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Exploring the treatment of Systemic Sclerosis with Danggui (Angelica sinensis, AS)-Sini Decoction using GEO combined with Network pharmacology and Molecular docking

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Competing interests

The authors declare no conflicts of interest.

Abbreviations

DGSND, Danggui-Sini Decoction; SSc, systemic sclerosis; PPI, Protein-protein interaction; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genomics; TNF, Tumor Necrosis Factor; STAT3, Signal transducer and activator of transcription 3; TNF, Tumor Necrosis Factor; VEGFA, Vascular endothelial growth factor; NFκB1, Nuclear factor kappa-B; MAPK, Mitogen-activated protein kinase; JAK, Janus Kinase.

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Abstract

Background: To explore the relevant targets of the Chinese herbal medicine compound Danggui-Sini Decoction (DGSND) for the treatment of systemic sclerosis (SSc). **Method:** We used TCMSP to enlist ingredients and Swiss Target Prediction for targets fishing, and the protein names by UniProt for organized as gene symbol. Strawberry Perl was used to integrate the active ingredients and the drugs action target of DGSND, with Cytoscape 3.9.0 software to construct "Active ingredient-Drug target" network. Then, GEO database, GeneCards database, TTDdatabase, DisGENent database and MalaCards database for SSc-related disease target prediction, and then analyzed the active drug targets of DGSND and SSc-related targets of Danggui-Sini Decoction treat SSc. Then, the above results we combined with STRING database to visualize the Protein-protein interaction (PPI) network for the core targets of SSc, and performed Gene ontology (GO) functional analysis and Kyoto Encyclopedia of Genomics (KEGG) signaling pathway enrichment analysis for SSc-related targets treated with DGSND **Result:** DGSND contains 223 active ingredients including Sitogluside, Benzoylpaeoniflorin, (-)-Asarinin Palbinone, Glycyro, 4'DMEP etc. which acts on Signal transducer and activator of transcription 3 (STAT3), Tumor Necrosis Factor (TNF), Vascular endothelial growth factor (VEGFA), Nuclear factor kappa-B (NFκB1), Interleukin (IL1β, IL17), Mitogen-activated protein kinase (MAPK) and Janus Kinase (JAK) and many other genes totaling 176, mainly involved in 4 more biological processes 3 molecular functions and 3 cellular components. **Conclusion:** DGSND is primarily used to treat SSc by regulating calcium homeostasis, inflammatory signaling pathways and neural cell repair and apoptosis-related pathways within the body.

Keywords: Danggui-Sini Decoction; systemic sclerosis; network pharmacology

Introduction

Systemic sclerosis (SSc), also known as scleroderma, belongs to the categories of “Pi Bi (skin paralysis)”, “Mai Bi (pulse paralysis)”, “Ji Bi (muscle paralysis)” and “Nei Bi (internal paralysis)” in Chinese traditional medicine as well as. “Bi (Paralysis)” was first published in the *Yellow Emperor’s Canon of Medicine Plain Conversation-Theory of Paralysis*. According to the records of *Yellow Emperor’s Canon of Medicine Plain Conversation-Theory of Paralysis* mentioned, “wind, cold and dampness are mixed together, which means Bi(paralysis).” It means Bi(paralysis) occurs due to external attack of wind, cold and dampness, and internal deficiency of spleen Yang and kidney Yang, resulting in loss of harmony between Qi and Blood [1]. In modern medicine, SSc is a chronic systemic autoimmune inflammatory disease, and is usually considered to belong to the category of “skin paralysis” among the “five body paralysis”. The clinical manifestations of scleroderma are in line with “paralysis of the veins”, “paralysis of the muscles”, “paralysis of the internal organs”, etc [2]. The pathogenesis of SSc remains unclear, and the disease is currently thought to be due to immune system dysfunction, hormonal abnormalities, environmental and genetic factors leading to microcirculatory disorders, inflammatory responses in the body, and fibrosis of the skin and multiple organs [3, 4]. Disturbances in the body’s internal environment lead to activation and secretion of multiple autoantibodies and cytokines, resulting in damage and activation of vascular endothelial cells, which in turn stimulate abnormalities in collagen synthesis by fibroblasts, leading to fibrosis of the vessel walls and tissues. SSc is characterized by extensive vascular lesions, collagen proliferation and fibrosis of the skin and multiple organs [5]. SSc starts insidiously and the first symptom in most patients is Raynaud's phenomenon [6], and the typical manifestation of itis the “triphasic” color change of pale, blue and flushed skin on the extremities when the patient is stimulated by cold or emotional changes. It often starts at the fingertips and then spreads to the entire finger and even the palm of the hand. It may be accompanied by pain or sensory abnormalities at a later stage, and in severe cases, even ulcers on the extremities. It also affects the skin, joints, muscles, and eventually the organs, and in severe cases can lead to death due to sclerosis of the organs and surrounding blood vessels. Furthermore, the disease also seriously affects the quality of everyday life, appearance and physical and mental health of patients.

