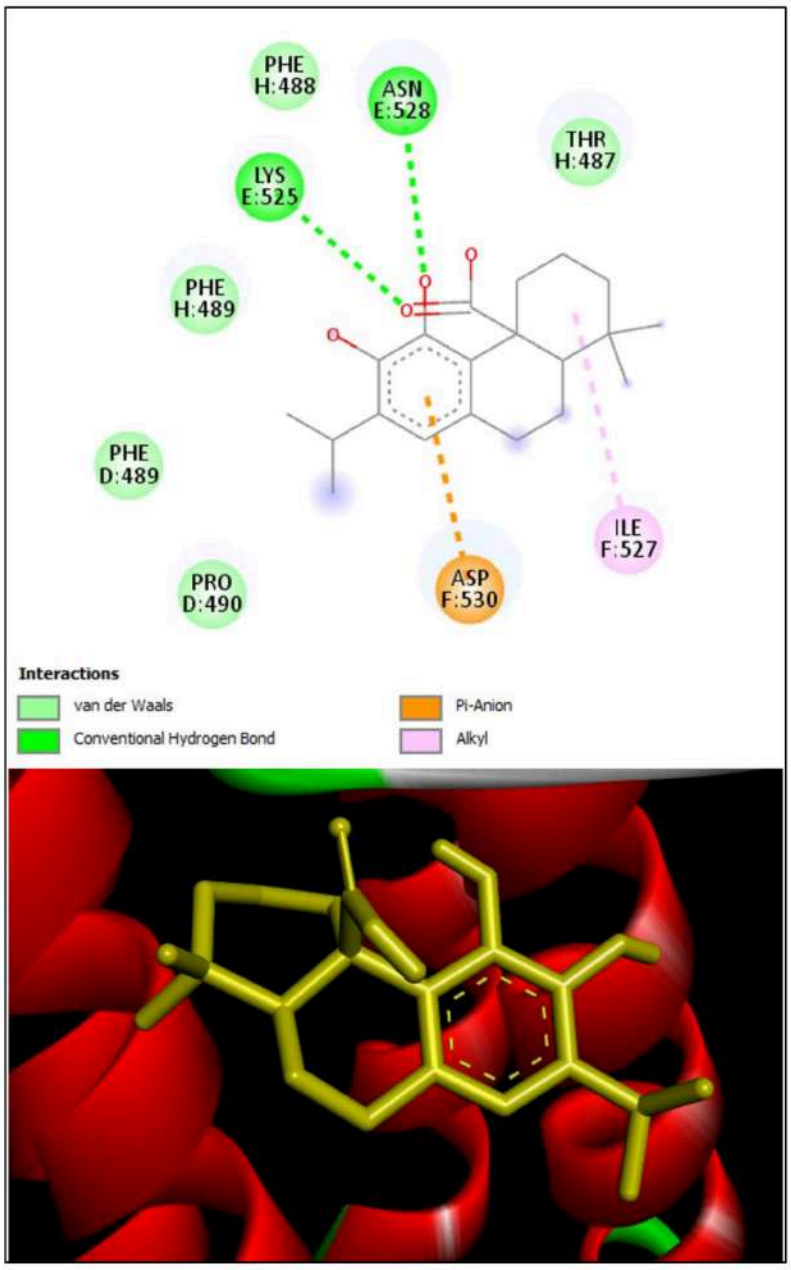

Fig 4.3: 3D and 2D interaction between Carnosic Acid (PubChem CID 65126) extracted from rosemary (Rosmarinus officinalis) leaves and to be used as a potential inhibitor of proteins of Nipah Virus Nucleoprotein (PDB ID: 4CO6)

