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Mechanistic Insight Flavonoids against Enoyl-ACP-Reductase for Effective Management of Mycobacterium Tuberculosis: *In-Silico* Validation

BY

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Abstract

Background:*Molecular evidence for tuberculosis (TB), one of the oldest human diseases, dates back more than 17,000 years. Despite improvements in identification and treatment, TB is remains one of the top 10 infectious diseases that kill people globally, second only to HIV. According to the World Health Organization (WHO), tuberculosis is a global pandemic. For people with HIV, it is the major cause of death. In India, the fight against TB has largely been divided into three historical periods: the early period, before the development of x-ray and chemotherapy; the post-independence era, when national TB control programmes were started and put into place; and the present period, when an ongoing WHO-assisted TB control programme is in place.*

Method:*In the current study, a molecular docking technique was used to try and identify enoyl ACP reductase (InhA) protein inhibitors. A grid-based docking strategy was used to determine the binding using the Auto Dock software. Merck Molecular Force Field was used to build the 2D structures of compounds, convert them to 3D, and then energetically reduce them up to arms gradient of 0.01. (MMFF).*

Result: *The molecular docking of baicalein, pectolinarin, myricetin, and hispidulin with enoyl ACP reductase (InhA) showed binding energy (Kcal/mol) -6.31, -6.12, -5.95 & -7.06 respectively.*

Conclusion: *Thefinal results of the existing research found out that the chosen molecule highlybounded with InhA thereby inhibiting the mycobacterial cell wall synthesis.*

Key words: *Mycobacterium tuberculosis, baicalein, pectolinarin, myricetin, hispidulin, Enyl ACP reductase (InhA) & molecular docking.*

INTRODUCTION

Tuberculosis (TB), one of the primogenital illnesses known to man, co-evolved with humans for at least a few million years before that. The oldest known tuberculosis DNA evidence was identified in 9000-year-old human bones unearthed in a Neolithic settlement in the Eastern Mediterranean and in a fossilised bison (Pleistocene bison) whose age was determined by radiocarbon dating at 17,870230 years [1]. Dr. Richard Morton first connected the pulmonary form of TB to "tubercles" in 1689, but the disease's vast spectrum of symptoms prevented it from being recognised as a single condition until the 1820s. J. L. Schönlein ultimately gave it the name "tuberculosis" in 1839. The bacillus that causes tuberculosis, Mycobacterium tuberculosis, was identified by Robert Koch in 1882. For this discovery, he was awarded the Nobel Prize in physiology or medicine in 1905. A group of bacterial species that cause tuberculosis is known as the Mycobacterium tuberculosis complex. Currently, Mycobacterium tuberculosis is the principal culprit behind tuberculosis in humans. Additional members of the M. TB complex that have been connected to tuberculosis include M. bovis, M. microti, and M. africanum. M. bovis has a greater host range and is the main cause of tuberculosis in other animal species, but M. africanum infections are relatively rare and M. microti is not known to cause TB in people. Milk, milk products, or meat from infected animals are the usual routes by which M. bovis infects people [2]. Despite improvements in diagnostic and therapeutic techniques, millions of people continue to have TB and die from it. Along with HIV/AIDS and malaria, which each claim the lives of 3 million people each year, TB is one of the top three infectious diseases that claim lives globally[3].

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Due to the wide range of health advantages flavonoids from plant sources have been shown to have in several epidemiological studies, there has been an increase in interest in their research. Since flavonoids are closely linked to dietary components and human health, it is important to assess how structure and function are related. The arrangement, quantity, and replacement of functional groups around the nuclear structure of flavonoids affects their bioavailability, metabolism, and biological activity. Along with tea and wine, fruits and vegetables are the primary dietary sources of flavonoids for people. The majority of recent studies have concentrated on flavonoids' effects on human health. Many flavonoids have been demonstrated to have antioxidative activity, the ability to scavenge free radicals, the ability to prevent coronary heart disease, the ability to protect the liver from damage, the ability to reduce inflammation, and the ability to combat cancer [4].

