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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH



# Network pharmacology and bioinformatics approach reveals the hypolipidemic mechanism of Dan Tian Jiang Zhi pill

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## Abstract

Dan Tian Jiang Zhi (DTJZ) pill is a traditional effective treatment for high blood lipid in China. It mainly consists of seven herbal medicines formulated according to traditional Chinese Medicine (TCM) practices. In this study, a network pharmacology-based strategy was used to predict the active ingredients, potential targets, signaling pathways, and investigate the "ingredient-target-pathway" mechanisms of action of DTJZ pill for the treatment of lowering blood lipids. Compounds of herbs in DTJZ pill were collected from TCMSP public database and literature. Furthermore, compounds with the oral bioavailability (OB) ≥30%, drug-likeness (DL) ≥0.18, and Caco-2 ≥0.40 were screened according to ADME features. Then, the potential targets of the active compounds were predicted by pharmacophore mapping approach and mapped with the target genes of the hypolipidemic. The compound–target network, protein–protein interaction (PPI), and compound–target–biological process network were built by Cytoscape software. The core targets were selected according to the degree values. Network analysis indicate that five target genes BMP2, APOA2, ALB, ESR1, and F2 are key nodes and play important roles in this prescription. Sixty-seven chemical compositions are screened from 909 total compounds in DTJZ pill. Among which, compounds arachidonate, Stigmasterol, Liquiritigenin, β-sitosterol, and 2-isopropyl-8-methylphenanthrene-3,4-dione play important roles in the hypolipidemic process. With the application of bioinformatics, signaling pathway enrichment, and GO biological analysis of targets were also performed. The main herb *Radix Salviae* could regulate blood lipid levels by participating in the adipocytokine signaling pathway, cholesterol homecostasis, and steroid metabolic biological process. Due to the interaction among diseases, genes, targets and medicines, the mechanism of multiple compounds, multitarget, and multipathway mechanisms in the cooperative treatment of hypolipidemic were demonstrated.

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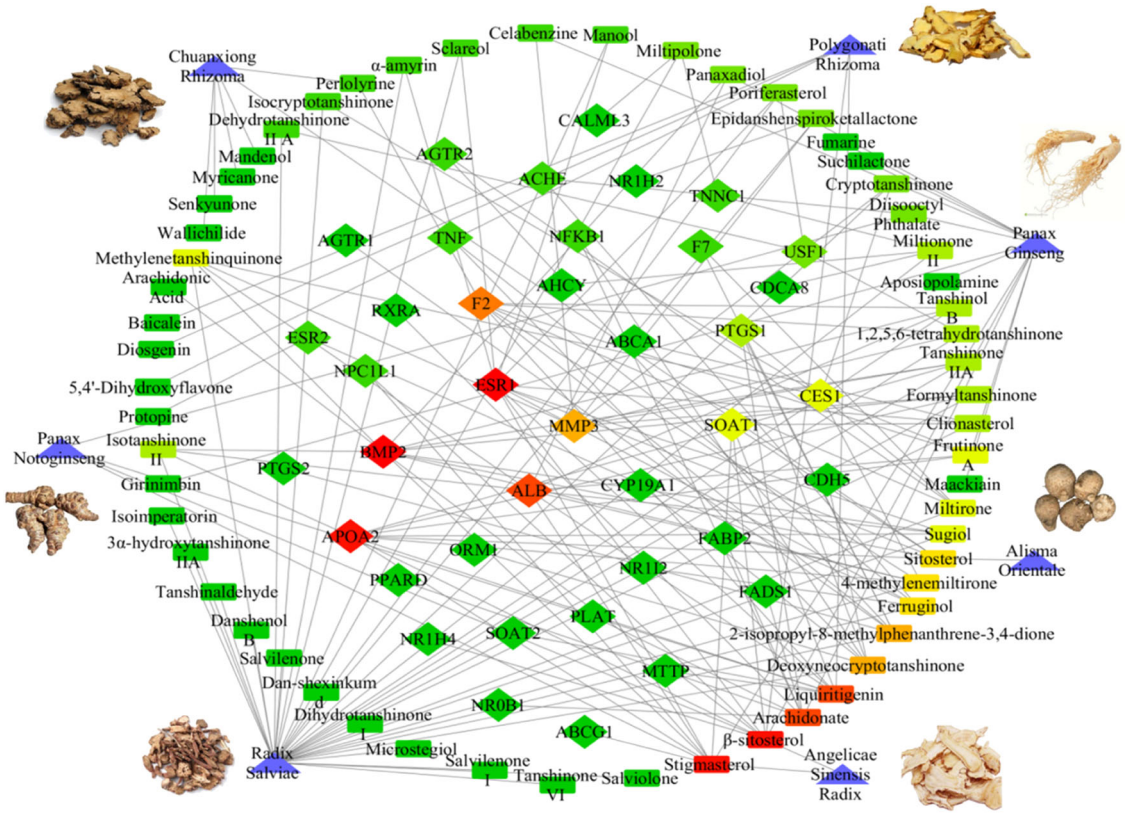
**Graphical Abstract**

Traditional Chinese medicine hypolipidemic DTJZ pill generates the hypolipidemic action by affecting multiple targets and multiple pathways.




**Keywords** Network pharmacology · Chinese materia medica · Bioinformatics · Hyperlipidaemia

## Introduction

With the development of economics and living conditions, the prevalence of obesity has become a global problem all over the world (Lunagariya et al. 2014). Nowadays, obesity has been recognized as an important risk factor for the development of metabolic diseases including hyperlipidemia, hypertension, arteriosclerosis, noninsulin-dependent diabetes mellitus, coronary heart disease, and some types of cancer (Aronne 2002; Kopelman 2000; Antonopoulos et al. 2016). Because pancreatic lipase is a predominant lipolytic enzyme in humans, which is responsible for the hydrolysis and absorption of 50–70% of total dietary fat in the intestinal lumen, there are a lot of potent inhibitors are produced (Embleton and Pouton 1997). However, they are accompanied with a lot of adverse effects, such as oily stools, flatulence, fecal urgency, and abdominal cramps. Thus, some other natural pancreatic lipase inhibitors with fewer side effects than synthetic drugs are needed. In China, Traditional Chinese Medicine (TCM) has been used for the effective treatment of hyperlipidemia for a long history. Dan tian Jiang zhi (DTJZ) pill is a commonly used hypolipidemic prescription that can reduce serum lipids and improve microcirculation. It is mainly composed of seven herbal medicines, namely *Radix Salviae*, *Panax Notoginseng*, *Chuanxiong Rhizoma*, *Alisma Orientale*, *Panax Ginseng*, *Angelicae Sinensis Radix*, and *Polygonati Rhizoma*. Unfortunately, the molecular mechanism of the constituents from this prescription against metabolic disorders has not been well investigated.

