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Identification Of Co-Expressed Genes In Meningitis And Its Risk Factors For Evaluation Of The Lead Phytochemical Drug Candidates Using Network Pharmacology Approach

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Abstract

Meningitis poses a significant health challenge worldwide, with its multifaceted forms caused by various pathogens. Addressing its complexity requires innovative therapeutic approaches beyond conventional treatments. Hence, I propose employing network pharmacology to identify promising phytochemical compounds derived from plants as potential therapeutics for meningitis and its associated risk factors.

In this study, we aim to leverage bioinformatics tools to analyze gene expression data obtained from patients with meningitis and individuals at risk of developing the disease. By conducting co-expression analysis, we anticipate identifying genes exhibiting altered expression patterns across both contexts. These genes are likely to play crucial roles in the development and susceptibility to meningitis. Subsequently, we will construct a network where co-expressed genes serve as nodes, interconnected by known interactions. This network will provide insights into the molecular mechanisms underlying meningitis and its risk factors.

Integrating information on phytochemical compounds with relevant properties, we will employ computational tools to predict interactions between co-expressed genes and phytochemicals. Through network analysis, we will prioritize lead phytochemical candidates based on their interactions with key genes implicated in the disease process. We anticipate that this study will unveil co-expressed genes that are pivotal in both meningitis and its risk factors. By establishing a network-based approach, we aim to identify promising phytochemical-based therapies for treating meningitis.

In conclusion, this investigation amalgamates co-expression analysis and network pharmacology to pave the way for novel phytochemical-based therapies against meningitis. Targeting co-expressed genes involved in both the disease and its risk factors holds promise for developing more effective and broadly therapeutic treatment strategies.

Keywords: Meningitis, Network Pharmacology, Co-expressed Genes, Phytochemicals, Drug Discovery

1. Introduction

Meningitis is a severe inflammation of the meninges, the protective membranes surrounding the brain and spinal cord, caused by bacteria, viruses, fungi, or parasites. Bacterial meningitis, often caused by *Streptococcus pneumoniae*, *Neisseria meningitidis*, or *Haemophiles influenzae type b*, can result in severe complications such as brain damage and death. **(Thigpen et al., 2011)** Viral meningitis, typically caused by enteroviruses or herpesviruses, is less severe but still significant. Diagnosis relies on clinical presentation and cerebrospinal fluid analysis, with treatment varying according to the causative agent. **(Durand et al., 1993)**

Recent studies (2014-2024) have utilized advanced gene expression technologies such as microarrays and RNA sequencing to identify co-expressed genes in meningitis. **(Wang et al., 2017)** These studies highlight genes involved in inflammatory responses, apoptosis regulation, fungal cell wall synthesis, and blood-brain barrier integrity. **(Montenegro, 2022**) Such findings pave the way for potential therapeutic targets and enhance our understanding of meningitis pathogenesis. Network pharmacology is emerging as a promising approach to discover novel, natural therapeutic agents.

1.1. Decoding the Language of Genes: Co-expression Analysis in Meningitis

Advanced technologies like microarrays and RNA sequencing enable the identification of genes that are co-expressed during meningitis, playing critical roles in disease processes. Co-expression analysis reveals genes involved in pathogenesis, immune response, and host susceptibility. **(Zhang et al., 2018)** & **(Seok et al., 2013)** Functional enrichment analysis further categorizes these genes, elucidating key biological pathways and potential therapeutic targets. **(Steffen Klasberg, Bitard-Feildel, & Mallet, 2016)** This comprehensive understanding of gene interactions and functions is essential for developing effective meningitis therapies.

1.2. Delving into the Gene Landscape of Meningitis

Microarray and RNA sequencing technologies are crucial for uncovering genes that exhibit coordinated expression patterns during meningitis. By analyzing the transcriptome, researchers can identify genes co-expressed during the disease state, providing insights into disease pathogenesis, immune responses, and host susceptibility. Once these genes are identified, functional enrichment analysis categorizes them based on biological functions and cellular processes. **(Steffen Klasberg, Bitard-Feildel, & Mallet, 2016)**

