Establishing Phylogeny, Functional Profile and Novel Drug for Nipah Virus Encephalitis

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Abstract. Background: Nipah virus encephalitis causing microbiome Nipah henipavirus was taken in this work which is the causative factor for encephalitis condition in humans. The fruit bats of *Pteropus* genus are the natural reservoir and acts as a carrier of this Nipah henipavirus. The phylogeny, functional profile of Nipah virus microbiome was identified by using metatranscriptomic sequencing. The receptor genes involved in the Nipah virus encephalitis mainly L protein and a P gene product – V protein were taken in this work and molecular docking studies were done using the phytocompounds from *Centella asiastica* with the target protein receptors taken. The computational drug designing was employed to prove the efficiency of the novel drug against the Nipah virus encephalitis disease. From the result of this study, the phytocompound- Arjunolic acid from *Centella asiatica* plant showed better interactions with the protein receptors with good docking score.

Methodology: Using metatranscriptomic sequencing, the taxonomic phylogeny and functional profile of the microbiome was identified. On using the Krona and GraPhlAn tools, the presence of the microbiome Nipah virus was confirmed. By using Normalised gene families, was able to get the functional profile of the microbiome. The receptor genes involved in the Nipah virus encephalitis mainly L protein and a P gene product – V protein were taken in this work. Using computer- aided drug designing the novel ligands from the medicinal plant – *Centella asiatica* was further docked with the gene receptors that was taken and the novel drug was designed against nipah virus.

Keywords: Nipah virus Encephalitis · *Pteropus* spp · Microbiome · Functional profile · Phylogeny · Metatranscriptomics sequencing · Gene receptors · Phytocompounds · Docking · Drug designing

1 Introduction

Nipah virus encephalitis is an emerging zoonotic disease caused by Nipah henipavirus (NiV). The Nipah henipavirus is known to cause encephalitis condition in humans. The natural reservoir of Nipah henipavirus is found to be fruit bats of *Pteropus* genus

R. Somashekhar et al. (Eds.): ICBDS 2022, AHSR 58, pp. 239–253, 2023.
https://doi.org/10.2991/978-94-6463-164-7_17

belonging to the family of *Pteropodidae* responsible in transmitting Nipah virus to both humans and animals. Transmission of NiV to man occurs mainly in the places where man, pigs and bats come in close proximity [1]. NiV transmission occurs via consumption of virus contaminated foods mainly fruits and contact with infected animals such as pigs or an infected human body fluid. NiV has been found in urine, kidney and uterus of infected wild bats and the virus has also been found in fruits or juice like date palm sap contaminated with the urine or saliva of bat [2].

The Nipah henipavirus (NiV) is a negative sense, single stranded, non-segmented and enveloped RNA virus belongs to the family of Paramyxovirus and genus *Henipavirus.* NiV particles are pleomorphic, spherical to filamentous and ranges in size from 40 to 1,900 nm. They contain a single layer of surface projections with an average length of 17 ± 1 nm [4]. The genome size of NiV is 18246 bp long. The RNA genome encoding six structural proteins and three non-structural proteins, consists a consecutive arrangement of six genes namely, nucleocapsid (N), phosphoprotein(P), matrix(M), attachment glycoprotein (G), fusion glycoprotein(F) and polymerase(L). The, N, P and L attaches to viral RNA forming the virus ribonucleoprotein. The synthesis of viral m RNA is catalysed 6by L and P. The F and G proteins are responsible for cellular attachment of the virion and subsequent host cell entry [5]. The M protein contributes to viral assembly and release. The non-structural protein C participates in the host immune response and serves as a virulence factor [6] (Fig. 1).

The Interactions between Class B ephrins (viral receptors) on host cells and the NiV glycoprotein (G) triggers changes in conformations in the latter, leading to the activation of F glycoprotein and membrane fusion [7]. It is thought that the replication strategies in addition to fusion of the ephrin receptors are responsible for greater pathogenicity of the Nipah viruses. The G glycoprotein mediates attachment to the receptors of host cell surface and the fusion (F) protein makes fusion of virus-cell membranes for cellular entry. The G protein of Nipah virus binds to the host ephrin B2/3 receptors and induces the conformational changes in G protein which triggers the F protein refolding [8]. The microRNA processing machinery along with the PRP19 complex are the host targets of the virus [9].

