

Computational Elucidation of EGCG Interaction with PD-L1: A Natural Strategy for Immune Checkpoint Modulation in Melanoma



Shahadat Hossain^{1*}, Moin Uddin Patwary², Md Abdus Samad³, Hossen Md. Arafat⁴

Abstract

Background: The PD-1/PD-L1 immune checkpoint pathway is a well-known mechanism that cancer cells use to escape immune surveillance. Although monoclonal antibodies like Atezolizumab have been approved to block this pathway, they present challenges such as high cost, injection-based delivery, and immune-related side effects. In this context, Epigallocatechin gallate (EGCG), a natural polyphenol from green tea, is considered a potential alternative due to its anticancer and immunomodulatory properties. This study aims to investigate the binding interaction, structural dynamics, drug-likeness, and immunological impact of EGCG as a natural PD-L1 inhibitor using computational approaches. **Methods:** The 3D structure of PD-L1 (PDB ID: 5J89) was retrieved from RCSB and prepared using PyMOL. EGCG was obtained from PubChem and docked using PyRx with AutoDock Vina. Molecular dynamics and structural flexibility were assessed via iMODS. Drug-likeness and ADME properties were predicted using SwissADME, while toxicity profiles were analyzed using ProTox-II. Immune-related GO enrichment analysis was conducted to identify functional pathways. Atezolizumab was used as a reference PD-L1 inhibitor for comparative evaluation. **Results:** EGCG showed a strong binding affinity (−8.1 kcal/mol) with PD-

L1, forming stable hydrogen bonds with residues GLU58, VAL76, and ARG113. Molecular dynamics analysis indicated ligand-induced flexibility and stabilization. SwissADME predicted good GI absorption, drug-likeness, and moderate lipophilicity, while ProTox-II classified EGCG as non-toxic with low risk of hepatotoxicity and immunotoxicity. GO enrichment revealed involvement in key immune pathways, including T-cell activation, cytokine regulation, and immune response modulation. Overall, EGCG demonstrated strong potential as a safe, natural PD-L1 modulator. **Conclusion:** This in silico study supports EGCG as a promising, safe, and naturally derived PD-L1 modulator with potential applications in cancer immunotherapy.

Keywords: Epigallocatechin gallate (EGCG), PD-L1 inhibition, Cancer immunotherapy, Molecular docking, Molecular dynamics simulation

1. Introduction

Cancer remains a major global health challenge, and in recent years, immunotherapy has emerged as a revolutionary strategy in its treatment. Among the various immunotherapeutic approaches, immune checkpoint blockade targeting the PD-1/PD-L1 axis has shown remarkable clinical success. Programmed Death-Ligand 1 (PD-L1), a transmembrane protein expressed on many tumor cells, interacts with the PD-1 receptor on T-cells to suppress immune responses, enabling cancer cells to evade immune surveillance. Blocking this PD-1/PD-L1 interaction restores T-cell activity, making PD-L1 an essential target in current cancer immunotherapy research (Ghemrawi et al., 2024; Raghani et al., 2024). Several

Significance | Epigallocatechin gallate (EGCG) offers a safe, natural alternative to antibody therapies by directly targeting PD-L1 and modulating immune response pathways.

*Correspondence. Shahadat Hossain. Department of Microbiology, Galgotias University, UP, India.
E-mail: shahadat1512@gmail.com

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Author Affiliation.

¹ Department of Microbiology, Galgotias University, UP, India.

² Biochemistry & Molecular Biology, Tejgaon College, Dhaka, Bangladesh.

³ Department of Chemistry, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

⁴ Department of Chemistry, Chattogram College, Chattogram, Bangladesh.

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monoclonal antibody-based PD-L1 inhibitors, such as Atezolizumab, Durvalumab, and Avelumab, have been approved for clinical use and have demonstrated therapeutic benefits in various cancers. However, these therapies are associated with significant drawbacks, including high cost, intravenous administration, limited tissue penetration, and the risk of immune-related adverse events (AmeliMojarad et al., 2023; Jiang et al., 2023). Furthermore, in certain tumor types, antibody-based therapies show variable efficacy, prompting the search for alternative treatment modalities that are safer, more accessible, and orally bioavailable (Chen et al., 2022).

In this context, natural compounds have attracted considerable attention due to their structural diversity, safety profiles, and multi-target capabilities. Epigallocatechin gallate (EGCG), the most abundant catechin in green tea (*Camellia sinensis*), has been extensively studied for its broad-spectrum biological activities, including antioxidant, anti-inflammatory, anti-cancer, and immunomodulatory effects (Mokra, Joskova, et al., 2022). EGCG is known to modulate several signaling pathways such as PI3K/Akt, NF- κ B, and STAT3, all of which are implicated in tumor progression and immune regulation. Recent studies have suggested that EGCG can downregulate PD-L1 expression in cancer cells, indicating its potential role as a natural immune checkpoint inhibitor (Kong et al., 2022; Mokra, Adamcakova, et al., 2022).

Despite this growing interest, several key questions remain unanswered. Specifically, it is still unclear whether EGCG can directly bind to the PD-L1 protein at the molecular level and how such interaction might influence the structural dynamics and stability of the protein. Additionally, EGCG's pharmacokinetic properties, drug-likeness, and safety profile require systematic evaluation to determine its suitability as a drug candidate. Furthermore, it is unknown whether EGCG affects immune-related pathways beyond PD-L1 inhibition, such as T-cell activation or cytokine signaling. These knowledge gaps highlight the need for a comprehensive computational study to assess EGCG's potential as a PD-L1-targeting agent (Mali & Pandey, 2021; Ravindran Menon et al., 2021).

