Computational Screening of Drug-like Properties of *Tinospora Cordifolia* Against Tuberculosis

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**ABSTRACT**

**Background:** Drug-Resistant Tuberculosis (TB) resurgence over the globe broadens the research towards its causative agent *Mycobacterium tuberculosis*. The diarylquinoline derivative drug Bedaquiline which inhibits ubiquitous enzyme ATP synthase was introduced in 2012. ATP synthase is an essential enzyme that helps in supplying energy to *Mycobacterium tuberculosis* even in the unfavourable conditions which helps them to survive in macrophages of human beings. Since 2017, resistance to bedaquiline has been reported worldwide which emphasises us for the discovery of the new drugs. The medicinal plant *Tinospora cordifolia* is used in various traditional healing systems worldwide to treat diabetes, leprosy, etc.

**Methods:** The current study summarizes the pharmacology of the phytocompounds of *Tinospora cordifolia*. In silico study was performed by using tools like CASTp, PubChem, ChemSpider, Swiss ADME, Pro Tox server, HEX dock, PyMol, and Lig plot+. ADMET study of the phytocompounds was also studied.

**Results:** The results obtained in the study indicated that the 2-Hydroxy-3-oxoicosanoate was the potent inhibitor which shows the highest affection towards ATP synthase of *Mycobacterium tuberculosis* and also accomplishes the entire criterion to be an effective drug.

**Conclusions:** Many of the phytocompounds of *Tinospora cordifolia* shows good ADMET analysis and docking result. Among all the phytocompounds 2-Hydroxy-3-oxoicosanoate shows the best result in terms of docking. This is only an in-silico study to fasten the drug discovery process, further validation of the proposed drug requires in-vitro and in-vivo study.

**Key Words:** ADMET, Docking Study, *Mycobacterium tuberculosis*, *Tinospora cordifolia*, Tuberculosis, 2-Hydroxy-3-oxoicosanoate

**INTRODUCTION**

*Mycobacterium tuberculosis* (Mt) bacteria are always being a challenge due to its resistance property towards the first-line drugs i.e. isonicotinic acid hydrazide (INH), Rimactane, ethambutol, and pyrazinamide, the condition is known as Multi-Drug Resistant (MDR) TB. In the worst case, it can lead to Extensive Drug Resistance (XDR) TB i.e. resistance to any fluoroquinolone drugs.¹ In the year 1993, the worldwide health crisis has been affirmed by the WHO (World Health Organisation) when 1.3-1.6 million deaths were estimated each year from tuberculosis.¹ Recently in 2014 WHO and United Nations (UN) launched the “End TB Strategy” at the World Health Assembly by adopting UN Sustainable Development Goals in 2015.² The present-day scenario of TB states that the drugs available in the market are inefficient against TB as there exist around 2.3 million cases of TB around the globe.³ After the rigorous clinical trials for new drugs against drug resistance TB, Bedaquiline (Sirturo) came in the market with a new hope. It is a diarylquinoline offshoot which inhibits the ε subunit of mycobacterium adenosine triphosphate (ATP) synthase.⁴ ATP synthase is a ubiquitous enzyme in which theatre crucial functions in chemiosmotic energy change. Mt is astute enough that it escapes the primary defence mechanism of the host, and deals with hostile conditions within the macrophages. ATP synthase is responsible for optimal development and metabolism in Mt. ATP synthase enzyme utilizes the power store in a transmembrane for the making of ATP. ATP synthase has two parts hydrophilic F1 complex which has 5 subunits.
α3 β3 γ δ ε and membrane entrenched F0 complex which has 3 subunits a1 b2 c10-15. The World Health Organization (WHO) said that the inappropriate utilize of bedaquiline can lead to the resistance and according to the current status the mutation in the AtpE gene of Mtb has been noticed in many cases which results in bedaquiline resistance and research is aimed to ascertain a new suitable inhibitor of ATP synthase. Medicinal plants and marine species have been in use for epochs to cure tuberculosis. Tinospora cordifolia is a deciduous climbing shrub known as “Guduchi” belonging to the family Menispermaceae which is found at higher altitudes. It has anti-diabetic antiperiodic, antispasmodic, and anti-inflammatory properties. The plant generally contains alkaloids, glycosides, steroids, and aliphatic compounds. According to the survey, there is a rapid increase in TB cases which suggests the development of an effective treatment. ATP synthase can serve as a probable target for drug and Tinospora cordifolia has been reported to be an effective medicinal plant. The amalgamation of these two factors aggravated us towards the in-silico study of phytocompounds of Tinospora cordifolia in computer-aided drug designing, which is expected to assist in craft a new antituberular drug. In the current study, the phytocompounds of Tinospora cordifolia was used as a ligand molecule against TB. The ADMET properties of phytocompounds were predicted for assessing efficiency, inhibition capacity, absorption, distribution, metabolism, and toxicity response of drugs.

