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Exploring the Therapeutic Potential of Danshen (Salvia Miltiorrhiza) and Ginkgo Biloba in Coronary Heart Diseases: A Network Pharmacology Investigation

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Research Article

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Abstract

Atherosclerosis is a leading cause of coronary heart disease (CHD), which imposes a significant health burden on the world. Historical formulations of traditional Chinese medicine (TCM), including ginkgo biloba-Danshen herb pair (GDHP) have been extensively applied in cardiovascular therapy where the molecular mechanisms involved in their effects are not well understood. To examine the possible targets and the pathways of GDHP in the treatment of CHD, network pharmacology was implemented. The BATMAN-TCM database was used to identify active compounds and related targets and GeneCards, OMIM, PharmGKB, and DrugBank were used to identify the CHD-associated genes. With the help of STRING and Cytoscape, a protein-protein interaction (PPI) network was created. DAVID was used to do gene ontology (GO) and KEGG pathway enrichment analysis. AutoDock Vina and Discovery Studio were used to validate the compound-target interactions using molecular docking. The screening was done to identify active compounds (34) and matched targets (199), and 16 of them were common to GDHP and CHD. Network analysis identified gallocatechin, carnosol, and tanshinone IIA as the important bioactive constituents. The key targets were TLR4, P2RY12, ITGB3, NOS3, PTGS1, SERPine1, and TNF. Enrichment analyses demonstrated that the prominent roles in inflammatory regulation, lipid metabolism, insulin resistance, and fluid shear stress pathways were involved. Stable binding between hub targets and active components was confirmed by molecular docking, which can be used to support their potential in therapy. The protective effects of GDHP against atherosclerosis and CHD could be through the control of inflammatory, metabolic and vascular shear stress response. This evidence gives a network-based explanation of why GDHP should be used in cardiovascular disease and that experimental confirmation of the predicted mechanisms is required to support this hypothesis.

Introduction

Around the globe, cardiovascular illnesses are the most common cause of death [1, 2]. Research has indicated that the prevalence of cardiovascular illnesses is almost three times higher in older adults than in other age groups [3]. The prevalence of cardiovascular illnesses has been significantly decreased by the use of statins and antiplatelet medications. However, it is yet unknown if long-term drug use and residual chronic heart disease (CHD) pose a concern [4]. Coronary heart disease (CHD) is a multifactorial disease. In the US, it is the most prevalent kind of heart disease. It is also known as ischemic heart disease or coronary heart disease. The buildup of plaque in the walls of the coronary arteries, which provide blood to the heart and other areas of the body, is the cause of coronary heart disease (Fig. 1). Deposits of cholesterol and other materials in the artery make up plaque. Over time, plaque accumulation narrows the lining of the arteries, obstructing blood flow either completely or partially. Atherosclerosis is the term used to describe this process. Drug resistance can arise as a result of consistent, long-term use of a particular medication. Therefore, finding new targets is essential for the synergistic use of treatments.

TCM has clear benefits for treating CHD. Danshen decoction, made from Salvia miltiorrhiza Bunge's root and rhizome, is mostly used in Traditional Chinese Medicine (TCM) to treat various abdominal and

cardiac ailments [5]. It can improve blood flow, break up blood clots, encourage the flow of essential energy, and reduce discomfort. As such, it is widely used in the medical treatment of angina pectoris, coronary heart disease, and gastric and duodenal ulcers. Clinical studies have shown its efficacy in reducing atherosclerosis and thrombosis, as well as its capacity to inhibit blood clotting and improve fibrin breakdown [5–7]. Still, TCM decoctions contain numerous potent substances that have proven to be useful in treating various ailments. Even while these substances clearly have therapeutic benefits, we still don't fully grasp which particular components have which pharmacological effects. Although studies on Salvia miltiorrhiza's potential for treating CHD, angina pectoris, and diabetic cardiomyopathy have been published [5, 8], there is still a need to further investigate the processes by which Salvia miltiorrhiza treats CHD. The Ginkgo biloba L. plant's leaves are used to make Ginkgo biloba extract (GBE).

The primary components of it are terpenoid lactones and flavonoids, which function as free radical accelerators and antioxidants. In addition, GBE promotes cognition, increases cerebral blood flow, and has other advantages [9]. GBE is utilized as a pharmaceutical treatment for enhancing cardiovascular and cerebrovascular conditions in European nations, While it is used as a dietary supplement in Japan and the United States [10]. GBLs are widely used as a natural dietary supplement in a variety of industries, including food additives, health foods, pharmaceuticals, and functional drinks [11, 12]. EGb has sparked significant interest in its therapeutic properties, leading to a greater focus on both fundamental research and practical use in clinical settings. In the fields of antiaging and therapeutic therapy for cardiovascular and cerebrovascular illnesses, EGb plays a vital role [13, 14]. This study investigated the potential mechanisms of Ginkgo biloba and Salvia miltiorrhiza on CHD using network pharmacology techniques. In order to clarify the relationship between the active ingredients in herbal medicine and diseases, we also incorporated literature research, which led to the development of a unique strategy for the prevention and treatment of coronary heart disease (CHD). As shown in Fig. 2, the study employed a particular network pharmacology and bioinformatics technique.

Methodology

Screening of active compounds:

The chemical composition of Salvia miltiorrhiza Bunge and Ginkgo biloba was investigated using the TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php) [15]. The screening criteria for compounds were established as having an oral bioavailability (OB) of at least 30% and a drug-likeness (DL) score of at least 0.18. OB indicates the relative amount of absorption of drug after taken orally while DL indicates the similarity between the active compound and the known drug. Furthermore, validated target proteins that were proven to be impacted by the active components were extracted from the TCMSP database. The UniProt Knowledge Base was used to transform the active ingredient's target protein names to their conventional target gene names (UniProtKB, http://www.uniprot.org/).

Retrieval of CHD-related genes:

The keyword "chronic heart disease" was used to obtain CHD-related genes from the Genecards database (https://www.genecards.org/), OMIM database (https://omim.org/), and DrugBank database (https://www.drugbank.ca/). The CHD-related genes were then combined, and any duplicates were eliminated. Moreover, the Venny v2.1 online tool (https://bioinfogp.cnb.csic.es/tools/venny/index.htm) was utilized to identify the common gene targets between CHD and herbs targets.

Construction of Herbal Compound-Target Network:

The "Herbal-compound-target" network was created with Cytoscape software (Version 3.10.1). A network diagram of the proteins and their interactomes was also generated. The topological metrics were used to identify the key active compounds and CHD-related targets in both plants. The PPI was analysed using the String database (https://string-db.org/cgi/input.pl). The "organism" parameter was set to "Homo sapiens," and the core genes were chosen using a protein-protein interaction (PPI) with a composite score greater than 0.5.

Construction of PPI Network of Common Targets genes

The common gene targets between CHD and herbs were imported into the STRING 11.0 database (https://string-db.org/) for PPI analysis. A protein that had a total value greater than 0.4 was chosen to build an interaction network [15]. Furthermore, the network was visualised with Cytoscape 3.7.1 software. The PPI network's parameters were analysed using a Network Analyzer. MCODE was then used to determine the most important module in the PPI network. An MCODE module was characterised as identifying strongly linked areas in a large-scale protein network. The degree of protein link inside the module was assessed using the following criteria: degree cutoff of 2, node score cutoff of 0.2, K-score of 2, and maximum depth of 100.

GO and KEGG enrichment analysis

The Database for Annotation, Visualisation, and Integrated Discovery (DAVID) is a functional annotation tool for understanding the biological importance of a gene list. The common CHD targets were used to populate the DAVID database, with the organism identified as "Homo sapiens." The barrier for GO keywords was set at an FDR of less than 0.05, and for KEGG pathways, it was set at a P-value less than 0.05.

Molecular Docking Studies

The target protein structures and drug compound structures were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/), respectively. The Biovia Discovery Studio was used to hydrogenate and remove water molecules for protein processing.