In recent years, network pharmacology was used to effectively and systematically study the mechanism of these compound preparations in traditional medicine (Hopkins 2008; Li and Zhang 2013). Compared with a single-target inhibitor, compound preparation in traditional medicine can act multiple targets by multiple component reaction. It plays an indispensable role in core pathways of diseases, and helps achieve the purpose of treating diseases (Wu et al. 2013). For example, Tang et al. have applied network pharmacology to study the mechanism of action of Xuan Hu Suo Powder in treating osteoarthritis (Tang et al. 2016). An Huang et al. have explored the mechanism of action of

Longzuan Tongbi Formula on rheumatoid arthritis (Huang et al. 2019). In contrast to the traditional "one drug, one target" principle of drug design, network pharmacology aims to investigate the influence or intervention of drugs on diseases as a whole, the relationship between drugs, their targets, and diseases can be studied through scientific calculations and displayed through visual networks (Hopkins 2007). Generally, the network pharmacological approach provides new insights into the systemic connection between Chinese herbal medicine, therapeutic targets and disease. It is also recognized as a powerful and promising tool to discover the potential bioactive ingredients (Sarkar et al. 2019; Song et al. 2019).

Although the lipid-lowering effect of DTJZ pill has been approved, network pharmacology-based prediction of the bioactive components and their target pathways has not been performed. Therefore, the aim of this work is to achieve the interaction between the bioactive compounds and targets and the interaction between various targets, and then find out and validate the key nodes based on network pharmacology analysis. The bioactive compounds and their candidate target genes were thoroughly retrieved from public databases. Interactions of target compounds with target genes based on ADME criteria resulted in a network of seven herbs, 67 compounds, and 39 target genes with 106 nodes and 113 edges. In addition, bioinformatics approaches have also been adopted to explore the mechanism of action of DTJZ pill in treating hyperlipidemia. Signaling pathway enrichment and GO analysis of targets were thoroughly performed. For the main herb *Radix Salviae* in the prescription, the chemical constituents involved in the biological process (BP) was also analyzed in detail. Finally, the multiple compounds, multitarget, and multipathway mechanisms related to DTJZ pill in the cooperative treatment of hypolipidemic were explained from the perspective of network pharmacology.

## Material and methods

### Chemical databases and evaluation of drug-likeness (DL)

The small molecules were obtained from the TCM database (http://tcm.cmu.edu.tw/), the ZINC databases (http://zinc.docking.org/), and pubchem compound (https://www.ncbi.nlm.nih.gov/pubmed/) were used as resource for the 2D and 3D chemical structures, chemical numbers and physicochemical properties. The systems-level pharmacological database for TCMSP server (http://lsp.nwu.edu.cn/tcmsp.php) was used to calculate the ADME-related properties of naturally occurring compounds of interest (Ru et al. 2014). It also provides an in silico ADME-systems evaluation model, which integrates DL, oral bioavailability (OB), Caco-2 permeability, and other features.

### Prediction of targets

In order to identify the target genes that are related to hyperlipidemia, TCMSP database, BATMAN-TCM database (http://bionet.ncpsb.org/batman-tcm/), and DrugBank database (https://www.drugbank.ca/) were applied to find out the potential targets. The predicted candidate targets are ranked via BATMAN-TCM according to the order of decreasing score given by the target prediction algorithm for the drug–target interaction prediction. Score cutoff was set at 80 and adjusted $P$ value was set at 0.05. Then, the human gene database GeneCards (http://www.genecards.org/), the OMIM database (http://www.ncbi.nlm.nih.gov/omim), the therapeutic targets database TTD (http://bidd.nus.edu.sg/BIDD-Databases/TTD/TTD.asp), and PharmMapper website (http://www.lilab-ecust.cn/pharmmapper/) were used to search for information about hypolipidemic target genes using only "Homo sapiens" proteins. The top ten target genes in target database of pharmacophore are considered according to the fit score. UniProt (http://www.uniprot.org/) was utilized for retrieving gene information including name, gene ID, and organism.

### Network construction

A protein–protein interaction was established by Cytoscape 3.5.1 (http://www.cytoscape.org/). The network parameters were calculated using the network analyzer plugin in Cytoscape. Cytoscape combined score of interactions was adopted for judging the importance of nodes in each given network. Definition of topological feature set for the network. As a Cytoscape plugin, CytoNCA was used to analyze the topological properties of every node in the interaction network in order to calculate two topological properties: betweenness centrality (BC) and degree centrality (Tang et al. 2015).

### Gene ontology (GO) and the KEGG pathway enrichment analysis

We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov/) (Huang et al. 2009), an online program that provides comprehensive data for high-throughput gene functional analysis for elucidation of biological characteristics, to obtain GO terms belonging to the BP, cellular component (CC), and molecular function (MF) categories. In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway functional enrichment analyses were performed for the above DEGs. Results with values of $P < 0.05$ were

considered to be statistically significant (Wu et al. 2019; Liang et al. 2019).

