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Proceeding Paper

Naturally Occurring Green Tea Polyphenols as Anti-Mycobacterial Agents †

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Abstract: Tuberculosis (TB) is a global health burden especially in tropical countries. Extensive increments in MDR (Multidrug resistance (MDR): Resistance to at least both isoniazid and rifampicin.) and XDR (Extensive drug resistance (XDR): Resistance to any fluoroquinolone, and at least one of three second-line injectable drugs (capreomycin, kanamycin, and amikacin), in addition to multidrug resistance) tuberculosis highlights the ineffectiveness of established anti-TB agents. There is an urgent necessity to identify potent anti-TB agents with unique mechanisms. Green tea and Black tea polyphenols have great potential to inhibit viruses including SARS-COV-2, bacterial strains, etc. In this context, we have screened and identified 65 Green tea bioactive compounds against four mycobacterial *pantothenate synthetase* and *enoyl acyl carrier* enzymes. Our molecular docking results revealed that *Theaflavin-3-gallate* had a higher binding affinity against 2X22 and 3IVX targets with docking scores of −134.13 and −135.592 Kcal/mol, respectively. Furthermore, our molecular dynamics simulations for 10 ns resulted better stabilities of these complexes. We also evaluated in silico drug-likeness and toxicity profiles for the studied polyphenols. Our in silico toxicity analysis suggested that these polyphenols would exhibit lesser toxicity such as eye corrosion, skin irritations, etc. Thus, our present study would provide better insights on studying naturally occurring polyphenols as potential anti-TB agents.

Keywords: Tuberculosis; *Mycobacterium*; EGCG; green tea polyphenols; enoyl reductase

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1. Introduction

Tuberculosis (TB), which is a communicable disease, is one of the top 10 causes of death worldwide, especially in low-income tropical countries, where there is a scarcity of healthcare facilities. As per the WHO estimates for the year 2019, a total of 1.4 million people died due to TB [1]. The rising cases of multidrug-resistant TB (MDR-TB) are alarming and present a global health security threat (206,030 people were found to have multidrug- or rifampicin-resistant TB (MDR/RR-TB) strains) [1,2]. The unusual cell wall, made up of α-alkyl-β-hydroxy fatty acids or mycolic acid (MA), acts as a major barrier for therapeutic drugs to reach inside mycobacterial cells. It is noteworthy to mention that the MA serve key roles in maintaining structural integrity and to provide protection against an oxidative stress. It is also worth noting, that targeting a 2-trans-enoyl-acyl carrier protein reductase, called InhA is not always a good idea. Although, it is a good target, which is vital, and is the target for isoniazid, but resistance to isoniazid is one of the criteria for classifying *M. tb* as MDR, though most of the mutations occur in katG gene, activating isoniazid. Therefore, new drugs active on InhA could only partly overcome MDR [3]. Green tea and Black tea are the most popular beverages consumed. These are particularly derived from the plant *Camellia sinensis* [4]. In vitro and animal studies provide strong evidence that

polyphenols derived from tea (polyphenols (the green tea polyphenols (GTPs)), especially flavanols, flavandiols, flavonoids, and phenolic acids, etc.) may possess the bioactivity that can affect the pathogenesis of several chronic diseases (Figure 1). The GTPs are also known for their wide pharmacological potentials, including anticarcinogenic, antioxidant, antituberculosis (anti-TB), and also, very recently, anti-SARS-Cov-2 properties [4,5]. It is interesting to note that these health-enhancing effects of GTPs were mainly attributed to the phytoconstituent present called '(−)-epigallocatechin-3-gallate' (EGCG). In a very recent study, GTP epigallocatechin-3-gallate was demonstrated to inhibit InhA, the enoyl-ACP reductase of mycobacterium. This has prompted us to screen in silico a set of GTPs against various pivotal targets of mycobacterium including InhA [6,7]. For the best-docked top three hits with higher docking scores, we listed down their drug-likeness assessment, and ADMET (absorption, distribution, metabolism, excretion, toxicity) properties. Furthermore, we examined molecular dynamics simulations for the best docked hit, i.e., target complexes for the duration of 10 ns each.