PyRx was used to calculate the docking binding energy and affinities of each complex. The Biovia Discovery Studio was used to analyze the interactions between proteins and chemicals.

Results

Active Compounds and disease targets

The active substances of herbs and their respective disease targets were obtained from the TCSMP database. (Supplementary Table S1). A total of 34 active components from both plants were extracted (Supplementary Table S2). Suppressing the canonical NF-κB pathway, one of the derivatives of gallocatechin known as epigallocatechin gallate exhibits antioxidative, anti-inflammatory, hypolipidemic, anti-thrombogenic, and anti-hypertensive effects, providing protection against CVDs [16]. Both are members of the family catechins. Salvia miltiorrhiza extract contains a compound called carnosol, which has anti-inflammatory and antioxidant properties. According to reports, carnosol possesses strong anti-microbial, neuroprotective, and anti-tumor qualities [17]. The biologically active substance tanshinone IIA (TS) is taken from the rhizome of the Salvia miltiorrhiza Bunge plant [18]. Numerous studies have revealed that TS can prevent or delay the course of a wide range of illnesses, including cancer, neurological ailments, and cardiovascular issues [19–22]. Based on their medicinal qualities, the following drugs have also been identified as potential CHD treatments: miltrone, succinic acid, ginkgetin, quercitrin, hyproside, sciadopitisin, techtochrysin, astragalin, bilobetin, isoginkgetin, gallocatechin, isoguanosine, isoimperatorin, dauricine, hexadecanoic acid, and chyrophanol.

Herbal Compound-Targets Network

The disease targets retrieved from the UniProt database were combined with CHD disease genes retrieved from Genecards (9248 genes), OMIM (64 genes), PharmGkb (27 genes), and DrugBank (211 genes) (Fig. 3a). The data included 34 active components that corresponded to 176 specific targets, 199 targets related to diseases, and 16 target genes that were common to both (Fig. 3b). The Cytoscape programme was loaded with the target genes and active substances. Figure 4 shows the network of target genes and herbal active substances that was created. The degree shows how many target genes and herbal active substances are associated with one another. As the degree value of a target point rises, so does the relevance of its constituent parts.

Screening of Key Genes

16 common gene targets were used to construct the PPI network (Fig. 5). The CytoNCA was employed to acquire the PPI network following two rounds of screening. A total of 14 nodes were acquired during the 1st screening, and 13 nodes (HLTF, MMP3, SERPINE1, IL6, TLR4, ITGB3, PTGS1, NOS3, TNF, SHARPIN, SPATA2, CYP3A4 and P2RY12) were identified in 2nd screening and finally 8 key genes (TNF, IL6, ITGB3, PTGS1, NOS3, SERPINE1, TLR4, and P2RY12) were identified based on degree and central connectivity (Fig. 6).

GO and KEGG Enrichment Analysis

Using the DAVID database, an analysis was done on the cellular components (CC), biological processes (BP), and molecular functions (MF) of the 16 primary targets in CHD treatment. We chose the top 30 results from the GO biological function analysis (Fig. 7(a-d)) based on a P-value < 0.05. The most important BP concepts discovered were blood coagulation, hemostasis, and platelet activation (Fig. 7a). Membrane rafts, membrane regions, membrane microdomains, and the plasma membrane's exterior area all had more CC terms. When it came to cytokine receptor binding and protease binding, MF keywords were highly enriched.

Using a significance level of P < 0.05, a KEGG pathway enrichment analysis was performed to assess the eight common targets. Forty-two pathways have highly enriched targets (Supplementary Table S3). Gene counts were used to identify the top 20 significantly enriched pathways (Table 1). We used Cytoscape 3.7.1 to create a compound target pathway network (Fig. 8A) by selecting the top 20 pathways based on the gene ratios and P values from the KEGG enrichment analysis findings, along with related targets and compounds. In most of the pathways, the main targets IL6, TNF, TLR4, P2RY12, SERPINE1, ITGB3, NOS3, and PTGS1 were implicated in different ways. Using a free web tool, a Sankey diagram (Fig. 8B) was created based on the relationships between target genes and enriched pathways (http://www.bioinformatics.com.cn). The GO and KEGG databases exhibit substantial enrichment for the TNF signalling pathway and fluid shear stress, indicating that GDHP may function through these two routes in the treatment of CHD.

Table 1
; Key genes' KEGG enrichment results.

IDs	Count	P values	Genes
hsa04933	4	5.05E-05	IL6, NOS3, SERPINE1, TNF
hsa05142	4	5.36E-05	IL6, SERPINE1, TNF, TLR4
hsa04066	4	6.54E-05	IL6, NOS3, SERPINE1, TLR4
hsa04611	4	9.61E-05	P2RY12, NOS3, ITGB3, PTGS1
hsa05417	4	9.61E-05	IL6, NOS3, TNF, TLR4
hsa04151	4	0.002182	IL6, NOS3, ITGB3, TLR4
hsa05144	3	6.73E-04	IL6, TNF, TLR4
hsa05134	3	8.44E-04	IL6, TNF, TLR4
hsa05321	3	0.001137	IL6, TNF, TLR4
hsa05133	3	0.001551	IL6, TNF, TLR4
hsa05323	3	0.002312	IL6, TNF, TLR4
hsa05410	3	0.002513	IL6, ITGB3, TNF
hsa04640	3	0.002616	IL6, ITGB3, TNF
hsa05146	3	0.002775	IL6, TNF, TLR4
hsa04931	3	0.003105	IL6, NOS3, TNF
hsa04620	3	0.003105	IL6, TNF, TLR4
hsa05135	3	0.004951	IL6, TNF, TLR4
hsa05418	3	0.005093	NOS3, ITGB3, TNF
hsa04936	3	0.00531	IL6, TNF, TLR4
hsa05161	3	0.006863	IL6, TNF, TLR4

Molecular Docking analysis

We conducted molecular docking analysis of the important genes (IL6, TNF, TLR4, P2RY12, SERPINE1, ITGB3, NOS3, and PTGS1) using the matching active molecules as the control in the analysis in order to confirm the research results of the network analysis. Key CB-related targets and the 12 compounds with the highest degree values in the herb-compound-target network analysis were chosen for molecular docking based on the degrees of common targets in the PPI network and the KEGG findings. The SwissADME web-based programme (http://www.swissadme.ch/) and the ADMET lab (https://admet.scbdd.com/) (Supplementary Table S4) were used to assess the absorption, distribution,

metabolism, excretion, and toxicity (ADMET) characteristics of the eight chosen drugs. Following molecular docking, the 18 compounds (out of 34) from GDHP were sorted according to their medicinal properties and binding affinity. These compounds included Gallocatechin, Carnosol, Tanshinone IIA Miltrone, Succinic Acid, Ginkgetn, Quercitrin, Hyproside, Sciadopitisin, Techtochrysin, Astragalin, Bilobetin, Isoginkgetin, Gallocatechin, Isoguanosine, Isoimperatorin, Dauricine, Hexadecanoic Acid, and Chrysophanol.

Using Discovery Studio 2020, the compound target interactions with the greatest free binding energy scores and their binding modes were shown (Fig. 10A–H). The docking data' free binding energies fell between − 4 and − 12 kcal/mol, suggesting stable binding.

The heat map in Fig. 11 displays the binding affinities of all compounds with target proteins. The red colour corresponds to the greatest binding values, the yellow colour to intermediate values, and the blue colour to lower binding values. One of the chosen compounds that exhibited the strongest binding value to every target protein and all binding interactions with every target protein is shown in Fig. 10. Binding affinities were ascribed to PI-PI Stacked interactions with TNF's TYR-59 residues, hydrogen bonding with TYR-151 residues, van der Waals forces with ILE-155, HIS-15, LEU-57, and TYR-119 residues.