Flavonoids	Pharmacological Activity
Baicalein	Anti-inflammatory,anti-tumor, cardioprotective, neuroprotective, anti-ocular disorders, and mitochondrial functions [5].
Pectolinarin	Anti-inflammatory, Anti-oxidant & antidiabetic Potential [6].
Myricetin	Anticancer, antidiabetic, anti-obesity, cardiovascular protection, osteoporosis protection, anti-inflammatory, and hepatoprotective [7].
Hispidulin	Flavones have recently attracted scientific and public attention due to their alleged protective effects against a number of malignancies, atherosclerosis, and osteoporosis [8].

Experimental Work

As per literature survey and various beneficial pharmacological potential baicalein,pectolinarin, myricetin, and hispidulin were selected as lead molecule for current investigation.

Docking Study of Mycobacterium tuberculosis Enoyl ACP reductase

Ligand Preparation:

2D Structure of ligands like baicalein, pectolinarin, myricetin, and hispidulinwere drawn using ChemSketch [9], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

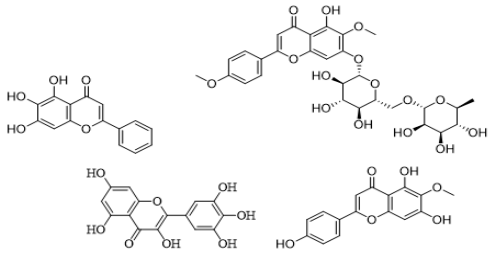

Figure 1: 2D structure of baicalein, pectolinarin, myricetin, and hispidulin.

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y, and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.347 Å and No. of points considered are 50, 50, and 50 points in the x, y, and z dimensions and 60.246, -1.91, and 85.861 as x, y, z centers [10].

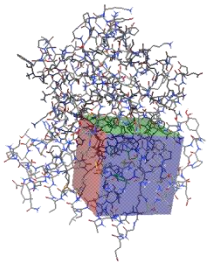

Figure 2: Grid box covering all active sites in Enoyl ACP reductase enzyme

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [11].

Docking of beta-tubulin with Quercetin

Crystal structure

The crystal structure of the protein consisting of Enoyl ACP reductase enzyme is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (4bqp.pdb) registered in the Protein data bank was used [12]. The complex Methyl-thiazole-based ligand was separated by using Chimera software.

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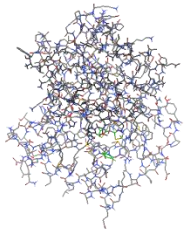

Figure 3: Crystal structure of Enoyl ACP reductase enzyme (PDB ID-4bqp)

Processing of Protein

The downloaded receptor protein is having five chains, i.e. chain A, B, C, D, and E. Out of these five chains, chain A was selected for experimental purpose, and other chains were removed from it. The bound ions were separated from the macromolecular complex by using software Chimera [13].

Molecular Docking Simulation Studies

Docking of ligands like baicalein, pectolinarin, myricetin, and hispidulin against tubercular Enoyl ACP reductase enzyme was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [14].

Toxicity & ADME-T Studies

The pharmacokinetics of selected flavonoids ligand molecules was studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME-T properties. [15-20]

Result and Discussion

The majority of antimycobacterial drug development initiatives over the previous two decades have centered on screening biochemically targeted inhibitors but regrettably have not resulted in novel TB medicines. Because of the complexity of the microenvironment experienced by the human host Mycobacterium TB, the causative agent of tuberculosis, recent anti-tuberculosis efforts have switched to the creation of whole-cell screening assays, however, this strategy is not practical. It's still quite challenging. As a result, several screening techniques have been created that more closely resemble the in vivo condition of MTB in a tuberculosis patient. Even using whole-cell screening methods, it is possible to identify the precise mycobacterial drug target of a promising hit or lead chemical to direct a prospective optimization process and create drug-like compounds. Ideally, a drug target should be important, drug-sensitive, and in vivo druggable so that it may be exploited to create therapeutically relevant antibiotics. Previous investigation revealed that there are many reports of essential targets for mycobacterial drugs. In this study, we selected enoyl ACP reductase (InhA) to serve as structural models for molecular docking studies. Selection criteria for putative targets were based on the availability of a resolved 3D crystal structure of the H37Rv-MTB strain and the essentiality of the target for mycobacterial growth and survival. Selection of individual 3D crystal structures was guided by consideration of crystal resolution, non-mutability (wild-type), overall quality of the crystal structure, and absence of missing loops.