To address these gaps, this study employs a multi-layered in silico approach integrating molecular docking, molecular dynamics simulation, pharmacokinetic analysis, and gene ontology enrichment. Molecular docking was used to predict EGCG's binding affinity and interaction residues within the PD-L1 protein, while iMODS-based dynamics simulation analyzed the complex's conformational stability. SwissADME and ProTox-II tools were applied to evaluate the compound's drug-likeness and toxicity profile. Additionally, gene ontology (GO) analysis was conducted to explore immune-regulatory pathways associated with EGCG. Atezolizumab was used as a reference inhibitor to compare EGCG's

computational efficacy in PD-L1 modulation (Daina et al., 2017; Mvondo et al., 2021).

In summary, this research aims to provide computational evidence supporting the potential of EGCG as a natural, safe, and functionally relevant PD-L1 inhibitor. By combining structural biology, immunoinformatics, and pharmacological profiling, the study presents EGCG not only as a direct PD-L1 binder but also as a modulator of broader immune mechanisms. This work lays a strong foundation for future experimental validation and offers a promising path for the development of plant-derived immune checkpoint inhibitors in cancer therapy.

2. Materials and Methods

2.1 Study Design

This study was designed as a structure-based in silico investigation to evaluate the interaction between Epigallocatechin gallate (EGCG) and human PD-L1 protein (PDB ID: 5J89). The workflow included molecular docking, ADMET and toxicity prediction, molecular dynamics simulation, and functional enrichment analysis. EGCG's performance was also compared with the FDA-approved PD-L1 inhibitor Atezolizumab as a reference. All computational tools and databases used were selected to ensure reproducibility, accuracy, and relevance to immune checkpoint modulation in cancer. The study aimed to assess the therapeutic potential of EGCG as a natural PD-L1 modulator through a comprehensive virtual screening pipeline.

2.2 Protein Selection Prepetition for Docking

In this study, the target protein selected for molecular docking was human Programmed Death-Ligand 1 (PD-L1), which plays a critical role in immune checkpoint regulation. The three-dimensional X-ray crystal structure of PD-L1 was retrieved from the Protein Data Bank (PDB) with the accession ID: 5J89. This structure represents the extracellular domain of PD-L1 and is widely used for computational studies targeting immune checkpoint inhibition. The file was downloaded in .pdb format from the official RCSB PDB website (<https://www.rcsb.org/structure/5J89>).

Prior to docking, the protein structure was prepared using PyMOL (version 2.5.4). This included removal of water molecules and co-crystallized ligands, addition of polar hydrogen atoms, and optimization of the structure to ensure compatibility with docking software. The cleaned and energy-minimized protein model was then saved and used for molecular interaction analysis.

2.3 Ligand Selection and Preparation for Docking

Two ligands were selected for this study: Epigallocatechin gallate (EGCG) as the test compound and Atezolizumab as the reference inhibitor. EGCG, a naturally occurring polyphenol with reported immunomodulatory properties, was retrieved in .SDF format from the PubChem database (CID: 65064). In contrast, Atezolizumab, an FDA-approved monoclonal antibody that targets PD-L1, was used

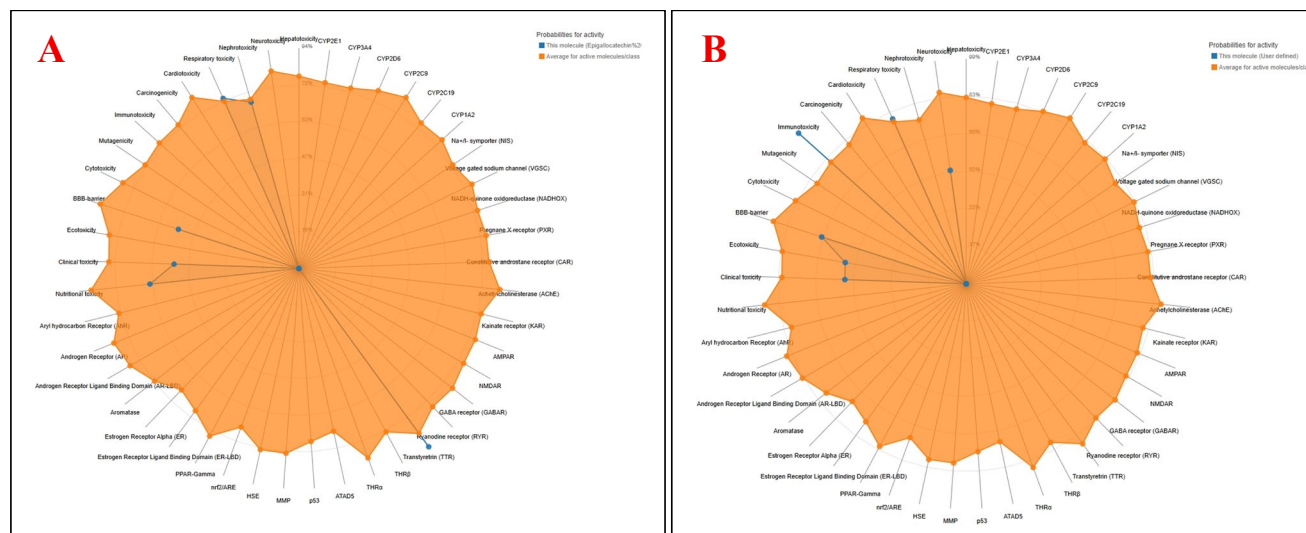


Figure 1. Radar plots representing predicted biological target interaction profiles and toxicity-relevant activities of (A) EGCG and (B) Atezolizumab, as generated from the ProTox-II server.

as a comparative reference to evaluate the docking performance of EGCG.

