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Unveiling Naturally Occurring Green Tea Polyphenol Epigallocatechin-3-Gallate (EGCG) Targeting *mycobacterium* DPRE1 for Anti-Tb Drug Discovery †

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Abstract: Increasing rates of multi-drug resistant (MDR) and extremely-drug resistant (XDR) cases of tuberculosis (TB) strains are alarming, which eventually hampered an effective control of the pathogenic disease. Epigallocatechin gallate (**EGCG**) is a major polyphenolic constituent of green tea, earlier demonstrated in-vitro potency against TB strains. However, efforts to elucidate the exact mechanism of interactions are still ongoing. Aiming to elucidate the probable mechanism of its anti-TB action as **Decaprenylphosphoryl-beta-D-ribose 2′-epimerase (DprE)** inhibition, we investigated molecular modeling analysis. Our Molecular docking analysis for a set of **65** Tea bioactive compounds was realized that EGCG has the highest binding affinity (docking score: **−142.98 Kcal/mol**) against DPRE (pdb id: 4p8c) from *Mycobacterium tuberculosis*. Further, molecular dynamics analysis for **100 ns** resulted in extreme stability of the ligand-protein complex. We further accessed **in-silico pharmacokinetics and toxicitie s** for top 3 green tea polyphenols based on docking scores. Our results provide critical insights into the mechanism of action of EGCG and other green tea polyphenols as a potential therapeutic agent (DprE1) against TB.

Keywords: tuberculosis; *Mycobacterium*; EGCG; green tea polyphenols; DprE1

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1. Introduction

Tuberculosis (TB) is considered as a public health crisis, which is hampering healthcare systems especially in low-economic countries [1–5]. As per the latest WHO statistics, 1.4 million people died from TB in 2019 (10 million people fell ill) [2]. Furthermore, considering increasing numbers of multidrug-resistant TB (MDR-TB) or multidrug- or rifampicin-resistant TB (MDR/RR-TB) cases, there is an urgent need to develop newer anti-TB agents with unique mechanisms of actions. Mycobacterial cell wall is made up of a mycolic acid, which are long fatty acids. The synthesis of mycolic acid is regulated via enzymes of the fatty acid synthase (FAS) complex. Enoyl reductase, pantothenate synthetase, and Decaprenylphosphoryl-β-d-ribose 2′-epimerase (DprE1) are key attractive targets for the discovery of newer anti-TB agents. From our literature analysis, it is clear that polyphenols (Figure 1) from the leaves of Green tea (GTPs) and Black tea (*Camellia sinensis*) have significant pharmacological potentials against varieties of biological targets [3]. Recently, Anand et al., 2006 showed the potential of GTP, epigallocatechin-3-gallate inhibiting *Mycobacterium tuberculosis* survival within human macrophages [5]. Their study suggested that epigallocatechin-3-gallate has ability to down-regulate tryptophan-aspartate containing coat protein gene transcription [5]. Sun et al., 2015 proposed probable mechanism of the effects of epigallocatechin gallate (EGCG) on the growth of *Mycobacterial smegmatis* mc(2)155 [4]. Moreover, their analysis revealed that EGCG had impact on

the integrity of *mycobacterial* cell wall. EGCG has not been studied in details for their probable targets for the inhibition of *Mycobacterium tuberculosis*, although few reports have mentioned some antimycobacterial activity based on enzyme inhibition studies in combination with compounds, such as triclosan. Considering these facts, we particularly aimed to explore the probable anti-TB drug target for Epigallocatechin gallate (EGCG), a major bioactive compound from green tea extracts [6]. Furthermore, we have also calculated docking affinity scores for other GTPs. Top 3 GTPs were subjected for in-silico absorption, distribution, metabolism, excretion (ADME) and toxicity (T) analysis. Finally, the best docked epigallocatechin gallate (EGCG): 4P8C complex was simulated and analyzed for 100 ns molecular dynamics analysis.

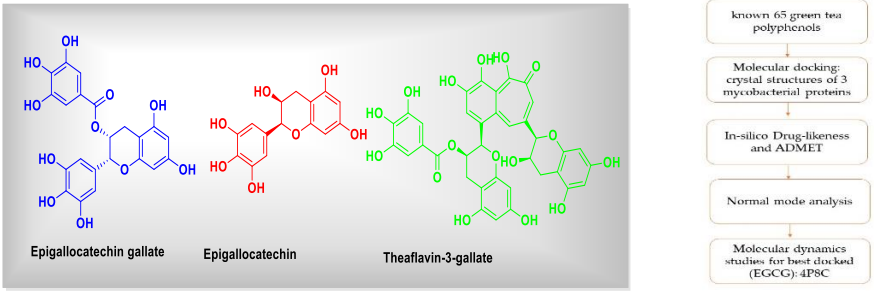

Figure 1. (**a**) Chemical structures of Green tea polyphenols (representatives) and (**b**) The graphical summary of workflow followed.