Stable complexes are formed when hydrophobic small molecules and active cavities of target proteins establish hydrogen bonds. In this investigation, we found that GDHP's gallocatechin, carnosol, and tanshinone IIA exhibited potent binding activity with IL6, TNF, TLR4, P2RY12, SERPINE1, ITGB3, NOS3, and PTGS1. These substances are therefore potential medication candidates. A method to evaluate the binding mechanisms between the herbal medicines and disease-associated targets is molecular docking technology. It should be mentioned, nonetheless, that further research is required to confirm the possible herbal constituents.

Discussion

Atherosclerosis is one of the primary causes of CHD around the world [23]. These days, the use of common synthetic drugs to treat congestive heart failure is limited because of their toxic nature and side effects. These treatments include statins, nicotine, angiotensin receptor blockers, antioxidants, antiplatelets, and anti-coagulants [24]. Chinese herbal therapy frequently offers a multicomponent, multitarget, affordable therapeutic alternative without overtly harmful or adverse side effects. This study has shown pertinent evidence suggesting that Danshen (Salvia Miltiorrhiza) and ginkgo biloba may be compatible, with potential benefits for treating atherosclerosis and possibly coronary heart disease [25, 26]. Consequently, the underpinning was comprehensively evaluated in this study using the network pharmacology analysis technique. The way GDHP functions as a CHD treatment. In this instance, hub targets were identified as IL6, TNF, TLR4, P2RY12, SERPINE1, ITGB3, NOS3, and PTGS1, based on the results of the network analysis. Tumor necrosis factor (TNF)-α's functional involvement in the heart has been extensively studied during the last 15 years. Each of these studies has demonstrated the many, potentially conflicting functions that TNF-α plays in cardiac disease and function.

[27]. TNF-α is produced by monocytes and macrophages, found in atherosclerotic plaques, and contributes to the onset of atherosclerosis. Elevated TNF-α levels in congestive heart failure (CHD) patients might worsen cardiac function and encourage heart remodeling. Two inflammatory mediators, TNF-α and IL-1, are produced under the regulation of NF-κB. NF-κB regulates two inflammatory mediators: TNF-α and IL-1. Concurrently, a positive and negative feedback loop that can activate NF-κB and cause a continuous rise in cytokines is created by the production of IL-1, TNF-α, and related cytokines. participate in the onset and progression of atherosclerosis. Clinical study indicates that the degree of coronary atherosclerosis is associated with the connection between TNF-α and oxidative stress, and this association may be used as a noninvasive diagnostic tool for coronary chronic total occlusions (CCTO) in senior CHD landmark patients [28]. Research has demonstrated that estrogen has a direct anti-atherosclerotic impact and can prevent atherosclerosis from developing [28, 29]. Furthermore, early estrogen treatment administration can prevent blood vessel damage by allowing estrogen to fully bind to its receptor in cases of atherosclerosis [30, 31]. Therefore, it is anticipated that in order to cure atherosclerosis, GDHP will target inflammation and the oestrogen receptor. To learn more about the mechanism by which GDHP prevents atherosclerosis, two analyses were conducted: the KEGG functional enrichment analysis and the GO biological function analysis.

According to the GO biological function analysis, blood pressure, hormone-mediated and intracellular receptor signaling pathways, the metabolism of fat and steroids, and the control of the inflammatory response were the main BPs implicated. MF's main research topics were inflammation and lipids, particularly steroid binding, steroid receptor activity, and nuclear receptor activity. The main locations of CC concentration were the transcription regulator complex, the RNA polymerase II transcription regulator complex, nuclear chromatin, and other elements. The KEGG analysis findings showed that hemodynamic issues, metabolic issues, and GDHP treatment strategies against atherosclerosis were largely connected with inflammatory signaling pathways, including fluid shear force as well as TNF and insulin signaling pathways (Fig. 9). Gallocatechin, Carnosol, and Tanshinone IIA were determined to be the most plausible therapeutic components based on the composition-to-target network and the ensuing molecular docking affinity analysis. Modern pharmacology has shown that tanshinone IIA has biological features such as anti-inflammatory and anti-tumor effects [32]. It is commonly used to treat arrhythmia, cardiomyopathy, and congenital heart abnormalities, among other cardiac disorders. Tanshinone IIA, often known as tan-IIA, is the main fat-soluble component of Salvia miltiorrhiza.

Tan-IIA has been found to possess anti-inflammatory and anti-oxidant properties through modern pharmacological research. Tan-IIA may be a useful treatment for cardiovascular diseases (CVD) since it increases angiogenesis, which has notable cardioprotective effects. Tan-IIA has been shown to have broad immunomodulatory effects and to be important for immune cell development and function These include beneficial effects like cardioprotection against ischemia, myocarditis, and pressure overload, as well as potentially detrimental consequences like the start of atherosclerosis, reperfusion damage, hypertrophy, and heart failure. Tan-IIA regulates the activation and activity of immune cells, reducing the production of inflammatory mediators and mending abnormal signalling pathways.

Because it can regulate signal transduction pathways like the TLR/NF-κB pathway and the MAPKs/NF-κB pathway, tan-IIA possesses anti-inflammatory, anticoagulant, antithrombotic, and neuroprotective qualities. It has a preventive effect against the onset of cardiovascular disorders including atherosclerosis and hypertension as well as Alzheimer's disease. Through the death of various tumour cells, the activation of differentiation and apoptosis, and maybe the suppression of the development of carcinomas, tan-IIA has also been demonstrated to have anti-tumor capabilities [33, 34]. Cranosol is a bioactive ortho-diphenolic diterpene that is linked to the abietane carbon skeleton.

Research has demonstrated that Salvia miltiorrhiza naturally possesses this intriguing polyphenol. Additionally, prior research has demonstrated that carnosol possesses a number of beneficial qualities, such as immunomodulatory effects, anti-oxidant, anti-microbial, anti-diabetic, anti-cancer, and modulation of peripheral pain signaling [35]. Furthermore, a wealth of data suggests that carnosol stimulates and raises the amounts of Nrf2, or nuclear factor erythroid-derived 2-related factor 2, which is displaced into the nucleus.[36]. Epigallocatechin-3-gallate, the primary polyphenolic component of green tea, is a derivative of gallocatechin and has been demonstrated to have several impacts on cardiovascular health by altering various pathways {Chan, 2014 #2}. Both gallocatechin and epigallocatechin-3-gallate belong to a family called catechins{Guo, 2020 #10}. Thus, Gallocatechin, Carnosol and Tanshinone IIA may be crucial elements in the treatment of CHD.

We looked further into the network pharmacology prediction results based on our team's prior relevant studies as well as other literature. Prior studies have demonstrated that GDHP can suppress the expression of inflammatory factors, such as IL-6, and prevent the activation of lipid and atherosclerosis, and TNF signaling pathways. Lowering the blood lipid levels of TG, TC, ApoB100, and Lp(a) is the main way to do this [26]. These results support the network pharmacology data and show how GDHP changes the expression of important genes and alters signaling pathways such as TNF, insulin resistance, lipid and atherosclerosis, fluid shear stress, and atherosclerosis. TNF is a key proinflammatory factor that mediates both immunological and inflammatory responses. IL-1 and IL-6 cells have an anti-inflammatory role and accelerate the development of atherosclerosis in addition to their inherent capacity to regulate lipid metabolism and stimulate the production of adhesion molecules [37, 38]. In high-fat diet mice, resveratrol can dramatically lower the amounts of TG, TC, and free fatty acids in the liver and plasma. Its mechanism of action may be connected to a decrease in lipid peroxidation and hepatic TNF-α production [39]. In a mouse model of ulcerative colitis, polydatin primarily targets the NF-κB-p65 pathway and reduces the production of TNF-α, IL-6, and IL-1β, the main inflammatory cytokines, resulting in an anti-inflammatory effect [40]. Furthermore, studies have demonstrated that the beneficial effects of polydatin on HFD-induced obese mice are caused by the lowering of TNF-α, MCP-1, IL-6, S100A8, and S100A9-triggered inflammation [41]. As demonstrated earlier, our study validates the TNF signaling pathway predicted by the PPI target gene and modifies the production of IL-6, an inflammatory factor downstream.