Infected individuals show symptoms such as initially with high fever, headache, vomiting, myalgia, sore throat followed by dizziness, drowsiness, mental confusion,

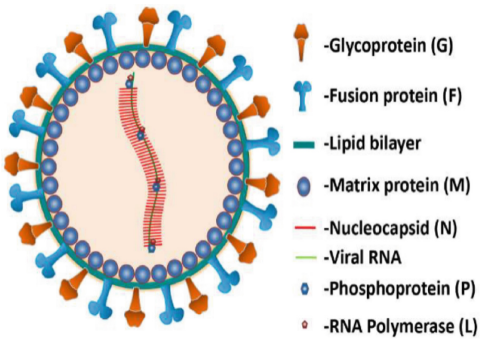


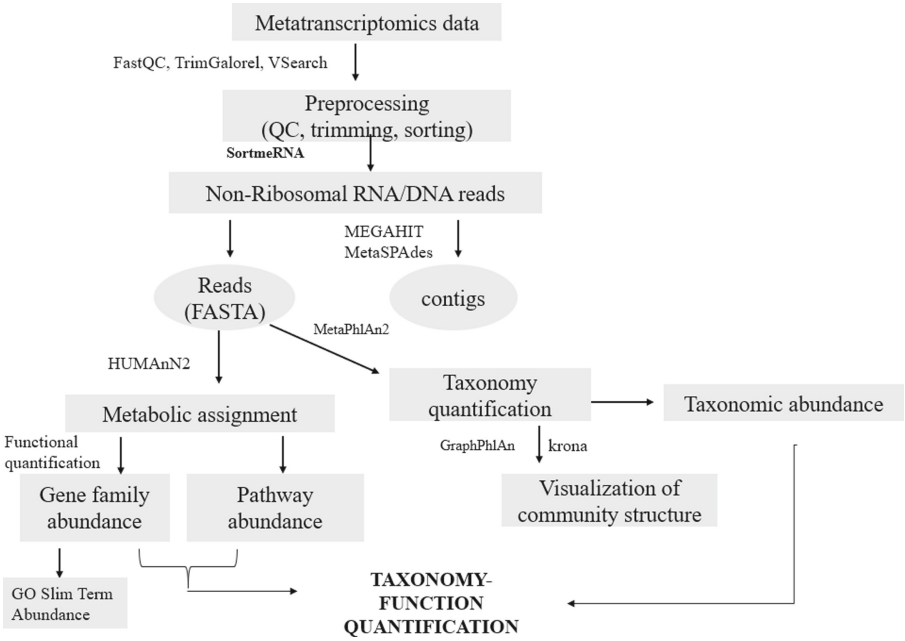
Fig. 1. Schematic representation of Nipah virus structural proteins.

disorientation, acute respiratory issues, acute encephalitis that eventually progresses towards leading to coma. The incubation period ranges from 4 to 14 days. The fatality rate of Nipah virus encephalitis is estimated at 40% to 75%. Currently no specific diagnostic test for Nipah virus. As Nipah virus is considered as one of the deadliest emerging zoonotic disease according to the world health organisation, the need of efficient drug against nipah virus to tackle the problem is highly essential in health concerns. Thus, using medicinal plants popularly native to India such as *Centella asiatica* commonly known as Indian pennywort or Brahmi was taken in this present study identifying the novel inhibitors of the Nipah virus infection.

2 Methodology

The present study was conducted in the Galaxy Australia platform. It is a web accessible platform to perform computational biological research.

The whole genome sequence of Nipah henipavirus was downloaded from Sequence read archive (SRA) database at NCBI for the IDs **SRR10027400.1.1** and **SRR10027400.1.2** for which Fast QC was done and executed to check the sequence quality.



FASTQC - Generates web report that aids in assessing the quality of data.
MultiQC - Aggregates multiple FastQC results into single report
Cutadapt - used for trimming and filtering the input reads.
SortMeRNA - Sorts the rRNA sequences with high accuracy and specificity.

FASTQC Interlacer - Joins paired end (forward & reverse) reads from two separate files creates single interlaced file.
MetaPhlAn - used for profiling microbiota relies on 1M clade-specific marker genes identified from ~100,000 reference genomes
Krona - Used to visualize results of metagenomic profiling as zoomable pie chart.
GraPhlAn -Tool used for producing high-quality circular representation of taxonomic & phylogenetic trees.
HUMAan - used to extract functional information. A pipeline for efficient & accurate profiling presence/absence & microbial pathways abundance in a community from metatranscriptomic sequencing data.
Molinspiration - It offers broad range of cheminformatics software tools supports molecule manipulation and processing.
PYMOL - User-sponsored open source model visualization tool creates high-quality 3D molecular pictures.

3 Results and Discussion

The whole genome sequence of **Nipah henipavirus** was downloaded from SRA database for the IDs **SRR10027400.1.1** and **SRR10027400.1.2** for which Fast QC was done and executed.

As per base sequence quality results of FASTQC and MultiQC the sequence quality is good after trimming using cutadapt. Cutadapt removed adapter sequences, primers, poly-A tails and other unwanted sequences from the input FASTQ files. The quality of the sequences of the genome data was checked before and after trimming. The quality report is given in the Fig. 2. A & B.

