Establishing the Taxa, Functional profile, and in-silico Ayurvedic Remedy of Microbiota implicated in West Nile Fever

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Abstract

West Nile fever causing microbiome is taken in this work. *Culex nigripalpus* mosquito is the causative factor for West Nile Virus. Using Metatranscriptomic sequencing, identified the taxa and functional profile of the microbiome is identified. Again, the receptor genes involved in West Nile fever is taken and using computer aided drug design, the novel ligands from Ayurvedic medicinal plants *Ginkgo biloba*, *Uncaria tomentosa*, *Lycoris radiata* and *Glycyrrhiza glabra*. Further, *in-vitro* and *in-vivo* studies can be done on the selected ligands to prove their efficiency as drugs for the disease

1. Introduction

West Nile fever is an infection caused by the West Nile virus, which is spread by mosquito, *Culex nigripalpus*. *Culex nigripalpus* is a primary vector for causing West Nile fever; they proliferate in the nutrient rich media and colonize in freshwater aquatic habitats. The microbiota of mosquitoes provides nutrition for the development and transmission of the pathogen [1]. Mosquitoes are the main vector for transmission of the virus to humans.

West Nile virus is a member of the Japanese encephalitis virus serogroup of genus Flavivirus, family Flaviviridae. It is a positive single stranded RNA virus having lipid bilayer membrane surrounded by a nucleocapsid core having length of about 11Kb which is translated to single polyprotein precursor [2]. The virus is maintained in a mosquito-bird mosquito transmission cycle primarily involving *Culex* species mosquitoes, transmitted primarily between avian hosts and mosquito vectors. The virus replicates affecting the brain causing neuro-virulence, it turns to be neuroinvasive when it gains access to the central nervous system. The viral serine protease, NS2B-NS3 plays a crucial role in viral replication. The distribution of the virus is seen throughout Africa, the Middle East, southern Europe, western Russia, southwestern Asia, and Australia [3].

The RNA genome consisting of 5 non-coding regions, a single open reading frame coding for three viral structural proteins (capsid, membrane, envelope) and seven non-structural (NS) proteins NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5, 3 non-coding regions of about 600 nucleotides. The important properties of the virus, including host range, tissue tropism, replication, assembly, and the stimulation of B and T cell immune responses. The Src family kinase c-Yes was recently reported to be important for maturation of West Nile virus particles.

Mosquito salivary components introduced at the site of infection initially target cells such as keratinocytes and dendritic cells. The virus initially replicates in dendritic cells, then spreads to regional lymph nodes and into the bloodstream and potentially to central nervous system. The expression of the central nervous system of the chemokine receptor CCR5 and its ligand CCL5 are prominently upregulated by the West Nile virus

The virus is transmitted to humans through non-vector mediated routes. the intrauterine route, through breast milk (from mother to child) blood transfusion, a bone marrow transplant and organ transplantation.

The individuals infected with this virus are asymptomatic. The incubation period is 2–14 days before the onset of symptoms. The severity ranges from mild encephalitic disease to a more severe from characterized by coma and death. Some individuals develop flu-like symptoms i.e., fever, headache, malaise, myalgia, fatigue, skin rash, lymph adenopathy, vomiting, and diarrhoea etc. Immuno-compromised individuals develop severe neuro-invasive diseases such as West Nile meningitis, West Nile encephalitis, and acute flaccid paralysis. Individuals with West Nile fever recover within days to months Whereas those with West Nile encephalitis have a poorer outcome to recover. In acute flaccid paralysis individuals develop symptoms headache, fever, malaise, gastrointestinal upset, skin rash, and some patients have neck rigidity and changes in mental status [4].

Metatranscriptomic analysis using Galaxy

Next-generation sequencing (NGS) is an advanced version of non-Sanger-based sequencing technology that offers ultra-high throughput, scalability, and speed. Galaxy is an open source, web-based platform for next generation computational

biomedical research [5]. Metatranscriptomics analysis enables understanding of how the microbiome responds to the environment by studying the functional analysis of genes expressed by the microbiome [6]. The genes from the Metagenomic analysis were transcribed from functional data, active metabolic pathways can be identified in our selected microbiome community [7].

Computer aided drug design

Drug design is the whole process of taking a newly discovered compound or drug molecule. Structure based drug designing technique is used here to build, display, simulate and analyze the molecular structure. Here we have used SWISS-MODEL tool [8] for modelling the proteins (gene receptors) responsible for West Nile fever CCR5, CLEC4M, IFITM2, IRF3. Selected models from homology modelling [9] output are docked with selected phytocompound from medicinal herbs. Selection of phytocompounds was done using Lipinski rule for drug which is based on the ADME properties. Molecular docking [10] was done using Patchdock tool and best interacting phytocompounds with the gene receptors can be selected as ligands.

2. Materials And Methods

West Nile viruses' fastq sequences SRR10017187.1.1 and SRR10017187.1.2 were retrieved from SRA database.

Sequence's quality was checked using FASTQC [11]. MultiQC [12] was done to aggregate results from FASTQC analyses into a single report.

Sequences were trimmed using cutadapt.

FASTQC followed by MultiQC was re-run using the results of cutadapt.

Next, using SortMeRNA tool [13–17] any reads identified as rRNA in dataset was removed.

Next, using FASTQ INTERLACE tool [18] paired end FASTQ reads from two separate files were joined.

MetaPhlAn tool [19] was used for profiling the composition of microbial communities (Bacteria, Archaea and Eukaryotes) from our microbiota.

Krona tool [20, 21] was used to visualize the results of a metagenomic profiling as a zoomable pie chart and GraPhlAn tool [22] for visualizing high-quality circular representations of taxonomic and phylogenetic trees.