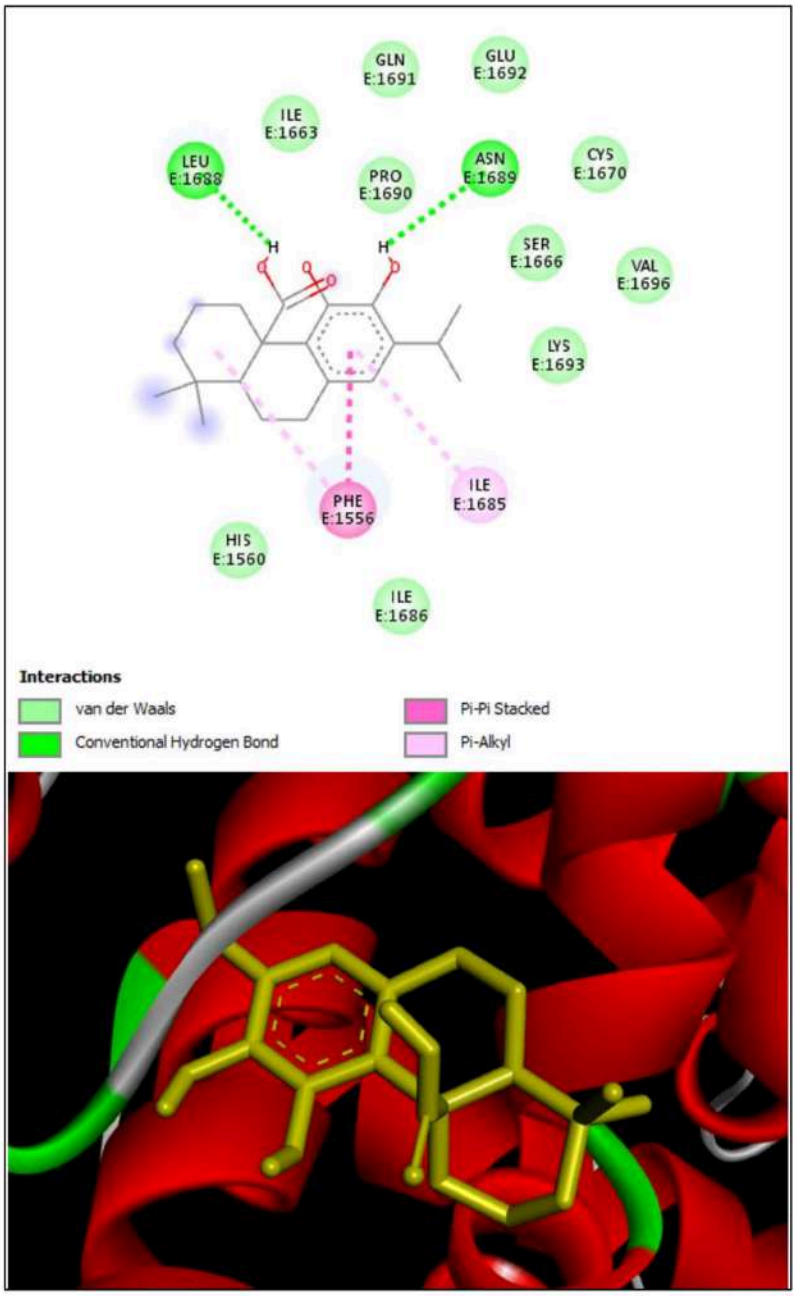

Fig 4.4: 3D and 2D interaction between Carnosic Acid (PubChem CID 65126) extracted from rosemary (Rosmarinus officinalis) leaves and to be used as a potential inhibitor of proteins of Nipah Virus L-polymerase (RdRp) (Modeled)