However, these findings also raised the possibility that the anti-atherosclerotic processes produced by GDHP include shear force and IR. At the blood vessel's bifurcation, branching out, and bending,

turbulence and low shear stress may promote cell division and apoptosis. This, in turn, may stimulate the synthesis and uptake of lipids by the endothelium, an inflammatory response, and the subcutaneous aggregation of monocytes and lipids, all of which increase the risk of plaque and thrombus formation [42]. IR is the primary pathway and crucial connector between different metabolic disorders and congestive heart failure (CHD) [43]. It is intimately linked to the onset and progression of CHD. For instance, research has demonstrated that individuals with CHD have IR [44]. Furthermore, endothelial damage, oxidative stress response, lipid metabolic disorder, inflammation, and other mechanisms can all contribute to and hasten atherosclerosis. By raising ROS levels, IR can trigger JNK and IKK, which in turn can intensify and start an inflammatory response that leads to endothelial diseases [45]. For example, JNK triggers the production of inflammatory molecules like interleukin-2 (IL-2) and matrix metalloproteinases, as well as TNF-α [46]. TNF-α and IL-6 may affect insulin signal transmission, fibrinolytic enzyme activator inhibitor 1, and the creation and secretion of extracellular matrix proteins. Additionally, these substances can trigger the migration and activation of intramural smooth muscle cells from the middle to the vascular endothelium, enhance the production of free fatty acids (FFA), and activate the MAPK, endothelial DAG, and PKC signal pathways [47].

Conclusion

This work established the binding capacities of the active components in herbs and their targets, and it used network pharmacology to anticipate the targets, mechanism, and related signal pathways of GDHP in the treatment of CHD. The effectiveness of GDHP's active components, including gallocatechin, carnosol, and tanshinone IIA, in binding to their main targets was assessed in this work using molecular docking. Therefore, it is plausible that HSHP plays a major role in the management of atherosclerosis, and that its main mode of action is through the modification of hub targets associated with IR, inflammation, blood flow shear force, and signalling pathways connected to metabolism. Further experimental validation is required to validate the efficacy of GDHP on CHD, and there are several limitations to the study concerning the biologically active component assessment. Therefore, further research is needed to elucidate the potential therapeutic mechanisms of GDHP.

Declarations

Conflict of Interest

Authors declare no conflict of interest.

Authors Contribution

Mubashra Asghar Bhatti: Data collection, Formal analysis, Review, and writing of the initial draft. **Rahman Mazhar**: Formal analysis, Review, and writing of the initial draft. **Hafiz Farhan**: Material and Methods, and Review and writing of the initial draft. **Muqaddas Mustafa**: Formal analysis. **Muhammad Sajid**: Conceptualization, investigation, and supervision. **Esha Altaf:** Review and writing of the final version of the manuscript. **Kinza Akhtar**: Formal Analysis. **M. Usama Tahir**: Review and writing of the final version of

the manuscript. **Misbah Shafique**: Review and writing of the final version of the manuscript. **Ayesha Amin**: review and writing of the final version of the manuscript. **Jaweria Zafar**: Formal Analysis. **Aqsa Tahir:** Formal Analysis. All authors reviewed the final version of the manuscript and approved it for publication.

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Supplementary Material

Supplementary Figure S1 and Supplementary Tables S1-S2 are not available with this version.

Figures

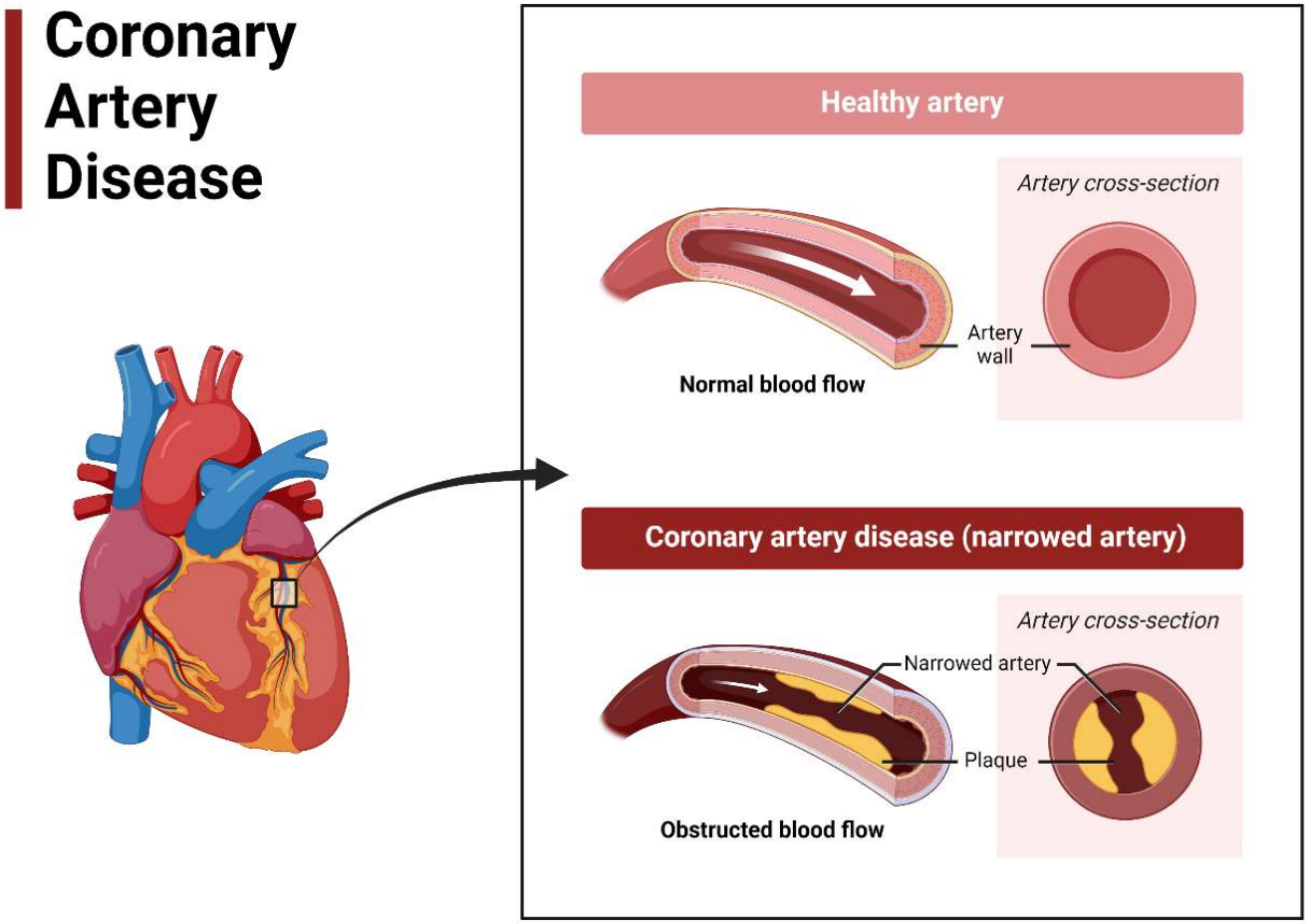


Figure 1

A comparison between a Coronary Artery without Coronary Heart Disease and one with Coronary Heart Disease. The picture highlights the plaque accumulation and arterial constriction in the diseased artery relative to the healthy, unobstructed artery, illuminating the structural variations and pathological alterations linked to coronary heart disease.