Further, HUMAnN [23] pipeline was used for efficiently and accurately profiling the presence/absence and abundance of microbial pathways in our microbiota.

Next, using the genes present in our microbiota, their 3d structure was modeled using SWISS-MODEL [8].

Phyto-compounds were downloaded from PUBCHEM.

Using, molinspiration software [24], following the principles of Lipinski's rule of five, phytocompounds were selected for docking.

Further, docking was performed using patchdock [26, 27].

3. Results And Discussion

Metagenome, having accession number SRR10017187, for West Nile virus was downloaded from SRA database.

As, per **Per base sequence quality** results of FASTQC and MultiQC, the sequence quality is not good hence we go ahead with trimming the sequence.

CUTADAPT tool [27] is used for trimming. It finds and removes adapter sequences, primers, poly-A tails, and other types of unwanted sequence from our data. It searches for the adapter in all reads and removes it when it finds it. Further, sequence quality of the cutadapt output is checked using FASTQC and MultiQC and it is found within the range.

SortMeRNA tool removes any reads identified as rRNA from our dataset. Fastq Interlace tool joins paired end FASTQ reads from two separate files. **Taxonomic profiling [28] was done using MetaPhlAn tool (Fig. 1, Table 1). The output is visualized using Krona and Graphlan (Fig. 2).**

Table 1

MetaPhlAn: Predicted taxon relative abundances at each taxonomic levels

kingdom	kingdom id	phylum	class	order	family	family id	genus	genus id	species	species id	strains	strains id
Viruses	10239					100.0						
Viruses	10239	Viruses unclassified					100.0					
Viruses	10239	Viruses unclassified	Viruses unclassified					100.0				
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified					100.0			
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified	Flaviviridae	11050				100.0		
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified	Flaviviridae	11050	Flaviviruses	11051			100.0	
Viruses	10239	Viruses unclassified	Viruses unclassified	Viruses unclassified	Flaviviridae	11050	Flaviviruses	11051	West Nile virus	11082		100.0

After generation of taxonomy, we move to functional information of our microbiome. Functional information of the above microbiome community [28] was done using **HUMAN pipeline (Table 2).**

Table 2

Functional information of microbiome

# Gene Family	humann_Abundance-RELAB
UNMAPPED	0.888769
UniRef90_D9MXB1	0.0073362
UniRef90_D9MXB1 unclassified	0.0073362
UniRef90_UPI0001DD3770	0.00495637
UniRef90_UPI0001DD3770 unclassified	0.00495637
UniRef90_A0A1B1WW31	0.00484378
UniRef90_A0A1B1WW31 unclassified	0.00484378
UniRef90_B9DF73	0.00459488
UniRef90_B9DF73 unclassified	0.00459488
UniRef90_A0A067ZQ74	0.00422115
UniRef90_A0A067ZQ74 unclassified	0.00422115
UniRef90_A0A077EZ14	0.00417222
UniRef90_A0A077EZ14 unclassified	0.00417222
UniRef90_A0A067ZS80	0.00390475
UniRef90_A0A067ZS80 unclassified	0.00390475
UniRef90_UPI0003F054D7	0.00376104
UniRef90_UPI0003F054D7 unclassified	0.00376104
UniRef90_F1BA45	0.0036982
UniRef90_F1BA45 unclassified	0.0036982
UniRef90_A0A1X3CH90	0.00318336
UniRef90_A0A1X3CH90 unclassified	0.00318336
UniRef90_UPI000D0C92C0	0.00302072
UniRef90_UPI000D0C92C0 unclassified	0.00302072
UniRef90_K9L2G3	0.00265002
UniRef90_K9L2G3 unclassified	0.00265002
UniRef90_A0A1B1WWN4	0.00258533
UniRef90_A0A1B1WWN4 unclassified	0.00258533
UniRef90_K7ET80	0.00256502
UniRef90_K7ET80 unclassified	0.00256502
UniRef90_Q91CD9	0.00251161
UniRef90_Q91CD9 unclassified	0.00251161
UniRef90_UPI00018E17CF	0.00240202
UniRef90_UPI00018E17CF unclassified	0.00240202

UniRef90_G7PXF9	0.00234066
UniRef90_G7PXF9 unclassified	0.00234066
UniRef90_F6Z663	0.00218081
UniRef90_F6Z663 unclassified	0.00218081
UniRef90_G7MFM1	0.00215974
UniRef90_G7MFM1 unclassified	0.00215974
UniRef90_A0A2K6NB76	0.0020891
UniRef90_A0A2K6NB76 unclassified	0.0020891
UniRef90_UPI000181CCFE	0.00203554
UniRef90_UPI000181CCFE unclassified	0.00203554
UniRef90_C9WPK8	0.00195453
UniRef90_C9WPK8 unclassified	0.00195453
UniRef90_P14335	0.00180304
UniRef90_P14335 unclassified	0.00180304
UniRef90_F7HJ82	0.00180269
UniRef90_F7HJ82 unclassified	0.00180269
UniRef90_A0A0D3MDX0	0.00160595
UniRef90_A0A0D3MDX0 unclassified	0.00160595
UniRef90_UPI0000E69E2F	0.00153697
UniRef90_UPI0000E69E2F unclassified	0.00153697
UniRef90_I3VPR6	0.00149368
UniRef90_I3VPR6 unclassified	0.00149368
UniRef90_G8E0A9	0.00146336
UniRef90_G8E0A9 unclassified	0.00146336
UniRef90_D3X888	0.0013421
UniRef90_D3X888 unclassified	0.0013421
UniRef90_UPI00003BECBC	0.00130469
UniRef90_UPI00003BECBC unclassified	0.00130469
UniRef90_UPI0002C39258	0.00127138
UniRef90_UPI0002C39258 unclassified	0.00127138
UniRef90_Q6VXX6	0.00120319
UniRef90_Q6VXX6 unclassified	0.00120319
UniRef90_D3X8F1	0.0011535
UniRef90_D3X8F1 unclassified	0.0011535