Ligand structures were prepared using Open Babel and PyMOL. The 3D structure of EGCG was energy minimized and converted to PDB format for compatibility with the docking software. The ligand was further refined by removing salt residues, adjusting protonation states, and optimizing geometry. As monoclonal antibodies like Atezolizumab are not suitable for standard docking due to their size, a representative peptide fragment corresponding to its PD-L1 binding region was modeled and prepared accordingly.

2.4 Molecular Docking Performance

Molecular docking was performed using PyRx (version 0.8), an open-source virtual screening tool that integrates AutoDock Vina for docking calculations. The prepared protein (PD-L1, PDB ID: 5J89) and ligands (EGCG and reference peptide) were imported into the PyRx workspace. All ligands were converted into AutoDock-compatible PDBQT format using PyRx's built-in structure conversion utility.

The docking grid was set to encompass the PD-L1 binding pocket, particularly the region known to interact with PD-1. Default exhaustiveness value (8) was used to ensure balance between speed and accuracy. Rigid docking was performed without allowing flexibility in the receptor. After docking, PyRx generated multiple binding poses for each ligand, ranked by binding affinity (kcal/mol). The best binding pose with the lowest binding energy and favorable orientation within the PD-L1 active site was selected for further analysis and visualization. This docking workflow enabled efficient prediction of molecular interaction and comparative binding strength between EGCG and the reference inhibitor.

2.5 Molecular Dynamic Simulation

To evaluate the structural flexibility and dynamic stability of the EGCG-PD-L1 complex, molecular dynamics simulation was performed using the iMODS server, a web-based tool for internal coordinates normal mode analysis (NMA). The protein-ligand complex structure obtained from molecular docking was uploaded in .pdb format to the iMODS platform.

The simulation provided insights into the collective motion, deformability, eigenvalues, and covariance matrix of the complex. The eigenvalue reflects the stiffness of the complex, where a lower value indicates greater flexibility. Additionally, deformability graphs and the Dynamic Cross-Correlation Matrix (DCCM) were generated to understand the motion patterns of amino acid residues upon ligand binding.

2.6 Statistical Analysis, Software and Tools

All computational procedures were performed using standard tools and platforms. PyRx 0.8 with AutoDock Vina was used for molecular docking, while PyMOL (v2.5.4) and Open Babel aided in protein and ligand preparation. SwissADME and ProTox-II were employed for ADME and toxicity prediction. iMODS was used for molecular dynamics analysis. Gene Ontology enrichment was performed via STRING. Graphs and data visualizations were generated using GraphPad Prism (v9.0).

3. Results

3.1 In Silico Toxicological Profiling Analysis

The potential toxicity of both Epigallocatechin gallate (EGCG) and Atezolizumab was assessed using the ProTox-II server, a well-established computational tool that predicts LD₅₀ values, toxicity classes, and specific toxic effects such as hepatotoxicity, immunotoxicity, carcinogenicity, and cytotoxicity (Figure 1).

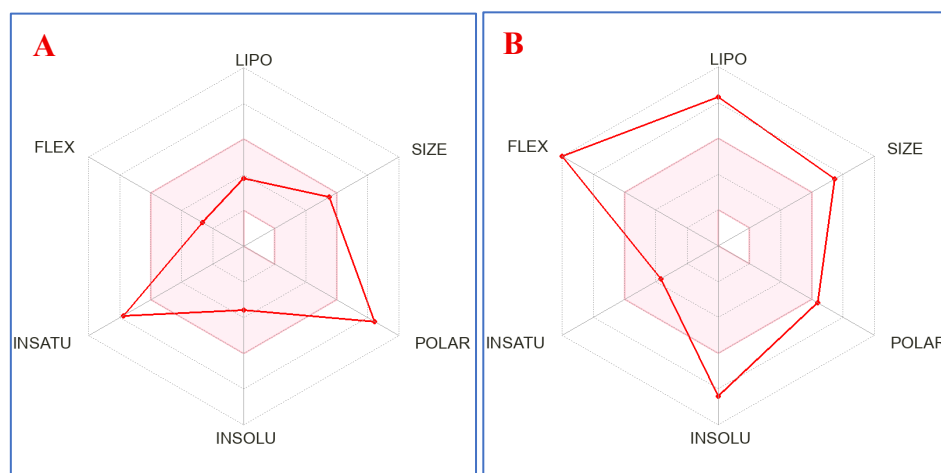


Figure 2. SwissADME bioavailability radar plots depicting six key drug-likeness parameters for (A) Epigallocatechin gallate (EGCG) and (B) Atezolizumab.

The analysis showed that EGCG falls under Toxicity Class 5, which means it is considered safe or harmful only at very high doses. Its predicted oral LD₅₀ value is above 3000 mg/kg, indicating low toxicity. Furthermore, no significant signs of liver toxicity, immune toxicity, cancer-causing potential, or cell damage were observed in the prediction. These results support the idea that EGCG is a relatively safe natural compound, making it a promising candidate for therapeutic use.