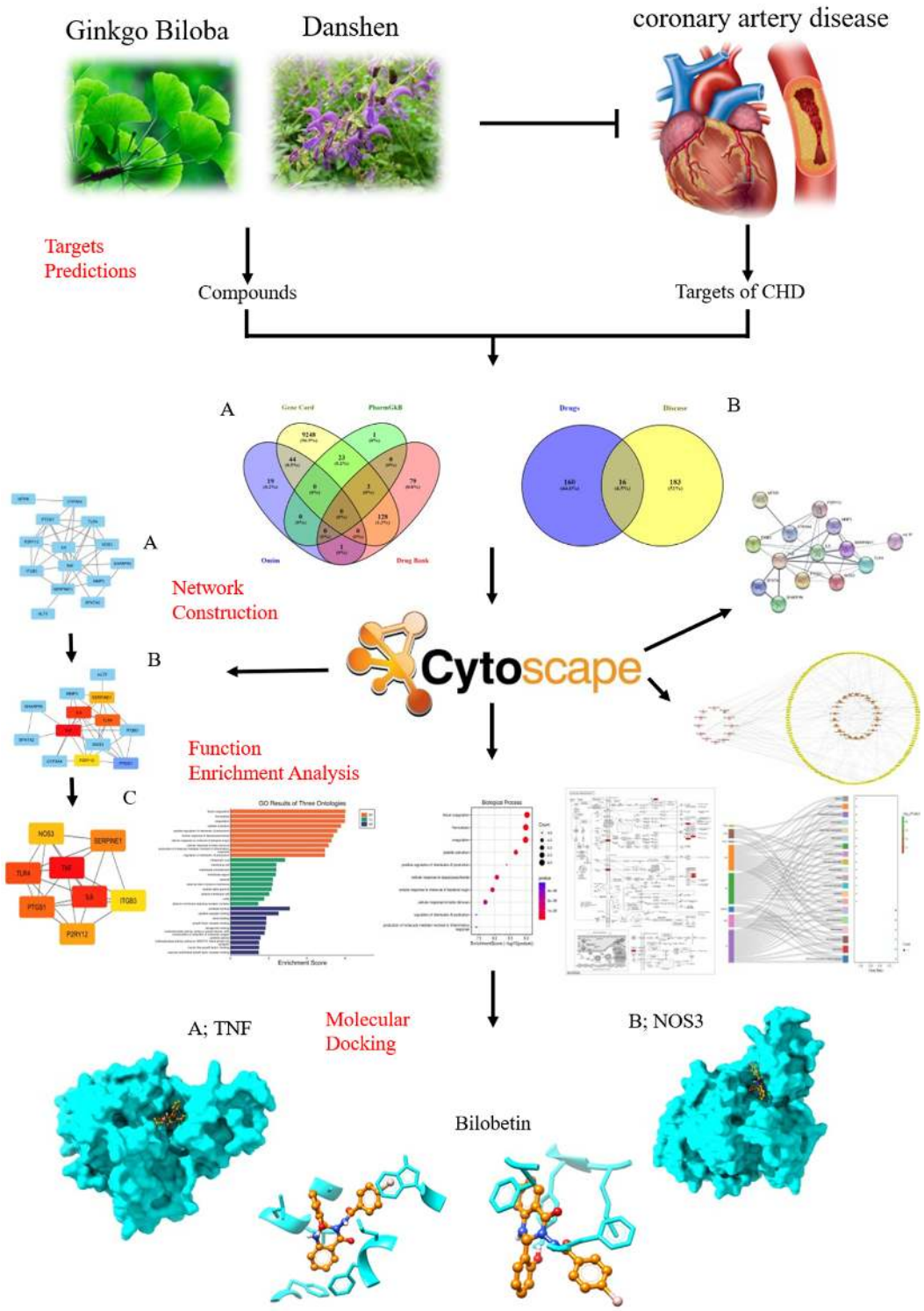


Figure 2

Workflow for studying the mechanisms in the therapy of CHD. The initial step was to screen the target, which was then followed by a network of compounds. The following steps included core gene screening and molecular docking.

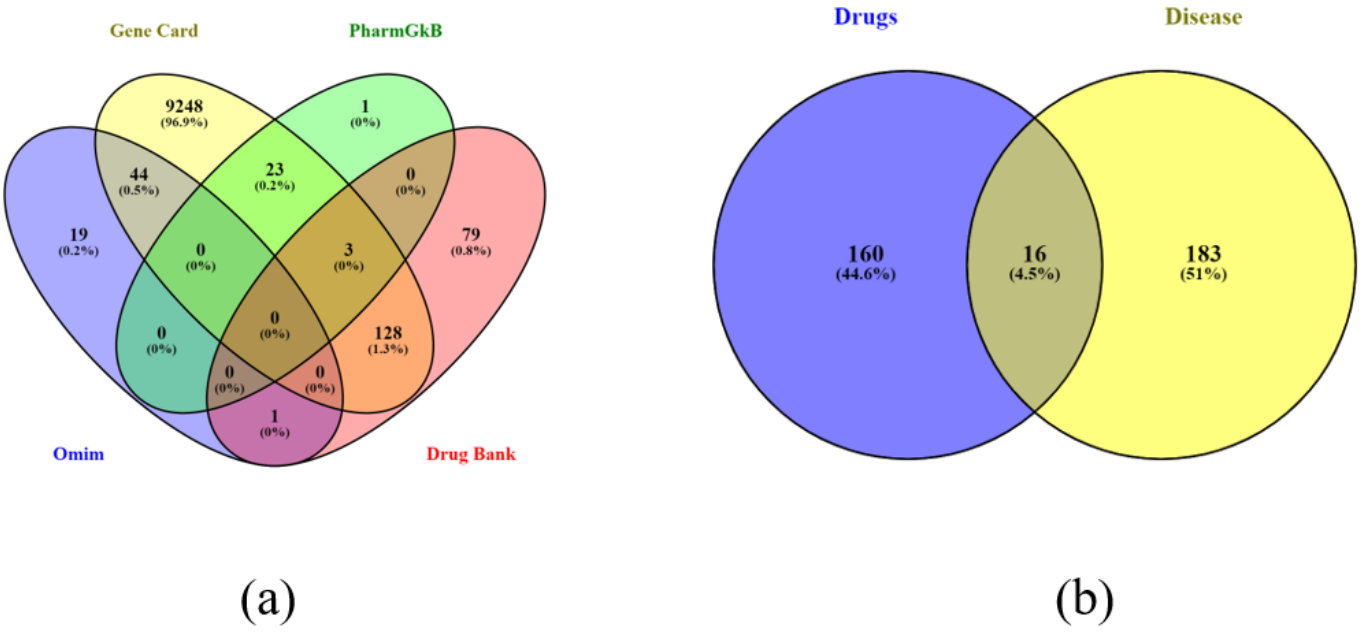


Figure 3

Venn Diagram of herbs targets **(a)** CHD disease targets **(b)**Common targets between CHD and herbs.