UniRef90_A0A1S6WNB4	0.00110115
UniRef90_A0A1S6WNB4 unclassified	0.00110115
UniRef90_D3X8F7	0.00102768
UniRef90_D3X8F7 unclassified	0.00102768
UniRef90_D3X8D8	0.000998791
UniRef90_D3X8D8 unclassified	0.000998791
UniRef90_Q91KP3	0.000989086
UniRef90_Q91KP3 unclassified	0.000989086
UniRef90_D9IFF5	0.000952321
UniRef90_D9IFF5 unclassified	0.000952321
UniRef90_F7EY45	0.000912381
UniRef90_F7EY45 unclassified	0.000912381
UniRef90_D5K1B9	0.00090469
UniRef90_D5K1B9 unclassified	0.00090469
UniRef90_Q67428	0.000861045
UniRef90_Q67428 unclassified	0.000861045
UniRef90_Q91AB0	0.000854561
UniRef90_Q91AB0 unclassified	0.000854561
UniRef90_D3X875	0.000850089
UniRef90_D3X875 unclassified	0.000850089
UniRef90_A0A2K5WQL4	0.000795493
UniRef90_A0A2K5WQL4 unclassified	0.000795493
UniRef90_B1P6F1	0.000689513
UniRef90_B1P6F1 unclassified	0.000689513
UniRef90_P29984	0.000618302
UniRef90_P29984 unclassified	0.000618302
UniRef90_A0A0B5H4C4	0.000582165
UniRef90_A0A0B5H4C4 unclassified	0.000582165
UniRef90_Q6YFU6	0.00051542
UniRef90_Q6YFU6 unclassified	0.00051542
UniRef90_A0A0P0RST2	0.000505187
UniRef90_A0A0P0RST2 unclassified	0.000505187
UniRef90_A0A0D5CWD5	0.000489091
UniRef90_A0A0D5CWD5 unclassified	0.000489091

UniRef90_A0A068AXW2	0.000463204
UniRef90_A0A068AXW2 unclassified	0.000463204
UniRef90_A0A1X3CWX3	0.000451315
UniRef90_A0A1X3CWX3 unclassified	0.000451315
UniRef90_I1TEC3	0.000443267
UniRef90_I1TEC3 unclassified	0.000443267
UniRef90_A0A229QXW6	0.000390111
UniRef90_A0A229QXW6 unclassified	0.000390111
UniRef90_UPI0000485C0B	0.000373856
UniRef90_UPI0000485C0B unclassified	0.000373856
UniRef90_Q6VXX7	0.000367549
UniRef90_Q6VXX7 unclassified	0.000367549
UniRef90_H2NBX3	0.000356109
UniRef90_H2NBX3 unclassified	0.000356109
UniRef90_UPI000642CDD8	0.000349761
UniRef90_UPI000642CDD8 unclassified	0.000349761
UniRef90_I3N2P9	0.000333919
UniRef90_I3N2P9 unclassified	0.000333919
UniRef90_D9IFG5	0.000299914
UniRef90_D9IFG5 unclassified	0.000299914
UniRef90_D3X874	0.000283626
UniRef90_D3X874 unclassified	0.000283626
UniRef90_Q9DP92	0.000282183
UniRef90_Q9DP92 unclassified	0.000282183
UniRef90_D3X8B2	0.000279068
UniRef90_D3X8B2 unclassified	0.000279068
UniRef90_A0A075FC35	0.000211602
UniRef90_A0A075FC35 unclassified	0.000211602
UniRef90_B7U2U9	0.000200363
UniRef90_B7U2U9 unclassified	0.000200363
UniRef90_C9WPM0	0.000194638
UniRef90_C9WPM0 unclassified	0.000194638
UniRef90_A0A0P7UTC2	0.000191691
UniRef90_A0A0P7UTC2 unclassified	0.000191691

UniRef90_F7FBV8	0.000191691
UniRef90_F7FBV8 unclassified	0.000191691
UniRef90_G3SQL6	0.000191691
UniRef90_G3SQL6 unclassified	0.000191691
UniRef90_H3BYH5	0.000191691
UniRef90_H3BYH5 unclassified	0.000191691
UniRef90_P60866	0.000191691
UniRef90_P60866 unclassified	0.000191691
UniRef90_D3X8C7	0.000186455
UniRef90_D3X8C7 unclassified	0.000186455
UniRef90_A0A2K6EGR5	0.000170037
UniRef90_A0A2K6EGR5 unclassified	0.000170037
UniRef90_A0A0B7JKQ7	0.000166418
UniRef90_A0A0B7JKQ7 unclassified	0.000166418
UniRef90_UPI0005215E94	0.000165638
UniRef90_UPI0005215E94 unclassified	0.000165638
UniRef90_G3I2D3	0.00016095
UniRef90_G3I2D3 unclassified	0.00016095
UniRef90_A0A2I0MVY6	0.000152329
UniRef90_A0A2I0MVY6 unclassified	0.000152329
UniRef90_L9LBB7	0.000143369
UniRef90_L9LBB7 unclassified	0.000143369
UniRef90_UPI0006B0C551	0.000133289
UniRef90_UPI0006B0C551 unclassified	0.000133289
UniRef90_A0A091DCD6	0.000130236
UniRef90_A0A091DCD6 unclassified	0.000130236
UniRef90_UPI000D3231B4	0.00012363
UniRef90_UPI000D3231B4 unclassified	0.00012363
UniRef90_UPI000359548B	0.000119308
UniRef90_UPI000359548B unclassified	0.000119308
UniRef90_UPI00051F13F9	0.000116856
UniRef90_UPI00051F13F9 unclassified	0.000116856
UniRef90_UPI0005216B30	0.000112244
UniRef90_UPI0005216B30 unclassified	0.000112244