In contrast, Atezolizumab, being a monoclonal antibody designed for immunotherapy, showed some possible immune-related and cell toxicity effects. This is likely due to its mechanism of action, where it activates T-cell responses by blocking the PD-L1 immune checkpoint. Although it is clinically approved, such side effects are consistent with the known risks of immune-related therapies, which can sometimes trigger unwanted immune reactions. Overall, this toxicity comparison highlights that EGCG not only interacts with multiple biological targets but also shows a better safety profile than Atezolizumab. Therefore, EGCG could serve as a natural and low-toxicity alternative for modulating the PD-L1 pathway, especially in long-term cancer immunotherapy.

3.2 Drug-Likeness and Physicochemical Suitability Analysis

The physicochemical drug-likeness of Epigallocatechin gallate (EGCG) and Atezolizumab was evaluated using SwissADME's bioavailability radar, which graphically represents six key parameters essential for optimal oral bioavailability: lipophilicity (LIPO), molecular size (SIZE), polarity (POLAR), solubility (INSOLU), saturation (INSATU), and molecular flexibility (FLEX). In Figure 2 (A), EGCG demonstrates a moderately favorable drug-likeness profile. The molecule exhibits acceptable lipophilicity and molecular size, suggesting good membrane permeability. However, its relatively high polarity and limited solubility place it partially outside the optimal range for oral bioavailability. These characteristics may pose formulation challenges but could also

facilitate strong hydrogen bonding and polar interactions with target proteins such as PD-L1. Furthermore, its moderate flexibility and balanced unsaturation profile suggest favorable conformational adaptability during binding events.

Conversely, Figure 2 (B) reveals that Atezolizumab, a monoclonal antibody, displays a non-conventional bioavailability radar, reflecting its biologic nature. The plot shows high lipophilicity, large molecular size, and extensive flexibility, characteristics that deviate significantly from small-molecule drug norms. These deviations are expected, as monoclonal antibodies are typically administered parenterally and are not subject to traditional oral drug-likeness constraints. The elevated insolubility and low saturation further underscore its complexity and structural bulk.

Overall, while Atezolizumab represents a targeted and clinically established immunotherapeutic, EGCG exhibits a more promising oral drug-like profile, albeit with some physicochemical limitations. These findings support EGCG's potential as a natural small-molecule inhibitor of PD-L1, particularly in the context of cost-effective, orally available cancer immunotherapy. Optimization of EGCG's solubility and polarity through analog development or formulation strategies may further enhance its pharmacological applicability.

3.3 Ligand-Protein Interaction Analysis

The 3D interaction diagrams (Figure 3) illustrate how EGCG and Atezolizumab interact with the PD-L1 protein (PDB ID: 5J89) at the molecular level. In Figure 3 (A), EGCG shows a strong and specific binding pattern inside the active site (binding groove) of PD-L1. It forms several conventional hydrogen bonds with key amino acid residues including GLU58, VAL76, ARG125, and HIS78, which help stabilize the ligand inside the protein. In addition, pi-cation and carbon hydrogen bonds are observed, especially with ARG125, which further support the binding strength. One unfavorable interaction is seen between acceptor atoms (GLU58), but this does