Lastly, we have signified a probable lead that could be developed as drug candidate against *mycobacterial* targets.

2. Materials and Methods

2.1. Molecular Docking Simulations

For the current study, we have taken a set of known 65 green tea polyphenols or compounds [7]. Overall, molecular docking comprises 5 main steps-1] protein preparation, 2] ligand preparation, 3] receptor grid generation, 4] ligand docking procedure and then 5] viewing the docking results. All necessary structures have been drawn using 'ChemSketch'. All the 3D crystal structures of 3 mycobacterial proteins (the enoyl reductase receptor protein (PDB IDs: 2X22, Decaprenylphosphoryl-β-d-ribose 2′-epimerase (DprE1), 4p8c and the pantothenate synthetase, 3IVX) were downloaded from the protein database bank (PDB database, www.rcsb.org, accessed on). The data set including the inbound (co-crystallized ligand) was docked into the binding pocket of pantothenate synthetase and DprE1 (PDB-ID: 3IVX, 4p8c) target enzymes. For the reliability of the docking protocol, we evaluated it through the RMSD value. The grid was centered around active binding site residues Gly158, Met195, Pro38, etc. (for case of 3ivx). We have performed our docking analysis using 'iGemDock' as per standard procedures by making 15 Å radius from binding pocket, followed by visualization using Discovery Studio 2020 Visualizer [8,9].

2.2. In-Silico Drug-Likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) Analysis

Top 3 higher affinity GTPs were subjected for an in-silico ADME analysis using SWISS tools (http://www.swissadme.ch, accessed on). For the toxicity assessments, we used 'admetSAR' (http://lmmd.ecust.edu.cn:8000/, accessed on).

2.3. Normal Mode Analysis

To gain more insights on the conformational flexibilities [8] of proteins with their best docked hits, we have performed the Normal Mode Analysis (NMA) with internal coordinates (IC) using a fast and easy server, iMODS (http://imods.chaconlab.org/, accessed on).

2.4. Molecular Dynamics Studies

The docking studies don't involve the flexible nature of the protein. For confirming the exact binding mode and stability we have to study MD simulations with the Desmond program. The stability of epigallocatechin gallate (EGCG): 4P8C complex was evaluated through 100 ns molecular dynamics simulations. The simulations provided exact binding interaction of the docking complex with system embedded with water molecules, temperature and pressure. The standard NTP conditions were followed for MD simulation setup. The complex was originated in all proper binding poses with an acceptable RMSD value (<3 Å). Molecular dynamics (MD) simulation for a period of 100 ns was carried out for best docked hit with epigallocatechin gallate (EGCG): 4P8C complex and it was achieved with the GROMACS simulation package, 2020 as per known literature protocols [9].

3. Results and Discussion

3.1. Molecular Docking Simulations

Our molecular docking analysis of 65 GTPs on 3 anti-TB targets suggested that epigallocatechin gallate (EGCG) had highest affinity towards DprE1 target rather than 2X22 and 3IVX. The docking interactions depicted that this compound had interactions with GLN A:328; TYR A:60; GLY A:334; LYS A:418; VAL A:365; LEU A:317 amino acids with 7 conventional hydrogen bonds at receptor site of target 4P8C (Figure 2) [9,10]. VAL A:365 formed Pi-sigma interaction with the aromatic portion. Alkyl and Pi-alkyl interactions were also observed for LEU A:363 and LEU A:317 amino acids. From Figure 2, it was also revealed that the binding mode of EGCG has favorable H-bond donors (purple coloured) and acceptable (green colored) regions in binding cavity. Tables 1 and 2 would give better insights on interaction profiles of studied green tea/black tea molecules. From our docking analysis of 65 tea bioactive on 4p8c, we found top 3 best docked hits as Theaflavin-3-gallate (docking score: −124.06 kcal/mol), Epigallocatechin Gallate (docking score: -142.98 kcal/mol), and Epigallocatechin (docking score: −127.73 kcal/mol). Docking affinities for these 3 bioactive were found to be greater than standard drug, Ciprofloxacin* (docking score: −109.20 kcal/mol). Standard Isoniazid was interacted with LYS A:367; VAL A:365; CYS A:387; ASN A:385 amino acid residues (docking score: −121.21 kcal/mol). The binding cavity residues for inbound were found to be VAL A: 365; LYS A: 418; ASN A: 385; LYS A: 418 (SALT BRIDGE). Moreover, our re-docking validation protocol also resulted RMSD value below 2 Å, suggesting valid docking results.