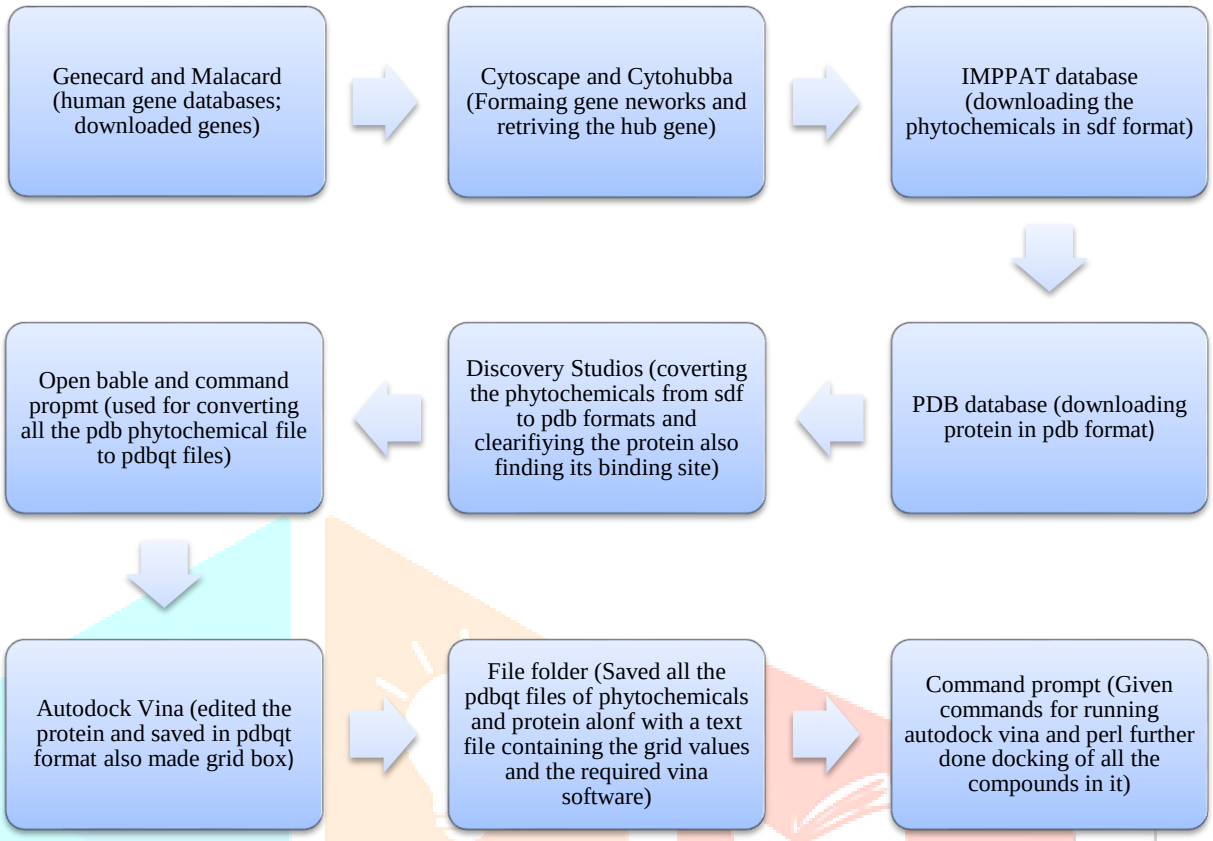
1.3. Risk Factors and Their Genetic Influence

Genetic predisposition and environmental factors significantly influence susceptibility to meningitis. Certain genetic variations can affect immune response and inflammation regulation, increasing an individual's risk of the disease. Understanding these genetic risk factors can improve early diagnosis and preventive measures. Environmental factors such as overcrowding, poor sanitation, and malnutrition also play a role by influencing gene expression and increasing infection risk. Addressing these factors through improved sanitation, vaccination programs, and nutritional interventions can help protect vulnerable populations. **(Borrow et al., 2006)** & **(Zhang, Li, Chen, & Su, 2013)**

1.4. Network Pharmacology: Unveiling Potential from Nature's Bounty

Network pharmacology bridges the gap between gene expression studies and therapeutic discovery. By constructing a network of interacting genes, proteins, and metabolites associated with meningitis, researchers can overlay this with data on phytochemicals—bioactive compounds from plants—to identify potential drug candidates. In silico docking simulations assess the binding efficacy of these phytochemicals to target genes or proteins, while in vitro assays validate their biological activity against meningitis. This integrative approach leverages natural compounds to uncover novel therapeutic agents for meningitis treatment. **(AN, 2023)** & **(Chandran, Neelay Mehendale, Patil, Rathnam Chaguturu, & Patwardhan, 2017)**

FLOW CHART OF MATERIALS AND METHODS



2. Materials and methods

2.1. Discovery and retrieval of genes: Target identification

A drug target is a biological entity, often a protein, that can modulate disease phenotypes. Identifying prime drug targets is crucial in drug discovery. Using databases like *GeneCards* and *MalaCards* databases. Like Genecard is a widely used, searchable database of human genes that provides integrated information on all known and predicted genes **(Rebhan, 1997)**. Developed and maintained by the Crown Human Genome Center at the Weizmann Institute of Science, in collaboration with LifeMap Sciences, it offers a wealth of resources for studying human genes and their roles in health and disease **(Stelzer et al., 2016).** Whereas, Malacard is a valuable resource for studying human diseases. It serves as an integrated platform offering diverse clinical and genetic annotations along with structured search functionalities **(Rappaport et al., 2016)**. Developed by researchers at the Weizmann Institute of Science, MalaCards provides a comprehensive view of human diseases and their associated genes. 3,856 genes associated with meningitis, were retrieved also the list was refined removing duplicates.