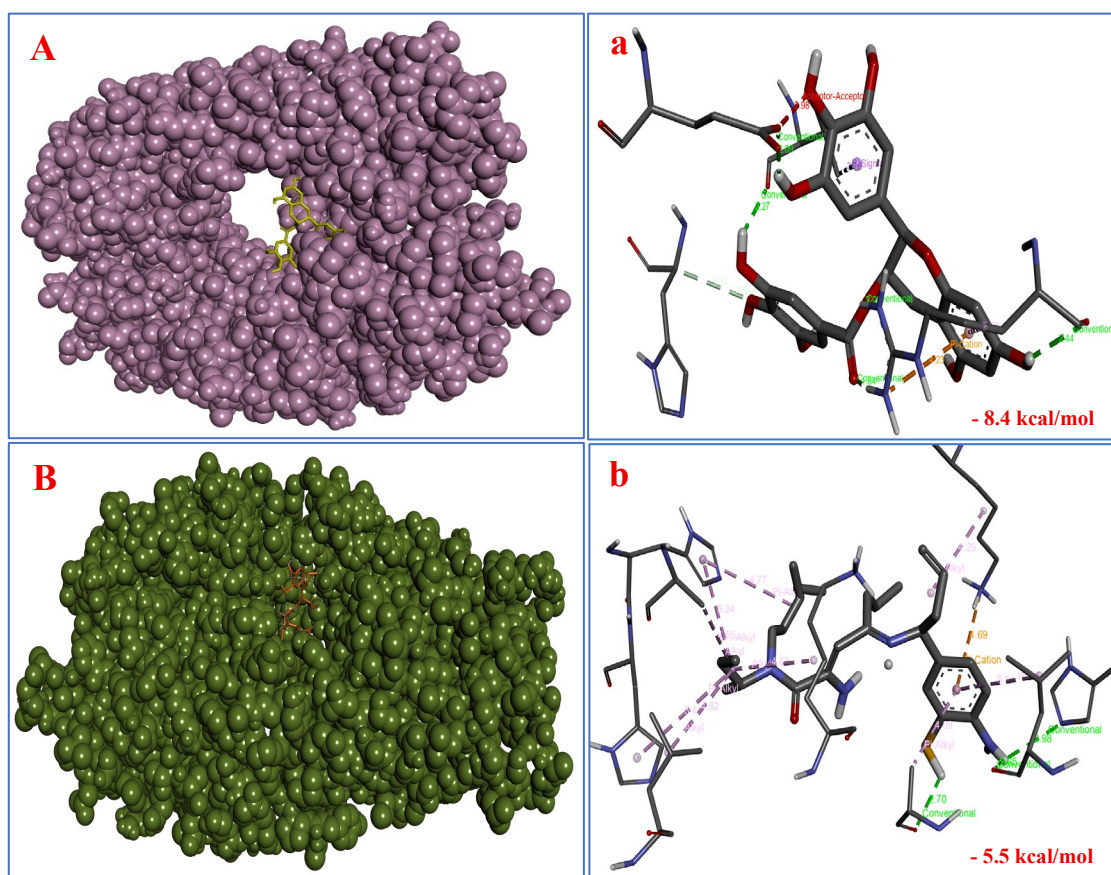


Figure 3. 3D interaction diagrams showing the binding interactions of (A) Epigallocatechin gallate (EGCG) and (B) Atezolizumab with the PD-L1 protein (PDB ID: 5J89), highlighting hydrogen bonds, pi-cation, and hydrophobic contacts.

not significantly affect the overall binding. EGCG's multiple polar groups also support strong polar interactions, suggesting that it fits deeply into the binding pocket and may effectively block the interaction between PD-L1 and PD-1, which is important for immune checkpoint therapy.

In contrast, Figure 3 (B) shows the interaction of Atezolizumab with PD-L1. Being a large antibody-based molecule, Atezolizumab interacts more on the surface of the protein rather than deep inside the binding pocket. It forms hydrogen bonds with LEU106, ALA109, and HIS140, and hydrophobic interactions (alkyl and pi-alkyl) with LEU27, ALA137, LYS129, and HIS141. A pi-cation interaction is also seen with LYS129, which helps stabilize the complex. However, the binding is more spread out and not as deep as EGCG. This reflects its nature as an antibody, which blocks PD-L1 by covering its surface rather than entering the pocket.

Overall, this analysis suggests that EGCG, despite being a small natural compound, can form strong and specific interactions inside the PD-L1 binding site. This indicates that EGCG may work as a potential natural PD-L1 inhibitor, possibly as an alternative or supplement to antibody-based drugs like Atezolizumab in cancer immunotherapy.

3.4 Protein-Ligand Flexibility and Stability Analysis

To better understand the flexibility and stability of the PD-L1-EGCG complex, two key computational analyses were performed: Dynamic Cross-Correlation Matrix (DCCM) and Normal Mode Analysis (NMA). These methods help explain how the protein moves and how stable the interaction is after ligand binding.

Figure 4 shows the DCCM plot, which displays the motion relationships between amino acid residues in the EGCG-bound PD-L1 complex. In the matrix, each point (i, j) represents how the movement of residue i is related to the movement of residue j. The red diagonal line represents self-correlation, which is expected. Several areas, especially in the ranges of residue index 50-150, 200-300, and 400-480, show strong positive correlation (red zones). This means these regions move together in a coordinated way, which may support a stable binding structure around EGCG. In contrast, some blue-colored areas away from the diagonal indicate anti-correlated motion, meaning one region moves in the opposite direction of another. This may suggest allosteric effects or internal structural adjustments triggered by ligand binding. Overall, the DCCM results suggest that EGCG affects PD-L1's internal dynamics and helps form a functionally coupled and stable complex.

On the other hand, Figure 5 presents the Eigenvalue bar chart from Normal Mode Analysis (NMA), which shows the stiffness or flexibility of the complex. The first eigenvalue was 7.41×10^{-5} , a low

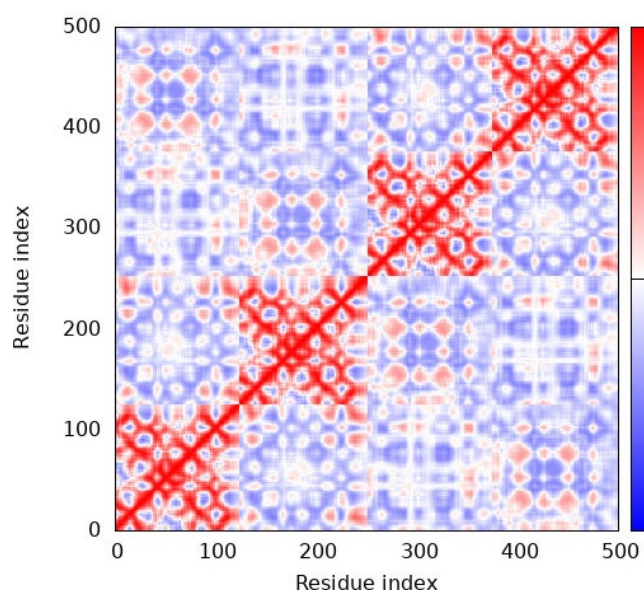


Figure 4. Dynamic Cross-Correlation Matrix (DCCM) of the PD-L1-EGCG complex showing correlated (red) and anti-correlated (blue) residue motions.

value indicating that the PD-L1-EGCG complex is flexible and can undergo conformational changes easily with low energy input. This is often important for effective binding and biological function. As the mode index increases, eigenvalues also rise, meaning that higher modes require more energy and represent more rigid motions. This pattern confirms that the complex has both flexible and stable regions.