Filter with Sort MeRNA tool removes any reads identified as r RNA from our dataset. The corresponding ribosomal RNA were selected. The strands were combined using FastQ Interlacer. Fastq Interlace tool joins paired end FASTQ reads from two separate

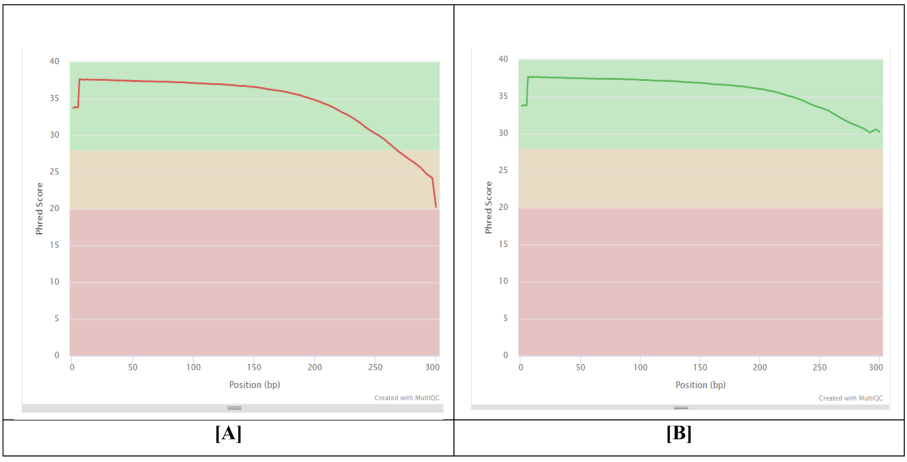


Fig. 2. FastA sequence: [A] Before trimming, [B]- After trimming.

files. Taxonomic profiling was done using MetaPhlAn tool Table 1. The phylogeny is seen using Krona pie chart & Graphlan. The output is visualized using Krona and Graphlan Fig. 3. A & B. The output of Phylogenetic tree is depicted in Fig. 3.

After generation of taxonomy, we move to functional information of our microbiome. Function information of the above microbiome community was done using HUMAnN pipeline given in Table 2.

Next, from the gene family information, we obtain the functional information of our microbiome using Superfamily server. The functional information of 15 families from Normalized gene families, pathway information as detected by superfamily is given in Table 3.

Homology Modelling- Selection and Retrieval of 3D Structures of Target Protein

In this study, long RNA polymerase (L protein) and P gene product- V protein was selected as the target proteins. The 3D structure of these two proteins were downloaded from swissmodel. Homology modelling of the receptors are done using SWISS-MODEL server. The receptor model and corresponding Ramachandran plot results are given in below Fig. 5.

Selection and Retrieval of 3D Structures of Ligands

About 25 phytocompounds were selected from the Indian medicinal herb, *Centella asiatica*, a herbaceous creeper proved to have excellent antiviral and anti-inflammatory properties. (Nora E. Gray et al.). The cheminformatics or molecular properties of all 25 compounds were obtained from Molinspiration property engine v2021.10.

Molinspiration is a web-based tool used to predict the bioactivity score of the compounds offers broad range of cheminformatics software tools supporting molecule manipulation and processing. Among 25 compounds, 5 compounds shortlisted showing nviolations as '0' were selected based on lipinski's rule of five whose structures were downloaded from protein data bank depicted in Fig. 6 below and the potential ability of these phytocompounds were studied by ADME (Adsorption, distribution and metabolism extraction) analysis shown in Table 4.

Virtual screening and molecular docking

Further patch docking was performed for the above 5 phytocompounds with the corresponding target protein receptors giving output of a list of potential complexes sorted by shape complementarity criteria. The PDB file of the complex was downloaded for which molecular docking studies was conducted using Pymol software tool. The Pymol software is an open source molecular visualisation tool creates high-quality 3D pictures of biological macromolecules.

Molecular docking results docked with phytocompounds of Centella asiatica

According to the docking studies it is clearly seen that, Arjunolic acid shows many numbers of interactions with both the target protein receptors i.e., RNA polymerase L protein and V protein and has a good docking score. Hence the present study concludes that Arjunolic acid phytocompound from the herbaceous creeper *Centella asiatica* has potential ability in inhibiting the target proteins mentioned above. Among all the five phytocompounds studied, Arjunolic acid acts as a best inhibitor for the infection caused by Nipah virus on basis of molecular docking studies.