UniRef90_K7P5A3	0.000108977
UniRef90_K7P5A3 unclassified	0.000108977
UniRef90_L5JT11	8.00319e-05
UniRef90_L5JT11 unclassified	8.00319e-05
UniRef90_Q6VYH3	7.08503e-05
UniRef90_Q6VYH3 unclassified	7.08503e-05
UniRef90_A0A2U3XJL7	5.7032e-05
UniRef90_A0A2U3XJL7 unclassified	5.7032e-05

Next, from the gene family information, we obtain the functional information of our microbiome using Superfamily server. The Functional information of 1st five families from Normalized gene families as detected by Superfamily (HMM library and genome assignments server) is given below (Fig. 3).

As per the information obtained from Superfamily database it is seen that the Domain D9MXB1 is seen in the region 296-430 and 3-284 of the superfamily P-loop containing nucleoside triphosphate hydrolases and in the Family of RNA helicase.

A0A1B1WW31 domain, Region 1 at position 291-590, and of the superfamily Viral glycoprotein, central and dimerisation domains of Family Viral glycoprotein, central and dimerisation domains, 2 Region 2 at position 1990-2124 corresponds to Superfamily P-loop containing nucleoside triphosphate hydrolases and Family RNA helicase and Region 3's position 582-696 Superfamily E set domains with Family of Class II viral fusion proteins C-terminal domain.

B9DF73 Domain is included in Region 1 at position 670-996 and 1024-1247 of the Superfamily DNA/RNA polymerases Family RNA-dependent RNA-polymerase. 2nd Region at position 370-621 of the Superfamily S-adenosyl-L-methionine-dependent methyltransferases and Family mRNA cap methylase.

A0A067ZQ74 Domain's 1st Region at 2831-3157, 3185-3408 of Superfamily DNA/RNA polymerases of Family RNA-dependent RNA-polymerase. 2nd Region at 291-590 of Superfamily Viral glycoprotein, central and dimerisation domains of Family Viral glycoprotein, central and dimerisation. 3rd Region at 1990-2124 of Superfamily P-loop containing nucleoside triphosphate hydrolases in the Family RNA helicase. 4th Region at 582-696 of Superfamily E set domains of Family Class II viral fusion proteins C-terminal domain. 5th Region at 1687-1978 of Superfamily P-loop containing nucleoside triphosphate hydrolases of the Family RNA helicase. 6th Region at 1510-1676 of the Superfamily Trypsin-like serine proteases of Family Viral proteases. 7th Region at 22-99 of Superfamily Flavivirus capsid protein C of Family Flavivirus capsid protein C and 8th Region of Superfamily S-adenosyl-L-methionine-dependent methyltransferases of Family mRNA cap methylase.

A0A077EZ14 Domain's 1 Region at 485-619 of the Superfamily P-loop containing nucleoside triphosphate hydrolases of Family RNA helicase. Region 2 at 182-473 of Superfamily P-loop containing nucleoside triphosphate hydrolases of Family RNA helicase and Region 3 at 5-171 of Superfamily Trypsin-like serine proteases of Family Viral proteases.

Structure based drug designing of West Nile Fever

Since, West Nile fever is a mosquito-borne disease, we further go ahead towards designing novel drug for the disease. From the MetaPhlAn: Bowtie2 output we get the gene ids. Corresponding gene receptors are taken from NCBI for our work (Table 3).

Table 3

Genes with their NCBI Accession number

Sl. No	Gene Receptors	NCBI Accession Number	Homologous Template
1.	CCR5	AAB57793.1	4MBS
2	CLEC4M	AAI10615.1	1SL6
3	IFITM2	CAG33175.1	3WXX
4	IRF3	AAH71721.1	7JFM

Abbreviations of genes:

1. CCR5: C-C Motif Chemokine Receptor 5
2. CLEC4M: C-Type Lectin Domain Family 4 Member M
3. IFITM2: Interferon Induced Transmembrane Protein 2
4. IRF3: Interferon Regulatory Factor 3

Homology modeling

Homology modeling of the above receptors are done using SWISS-MODEL server. The receptor model and corresponding ramachandran plot results are given in Fig. 4. Template used for modeling is given in Table 3.

Ayurvedic Medicinal plants *Ginkgo biloba*, *Uncaria tomentosa*, *Lycoris radiata* and *Glycyrrhiza glabra* are traditionally used to treat many diseases, such as respiratory disorders and fever. The potency of their phytochemicals in treating West Nile Fever is studied here.

As per Lipinski's rule of five [ADME(Adsorption, distribution and metabolism extraction)] we check the drug likeliness of the above phytochemicals (Table 4-7).