## Results

### Selection of constituents using ADME screening

According to Lipinski's rule "rule of five", a molecule is likely to have good absorption and/or permeation if it possesses less than five hydrogen-bond donors, less than ten hydrogen-bond acceptors, a calculated log P (Alog P) lower than five, and a molecular weight (MW) lower than 500 Da. In this text, effective chemical composition that are satisfied with OB ≥ 30%, DL ≥ 0.18, ALog P ≤ 5, Caco-2 ≥ 0.40, and MW ≤ 500 were screened. Where, OB signifies the percentage of an orally administered dose of unchanged drug reaching the systemic circulation, and is an indicative of conjunction of the ADME process. High OB is often a key indicator to determine the drug-like property of bioactive molecules as therapeutic agents (AboulFotouh et al. 2019). DL is a qualitative concept used in drug design for an estimate on how "drug-like" a prospective compound is, which helps to optimize pharmacokinetic and pharmaceutical properties, such as solubility and chemical stability (Tao et al. 2019). The human intestinal cell line Caco-2 is generally used as an efficient in vitro model to study the passive diffusion of drugs across intestinal epithelium (Nozari et al. 2019). DTJZ pill is mainly composed by seven herbal medicines, namely *Radix Salviae*, *Panax Notoginseng*, *Chuanxiong Rhizoma*, *Alisma Orientale*, *Panax Ginseng*, *Angelicae Sinensis Radix*, and *Polygonati Rhizoma*. A total of 909 compounds were identified, of which the structure and names were confirmed via pubchem database. Based on the screening criteria, finally, 67 chemical compositions are screened in Table 1. These compounds are the major effective components of DTJZ pill herbal products.

### Potential target genes and network analysis

The target genes related to hyperlipidaemia were searched in TCMSP database, BATMAN-TCM database, and DrugBank database. Then, OMIM database, the human gene database GeneCards, and the therapeutic targets database TTD were further used to search for only "Homo sapiens" information. As a result, 311 target genes related to Homo sapiens hypolipidemic were obtained. The final selected genes that are related to these 67 screened chemical compositions are listed in Table 2. Further linking of target compounds with target genes based on ADME criteria resulted in a network of seven herbs, 67 compounds, and 39 target genes with 106 nodes and 113 edges (Fig. 1). Herbs are indicated by slate triangle nodes, compounds are represented by rectangle nodes, target genes are shown as diamond nodes. The nodes are filled in order of degrees from green-yellow-red except the herbs. After analyzing the topological properties of every node in the interaction network, it shows that the degrees of five genes BMP2, APOA2, ALB, ESR1 and F2 are more than eight, which implied that these genes might be key nodes in this network. Among which, APOA2 (Apolipoprotein A-II) is the second most abundant protein in high-density lipoprotein particles and is involved in lipid metabolism (Blanco-Vaca et al. 2001). In this text, it was shown that Sugiol, 4-methylenemiltirone, Formyltanshinone, Isotanshinone II and Tanshinone IIA could interact directly with the APOA2 during the process of treatment.

In addition, five compounds are also considered to be important elements in the hypolipidemic process, namely Arachidonate, Stigmasterol, Liquiritigenin, β-sitosterol, and 2-isopropyl-8-methylphenanthrene-3,4-dione. Actually, it was showed that supplementation of phytosterols stigmasterol to high-fat western-style diet significantly reduced the body weight gain, serum cholesterol, histological score, and alanine aminotransferase levels (Feng et al. 2018). Compared with β-sitosterol, stigmasterol is more effective in decreasing hepatic lipid accumulation and nonalcoholic fatty liver disease in mice fed a high-fat western-style diet (Kim et al. 2011). Liquiritigenin treatment can completely abolish the formation of thiobarbituric acid reactive substances in liver tissue, which is induced by high-fat diet feeding. This demonstrated that Liquiritigenin has the ability to inhibit liver fat accumulation, thereby protecting the liver from liver damage and lipid peroxidation (Yuan et al. 2019). In addition, study shows that β-sitosterol could effectively reduce cholesterol concentration in dietary model through competitive mechanism in vitro (Lei et al. 2016). β-sitosterol was also suggested as a health supplement in management of hypercholesterolemia due to its potential in decreasing plasma cholesterol in hamster model (Yang et al. 2009).

As the main drug in the DTJZ pill, *Radix Salviae* contains the most effective chemical components (Fig. 1). In order to clarify the hypolipidemic role of *Radix Salviae*, the components and the corresponding gene targets were further analyzed in detail. From Fig. 2, there are 60 nodes and 136 edges in this figure. Based on the STRING database, the target and the target interaction is obtained and indicated by the dotted line with arrows. From this figure, it can be seen that genes ESR1, BMP2, MMP3, APOA2, and F2 are important targets and can interact with multiple chemical components of *Radix Salviae*. Meanwhile, the Cryptotanshinone, 2-isopropyl-8-methylphenanthrene-3,4-dione, and Deoxyneocryptotanshinone are important active ingredients that can interact with a lot of targets. As the most pivotal compound, cryptotanshinone

