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 increases 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

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, flavandiol, 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.

\[\text{Chemical structures of Green Tea bioactive compounds (representative).}\]

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

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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                    |
| Theasinsenin D                | –72.1619                   | Barrigendol R1                | –86.4843                    |
| Theaflavin-3-gallate          | –134.13                    | Barrigendol                  | –89.0693                    |
| Isotheaflavin                 | –72.621                    | Camelliagenin                | –95.1799                    |
| Epigallocatechin-3,5-Di-O-Gallate | –72.0176            | Galloclatechin                | –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                    |
| Epigallocatechin-3-Gallate    | –65.361                    | Theanine                      | –83.9851                    |
| Epigallocatechin Gallate      | –122.3403                  | Ascorbic Acid                 | –80.1271                    |
| Theasinsenin 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                    |
| Kaempferitin                  | –72.7401                   | Caffeine                      | –84.4502                    |
| Isoqueretin                   | –89.9038                   | Xanthine                      | –86.7595                    |
| Epiafzelechin 3-O-Gallate     | –79.4119                   | Linalool Oxide                | –83.9907                    |
| Phosphorhodite                | –71.1657                   | Phenylacetaldehyde            | –87.8044                    |
| Epigallocatechin 3-O-P-Coumarate | –78.8643               | Methylxanthine                | –79.6185                    |
| Phosphorhodite                | –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).
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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 (LD50, 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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