Table 4

ADME studies of *Ginkgo biloba*

SL. NO	Compounds	Mi Log p	TSPA	Natoms	mw	nON	nOHNH	N-Violations	nROTB	VOLUME
1	Ginkgolide A	-1.46	128.60	29	408.40	9	2	0	1	339.84
2	Ginkgolide B	-2.38	148.83	30	424.40	10	3	0	1	347.88
3	Ginkgolide C	-3.29	169.06	31	440.40	11	4	1	1	355.93
4	Isorhamnetin	1.99	120.36	23	316.26	7	4	0	2	257.61
5	Quercetin	1.68	131.35	22	302.24	7	5	0	1	240.08
6	Protocatechuic acid.	2.10	118.21	20	276.24	6	5	0	3	231.33
7	Luteolin	1.97	111.12	21	286.24	6	4	0	1	232.07

Table 5

ADME studies of *Uncaria tomentosa*

SL. NO	Compounds	Mi Log p	TSPA	Natoms	mw	nO N	nOHNH	N Violations	nROTB	VOLUME
1	Uncarine C	2.18	67.88	27	368.43	6	1	0	2	333.36
2	Ajmalicine	3.41	54.57	26	352.43	5	1	0	2	325.29
3	Isomitraphylline	2.18	67.88	27	368.43	6	1	0	2	333.36
4	Isopteropodine	2.18	67.88	27	368.43	6	1	0	2	333.36
5	Mitraphylline	2.18	67.88	27	368.43	6	1	0	2	333.36
6	Isorhynchophylline	2.83	67.88	28	384.48	6	1	0	5	360.74
7	Speciophylline	2.18	67.88	27	368.43	6	1	0	2	333.36

Table 6

ADME studies of *Lycoris radiata*

SL. NO	Compounds	Mi Log p	TSPA	Natoms	mw	nON	nOHNH	N Violations	nROTB	VOLUME
1	Caranine	1.45	41.93	20	271.32	4	1	0	0	241.36
2	galanthamine	1.54	41.93	21	287.36	4	1	0	1	268.19
3	Hippeastrine.	1.23	68.24	23	315.32	6	1	0	0	269.11
4	Homolycorine	1.90	48.01	23	315.37	5	0	0	2	288.23
5	Lycoramine	1.56	41.93	21	289.38	4	1	0	1	274.38
6	lycorine	0.54	62.16	21	287.31	5	2	0	0	249.40
7	Tazettine	-2.45	57.16	25	346.40	6	1	0	1	311.82

Table 7

ADME studies of *Glycyrrhiza glabra*

SL. NO	Compounds	Mi Log P	TSPA	Natoms	mw	nON	nOHNH	N Violations	nROTB	VOLUME
1	phloretin	2.66	97.98	20	274.27	5	4	0	4	240.11
2	Rosmarinic acid	1.63	144.52	26	360.32	8	5	0	7	303.54
3	Isocoumarin	1.82	30.21	11	146.15	2	0	0	0	128.59
4	Cortison	1.43	91.67	26	360.45	5	2	0	2	337.20
5	Liquiritigenin.	2.20	66.76	19	256.26	4	2	0	1	222.24
6	Liquiritin	0.41	145.91	30	418.40	9	5	0	4	354.37
7	Glabridin	4.20	58.92	24	324.38	4	2	0	1	295.25
8	Chlorozotocin	-1.48	159.75	20	313.69	10	5	0	9	253.94
9	hispaglabridinA	6.24	58.92	29	392.50	4	2	1	3	372.59
10	Isoliquiritin	0.98	156.91	30	418.40	9	6	1	6	358.03
11	O-methylglabridin	4.04	68.16	26	354.40	5	2	0	2	320.79

It is seen that phytocompounds Ginkgolide C, hispaglabridinA and Isoliquiritin have 1 violations each in Lipinski's rule of 5 and hence can't be considered as ligands.

Molecular Docking:

Further docking is performed with the receptors in Table 4 with the above phytocompounds (Table 4-7). Docking scores, interacting amino acids along with number of interactions are noted in Table 8-11.

Table 8
Docking of CCR5 receptor

Plant: <i>Ginkgo biloba</i>			
COMPOUND	DOCKING SCORE (Kcal/mol)	INTERACTING AMINO ACID	NO. OF INTERACTIONS
Ginkgolide A	-4.234	THR-107, GLU-336	2
Ginkgolide B	-4.342	ASN-311, THR- 312, THR-197, TYR-304,	5
Ginkgolide C	-4.248	ASP-286, THR-67, ASP-68, ARG- 128	7
Isorhamnetin	-4.236	THR-337, TYR-39, CYS-180	4
Quercetin	-3.880	THR-107	1
Protocatechuic acid.	-3.840	ASP-68, ARG-128, ARG-142, THR- 67, SER-65	7
Luteolin	-3.822	ASP-68, GLU-355	3
Plant: <i>Uncaria tomentosa</i>			
Uncarine c	-4.742	TRY-110, GLU-335	2
Ajmalicine	-4.824	GLU-336	1
Isomitraphylline	-4.694	GLU-336, THR-107	2
Isopteropodine	-5.038	ASP-286	1
Mitraphylline	-4.610	THR-107	1
Isorhynchophylline	-5.204	GLU-336	1
Speciophylline	-4.586	GLU-336, TYR-304	2
Plant: <i>Lycoris radiata</i>			
Caranine	-3.740	ARG-128, ARG-142	3
Tazettine	-4.428	TYR-110	1
Lycorine	-3.952	GLU-355	1
Lycoramine	-4.178	SER-65	1
Homolycorine	-4.332	SER-182	1
Hippeastrine	-3.980	ASP-286, LYS-64	2
galanthamine	-4.042	ASN-311	1
Plant: <i>Glycyrrhiza glabra</i>			
phloretin	-3.852	THR-107	1
Rosmarinic acid	-4.824	TYR-304, ASN-311, THR-312, THR-197	5
Isocoumarin	-2.728	SER-65, THR-67	2
Cortison	-4.368	TYR-304, GLU-336, TYR-110	3
Liquiritigenin	-3.666	ASP-68, ARG-142	3

Liquiritin	-4.958	SER-65, ASP-68, GLU-355, ARG-288	4
Glabridin	-4.614	GLU-355	1
Chlorozotocin	-4.074	GLU-356, SER-65	3
hispaglabridinA	-5.512	ASP-68, ARG-285	2
Isoliquiritin	-5.302	VAL-353, ARG-288, ARG-285, ASP-68, SER-65, LEU-289	8
O-methylglabridin	-4.952	ARG-142	2