3.2. Molecular Dynamics Simulation and Normal Mode Analysis

The best docked; Epigallocatechin Gallate (EGCG) with target 4P8C was simulated for 100 ns molecular dynamics and normal mode analysis. MD simulations showed that Root Mean Square Fluctuation (RMSF) values were obtained within tolerable ranges (0.4 nm). The Root mean square deviation (RMSD) value was obtained below 0.25 nm, suggesting stability of complex (Figure 3). From our NMA results, we observed that Epigallocatechin Gallate with protein 4P8C complex was retained with good deformability, and eigenvalue value profiles (Figure 3). The radius of gyration value was also retained below 2.15 nm. The solvent accessible surfaces areas were found to be around180 nm^2.

3.3. In-Silico ADMET Studies

Cytochrome P450 (CYPs) enzymes are key enzymes responsible for various metabolism. Our in-silico calculated ADMET (absorption, distribution, metabolism, excretion, toxicity) in our body properties for the top best docked 3 hits are represented in Table 3. Three GTPs, Theaflavin-3-gallate, Epigallocatechin and Epigallocatechin Gallate (EGCG)

exhibited positive human intestinal absorption profiles, negative the Blood Brain Barrier passage, non-carcinogenic, non-AMES toxic, and class IV acute oral toxicity profiles.

Table 1. Docking interaction energies* of selected **65** bio-active molecules and **3** FDA approved drugs for target protein 4P8C.

Molecules	-iGemDock Interaction Energy	Molecules	-iGemDock Interaction Energy
Oolonghomobisflavan A	−62.2219	Theaflavic Acid	−82.4934
Theasinensin D	−70.1619	Barrigenol R1	−86.4843
Theaflavin-3-gallate	**−124.06**	Barringtogenol	−52.0693
Isotheaflavin	−62.621	Camelliagenin	−95.1799
Epigallocatechin-3,5-Di-O-Gallate	−71.0176	Gallocatechin	−85.7374
Oolonghomobisflavan B	−75.4779	Catechin	−101.992
Cis-3-Hexenol	−63.5566	Epicatechin	−98.6033
Epigallocatechin-3,4-Di-O-Gallate	−92.6784	Epiafzelechin	−91.5357
Vicenin 2	−96.9806	Quercetin	−102.834
Epicatechin-3,5-Di-O-Gallate	−101.495	Cryptoxanthin	−92.1799
Rutin	−87.1416	Myricetin	−82.5936
Proanthocyanidin	−84.8129	Apigenin	−63.6163
Pheophytin	−90.2865	Nerolidol	−82.584
Benzaldehyde	−91.9877	Kaempferol	−89.1838
Epitheaflavic Acid 3′-Gallate	−65.361	Theanine	−73.9851
Epigallocatechin Gallate	**−142.98**	Ascorbic Acid	−80.1271
Theasinensin E	−62.6409	Quinic Acid	−75.3299
Myricitrin	−61.915	Succinic Acid	−85.5696
Theaflavin	−55.9704	Methyl Salicylate	−81.1848
Epicatechin Gallate	−72.5287	Theobromine	−72.7269
Kaempferitrin	−71.7401	Caffeine	−84.4502
Isoquercetin	−73.9058	Xanthine	−75.7595
Epiafzelechin 3-O-Gallate	−73.4119	Linalool Oxide	−83.9907
Pheophorbide	−71.1657	Phenylacetaldehyde	−87.8044
Epigallocatechin 3-O-P-Coumarate	−76.8643	Methylxanthine	−79.6185
Pheophorbide	−67.9266	Theophylline	−88.1319
Oxalic Acid	−82.9277	Geraniol	−95.2378
Cryptoxanthin	−81.2634	Hexanal	−95.8974
Isovitexin	−72.924	Diphenylamine	−93.4455
Vitexin	−55.6638	Trans−2−Hexenal	−94.076
Chlorogenic Acid	−49.7604	Linalool	−86.4307
Coumaroyl Quinic Acid	−94.7189	Phenylethanol	−101.468
Epigallocatechin	**−127.73**	**Ciprofloxacin ***	**−109.20**

* Docking scores have been provided only for the higher affinity scored target protein.

Table 2. Energy contribution of the key residues computed by docking methodology.

