

Identification of phyto-immunomodulators of CD8A and PTPRC from *Schinus molle* as potential therapeutics of chronic chagasic cardiomyopathy via integrative transcriptomics and molecular dynamic simulation approaches

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Abstract: The most terrifying cardiac complication of *Trypanosoma cruzi* infection is Chronic Chagasic Cardiomyopathy (CCC), which is the complication that occurs in about one-third of the infected individuals and is one of the major causes of non-ischemic heart failure in Latin America. Although fairly disease-burdening, the molecular pathways of CCC immunopathology are not fully studied, and disease-modifying therapy is currently lacking. This study used an integrative computational strategy involving RNA-seq transcriptomics, protein-protein interaction (PPI) network analysis, gene ontology and KEGG pathway enrichment, molecular docking, ADMET profiling, and molecular dynamics simulations to define the host immune transcriptional environment of CCC and determine which phytochemicals may regulate its major regulation targets. RNA-seq analysis of severe CCC cardiac tissue compared to healthy controls (GEO: GSE191081) found 2,973 significantly differentially expressed genes (DEGs), 2,239 of which were upregulated, and 734 of which were downregulated. The analysis of functional enrichment showed that the upregulated genes were most linked to T cell activation, antigen receptor-mediated signaling, cytokine production, and MHC complex assembly, whereas downregulated genes were enriched in potassium ion transport, sarcoplasmic reticulum functioning and cardiomyocyte survival pathways. Multi-algorithm hub gene analysis with cytoHubba on PPI networks revealed 5 core regulatory genes, including CD4, PTPRC, CD8A, IFNG and FCGR3A, that were invariably within the top five network topologies of all five topological models (Degree, MCC, MNC, Closeness, and EPC). Among them, CD8A (PDB: 1CD8) and PTPRC (PDB: 1YGR) were chosen to be screened virtually against 110 drug-like phytochemical compounds of *Schinus molle* downloaded in the IMPPAT database with and without structure. The highest-ranking compound found in molecular docking relative targeting PTPRC (−7.70 kcal/mol) and CD8A (−6.94 kcal/mol), IMPHY006486, was also found to have strong binding affinities with diverse interactions with the essential active site residues, such as Ser928, Gln882, Phe890 (PTPRC) and Thr30, Phe48, Tyr51 (CD8A). Favorable pharmacokinetic properties of the lead compounds including high intestinal absorption, lack of risk of CYP-mediated

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drug interaction and negative AMES mutagenicity were confirmed by ADMET profiling. All four protein-ligand complexes were found to be structurally stable at 100 nanoseconds in molecular dynamics simulations, with moderate backbone RMSD (1.52–2.82 Å), maintained components of secondary structure, and maintained protein-ligand interaction fractions over the trajectories. Such results indicate that IMPHY006486, IMPHY004111, and IMPHY003082 emerged as computationally prioritized candidates for further experimental evaluation as potential modulators of CD8A and PTPRC. They should be validated experimentally to demonstrate their therapeutic potential. This is a systems-level and structure-based study that points out important immune hubs in the development of CCC pathogenesis and shows how combined transcriptomics and molecular modeling can identify immunomodulatory candidates faster in the context of Chagas heart disease.

Keywords: Chronic chagasic cardiomyopathy, *Trypanosoma cruzi*, differential gene expression, protein-protein interaction network, hub genes, molecular docking

1 Introduction

Chagas disease or American trypanosomiasis is a neglected tropical disease that can be life-threatening and is caused by a protozoan parasite called *Trypanosoma cruzi*. It is estimated that over 7 million individuals across the world are infected with *T. cruzi* causing over 10,000 deaths annually with the disease being prevalent in endemic regions of 21 continental Latin American nations (Agbajelola et al., 2025). Since then, the disease has become a worldwide public health concern because of migration and environmental modification with its burden worldwide approximated at more than 6.4 million cases as of 2019 (Pérez-Molina & Molina, 2018). Transmission is mainly caused by the feces of the infected triatomine insects (kissing bugs) in contact with the vectors of the disease, but congenital transmission, blood transfusion, organ transplantation, and oral intake of contaminated food also form part of the known routes of infection in endemic regions throughout the Americas (Gómez-Ochoa et al., 2022).

Chagas disease is a biphasic clinical disease. The acute phase, which is typified by high parasitemia, is usually asymptomatic or presents with generic symptoms such as fever, malaise and tachycardia. In 90% of cases these manifestations disappear spontaneously and in the indeterminate form about 60–70 percent of infected individuals do not develop signs and symptoms of the disease. The rest of the patients (30–40%) can enter chronic stage, which covers cardiac, digestive, or neurological problems (Norman & López-Vélez, 2019). The most severe form of the disease is Chronic Chagasic Cardiomyopathy (CCC) that occurs in one-third of positive serology cases and in severe cases, transplant is the only treatment method (Nunes et al., 2021). The most prevalent etiology of myocarditis in the world is the CCC, which is the most significant cause of non-ischemic cardiomyopathy in endemic areas of Latin America with the mortality rate varying between 10–1% and up to 84% in high-risk patients (Bilate & Cunha-Neto, 2008).

The CCC pathogenesis is also multifactorial and not fully understood, an intricate combination of persistence of the

parasite, auto-immune mechanisms, micro-vascular dysfunction, and neuro-genic dysregulation of the myocardium. Both murine model and human evidence indicate that an autoimmune response, and an immune response evoked by a parasite which entails the production of inflammatory cytokines and chemokines, may contribute to the production of the heart lesions which result in CCC (Cunha-Neto & Chevillard, 2014). Histological examinations of the CCC show that relative to non-Chagas dilated cardiomyopathy, CCC is linked with many CD8+, memory T cells, B cells, macrophages, and mast cells that infiltrate cardiac tissue, all capable of causing tissue damage (Duque et al., 2025). In the peripheral blood, an increased concentration of proinflammatory cytokines such as IFN- γ , TNF- γ , IL-1 β as well as nitric oxide has consistently been detected in patients with CCC, which once again supports the immunopathological status of the disease and the important role of innate and adaptive immune cascades in CCC progression (do Vale et al., 2024).

One of the main immune regulators was identified as a highly upregulated cytokine in CCC pathogenesis, which IFN γ , with a maximum increase of 56-fold twice as high as in healthy individuals, is attracted by differentiation of CD4+ Th1, and CD8+ T cells and at the same time contributes to heart damage by various pathological mechanisms (Gómez et al., 2023). CD4+ and CD8+ T lymphocytes are the major effector populations in this immunopathological process, where *T. cruzi* infection induces the changes in CD4+ T and B cell compartments that are more significant in the mild CCC clinical form, implying the existence of orderly cellular communication in Chagas disease (Pereira et al., 2018). (CD45) an important controller of T cell receptor signaling and lymphocyte activation, and FCGR3A (CD16a), an Fc receptor found on natural killer cells and monocytes, have also become molecules of interest in the immunoinflammatory cascades that are typical of CCC. CCC cardiac tissue weighted gene co-expression network analysis has indicated that differentially expressed genes mainly concern T-cell signaling pathways and are closely related to the availability of T-cells (CD8+) in the myocardium (Zhou et al., 2021).

Although there are antiparasitic agents including benznidazole and nifurtimox, which have been proven to be most effective when used in the acute phase, there is no disease-modifying therapy now in relation to CCC. Compared to other types of cardiomyopathies, the prognosis of patients with Chagas heart disease is unfavorable and sudden cardiac death is a significant cause of mortality, and diffuse myocarditis is fibrotic and hypertrophic in most patients (Bocchi et al., 2017). These constraints highlight an urgent requirement of innovative immunomodulatory therapeutic protocols against the molecular pathways of cardiac immunopathology.

Phytochemicals that are of plant origin have become a promising source of bioactive substances with immunomodulatory, anti-inflammatory, and antiparasitic effects. *Schinus molle* (Peruvian pepper tree) is a common plant throughout Latin America that has been thoroughly researched due to the wide range of secondary metabolites found, and these include terpenoids, flavonoids, and polyphenols. Phytochemicals of *Schinus molle* have been shown to possess the ability to inhibit disease-relevant protein targets in silico and the compounds have good drug-likeness and pharmacokinetic properties, which can be developed into new drugs (Brochet et al., 2022; Kumar et al., 2023). Computational methodology has revolutionized drug discovery at early stages through the combination of computational techniques such as molecular docking, ADMET profiles and molecular dynamics of drug-target interactions at atomic scale, have been facilitated by computational technology (Sliwoski et al., 2014).

The present work has successfully used an integrative computational model to decipher the host immune transcriptional pattern of CCC and provide its important points of therapeutic targets at the molecular level. Based on publicly available bulk RNA-sequencing of severe CCC cardiac tissue and healthy controls (Gene Expression Omnibus GSE191081 accession), the data was used to identify and characterize differentially expressed genes based on gene ontology and KEGG pathways enrichment analysis. Topological analysis and protein-protein interaction (PPI) network construction using cytoHubba revealed that five hub genes CD4, PTPRC, CD8A, IFNG and FCGR3A were core central immune regulatory nodes in CCC pathogenesis. Among them, CD8A and PTPRC were chosen, which was used in the structure-based molecular docking with phytochemical compounds provided in the IMPPAT database by *Schinus molle*. Possibly promising lead compounds were then screened based on ADMET pharmacokinetic properties and 100-nanosecond molecular dynamics studies were performed to determine the structural stability of protein-ligand complexes. This pipeline of integration offers a basis on which the development of phytochemical-based immunomodulatory therapeutics can be rational towards the key immune effector nodes of Chronic Chagasic Cardiomyopathy.

2 Materials and Methods

2.1 Source and Preprocessing of RNA-seq Expression Data

The RNA-seq dataset used in this study (GEO accession: GSE191081) includes human cardiac tissue samples from control individuals and patients with Chronic Chagasic Cardiomyopathy (Edgar et al., 2002). For the present analysis, a subset of fourteen samples (6 controls and 8 severe CCC cases) was selected to ensure a biologically consistent comparison between healthy and advanced disease states. Although the sample size is relatively limited, this reflects the practical challenges associated with obtaining high-quality human cardiac tissue biopsies. Similar sample sizes have been reported in previous CCC transcriptomic studies (Brochet et al., 2022; Love et al., 2014). To ensure robustness, stringent statistical thresholds and downstream validation analyses were applied. Before any further analysis, the raw count data matrices were processed for quality control to filter out genes that are persistently low in expression across samples, thus eliminating noise in the data. Normalization of the library size differences among samples was done using the DESeq2 package in R to correct for any differences in library sizes among samples (Conesa et al., 2016; Love et al., 2014). Principal component analysis (PCA) was then carried out to determine the distribution of the data and the clustering of the samples (Jolliffe & Cadima, 2016).

2.2 Identification of Differentially Expressed Genes

The analysis of differential gene expression was performed using the DESeq2 package (Love et al., 2014), which uses a negative binomial generalized linear model to identify statistically significant differences in gene expression between experimental groups. Genes that fulfilled the threshold criteria of an adjusted p-value < 0.05 and an absolute log₂ fold change ≥ 1 were identified as differentially expressed genes (DEGs). DEGs were further categorized as up-regulated or down-regulated based on the sign of the fold change. The expression profiles were then represented using volcano plots and hierarchical heatmaps to aid in the interpretation of expression profiles (Gu et al., 2016; Villanueva & Chen, 2019).

2.3 Gene Ontology and Pathway Enrichment Analysis

To better understand the biological implications of the identified DEGs, functional annotation and pathway enrichment analysis was performed using the Enrichr tool (Kuleshov et al., 2016). The study included Gene Ontology (GO) terms, which included biological processes (BP), molecular functions (MF), and cellular components (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment (Ashburner et al., 2000; Kanehisa & Goto, 2000). GO terms with a false discovery rate (FDR) of <0.05 were considered statistically significant and were used for biological interpretation. The results of the pathway enrichment analysis were visualized using bar charts and bubble plots to give a clear insight into the overrepresented biological themes (Yu et al., 2012).

2.4 Construction and Topological Analysis of the Protein–Protein Interaction Network

To explore possible functional interactions between proteins encoded by the up-regulated DEGs, a protein-protein interaction (PPI) network was built using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (Szkarczyk et al., 2021). Only interactions with a combined confidence score of ≥ 0.7 were considered to ensure that high-confidence interactions were included. The resulting network was then imported into Cytoscape software (version 3.9.1) for visualization and topological analysis (Shannon et al., 2003). Important topological parameters, such as degree centrality, betweenness centrality, and closeness centrality, were calculated to assess the relative importance of individual nodes and to identify proteins that may have critical regulatory functions in the network (Chin et al., 2014).

2.5 Hub Gene Prioritization Using Multi-Algorithm Network Scoring

The hub genes were obtained from the PPI network developed using the CytoHubba tool in Cytoscape (Chin et al., 2014). To make the identification of hub genes more reliable, five different algorithms for network topology were used: Degree, Maximal Clique Centrality (MCC), Maximum Neighborhood Component (MNC), Closeness Centrality, and Edge Percolated Component (EPC) (Chin et al., 2014). Genes that were ranked as top candidates in the majority of these algorithms were considered as hub genes, indicating their potential role in regulating important biological processes. The biological relevance of the hub genes identified was further validated by a literature search of published studies (Oulas et al., 2019).