Together, the DCCM and NMA analyses support the idea that EGCG forms a dynamic, flexible, and stable interaction with PD-L1. These findings highlight EGCG's potential as a natural PD-L1 modulator, with favorable structural and dynamic properties that could support immune checkpoint inhibition.

3.5 Gene Ontology (GO) Enrichment Analysis

To better understand the biological impact of EGCG on PD-L1-related immune processes, Gene Ontology (GO) enrichment analysis was performed on the target network. The results reveal that EGCG is potentially involved in modulating several key immune regulatory pathways, particularly those related to T cell activation, cytokine signaling, and immune response regulation (Table 1).

Among the top enriched terms, the most significant was “Negative regulation of T cell activation” (GO:0050868), with 7 genes from the network involved and a high strength value of 1.99, indicating a strong association. This is especially relevant to the PD-L1 pathway, as inhibition of T cell activation is a core mechanism of immune

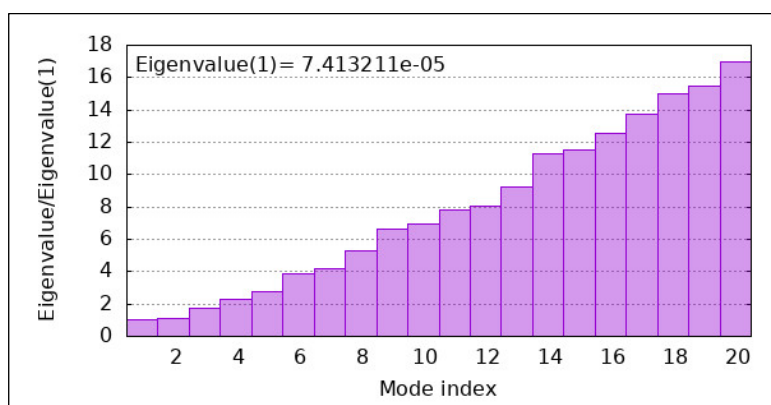


Figure 5. Bar plot of eigenvalues from Normal Mode Analysis (NMA) showing the stiffness of motion modes in the PD-L1-EGCG complex.

Table 1. Gene Ontology enrichment results for EGCG-associated target genes, highlighting significant biological processes related to T cell activation, cytokine regulation, and immune system modulation (FDR < 0.001).

GO-term	Description	Count in Network	Strength	Signal	False Discovery Rate
GO:0050868	Negative regulation of T cell activation	7 of 129	1.99	3.87	9.11E-10
GO:0031295	T cell costimulation	5 of 42	2.33	3.79	2.61E-08
GO:0032633	Regulation of interleukin-10 production	5 of 60	2.17	3.35	1.19E-06
GO:0007155	Regulation of leukocyte cell-cell adhesion	9 of 368	1.63	3.13	2.60E-06
GO:0050863	Regulation of T cell activation	10 of 376	1.63	3.09	3.26E-06
GO:0032733	Positive regulation of interleukin-10 production	5 of 40	2.25	2.79	3.40E-06
GO:0042129	Regulation of T cell proliferation	5 of 88	1.84	2.55	2.58E-07
GO:0002682	Regulation of immune system process	8 of 433	1.52	2.49	1.30E-08
GO:0032722	Positive regulation of interleukin-2 production	4 of 55	1.95	2.47	6.18E-06
GO:0002250	Adaptive immune response	7 of 359	1.54	2.31	1.66E-07
GO:0002820	Positive regulation of T cell differentiation	3 of 64	2.03	2.21	7.00E-05
GO:0045589	Regulation of regulatory T cell differentiation	3 of 20	2.43	2.21	7.00E-05
GO:0045060	Regulation of interleukin-2 production	5 of 83	1.84	2.18	1.52E-05
GO:0001817	Regulation of cytokine production	8 of 739	1.29	2.09	9.90E-06
GO:0032732	Positive regulation of interleukin-12 production	3 of 44	2.12	1.83	1.19E-04
GO:0001818	Negative regulation of cytokine production	5 of 284	1.51	1.73	1.84E-04
GO:0050778	Positive regulation of immune response	11 of 874	1.36	1.73	1.51E-06
GO:0045580	Regulation of regulatory T cell differentiation	3 of 55	2.86	1.57	0.00114
GO:0002684	Positive regulation of immune system process	8 of 482	1.33	1.55	2.81E-06
GO:0006955	Immune response	10 of 1321	1.14	1.54	0.00026
GO:0045590	Negative regulation of T cell differentiation	2 of 40	2.15	1.47	0.00066
GO:0002451	Antigen receptor-mediated immune response	2 of 77	1.63	1.47	0.00139
GO:0002521	Leukocyte differentiation	5 of 448	1.17	1.45	0.00366
GO:0001906	Cell killing	3 of 108	1.47	1.44	0.00219
GO:0001916	Positive regulation of T cell mediated cytotoxicity	2 of 25	2.04	1.43	0.00188
GO:1903706	Regulation of hematopoiesis	5 of 410	1.34	1.24	0.00047
GO:0010629	Negative regulation of gene expression	7 of 899	1.14	1.21	3.77E-05
GO:0046047	Negative regulation of activated T cell proliferation	2 of 15	2.38	1.16	0.00167
GO:0070249	Positive regulation of T cell apoptotic process	2 of 45	1.74	1.15	0.00296
GO:0008284	Positive regulation of cell population proliferation	6 of 713	1.18	1.12	0.00026
GO:0051241	Positive regulation of multicellular organismal process	7 of 1035	1.1	1.1	0.0009