Sr. No.	Molecules	Residues with Contribution Energy
1.	Isoniazide	LYS A:367; VAL A:365; CYS A:387; ASN A:385

2.	Pyrazinamide	VAL A: 365; LYS A: 418; ASN A: 385
3.	Ciprofloxacin	GLN A:117; VAL A:365; CYS A:387; LYS A:367; HIS A:132
4	Theaflavin-3-gallate (Best docked)	TRP A:16; LEU A:363; HIS A:315; THR A:118; LEU A:317
5	Epigallocatechin	GLN A:334; TYR A:60; CYS A:387; LYS A:418
6	**Epigallocatechin Gallate (EGCG)**	**GLN A:328; TYR A:60; GLY A:334; LYS A:418; VAL A:365; LEU A:317**
7	Inbound ligand (y22)	VAL A: 365; LYS A: 418; ASN A: 385; LYS A: 418 (SALT BRIDGE)

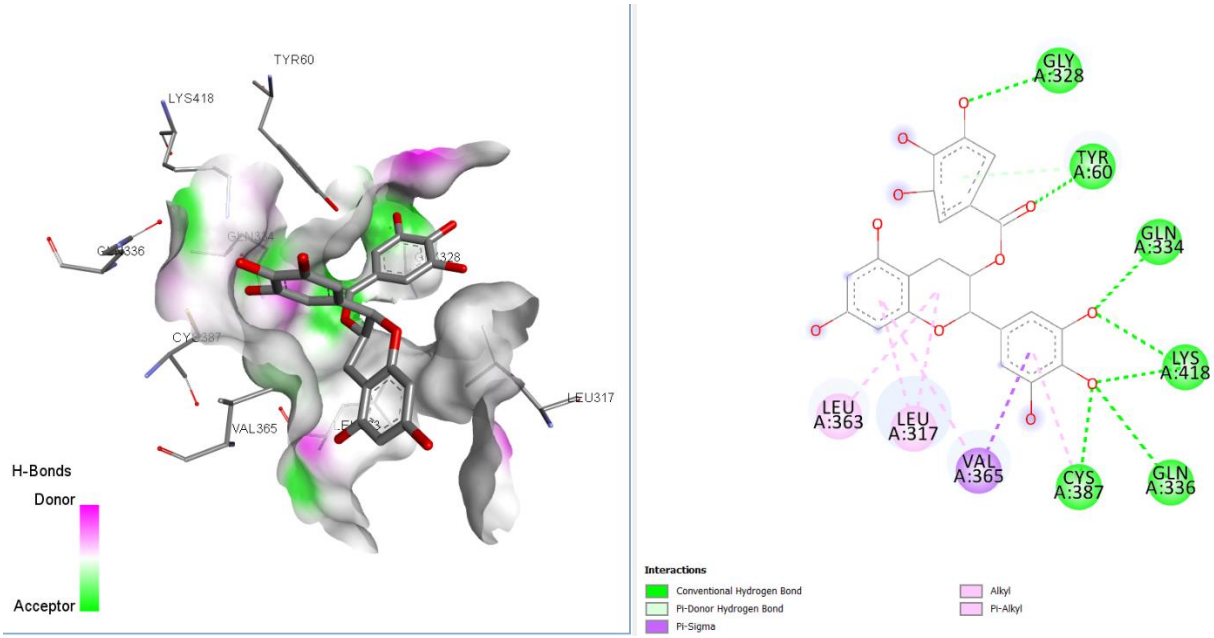

Figure 2. 2D and 3D-interaction profiles for best docked *Epigallocatechin Gallate* with **4p8c**.