Table 1. MetaPhlAn: Predicted taxon relative abundances at each taxonomic levels

#SampleID	Metaphlan_Analysis	
#clade_name	NCBI_tax_id	relative_abundance
k__Viruses	10239	100.0
k__Viruses|p__Negarnaviricota	10239|2497569	99.93937
k__Viruses|p__Viruses_unclassified	10239|	0.06063
k__Viruses|p__Negarnaviricota|c__Monjiviricetes	10239|2497569|2497574	99.93756
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified	10239||	0.06063
k__Viruses|p__Negarnaviricota|c__Ellioviricetes	10239|2497569|2497576	0.00181
k__Viruses|p__Negarnaviricota|c__Monjiviricetes|o__Mononegavirales	10239|2497569|2497574|111157	99.93756
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Viruses_unclassified	10239|||	0.0546
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Ortervirales	10239||2169561	0.00603
k__Viruses|p__Negarnaviricota|c__Ellioviricetes|o__Bunyavirales	10239|2497569|2497576|1980410	0.00181
k__Viruses|p__Negarnaviricota|c__Monjiviricetes|o__Mononegavirales|f__Paramyxoviridae	10239|2497569|2497574|111157|11158	99.93756
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Viruses_unclassified|f__Flaviviridae	10239|||11050	0.0546
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Ortervirales|f__Retroviridae	10239||2169561|11632	0.00603
k__Viruses|p__Negarnaviricota|c__Ellioviricetes|o__Bunyavirales|f__Phenuiviridae	10239|2497569|2497576|1980410|1980418	0.00181
k__Viruses|p__Negarnaviricota|c__Monjiviricetes|o__Mononegavirales|f__Paramyxoviridae|g__Henipavirus	10239|2497569|2497574|111157|11158|260964	99.93756
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Viruses_unclassified|f__Flaviviridae|g__Flavivirus	10239|||11050|11051	0.0546
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Ortervirales|f__Retroviridae|g__Lentivirus	10239||2169561|11632|11646	0.00332
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Ortervirales|f__Retroviridae|g__Retroviridae_unclassified	10239||2169561|11632|	0.00271
k__Viruses|p__Negarnaviricota|c__Ellioviricetes|o__Bunyavirales|f__Phenuiviridae|g__Phlebovirus	10239|2497569|2497576|1980410|1980418|11584	0.00181
k__Viruses|p__Negarnaviricota|c__Monjiviricetes|o__Mononegavirales|f__Paramyxoviridae|g__Henipavirus|s__Nipah_henipavirus	10239|2497569|2497574|111157|11158|260964|121791	99.93113
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Viruses_unclassified|f__Flaviviridae|g__Flavivirus|s__Zika_virus	10239|||11050|11051|64320	0.05094
k__Viruses|p__Negarnaviricota|c__Monjiviricetes|o__Mononegavirales|f__Paramyxoviridae|g__Henipavirus|s__Hendra_henipavirus	10239|2497569|2497574|111157|11158|260964|63330	0.00644
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Viruses_unclassified|f__Flaviviridae|g__Flavivirus|s__Yellow_fever_virus	10239|||11050|11051|11089	0.00366
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Ortervirales|f__Retroviridae|g__Lentivirus|s__Equine_infectious_anemia_virus	10239||2169561|11632|11646|11665	0.00332
k__Viruses|p__Viruses_unclassified|c__Viruses_unclassified|o__Ortervirales|f__Retroviridae|g__Retroviridae_unclassifiedls__Human_endogenous_retrovirus_K	10239||2169561|11632||45617	0.00271
k__Viruses|p__Negarnaviricota|c__Ellioviricetes|o__Bunyavirales|f__Phenuiviridae|g__Phlebovirus|s__Rift_Valley_fever_phlebovirus	10239|2497569|2497576|1980410|1980418|11584|1933187	0.00181

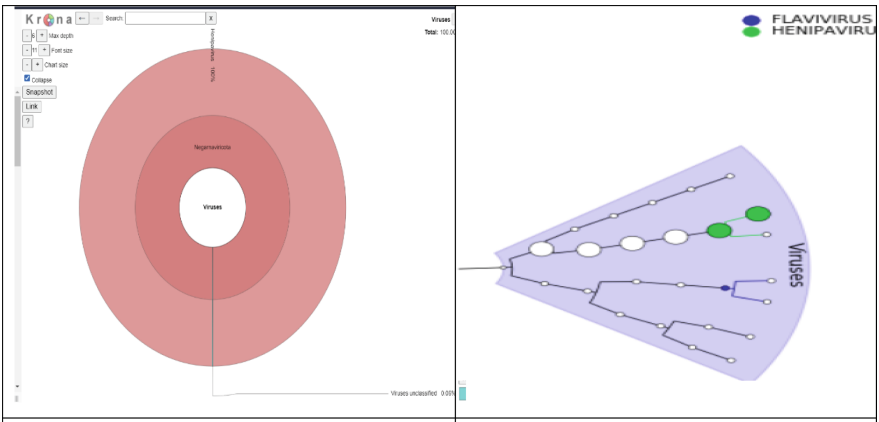


Fig. 3. Taxonomical profiling A- Krona visualization, [B]- GraPhlAn visualization.

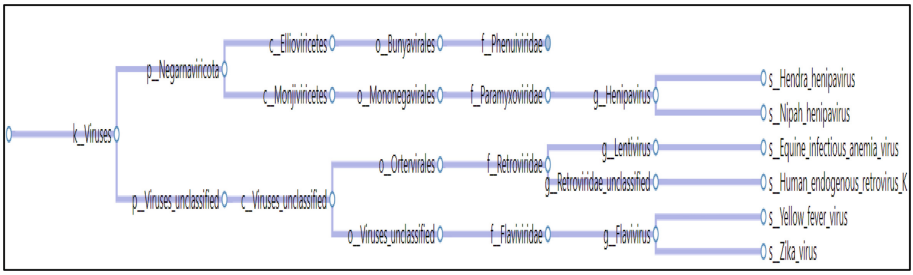


Fig. 4. Phylogenetic tree.