Table 9

Docking of IFITM2 receptor

Plant: <i>Ginkgo biloba</i>			
COMPOUND	DOCKING SCORE (Kcal/mol)	INTERACTING AMINO ACID	NO. OF INTERACTIONS
Ginkgolide A	- 4.556	THR-215, VAL-145, VAL-211, VAL-131	4
Ginkgolide B	- 4.546	GLU-61	1
Ginkgolide C	- 4.592	VAL-131, THR-215	2
Isorhamnetin	- 4.654	VAL-145, THR-215	2
Quercetin	- 4.372	ALA-60	1
Protocatechuic acid	-4.660	THR-195	1
Luteolin	-4.378	VAL-145, ILE-150	3
Plant: <i>Uncaria tomentosa</i>			
Uncarine C	- 5.064	VAL-131, ILE-127	2
Ajmalicine	- 5.336	ALA-16, THR-14, VAL-21, ASN-90	4
Isomitraphylline	- 4.876	GLU-58	1
Isopteropodine	- 4.966	VAL-211, THR-215	2
Mitraphylline	-4.686	THR-215	1
Isorhynchophylline	-5.304	ILE-127, VAL-211	2
Speciophylline	- 4.832	ILE-127	1
Plant: <i>Lycoris radiata</i>			
Caranine	-4.498	ILE-50	1
galanthamine	- 4.754	THR-14, THR-23	2
Hippeastrine.	- 4.716	ASP-90, TYR-85, HIS-57, ALA-60	5
Homolycorine	-4.780	VAL -211	1
Lycoramine	-4.428	ASN-90, TSR-14, GLY-12	3
lycorine	-4.668	ILE-150	1
Tazettine	-4.620	TYR-85, ASN-90	2
Plant: <i>Glycyrrhiza glabra</i>			
Cortison	-4.972	ILE-150, ALA-126, GLU-187	3
Glabridin	-5.038	LYS-102	1
Hispaglabridin A	-5.848	VAL-131	1
Isocoumarin	-2.986	GLY-152	1
Isoliquiritin	-5.744	THR-193, ILE-150, ASP-81, GLU-187	4
Liquiritigenin	-4.244	ASN-97	1
Liquiritin	-5.582	THR-198, ILE-88, GLY-146, VAL-145	3

O-methylglabridin	-5.378	GLY-152	1
phloretin	-4.852	TYR-85, ASP-90	2
Rosmarinicacid	-5.508	LYS-102, ASP-90	2
Chlorozotocin	-4.668	TYR-85, SER-84, ASP-90	5

Table 10

Docking of CLEC4M receptor

Plant: <i>Ginkgo biloba</i>			
COMPOUND	DOCKING SCORE (Kcal/mol)	INTERACTING AMINO ACID	NO. OF INTERACTIONS
Ginkgolide A	-3714	GLN-87, ASN-63	2
Ginkgolide B	-3776	GLN-87, GLN-10	3
Ginkgolide C	-3686	GLN-87, LYS-175	3
Isorhamnetin	-3420	ARG-51	1
Quercetin	-3422	THR-40, THR-17, THR-13, LYS-43	4
Protocatechuic acid	-3076	THR-40, LYS-20	2
Luteolin	-3330	LYS-176	1
Plant: <i>Uncaria tomentosa</i>			
Uncarine C	-4182	ASN-63	1
Ajmalicine	-4178	GLN-87	1
Isomitraphylline	-4390	THR-58, ASP-56	1
Isopteropodine	-4178	GLN-87	1
Mitraphylline	-4086	GLM-84	1
Isorhynchophylline	-4332	ASN-63	1
Speciophylline	-3936	GLN-10,LYS-175, ASN-63	4
Plant: <i>Lycoris radiata</i>			
Caranine	-3340	ASN-69,MET-67.	2
galanthamine	-3602	THR-17	1
Hippeastrine.	-3484	LYS-175	1
Homolycorine	-3758	LYS-20	1
Lycoramine	-3662	LYS-20, THR 40	2
lycorine	-3458	MET-67, THR-58	2
Tazettine	-3770	LYS-92	1
Plant: <i>Glycyrrhiza glabra</i>			
Cortison	-3714	ASN-63, GLN-87, TYR-13	3
Glabridin	-3906	LYS-176, GLU-5, LYS-8	3
HispaglabridinA	-4884	ASN-63	1
Isocoumarin	-2296	THR-40	1
Isoliquiritin	-4250	CYS-180, ARG-182	2
Liquiritigenin	-3310	LYS-8,LYS-176,PHE-181,ALA-178	4
Liquiritin	-4408	GLN-87, ARG-85	2

O-methylglabridin	-4268	ARG-109, THR-58	2
phloretin	-3390	GLU-5, ARG-85, VAL-84	3
Rosmarinicacid	-4488	LYS-176	1
Chlorozotocin	-3532	LYS-176, GLU-5, LYS-8	3