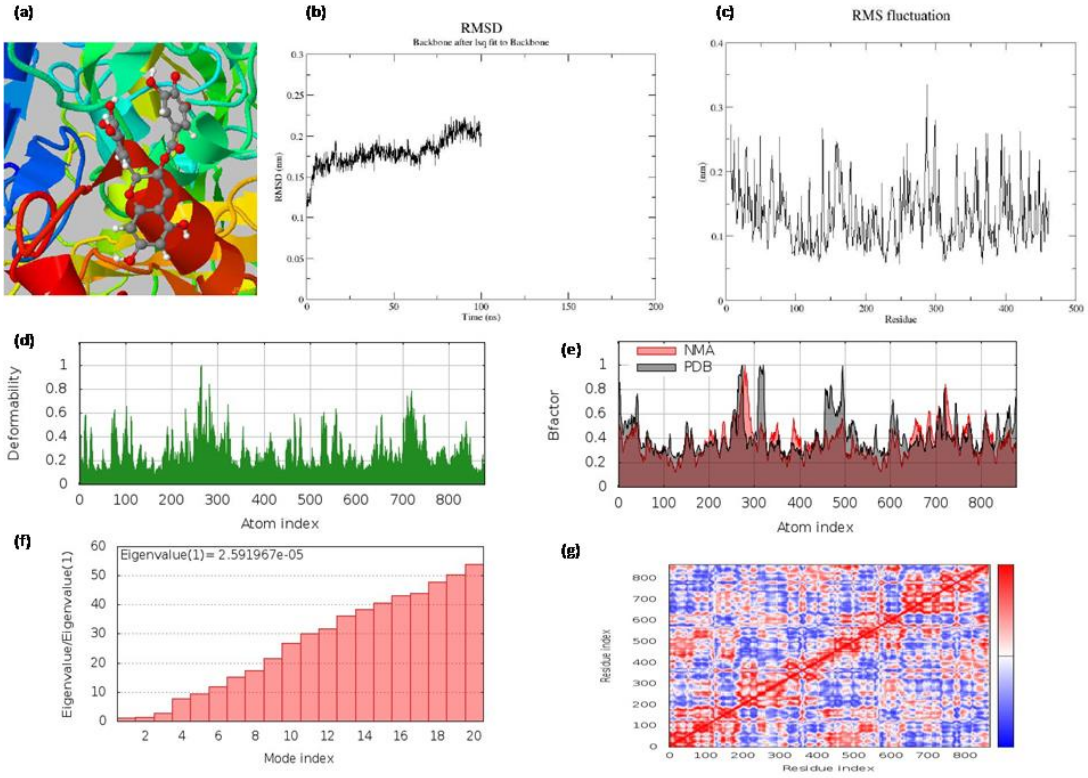

Figure 3. (**a**) The binding pocket; (**b**) The Root Mean Square Deviations (RMSD) of backbone atoms relative to the starting complexes during **100** ns MD; (**c**) Protein RMSF plot (On this plot, peaks indicate areas of the protein that fluctuate the most during the simulation and Protein residues that interact with the ligand are marked with green-coloured vertical bars.); (**d**) Deformability; and (**e**) B-factor profiles; (**f**) Eigenvalue and (**g**) Covariance matrix of the complex for *Epigallocatechin Gallate* with **4p8c,** respectively.

Table 3. In-silico ADMET profiling for top **3** best docked hits against target **4P8C**.

Properties	Theaflavin-3-Gallate	Epigallocatechin	Epigallocatechin Gallate (EGCG) *
CYP450 2C9 Substrate	Non-substrate	Non-substrate	**Non-substrate**
CYP450 2D6 Substrate	Non-substrate	Non-substrate	**Non-substrate**
CYP450 3A4 Substrate	Non-substrate	Non-substrate	**Non-substrate**
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	Weak inhibitor	**Weak inhibitor**
AMES Toxicity	Non-AMES toxic	Non-AMES toxic	**Non-AMES toxic**
Carcinogens	None	None	**None**
Acute Oral Toxicity	IV	IV	**IV**
P-glycoprotein Inhibitor	Non-inhibitor	Non-inhibitor	**Non-inhibitor**
Rat Acute Toxicity (LD_{50}, mol/kg)	2.6693	1.8700	**2.6643**
Human Intestinal Absorption	+	+	**+**
AlogP	3.19	1.25	**2.23**
H-Bond Acceptor	16	7	**11**
H-Bond Donor	11	6	**8**
Tetrahymena pyriformis (pIGC50 (ug/L))	0.595	0.792	**0.913**

Blood Brain Barrier	-	-	**-**

* Best docked.

4. Conclusions

From our current study, we noticed that Epigallocatechin Gallate (EGCG) has strong interactions with 4p8c enzyme (docking score: -142.98 kcal/mol) (amino acid residues: GLN A:328; TYR A:60; GLY A:334; LYS A:418; VAL A:365; LEU A:317). The binding energy for EGCG was obtained higher when compared with std. drug ciprofloxacin (docking score: -109.20 kcal/mol). Moreover, in-silico ADMET analysis revealed that this compound has low Human Ether-a-go-go-Related Gene Inhibition, No AMES Toxicity and No Carcinogens. Considering previous literature report on EGCG as antimycobacterial, we investigated same against DprE1 enzyme via molecular docking analysis. Considering benefits of GTPs, this study may provide further directions to develop more potent anti-TB compounds. Moreover, we also believe that synthetic analogues of EGCG could also be tested for in-vitro anti-TB potentials. The tea extract containing EGCG, could also be tested in-vitro for anti-TB enzymatic assessments.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization, S.M. and A.P.; methodology, S.M.; software, S.M.; writing—review and editing, S.M. and A.P.; visualization, S.M. and A.P.; supervision, A.P.

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Conflicts of Interest: The authors declare no conflict of interest.

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