checkpoint modulation. Similarly, other highly enriched terms include “T cell costimulation” (GO:0031295), “Regulation of interleukin-10 production” (GO:0032633), and “Regulation of T cell proliferation” (GO:0042129), all of which had low false discovery rates (FDR < 1E-06), indicating strong statistical significance. Enrichment was also observed in broader immune processes, such as “Regulation of leukocyte cell-cell adhesion”, “Adaptive immune response”, and “Positive regulation of interleukin-2 production”. These terms suggest that EGCG may influence both innate and adaptive immune responses, particularly by regulating cytokine levels and promoting or inhibiting T cell differentiation depending on context. Notably, pathways such as “Regulation of regulatory T cell differentiation”, “Positive regulation of T cell apoptotic process”, and “Antigen receptor-mediated immune response” further support the dual immunoregulatory role of EGCG. Together, these enriched GO terms suggest that EGCG may not only function as a PD-L1 inhibitor, but also as a broader immunomodulatory agent, influencing multiple levels of immune signaling and homeostasis. The strong enrichment signals and low FDR values confirm the robustness of these findings and support

EGCG’s potential role in natural immune checkpoint modulation, particularly relevant in contexts such as cancer immunotherapy or autoimmune regulation.

4. Discussion

Immune checkpoint therapy has emerged as a revolutionary advancement in modern cancer treatment. Among various targets, the PD-1/PD-L1 pathway is widely recognized as a key mechanism by which cancer cells escape immune surveillance (Parvez et al., 2023). While antibody-based PD-L1 inhibitors, such as Atezolizumab, have shown clinical effectiveness, their high cost, injectable administration, and potential side effects highlight the need for alternative therapeutic strategies. In this context, naturally derived small molecules like Epigallocatechin gallate (EGCG) are being explored as promising alternatives (Gonçalves et al., 2021). EGCG is already known for its anti-cancer, anti-inflammatory, and immunomodulatory properties. Recent studies have reported that EGCG can downregulate PD-L1 expression, suggesting its potential role as a natural immune checkpoint inhibitor (Messeha et al., 2021; Ravindran Menon et al., 2021). However, direct interactions

between EGCG and PD-L1, along with their functional outcomes, remain underexplored. This study therefore aims to computationally investigate the interaction between EGCG and PD-L1, along with a detailed analysis of EGCG's drug-likeness, toxicity, molecular stability, and functional immunological relevance (Trivedi et al., 2022).

In this study, EGCG showed strong and specific binding to the PD-L1 active site, with a docking score of -8.1 kcal/mol. The ligand formed multiple hydrogen bonds with key residues such as GLU58, VAL76, and ARG113, which are located near the PD-1 interaction interface. This suggests that EGCG may effectively block PD-L1 from binding to PD-1, thereby disrupting immune evasion by cancer cells (Manica et al., 2022; Guo et al., 2023). Compared to Atezolizumab, which covers a broader surface area as a monoclonal antibody, EGCG fits precisely into the binding groove, allowing site-specific interactions with important residues (Li et al., 2021). Such binding makes EGCG a promising candidate for non-antibody immune checkpoint inhibition. Similar results were reported by Tang et al. (2024) and Manica et al. (2023), they observed stable hydrogen bonding and PD-L1 conformational shifts induced by EGCG, supporting its potential as a natural modulator. These consistent findings reinforce that EGCG may serve as a cost-effective, natural inhibitor of PD-L1 with therapeutic relevance in cancer immunotherapy (Manica et al., 2023; Tang et al., 2024).

The SwissADME analysis of EGCG revealed favorable drug-like properties, including moderate lipophilicity, an acceptable topological polar surface area (TPSA), and good gastrointestinal (GI) absorption. These parameters are essential for oral bioavailability, and EGCG demonstrated compatibility with Lipinski's Rule of Five, which supports its drug-likeness (Daina et al., 2017; Akter et al., 2022; Islam et al., 2023). Despite EGCG's relatively high polarity, which might slightly hinder passive membrane diffusion, its compatibility with PD-L1's polar binding site suggests a beneficial interaction that compensates for this limitation.

Studies also confirm EGCG's potential for oral administration. Kang et al. (2021) and Manica et al. (2024) noted that EGCG has promising bioavailability and formulation adaptability, making it suitable for drug development (Kang et al., 2021; Manica et al., 2024). Further, research by Mehmood et al. (2022) observed that flavonoids like EGCG often exhibit sufficient GI absorption when appropriately formulated (Mehmood et al., 2022). These computational predictions align with the present findings, reinforcing EGCG's suitability as a natural PD-L1 modulator with favorable ADME (absorption, distribution, metabolism, and excretion) profiles.

The ProTox-II server classified Epigallocatechin gallate (EGCG) as Toxicity Class 5, with an estimated LD₅₀ > 3000 mg/kg, indicating low acute toxicity and a favorable safety profile. Importantly, no

signs of hepatotoxicity, immunotoxicity, carcinogenicity, or cytotoxicity were predicted-factors that are essential when considering drug-likeness for long-term immunotherapy.