Table 11

Docking of IFR3 receptor

Plant: <i>Ginkgo biloba</i>			
NO. OF INTERACTIONS	NO. OF INTERACTIONS	NO. OF INTERACTIONS	NO. OF INTERACTIONS
Ginkgolide A	-4966	PRO-153, SER-152, HIS-151, ARG-25, ARG-193	6
Ginkgolide B	-5054	SER-152, GLU-110, ARG-193, SER-152, GLY-26, ARG-25	6
Ginkgolide C	-4842	TYR-155, GLU-111, GLY-26, VAL-172, ARG-25,	6
Isorhamnetin	-4672	ARG-25, ARG-27, SER-152, LEU-200	4
Quercetin	-4388	LEU-200, ARG-193, ARG-27, ARG-25, TYR-155, THR-156	6
Protocatechuic acid	-4052	GLY-26, TYR-155, SER-152 ARG-193	4
Luteolin	-4300	ARG-27, LYS-173, GLU-111, LEU-200, ARG-25	6
Plant: <i>Uncaria tomentosa</i>			
Uncarine c	-5362	GLU-110, SER-152, LEU-200, LYS-173, ARG-25	5
Ajmalicine	-5208	GLU-111, SER-152, GLU-110, TYR-155	4
Isomitraphylline	-5246	SER-152, GLU-110, TYR-155, ARG-25	4
Isopteropodine	-4892	ARG-25, ARG-193, GLU-110	3
Mitraphylline	-5292	LYS-152, SER-152	2
Isorhynchophylline	-5338	ARG-25, GLU-110, TYR-155, ARG-193	5
Speciophylline	-5068	LEU-200, GLU-110, ARG-25	3
Plant: <i>Lycoris radiata</i>			
Caranine	-4180	ARG-25	1
galanthamine	-4632	LYS-173, ARG-25	2
Hippeastrine.	-4644	SER-152	1
Homolycorine	-5034	SER-152, GLU-110, LYS-173,	1
Lycoramine	-4508	LEU-200, GLU 110	2
lycorine	-4418	SER-152, VAL-172, ARG-25, GLU-110	4
Tazettine	-5380	SER-152, ARG-25	2
Plant: <i>Glycyrrhiza glabra</i>			
Cortison	-5186	GLY-150, TYR-155, ARG-25, ARG-27, GLU-111	6
Glabridin	-5092	ARG-193, SER-152, LEU-200, ARG-25	2
Hispaglabridin A	-6154	ARG-25	1
Isocoumarin	-2978	LYS-173, GLU-110	2
Isoliquiritin	-5440	ARG-154, ARG-27, ARG-193, TYR-155	4
Liquiritigenin	-4388	GLY-26, LYS-173, GLY-150, LEU-200, TYR-155	5
Liquiritin	-5706	HIS-151, GLU-110, TYR-155, ARG-25,	6

O-methylglabridin	-5594	TYR-155, ARG-193	2
phloretin	-4576	LEU-200, GLU-111, ARG-193, ASP-67, ARF-25, SER-152	5
Rosmarinicacid	-5214	ASP-67, ARG-193, SER-198, LEU-200,LYS-173, GLU-111	6
Chlorozotocin	-4648	ARG-25, TYR-155, SER-152, ASP-67	4

As per docking studies it is seen that phytochemicals Ginkgolide A, Ginkgolide B, Luteolin and Protocatechuic acid from Ginkgo biloba and Liquiritin, Cortison and Chlorozotocin from Glycyrrhiza glabra have good interactions with the West Nile fever gene receptors.

Conclusion

The taxonomy and functional information of West Nile fever microbiome are identified. The Human abundance table and superfamily database identified superfamilies as P-loop containing nucleoside triphosphate hydrolases, Viral glycoprotein Family, DNA/RNA polymerases Family, E set domains, S-adenosyl-L-methionine-dependent methyltransferases,

Trypsin-like serine proteases, Flavivirus capsid protein C and S-adenosyl-L-methionine-dependent methyltransferases and of the families RNA helicase, Viral glycoprotein, Class II viral fusion proteins C-terminal domain, RNA-dependent RNA-polymerase, mRNA cap methylase, Viral proteases, Flavivirus capsid protein C and mRNA cap methylase.

Again, as per docking studies and ADME analysis it is seen that phytochemicals Ginkgolide A, Ginkgolide B, Luteolin and Protocatechuic acid from Ginkgo biloba can be potential ligands for the receptors implicated in West Nile Fever. Again, phytochemicals Liquiritin, Cortison and Chlorozotocin from Glycyrrhiza glabra can be potential ligands for the receptors implicated in West Nile Fever as per ADME analysis and docking studies. Further, in-vitro and in-vivo studies can be done on the above phytochemicals to establish their potential as drugs in treating West Nile Fever.

Declarations

1. Ethics approval and consent to participate: Not applicable
2. Consent for publication: Yes
3. Availability of data and materials:

Item. No.	Data	Resource
1.	Microbiome with accession number SRR10017187	https://www.ncbi.nlm.nih.gov/sra/?term=SRR10017187
2.	CCR5 with accession number AAB57793.1	https://www.ncbi.nlm.nih.gov/protein/AAB57793.1
3.	CLEC4M with accession number AAI10615.1	https://www.ncbi.nlm.nih.gov/protein/AAI10615.1
4.	IFITM2 with accession number CAG33175.1	https://www.ncbi.nlm.nih.gov/protein/CAG33175.1
5.	IRF3 with accession number AAH71721.1	https://www.ncbi.nlm.nih.gov/protein/AAH71721.1
6.	4MBS	https://www.rcsb.org/structure/4MBS
7.	1SL6	https://www.rcsb.org/structure/1SL6
8.	3WXX	https://www.rcsb.org/structure/3WXX
9.	7JFM	https://www.rcsb.org/structure/7JFM