MATERIAL AND METHODS

Target and Cavity Prediction
3D structure file of Mtb F-ATP synthase subunit epsilon (ATPε) (PDB file: 5YIO) was retrieved from Protein Database (PDB) (https://www.rcsb.org). 5YIO is an NMR determined structure. CASTp (Computed Atlas of Surface Topography of proteins) is an online source that makes available the detailed quantitative analysis of voids and pockets of proteins present on the surface which are often allied with protein binding sides. It is used for ascertaining surface features and functional regions of proteins. Access to CASTp is four-letter PDB file (http://sts.bioe.uic.edu).

Ligand Preparation
The phytocompounds of Tinospora cordifolia were retrieved from the chemical structure databases: PubChem and ChemSpider. PubChem database gives information about chemical substances and their biological activities. It has three interlinked databases i.e. compound, substance, and biosays (https://pubchem.ncbi.nlm.nih.gov/). ChemSpider is a chemical database offering access to properties of a compound like its physiochemical properties, 2D, and 3D structure (http://www.chemspider.com). The configuration of the compounds was taken from both the data bank and transformed to .pdb format by Open Babel tool version 2.4.1. It is an online toolbox that can verbalize many languages of the chemical data and can convert it in a user suitable form.

ADMET prediction
ADMET prediction is indispensable to know whether the ligand can be taken via the oral route by human beings. In-silico ADMET predictions of the ligands were done by using the SwissADME predictor and ProTox-II. Swiss ADME is a web tool that assesses a compound on the analysis of its absorption, distribution, metabolism, and excretion (ADME). It is free to access to a predictive model of a compound to be a potent drug(http://www.swissadme.ch). ProTox-II is an online net server for the in-silico toxicity calculation of compounds (http://tox.charite.de). The ADMET analyses of the compounds were done and the best compounds which passed ADMET analyses were taken further for molecular docking.

Docking
Mycobacterium tuberculosis F-ATP synthase subunit epsilon (ATPε) (PDB file: 5YIO.A) was docked with different ligands using Hex 8.0.0. Hex is a docking software that works in the FFT algorithm (Fast Fourier transform) and docking energy was tabulated using the default parameters. Docking visualization was done with the help of PyMol and LigPlot+. PyMol is a molecular visualization tool that works in horizontal and vertical scripts. It shows the 3D visualization of a compound. LigPlot+ is also visualization software that shows the intermolecular attraction, hydrogen bonding, and hydrophobic interactions.

RESULT AND DISCUSSION

Cavity prediction and Ligand preparation
Structural pockets and cavities determine the binding sites and active sites of the protein. CASTp server is an online service that works by using the alpha complex, Delaunay triangulation, and default probe sphere 1.4 Å. PDB file of Mtb F-ATP synthase subunit epsilon (ATPε), 5YIO_A was uploaded to the CASTp server which isolates and measures the surface pockets and cavities. The results of CASTp calculation show the presence of one large pocket. The volume and Area of solvent accessible surface (SA) of pocket and the corresponding amino acid was shown in Table 1. In the current study chemical structure databases, ChemSpider and PubChem were used to retrieve the 2D and 3D structure of all the phytocompounds present in Tinospora cordifolia. The structures were downloaded and transformed into .pdb layout by Open Babel tool version 2.4.1 (figure 1).
Table 1: Volume and Area of solvent accessible surface (SA) of pocket and corresponding amino acid

