Comparative *Insilico* Analysis and Identification of *Anisomeles* derived inhibitors against Lex A repressor & Rv0098 of H37Rv-*Mycobacterium tuberculosis*

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ABSTRACT:

Tuberculosis (TB) is an infectious airborne disease caused by the Pathogen *Mycobacterium tuberculosis*. It is the major cause of human mortality rate from a curable infectious disease, attacking mainly in developing countries. Vaccination against tuberculosis is available but proved to be unsuccessful against emerging multidrug and extensive drug resistant bacterial strains. This in turn raises the pressure to speed up the research on developing new and more efficient anti-tuberculosis drugs. There is a growing focus on the importance of medicinal plants to cure these type of infectious disease. *Anisomeles malabarica* is perhaps the most useful traditional medicinal plant. Thus, the development of a plant- based antibacterial preparation promises a more potential alternative when compare to synthetic drugs against Tuberculosis. The pharmacological studies of *Anisomeles malabarica* had revealed that it possess antibacterial activity and huge potential to cure Tuberculosis. The 34 phytochemical compounds from *Anisomeles malabarica* were identified through literature survey and also the protein Lex A repressor and Hypothetical protein Rv0098 of the strain H37Rv with the PDB ID 2PFC, 6A2Q were identified to be more pathogenic and responsible for Tuberculosis disease in human. We carried out Molecular Docking studies for the above 34 Compounds and 2 Proteins. Docking was performed for both synthetic compounds and *Anisomeles* compounds. After Comparative studies the best docking interactions were reported. Finally through our current study we identified that the above 2 proteins interacting with *Anisomeles* compounds showed best results while comparing with synthetic compounds interaction.

KEYWORDS:

Tuberculosis, *Anisomeles malabarica*, Antibacterial studies, phytochemical compounds, Docking.

INTRODUCTION:

Anisomeles malabarica with common name Malabar catmint is a species of herbaceous plant native to India, Bangladesh, Srilanka, Andaman &Nicobar Islands, Thailand, Malaysia, Indonesia, New Guinea, and Northern Australia. It belongs to the family Lamiaceae (Mint family). The Species of *Anisomeles* are *Anisomeles indica, Anisomeles salviifolia, Anisomeles candicans, and Anisomeles heyneana.* The natural compounds present in *Anisomeles malabarica* were identified to have the anti-bacterial activity to cure the infectious disease such as tuberculosis. The other Pharamacological activity of *Anisomeles malabarica* were found to be Antibacterial, Analgesic, Anticancer, Antipyretic, Anti allergic, Anti-inflammatory, Antirheumatic, Antiphlogistic, Antidiabetic and febrifuge actions. (KavithaR, Nelson, 2012). Due to this pharmacological activity the *Anisomeles malabarica* species is identified to cure the Tuberculosis disease. Tuberculosis is an infectious disease caused by several species of Mycobacteria including *Mycobacterium tuberculosis* (MTB), *M.bovis, M.africanum, M.microti, M.avium and M.leprae* that are intracellular. Gram-positive, non-motile and rod-shaped obligate aerobic pathogens of higher vertebrates. Tuberculosis(TB) causes considerable morbidity among millions of people each year worldwide and ranks as the second leading cause of death from infectious disease, after the human immunodeficiency virus(HIV)(Andreas Sandgren ,Michael Strong,2009).