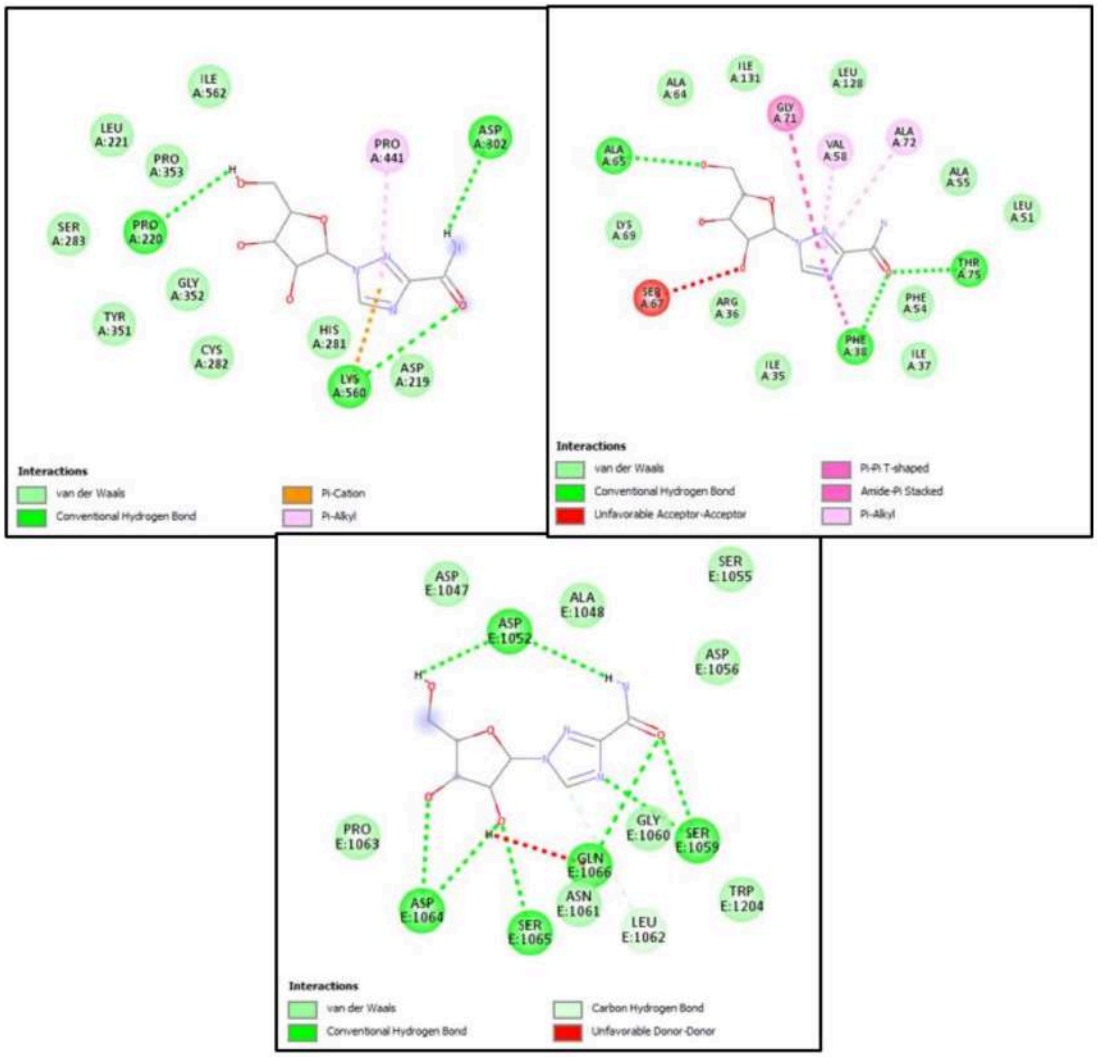

Fig 4.5:2-D Interaction of Rabvirin as a positive control with Nipah Virus

a) Nucleoprotein (PDB ID: 4CO6)

b) Phosphoprotein (PDB ID: 4N5B), and

c) L-polymerase (RdRp) (Modeled)

CHAPTER 5

DISCUSSION

Through the computational investigation it identified that carnosic acid obtained from a natural plant leaves of Rosemary (*Rosmarinus officinalis*) act as a potential inhibitor for the proteins of Nipah Virus Nucleoprotein and Nipah Virus phosphoprotein as the molecular docking studies suggested. The binding affinity of NiV-N is -7.6 kcal/mol and with NiV-P is -7.4 kcal/mol which is greater then control used for this study. Thereby indicating that the lead compound has more affinity towards target protein than the standard control i.e. Ribavirin. Carnosic acid interacted with the N-terminal domain of Nucleoprotein and the binding residues were VAL 58, ALA 64, ALA 65, LYS 69, ILE 131 out of which 2 were conventional hydrogen bonds (ALA 65 and LYS 69). In Phosphoprotein, Carnosic acid interacted with the C terminal domain at residues LYS 525, ILE 527, ASN 528, ASP 530 and formed 2 conventional hydrogen bonds (LYS 522 and ASN 528). Binding residues in large protein were PHE 1556, ILE 1685, LEU 1688, ASN 1689.

Pharmacokinetic studies after molecular docking showed that most of the drugs had favorable bioavailability and bioactivity ratings. The majority of them also complied with

Lipinski's rule of five, demonstrating the test compounds' characteristics that make them potentially useful drugs.