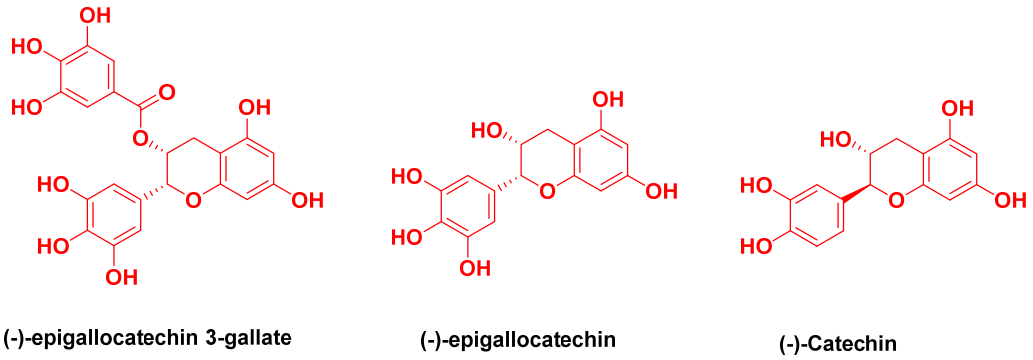

Figure 1. Chemical structures of Green Tea bioactive compounds (representative).

Herein, we had three objectives to screen a set of known 65 bioactive molecules from tea against known anti-TB targets. Secondly, we also compared molecular docking simulation and molecular dynamics results with standard anti-TB drugs (Pyrazinamide, Ethambutol and Isoniazid) against mycobacterial targets. Lastly, we signified a probable lead that could be developed as a drug candidate against mycobacterial targets.

2. Materials and Methods

2.1. Molecular Docking Analysis

A set of 65 reported tea bioactive compounds was retrieved from the study reported by Bhardwaj et al. 2021 [7]. All the structures were then drawn using 'ChemDraw V. 12.1'. All the 3D crystal structures of 4 mycobacterial proteins (the enoyl reductase receptor protein (PDB IDs:2NV6; 2X22 (crystal structure of *M. tuberculosis* InhA inhibited by PT70); 1BVR; and the pantothenate synthetase, Crystal structure of pantothenate synthetase in complex with 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1H-indol-1-yl)acetic acid, i.e., 3IVX) were downloaded from the protein database bank (PDB database, www.rcsb.org accessed on 20 April 2021). For the protein preparations and ligand preparations, we followed the known protocols. Finally, molecular docking simulations were performed with popular software, including, 'Molegro Virtual Docker v. 6.0.1' and 'iGemDock' as per standard procedures. The best docked hits were identified via higher docking scores and were then visualized with Discovery Studio 2020 Visualizer (BIOVIA, Dassault Systèmes) or with Pymol (GLSL version 4.60, for educational use).

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

For the best docked top three hits, we predicted their ADME properties using SWISS tools (http://www.swissadme.ch accessed on 20 April 2022). In order to access a drug-likeness nature of obtained best docked hits, we used Lipinski's rule of five criteria. The

assessments for toxicities were predicted by using online platform, 'admetSAR'(http://lmmd.ecust.edu.cn:8000/ accessed on 20 April 2022).

2.3. Normal Mode Analysis

To gain more insights into the conformational flexibilities [8] of proteins with their best docked hits, we performed the Normal Mode Analysis (NMA) with internal coordinates (IC) using a fast and easy server, iMODS (http://imods.chaconlab.org/ accessed on 20 April 2022). This server also guides medicinal chemists by providing more details on co-variance map, eigenvalues, deformability, variance, the collective motions of proteins, B-factor, etc. Deformations in proteins were depicted by the term deformability, while mobility profile was denoted by the B-factor.