2.6 Structure Retrieval and Preparation

Among the hub genes identified, CD8A and PTPRC were chosen for structure-based molecular docking due to their significant immunoregulatory functions in T-cell-mediated myocardial inflammation associated with Chronic Chagasic Cardiomyopathy (CCC). The choice was further supported by the availability of suitable experimentally resolved crystal structures in the Protein Data Bank for computational analysis (Li et al., 2023). CD8A was prioritized due to its significant role in activating cytotoxic T-lymphocytes and mediating MHC class I-related immune responses tied to cardiomyocyte injury in CCC. Similarly, PTPRC, an essential regulator of leukocyte signaling and T-cell activation, was selected due to its involvement in immune cell regulation during chronic inflammatory responses (Connolly et al., 1990). Although other hub genes such as CD4, IFNG, and FCGR3A exhibited biological relevance, they were not included in downstream docking analyses because of limitations related to structural completeness, availability of suitable experimentally resolved structures, or the absence of well-defined regions amenable to reliable small-molecule interaction analysis. The structures of the hub gene proteins were sourced from the Protein Data Bank (PDB) under IDs 1CD8 and 1YGR. For CD8A (PDB ID: 1CD8), the crystallographic structure comprises a monomeric single chain (chain A; 114 amino acids),

utilized for both molecular docking and dynamics simulations. While CD8 operates as a dimeric co-receptor on T cells, this specific structure offers a well-resolved extracellular domain ideal for structure-based virtual screening. Water molecules and heteroatoms were removed, followed by optimization which involved adding polar hydrogens and Gasteiger charges using AutoDockTools (Huey & Morris, 2008). The optimized structures were then saved in pdbqt format for further experiments.

2.7 Compound Retrieval and Preparation

Phytochemical compounds from *Schinus molle* were sourced from the IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database (Mohanraj et al., 2018). A total of 407 compounds were initially gathered; however, after removing duplicates, the final count was reduced to 200 unique compounds. These compounds underwent further screening utilizing drug-likeness filters that are in accordance with Lipinski's rule of five. This screening process effectively narrowed the selection down to 110 potential ligands that are deemed appropriate for subsequent docking studies.

2.8 Molecular Docking

Molecular docking was conducted using Autodock Vina (Trott & Olson, 2010; Morris et al., 2008) to analyze the interaction of compounds with hub genes CD8A and PTPRC. For CD8A, the docking grid was specifically focused on the protein–protein interface to precisely evaluate ligand binding at the active site, with the grid defined at coordinates ($x = 12.450$, $y = -4.210$, $z = 18.630$) and a size of $40 \times 40 \times 40$ Å for comprehensive coverage of the interface binding residues.

This grid size was designed to cover the entire interaction interface related to CD8A activity, enabling adequate conformational sampling of ligands in the key binding area. Molecular docking for PTPRC was also conducted, focusing on its binding interface region. The docking grid was centered on key PTPRC interface residues critical for receptor recognition and signal transduction, with a grid size configured to encompass the PTPRC binding interface at coordinates $x = -15.720$, $y = 22.310$, and $z = 5.840$. An exhaustiveness value of 16 was assigned, generating 10 conformations for each compound. Post-docking, compounds were ranked according to their binding affinity (kcal/mol).

2.9 ADMET

ADMET analysis was conducted to assess the absorption, distribution, metabolism, excretion, and toxicity of selected compounds, as well as their drug-likeness and pharmacokinetic properties, utilizing the pkCSM web server (Pires & Ascher, 2015).

2.10 Molecular Dynamics (MD) Simulations

Molecular dynamics simulations were conducted to evaluate the conformational stability and dynamic interaction of protein–ligand complexes featuring CD8A and PTPRC. The chosen phytochemicals were selected based on their advantageous docking and ADMET profiles, serving as

the initial ligand structures. Simulations utilized GROMACS 2025.4 (Van Der Spoel et al., 2005) alongside the CHARMM36 March 2025 force field, (Huang & MacKerell, 2013) with ligand topologies and parameters generated via the CGenFF server (Vanommeslaeghe et al., 2012). Each protein–ligand complex underwent a series of preparatory steps prior to molecular dynamics (MD) simulations. Initially, the complexes were placed in a cubic simulation box and solvated with an explicit water model, incorporating counter ions to achieve system neutrality. Following this setup, energy minimization was executed to eliminate steric clashes and unfavorable interactions. Equilibration of the system was conducted using NVT and NPT ensembles to stabilize temperature and pressure. Finally, the production MD simulations were carried out for 100 ns under periodic boundary conditions. For post-simulation trajectory analysis and protein–ligand interaction profiling, the generated GROMACS trajectories were further processed using the DESMOND Simulation Interaction Diagram analysis tool. The MD simulations in this study served as a preliminary computational assessment aimed at evaluating complex stability and the persistence of interactions.

3 Results

3.1 Identification of Differentially Expressed Genes

To identify the transcriptional changes that occur in Chronic Chagasic Cardiomyopathy (CCC), publicly available bulk RNA-seq data were obtained from the NCBI Gene Expression Omnibus (GEO) with accession number GSE191081.

This dataset includes heart tissue RNA-seq profiles of patients with severe CCC (sevCCC) and healthy controls, which were sequenced using the Illumina NovaSeq 6000 platform. For this analysis, a subset of fourteen biologically relevant samples was selected: six control samples (GSM5738425–GSM5738430) and eight severe CCC samples (GSM5738439–GSM5738446), which correspond to the condition labels “Control” and “CCC,” respectively. All analyses were limited to these fourteen wild-type samples to provide a biologically meaningful comparison between healthy cardiac tissue and cardiac tissue of patients with advanced disease.

Differential expression analysis was conducted using the DESeq2 package in R on the raw count matrix. Genes with low expression across all samples were filtered before analysis. Statistical inference was performed using a negative binomial generalized linear model, with p-values adjusted for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) procedure. Genes that satisfied the criteria of adjusted p-value < 0.05 and $|\log_2 \text{fold change}| \geq 1$ were defined as statistically significant and biologically meaningful differentially expressed genes (DEGs).

A total of 2973 genes were found to be significantly differentially expressed in severe CCC compared to control heart tissue. To better understand the distribution and intensity of the transcriptional response, a volcano plot was generated with the $\log_2$ fold change on the x-axis and the $-\log_{10}$ adjusted p-value on the y-axis (Figure 1). Genes that are upregulated in CCC tissue are colored red (right side), while genes that are downregulated are colored blue (left side), and non-significant genes are colored grey. The extreme bias of the plot, where the majority of the significant genes are

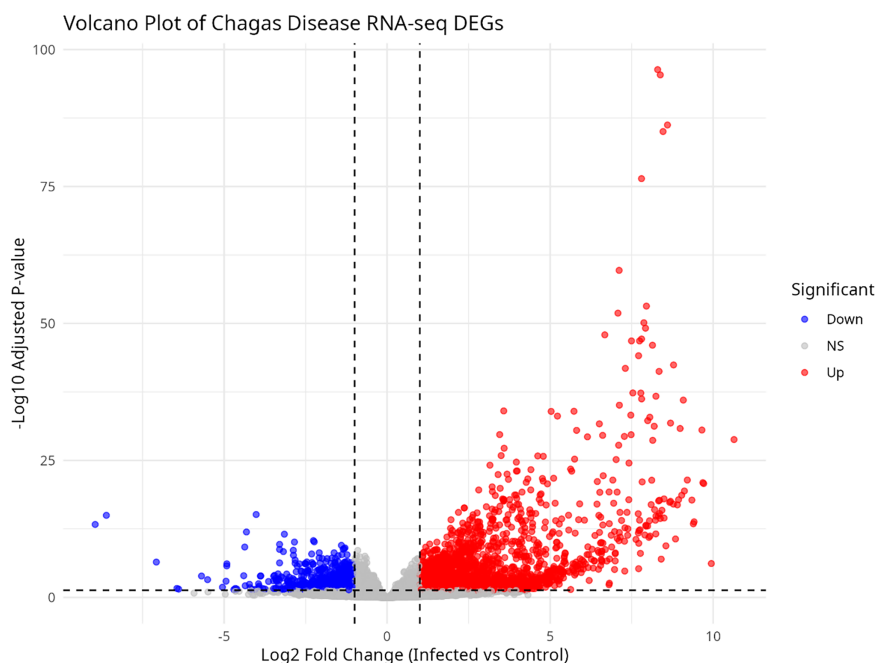


Figure 1. Volcano plot of differential gene expression between severe Chronic Chagasic Cardiomyopathy (CCC) and control heart tissue samples. Genes that are upregulated in CCC are represented in red, downregulated genes are represented in blue, and non-significant genes are represented in gray. The dashed vertical lines represent the threshold $|\log_2 \text{FC}| \geq 1$, and the dashed horizontal line represents the significance threshold for the adjusted p-value

on the upregulated side, suggests that a predominantly pro-inflammatory transcriptional response is present in cardiac tissue in CCC. Some genes with high fold change and high significance are visible at the top of the plot, suggesting an intense transcriptional response to the pathological insult.

3.2 Classification of Upregulated and Downregulated Genes

Of the 2973 DEGs, 2239 genes showed significant upregulation ($\log_2FC > 1$) in CCC heart tissue compared to controls, and 734 genes showed significant downregulation ($\log_2FC < -1$). The strong preponderance of upregulated genes indicates a high degree of transcriptional activation in the diseased myocardium, as would be expected in the chronic inflammatory environment that defines CCC. It is important to note that the DEGs be divided into these directional categories, since upregulated genes may represent the activation

of immune effector and inflammatory pathways, while down-regulated genes may represent the repression of processes that are critical to normal cardiac physiology.

3.3 Functional Annotation of Differentially Expressed Genes

To explore the biological meaning of the identified DEGs, Gene Ontology (GO) enrichment analysis was conducted separately for the upregulated and downregulated gene lists under the three major GO terms: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF).

3.3.1 Biological Process (BP) Enrichment

Biological process GO enrichment of upregulated genes showed a prominent pattern of adaptive and innate immune response activation (Figure 2A). The most significantly enriched terms included antigen receptor-mediated

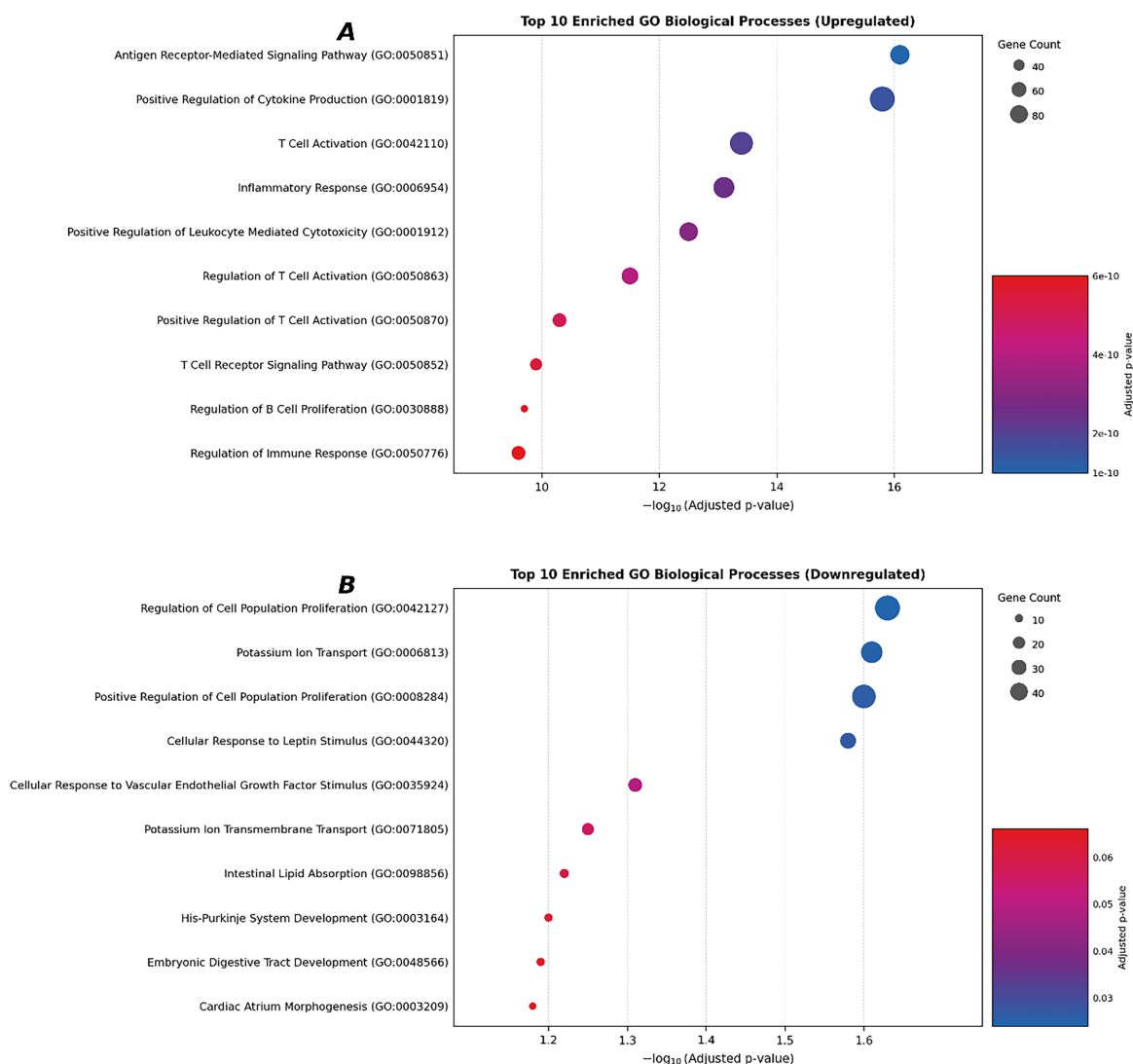


Figure 2. Gene Ontology (GO) biological process enrichment analysis of differentially expressed genes. (A) Top 10 most significantly enriched GO biological processes for upregulated genes in CCC, showing prominent activation of immune and inflammatory pathways. (B) Top 10 most enriched GO biological processes for downregulated genes, showing suppression of proliferative and cardiac development processes. The size of the dots corresponds to the number of genes, and the intensity of the color corresponds to the $-\log_{10}$ adjusted p-value

signaling pathway (GO:0050851), positive regulation of cytokine production (GO:0001819), T cell activation (GO:0042110), and inflammatory response (GO:0006954). Other terms such as positive regulation of leukocyte-mediated cytotoxicity (GO:0001912) and T cell receptor signaling pathway (GO:0050852) further emphasize the pivotal role of lymphocyte-mediated immunity in CCC pathogenesis. These data are consistent with the known role of T cell-mediated cardiac inflammation in the pathogenesis of myocardial damage in Chagas disease.