Table 2. Normalized gene families.

# Gene Family	humann_Abundance-RELAB
UNMAPPED	0.883335
UniRef90_Q997F0	0.024577
UniRef90_Q997F0|unclassified	0.024577
UniRef90_Q9IK91	0.0158699
UniRef90_Q9IK91|unclassified	0.0158699
UniRef90_Q9IH63	0.013786
UniRef90_Q9IH63|unclassified	0.013786
UniRef90_Q997F1	0.00812108

(*continued*)

Table 2. (*continued*)

# Gene Family	humann_Abundance-RELAB
UniRef90_Q997F1|unclassified	0.00812108
UniRef90_Q997F2	0.00792085
UniRef90_Q997F2|unclassified	0.00792085
UniRef90_Q9IK92	0.00697308
UniRef90_Q9IK92|unclassified	0.00697308
UniRef90_Q9IK90	0.00619471
UniRef90_Q9IK90|unclassified	0.00619471
UniRef90_UPI0004E5BCD6	0.00601413
UniRef90_UPI0004E5BCD6|unclassified	0.00601413
UniRef90_Q9IH62	0.00354968
UniRef90_Q9IH62|unclassified	0.00354968
UniRef90_UPI000181D329	0.00216587
UniRef90_UPI000181D329|unclassified	0.00216587
UniRef90_UPI0003ED1A6F	0.00180258
UniRef90_UPI0003ED1A6F|unclassified	0.00180258
UniRef90_A0A391HKJ0	0.00150724
UniRef90_A0A391HKJ0|unclassified	0.00150724
UniRef90_UPI00076B9105	0.00135851
UniRef90_UPI00076B9105|unclassified	0.00135851
UniRef90_A0A090FKU9	0.00126407
UniRef90_A0A090FKU9|unclassified	0.00126407
UniRef90_A0A090FEL8	0.00113837
UniRef90_A0A090FEL8|unclassified	0.00113837
UniRef90_A0A0A1YV31	0.000777327
UniRef90_A0A0A1YV31|unclassified	0.000777327
UniRef90_A0A2H1T2D3	0.000725857
UniRef90_A0A2H1T2D3|unclassified	0.000725857

Table 3. The functional information of 15 families from Normalized gene families, pathway information.

Sl. No.	Superfamily id	Pathway information
1	Q997F0	(sp|Q997F0|L_NIPAV) Cap-specific mRNA (nucleoside-2'-O-) -methyltransferase 2 KW=Complete proteome OX=121791 OS=Nipah virus. GN=L; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
2	Q9IK91	(sp|Q9IK91|PHOSP_NIPAV) Phosphoprotein KW=Complete proteome OX=121791 OS=Nipah virus. GN=P/V/C; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
3	Q9IH63	(sp|Q9IH63|FUS_NIPAV) Fusion glycoprotein F1 KW=Complete proteome OX=121791 OS=Nipah virus. GN=F; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
4	Q997F1	(sp|Q997F1|C_NIPAV) Protein C KW=Complete proteome OX=121791 OS=Nipah virus. GN=P/V/C; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
5	Q997F2	(sp|Q997F2|V_NIPAV) Non-structural protein V KW=Complete proteome OX=121791 OS=Nipah virus. GN=P/V/C; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
6	Q9IK92	(sp|Q9IK92|NCAP_NIPAV) Nucleocapsid protein KW=Complete proteome OX=121791 OS=Nipah virus. GN=N; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
7	Q9IK90	(sp|Q9IK90|MATRX_NIPAV) Matrix protein KW=Complete proteome OX=121791 OS=Nipah virus. GN=M; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
8	Q9IH62	(sp|Q9IH62|GLYCP_NIPAV) Glycoprotein G KW=Complete proteome OX=121791 OS=Nipah virus. GN=G; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=58060,58065,9606,9405,132908,153297,9823
9	P03631	(sp|P03631|REPA_BPPHS) RepA KW=Complete proteome; Reference proteome OX=1217068 OS=phi-X174). GN=A; OC=Viruses; ssDNA viruses; Microviridae; Bullavirinae; Phix174microvirus. OH=498388

(*continued*)

Table 3. (*continued*)