One of the natural compounds that has been investigated the most thoroughly is flavonoids, which are found in plants. Flavonoids have been found to have several medical benefits, including antibacterial, antioxidant, anti-inflammatory, anti-cancer, and antiviral characteristics. The human immune system is boosted by these natural substances, which are also known to prevent infection by inhibiting the development of harmful microorganisms, including drug-resistant Mtb strains. Flavonoids are phenolic chemicals that typically include two phenyl rings connected to a heterocyclic ring. They are a subclass of phenolic compounds. Nevadensine, naringenin, isoflavanquinone, epicatechin, isoharmnetin, kaempferol, luteolin, myricetin, and quercetin are a few examples of flavonoids having anti-tuberculosis action. Rutin can be administered intravenously to treat pulmonary TB, according to studies. Synthetic flavanones have also been shown to have anti-tuberculosis action against Mtbas per Rabaan AA et al;2022. Based on the aforementioned factors, the current study was created to evaluate the anti-tuberculosis efficacy of various pharmacologically active flavonoids, including baicalein, pectolinarin, myricetin, and hispidulin, by computational analysis.

The molecular docking of baicalein, pectolinarin, myricetin, and hispidulin with enoyl ACP reductase (InhA) showed binding energy (Kcal/mol) -6.31, -6.12, -5.95 & -7.06 respectively. The result was tabulated in Table 1 and binding mode of flavonoids displayed in fig.4-7. The molecular interaction of selected compound shown in fig.12-15(2D) &16-19 (3D). As per outcome of the investigation binding energy of hispidulin shown good interaction with enoyl ACP reductase having conventional hydrogen bonding at MET A:98, PRO A:98, PHE A:97, GLY A:96, MET A:161 THR A:196, ILE A:21, PRO A:193, ALA A:191, THR A:158 &PHE A:149 along with Pi- sigma bonding with MET A:103, ALA A:198 & MET A199.The outcome of investigation showed that all selected flavonoids is more or less potent enoyl ACP reductase inhibitor and effectively with selected ligand but hispidulin exhibited potent inhibitor of enoyl ACP reductase.

The pharmacokinetic profile of the selected flavonoid ligand was shown to have a good pharmacokinetic profile, not associated with significant toxic effects such as reproductive effects, irritant effects, and tumorigenic properties, but with some mutagenicity. The results of pharmacokinetic and toxicity profile analysis of flavonoids are shown in Figures 8-11.

Table 1: Results of docking of ligands like baicalein, pectolinarin, myricetin, and hispidulin against tubercular Enoyl ACP reductase enzyme.

S. No	CompoundName	Structure	BindingEnergy (Kcal/mole)

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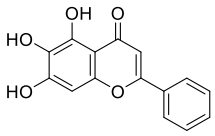
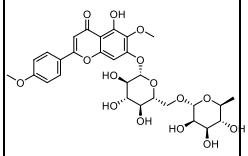
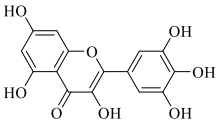
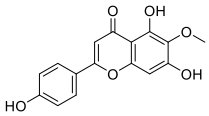
1	Baicalein		-6.31
2	Pectolinarin		-6.12
3	Myricetin		-5.95
4	Hispidulin		-7.06

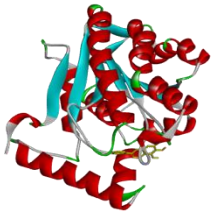


Figure 4: Binding mode of baicalein within the active site of tubercular Enoyl ACP reductase enzyme.

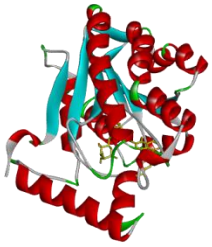


Figure 5: Binding mode of pectolinarin within the active site of tubercular Enoyl ACP reductase enzyme.