**Table 1** A list of the final selected compounds among the five herbal medicines in DTJZ pill for network analysis

| No. | Herbal medicine | Chemical | No. | Herbal medicine | Chemical |
|---|---|---|---|---|---|
| 1 | *Panax Notoginseng* | Mandenol | 35 | *Radix Salviae* | Miltirone |
| 2 | *Panax Notoginseng* | Liquiritigenin | 36 | *Radix Salviae* | Deoxyneocryptotanshinone |
| 3 | *Panax Notoginseng* | Diisooctyl Phthalate | 37 | *Radix Salviae* | Salvilenone I |
| 4 | *Panax Notoginseng* | Beta-sitosterol | 38 | *Radix Salviae* | Salviolone |
| 5 | *Panax Notoginseng* | Stigmasterol | 39 | *Radix Salviae* | Sugiol |
| 6 | *Radix Salviae* | 1,2,5,6-Tetrahydrotanshinone | 40 | *Radix Salviae* | Tanshinone IIA |
| 7 | *Radix Salviae* | Poriferasterol | 41 | *Radix Salviae* | Tanshinone VI |
| 8 | *Radix Salviae* | Clionasterol | 42 | *Panax Ginseng* | Diisooctyl phthalate |
| 9 | *Radix Salviae* | Isoimperatorin | 43 | *Panax Ginseng* | Stigmasterol |
| 10 | *Radix Salviae* | Sugiol | 44 | *Panax Ginseng* | Beta-sitosterol |
| 11 | *Radix Salviae* | Dehydrotanshinone IIA | 45 | *Panax Ginseng* | Maackiain |
| 12 | *Radix Salviae* | α-Amyrin | 46 | *Panax Ginseng* | Aposiopolamine |
| 13 | *Radix Salviae* | 2-Isopropyl-8-methylphenanthrene-3,4-dione | 47 | *Panax Ginseng* | Celabenzine |
| 14 | *Radix Salviae* | 3α-Hydroxytanshinone IIA | 48 | *Panax Ginseng* | Arachidonate |
| 15 | *Radix Salviae* | 4-Methylenemiltirone | 49 | *Panax Ginseng* | Frutinone A |
| 16 | *Radix Salviae* | Formyltanshinone | 50 | *Panax Ginseng* | Girinimbin |
| 17 | *Radix Salviae* | Methylenetanshinquinone | 51 | *Panax Ginseng* | Panaxadiol |
| 18 | *Radix Salviae* | Tanshinol B | 52 | *Panax Ginseng* | Suchilactone |
| 19 | *Radix Salviae* | Sclareol | 53 | *Panax Ginseng* | Fumarine |
| 20 | *Radix Salviae* | Tanshinaldehyde | 54 | *Angelicae Sinensis Radix* | Beta-sitosterol |
| 21 | *Radix Salviae* | Danshenol B | 55 | *Angelicae Sinensis Radix* | Stigmasterol |
| 22 | *Radix Salviae* | Salvilenone | 56 | *Chuanxiong Rhizoma* | Mandenol |
| 23 | *Radix Salviae* | Cryptotanshinone | 57 | *Chuanxiong Rhizoma* | Myricanone |
| 24 | *Radix Salviae* | Dan-shexinkum d | 58 | *Chuanxiong Rhizoma* | Perlolyrine |
| 25 | *Radix Salviae* | Deoxyneocryptotanshinone | 59 | *Chuanxiong Rhizoma* | Senkyunone |
| 26 | *Radix Salviae* | Dihydrotanshinone I | 60 | *Chuanxiong Rhizoma* | Wallichilide |
| 27 | *Radix Salviae* | Epidanshenspiroketallactone | 61 | *Chuanxiong Rhizoma* | Sitosterol |
| 28 | *Radix Salviae* | Ferruginol | 62 | *Alisma Orientale* | Sitosterol |
| 29 | *Radix Salviae* | Isocryptotanshinone | 63 | *Polygonati Rhizoma* | Liquiritigenin |
| 30 | *Radix Salviae* | Isotanshinone II | 64 | *Polygonati Rhizoma* | Baicalein |
| 31 | *Radix Salviae* | Manool | 65 | *Polygonati Rhizoma* | Beta-sitosterol |
| 32 | *Radix Salviae* | Microstegiol | 66 | *Polygonati Rhizoma* | Diosgenin |
| 33 | *Radix Salviae* | Miltionone II | 67 | *Polygonati Rhizoma* | 5,4′-Dihydroxyflavone |
| 34 | *Radix Salviae* | Miltipolone | | | |

remains the relationships between MMP3, NFKB1, ORM1, PTGS1, TNF, CDH5, CES1, and CYP19A1. Among which, the important MMP3 gene is the major enzyme system responsible for the collagen content in several tissues including the aorta (Yang et al. 2009, 2010). Studies have shown that increased serum MMP3 level is associated with increased future risk of atherothrombotic events (Blankenberg et al. 2003; Beyzade et al. 2003). In addition, recent studies have shown that Cryptotanshinone can also reduce blood lipids by inhibiting the target gene pancreatic lipase (Marrelli et al. 2019). It indicates from above analysis that the same chemical component can interact with different targets to achieve the purpose of lowering blood lipids.

These gene–gene interactions indicate that RXRA, ALB, F2, APOA2, and ESR1 can be more closely linked to other targets. The node degree and BC of RXRA are 9 and 0.765, respectively, which remain the highest values. Data suggested that RXRA gene is associated individually or jointly with lipid metabolism (Kalaany and Mangelsdorf 2006). It was also suggested that RXRA is involved in the genetic architecture of dyslipidaemia in hemodialysis patients (Grzegorzewska et al. 2018). From this analysis, the target RXRA gene has multiple interactions with other genes, such as ABCA1, ALB, APOA2, FADS1, NR1H2, NR1H4, NR1I2, PPARD, and TNF. The most important ABCA1 protein is involved in protecting the function of pancreatic

**Table 2** Hypolipidemic gene targets and their symbols

| No. | Entry ID | Gene name | Protein name |
|---|---|---|---|
| 1 | O95477 | ABCA1 | Phospholipid-transporting ATPase ABCA1 |
| 2 | P45844 | ABCG1 | ATP-binding cassette subfamily G member 1 |
| 3 | P22303 | ACHE | Acetylcholinesterase |
| 4 | P30556 | AGTR1 | Type-1 angiotensin II receptor |
| 5 | P50052 | AGTR2 | Type-2 angiotensin II receptor |
| 6 | P23526 | AHCY | Adenosylhomocysteinase |
| 7 | P02768 | ALB | Serum albumin |
| 8 | P02652 | APOA2 | Apolipoprotein A-II |
| 9 | Q92731 | ESR2 | Estrogen receptor beta |
| 10 | P03420 | F2 | Fusion glycoprotein F2 |
| 11 | P12643 | BMP2 | Bone morphogenetic protein 2 |
| 12 | P27482 | CALML3 | Calmodulin-like protein 3 |
| 13 | Q53HL2 | CDCA8 | Borealin |
| 14 | P33151 | CDH5 | Cadherin-5 |
| 15 | P23141 | CES1 | Liver carboxylesterase 1 |
| 16 | P11511 | CYP19A1 | Aromatase |
| 17 | Q9SAD4 | ESR1 | Ethylene-responsive transcription factor ESR1 |
| 18 | Q96AV8 | F7 | Transcription factor E2F7 |
| 19 | P12104 | FABP2 | Fatty acid-binding protein |
| 20 | O60427 | FADS1 | Acyl-CoA (8-3)-desaturase |
| 21 | P08254 | MMP3 | Stromelysin-1 |
| 22 | P55157 | MTTP | Microsomal triglyceride transfer protein large subunit |
| 23 | P19838 | NFKB1 | Nuclear factor NF-kappa-B p105 subunit |
| 24 | Q9UHC9 | NPC1L1 | NPC1-like intracellular cholesterol transporter 1 |
| 25 | P51843 | NR0B1 | Nuclear receptor subfamily 0 group B member 1 |
| 26 | P55055 | NR1H2 | Oxysterols receptor LXR-beta |
| 27 | Q96RI1 | NR1H4 | Bile acid receptor |
| 28 | O75469 | NR1I2 | Nuclear receptor subfamily 1 group I member 2 |
| 29 | P02763 | ORM1 | Alpha-1-acid glycoprotein 1 |
| 30 | P00750 | PLAT | Tissue-type plasminogen activator |
| 31 | Q03181 | PPARD | Peroxisome proliferator-activated receptor delta |
| 32 | P23219 | PTGS1 | Prostaglandin G/H synthase 1 |
| 33 | P35354 | PTGS2 | Prostaglandin G/H synthase 2 |
| 34 | P19793 | RXRA | Retinoic acid receptor RXR-alpha |
| 35 | P35610 | SOAT1 | Sterol O-acyltransferase 1 |
| 36 | O75908 | SOAT2 | Sterol O-acyltransferase 2 |
| 37 | P01375 | TNF | Tumor necrosis factor |
| 38 | P63316 | TNNC1 | Troponin C, slow skeletal and cardiac muscles |
| 39 | P22415 | USF1 | Upstream stimulatory factor 1 |