The 34 Phytochemical compounds of *Anisomeles malabarica* were identified through Literature survey in PubMed. The *Mycobacterium tuberculosis* strains H37Rv (P. Bifani,S. Moghazeh,2000) are the most commonly used controls for M. tuberculosis identification in the clinical and research laboratory setting. It was first isolated by Dr.EdwardR.Baldwin in 1905. The strain came from a 19 year old patient with chronic pulmonary tuberculosis at the Trudeau Sanatorium in Saranac Lake, New York. It was maintained for many years by serial passage of cultures at the Trudeau Sanatorium and initially named strain H37.Over time it was found to have variable virulence in animal models based on which medium it was grown . Strains with different virulence were then intentionally produced, with H37R being less virulent after growing in acidic media and H37S was more virulent in guinea pigs after being grown in alkaline media (with R standing for resistant to environment, and S for sensitive to environment). The more virulent strain was later renamed H37Rv, with R standing for rough morphology and v standing for virulent. The strain was used for many laboratory studies and became the standard for tuberculosis. It was later designated as the neotype for the species. Koch first discovered *Mycobacterium tuberculosis* as the cause of tuberculosis in 1892 but the strains he studied were not preserved and it is unclear how related H37Rv may be to those strains. H37Rv has continued to be the strain of tuberculosis most used in laboratories, and was the first to have its complete genome published in 1998.The structures of protein LexA repressor and Hypothetical protein Rv0098of the strain H37Rv were identified to be the target through previous reports and they were retrieved from pdb. The pdb ID are 6A2Q and 2PFC. Molecular docking was carried out for these 2 proteins with the 34 compounds. The goal of ligand-protein docking is to predict the predominating binding mode(s) of a ligand with a protein of known three-dimensional structure.

MATERIALS AND METHODS:

Selection of small molecules:

Through literature survey, 34 phytochemical compounds were identified from the plant *Anisomeles malabarica* with the help of PubMed database. PubMed is a free resource developed and maintained by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (NLM). It is free search engine accessing primarily the MEDLINE database of references and extracts on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health maintains the database as part of the Entrez system of information retrieval. For Computational analysis, the compound should possess at least 2 dimensional structures. .Using the pubChem database the 2dimensional structures of 34 compounds were retrieved. PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely retrieved via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing PubChem database.

Selection of Proteins:

The Mycobacterium tuberculosis strains H37Rv are the most commonly used controls for M. tuberculosis identification in the clinical and research laboratory setting. It was first isolated by Dr.EdwardR.Baldwin in 1905. The structures of protein Lex A repressor and Hypothetical protein Rv0098 of the strain H37Rv were predicted and retrieved from pdb. The pdb ID are 6A2Qand 2PFC.The three dimensional structure of the protein were retrieved using Protein Data Bank (PDB) (https://www.rcsb.org/) which was determined by experimental studies by X-Ray Diffraction. The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy. The PDB is a key in areas of structural biology, such as structural genomics.

Molecular Docking using Argus Lab:

After the preparation of the protein and ligand, molecular docking studies were performed to evaluate the interactions using Argus lab. Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominating binding mode(s) of a ligand with a protein of known three-dimensional structure. Argus lab protocol was carried to find the best interactions between the protein and the ligand. Argus lab is a free molecular package that runs under windows. It is installed on all public computers and can be retrieved from www.arguslab.com/downloads.htm/ . It is a molecular modeling, graphics, and drug design program. The Argus lab docking engine, implemented in it, approximates an exhaustive search method with similarities to Dock and Glide. Flexible ligand docking is possible with Argus lab, where the ligand is described as a torsion tree and grids are constructed that overlay the binding site. Ligand's root node is placed on a search point in the binding site and a set of diverse and energetically favorable rotations is created. The docking was carried out for 2proteins of strain H37Rv with 34 phytochemical compounds of *Anisomeles malabarica* through Argus lab.

Comparative Studies:

The best interaction of the phytochemical compounds from *Anisomeles malabarica* were compared to the interactions of synthetic compounds such as Isoniazid and Rifampicin for the disease Tuberculosis. The synthetic compounds was docked with the same proteins Lex A repressor and Hypothetical protein Rv0098 of the strain H37Rv of the PDB ID of 2PFC and 6A2Q. The comparative Docking studies were performed to prove that the phytochemical compounds has the best interactions than the synthetic compounds. After observing the results from the current study, we strongly recommend that the phytochemical compounds of *Anisomeles malabarica* can lead to the discovery and development of potential drugs against bacterial diseases such as drug resistant tuberculosis.

RESULTS AND DISCUSSIONS:

Preparation of small molecules:

The 34 phytochemical compounds through literature were identified from the plant *Anisomeles malabarica* with the help of PubMed database.