Conversely, the downregulated gene list was significantly enriched for terms associated with cellular proliferation and cardiac development (Figure 2B). Highly enriched terms included regulation of cell population proliferation (GO:0042127), potassium ion transport (GO:0006813), positive regulation of cell population proliferation (GO:0008284), and cellular response to leptin stimulus (GO:0044320). The downregulation of potassium ion transport and cardiac development indicates an impairment in the electrophysiological integrity and structural integrity of the failing CCC heart.

3.3.2 Cellular Component (CC) Enrichment

The enrichment analysis of upregulated genes for cellular components revealed the prominent enrichment of immune cell-related structures (Figure 3A). The topmost enriched term was MHC protein complex (GO:0042611), followed by MHC class II protein complex (GO:0042613), T cell receptor complex (GO:0042101), and phagocytic vesicle (GO:0045335). The enrichment of MHC protein complexes in upregulated DEGs indicates the increased antigen presentation function of immune infiltrating cells in the diseased myocardium. The enrichment of vesicular components like lumenal side of endoplasmic reticulum membrane (GO:0098553), specific granule membrane (GO:0035579), and secretory granule membrane (GO:0030667) indicates the increased immune cell secretory function.

The down-regulated DEGs were enriched in components typical of cardiomyocyte physiology (Figure 3B). The most significant term was voltage-gated potassium channel complex (GO:0008076), followed by transcription factor AP-1 complex (GO:0035976), cell-cell junction (GO:0005911), basolateral plasma membrane (GO:0016323), and sarcoplasmic reticulum membrane (GO:0033017). The reduction of sarcoplasmic reticulum and ion channel-related components is particularly striking, as these components are critical for the regulation of intracellular calcium dynamics and cardiac contractility. These findings suggest that CCC is linked to a progressive loss of cardiomyocyte-specific structural and functional compartments.

3.3.3 Molecular Function (MF) Enrichment

Enrichment of upregulated genes for GO molecular function also supported the prominent presence of immune receptor and antigen presentation pathways (Figure 4A). The most prominently enriched molecular functions were MHC class II protein complex binding (GO:0023026), cytokine

receptor activity (GO:0004896), and MHC protein binding (GO:0042287), together with non-membrane spanning protein tyrosine kinase activity (GO:0004715) and C-C chemokine receptor activity (GO:0016493). The prominent enrichment for MHC-related molecular functions supports the cellular component analysis and suggests that antigen presentation by infiltrating immune cells is a hallmark molecular process of CCC.

The downregulated genes were significantly enriched in molecular functions related to growth factor and hormone signaling, as well as ion channel activity (Figure 4B). The most important enriched terms included growth factor activity (GO:0008083), receptor ligand activity (GO:0048018), potassium channel activity (GO:0005267), DNA-binding transcription activator activity (GO:0001228), and vascular endothelial growth factor receptor 2 binding (GO:0043184). The reduction of potassium channel activity is in line with the electrophysiological defects found in CCC, while the reduction of transcription activator activity and growth factor-related functions may indicate a lack of compensatory myocardial remodeling capabilities.

Taken together, the results of the integrated GO enrichment analysis with respect to the BP, CC, and MF ontologies demonstrate a consistent and biologically valid transcriptional profile in CCC. The upregulated genes point to immune activation, antigen presentation, and inflammatory cytokine signaling pathways, whereas the downregulated genes indicate a systemic suppression of cardiomyocyte-specific electrophysiological, structural, and proliferative functions. This bidirectional gene expression profile is consistent with the immunopathological hypothesis of CCC, where the immune system causes progressive damage to the myocardium.

3.4 KEGG Pathway Enrichment Analysis

To better understand the functional significance of the identified DEGs in the context of known biological pathways, KEGG pathway enrichment analysis was carried out for the upregulated and downregulated gene lists separately (Figure 5).

Enrichment analysis of the upregulated gene list showed a preponderance of activation of immune recognition and inflammation pathways (Figure 5A). The most significantly enriched pathway was hematopoietic cell lineage, indicating a general differentiation and proliferation of immune cells within the diseased myocardium. Other highly enriched pathways included graft-versus-host disease, cell adhesion molecule (CAM) interaction, allograft rejection, intestinal immune network for IgA production, and antigen processing and presentation. The simultaneous enrichment of graft-versus-host disease and allograft rejection pathways, which are characteristic of alloreactive T cell responses, further supports the hypothesis that autoreactive lymphocyte responses play a role in cardiomyocyte injury in CCC. Type I diabetes mellitus, Th1/Th2 cell differentiation, and natural killer cell-mediated cytotoxicity pathways were also significantly enriched, underscoring the complex nature of the adaptive immune response in this disease.

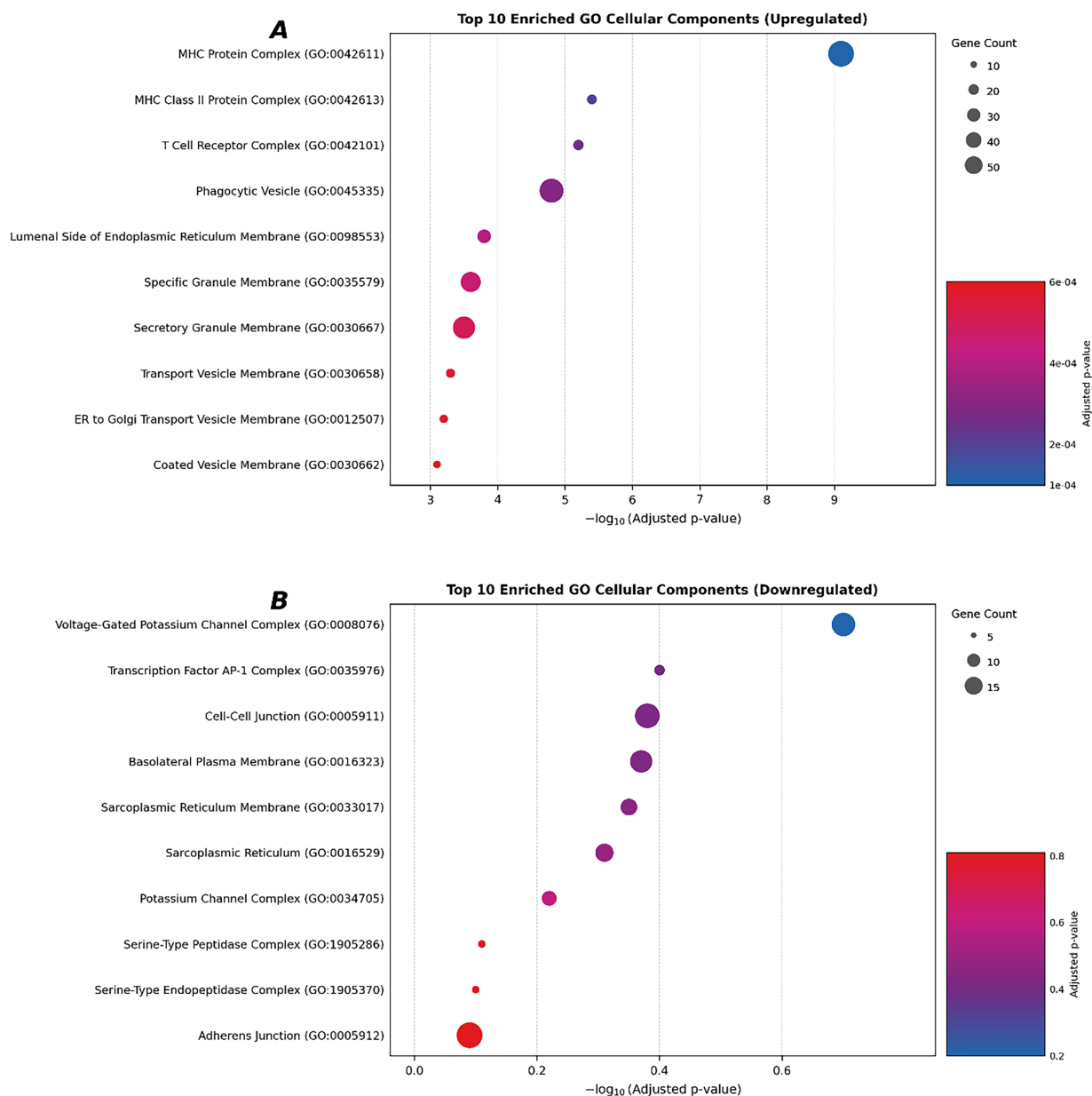


Figure 3. Gene Ontology (GO) cellular component enrichment analysis of differentially expressed genes. (A) Top 10 enriched GO cellular components for upregulated genes in CCC, characterized by immune cell antigen presentation and secretion. (B) Top 10 enriched GO cellular components for downregulated genes, indicating the reduction of cardiomyocyte ion channel and contractile apparatus components. Dot size corresponds to gene expression count, and color corresponds to $-\log_{10}$ adjusted p-value

Conversely, KEGG pathway enrichment for the downregulated genes showed that the pathways were enriched for processes that are central to cardiac function and signaling (Figure 5B). The most significantly enriched pathway was the MAPK signaling pathway, followed by the PI3K-AKT signaling pathway, both of which are crucial for cardiomyocyte survival and hypertrophic responses. Other significantly enriched terms included adrenergic signaling in cardiomyocytes, cytoskeleton in muscle cells, dilated cardiomyopathy, and hypertrophic cardiomyopathy, which directly relate the transcriptional downregulation seen in CCC to the structural and functional deficits that are characteristic of the end-stage disease. The downregulation of the cAMP signaling pathway

and the TNF signaling pathway also indicates the failure of neurohumoral and inflammatory resolution pathways in the CCC myocardium.

3.5 Protein-Protein Interaction Network Construction and Hub Gene Identification

To determine the most important regulatory nodes in the transcriptional response to CCC, a protein-protein interaction (PPI) network was built with the upregulated DEGs. The STRING database was queried to gather known and predicted interactions between the proteins encoded by the upregulated DEGs, and the resulting network was then loaded into Cytoscape. The upregulated gene set was chosen

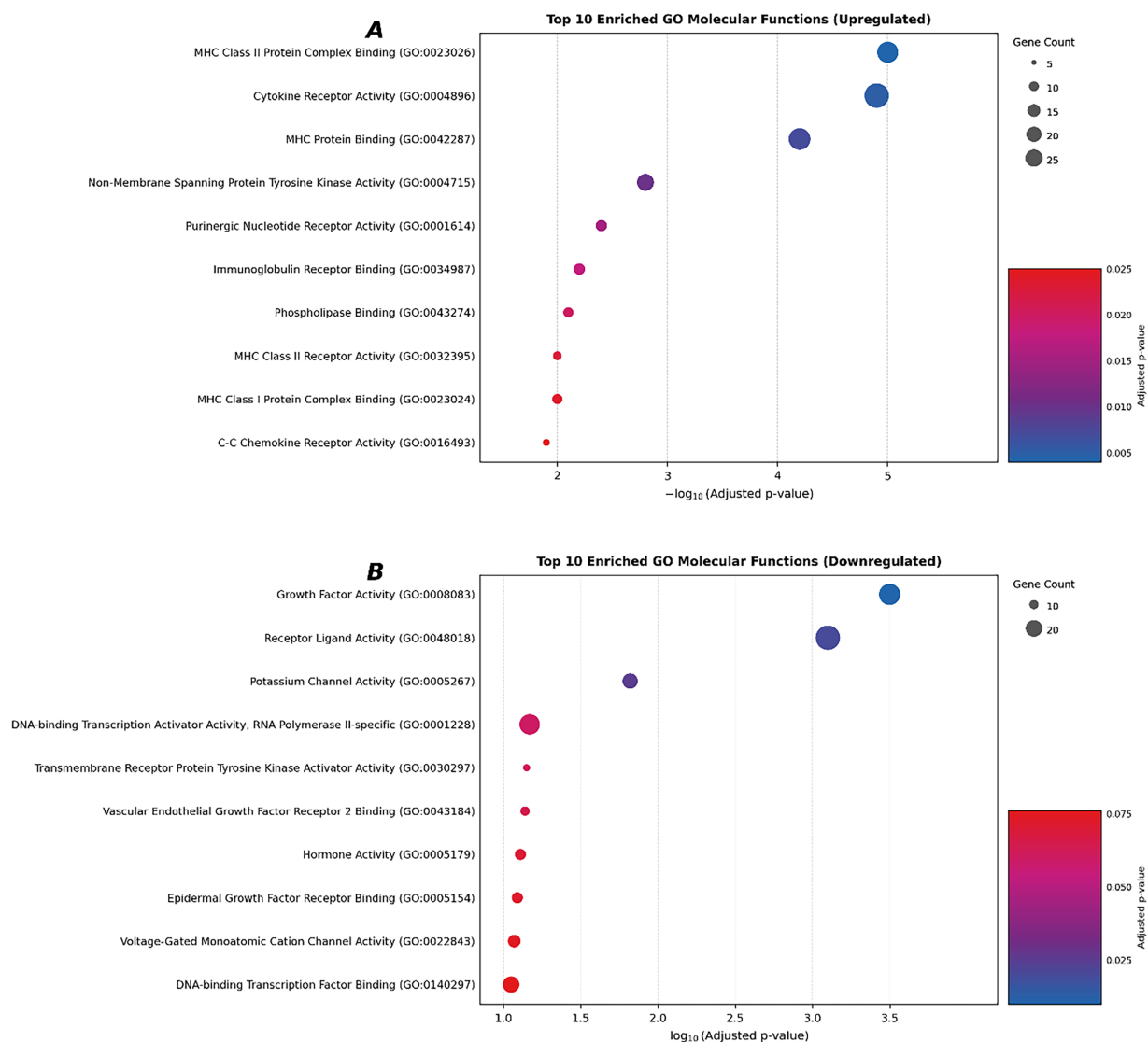


Figure 4. Gene Ontology (GO) molecular function enrichment analysis of differentially expressed genes. (A) Top 10 enriched GO molecular functions for upregulated genes, which are predominantly involved in MHC-related antigen presentation and cytokine receptor functions. (B) Top 10 enriched GO molecular functions for downregulated genes, which are suppressed for growth factor signaling and ion channel molecular functions. Dot size indicates gene number, and color indicates $-\log_{10}$ adjusted p-value

for network analysis because these genes represent the active transcriptional response to the pathological condition and are therefore more likely to encode proteins that interact functionally with one another in the context of immune activation and inflammation.