This *in silico* result is strongly supported by prior *in vivo* and *in vitro* findings. Hwang et al. (2023) showed that EGCG was non-toxic in animal models at therapeutic doses (Hwang et al., 2023). Similarly, Manica et al. (2024) and Yuliati et al. (2024) reported its non-mutagenic and non-carcinogenic nature, even under prolonged exposure (Manica et al., 2024; Yuliati et al., 2024). García-Rodríguez et al. (2021) demonstrated that EGCG exhibits no genotoxic effects in human cells, while Xu et al. (2021) observed high safety margins in sub-chronic toxicity studies (García-Rodríguez et al., 2021; Xu et al., 2021).

The Dynamic Cross-Correlation Matrix (DCCM) analysis revealed significant correlated motions within the EGCG-bound PD-L1 complex, particularly between residues 50-150 and 200-300. These regions exhibited intense red coloration, indicating that EGCG binding induced coordinated atomic fluctuations in key protein domains, which is often associated with ligand-mediated structural stability. Supporting this, the Normal Mode Analysis (NMA) showed a very low first eigenvalue of 7.41×10^{-5} , suggesting that the complex possesses high flexibility and can undergo conformational adjustments with minimal energy input. Such dynamic behavior is critical for functional modulation of immune checkpoint proteins.

These findings align with those of Guo et al. (2021) and Biswash et al. (2024), they reported that lower eigenvalues reflect greater dynamic adaptability in protein-ligand complexes, often leading to enhanced biological functionality (Journal of Molecular Modeling) (Guo et al., 2021; Biswash et al., 2024). Additionally, Gonzalez Suarez et al. (2022) emphasized that motion correlation between structural domains often stabilizes ligand binding and promotes allosteric effects-strengthening the interpretation that EGCG may modulate PD-L1 through both direct binding and dynamic reshaping (Gonzalez Suarez et al., 2022).

The GO-term enrichment analysis of genes associated with EGCG treatment revealed significant involvement in immune regulatory pathways, notably in "negative regulation of T cell activation," "T cell costimulation," and "regulation of interleukin-10 (IL-10) production." These enriched terms indicate that EGCG not only binds to PD-L1 but may also influence broader immunological processes, such as T cell differentiation and cytokine signaling.

This dual activity highlights EGCG's potential as both a checkpoint inhibitor and a functional immunomodulator. Yang et al. (2024) previously described EGCG as a potent natural compound capable of regulating T cell dynamics and cytokine-mediated responses, supporting its role beyond direct molecular interaction (Yang et al., 2024). Additionally, Singh et al. (2021) and Siddique et al. (2025) demonstrated that polyphenols like EGCG can modulate immune gene expression, particularly in pathways linked to adaptive

immunity and anti-inflammatory responses (Yahfoufi et al., 2018; Siddique et al., 2025). Taken together, the GO-enrichment data suggest that EGCG may exert multi-level immunological effects, providing not only PD-L1 inhibition but also reshaping immune responses at the gene regulation level.

In the growing field of cancer immunotherapy, the search for safe, accessible, and cost-effective immune checkpoint inhibitors remains a pressing challenge. This study addresses that gap by exploring the potential of EGCG, a naturally derived small molecule, as an alternative to antibody-based PD-L1 blockers. Instead of relying solely on traditional monoclonal antibodies, which are often expensive and require injectable administration, this research highlights the feasibility of plant-derived compounds for immunomodulation. The integrative computational approach—spanning structural interaction, dynamic behavior, toxicity, and functional annotation—provides a holistic view of how natural compounds can influence immune signaling. Importantly, this study opens a promising avenue for developing orally active, biocompatible therapeutics that align with both efficacy and safety requirements.

5. Limitations

While the study offers valuable computational insights into EGCG's interaction with PD-L1, it is limited by the absence of experimental validation. All analyses were performed *in silico*, and do not account for the complex biological environment *in vivo*. Factors like metabolic stability, bioavailability in tumor tissue, and immune system variability remain unaddressed. Additionally, the GO-term associations are predictive, not experimentally confirmed. Future work involving cell-based assays, animal models, and clinical data correlation is essential to validate and extend these findings toward therapeutic application.

6. Conclusion

This study highlights Epigallocatechin gallate (EGCG) as a promising natural small-molecule inhibitor of PD-L1, with potential application in cancer immunotherapy. Through integrated computational analyses, EGCG was shown to exhibit strong binding affinity, favorable drug-like properties, low toxicity, and possible roles in immune pathway modulation. Its interaction with key PD-L1 residues and dynamic behavior suggests functional relevance in checkpoint blockade. While these findings support EGCG's potential as a cost-effective and biocompatible immunomodulator, experimental validation is necessary. Overall, this research lays a solid foundation for the development of plant-based, non-antibody therapeutics targeting immune checkpoints in oncology.

S. H. conceptualized the study, designed the computational workflow, performed molecular docking and ADME/toxicity analyses, and drafted the manuscript. M.U. P. conducted molecular dynamics evaluation, functional annotation, and contributed to data interpretation and manuscript revision. M.A. S. assisted in structural preparation, ligand optimization, and validated the docking methodology. H.M. A. contributed to results analysis, visualization, and critical revision of the final manuscript. All authors reviewed, edited, and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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