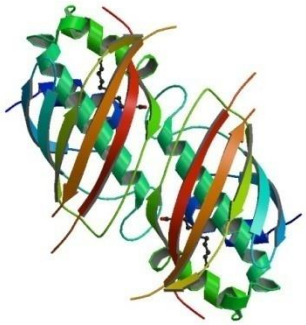
Table 1: Pytochemical Compounds from *Anisomeles malabarica*

S.No	Pytochemical Compounds
1.	Alpha pinene
2.	Camphene
3.	Beta pinene
4.	3-Octanol
5.	1,8-cineole
6.	Cis-sabinene hydrate
7.	Linalool
8.	Camphor
9.	Borneol
10.	Myrtenol
11.	Alpha-thujone
12.	Linalyl acetate
13.	Nerol
14.	Geraniol
15.	Geranial
16.	Thymol
17.	Bornyl acetate

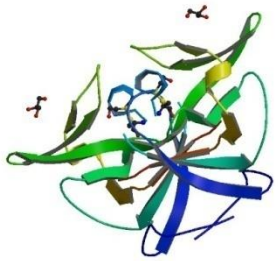
18.	Terpenyl acetate
19.	Anisole
20.	2-Isopropylbenzaldehyde
21.	Eugenol
22.	N-nonanyl acetate
23.	Delta-cadinene
24.	Isocaryophyllene
25.	Caryophyllene oxide
26.	Epiglobulol
27.	Globulol
28.	Nerolidyl acetate
29.	Farnesyl acetate
30.	Alpha bisabolol
31.	Trans-phytol
32.	Citronellol
33.	Isomenthol
34.	Azulene

Preparation of proteins:

The structures of protein Lex A repressor and Hypothetical protein Rv0098 of the strain H37Rv were predicted and retrieved from PDB. The PDB ID are 6A2Q and 2PFC.The three dimensional structure of the protein were retrieved using Protein Data Bank (PDB). The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids.



3D structure of Hypothetical protein Rv0098 2pFC



3D structure of protein Lex A repressor 6A2Q

Docking Interactions:

Molecular Docking was performed to find the interactions between the proteins Lex A repressor and Hypothetical protein Rv0098 of the strain H37Rv with the PDB ID of 6A2Q and 2PFC. Docking was carried out using the Argus lab protocol to find out the best interactions between the protein and the ligand. The best binding interactions for the protein 6A2Q is **-13.44 Kcal/mol** for the compound Delta Cadinene. The best binding interactions for the protein 2PFC is **-13.30 Kcal/mol** for the compound Trans Phytol.

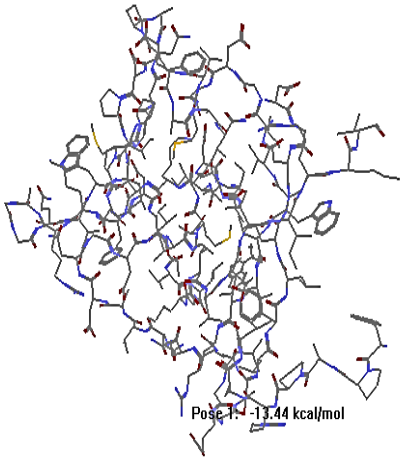
Table 2: Docking Interactions of the ligands and proteins.

S.No	Phytochemical Compounds	6A2Q Kcal/mol	2PFC Kcal/mol
1.	Alpha pinene	-10.19	-9.22
2.	Camphene	-8.22	-8.24
3.	Beta pinene	-10.69	-9.11
4.	3-Octanol	-6.07	-10.10
5.	1,8-cineole	-7.73	-7.45
6.	Cis-sabinene hydrate	-11.06	-8.48
7.	Linalool	-4.97	-8.68
8.	Camphor	-9.89	-12.15
9.	Borneol	-8.20	-7.94
10.	Myrtenol	-8.03	-7.90
11.	Alpha-thujone	-10.22	-8.94
12.	Linalyl acetate	-8.33	-7.53
13.	Nerol	-6.96	-8.48
14.	Geraniol	-9.75	-7.42