2.2. Network Construction

After retrieving genes from genecard and malacard databases, network of all the genes is made using bioinformatics tools *STRING* (Search Tool for the retrieval of interacting genes/proteins) on cytoscape. *Cytoscape* is a tool for viewing and analyzing very large networks. The network contains nodes and edges. The

nodes are used to represent genes/proteins and edges represent the interactions between the nodes. The network was formed at different confidence levels and analyzed for example at 0.40, 0.70, 0.90, and 0.95.

2.3. Hub gene identification

Cytohubba is plugin for Cytoscape, a popular software platform for visualizing and analyzing biological networks. CytoHubba is used specifically for identifying key nodes or hub proteins within a network based on various topological algorithms. It's commonly employed in network construction and analysis to understand the significance of specific nodes in a biological context.

Different parameters like MCC, MNC, DEGREE, STRESS etc. was analyzed for top 20 genes at 0.95 confidence level. For getting the hub gene/target gene a list was made of all the parameters and most repeating gene was listed out. Two genes NDUFS3 AND NDUFS8 were the most repeating genes satisfying the majority of parameters.

2.4.Ligand identification

After several literature studies it was found that Allium Sativum (garlic) has such medicinal properties which can fight against the disease meningitis. 36 ligands/phytochemicals were downloaded in sdf format from *IMPPAT* database (is a valuable resource for researchers exploring the potential of natural products from Indian medicinal plants for drug discovery).

2.5.Ligand preparation

All the ligands/phytochemicals were downloaded in sdf format and for docking they should be converted into pdbqt format. 36 downloaded phytochemicals from IMPPAT Database in sdf format were further converted to pdb format using *Discovery Studios* (a powerful software suite widely used in various fields of computational drug discovery and materials science. Developed by Dassault Systems Biovia, it offers a comprehensive set of tools for researchers to model, simulate, and analyze molecules and their interactions), command prompt and *Open Bable* were then converted to PDBQT files (the PDBQT file format, an extension of the PDB format, is essential for molecular modeling and docking simulations. It stores 3D structural data of macromolecules, aiding in predicting ligand binding to target proteins). Open bable is a free and open-source software toolkit designed to bridge the gap between different chemical data formats **(Trott & Olson, 2009)**. Developed and maintained by a collaborative community, it acts as a translator, allowing researchers to seamlessly convert, analyze, and manipulate chemical data stored in various formats.

2.6. Protein preparation

The genes /proteins were downloaded from protein data bank (PDB database) in pdb format then using discovery studios software protein preparation was done. Protein preparation is done to improve polarity of structure, addition of charges, AD4 atoms in order to minimize electron density. Lastly the protein is converted into pdbqt format using autodock vina as required for docking. The PDB database is particularly for protein searches, I got the protein (5XTB) by inputting the gene in the search column.

2.7. Grid generation

Grid generation is necessary for finding out the active sites in the receptor needed for docking. The grid box size was chosen to be large enough to cover the ligand but as small to ensure sufficient conformational sampling within available searching space. The value of search space should be no bigger than 30 Å × 30 Å × 30 Å, otherwise the search algorithm may suffer from insufficient sampling.

Preparation of the grid parameter file was done by using *AutoDock Tools*.

Adjust the grid box manually to get the suitable parameters which cover the entire protein or the binding site, and taking note the x, y, z coordinates of the center and sizes in the x, y, z dimension (Å) of the grid box. The preparation of the grid parameter file was done by using AutoDock Tools. Adjust the grid box manually to get the suitable parameters which cover the entire

Protein or the binding site, and taking note the x, y, z coordinates of the center and sizes in the x, y, z dimension (Å) of the grid box.

2.8. Preparation of configuration file (conf_txt):

The "conf_txt" is the configuration file text file used to specify the parameters and settings for a molecular docking simulation. This file contains various parameters that control the behavior of *AutoDock Vina* during the docking simulation.

File configuration includes protein and ligand file inputs (*. pdbqt), the values of center and size of a grid box (centre_x, centre_y, centre_z; and size_x, size_y, size_z), and Docking parameters.

3. Result

3.1. Discovery and retrieval of genes

The genes were downloaded using malacard and genecard databases, a total number of 3,836 (after removing the duplicates) genes were retrieved from the databases.