The identification of the hub gene was carried out using five different algorithms available in the cytoHubba Cytoscape tool: Degree, MCC (Maximal Clique Centrality), MNC (Maximum Neighborhood Component), Closeness, and EPC. These algorithms measure the centrality of nodes in the network based on different parameters. To identify the genes that are most likely to be central in the network based on different parameters, an UpSet plot was created to display the individual gene scores for each algorithm or their intersections (Figure 6).

Analysis of the UpSet plot showed that the Degree algorithm was the most successful in identifying the largest number of hub candidates (~10 genes), as shown in the horizontal bar chart on the left side of the UpSet plot. The intersection analysis showed that five genes were identified by all five centrality algorithms simultaneously, as shown by the tallest vertical bar (intersection size = 5) representing the connected dots for all five centrality algorithms CD4, PTPRC, CD8A, IFNG, FCGR3A. A second large intersection of four genes was also found among a subset of the algorithms (intersection size = 4), while each algorithm alone identified one additional unique gene (intersection size = 1 for EPC-degree, closeness-degree, and MNC-degree pairwise intersections). The genes identified by all five algorithms represent the most reliable candidates for hub genes, since their centrality is not dependent on any one algorithmic

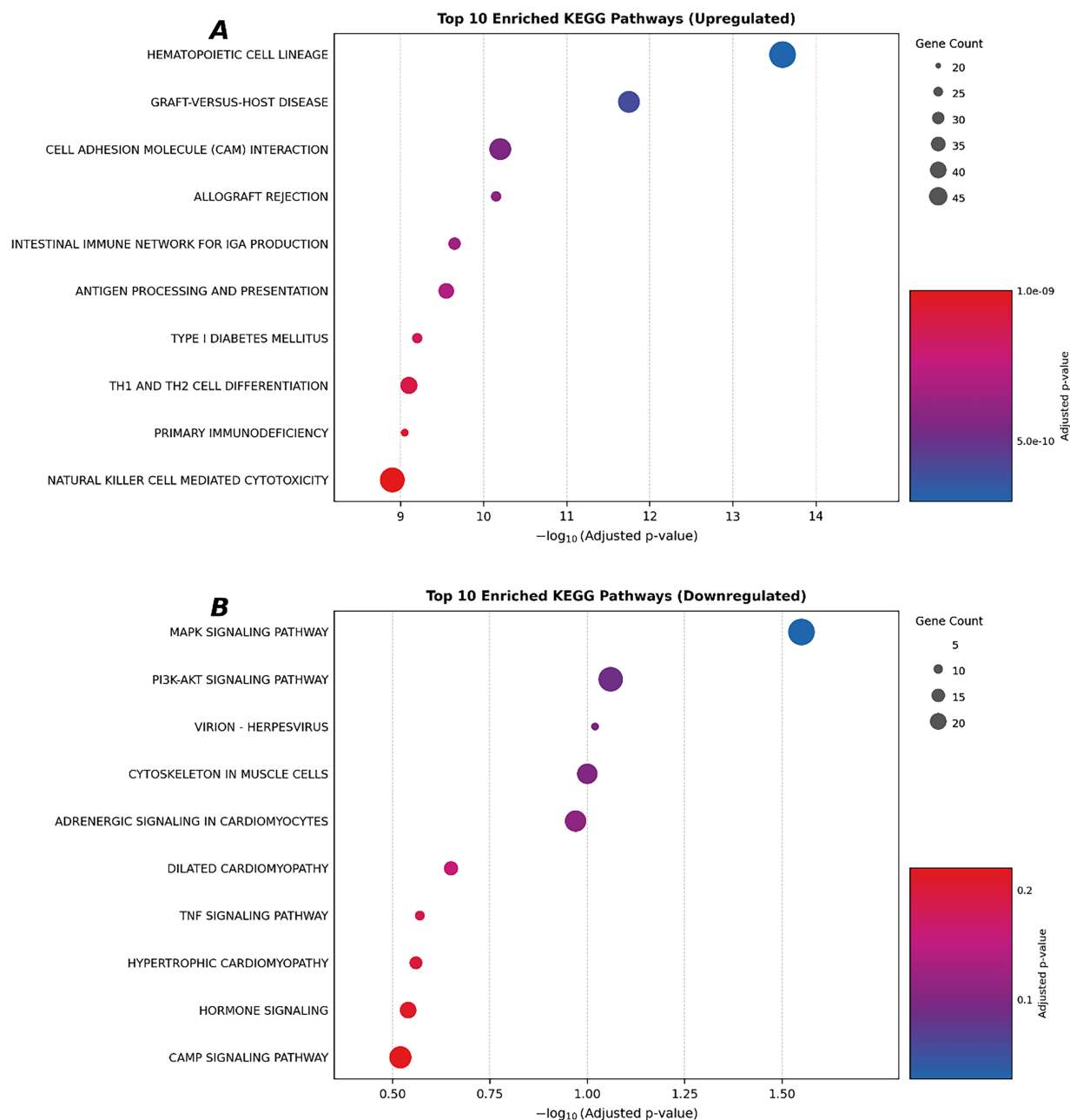


Figure 5. KEGG pathway enrichment analysis of differentially expressed genes in CCC. (A) Top 10 significantly enriched KEGG pathways for upregulated DEGs, focusing on immune cell differentiation and alloreactive immune responses. (B) Top 10 enriched KEGG pathways for downregulated DEGs, indicating the repression of key cardiomyocyte signaling and structural pathways. The size of the bubble corresponds to the number of genes, and the color corresponds to the $-\log_{10}$ adjusted p-value

assumption. These hub genes are topologically strategic in the upregulated PPI network and thus are most likely to play a coordinative role in the immune and inflammatory gene expression programs seen in CCC.

3.6 Molecular Docking

Molecular docking was utilized to evaluate the binding potential of selected phytochemicals sourced from the IMPPAT database against the crystal structures 1YGR (PTPRC/CD45) and 1CD8 (CD8A). The docking results indicated moderate to favorable predicted binding affinities for

the tested compounds, implying their potential interaction with the target regions of the proteins (Table 1). However, these docking scores are preliminary computational estimates and should not be considered as definitive evidence of stable biological binding or therapeutic efficacy. Details of the top screened phytochemicals are enlisted in Table S1 (Additional File 1). The reported RMSD values reflect the deviations of the docking poses generated by AutoDock Vina when compared to the top-ranked conformation. These values were included to evaluate the consistency of the predicted docking poses.

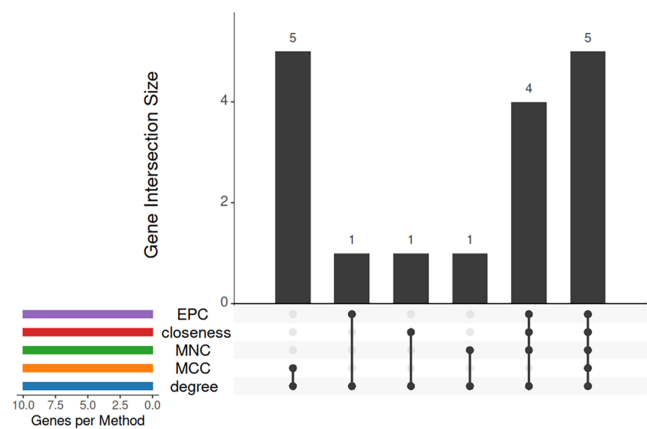


Figure 6. UpSet plot showing the intersection of hub genes identified by five cytoHubba network centrality algorithms (Degree, MCC, MNC, Closeness, EPC) on the upregulated DEG protein-protein interaction network. Vertical bars indicate the number of genes intersected by each combination of algorithms, while connected dots show the sets of algorithms that contributed to each intersection. Horizontal bars indicate the total number of hub candidates identified by each algorithm. The genes identified by all five algorithms represent the most consistently identified hub nodes in the CCC-associated transcriptional network

Table 1. Docking results of selected compounds with 1YGR and 1CD8 showing binding affinity, docking pose RMSD, and key protein-ligand interactions

1YGR			
Compound names	Binding affinity (kcal/mol)	RMSD	Interactions
IMPHY006486	−7.70	1.0	Leu926 (H-bond), Glu886 (H-bond), Gln882 (H-bond), Phe923 (π-alkyl), Pro927 (alkyl), Leu614 (van der Waals), Tyr617 (van der Waals), Lys618 (van der Waals)
IMPHY004111	−7.18	0.9	Ser928 (H-bond), Gln882 (H-bond), Phe890 (alkyl), Pro927 (alkyl), Leu614 (alkyl), Glu886 (van der Waals), Lys1181 (van der Waals), Tyr929 (van der Waals), Val885 (van der Waals)
IMPHY003082	−6.84	1.2	Ser928 (H-bond), Tyr929 (H-bond), Glu886 (H-bond), Arg925 (π-π T-shaped), Pro927 (amide-π stacked), Phe890 (π-alkyl), Leu614 (π-alkyl), Val885 (van der Waals), Gln882 (van der Waals)
IMPHY003696	−6.64	1.8	Leu926 (π-alkyl), Pro927 (π-alkyl), Pro803 (π-alkyl), Phe923 (alkyl), Arg925 (van der Waals), Gln924 (van der Waals), Ser928 (van der Waals), Tyr929 (van der Waals), Lys1177 (van der Waals)
1CD8			
IMPHY006486	−6.94	2.0	Thr30 (H-bond), Leu36 (π-alkyl), Tyr51 (π-alkyl), Phe48 (π-alkyl), Leu97 (van der Waals), Pro46 (van der Waals), Thr47 (van der Waals)
IMPHY003082	−6.74	2.6	Ser34 (H-bond), Met102 (H-bond), HOH229 (water-mediated H-bond), Phe48 (π-π stacked), Tyr51 (π-π stacked), Phe48 (π-lone pair), Leu97 (π-alkyl), Ser95 (van der Waals), Ala96 (van der Waals), Thr30 (van der Waals), Phe104 (van der Waals)
IMPHY006751	−6.44	1.2	Phe104 (π-alkyl), Leu36 (π-alkyl), Leu97 (π-alkyl), Tyr51 (π-alkyl), Pro46 (alkyl), Ser31 (van der Waals), Ser34 (van der Waals), Ala96 (van der Waals), Gly32 (van der Waals), Thr30 (van der Waals), Gln53 (van der Waals), Gln54 (van der Waals)
IMPHY004111	−6.32	1.1	Thr30 (H-bond), Leu36 (π-alkyl), Leu97 (π-alkyl), Tyr51 (π-alkyl), Met102 (π-alkyl), Ser34 (van der Waals), Gly32 (van der Waals), Phe48 (van der Waals), Thr47 (van der Waals)

For the 1YGR receptor, binding energies ranged from −7.70 kcal/mol to −6.64 kcal/mol. Notably, the compound IMPHY006486 exhibited the highest binding affinity of

−7.70 kcal/mol, coupled with a low RMSD of 1.02 Å, indicating a consistent docking pose within the docking protocol. In detailed interaction analysis, a significant