| Poc Id | PDB | Chain | Area (SA) | Volume (SA) | Amino acids          |
|--------|-----|-------|-----------|-------------|----------------------|
| 1      | 5YIO| A     | 107.696   | 49.224      | 24 PHE, 25 THR, 26 ARG, 31 GLU, 32 ILE, 45 LEU, 46 VAL, 48 ASP, 49 ALA, 50 MET, 51 VAL, 65 VAL, 112 ALA, 115 ARG, 116 ARG |

ADMET analysis and Docking

In advance of launching a drug to the market it faces several clinical trials. ADMET analysis is an indispensable phase of pharmacokinetics that provides substantial information about the drug’s ability of a compound. Swiss ADME and ProTox-II were used for ADMET analysis of the phytocompounds. Phytocompounds meeting all the values of the ADMET analysis are shown in Tables 2 and 3. Swiss ADME analyzes the physicochemical (PC) properties of the phytocompounds.

Table 2: ADMET analysis of the phytocompounds

| CID    | Compound                        | ESOL | Ali Class | SIT Class | BBB | Pgp S | CI     | Tox               |
|--------|---------------------------------|------|-----------|-----------|-----|-------|--------|-------------------|
| 192176 | Cholic acid                     | SOL  | SOL       | SOL       | NO  | YES   | NO     | Predicted LD50: 2000mg/kg |
| 85597  | Diffractaic acid                | M SOL| P SOL     | M SOL     | NO  | NO    | NO     | Predicted LD50: 300mg/kg |
| 431604 | 3,3-Bis(3,4-dimethoxyphenyl)propanoate | SOL | M SOL     | M SOL     | NO  | NO    | NO     | Predicted LD50: 1300mg/kg |
| 5320699| 3-(1-Naphthoyl)benzoate         | M SOL| M SOL     | M SOL     | YES | NO    | NO     | Predicted LD50: 200mg/kg |
| 13902409| 2-Hydroxy-3-oxoicosanoate       | M SOL| P SOL     | M SOL     | NO  | YES   | NO     | Predicted LD50: 3400mg/kg |
| 5461627| (4-Cinnamoyl-3,5-dihydroxyphenoxy)acetate | SOL | M SOL     | M SOL     | NO  | NO    | NO     | Predicted LD50: 3800mg/kg |

*CID: PubChem Compound ID, Sol: Soluble, M Sol: Moderately Sol, P Sol: Poorly Soluble, SIT Class: Silicos-IT class, BBB: Blood Brain Barrier permeant, Pgp S: Pgp substrate, CI: CYP3A4 inhibitor, Tox: Toxicity

Table 3: Computed properties of screened compounds based on Lipinski’s Rule of Five

| Phytocompound Name                        | Formula     | MW            | #H-bond acceptors | #H-bond donors | Lipinski #violations | MLOGP |
|-------------------------------------------|-------------|---------------|-------------------|----------------|----------------------|-------|
| Cholic acid                               | C24H40O5    | 408.57 g/mol  | 5                 | 4              | Yes; 0 violation     | 3.05  |
| Diffractaic acid                          | C20H22O7    | 374.38 g/mol  | 7                 | 2              | Yes; 0 violation     | 2.76  |
| 3,3-Bis(3,4-dimethoxyphenyl)propanoate    | C19H21O6    | 345.37 g/mol  | 6                 | 0              | Yes; 0 violation     | 2.06  |
| 3-(1-Naphthoyl)benzoate                   | C18H11O3    | 275.28 g/mol  | 3                 | 0              | Yes; 0 violation     | 3.26  |
| 2-Hydroxy-3-oxoicosanoate                 | C20H37O4    | 341.51 g/mol  | 4                 | 1              | Yes; 0 violation     | 3.30  |
| (4-Cinnamoyl-3,5-dihydroxyphenoxy)acetate | C17H13O6    | 313.28 g/mol  | 6                 | 2              | Yes; 0 violation     | 1.17  |