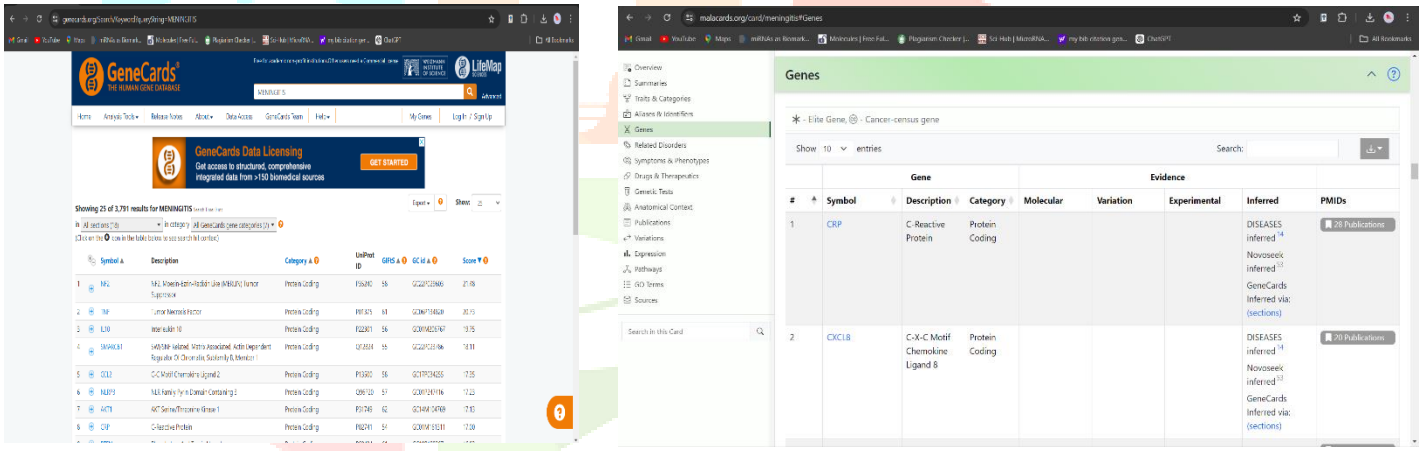


Fig.1: The above pictures show the searched page of genecard and malacard (in results has the gene name and its information)

3.2. Network Construction

Network of all the gens was created on cytoscape at confidence level 0.40, 0.60, 0.90 and 0.95. All 3,836 genes were copied from the excel sheet and were pasted in the string search column. The confidence levels in Cytoscape while creating a network is a strategic decision that directly influences the quality, reliability, and relevance of the resulting network. It enables us to tailor the network construction to the specific research needs and ensures that the network is both useful and scientifically valid for the questions being investigated.

Table 1: The table showing values of edges and nodes at different confidence levels.

Confidence level	Edges	Nodes
0.40	3095	557
0.70	1228	557
0.90	594	557
0.95	447	557