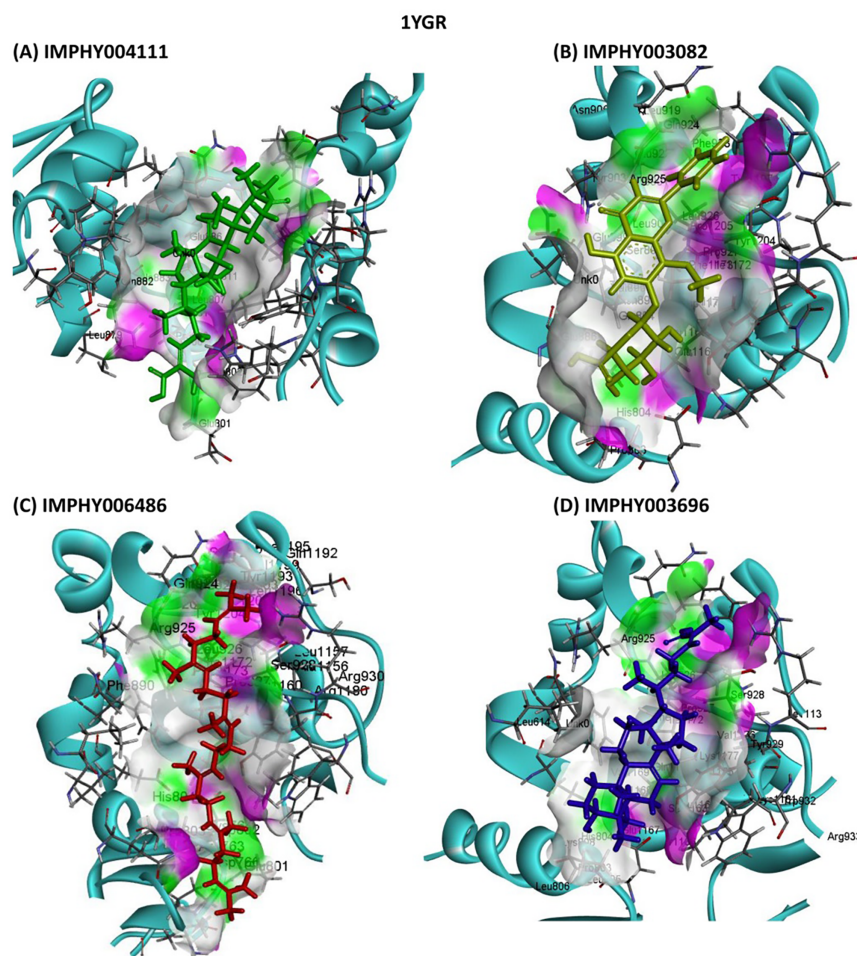


Figure 7. 3D interaction profile of the selected compounds against 1YGR based on the docking scores (Kcal/mol) and interactions. (A) IMPHY004111 (B) IMPHY003082 (C) IMPHY006486 and (D) IMPHY003696

amount of binding is seen for compound IMPHY004111 (−7.18 kcal/mol). Hydrogen bonds with Ser928 and Gln882, along with hydrophobic interactions with Phe890, Pro927, and Leu614, contribute to this interaction. In addition, Glu886, Lys1181, Tyr929, and Val885 provide van der Waals interactions in the binding pocket (Figures 7A, 8A). In a similar manner, IMPHY003082 showed various types of stabilizing interactions, including hydrogen bonding with Ser928, Tyr929, and Glu886, as well as a π - π T-shaped interaction with Arg925, amide- π stacking with Pro927, and π -alkyl interactions with Phe890 and Leu614, which altogether suggest a good level of ligand stabilization within the receptor binding cavity (Figures 7B, 8B). Similarly, it was found that IMPHY006486 forms several conventional hydrogen bonds with Leu926, Glu886, and Gln882. In addition, a π -alkyl interaction with Phe923 and an alkyl interaction with Pro927, along with van der Waals interactions with Leu614, Tyr617, and Lys618, contribute to the stability of the complex (Figures 7C, 8C). On the other hand, IMPHY003696 showed a slightly lower level of binding affinity, i.e., −6.64 kcal/mol, but was stabilized mainly through hydrophobic interactions, such as π -alkyl interactions with

Leu926, Pro927, and Pro803, as well as an alkyl interaction with Phe923, while Arg925, Gln924, Ser928, Tyr929, and Lys1177 showed van der Waals interactions (Figures 7D, 8D). Despite indications of potential ligand accommodation in the receptor interface from the interactions observed, the docking energies obtained are moderate, necessitating further computational and experimental validation.

The docking analysis with the ICD8 receptor indicated stable ligand-receptor interaction, with the binding affinity ranging from −6.94 to −6.32 kcal/mol. Among the ligands, IMPHY006486 recorded the highest affinity with the receptor, with a binding energy of −6.94 kcal/mol and was found to form conventional hydrogen bonds with Thr30, along with hydrophobic π -alkyl interactions with Leu36, Tyr51, and Phe48. The ligand was also found to be stabilized through van der Waals interactions with the surrounding amino acid residues such as Leu97, Pro46, and Thr47 (Figures 9C, 10C). The compound IMPHY003082 displayed different types of interactions, including hydrogen bonding with Ser34 and Met102, and a water-mediated hydrogen bond with HOH229. Additionally, π - π stacking interactions were observed with Phe48 and Tyr51, and a π -lone pair interaction with Phe48,

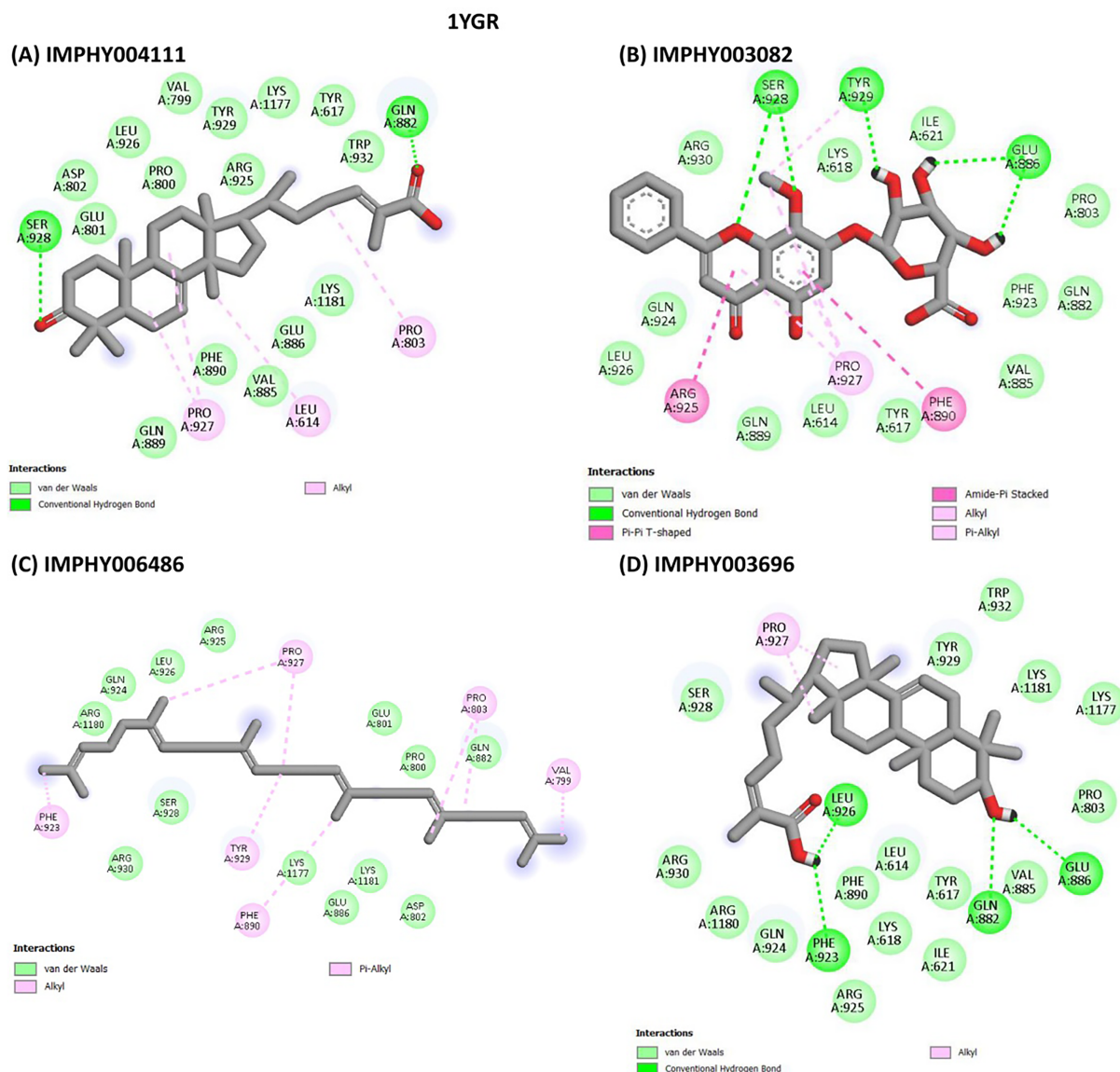


Figure 8. 2D interaction profile of the selected compounds against 1YGR based on the docking scores (Kcal/mol) and interactions. (A) IMPHY004111 (B) IMPHY003082 (C) IMPHY006486 and (D) IMPHY003696

indicating significant aromatic complementarity in the binding pocket (Figures 9B, 10B). The IMPHY006751 ligand binds through hydrophobic interactions such as π -alkyl interactions with Phe104, Leu36, Leu97, and Tyr51, as well as an alkyl interaction with Pro46. Other amino acid residues such as Ser31, Ser34, Ala96, Gly32, Thr30, Gln53, and Gln54 contribute through van der Waals interactions (Figures 9D, 10D). In the same way, the IMPHY004111 ligand is involved in a hydrogen bond with Thr30 and hydrophobic π -alkyl interactions with Leu36, Leu97, Tyr51, and Met102, as well as other van der Waals interactions with Ser34, Gly32, Phe48, and Thr47 (Figures 9A, 10A). The analysis of 1CD8 complexes indicates that while there is potential ligand compatibility with the receptor surface, the moderate binding affinities do not confirm robust inhibitory activity.

From the docking analysis, it is evident that the selected phytochemicals showed favorable predicted interactions

with both receptors through the formation of hydrogen bonds, hydrophobic interactions, π -stacking, and van der Waals forces. These findings offer initial computational evidence for potential interactions between phytochemicals and immune-related targets linked to Chronic Chagasic Cardiomyopathy. However, additional experimental validation and enhanced computational analyses are necessary before making claims about therapeutic applicability or biological efficacy.

3.7 ADMET Analysis

The ADMET profiling of five selected phytochemicals IMPHY006486, IMPHY004111, IMPHY003082, IMPHY003696, and IMPHY006751 showed notable differences in their pharmacokinetic and toxicity characteristics (Table 2). Among them, IMPHY003082 had the highest water solubility (log mol/L: -3.34), while

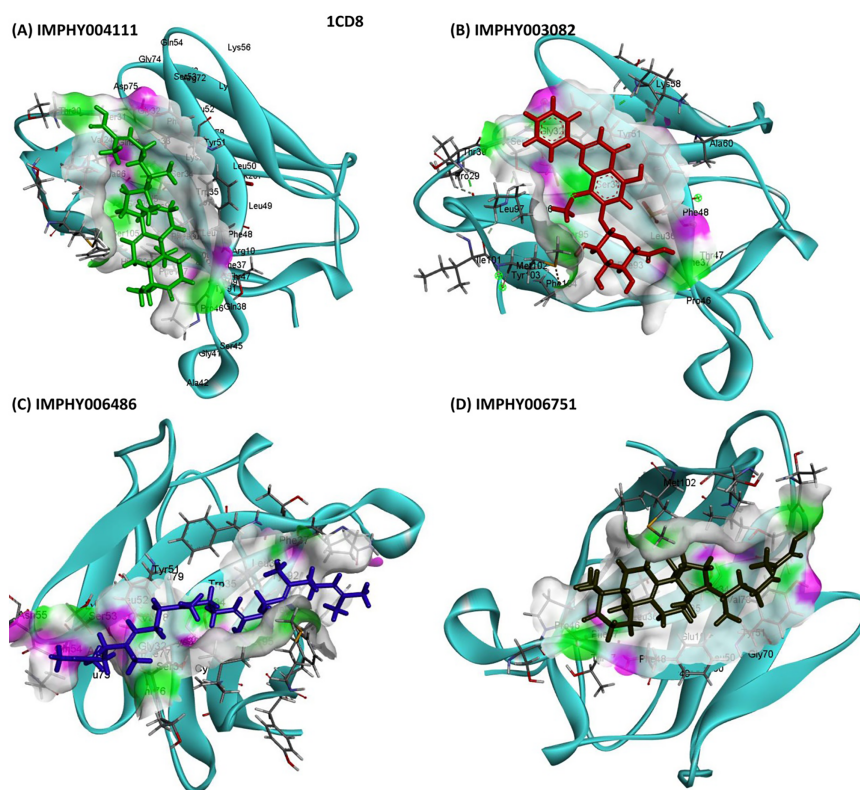


Figure 9. 3D interaction profile of the selected compounds against 1CD8 based on the docking scores (Kcal/mol) and interactions. (A) IMPHY004111 (B) IMPHY003082 (C) IMPHY006486 and (D) IMPHY006751

IMPHY006486 exhibited very low solubility (-8.244), which could negatively impact its dissolution, oral absorption, and bioavailability, indicating a need for formulation optimization or structural modification to enhance its pharmaceutical properties. The study on intestinal absorption of various compounds revealed that most compounds had satisfactory absorption rates, with IMPHY004111 exhibiting a complete 100% absorption rate. IMPHY003696 followed closely with an absorption rate of 97.41%, and IMPHY006751 at 97.616%. In contrast, IMPHY003082 demonstrated a significantly lower absorption rate of 46.181%. Notably, IMPHY003082 was identified as a substrate for P-glycoprotein, which may impact its bioavailability, unlike the other compounds which were found not to be substrates.

The predictions regarding blood–brain barrier (BBB) permeability indicate that IMPHY006486 has a high permeability score of 0.972, suggesting its ability to traverse the barrier effectively. In contrast, other tested compounds, such as IMPHY004111 (-0.279), IMPHY003082 (-1.501), IMPHY003696 (-0.323), and IMPHY006751 (-0.149), demonstrate significantly lower scores, implying they are likely restricted from entering the central nervous system (CNS). Cytochrome P450 profiling indicated that none of the tested compounds were identified as substrates or inhibitors for CYP2D6, and no inhibitory effects were noted for CYP1A2, CYP2C19, or CYP2C9. This suggests a lower likelihood of CYP-mediated drug–drug

interactions. Furthermore, the predicted total clearance values varied significantly, ranging from 0.287 to 1.791 log mL/min/kg, reflecting differences in metabolic handling across the compounds.

Toxicity predictions indicated that all compounds tested were non-mutagenic in the AMES test and did not inhibit hERG I. However, IMPHY006486 showed potential inhibition of hERG II, indicating a possible cardiotoxicity liability that should be carefully investigated through further in vitro or in vivo safety studies before therapeutic development. Furthermore, compounds IMPHY004111, IMPHY003696, and IMPHY006751 were predicted to possess hepatotoxic potential, suggesting a risk of liver-related adverse effects that may limit their clinical applicability unless confirmed and mitigated through subsequent optimization and experimental evaluation. In contrast, IMPHY006486 and IMPHY003082 were predicted to be non-hepatotoxic.