2.4. Molecular Dynamics Analysis

Molecular dynamics (MD) simulation for a period of 10 ns was performed for best docked hit with Theaflavin-3-gallate: target protein, 2X22 complex and it was achieved with Desmond module implemented in a Schrödinger package, 2020. For setting up initial systems, we used the OPLS-2005 molecular mechanic's force field. We kept ensemble class at NPT (temperature: 300 k, pressure: 1.01325 bar). Then, system was simulated further through the multistep MD protocols.

3. Results and Discussion

3.1. Molecular Docking Simulations

In order to gain more insights on binding mechanisms, we docked a set of 65 green tea bioactive compounds into 4 mycobacterial target proteins using 'iGemDock' tool. The docking protocol was validated via a redocking approach and was obtained with RMSD below 2 Å [9–11]. A dataset molecule, Theaflavin-3-gallate interacted with target proteins 2X22 and 3IVX with highest binding scores of −134.13 and −135.592 Kcal/mol, respectively. Compound, Theaflavin-3-gallate interacted with key amino acid residues, TRP A:160; MET A:103; GLN A:100; ASN A:159; MET A:155; THR A:162; PRO A:156, etc. (Figure 2). The results for the remaining green tea/black tea biomolecules are listed in Tables 1 and 2.

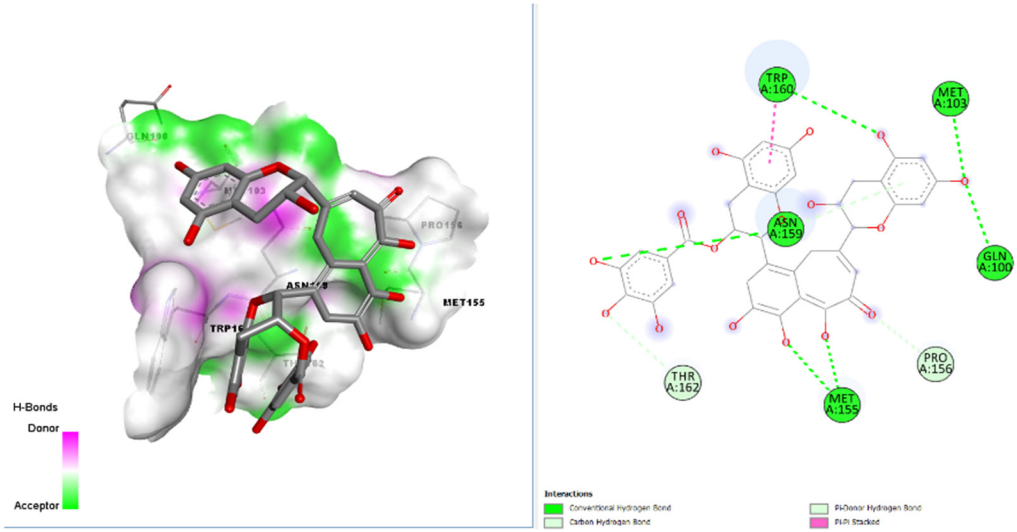

Figure 2. 2D and 3D-interaction profiles for best docked *Theaflavin-3-gallate* with 2X22.

Table 1. Docking interaction energies * of selected **65** bio-active molecules and **3** FDA approved drugs for target protein 2X22.