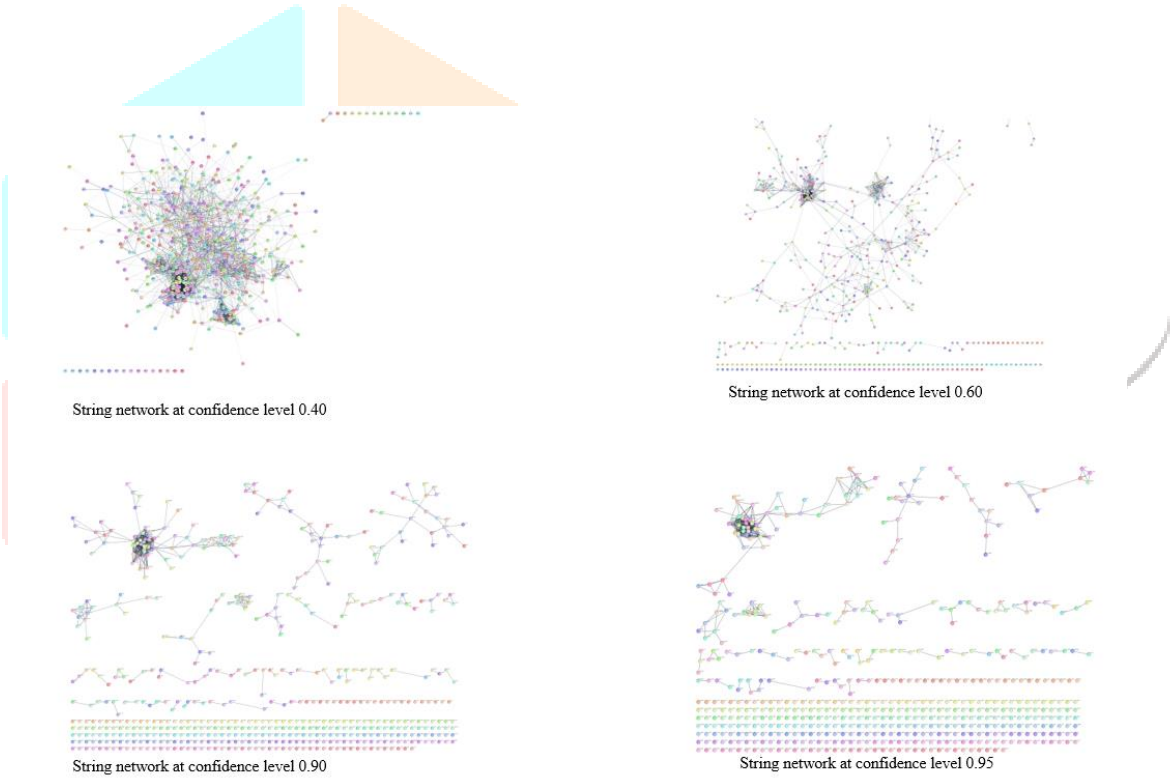


Fig. 2: Networks created at different confidence level made on Cytoscape.

3.3. The Hub gene

The genes NDUFS3 AND NDUFS8 were the most repeating genes satisfying the majority of parameters. Further degree of both the genes were checked from the node table present along with the networks and the gene NDUFS3 was finalized as the hub gene, as it had the highest degree and was common in all the parameters.

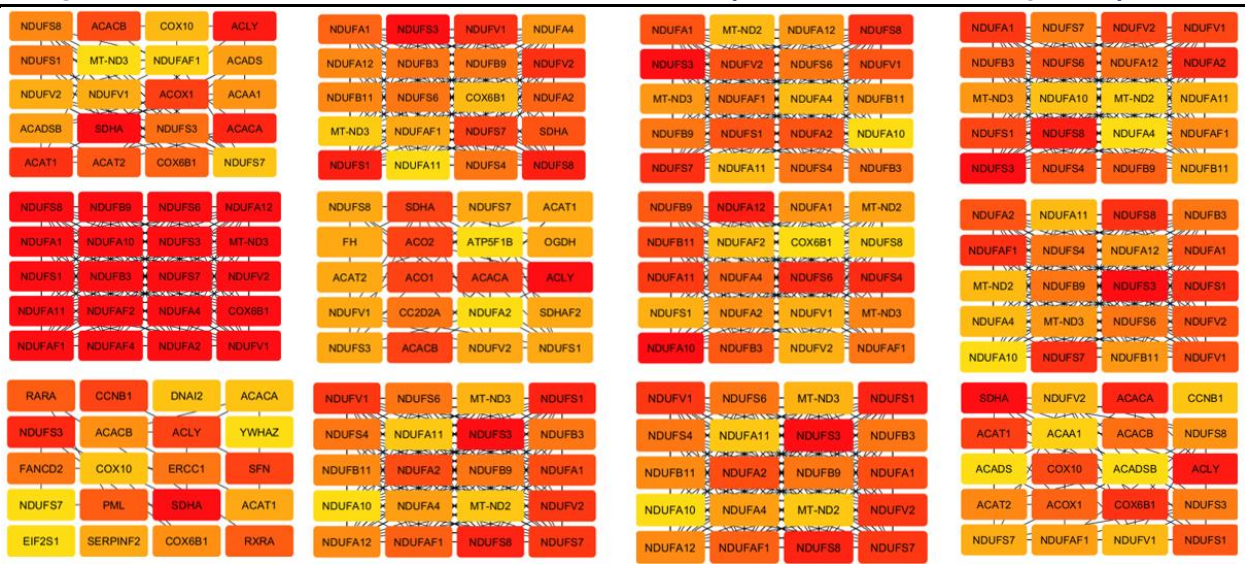


Fig. 3: Using Cytohubba different parameters (MCC, MNC, DEGREE, STRESS, DMNC, EPC, BOTTLENECK, ECCENTRICITY, CLOSENESS, RADIALITY, BETWEENESS & CLUSTRING COEFFICIENT) were analyzed for top 20 genes at 0.95 confidence level. In all total I got my hub gene NDUFS3

Table 2: The table is showing nodes and edges of all the parameters of CytoHubba made for getting our hub gene

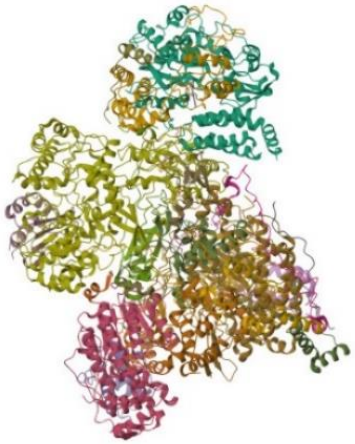
Parameter	Nodes	edges
MCC	20	189
MNC	20	179
DMNC	20	189
DEGREE	20	189
EPC	20	173
BOTTLENECK	20	15
ECCENTRICITY	20	43
CLOSENESS	20	189
RADIALITY	20	173
BETWEENESS	20	52
STRESS	20	59
Clustering Coefficient	20	5

3.4. The Protein

5XTB was the protein, which was retrieved by browsing PDB database and from there was downloaded in pdb format. Further, it's preparation was done improving polarity of the structure, adding charges, AD4 atoms in order to minimize electron density. Lastly it was converted into pdbqt format using autodock vina as required for docking.