Overall, the ADMET analysis indicates that the investigated phytochemicals exhibit mixed pharmacokinetic and safety profiles suitable for preliminary computational screening. While certain compounds showed favorable absorption and low predicted mutagenicity, important liabilities were also identified. Specifically, IMPHY006486 demonstrated very low aqueous solubility and potential hERG II inhibition, whereas IMPHY004111, IMPHY003696, and IMPHY006751 exhibited predicted hepatotoxicity alerts. These findings highlight challenges related to bioavailability, cardiotoxicity, and hepatic safety that should not be overlooked during

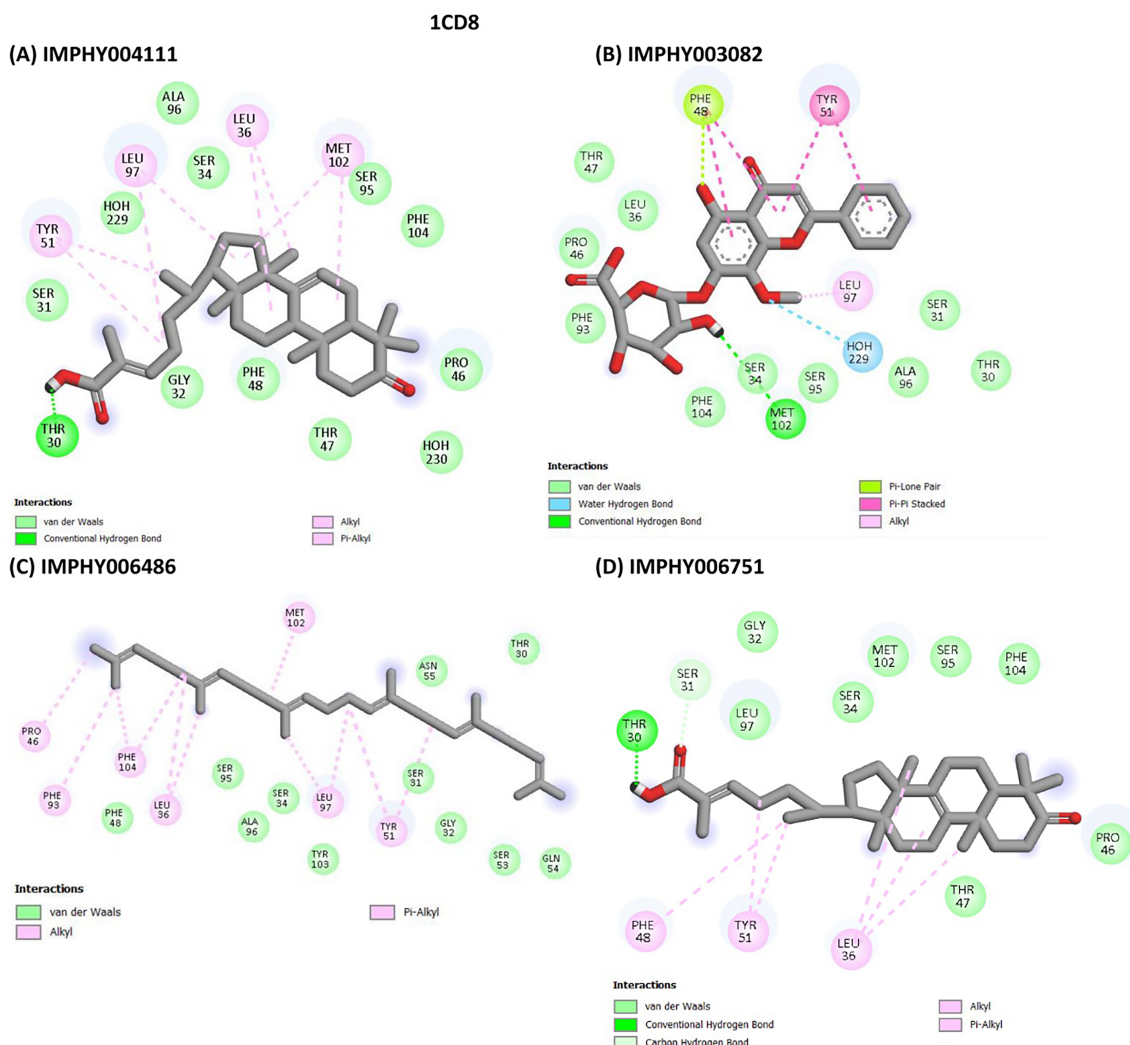


Figure 10. 2D interaction profile of the selected compounds against 1CD8 based on the docking scores (Kcal/mol) and interactions. (A) IMPHY004111 (B) IMPHY003082 (C) IMPHY006486 and (D) IMPHY006751

Table 2. ADMET profile of the selected compounds

ADMET properties	IMPHY006486	IMPHY004111	IMPHY003082	IMPHY003696	IMPHY006751
Water solubility numeric (log mol/L)	-8.244	-5.049	-3.34	-4.297	-5.165
Intestinal absorption (Numeric % Absorbed)	89.581	100	46.181	97.41	97.616
P-glycoprotein substrate	No	No	Yes	No	No
BBB permeability	0.972	-0.279	-1.501	-0.323	-0.149
CYP2D6 substrate CYP2D6 inhibitor	No	No	No	No	No
CYP1A2 CYP2C19 CYP2C9 inhibitor	No	No	No	No	No
Total clearance numeric (log mL/min/kg)	1.791	0.332	0.287	0.384	0.338
AMES toxicity	No	No	No	No	No
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	Yes	No	No	No	No
Hepatotoxicity	No	Yes	No	Yes	Yes

lead optimization. Consequently, these compounds should be regarded as preliminary computational candidates that necessitate further optimization and experimental pharmacological validation rather than being deemed established therapeutic agents.

3.8 Molecular Dynamics (MD) Simulations

To evaluate the dynamic characteristics of docked complexes, molecular dynamics simulations lasting 100 nanoseconds were conducted on ligand–protein systems that included IMPHY004111 and IMPHY006486, utilizing the crystal

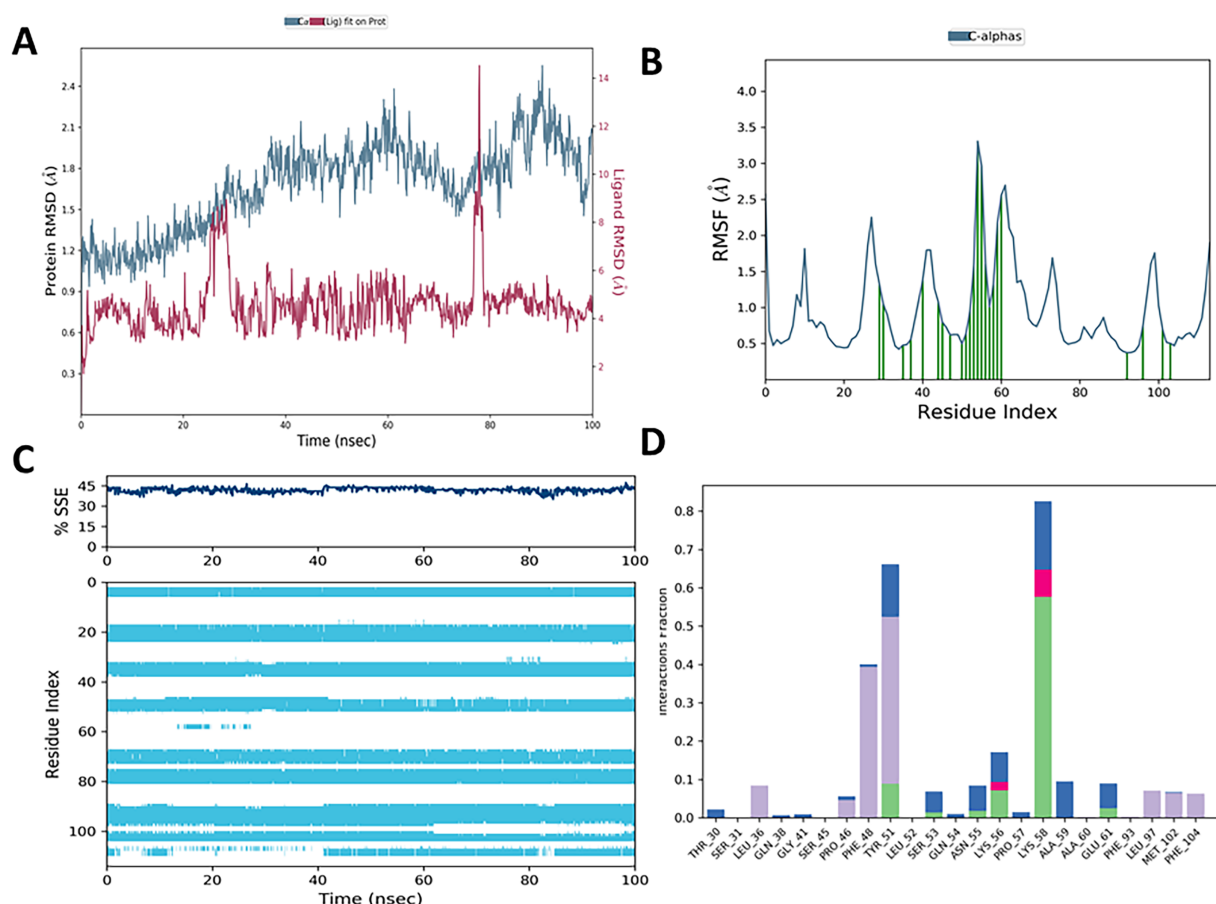


Figure 11. MD simulation of IMPHY004111-1CD8 complex. (A) RMSD showing stability of the protein-ligand complex during the 100 ns simulation (B) RMSF indicating residual flexibility (C) Secondary structure analysis showing structural stability of the protein (D) Protein-ligand interactions fractions highlighting key residues involved in binding

structures 1CD8 and 1YGR. The analysis included metrics such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), secondary structure element (SSE) stability, and profiling of protein-ligand interactions. The MD simulations aimed to preliminarily assess conformational stability and interaction persistence under simulated physiological conditions.

3.8.1 MD Simulation of IMPHY004111-1CD8 Complex

The RMSD profile of the IMPHY004111-1CD8 complex demonstrated stable structural behavior during a 100 ns simulation trajectory. The protein backbone RMSD initially increased then stabilized between 1.5–2.2 Å during the equilibration phase, indicating that the receptor preserved structural integrity while binding the ligand. The ligand's RMSD values showed minimal fluctuations, suggesting a consistent binding to the receptor throughout the simulation (Figure 11A).

The RMSF analysis indicated that most protein residues had fluctuations under 1.5 Å, reflecting minimal backbone flexibility throughout much of the structure. Some regions, particularly flexible loop segments, showed slightly higher RMSF peaks. In contrast, residues in the ligand-binding area

demonstrated lower fluctuations, implying stable interactions between the ligand and the protein (Figure 11B).

SSE analysis also confirmed the structural stability of the receptor during the entire simulation period. The relative ratio of α -helix and β -strand components remained between 40–45%, which confirmed the stability of secondary structural topology during the entire simulation period (Figure 11C).

Analysis of the protein-ligand interactions revealed that Thr30, Leu36, Phe48, Tyr51, Met102, and Phe104 are involved in the stabilization of the ligand within the binding pocket, as they are responsible for hydrophobic, van der Waals, and π interactions, respectively (Figure 11D). However, these interpretations should be interpreted as indicative of preliminary interaction stability rather than definitive evidence of strong or irreversible binding.

3.8.2 MD Simulation of IMPHY006486-1CD8 Complex

The RMSD evaluation for the IMPHY006486-1CD8 complex showed moderate fluctuations throughout the simulation process. The RMSD values for the protein backbone showed stability in the range of 1.6–2.1 Å, suggesting that the conformation integrity of the receptor was well maintained throughout the simulation process.

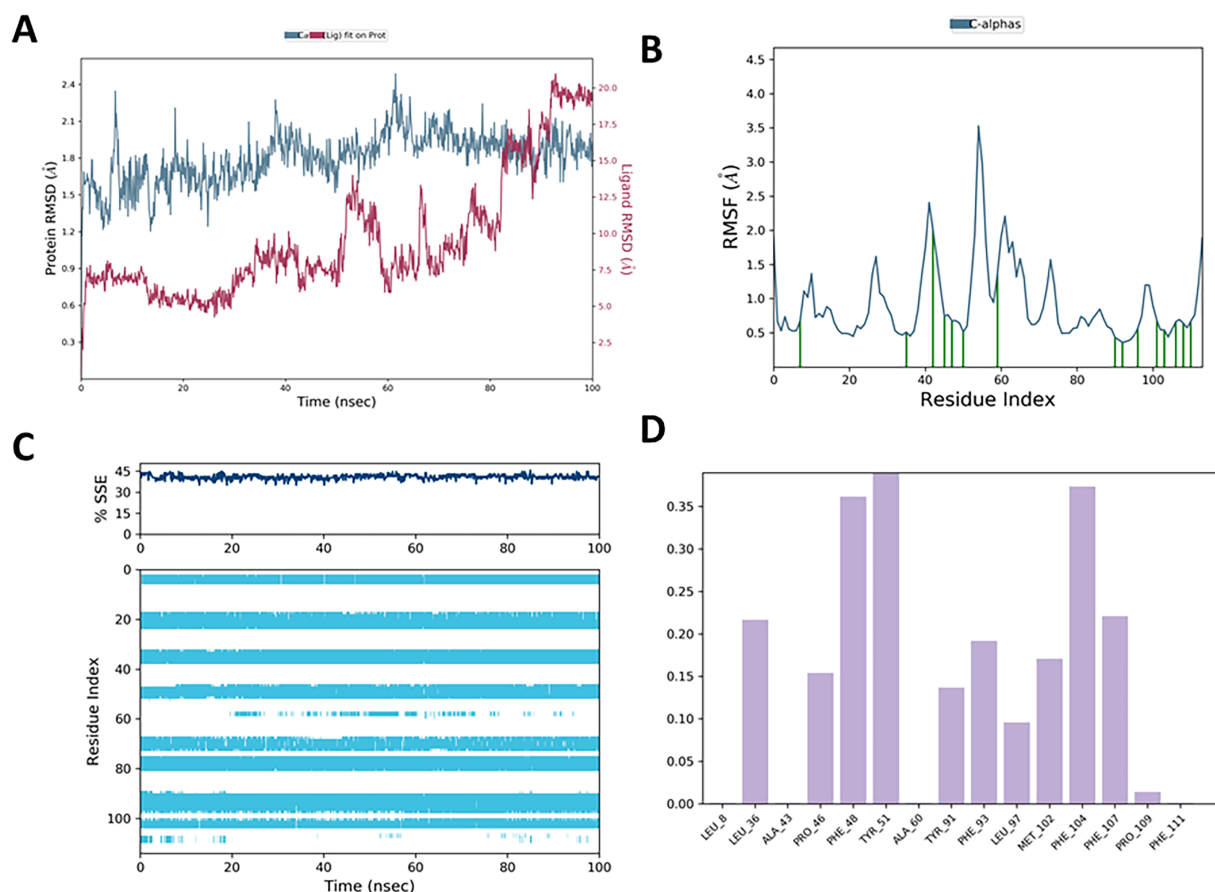


Figure 12. MD simulation of IMPHY006486-1CD8 complex. (A) RMSD showing stability of the protein-ligand complex during the 100 ns simulation (B) RMSF indicating residual flexibility (C) Secondary structure analysis showing structural stability of the protein (D) Protein-ligand interactions fractions highlighting key residues involved in binding

On the other hand, the ligand RMSD showed greater fluctuations, especially in the later stages of the simulation, suggesting conformation changes or repositioning of the ligand in the binding site (Figure 12A).