The findings indicate that carnosic acid is a powerful inhibitor of Nipah Virus replication because it binds to the multimerization domain of the phosphoprotein, which is required for genome replication. The multimerization region is necessary for the development of the L-P complex, as well as P stable binding to the N-RNA template. This protein interaction is required for both transcription and replication to proceed. It can also attach to the N-terminal domain of nucleoproteins, which is required for interaction with phosphoproteins to form the N-P complex prior to encapsulation. However, carnosic acid does not interact with big proteins in the RdRp domain. Thus, we may conclude that it does not directly block its catalytic activity.

Our findings validate Shin et al.'s observation that carnosic acid inhibits respiratory syncytial virus replication. This paper describes how carnosic acid can suppress the reproduction of another paramyxovirus, the Nipah virus. We propose that this ligand exerts its antiviral effect by interfering with phosphoprotein and nucleoprotein activities, limiting viral replication and transcription.

CHAPTER 6

CONCLUSION

Molecular Docking, protein-ligand interaction analysis and in silico ADMET predictions of phytochemical Carnosic acid on NiV proteins has been performed. The test ligand showed a strong binding to the target proteins as compared to the standard drug Ribavirin. There is no licensed treatment for Nipah Virus infection and the patients are given symptomatic treatments. Furthermore, being a BSL-4 agent, the research done on the virus in vivo is limited. Therefore, there is an immense need for more research on finding appropriate therapeutics and vaccines against the virus to prevent any possible pandemic in future. Overall, the current findings highlight the potential of carnosic acid as lead molecule for development of anti-NiV therapeutics.

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LIST OF PUBLICATION

Article Title: Assessing the Potentiality of Natural Inhibitors from Nuphar Lutea against Nipah Virus through Molecular Docking

Author(s): Ayushi Gupta, Sanya Arora, Yasha Hasija

Conference Name: 6th International conference on Manufacturing, Material Science and Engineering (ICMMSE-2024)

Conference Date and Venue: August 16th & 17th 2024 at Vignan Institute of Technology and Science, Hyderabad, Telangana,

Status of Paper: Accepted

Registration: Done

Date of Paper Acceptance: 6 June 2024

Article Title: Unlocking the Therapeutic Potential of Carnosic Acid through Computational Investigation: A Multitarget Inhibition Strategy Against Nipah Virus Replication

Author(s): Ayushi Gupta, Sanya Arora, Yasha Hasija

Conference Name: IEEE International Conference on Intelligent Systems and Advance Applications (ISAA-2024)

Conference Date and Venue: 25 and 26 October 2024 at Dr. D. Y. Patil Institute of Technology, Pimpri, Pune

Registration: pending

Status of Paper: yet to come

Date of Paper Communications:

Date of Paper Acceptance:

Place: Delhi Ayushi Gupta

Acceptance Letter

Date 06/06/2024

To,
Ms Ayushi Gupta
Department of Biotechnology,
Delhi Technological University, Delhi-110042ate 06/06/2024,

Dear Author,

It is our pleasure to inform you that your paper entitled **Assessing the Potentiality of Natural Inhibitors from Nuphar Lutea against Nipah Virus through Molecular Docking** (Paper Id: ICMMSE-50) has been accepted for Virtual oral paper presentation at ICAAIML-2024 on 30th and 31st August 2024 and the paper has been accepted for publication in WoS and **SCOPUS** indexed journal ***AIP Conference Proceedings*** (e-ISSN No. 1551-7616).

The program for the upcoming conference will be loaded on the website as soon as it is finalized.

We are looking forward to your participation at the conference.

Thanks and Regards,

AIP Conference Proceedings
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Scopus WEB OF SCIENCE

Dr. B Sridhar Babu,

Editor

ICMMSE 2024

PAPER NAME

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A Y U S H I G U P T A

+91 7428872677,
2K22/MSCBIO/15
ayushig575@gmail.com

EDUCATION

MSc (Biotechnology)	2022-2024	Delhi Technological University, New Delhi	8.18 CGPA
B.Sc. (Hons.) Biochemistry	2019-2022	Daulat Ram College, University of Delhi	8.36 CGPA
CBSE (Class XII)	2018	Kendriya Vidyalaya AFS Tughlakabad, New Delhi	75%
CBSE (Class X)	2015	Kendriya Vidyalaya AFS Tughlakabad, New Delhi	8.8 CGPA

INTERNSHIPS

Marketing Associate Intern, **Prep Junction,** New Delhi Dec 2021-Feb 2022

- Developed marketing strategies through market analysis and competitive insights.
- I worked on designing and executing marketing strategies by digital advertising

PROJECT WORK

Conducted in silico analysis and molecular docking studies on carnosic acid from Rosemary leaves to evaluate its potential as a multi-target therapeutic against Nipah virus replication. Performed comprehensive literature review, pharmacokinetic analysis, toxicity study and evaluate the binding affinity to support research findings.