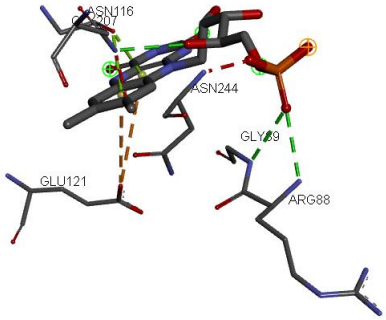
Table 3: The above data shows the gasterior and kollman charges on the protein we got while the preparation of protein in autodock vina

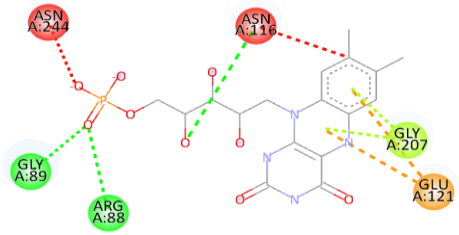
Receptor	Gasterior charges	Kollman charges
5XTB	-11.9899	-720.249



a

b





c

Fig. 4: The above images are of protein, which firstly was downloaded from PDB database further its preparation was done in discovery studios.

a. It's the actual 3D structure of the protein 5XTB.

b. The structure displays the ligand interactions showing the binding sites of the protein.

c. It is the 2D diagram of the receptor ligand interaction.

(b and c image is from discovery studios)

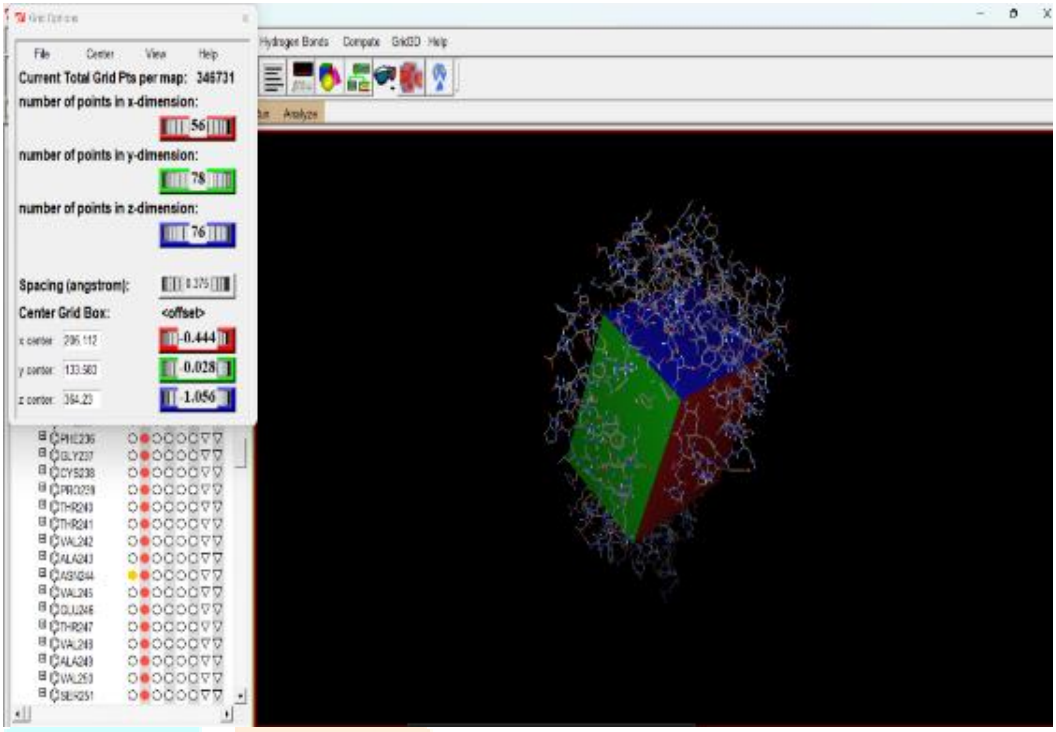


Fig. 5: The picture shows the grid prepared in the protein, along with the configuration values in the side box. The grid is made covering all the binding sites targeted in the protein. The x, y, z dimensions and the center x, y, z values are further used as text file for docking.