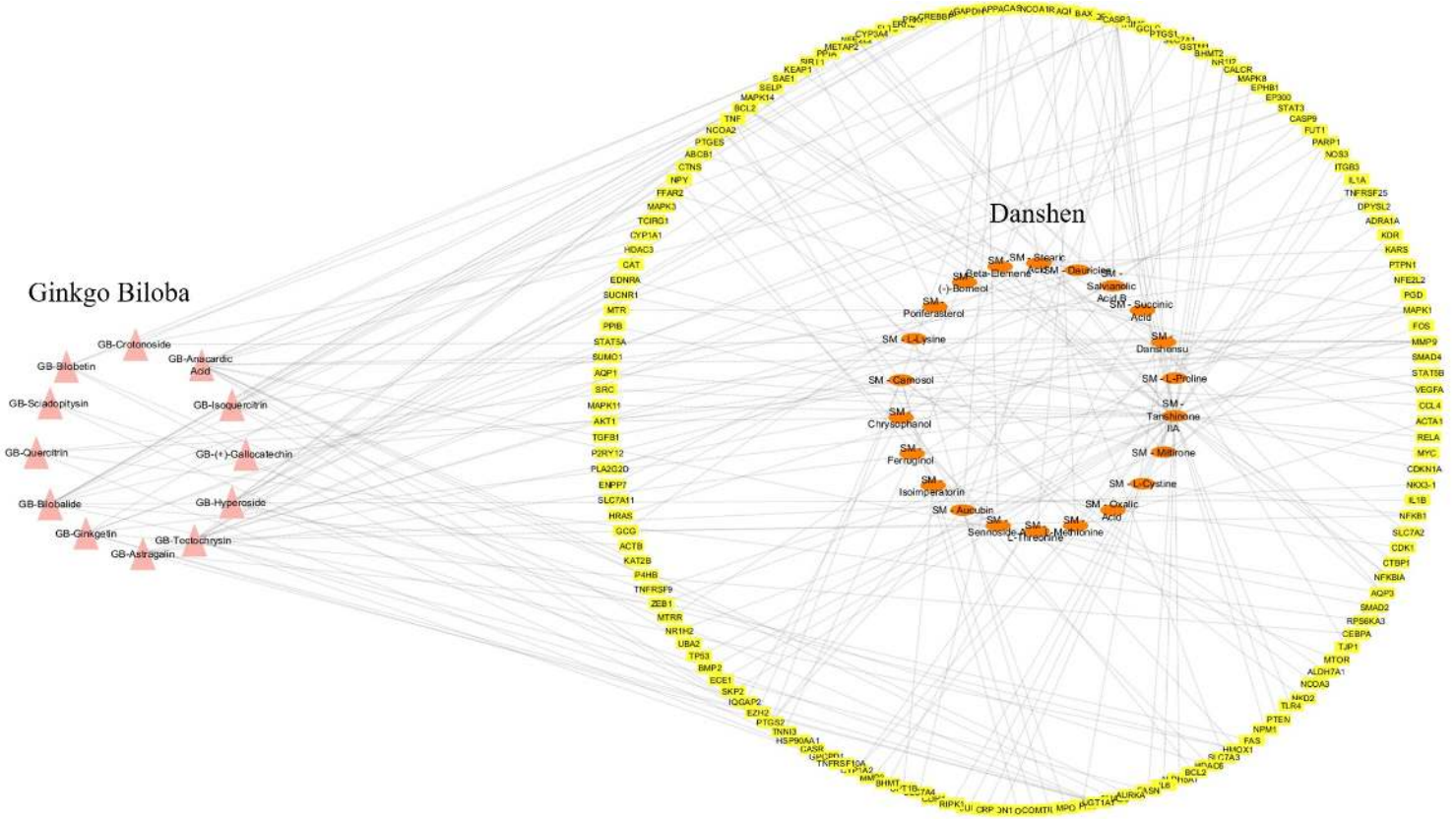


Figure 4

The interaction between the active cmpounds of Salvia miltiorrhiza and Ginkgo biloba and target genes. The pink and orange knots show the primary active components of Danshen (Salvia miltiorrhiza) and Ginkgo biloba, respectively, while the yellow knots represent the corresponding gene targets for treating CHD.