β-cells and insulin secretion by cholesterol homeostasis. A number of studies have proven that inhibition of ABCA1 degradation leads to an increase in the HDL biogenesis (Zhao et al. 2012). ABCA1 is considered to be the main actor in reverse cholesterol transport from plaque to liver, which is considered as a main mechanism for regression of atherosclerosis (Bogomolova et al. 2019).

## Potential target gene-related GO functional analysis

To analyze the biological classifications of these related genes, functional enrichment analysis was performed using the DAVID server. A total of 39 key potential genes that involved in antihyperlipidemic effect were uploaded. As shown in Fig. 3, GO functional analysis listed 20 GO terms with low *P* values and more targets enrichment. The red dotted line indicates the count of the terms, and the corresponding coordinates are shown on the right side of the graph. These targets have strong associations with multiple BPs, such as steroid metabolic process, lipid homeostasis, lipid localization, cholesterol homeostasis, sterol homeostasis, lipid transport, cholesterol metabolic process, sterol metabolic process, cholesterol transport, and so on. In the CC category, they were mostly enriched in the endoplasmic reticulum, membrane fraction, insoluble fraction, cell fraction, extracellular space and extracellular region part. In the MF category, they were mainly enriched in lipid binding, steroid binding, steroid hormone receptor activity, ligand-dependent nuclear receptor activity, cholesterol binding, sterol binding, monocarboxylic acid binding, fatty acid binding and lipid transporter activity. These results indicated that DTJZ pill plays a critical role in antihyperlipidemic effect by manipulating these functional processes which may lead to the high blood fat.

For the main herbal *Radix Salviae*, there are about 20 ingredients related to these hypolipidemic genes, which are related to important BPs, such as cholesterol homecostasis, steroid metabolic process, sterol metabolic process, cholesterol metabolic process, lipid localization, lipid transport, sterol homecostasis, lipid homecostasis, cholesterol transport, and sterol transport (Fig. 4). Among them, the most important BP is steroid metabolic process ($P$ value $= 3.8 \times 10^{-12}$), which includes a lot of target genes, such as ABCA1, ABCG1, NPC1L1, APOA2, CYP19A1, NR0B1, NR1H4, NR1I2, PPARD, RXRA, SOAT1, and SOAT2. In addition, the effective chemical compositions that are closely related to these hypolipidemic BPs are Clionasterol, 4-methylenemiltirone, Formyltanshinone, Isotanshinone II, Sugiol, Tanshinone IIA, Sugiol, Cryptotanshinone, Deoxyneocryptotanshinone, Ferruginol, and Clionasterol. As the most important chemical component node, Cryptotanshinone can lower blood lipids by

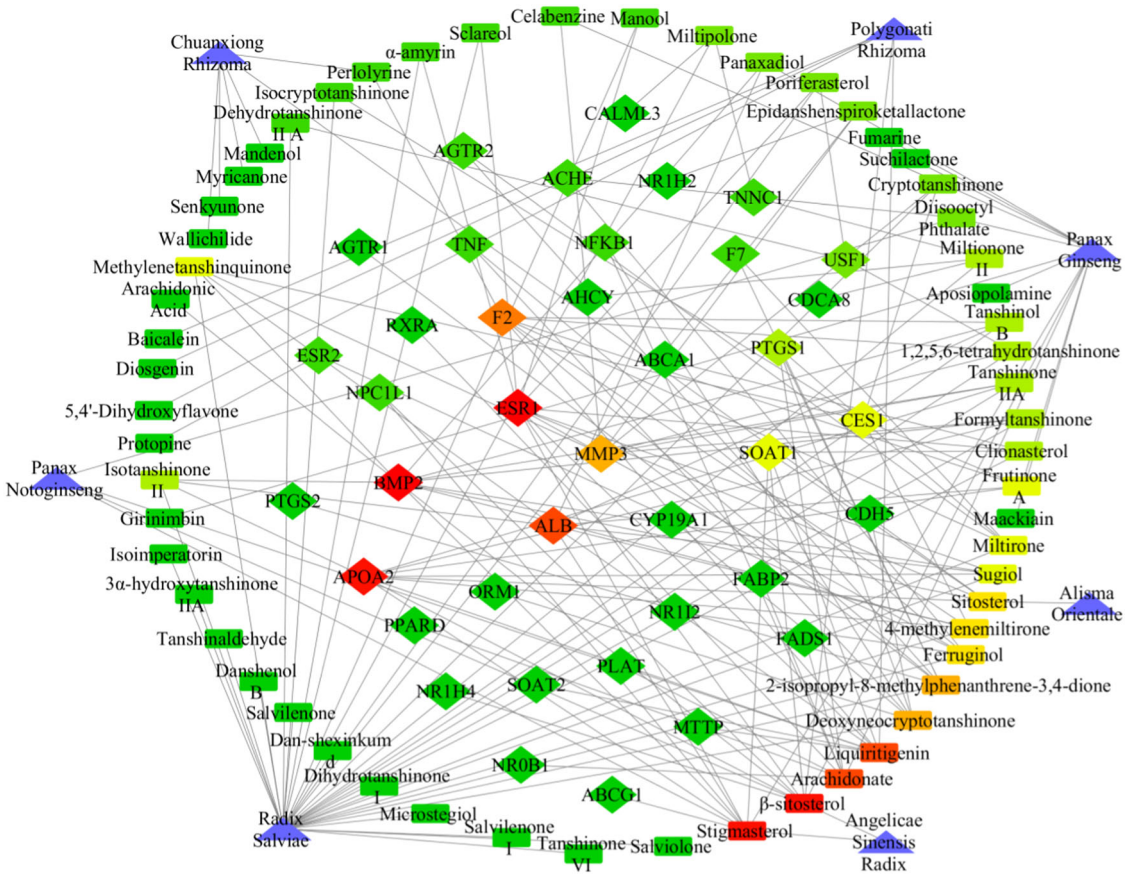



**Fig. 1** The herb-compound-target gene network of DTJZ pill. Herbs are indicated by slate triangle nodes; compounds are represented by rectangle nodes. Target genes are shown as diamond nodes