Molecules	iGemDock Interaction Energy	Molecules	-iGemDock Interaction Energy
Oolonghomobisflavan A	−66.2219	Theaflavic Acid	−84.4934
Theasinensin D	−72.1619	Barrigenol R1	−86.4843
Theaflavin-3-gallate	−134.13	Barringtogenol	−89.0693
Isotheaflavin	−72.621	Camelliagenin	−95.1799
Epigallocatechin-3,5-Di-O-Gallate	−72.0176	Gallocatechin	−86.7374
Oolonghomobisflavan B	−75.4779	Catechin	−102.992
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	−95.1799
Rutin	−87.1416	Myricetin	−83.5936
Proanthocyanidin	−84.8129	Apigenin	−83.6163
Pheophytin	−90.2865	Nerolidol	−84.584
Benzaldehyde	−91.9877	Kaempferol	−89.1838
Epitheaflavic Acid 3′-Gallate	−65.361	Theanine	−83.9851
Epigallocatechin Gallate	−122.3403	Ascorbic Acid	−80.1271
Theasinensin E	−62.6409	Quinic Acid	−85.3299
Myricitrin	−61.915	Succinic Acid	−85.5696
Theaflavin	−65.9704	Methyl Salicylate	−81.1848
Epicatechin Gallate	−75.5287	Theobromine	−84.7269
Kaempferitrin	−72.7401	Caffeine	−84.4502
Isoquercetin	−89.9058	Xanthine	−86.7595
Epiafzelechin 3-O-Gallate	−79.4119	Linalool Oxide	−83.9907
Pheophorbide	−71.1657	Phenylacetaldehyde	−87.8044
Epigallocatechin 3-O-P-Coumarate	−78.8643	Methylxanthine	−79.6185
Pheophorbide	−68.9266	Theophylline	−88.1319
Oxalic Acid	−87.9277	Geraniol	−95.2378
Cryptoxanthin	−81.2634	Hexanal	−95.8974
Isovitexin	−82.924	Diphenylamine	−93.4455
Vitexin	−85.6638	Trans−2-Hexenal	−94.076
Chlorogenic Acid	−89.7604	Linalool	−86.4307
Coumaroyl Quinic Acid	−94.7189	Phenylethanol	−101.468
Epigallocatechin	−115.6776	Ciprofloxacin *	−108.9558

* 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 (kcal/mol)
1	Isoniazide	TYR A:158 (PI-PI STACKING); VAL A:203; MET A:199; LYS A:165
2	Pyrazinamide	TYR A:158; MET A:161; ALA A:198
3	Ciprofloxacin	PRO A:156; MET A:199; TYR A:158; VAL A:203
4	Theaflavin-3-gallate (Best docked)	TRP A:160; MET A:103; GLN A:100; ASN A:159; MET A:155; THR A:162; PRO A:156
5	Epigallocatechin	ALA A:198; MET A:162; PRO A:193; PHE A:149; MET A:199; TYR A:158
6	Epigallocatechin Gallate (EGCG)	ALA A:198; MET A:162; PRO A:193; PHE A:149; MET A:199; TYR A:158
7	Inbound ligand	ALA A:198; MET A:162; PRO A:193; PHE A:149; MET A:199; TYR A:158

3.2. Molecular Dynamics Simulation and Normal Mode Analysis

The highest scored biomolecule, Theaflavin-3-gallate with protein 2X22 was simulated for molecular dynamics and normal mode analysis. MD simulations depicted that Root Mean Square Fluctuation (RMSF) values were obtained within tolerable ranges. The Root mean square deviation (RMSD) value was obtained below 3 Å, suggesting stability of complex (Figure 3). From our NMA results, we noticed that Theaflavin-3-gallate with protein 2X22 complex was retained with good deformability, and eigenvalue value profiles (Figure 3).