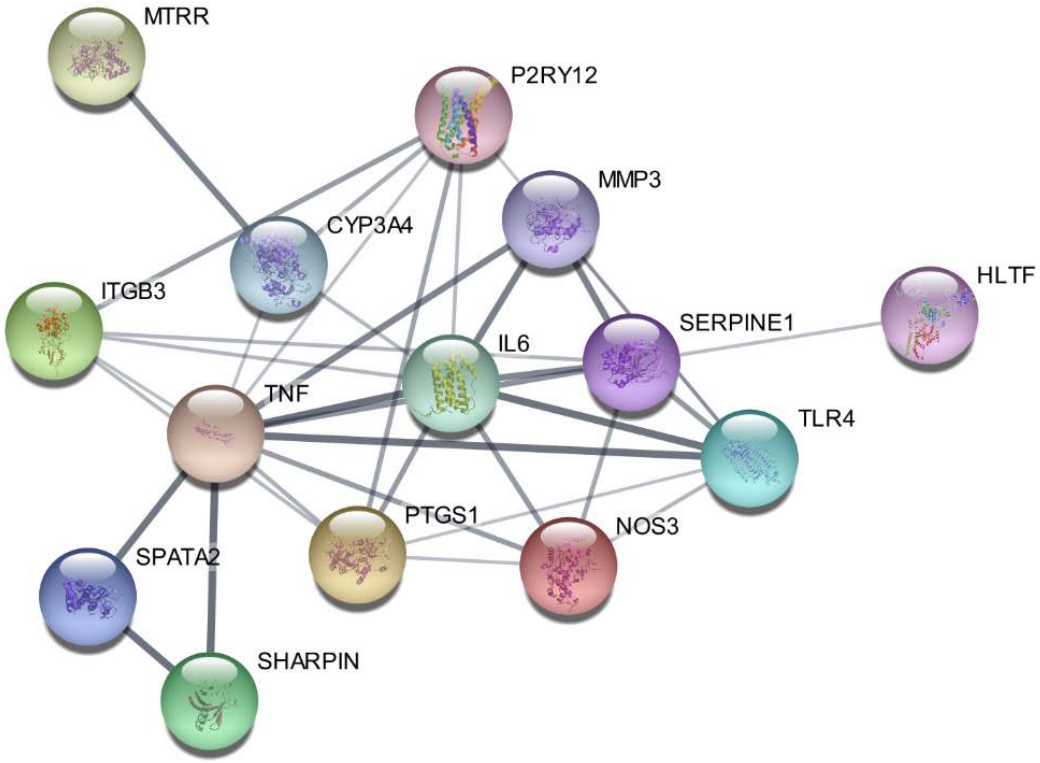


Figure 5

Common gene targets for CDH and the PPI network of herbs

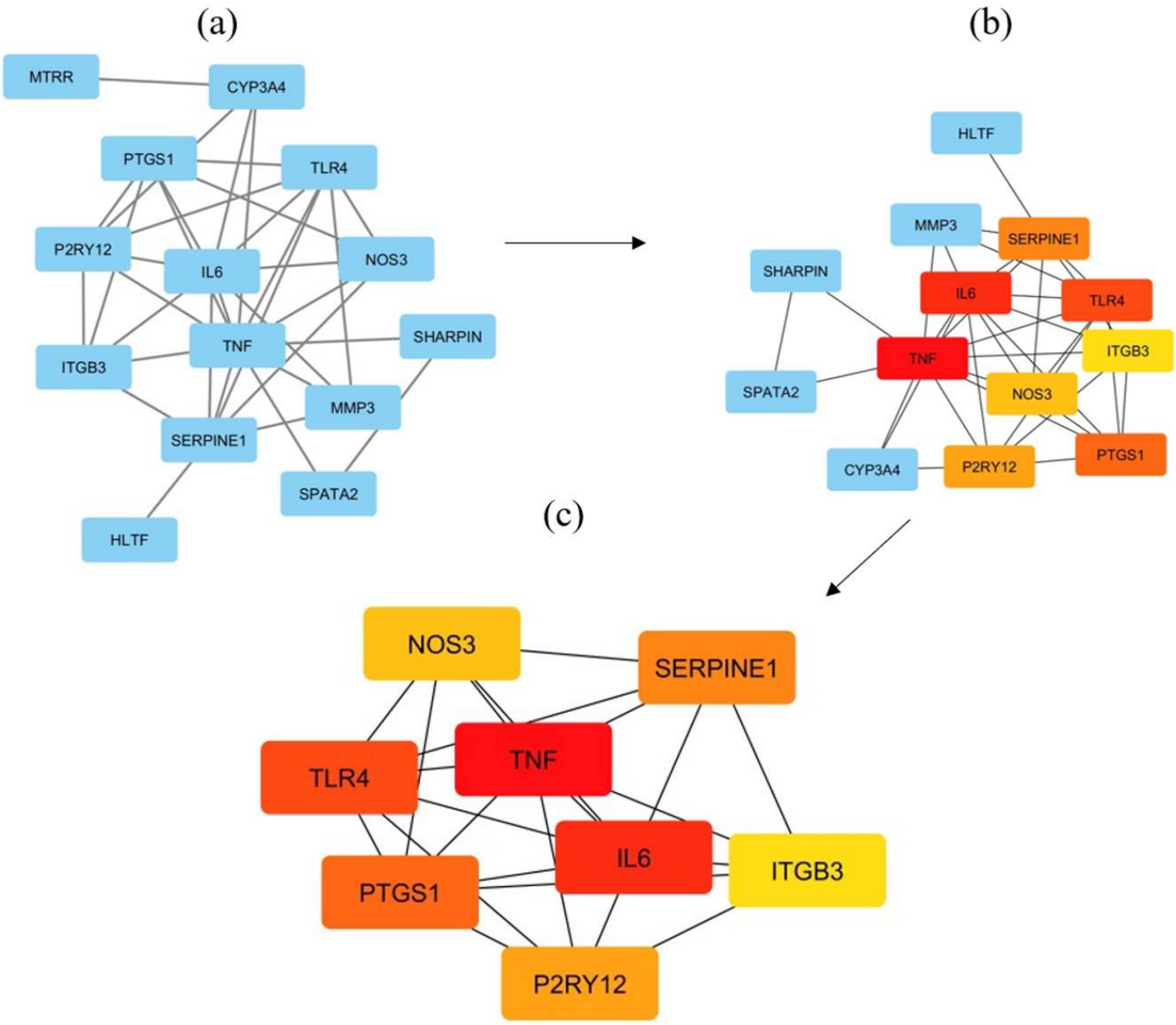


Figure 6

Screening of the key genes (a) 1st Screening (b) 2nd Screening (c) 8 key genes identified.



Figure 7

GO enrichment study of CHD-related gene targets. The top 10 words of the GO enrichment analysis are displayed as green, orange, and purple bars, respectively, for (a) biological process (BP), (b) cellular component (CC), (c) MF, and (d) molecular function (MF).

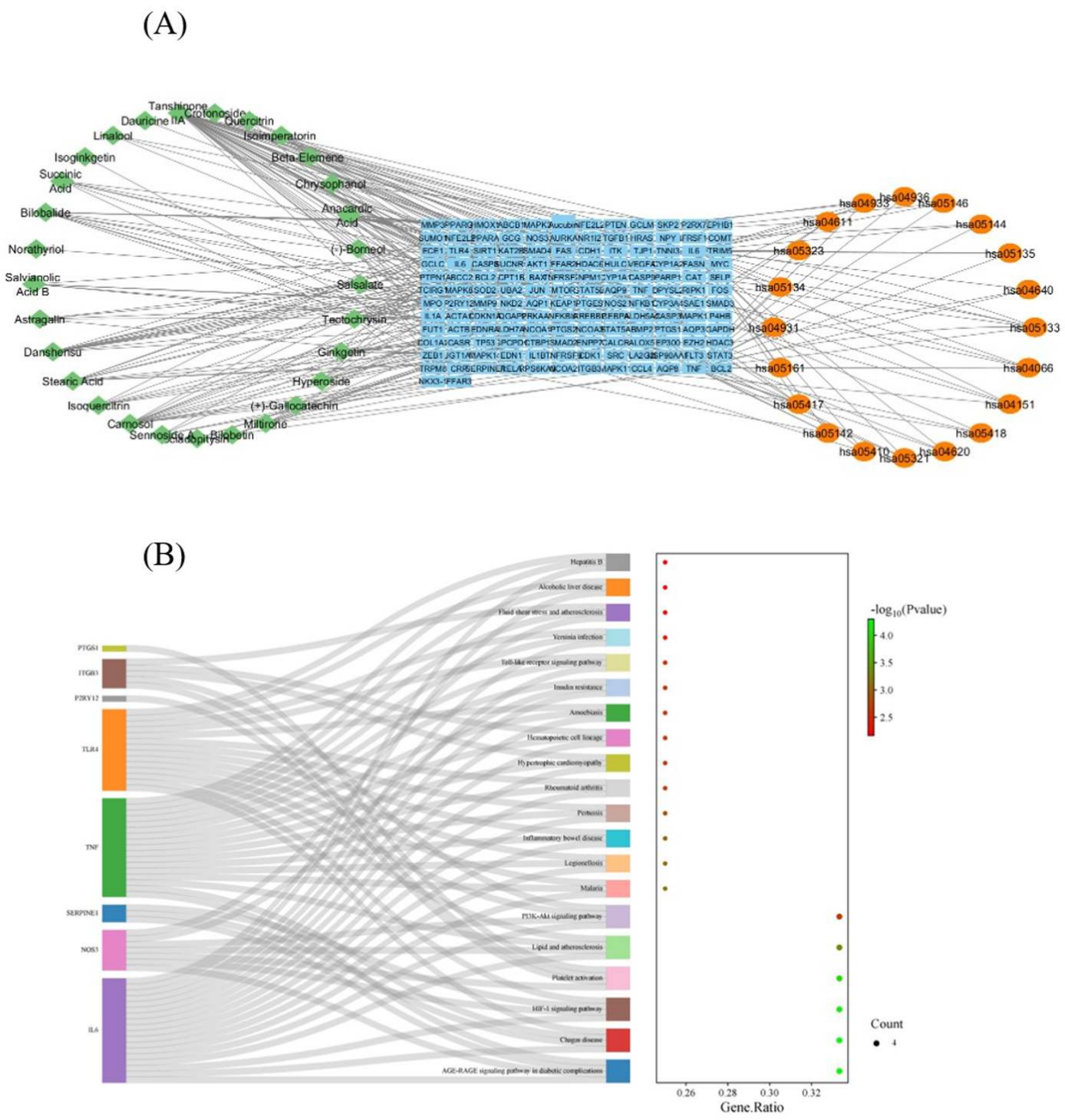


Figure 8

(A) The network of compounds, targets, and pathways linked to the mechanism of GDHP in the therapy of CHD. The routes are represented by the green nodes, the compounds by the pink nodes, and the targets by the purple nodes (B). The KEGG pathway analysis of the therapeutic targets of GDHP in the treatment of CHD is shown in the sankey diagram. The ownership of targets and pathways is shown by

the lines in the sankey diagram, while the therapeutic targets are represented by the left rectangle nodes and the KEGG pathways by the right rectangle nodes.