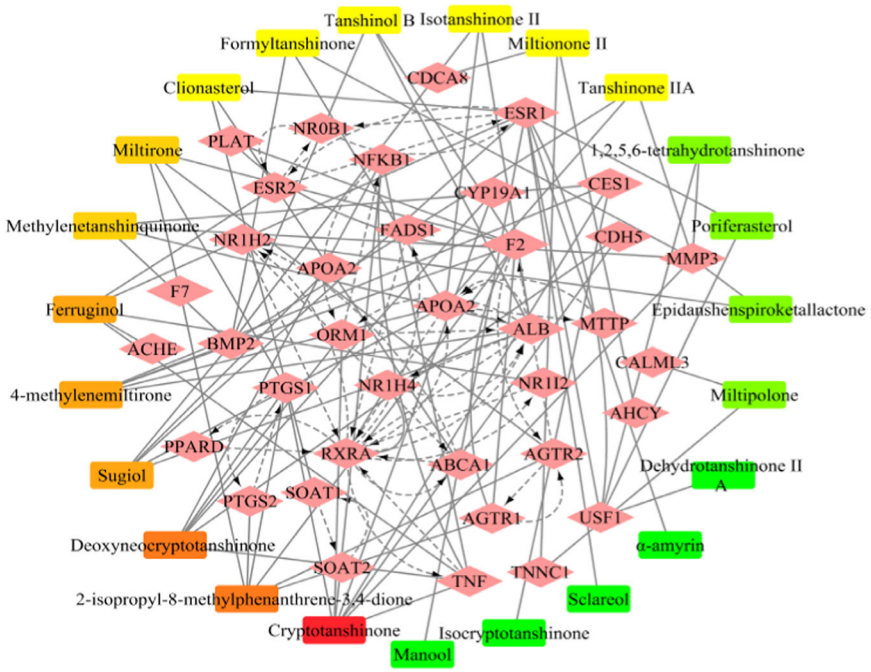



**Fig. 2** Network of chemical compositions of *Radix Salviae*-target gene. Compounds are represented by rectangle nodes and filled with different colors from green–yellow–red according to the numbers of degrees. Target genes are shown as pink diamond nodes. The interactions between composition and gene are marked as gray solid lines, whereas the gray dotted lines with arrows indicate relationships between gene and gene

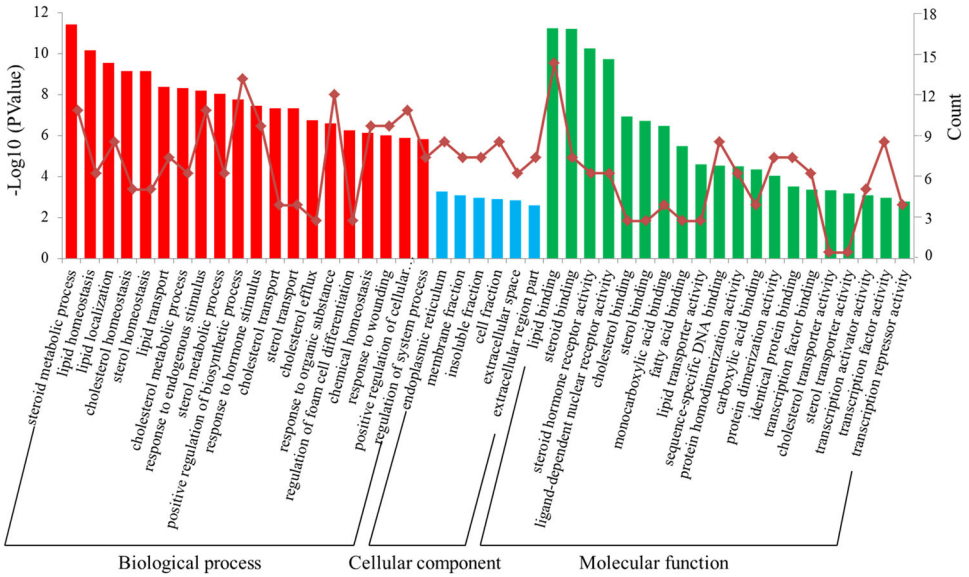



**Fig. 3** GO analysis for biological process, cellular component, and molecular function terms was performed on these genes. The top ten terms with $P < 0.05$ are shown