Sl. No.	Superfamily id	Pathway information
10	G7PQJ1	(tr|G7PQJ1|G7PQJ1_MACFA) Uncharacterized protein {ECO:0000313|EMBL:EHH56381.1} KW=Complete proteome; Reference proteome OX=9541 OS=Macaca fascicularis (Crab-eating macaque) (Cynomolgus monkey). GN=EGM_05775 OC=Catarrhini; Cercopithecidae; Cercopithecinae; Macaca.
11	P03641	(sp|P03641|CAPSD_BPPHS) GPF KW=Complete proteome; Reference proteome OX=1217068 OS=phi-X174). GN=F; OC=Viruses; ssDNA viruses; Microviridae; Bullavirinae; Phix174microvirus. OH=498388
12	P03643	(sp|P03643|G_BPPHS) GPG KW=Complete proteome; Reference proteome OX=1217068 OS=phi-X174). GN=G; OC=Viruses; ssDNA viruses; Microviridae; Bullavirinae; Phix174microvirus. OH=498388
13	F6VHV6	(tr|F6VHV6|F6VHV6_MACMU) Uncharacterized protein {ECO:0000313|Ensembl: ENSMMUP00000033787} KW=Complete proteome; Reference proteome OX=9544 OS=Macaca mulatta (Rhesus macaque). GN=OC=Catarrhini; Cercopithecidae; Cercopithecinae; Macaca.
14	O89342	(sp|O89342|FUS_HENDH) Fusion glycoprotein F1 KW=Complete proteome; Reference proteome OX=928303 OS=Hendra virus (isolate Horse/Autralia/Hendra/1994). GN=F; OC=Mononegavirales; Paramyxoviridae; Henipavirus. OH=9796,9606,9402,9403,94117
15	N6W0F0	(tr|N6W0F0|N6W0F0_9GAMM) Uncharacterized protein {ECO:0000313|EMBL:ENN96852.1} KW=Complete proteome OX=1307437 OS=Pseudoalteromonas agarivorans S816. GN=J139_20479 OC = Pseudoalteromonadaceae; Pseudoalteromonas.

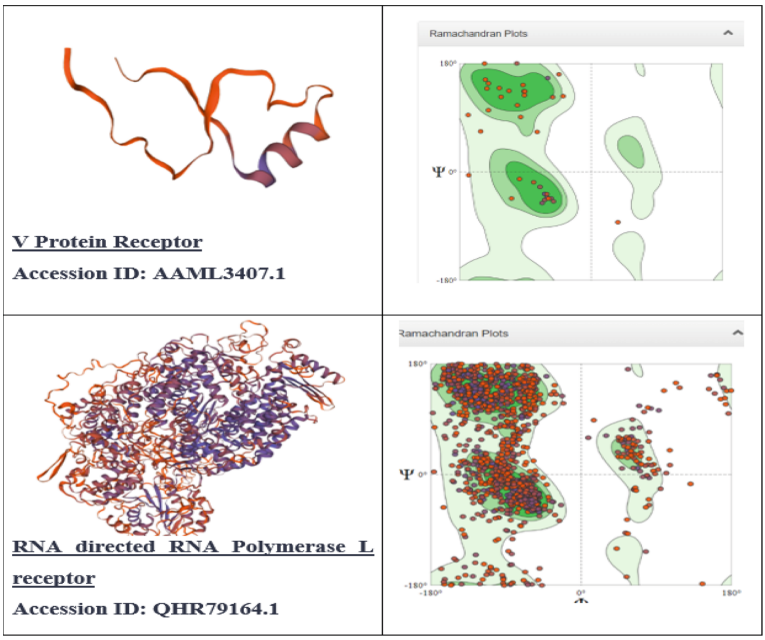


Fig. 5. Swiss-model generated receptor models with their Ramachandran plot.

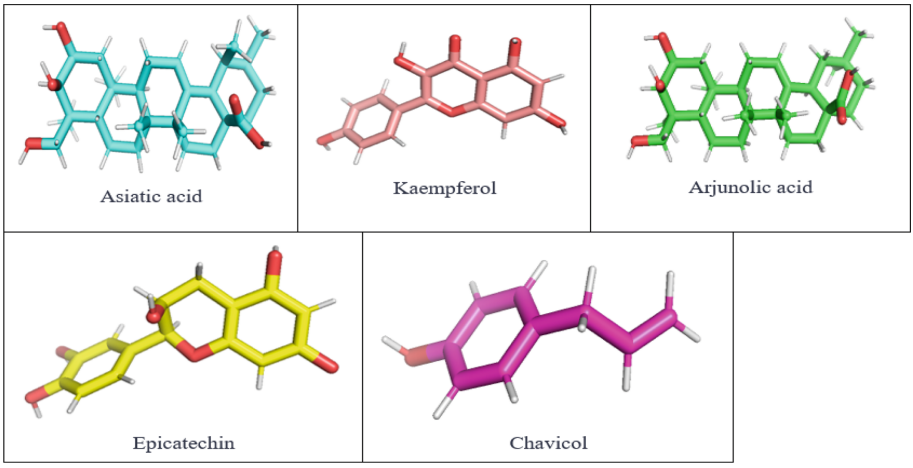


Fig. 6. Screening of Phytocompounds from *Centella asiatica*

Table 4. ADME results of *Centella asiatica*

Sl. no	Compounds	Mi Log p	TSPA	Natoms	mw	nO N	nO H N H	N Viol-atio ns	nROTB	VOLUME
1	Arjunolic acid	4.63	97.98	35	488.71	5	4	0	2	487.44
2	Asiatic acid	4.70	97.98	35	488.71	5	4	0	2	487.79
3	Chavicol	2.28	20.23	10	134.18	1	1	0	2	136.59
4	Epicatechin	1.37	110.37	21	290.27	6	5	0	1	244.14
5	Kaempferol	2.17	111.12	21	286.24	6	4	0	1	232.07