4. Docking

Docking is done using autodock vina. Auto Dock Vina, a free and fast open-source program, predicts how small molecules bind to proteins. Compared to other docking programs, Vina excels in speed while maintaining reasonable accuracy. Its user-friendly interface and flexibility in handling conformational changes make it accessible to researchers of varying experience levels. Additionally, Vina can utilize multiple processors for faster calculations, especially beneficial for large-scale screening in drug discovery. Overall, AutoDock Vina's speed, accuracy, ease of use, and open-source nature make it a valuable tool for researchers worldwide. **(Trott & Olson, 2009)**

Perl program files (.pl extension) contain human-readable code for various tasks. They typically include an optional interpreter line (#! /usr/bin/perl) followed by Perl statements and comments. To run these scripts, you make them executable and use perl script_name.pl on the command line. Perl excels in system administration, text processing, bioinformatics, and more, making it a versatile general-purpose programming language. We used this program file along with autodock vina for docking. Steps for docking:

- A folder is made containing the following:
  - Vina three files
  - Ligand pdbqt file
  - Receptor pdbqt file
  - Perl script file
  - Config.txt

- Further, using perl along with autodock vina, we gave commands accordingly and completed docking of all the 36 phytochemicals compounds. After complete docking of all the compounds, results were analyzed by comparing their binding energies/affinity, it was found that affinity of sativosider2 was the highest (-14.8) and so it was considered to be the best phytochemical compound docked with the receptor 5XTB and could be used for the treatment of meningitis.

- **[Sativosider2**: Sativosider2 is found to have properties relevant to meningitis treatment, it might work through several mechanisms like antimicrobial activity, anti-inflammatory effects, immunomodulatory effects and neuroprotective effects.

Table 4: The table above shows all the phytochemicals which were downloaded from IMPPAT database; when these ligands were further docked with the receptor, we got Sativosider2 as the ligand with the highest affinity and further was considered to be the best phytochemicals among all the 36 phytochemicals and can be used in the treatment of meningitis disease.

S. No.	Phytochemical Name	Affinity
1	(E)-alpha-bisabolene	-6.6
2	Allicin	-4.2
3	Allixin	-6.3
4	Allyl alcohol	-3.1
5	Allyl isothiocyanate	-3.4
6	Allyl methyl disulfide	-3.1
7	Allyl methyl trisulfide	-3.2
8	Allyl propyl disulfide	-3.5
9	Alpha-Guanine	-6.8
10	Ascorbic acid	-6.0
11	beta-Bisabolene	-6.3
12	Carvacrol	-5.8
13	Carvacrol methyl ether	-5.8
14	Diallyl sulfide	-3.5
15	Diallyl tetra sulfide	-3.6
16	Diallyl trisulfide	-3.5
17	Elemicin	-5.4
18	Eugenol	-5.6
19	Folic	-9.0
20	Gamma-terpinene	-5.3
21	Giberellina7	-8.3
22	Hypermellosum	-7.3
23	Isobutyl isothiocyanate	-4.0
24	Myrcene	-4.8
25	Nicotinic acid	-5.2
26	p-Cymene	-5.4
27	Phenethyl isothiocyanate	-6.0
28	Protopine	-9.5
29	Quercetin	-8.4
30	Riboflavin	-8.7
31	Sativosider2	-14.8
32	Sativosideb1	-7.1
33	Thiamine	-5.9
34	Thymol	-5.6
35	Thymol methyl ether	-5.8
36	Thymoquinone	-5.8