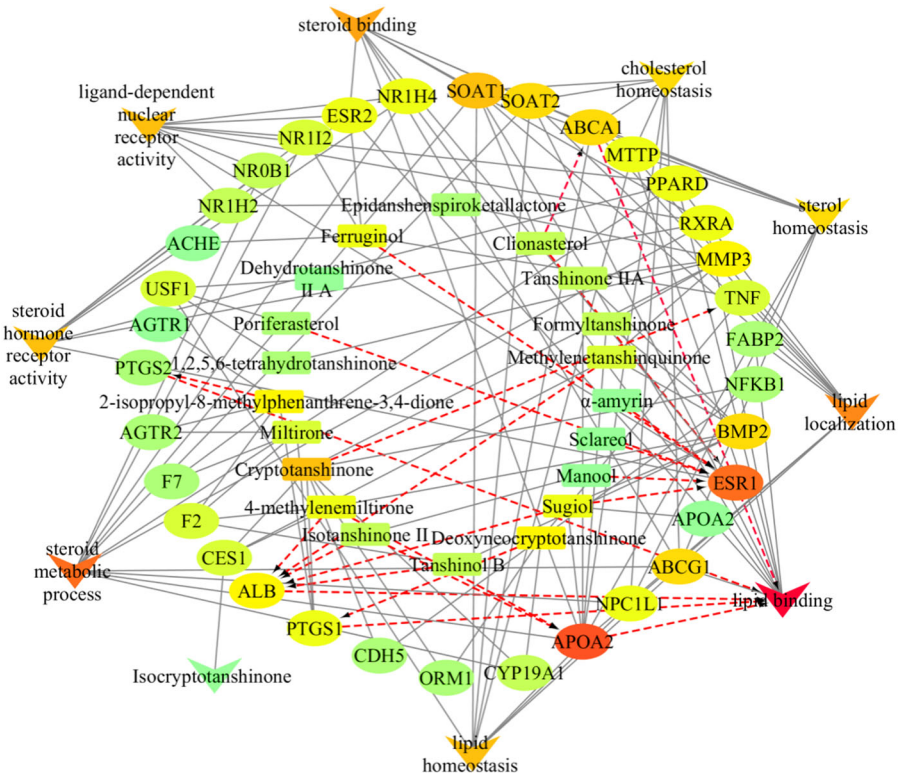



**Fig. 4** The network of *Radix Salviae*-components-target GO. Compounds are represented by rectangle nodes. Target genes are shown as ellipse nodes. Biological process and molecular function are marked as letter "V" type nodes. All colors are continuously filled from green to yellow–red in degrees

interacting simultaneously with these target genes MMP3, NFKB1, ORM1, PTGS1, TNF, CDH5, CES1, and CYP19A1. Owning higher node degrees, gene targets APOA2, ABCA1, ABCG1, SOAT1, ESR1, and SOAT2 can participate in the most BPs, such as steroid metabolic process, lipid homeostasis, lipid localization, cholesterol homeostasis and sterol homeostasis (the red dotted line in Fig. 4). In addition, they are all involved in the same MFs, such as lipid binding and steroid binding.

For MF, the important lipid binding (shown as red dotted line in Fig. 4) includes 15 target genes: ABCA1, ABCG1, ALB, APOA2, ESR1, ESR2, FABP2, MTTP, NR1H4, PPARD, PTGS1, PTGS2, RXRA, SOAT1, and SOAT2. During which, there are 18 compounds played critical roles in these processes: clionasterol, sotanshinone II, methylenetanshinquinone, Tanshinol B, 4-methylenemiltirone, formyltanshinone, isotanshinone II, tanshinone IIA, manool, poriferasterol, sclareol, sugiol, ferruginol, 2-isopropyl-8-

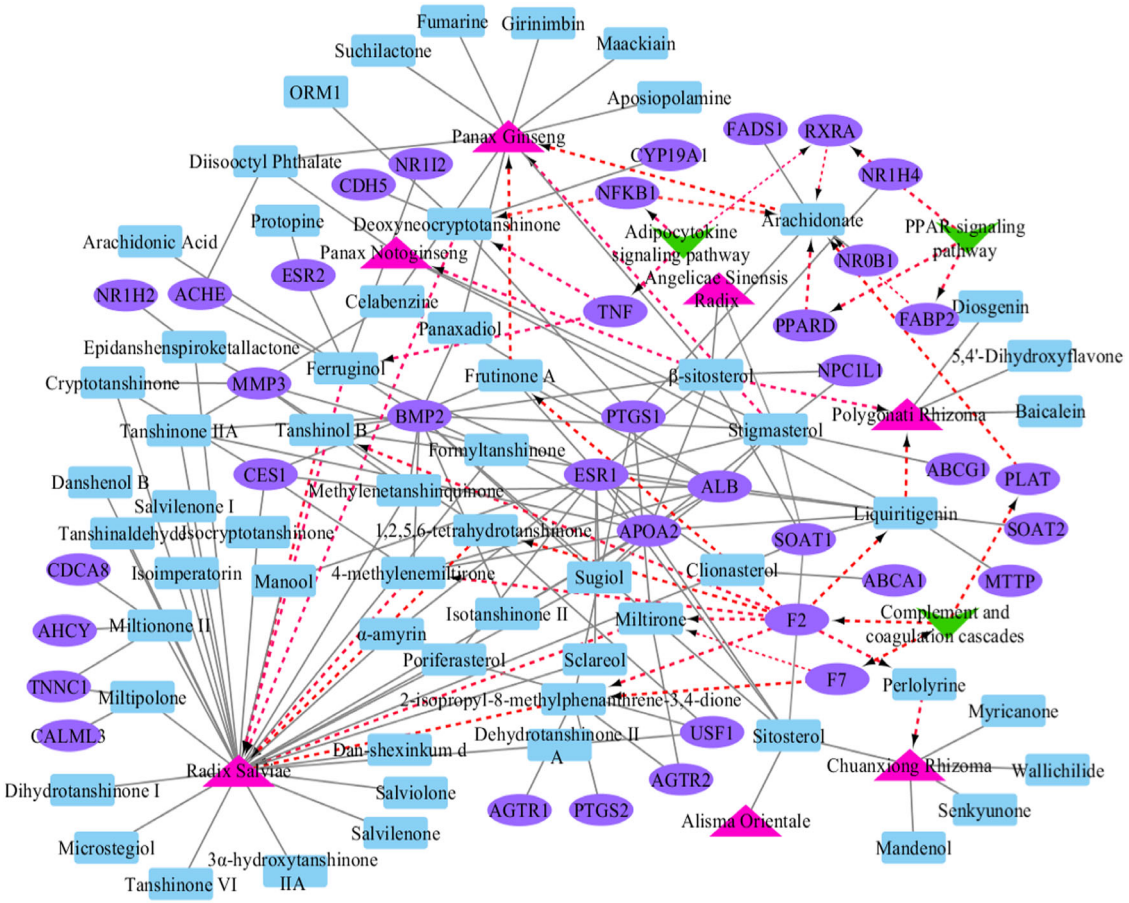



**Fig. 5** The herb-composition-target-pathway network for DTJZ pill. Herb is indicated by magenta triangle nodes; compound is represented by teal rectangle nodes. Target gene is shown as purple–blue ellipse nodes. Pathway is marked as green "V" shape symbol