Table 5. RNA Polymerase L protein Receptor Docked with Phytocompounds of *Centella asiatica*

Phytocompound	Interacting amino acids	No of interactions	Docking score	Docking
Asiatic acid	ASP-2073, LEU-2180, GLU-2179, ARG-2118	4	-6210kcal/mol	Yes
Kaempferol	SER-37, LYS-39, THR-42	3	-4342kcal/mol	Yes
Arjunolic acid	ARG-1600, ASP 1749, ARG 1596, LYS 2067, LYS 2068	5	-5986kcal/mol	Yes
Epicatechin	LEU-1363, ARG-944, VAL-1009	3	-4546kcal/mol	Yes
Chavicol	GLN-597	1	-3200kcal/mol	Yes

Table 6. V Protein (P gene product) Receptor Docked with Phytocompounds of *Centella asiatica*

Asiatic acid	LYS-39, GLN-41	2	-5002kcal/mol	Yes
Kaempferol	LYS- 39, ASP- 9	2	-2988kcal/mol	Yes
Arjunolic acid	SER-37, LYS 39, GLN 41, THR 42	4	-4736kcal/mol	Yes
Epicatechin	GLU-41, THR-42	2	-3276kcal/mol	Yes
Chavicol	-	0	-	No

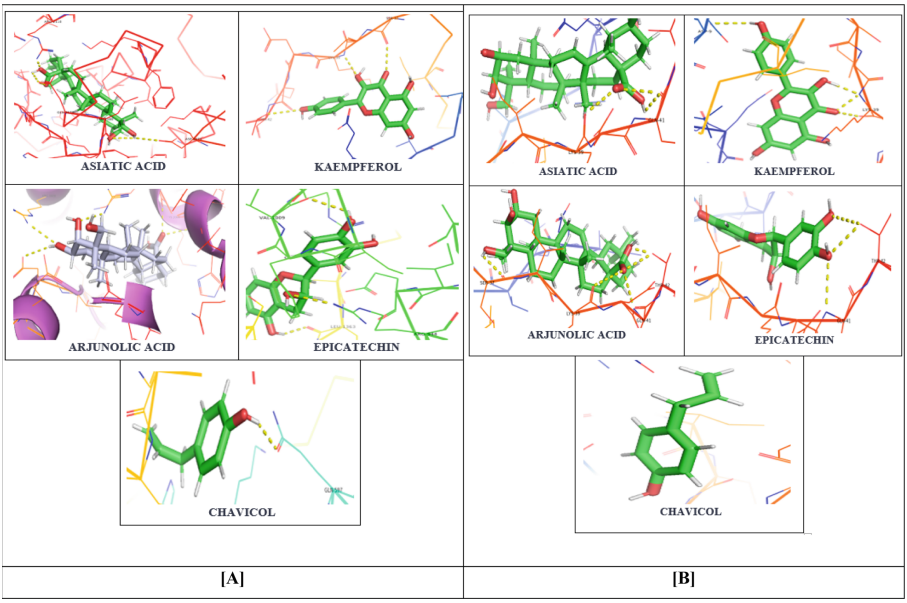


Fig. 7. Molecular docking results docked with phytocompounds of *Centella asiatica,* [A] - RNA Polymerase L protein docked with Phytocompounds, [B] - V protein docked with Phytocompounds.

4 Conclusion

The taxonomy and functional information of Nipah virus encephalitis microbiome was identified. Again, as per docking studies and ADME analysis it is seen that phytocompound ARJUNOLIC ACID shows most interactions with the proteins and it has good docking score also. Hence this phytocompound can be taken as novel drug lead for the Nipah virus encephalitis. Further in-vitro and in vivo studies can be done on the above phytocompounds to establish their potential as drugs in treating Nipah virus encephal.

Bibliography

1. Pulliam, J. R. C., Epstein, J. H., Dushoff, J., Rahman, S. A., Bunning, M., … Jamaluddin, A. A. (2011). Agricultural intensification, priming for persistence and the emergence of Nipah virus: a lethal bat-borne zoonosis. Journal of The Royal Society Interface, 9(66), 89–101. doi: https://doi.org/10.1098/rsif.2011.0223
2. WOAH (World Organisation for Animal Health) (Office International des Epizooties: OIE). 2009. Nipah virus encephalitis. Technical Disease Cards, OIE: Paris.
3. Raja, T., P. Ravikumar, M. R. Srinivasan, K. Vijayarani and Kumanan, K. 2020. Identification of Potential Novel Inhibitors for Nipah Virus – An in silico Approach. Int.J.Curr.Microbiol.App.Sci. 9(09): 3377-3390. doi: https://doi.org/10.20546/ijcmas.2020.909.420
4. Wang LF, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 286 –313. In Knipe DM, Howley PM (ed), Fields virology, 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA. https://doi.org/10.1128/JVI.74.21.9972-9979.2000.