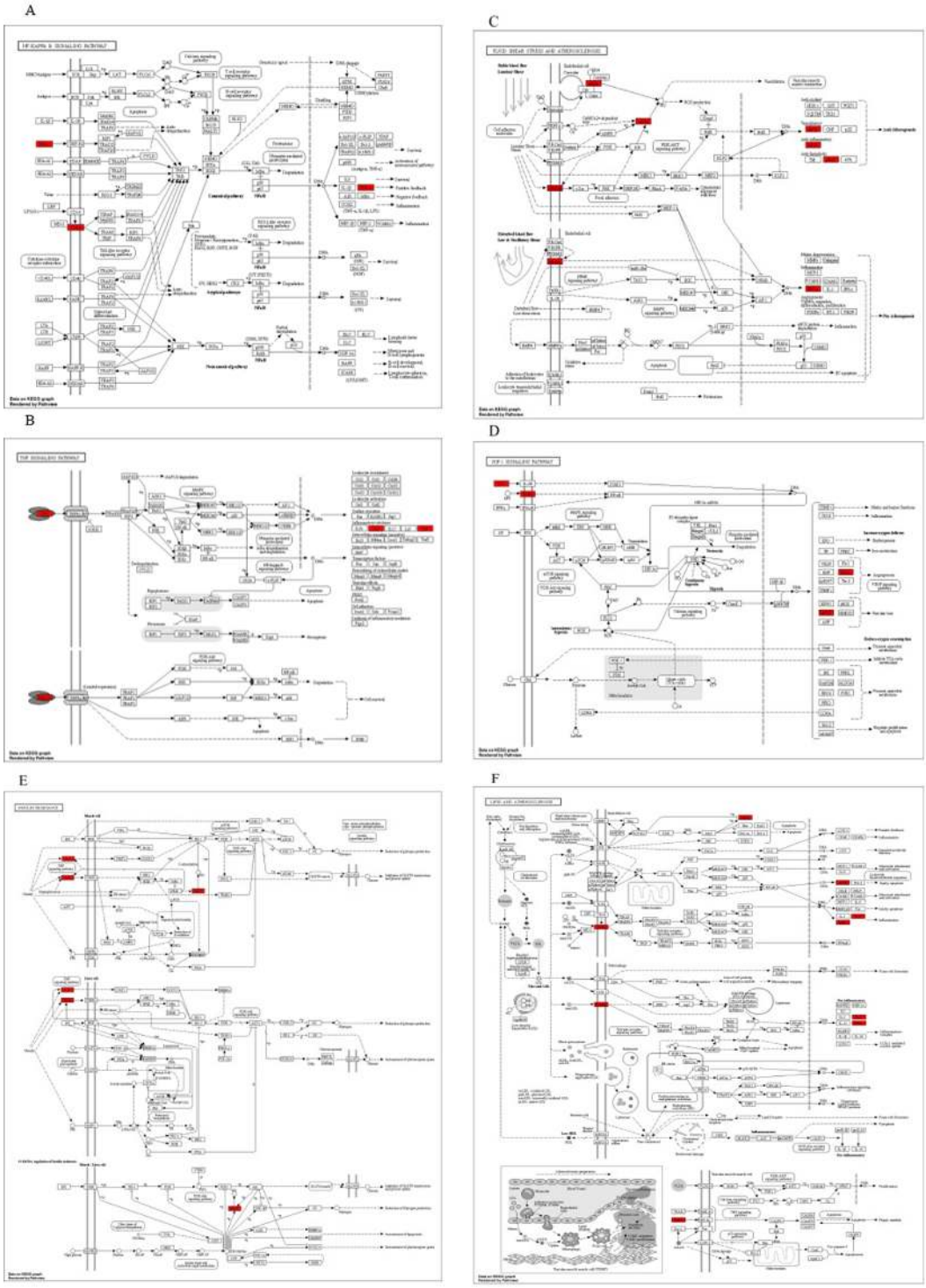


Figure 9

Signalling pathway in CHD

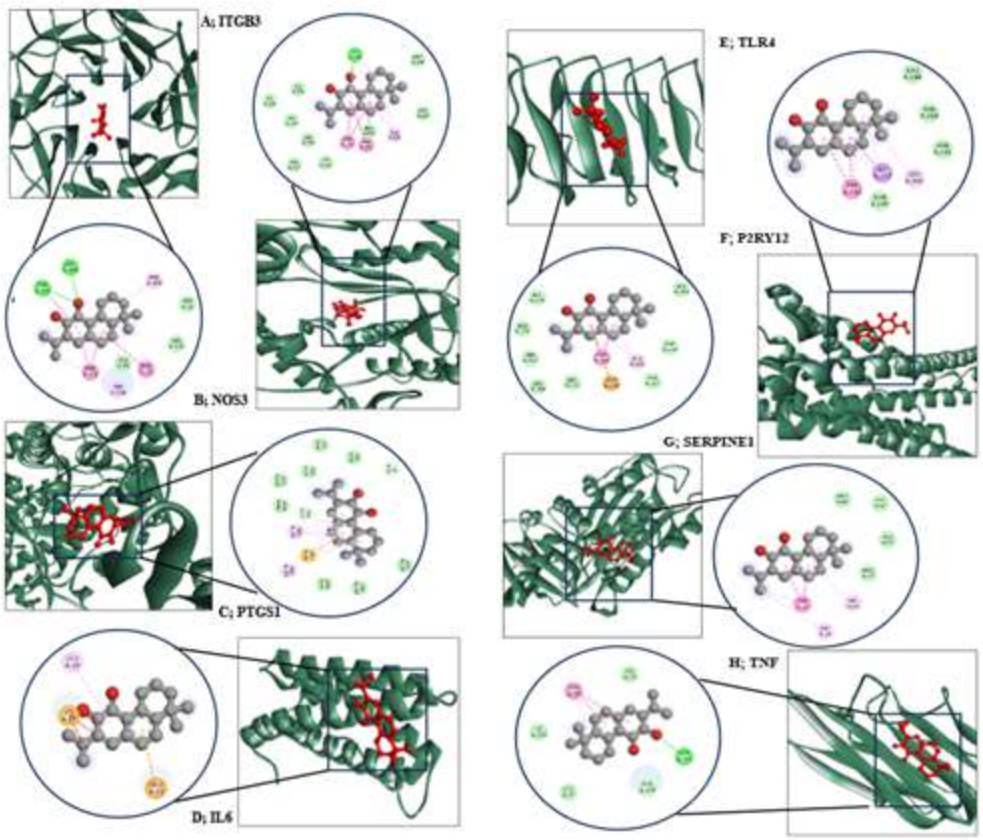


Figure 10

The docking simulation includes detailed interactions between target compounds.

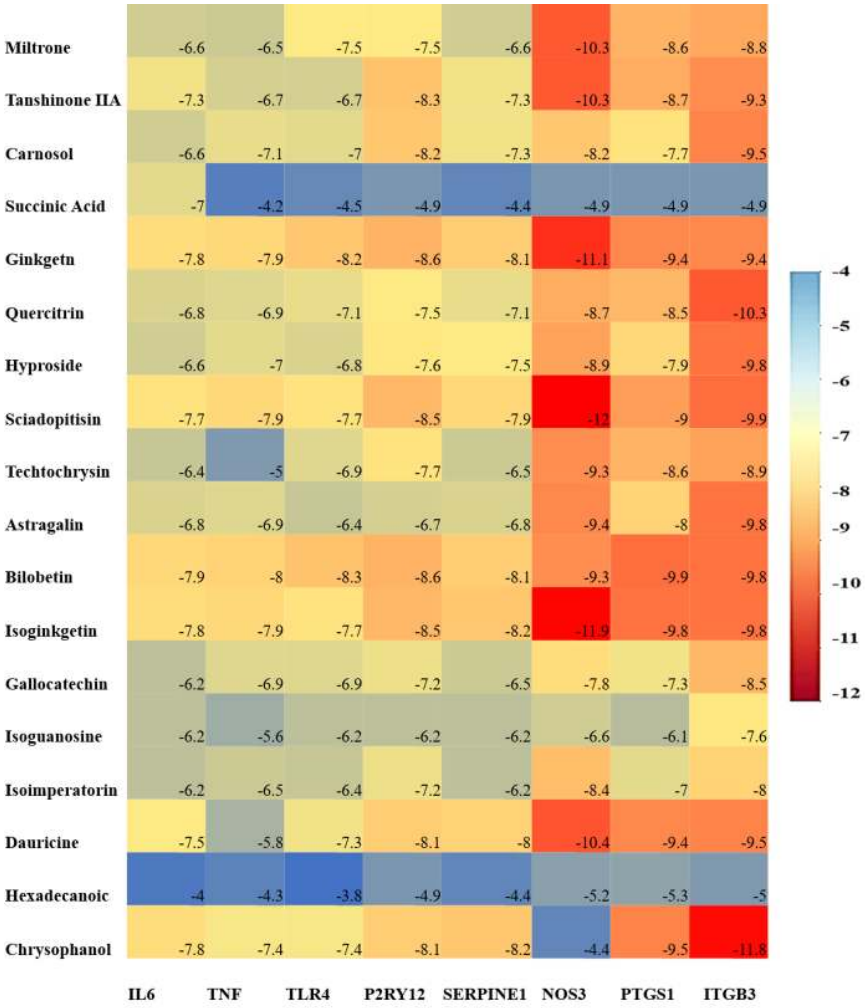


Figure 11

Heatmap representing molecular docking results showing the binding affinities of compounds with proteins. Red color indicates the highest binding values, yellow color represents medium binding values, and blue color indicates lower binding values. The heatmap visualizes the interaction strengths between compounds and proteins, providing insights into potential molecular interactions.