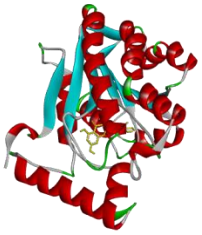


Figure 6: Binding mode of myricetin within the active site of tubercular Enoyl ACP reductase enzyme.

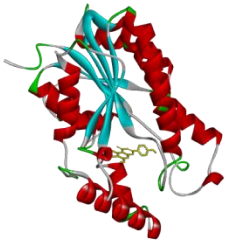


Figure 7: Binding mode of hispidulin within the active site of tubercular Enoyl ACP reductase enzyme.

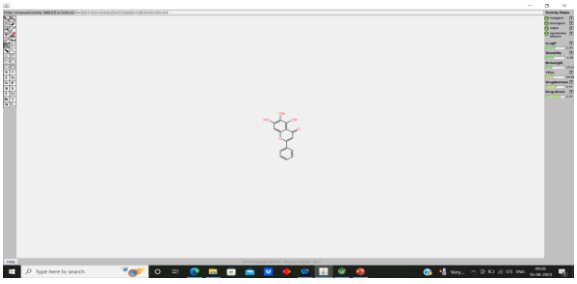


Figure 8: Pharmacokinetic and toxicity profiling of baicalein.

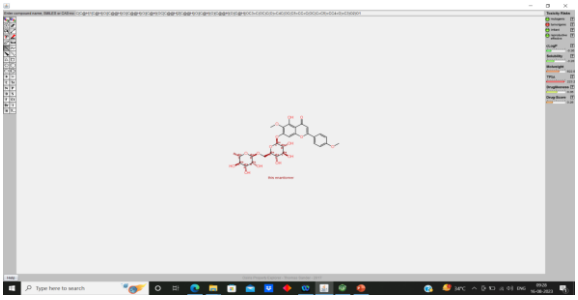


Figure 9: Pharmacokinetic and toxicity profiling of pectolinarin.

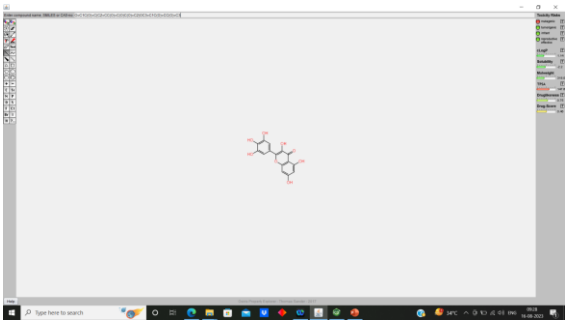


Figure 10: Pharmacokinetic and toxicity profiling of myricetin.

Figure 11: Pharmacokinetic and toxicity profiling of hispidulin.

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Interaction

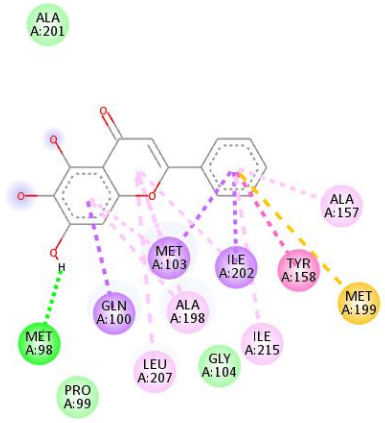

Figure 12: Two-dimensional binding mode of baicalein within the active site of tubercular Enoyl ACP reductase enzyme.

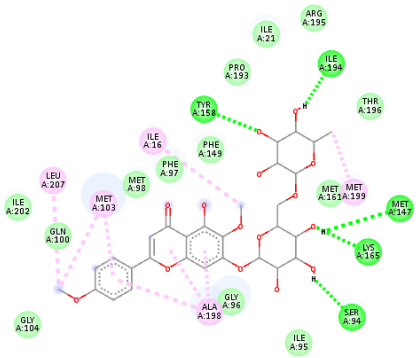

Figure 13: Two-dimensional binding mode of pectolinarin within the active site of tubercular Enoyl ACP reductase enzyme.