4. Competing interests: None

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References

1. Duguma, D., Hall, M. W., Smartt, C. T., Debboun, M., & Neufeld, J. D. (2019). Microbiota variations in *Culex nigripalpus* disease vector mosquito of West Nile virus and Saint Louis Encephalitis from different geographic origins. *PeerJ*, 6, e6168.
2. Ezgimen, M., Lai, H., Mueller, N. H., Lee, K., Cuny, G., Ostrov, D. A., & Padmanabhan, R. (2012). Characterization of the 8-hydroxyquinoline scaffold for inhibitors of West Nile virus serine protease. *Antiviral research*, 94(1), 18-24.
3. Petersen, L. R., Brault, A. C., & Nasci, R. S. (2013). West Nile virus: review of the literature. *Jama*, 310(3), 308-315.
4. Kramer, L. D., Li, J., & Shi, P. Y. (2007). West Nile virus. *The Lancet Neurology*, 6(2), 171-181.
5. Blankenberg, D., & Hillman-Jackson, J. (2014). Analysis of next-generation sequencing data using Galaxy. In *Stem cell transcriptional networks* (pp. 21-43). Humana Press, New York, NY.
6. Bashiardes S, Zilberman-Schapira G, and Elinav E, (2016), Use of Metatranscriptomics in Microbiome Research, *Bioinform Biol Insights.* ; 10: 19–25.
7. Handelsman J, (2004), Metagenomics: Application of Genomics to Uncultured Microorganisms, *Microbiol Mol Biol Rev.* 68(4): 669–685.
8. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, A P de Beer T, Rempfer C, Bordoli L, Lepore R and Schwede T, (2018), SWISS-MODEL: homology modelling of protein structures and complexes, *Nucleic Acids Res.*; 46(Web Server issue): W296–W303.
9. Vyas VK, Ukawala RD, Ghate M, and Chintha C (2012), Homology Modeling a Fast Tool for Drug Discovery: Current Perspectives, *Indian J Pharm Sci.*74(1): 1–17.
10. Morris GM, Lim-Wilby M, (2008), Molecular docking, *Methods Mol Biol*, 443:365-82.
11. Andrews, S. (n.d.). FastQC A Quality Control tool for High Throughput Sequence Data. Retrieved from <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

12. Ewels, P., Magnusson, M., Lundin, S., & Ållner, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, 32(19), 3047–3048. <https://doi.org/10.1093/bioinformatics/btw354>
13. Kopylova, E., Noé, L., & Touzet, H. (2012). SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics*, 28(24), 3211–3217. <https://doi.org/10.1093/bioinformatics/bts611>
14. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590–D596. <https://doi.org/10.1093/nar/gks1219>
15. Burge, S. W., Daub, J., Eberhardt, R., Tate, J., Barquist, L., Nawrocki, E. P., ... Bateman, A. (2012). Rfam 11.0: 10 years of RNA families. *Nucleic Acids Research*, 41(D1), D226–D232. <https://doi.org/10.1093/nar/gks1005>
16. Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460–2461. <https://doi.org/10.1093/bioinformatics/btq461>
17. Loman, N. J., Misra, R. V., Dallman, T. J., Constantinidou, C., Gharbia, S. E., Wain, J., & Pallen, M. J. (2012). Performance comparison of benchtop high-throughput sequencing platforms. *Nature Biotechnology*, 30(5), 434–439. <https://doi.org/10.1038/nbt.2198>
18. Blankenberg, D., Gordon, A., Von Kuster, G., Coraor, N., Taylor, J., & and, A. N. (2010). Manipulation of FASTQ data with Galaxy. *Bioinformatics*, 26(14), 1783–1785. <https://doi.org/10.1093/bioinformatics/btq281>
19. Beghini, F., McIver, L. J., Blanco-Míguez, A., Dubois, L., Asnicar, F., Maharjan, S., ... Segata, N. (2021). Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *ELife*, 10. <https://doi.org/10.7554/elife.65088>
20. Ondov, B. D., Bergman, N. H., & Phillippy, A. M. (2011). Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics*, 12(1). <https://doi.org/10.1186/1471-2105-12-385>
21. Cuccuru, G., Orsini, M., Pinna, A., Sbardellati, A., Soranzo, N., Travaglione, A., ... Fotia, G. (2014). Orione, a web-based framework for NGS analysis in microbiology. *Bioinformatics*, 30(13), 1928–1929. <https://doi.org/10.1093/bioinformatics/btu135>
22. Asnicar, F., Weingart, G., Tickle, T. L., Huttenhower, C., & Segata, N. (2015). Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *PeerJ*, 3, e1029. <https://doi.org/10.7717/peerj.1029>
23. Abubucker, S., Segata, N., Goll, J., Schubert, A. M., Izard, J., Cantarel, B. L., ... Huttenhower, C. (2012). Metabolic Reconstruction for Metagenomic Data and Its Application to the Human Microbiome. *PLoS Computational Biology*, 8(6), e1002358. <https://doi.org/10.1371/journal.pcbi.1002358>
24. <https://www.molinspiration.com>, SlovenskyGrob, Slovakia
25. Duhovny D, Nussinov R, Wolfson HJ. Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics (WABI) Rome, Italy, Lecture Notes in Computer Science 2452, pp. 185-200, Springer Verlag, 2002
26. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucl. Acids. Res.* 33: W363-367, 2005.
27. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, 17(1), 10. <https://doi.org/10.14806/ej.17.1.200>
28. Jagtap P, Mehta S, Sajulga R, Batut B, Leith E, Kumar P, Hiltmann S, 2021 Metatranscriptomics analysis using microbiome RNA-seq data (Galaxy Training Materials).
29. Gough, J., Karplus, K., Hughey, R. and Chothia, C. (2001). Assignment of Homology to Genome Sequences using a Library of Hidden Markov Models that Represent all Proteins of Known Structure. *J. Mol. Biol.*, 313(4), 903-919

Figures

Figure 1

Generation, personalization and annotation of tree: Tree in PhyloXML

Figure 2

Visualization of **Taxonomic profile** in Krona and GraPhlAn

Figure 3

Functional information of 1st five gene families as detected by Superfamily server [29]

Figure 4

Swiss-model generated receptor models with their ramachandran plot