ACADEMIC ACHIEVEMENTS AND AWARDS

Qualified Graduate Aptitude Test in Biotechnology (GAT-B): Rank 74

Dry Lab skills	Wet lab skills	technical skills	Interpersonal skill
Bioinformatics tool- BLAST, RasMol, SWISS-MODEL, PDB, Swiss PDB Viewer, homology modeling Molecular Docking- AutoDock, PyRx, Chimera, PyMOL, BIOVIA Microscopic examination	Molecular Biology & Immunological techniques (genomic and plasmid DNA isolation and Quantification, Protein isolation, Immunodiffusion) All types of chromatography UV-VIS spectroscopy	MS Excel, MS PowerPoint MS Office, Canva,	Communication skills Marketing skills Management skills Teamwork Time management

WORKSHOPS

Bioinformatics workshop on- How to calculate accurate binding free energy for drug design, SCIS, JNU

- Free energy calculation techniques: Umbrella Sampling method for calculating the PMF for Alanine Dipeptide Phi/Psi Rotation | 17 Feb'24

2 weeks Hands – on training workshop on Techniques for manipulation of Nucleic acids for Application in Genomics, CIIDRET, UDSC | 26 Dec-7Jan'23

- Genomic DNA & Plasmid DNA Extraction by using Column method, PEG and Magnetic Nanoparticles
- Principle & Applications of Agarose Gel Electrophoresis of DNA,
- Tracking Dye & Visualization Methods. Molecular Marker & Ladder for Size DNA
- Automated DNA sequencing using Sanger Sequencing and analysis of data

A Week Hands -on Workshop on Zebrafish Model system, Daulat Ram College, DU | 14-22 Dec 2022

- Learned Zebrafish husbandry and breeding set up, collection of eggs
- Learned development and behaviour of zebrafish, Learned behavioural assay and toxicity assay

A six–days Online Training on Model Organism, Zebrafish Lab Facility (DRC) | 3-8 Jan 2022

- Learned about E. coli, Zebrafish and Drosophila as model organism

A six- days Online Training on R – Language, Daulat Ram College | 21-28 Dec 2021

- worked with data objects and tabulation
- learned graphical analysis and distribution of data
- learned data manipulation and visualization
- hypothesis testing and Anova application

POSITIONS OF RESPONSIBILITY

BioSoc-DTU, Co-Head Technical Affairs | Aug'22- present

- Represent the society in all the administrative affairs between the college and the society. Coordinate among corporate, PR, Publicity, Logistics and other departments of society.
- Part of core team during orientation and recruitment of new members of society

IWSC-DRC, Graphics Head | Aug'21- April'22

- Leading Graphics team of five member, Designing posters and reels for various social media handles
- Managing and coordinating all the events and annual fest, Spreading awareness of various aspects of society

EXTRA-CURRICULAR ACTIVITIES AND VOLUNTEER

NSS VOLUNTEER

Engaged in volunteer work such as Community service, Environmental Conservation, Social awareness, Skill development, Enforcing safety measures (during COVID-19)

DHARA (THE ECO CLUB) VOLUNTEER

Activities included Recycling paper waste, planting trees, SAVE SOIL movement and attending seminars concerning awareness about Environmental issues

CERTIFICATE COURSE

- **Precision Medicine by University of Geneva.** Completed in May 2024 at coursera.org
- **Big Data, Genes, and Medicine by The State University of New York.** Completed in May 2024 at coursera.org
- Online Certification course on "**Molecular Docking**" offered by BioGrademy

OTHER INFORMATION

Linkedin profile: https://www.linkedin.com/in/ayushi-gupta575

DECLARATION

I hereby declare that the details furnished above are true and correct to the best of my knowledge and belief.