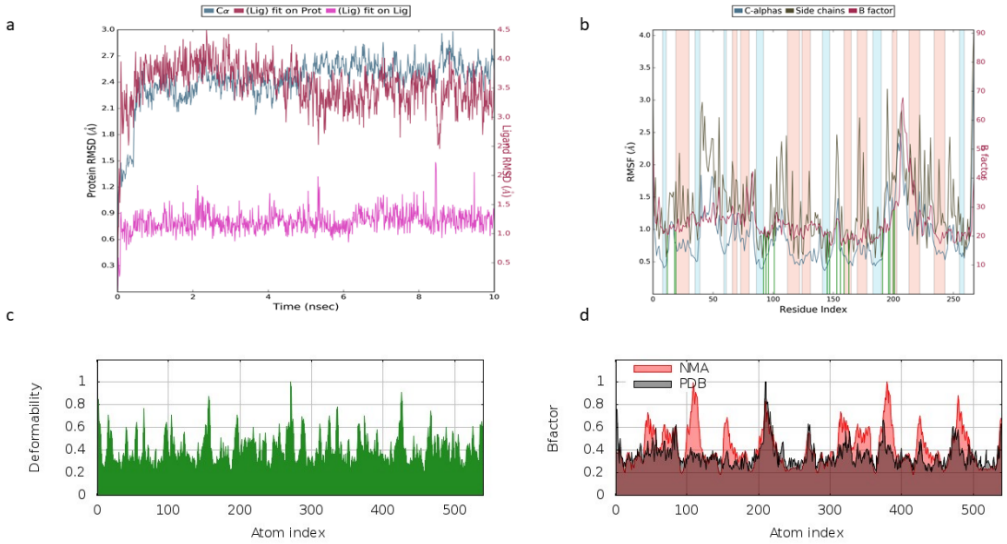

Figure 3. (**a**) The Root Mean Square Deviations (RMSD) of backbone atoms relative to the starting complexes during 10 ns MD; (**b**) Protein RMSF plot (On this plot, peaks indicate areas of the protein that fluctuate the most during the simulation and Protein residues Table 3. *gallate* with 2X22, respectively; (**c**) Normal mode analysis-Deformability; (**d**) B-factor analysis.

3.3. In Silico ADME Studies

Cytochrome P450 (CYPs) enzymes are a family metabolic enzymes responsible for bio-transformations of almost ~90% FDA approved drugs. Phase I and Phase II are two important pathways involved in the metabolism of xenobiosis. Our in silico calculated ADMET (absorption, distribution, metabolism, excretion, toxicity) properties for the top best-docked three hits are represented in Table 3. Compounds, Theaflavin-3-gallate, Epigallocatechin and Epigallocatechin Gallate (EGCG) exhibited non-carcinogenic, non-AMES toxic, and class IV acute oral toxicity profiles. All 3 of our proposed hits were found to have positive human intestinal absorption profiles and negative the Blood–brain barrier passage profiles.

Table 3. In silico ADMET profiling for top **3** best docked hits against target 2X22.

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	+	+	+
Blood–brain barrier	-	-	-

4. Conclusions

It is noteworthy to mention that green tea polyphenols have significant prooxidant properties and a great potential to inhibit in vitro SARS-Cov-2, bacterial and mycobacterial growths. However, our in silico methodology used herein indicates four probable therapeutic targets involved in anti-TB potentials. Furthermore, we also wish to note that apart from the reported potential of EGCG, Theaflavin-3-gallate may have strong interaction with

InhA target. The tea extract containing Theaflavin-3-gallate could also be tested in vitro for anti-TB assessments. Moreover, we believe that the core structure of Theaflavin-3-gallate could also be explored further to develop more potent synthetic analogues for TB. Our in silico ADMET analysis suggested safer probable pharmacokinetics for GTPs.

Many of the virtually screened compounds are usually inactive on mycobacterial cells due to their cell wall permeability. For better screening of virtually screening hits, a deeper understanding of the cell biology of mycobacteria and a thorough structure analysis of selected hits is required. Indeed, major limitations characterizing docking include a restricted sampling of both ligand and receptor conformations in pose prediction, and the use of approximated scoring functions, which very often provide results that do not correlate with the experimental binding affinities. Thus, the proper selection of a protein and binding site along with the best docking software will increase the likelihood of retaining the correct hits.

However, despite the success of molecular docking or drug repurposing via in silico methodologies, one must take into considerations the usage of an appropriate scoring function and algorithm, which may otherwise jeopardize molecular screening.

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