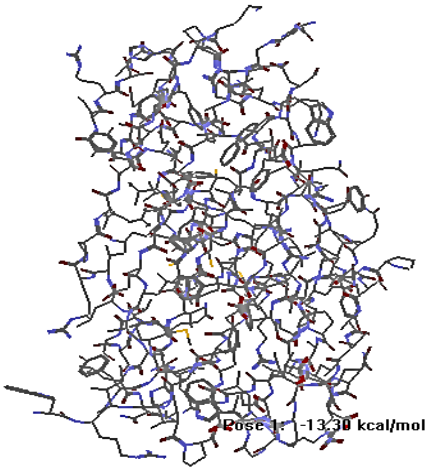
15.	Geranial	-10.66	-6.50
16.	Thymol	-9.90	-9.31
17.	Bornyl acetate	-8.39	-9.81
18.	Terpenyl acetate	-10.27	-7.98
19.	Anisole	-7.90	-7.60
20.	2-Isopropylbenzaldehyde	-9.27	-8.24
21.	Eugenol	-8.30	-7.91
22.	N-nonanyl acetate	-8.65	-7.11
23.	Delta-cadinene	**-13.44**	-10.16
24.	Isocaryophyllene	-12.41	-11.41
25.	Caryophyllene oxide	-13.14	-9.68
26.	Epiglobulol	-11.38	-10.86
27.	Globulol	-12.92	-11.37
28.	Nerolidyl acetate	-7.96	-8.96
29.	Farnesyl acetate	-11.18	-9.25
30.	Alpha bisabolol	-10.62	-9.31
31.	Trans-phytol	-10.27	**-13.30**
32.	Citronellol	-9.11	-8.06
33.	Isomenthol	-9.38	-8.47
34.	Azulene	-10.18	-8.33

Best binding energies of Phytochemical and Synthetic Compounds:

Phytochemical Compounds:

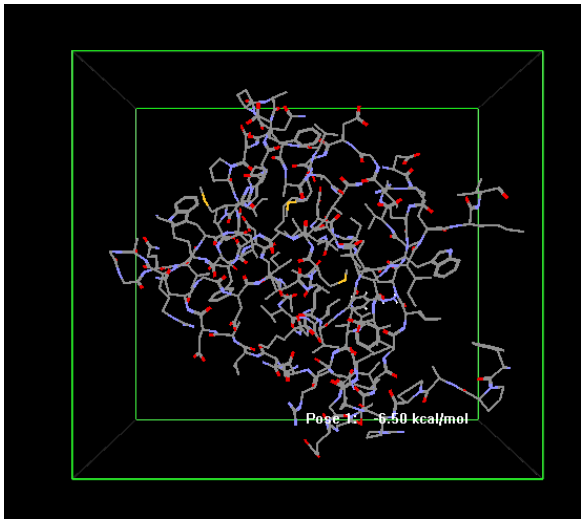


6A2Q for Delta-cadinene

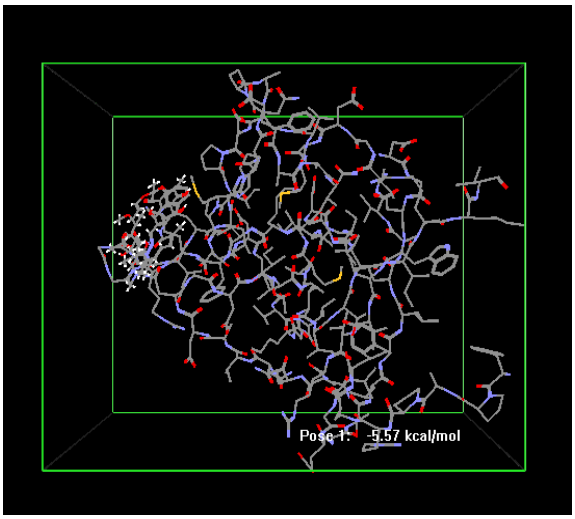


2PFC for Trans-phytol

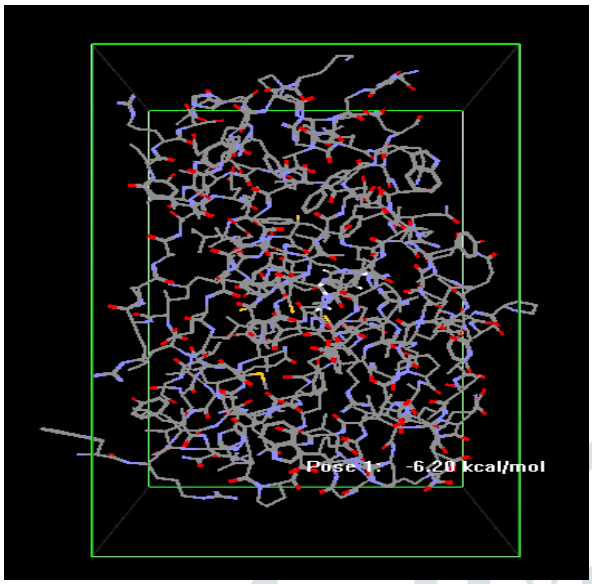
Synthetic Compounds:



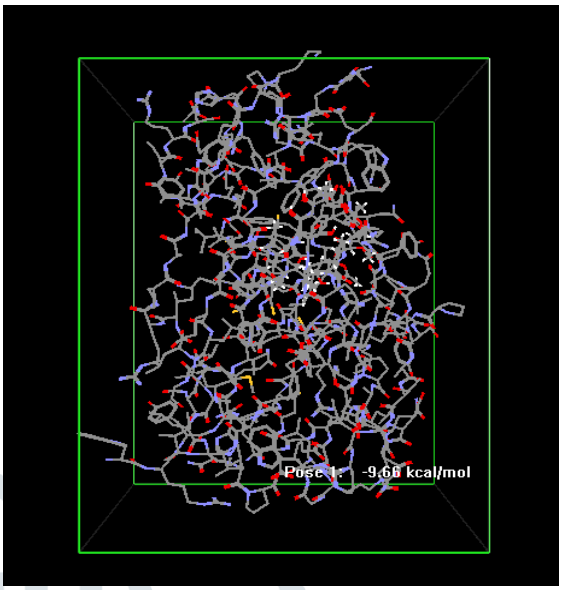
6A2Q for Isoniazid



6A2Q for Rifampicin



2PFC for Isoniazid



2PFC for Rifampicin

Comparative Studies:

The synthetic compounds were identified for the disease Tuberculosis through literature survey using PubMed. The compounds are Isoniazid and Rifampicin. These proteins were carried out for the Molecular docking to predict the interactions. It was performed to compare the interactions between synthetic compounds and phytochemical compounds. The synthetic compounds Isoniazid and Rifampicin were docked with the same proteins Lex A repressor and Hypothetical protein Rv0098 of the strain H37Rv of the PDB ID of 2PFC and 6A2Q.Thus the interactions of synthetic compounds were lesser than the phytochemical compounds.

Table 3: Docking interactions of Phytochemical and synthetic compounds.

S.No	Proteins	Phytochemical Compounds	Ligand Pose Kcal/mol
1	6A2Q	Delta-Cadinene	-13.44
2	2PFC	Trans-phytol	-13.30

S.No	Proteins	Synthetic Compounds	Ligand Pose Kcal/mol
1	6A2Q	Isoniazid	-6.50
2	6A2Q	Rifampicin	-5.57
3	2PFC	Isoniazid	-6.19
4	2PFC	Rifampicin	-9.66

CONCLUSION:

Docking studies was performed to prove that the phytochemical compounds derived from *Anisomeles malabarica* has the best interactions than the synthetic compounds. By comparing the both docking interactions of phytochemical compounds and synthetic compounds, we can conclude that the phytochemical compound Delta-cadinene of 6A2Q and Trans-phytol of 2PFC has more potential to cure the bacterial diseases such as tuberculosis. Hence we strongly recommend that the phytochemical compounds of *Anisomeles malabarica* could further lead to the discovery and development of potential drugs against bacterial disease such as tuberculosis. Although synthetic compounds were reported to be very powerful anti-TB drugs, it is also reported to possess huge serious side effects in patients undergoing treatment. Hence we strongly recommend these natural compounds could be further subjected to various clinical studies. Further we conclude that *in vitro* studies on these natural compounds of *Anisomeles malabarica* would lead to discovery of novel potential drugs against Tuberculosis disease.

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