methylphenanthrene-3,4-dione, α-amyrin, cryptotanshinone, deoxyneocryptotanshinone, and miltirone. It can be concluded that the same chemical component can affect different genes that are involved in the same or different BPs. The color of the nodes in the figure is filled in the order of green–yellow–red according to the node degree from high to low. Owning the highest degree in Fig. 4, Cryptotanshinone can influence the steroid metabolic process, lipid localization, and lipid binding via target genes CDH5, CES1, CYP19A1, MMP3, NFKB1, ORM1, PTGS1 and TNF. Actually, cryptotanshinone is the major lipophilic constituents of *Radix Salviae* (Marrelli et al. 2019). This is in accordance with previous study, which indicated that cryptotanshinone is able to reduce the concentration of body fat, serum cholesterol, and triglyceride levels in mice (Kim et al. 2007). It was also showed that cryptotanshinone can effectively inhibit adipogenesis process (Rahman et al. 2016).

## Potential target gene-related KEGG analysis

To examine the signaling pathways and functions of these target genes, we conducted pathway enrichment analysis using KEGG pathways. As shown in Fig. 5, the herb is represented by magenta triangle, the chemical composition is showed by teal rectangle, the gene target is represented by purple–blue ellipse, and the KEGG pathway is represented by green "V" shape symbol. A total of 39 targets obtained only seven KEGG signaling pathways, and all of the channels were significantly enriched ($P < 0.05$). Among them, four compounds miltirone, 4-methylenmitirone, 2-isopropyl-8-methylphenanthrene-3,4-dione,1,2,5,6-tetrahydrotanshinone and tanshinol B, separated from the main herb *Radix Salviae*, can affect the complement and coagulation cascades pathway by interacting with the F2 gene. In addition, the lipophilic compounds miltirone and 2-isopropyl-8-methylphenanthrene-3,4-dione can influence the complement and coagulation cascades pathway by affecting the target gene F7. The results of KEGG pathways enrichment analysis indicated the multiple channels and mechanisms of action of DTJZ pill against hyperlipidemia.

In addition, ingredients ferruginol and deoxyneocryptotanshinone of *Radix Salviae* could regulate blood lipid levels by participating in the adipocytokine

signaling pathway. For the *Chuanxiong rhizoma*, only perlolyrine reduces blood lipids by taking part in the complement and coagulation cascades pathway. The chemical component involved in the PPAR signaling pathway is arabiconate of *Panax ginseng*, which works primarily by interacting with targets RXRA, PPARD, and FABP2. For *Panax ginseng*, the chemical component involved in the PPAR signaling pathway is arachidonate, which plays an important role in the interaction with targets RXRA, PPARD, and FABP2. Study indicated that the metabolite arachidonate is important in lipid metabolism, and can be used as male biomarkers in O. potamophila (Wang et al. 2019a, 2019b). The drugs involved in the adipocytokine signaling pathway are deoxyneocryptotanshinone and ferruginol of *Radix Salviae*, and arachidonate of *Panax ginseng*. Taken together, these signaling pathways seem to be closely related to the beneficial effects of DTJZ pill against hypolipidemic.

## Discussion

Due to the serious adverse effects, the approved and marketed antiobesity drugs are restricted at a certain degree. Thus, more and more patients suffering from hyperlipidemia utilize traditional pharmacopeia to manage their obesity problems. In this regard, many medicinal plants have become very important and have shown potent plasma lipid levels lowering activities (Khanna et al. 2002; Bekkouch et al. 2019; Zhu et al. 2018; Pirillo and Catapano 2015). For a long time, many scholars have been working to explore the certain mechanism of their hypolipidemic effect. As a Chinese classic prescription, DTJZ pill is famous all over the country for its good clinical blood lipid-lowering effect. However, the bioactive components and their hypolipidemic mechanism have not been performed until now. In this text, 67 chemical compositions are screened from 909 total compounds in DTJZ pill based on our screening criteria. Among which, compounds Arachidonate, Stigmasterol, Liquiritigenin, β-sitosterol and 2-isopropyl-8-methylphenanthrene-3,4-dione play important roles in the hypolipidemic process. As the main drug in the DTJZ pill, *Radix Salviae* plays a pivotal role in reducing the blood lipid. Analysis indicated that Cryptotanshinone and Deoxyneocryptotanshinone can lower blood lipids by interacting simultaneously with target genes NFKB1, ORM1, PTGS1, TNF, CDH5 and CYP19A1. In addition, compared with the parent tanshinone IIA, it was reported that cryptotanshinone showed a higher inhibitory activity against pancreatic lipase ($IC_{50} = 6.86 \pm 0.43$ μM) (Marrelli et al. 2019). However, the target pancreatic lipase was not found through all of the databases used the in this text based on this higher screening criterion. Therefore, comparative interactions between cryptotanshinone and other hypolipidemic targets may be comprehensively analyzed both in theory and experiment in future.

Actually, for the main herb *Radix Salviae*, 55.6% of the ingredients have played important roles in lowering the blood fat via many BPs, such as cholesterol homecostasis, steroid metabolic process, sterol metabolic process, cholesterol metabolic process, lipid localization, lipid transport, sterol homecostasis, lipid homecostasis, cholesterol transport and sterol transport. As a quinoid diterpene, compound Cryptotanshinone was reported to be a good agent with multiple pharmacological benefits, involving anticancer (Wang et al. 2019a, 2019b; Li et al. 2015), antioxidative stress (Jin et al. 2013), anticardiac fibrosis properties (Ma et al. 2012), and so on. In addition, compounds ferruginol and deoxyneocryptotanshinone of *Radix Salviae* could regulate blood lipid levels by participating in the adipocytokine signaling pathway. Through BPs analysis, it showed that these potential genes that are related to this prescription have a strong association with steroid metabolic process, lipid homeostasis, lipid localization, cholesterol homeostasis, sterol homeostasis, lipid transport, cholesterol metabolic process, and so on. Based on the network analysis, DTJZ pill was confirmed to generate the hypolipidemic action by affecting multiple targets and multiple pathways, which reflects the multicomponent, multitarget, and multichannel characteristics of TCM. It provided a novel basis to clarify the mechanisms of hypolipidemic effect and a useful idea for further new drug design and development.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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