5. Ternhag A, Penttinen P. 2005. Nipah virus–another product from the Asian virus factory. Lakartidningen. 102(14): 1046–1047.
6. Nonstructural Nipah Virus C Protein Regulates both the Early Host Proinflammatory Response and Viral Virulence, Cyrille Mathieu,et al, doi: https://doi.org/10.1128/JVI.01203-12.
7. Steffen DL, Xu K, Nikolov DB, Broder CC. 2012. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses. 4(2):280–308. doi: https://doi.org/10.3390/v4020280
8. Liu Q, Bradel-Tretheway B, Monreal AI, Saludes JP, Lu X, Nicola AV, Aguilar HC. 2015. Nipah virus attachment clycoprotein stalk C-terminal region links receptor binding to fusion triggering. J Virol. 89(3):1838–1850. doi: https://doi.org/10.1128/JVI.02277-14
9. Martinez-Gil L, Vera-Velasco NM, Mingarro I. 2017. Exploring the Human-Nipah virus protein-protein Interactome. J Virol. 91(23):e01461–e01417. https://doi.org/10.1128/JVI.01461-17
10. Eaton BT, Broder CC, Wang LF. 2005. Hendra and Nipah viruses: pathogenesis and therapeutics. Curr Mol Med. 5(8):805–816. doi: https://doi.org/10.2174/156652405774962308
11. Bossart KN, Crameri G, Dimitrov AS, Mungall BA, Feng Y-R, Patch JR, Choudhary A, Wang L-F, Eaton BT, Broder CC. 2005. Receptor binding, fusion inhibition and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. J Virol. 79(11):6690–6702. doi: https://doi.org/10.1128/JVI.79.11.6690-6702.2005
12. Raj Kumar Singh, Kuldeep Dhama, Sandip Chakraborty, Ruchi Tiwari, Senthilkumar Natesan, Rekha Khandia, Ashok Munjal, Kranti Suresh Vora, Shyma K. Latheef, Kumaragurubaran Karthik, Yashpal Singh Malik, Rajendra Singh, Wanpen Chaicumpa & Devendra T. Mourya (2019) Nipah virus: epidemiology, pathology, immunobiology and advances in diagnosis, vaccine designing and control strategies – a comprehensive review, Veterinary Quarterly, 39:1, 26-55, DOI: https://doi.org/10.1080/01652176.2019.1580827
13. Sudeep et al. BMC Infectious Diseases (2021) 21:162 Detection of Nipah virus in Pteropus medius in 2019 outbreak from Ernakulam district, Kerala, India A. B. Sudeep1, Pragya D. Yadav2*, Mangesh D. Gokhale1, R. Balasubramanian3, Nivedita Gupta4, Anita Shete2, Rajlaxmi Jain2, Savita Patil2, Rima R. Sahay2, Dimpal A. Nyayanit2, Sanjay Gopale2, Prachi G. Pardeshi2, Triparna D. Majumdar2, Dilip R. Patil1, A. P. Sugunan3 and Devendra T. Mourya. https://doi.org/10.1186/s12879-021-05865-7.
14. Computational prediction of miRNAs in Nipah virus genome reveals possible interaction with human genes involved in encephalitis Sandeep Saini*, Chander Jyoti Thakur, Varinder Kumar, Suchita Tandon, Varuni Bhardwaj, Sonia Maggar, Stanzin Namgyal, Gurpreet Kaur
15. Structure of Nipah virus: https://www.researchgate.net/figure/Structure-of-Nipah-virus_fig1_332558975.
16. Colorized transmission electron micrograph of a mature extracellular Nipah Virus particle (green) near the periphery of an infected VERO cell (blue). Image captured and color-enhanced at the NIAID Integrated Research Facility in Fort Detrick, Maryland. Credit: NIAID.
17. Clayton BA, Wang LF and Marsh GA (2013) Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. Zoonoses and Public Health **60**, 69–83.
18. Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all, Kashmira J. Gohil,* Jagruti A. Patel, and Anuradha K. Gajjar[1], doi: https://doi.org/10.4103/0250-474X.78519
19. Marsh GA et al. (2012) Cedar virus: a novel henipavirus isolated from Australian bats. PLoS Pathogens **8**, e1002836.
20. WHO (2018) WHO | Nipah Virus Infection. World Health Organization; Available at http://www.who.int/csr/disease/nipah/en/ (Accessed 17 June 2018).

21. Luby SP (2013) The pandemic potential of Nipah virus. Antiviral Research 100, 38–43.
22. Chatterjee P (2018) Nipah virus outbreak in India. The Lancet 391, 2200.
23. Chua KB (2003) Nipah virus outbreak in Malaysia. Journal of Clinical Virology **26**, 265–275.
24. Luby SP and Gurley ES (2012) Epidemiology of henipavirus disease in humans. Current Topics in Microbiology and Immunology **359**, 25–40.
25. Chua KB et al. (1999) Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. The Lancet **354**, 1257–1259.