Several compounds failed in ADMET analysis. For drug discovery, many guiding principles and rules were made to analyze the physicochemical properties. The first and the most important rule was the rule of 5 which was discovered by Lipinski in 1997. The rule of five states that all the compounds are supposed to follow these five criteria so that they are membrane penetrable and have passive diffusion in the human intestine. The five criteria were log P < 5; Molecular weight (MW) ≤ 500; hydrogen bond acceptor (HBA) ≤ 10; hydrogen bond donor (HBD) ≤ 5 and no. of rotatable bond ≥ 10. Few phytocompounds have a molecular weight of more than 500, violation in MW can impact the changes in molecular events such as blood brain barrier penetration, absorption and metabolism, and hard to get penetrated in the human body. Some phytocompounds have HBA and HBD higher violating Lipinski rule, both the considerations enhance the bioavailability and permeability of the drug. An increase in HBD can lead to poor penetration and bioavailability. It was also found that some phytocompounds have a high value of log p and some have a low value of log p. Lipophilicity log P should be less than or equal to 5. If there is a high value of log p solubility and metabolism get affected whereas the low value of log p results in decreased permeability. Highly hydrophilic compounds cannot enter the membrane because they are not capable of penetrating the hydrophobic part of the bilayer membrane whereas highly lipophilic get trapped.
Cholic acid - The phytocompounds which plays the important role in DDI and also regulate the drug metabolism and the server ProTox-II was also used, which provides the toxicity of the phytocompounds LD50 in mg/kg. This server classifies the toxicity of a compound in 6 different classes. All the compounds qualified the ADMET analysis mainly falls under class 5 and 6. In class 5 LD50 is greater than in 2000 but less than 5000 which means it may be harmful if used and in class 6 LD50 is greater than 5000 which means it is nontoxic. The phytocompounds which pass all the ADMET analysis further docked with the target. In the ADMET analysis the phytocompounds Cholic acid, Diffractaic acid, 3,3-Bis(3,4-dimethoxyphenyl)propanoate, 3-(1-Naphthoyl)benzoate, 2-Hydroxy-3-oxoicosanoate, (4-Cinnamoyl-3,5-dihydroxyphenoxy)acetate, re-found to qualify the ADMET analysis and further docked with the target. 8 phytocompounds of Tinospora cordifolia docked with 5YIO_A. Docking analysis was executed by using HEX software. HEX works in FFT algorithm Fast Fourier Transform (FFT) and the docking energy of the 2-Hydroxy-3-oxoicosanoate with ATP synthase displayed the best docking as it shows the least binding energy i.e. -320.27 when compared with the bedaquiline i.e. -288.75. The docking energy of various compounds is tabulated in table 4.

### CONCLUSION

Mtbc bacteria are always being a challenge due to its Multi-Drug Resistant(MDR) and Extensive Drug Resistance(XDR) property. Bedaquiline drug is used against Mtbc but recently many cases are reported globally showing resistance towards bedaquiline, so the urge of new drug discovery came into projection. In the current study, all the phytocompounds of Tinospora cordifolia is been identified and analyzed to be used as a drug against a target 5YIO protein present in Mtbc. Many of the phytocompounds show good ADMET analysis and docking results. Among all the phytocompounds 2-Hydroxy-3-oxoicosanoate shows the best result in terms of docking. This study can be validated further in the wet lab and can be a potential drug for the treatment of Tuberculosis.

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### Conflicts of Interest

All authors have none to declare.

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Figure 1: 2D structure of the phytocompounds retrieved from PubChem and ChemSpider.

Figure 2: Visualization of docking 5YIO_A with the ligand 2-Hydroxy-3-oxoicosanoic acid.