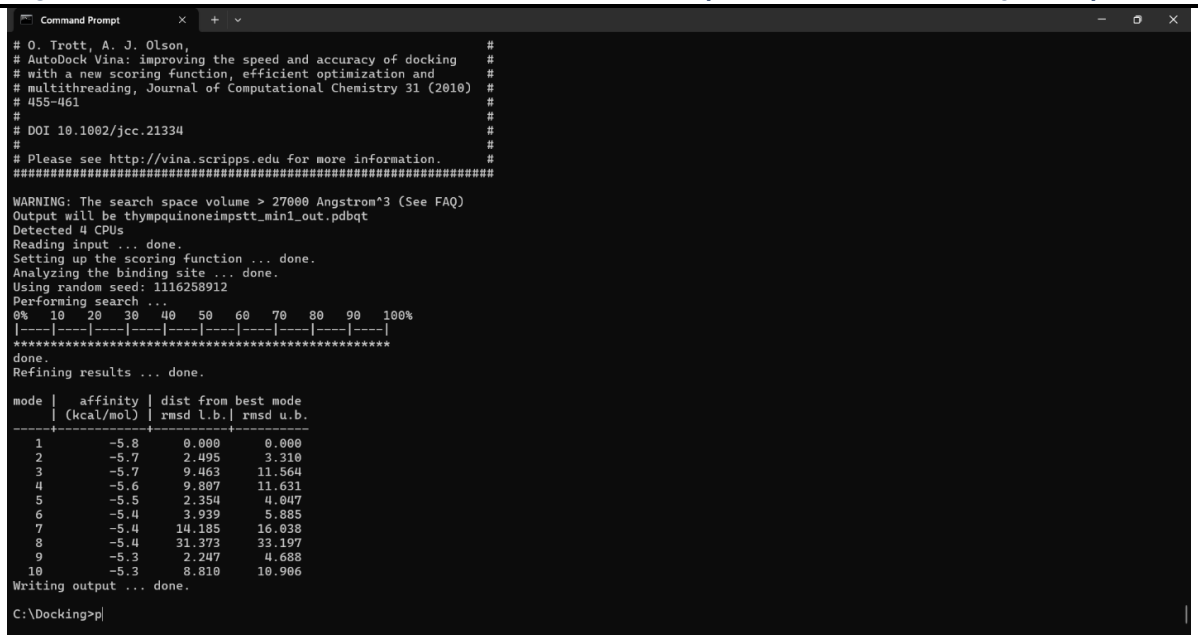


Fig. 6: The above image is the command prompt page showing the docking results.

5. Discussion

This study aimed to identify potential drug targets for meningitis through gene expression analysis and network analysis techniques. Using Cytoscape and CytoHubba, NDUFS3 was prioritized as a crucial hub gene due to its role in mitochondrial complex I, essential for cellular energy production.

NDUFS3 is vital for the assembly and stability of mitochondrial complex I, contributing to cellular respiration and ATP production. Mutations in NDUFS3 are linked to mitochondrial diseases like Leigh syndrome and mitochondrial complex I deficiency, characterized by muscle weakness and developmental delays. **(Yang et al., 2023)**

To explore NDUFS3, I employed gene expression and protein analysis, alongside animal models. Subsequently, natural compounds potentially interacting with NDUFS3 were identified using the IMPPAT database. These compounds underwent docking simulations to predict binding interactions, with sativosider2 emerging as a promising candidate.

Docking Simulations Workflow:

1. Protein Preparation: Using Discovery Studio, proteins were prepped by removing unnecessary atoms and assigning charges.

2. Ligand Preparation: Ligands were converted to a compatible format using Discovery Studio and Open Babel.

3. Docking: AutoDock Vina performed docking simulations, creating a grid around the active site to define potential binding interactions.

[The Ramachandran plot was used to validate protein structures by analyzing backbone dihedral angles (phi and psi), helping identify secondary structures, protein folding quality, and potential binding pockets.] **(Hollingsworth & P. Andrew Karplus, 2010)**

The current study has identified sativosider2 as a promising phytochemical for targeting NDUFS3 in meningitis. Future research should focus on identifying co-expressed genes in meningitis using transcriptomic data and co-expression network analysis, considering risk factors such as age and immune status. Employing a

network pharmacology approach will help evaluate sativosider2 and other phytochemicals by constructing interaction networks and predicting mechanisms of action and potential side effects. In vitro and in vivo validation through cell line experiments and animal models will assess biological activity and efficacy. Successful results could lead to well-controlled clinical trials to evaluate the safety and efficacy of sativosider2 in meningitis patients, ultimately aiding in the development of effective treatments. **(Nasim, Inavolu Sriram Sandeep, & Mohanty, 2022)**

5. Conclusion

Meningitis is a severe inflammation of the meninges, posing a significant global health threat, especially in developing countries. The emergence of antibiotic-resistant strains necessitates novel therapeutic strategies. This study identified sativosider2, a phytochemical from the IMPPAT database, as a potential drug targeting NDUFS3, implicated in mitochondrial dysfunction in meningitis. Future research should focus on identifying co-expressed genes in meningitis using transcriptomic data and bioinformatics tools, considering risk factors such as age and immune status. **(Hersi, Gonzalez, & Kondamudi, 2023)**

Network pharmacology can systematically evaluate sativosider2 and other natural products by constructing interaction networks of genes, proteins, and pathways associated with meningitis, predicting mechanisms of action and potential side effects. In vitro and in vivo validation will assess biological activity, efficacy, and pharmacokinetic properties of sativosider2. Successful results will lead to well-controlled clinical trials to evaluate safety and efficacy in patients. **(Chandran, Neelay Mehendale, Patil, Rathnam Chaguturu, & Patwardhan, 2017)**

The integration of in silico docking, network pharmacology, and experimental studies paves the way for developing novel and effective treatments for meningitis. Despite the advancements, future research should incorporate molecular dynamics simulations for a more realistic assessment of ligand-protein interactions. This comprehensive approach aims to harness natural products' potential, offering promising therapeutic options for meningitis. **(Zhang et al., 2021)**

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cytoHubba - Cytoscape App Store https://apps.cytoscape.org/apps/cytohubba

The National Center for Biotechnology Information. Gene - NDUFS3 NADH:ubiquinone oxidoreductase core subunit S3 [Homo sapiens (human)]
NDUFB11 and NDUFS3 play a role in atherosclerosis and chronic stress. National Institutes of Health (.gov)

Online Mendelian Inheritance in Man (OMIM). Leigh Syndrome National Institutes of Health (.gov)(https://omim.org/entry/256000)

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