RMSF analysis indicated that the majority of residues exhibited fluctuations of less than 1.5 Å, reflecting stable backbone dynamics. Certain regions displayed higher RMSF values, which were associated with flexible loops that do not directly impact ligand binding. Notably, residues situated within the binding pocket demonstrated lower RMSF values, suggesting reduced local flexibility and preliminary conformational stability of the protein-ligand complexes throughout the simulation period (Figure 12B).

Analysis of secondary structure elements indicated that the proportion of these elements remained stable throughout the simulation, suggesting that the ligand binding process did not significantly alter the protein structure (Figure 12C).

The protein-ligand interaction study highlighted specific residues Leu36, Pro46, Phe48, Tyr51, Met102, Phe104, and Pro109 as critical for ligand binding, primarily through hydrophobic and van der Waals forces. Furthermore, aromatic residues Phe48 and Tyr51 were identified as essential for sustaining ligand binding via π -interactions, clarifying how ligand binding is maintained despite ligand mobility

(Figure 12D). The observed fluctuations of ligands suggest a dynamic interaction behavior, typical during MD simulations of flexible protein-ligand systems.

3.8.3 MD Simulation of IMPHY004111-1YGR Complex

The MD simulation of the IMPHY004111-1YGR complex, conducted over 100 ns, demonstrated stable structural dynamics. Initially, the protein backbone RMSD increased before stabilizing, with fluctuations between 2.3 and 2.8 Å, suggesting that the receptor structure-maintained stability despite its size and flexibility. In contrast, the ligand RMSD showed moderate fluctuations but stayed within a consistent range, indicating that the ligand maintained a similar conformational and spatial arrangement in the binding region (Figure 13A). This suggests preliminary conformational stability of the protein-ligand complex, though not definitive proof of stable binding.

RMSF analysis revealed that most residues exhibited fluctuations below 2 Å, with a few specific regions showing peaks near 4 Å, particularly in the protein's flexible loops. These fluctuations are typical for flexible regions, which are commonly solvent-exposed. However, they do not significantly impact the stability of the ligand-binding site, where residues displayed relatively low RMSF values (Figure 13B).

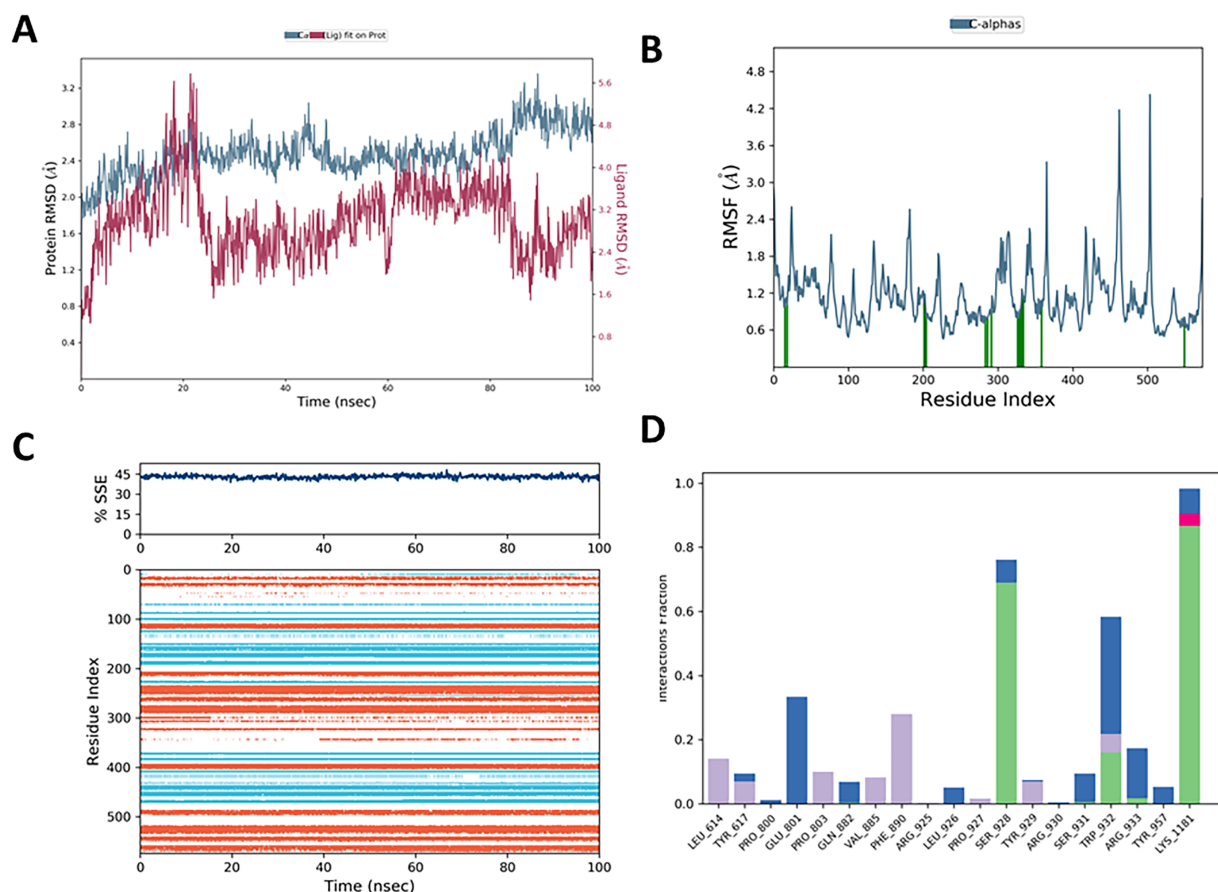


Figure 13. MD simulation of IMPHY004111-1YGR complex. (A) RMSD showing stability of the protein-ligand complex during the 100 ns simulation (B) RMSF indicating residual flexibility (C) Secondary structure analysis showing structural stability of the protein (D) Protein-ligand interactions fractions highlighting key residues involved in binding

The SSE analysis indicated that all secondary structural elements of the protein were highly conserved during the simulation period. Both α -helices and β -strands maintained a constant proportion of approximately 40–45%, demonstrating the preservation of the protein's structural integrity (Figure 13C).

Protein-ligand interaction analysis revealed the significant contribution of a group of residues, including Val885, Phe890, Arg925, Pro927, Ser928, Tyr929, Thr932, Arg933, Tyr931, and Lys1181, in the stabilization of the ligand. Among the identified residues, the higher fraction of the interaction was observed for Lys1181 and Ser928, indicating the hydrogen bonding and electrostatic interactions. Additional hydrophobic interactions with the amino acids Val885, Phe890, and Pro927 play a significant role in enhancing the stability of the ligand within the catalytic domain of the receptor (Figure 13D). These findings indicate that there was sustained interaction behavior throughout the simulation timeframe; however, this should not be interpreted as definitive proof of therapeutic efficacy.

3.8.4 MD Simulation of IMPHY006486-1YGR

The RMSD analysis of the IMPHY006486-1YGR complex indicated that the protein backbone achieved rapid equilibration within the first 10 ns of simulation, maintaining stability thereafter with fluctuations between 2.0–2.8 Å, suggesting maintenance of overall structural integrity during the simulation. Additionally, the RMSD results for the ligand demonstrated an initial increase as it bound to the target, followed by a period of stability, signifying sustained binding throughout the trajectory (Figure 14A).

Additionally, RMSF analysis indicated that most amino acids exhibited minimal fluctuations, generally below 1.5 Å. Increased flexibility was noted primarily in the loop and terminal regions, which are naturally more dynamic. In contrast, amino acids situated in the binding pocket showed minimal fluctuations, signifying stable ligand binding (Figure 14B).

Secondary structure analysis indicated that the protein preserved approximately 40–45% of its secondary structure elements throughout the simulation, with no notable unfolding events detected (Figure 14C).

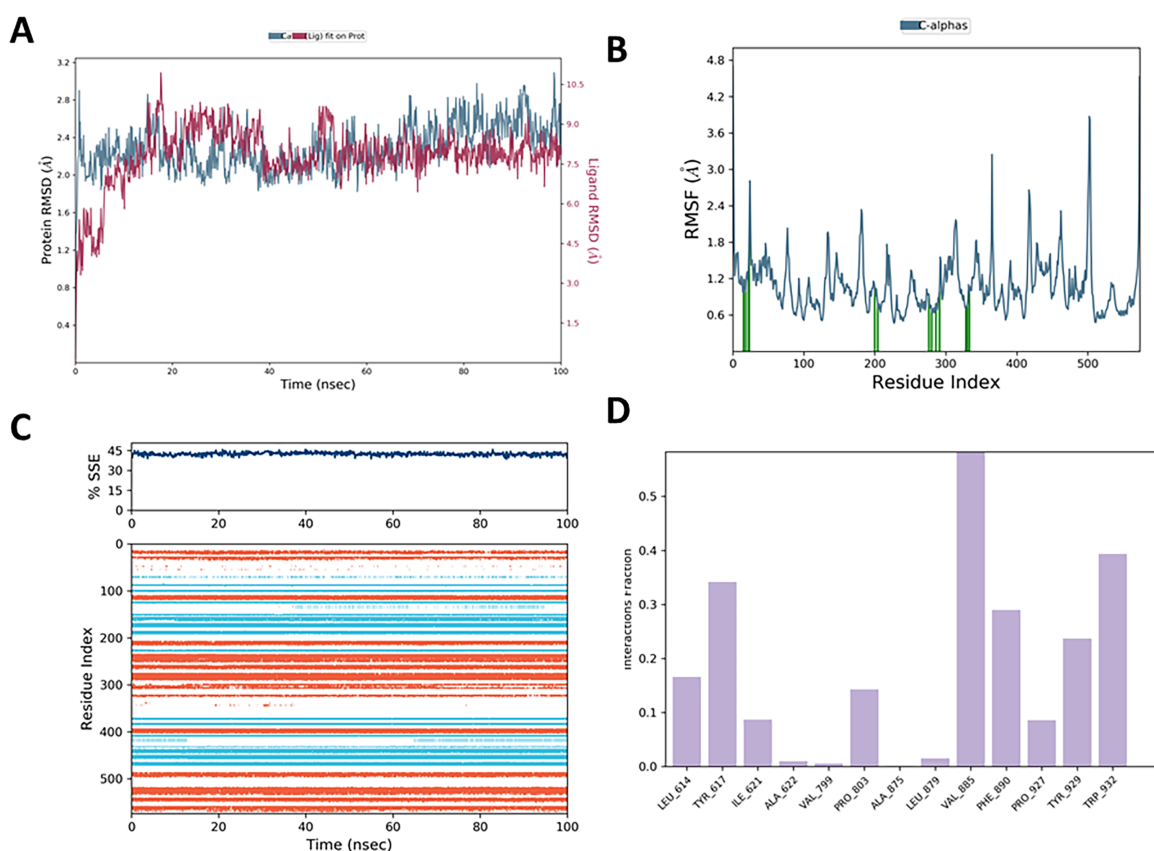


Figure 14. MD simulation of IMPHY006486-1YGR. (A) RMSD showing stability of the protein-ligand complex during the 100 ns simulation (B) RMSF indicating residual flexibility (C) Secondary structure analysis showing structural stability of the protein (D) Protein-ligand interactions fractions highlighting key residues involved in binding

The interaction analysis highlighted that IMPHY006486 consistently interacts with key residues including Leu64, Tyr61, Ile62, Val865, Phe869, Pro912, Tyr920, and Trp932, with Val865 showing the most significant interaction relevant to ligand binding. Overall, the data suggests that IMPHY006486 exhibits stable and persistent interaction with the 1YGR protein, suggesting persistent interactions during the simulation timeframe, which warrant further investigation through experimental validation (Figure 14D).

Overall, MD simulation results revealed that the complexes remained stable throughout the 100 ns simulation trajectory. Evidence of stability includes moderate root-mean-square deviation values, limited residue fluctuations, and maintained secondary structural elements, suggesting that ligand binding did not substantially compromise protein structural integrity. However, the present MD analyses represent preliminary computational observations based on single simulation trajectories, and therefore the observed interaction stability should be interpreted cautiously pending further replicate simulations, advanced free-energy analyses, and experimental validation.