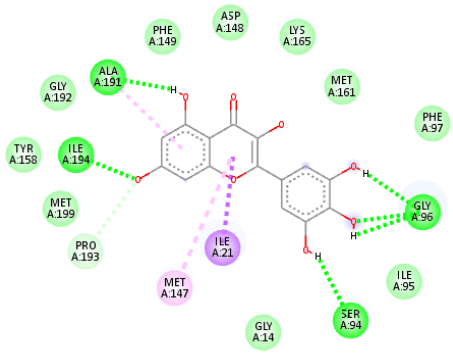

Figure 14: Two-dimensional binding mode of myricetin within the active site of tubercular Enoyl ACP reductase enzyme.

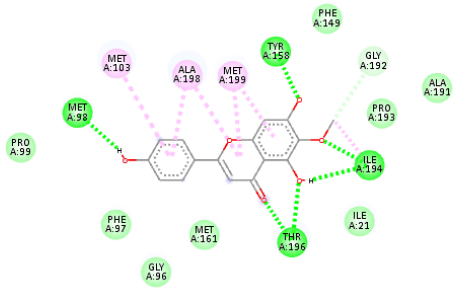

Figure 15: Two-dimensional binding mode of hispidulin within the active site of tubercular Enoyl ACP reductase enzyme.

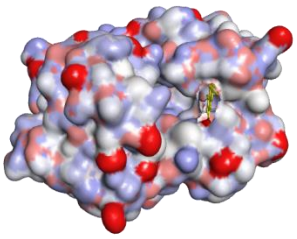

Figure 16: Three-dimensional binding conformation of baicalein within the active site of tubercular Enoyl ACP reductase enzyme.

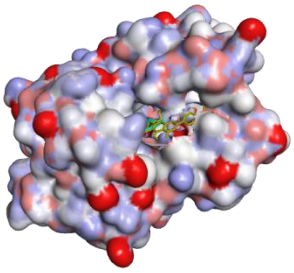

Figure 17: Three-dimensional binding conformation of pectolinarin within the active site of tubercular Enoyl ACP reductase enzyme.

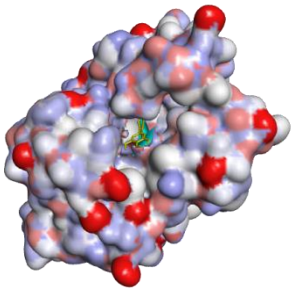

Figure 18: Three-dimensional binding conformation of myricetin within the active site of tubercular Enoyl ACP reductase enzyme.

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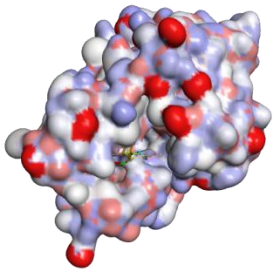

Figure 19: Three-dimensional binding conformation of hispidulin within the active site of tubercular Enoyl ACP reductase enzyme.

Conclusion

The final results of the existing research found out that the chosen molecule highlybounded with InhA thereby inhibiting the mycobacterial cell wall synthesis. The molecular docking of ligands like baicalein, pectolinarin, myricetin, and hispidulin with tubercular Enoyl ACP reductase enzyme exhibited the chemical interaction with the amino acids in the active pockets. Theoretically, all the ligand molecules have shown encouraging docking score. The binding pattern of the flavonoids as per binding energy are shown as ***hispidulin*** > ***baicalein*** >***pectolinarin***> ***myricetin.*** The docking result of hispidulin revealed that their docking scores was 7.06 kcal mol^{-1}, and it can be predicted as good inhibitor of tubercular Enoyl ACP reductase enzyme.

Divulgence Investigation

In the current scenario of anti-tubercular drug therapy there are frequent dosing of drug as per body of patient which leads several side effects along with development of drug resistant, so researchers have been interested in identifying flavonoids and their derivates with anti-mycobacterial properties. The outcome of the present investigation showed that the selected flavonoids has been potent inhibitor of *M.tuberculosis* bacteria via inhibition of InhA disrupts the biosynthesis of the mycolic acids which might be critical components of the mycobacterial cell wall. The selected flavonoids may be used as nutritional supplement for tubercular patient which helps to increases the curing frequency and prevent the drug resistant.

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