4 Discussion

Trypanosoma cruzi is a compulsory intracellular protozoa parasite or pathogen that in the form of a persistent

infection in cardiomyocytes and in immune cells causes a chronic and pathological inflammatory response which changes the myocardium remodeling. Understanding the processes that regulate the shift between the asymptomatic indeterminate infection to overt Chronic Chagasic Cardiomyopathy (CCC) is not completely understood yet, and a thorough comprehension of host transcriptional dysregulation and its coexistence with parasite-induced immune evasion is crucial to finding viable targets of therapy. In the current study, we were using an integrative computational paradigm that involved RNA-seq transcriptomics, protein-protein interactions (PPI) network analysis, gene ontology and pathway enrichment, molecular docking, ADMET profiling, and molecular dynamics simulation to define the immunopathological environment of CCC and determine phytochemical regulators of the major immune hubs (Brochet et al., 2022; Oulas et al., 2019).

Transcriptomic comparison of severe CCC cardiac tissue as compared to healthy controls identified 2,973 significantly differentially expressed genes with a significant imbalance of upregulated transcripts (2,239 upregulated and 734 downregulated). Such widespread pattern of transcriptional activation is in line with previous transcriptomic studies of CCC myocardium, which have repeatedly demonstrated that pro-inflammatory immune gene expression programs are predominant in advanced disease. Upregulation of genes

linked to antigen receptor-mediated signaling, T cell activation and positive regulation of cytokine production in our dataset are also highly upregulated by previous RNA-seq studies of CCC cardiac tissue, which also emphasized the role of adaptive immune response pathways in disease pathogenesis (Duque et al., 2025; Gómez et al., 2023). On the other hand, down-regulation of genes linked to potassium ion transport, cardiac development, sarcoplasmic reticulum functioning, and MAPK and PI3K-AKT signaling reflects the gradual loss of cardiomyocyte homeostatic machinery which is present in end-stage Chagas heart disease, which is in line with the electrophysiological and contractile dysfunction that defines CCC (Cunha-Neto & Chevillard, 2014; Marin-Neto et al., 2023).

Enrichment of gene ontology on the categories of biological process, cellular component, and molecular function, gave a consistent and anatomically sound description of CCC immunopathology. The upregulation of the genes which include MHC class II protein complex, T cell receptor complex, cytokine receptor activity and antigen processing and presentation is evidence of a high level of immune infiltration and increased antigen presentation activity in the diseased myocardium. The histopathological experiments have supported these findings, as reported cases of a severe inflammatory infiltrate, mediated by CD8⁺ T cells, in CCC explanted hearts, with an extensive interstitial fibrosis, and cardiomyocyte degeneration also observed (Díaz et al., 2022; de Higuchi et al., 1993). The concomitant activation of graft-versus-host disease and allograft rejection signaling in the activated KEGG gene set further validates the idea that the autoreactive response of lymphocytes is a potential cause of damage to cardiomyocytes in CCC, which has been endorsed by immunological and molecular evidence during the last 20 years (Bocchi et al., 2017; Puerta et al., 2023).

To find the most central regulatory nodes in the upregulated immune transcriptional network, a PPI network was built, and topological analysis was done with five cytoHubba algorithms: Degree, MCC, MNC, Closeness and EPC. All five algorithms identified five genes which were common hub nodes: CD4, PTPRC, CD8A, IFNG, and FCGR3A. Their strong showing by being convergently found across independent centrality measures highlights the strength of these genes as topological regulators of the immune interaction network within CCC. Not only are these hub genes topologically pivotal, but they are also highly entrenched in the biology of CCC-linked immunopathology. CD8A (Gene ID: 925) is the gene that expresses alpha chain of the CD8 co-receptor that is pivotal in cytotoxic T lymphocytes (CTLs) expression and plays a central role in T cell activation at MHC class I. Histopathological characterization of CCC cardiac explants has reported that the inflammatory infiltrate comprising of T cells is the most common in the diseased myocardium with CD8⁺CD45RO⁺ memory T cells preferentially enriched between CCC and no-Chagas cardiomyopathies (Díaz et al., 2022). The skewed preeminence of CD8⁺ T cells in CCC, which can equally be seen in the transcriptomic data of the current study, can be linked to the fact that CTL-based cytotoxicity is a primary event in

the chronic *T. cruzi* infection, implying the destruction of cardiomyocytes (Puerta et al., 2023).

In all hematopoietic cells, PTPRC (CD45, Gene ID: 5788) is an abundant transmembrane protein tyrosine phosphatase that is essential in TCR signal transduction: upon antigen interaction, the Lck kinase undergoes dephosphorylation and activation of the cascade of tyrosine phosphorylation (Weiss & Littman, 1994). Its role as a gatekeeper of T cell receptor signaling as a hub gene in CCC transcriptional network is biologically plausible since increased PTPRC activity in infiltrating T cells would be predicted to increase and maintain TCR-dependent inflammatory signaling in the myocardium. This makes PTPRC a promising pharmacological target to suppress pathological T cell activation without complete inhibition of adaptive immunity. It was also reported that CD4 (Gene ID: 920), which is a gene whose protein counterpart is the T helper co-receptor was also found to be a hub, which aligns with findings that CD4⁺ T cells have a significant role to play in the inflammatory environment of CCC, including cytotoxic CD4⁺ T cells that co-express granzyme B and perforin (Almeida et al., 2022). IFNG (Gene ID: 3458) which is an interferon-gamma encoding gene and a canonical Th1 cytokine and strong macrophage activator has been defined as one of the most highly upregulated mediators in CCC cardiac tissue and peripheral blood, and both facilitating parasite clearance and fueling myocardial immunopathology in tandem [13]. FCGR3A (CD16a, Gene ID: 2214) is a natural killer cell and monocyte antigen and a mediator of antibody-dependent cellular cytotoxicity (ADCC) and a pathway in the innate cytotoxic effector arm of the anti-*T. cruzi* immune response (doVale et al., 2024).

Despite their attractiveness as therapeutic targets, CD8A and PTPRC play fundamental roles in protective anti-parasite community. Consequently, pharmacological inhibition of these molecules could theoretically impair cytotoxic, T-cell function, antigen-dependent immune activation, and parasite control, potentially compromising host defense against persistent *T. cruzi* infection. Therefore, any therapeutic strategy directed against CD8A or PTPRC would require careful experimental evaluation of the balance between reducing harmful myocardial immunopathology and preserving sufficient anti-parasitic immune activity to prevent disease progression (Freire et al., 2022; Martin & Tarleton, 2004).

Molecular docking of drug-like phytochemicals of *Schinus molle* that are accessed in the IMPPAT database was carried out against CD8A (PDB: 1CD8) and PTPRC (PDB: 1YGR). Among the five hub genes (CD8A and PTPRC in particular) molecular docking was performed based on the two criteria of biological mechanistic centrality in CCC immunopathology and the possibility to use high-resolution crystal structures (PDB: 1CD8 and 1YGR) as the basis of virtual screening. The other hub genes, CD4, IFNG, and FCGR3A were not included because they lack clear small-molecule binding pockets, or because in this case the experimentally determined structure was not suitable to do the computational ligand screening. *Schinus*

molle is an Andean medicinally significant plant with anti-inflammatory, antioxidant and cardioprotective properties that are well-documented and are attributed to its diverse terpenoid, flavonoid and phenolic constituents (Pérez-Molina & Molina, 2018; doVale et al., 2024). In the case of PTPRC (1YGR), four lead compounds showed good binding affinities of between -6.64 and -7.70 kcal/mol with IMPHY006486 having the largest affinity (-7.70 kcal/mol) and hydrogen bonds with Leu926, Glu886, Gln882 along with π -alkyl interaction with Phe923. The enzyme responded to IMPHY004111 (-7.18 kcal/mol) by forming hydrogen bonds with Ser928 and Gln882 and had other hydrophobic interactions at Phe890, Pro927, and Leu614. The most diverse profile of interaction, including hydrogen bonds, π - π T-shaped, amide- π stacking, and π -alkyl interactions between various active sites residues, was provided by IMPHY003082. In the case of CD8A (1CD8) the binding affinities included -6.32 to -6.94 kcal/mol with IMPHY006486 taking the top position (-6.94 kcal/mol) binding Thr30 with a hydrogen bond and hydrophobic π -alkyl contact with Leu36, Tyr51 and Phe48 between the proteins. These binding energies are like those that were obtained in similar computational experiments using plant-derived phytochemicals on immune-regulatory targets (Feriani et al., 2022; Kumar et al., 2023).

Profiling of the five phytochemicals of interest using ADMET gave mostly positive results in terms of pharmacokinetic and safety profiles. IMPHY004111 is the best absorber by intestine (100%), and all the compounds were negative in AMES mutagenicity and hERG I inhibition, indicating that chances of genotoxicity and cardiac ion channel toxicity are low. None of the compounds were either CYP2D6 substrates or inhibitors, or CYP1A2, CYP2C19, CYP2C9, indicating a low possibility of drug-drug interaction via cytochrome P450. The hepatotoxicity alerts on IMPHY004111, IMPHY003696, and IMPHY006751 require special attention in the further preclinical trials. The implications of the ADMET findings, in general, are that these compounds are suitable candidates to be utilized in further development of drugs, as is generally also the case with similar IMPPAT-based phytochemical screening assays (Qazi et al., 2021; Vivek-Ananth et al., 2023).

The structural stability of all the protein-ligand complexes was verified by the 100-nanosecond molecular dynamics simulations. The backbone RMSD values of the 1CD8 complexes were stabilized at 1.5–2.2 Å and those of the 1YGR complexes were a bit higher (2.3–2.8 Å) which was anticipated due to the more complicated domain architecture of PTPRC. RMSF analysis revealed that binding pocket residues were found to be less flexible than distal loops in all systems, and SSE analysis showed that 40–45% ratio of 4045 percent of the 3-dimensional structure of the folds remained conserved along all trajectories, which demonstrated that the binding of ligands did not affect the structural integrity of the proteins. The repeated protein-ligand interaction fractions revealed that Lys1181 and Ser928 were the most occupied contact residues in the PTPRC active site with IMPHY004111 and Val865 was the most occupied contact residue in 1YGR. These findings

are in agreement with other MD simulation experiments of phytochemical-immune target complexes when an RMSD of less than 3.0 Å and a preserved secondary structure are considered to be good signs of stable binding (Mundhe et al., 2026; Prakash et al., 2023). Combining target identification by transcriptomics with structure-based virtual screening and dynamic simulation is a highly rigorous multi-scale strategy that substantially enhances the translational applicability of the identified lead compounds (Ghosh et al., 2025; Oulas et al., 2019; Parrinello & Rahman, 1981).

A limitation of this study is the relatively small number of transcriptomic samples analyzed ($n = 14$). However, this reflects the inherent difficulty in obtaining human cardiac tissue samples from CCC patients. The dataset used has been previously validated and published (Brochet et al., 2022), supporting its reliability. In addition, widely accepted RNA-seq analysis frameworks such as DESeq2 enable robust identification of differentially expressed genes even with moderate sample sizes when appropriate normalization and statistical controls are applied (Conesa et al., 2016; Love et al., 2014). Nevertheless, future studies incorporating larger cohorts and single-cell transcriptomics would further strengthen these findings. Limitations also include the inability of bulk RNA-seq to resolve cellular heterogeneity within the cardiac immune infiltrate and the inherent constraints of in silico methods in replicating the full complexity of intracellular signaling environments; experimental validation remains essential.

5 Conclusion

This study provides a systems-level computational analysis of host immune responses in Chronic Chagasic Cardiomyopathy, integrating RNA-seq profiling, functional enrichment, PPI network biology, molecular docking, ADMET evaluation, and molecular dynamics simulations. Five hub genes CD4, PTPRC, CD8A, IFNG, and FCGR3A were identified as the central immune regulatory nodes of the CCC-associated transcriptional network. Of these, CD8A and PTPRC were selected for structure-based virtual screening against *Schinus molle* phytochemicals from IMPPAT. Molecular docking identified IMPHY006486 as the top-ranked compound against both targets (1YGR: -7.70 kcal/mol; 1CD8: -6.94 kcal/mol), with IMPHY004111 and IMPHY003082 also demonstrating strong binding affinities and diverse interaction profiles with key active site residues. ADMET analysis confirmed favorable pharmacokinetic and safety profiles for the lead compounds. 100 ns molecular dynamics simulations indicated that the four protein-ligand complexes exhibited moderate conformational stability and consistent interaction patterns throughout the simulation period. Additionally, the secondary structure elements and overall structural integrity of the receptor proteins were preserved. However, these findings should be regarded as preliminary computational behavior derived from single-trajectory simulations and interpreted with caution, serving as indicative rather than definitive evidence of binding stability. These results

suggest that compounds IMPHY006486, IMPHY004111, and IMPHY003082 could act as phytochemical modulators for T cell-mediated immunopathology in CCC by interacting with CD8A and PTPRC. However, it is important to note that these findings are preliminary, and conclusive statements about their therapeutic effectiveness require additional experimental verification, such as in vitro assays, cellular immune models, and in vivo studies.

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Author Contributions

Sultan K. Al-Umairi and Sana Abdul Ghaffar contributed to conceptualization, and study design. Farah Shahid, Adeena Naeem, Muhammad Sadaqat, and Asma Khalil performed data curation, formal analysis, and experimental work. Mir Mohammad Shahroz and Fareeda Athar contributed to methodology development, validation, and software/technical support. Kishver Tusleem assisted in data interpretation, visualization, and manuscript editing. Mubarak A. Alamri conceived the study, secured resources, supervised the project, and wrote the original draft of the manuscript. All authors contributed to manuscript review, editing, and have approved the final version for publication.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journals/records-of-natural-products>.

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