The DGSND created by Zhang Zhongjing’s Who wrote *Treatise on Cold-induced and Miscellaneous Diseases* (Shang han za bing lun), which states, “For those with syncope in the hands and feet and a thin pulse that wants to stop, Danggui-Sini Decoction is the mainstay.” This compound formula is mainly composed of Danggui (Angelica sinensis), Baishao (Radices paeoniae alba), Guizhi (Cmnamomi mmulus), Xixin (Asarum sieboldii), Tongcao (Medulla tetrapanacis), DaZao (Fructus Jujubae), and Gancao (Licorice). This formula has the effect of warming the meridians and dispersing cold, nourishing blood and opening the veins, with analgesic, anti-inflammatory and antispasmodic effects [7, 8].

Chinese medicines are mostly compounded, with a large number of ingredients, each containing a large number of monomers, and it is a multi-targets and mult-pathway treatment of diseases. Therefore, in this study, we used the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) to screen the drug compounds by bioinformatics methods, and Swiss Target Prediction for targets fishing which corrected the protein names by using the Uniprot database. It is used to construct an “Active ingredient-Drug target” network using Cytoscape3.9.0 software. However, we retrieved the SSc disease-related genes from the GEO databas (https://www.ncbi.nlm.nih.gov/geo/), GeneCards database [9], TTDdatabase [10], DisGENent database [11] and MalaCards database [12]. Then, we combined with STRING database to visualize the Protein-protein interaction (PPI) network for the core targets of SSc, and performed Gene ontology (GO) functional analysis and Kyoto Encyclopedia of Genomics (KEGG) signaling pathway enrichment analysis for SSc-related targets treated with DGSND. Finally, molecular docking technology was used to verify the core targets(receptor) and core active ingredients(ligand). An outline of the method is shown in Figure 1. Our aim is to provide a reference for the extraction of active ingredients to improve the quality of life and survival rate of SSc patients.

Materials and methods

Find and obtain core active ingredients of DGSND

The traditional Chinese medicine system pharmacology database and analysis platform (TCMSP, https://tcmspw.com/tcmsp.php) [13]: TCMSP to enlist main active ingredients according to the optimal toxicokinetic ADME (Absortion, Distribution, Metabolism, Elimination) rules reported in the literature Oral bioavailability (OB) ≥ 20%, Drug-likeness (DL) ≥ 0.10 [14]. The related active ingredients were input into PubChem (https://pubchem.ncbi.nlm.nih.gov/) to obtain the molecular structure (Canonical SMILES) and downloaded SDF file of the related active ingredients.

Obtain potential drug targets of DGSND

The active ingredients of a drug perform related biological functions through the relevant targets. In addition to obtaining the targets of the core active ingredients of DGSND directly from the TCMSP database, information on the small molecule structure of the core active ingredient (Canonical SMILES) and SDF file was used for target identification. After that, import core active ingredient (Canonical SMILES) and PDB file into Swiss Target Prediction (https://new.swisstargetprediction.ch/) [15] identified and obtained a large number of possible targets for DGSND. And then, the species was limited to “*Homo sapiens*”. Then, normalization of targets was done by using the in combination with certified human genes from the Uniprot database (https://www.uniprot.org/) [16], by transferring target names to gene symbols. Moreover, Strawberry Perl (https://strawberryperl.com/) was then used to integrate the Methods subsection 2.1 active ingredients and the targets of DGSNT on which the drug ingredients act.

Construction of a SSc-related targets database

First, SSc-related disease targets were acquired in the following database, including the: Gene Cards database (https://www.genecards.org/) [9], TTD database (https://db.idrblab.org/ttd/) [10], DisGENent database (https://www.disgenet.org/web/DisGeNET/menu/home) [11] and MalaCards (https://www.malacards.org) [12] using “Systemic sclerosis” or “Scleroderma” as the keyword, were used to collect the SSc-related targets. Also, use a microarray data of different expressed genes in the Systemic sclerosis between the normal group and the SSc group were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), to obtain the GEO probe matrix series: GSE33463 and GSE117928, then, use “GEOquery” “Limma” (https://www.bioconductor.org/) package of Rmur4.0.2 were used to carry out joint analysis of multiple chips and correct data batches. The microarray data contained gene expression in skin and lung tissues from 44 healthy subjects and 61 SSc patients. The difference in genes between healthy persons and active SScGene patients with $P < 0.05$ adjusted and log2 (fold change) > 2 or log2 (fold change) < –2. After that, a “ggplot2” package was using to plot the volcanoes of differential gene expression. Finally, the results of the above four databases and GEO microarrays were used to eliminate repeat disease targets and to establish the database of SSc-related targets. Based on the preceding steps, “Venn” packages in Rmur4.0.2 was used to screen the overlapping targets and plotting a Venn diagram, which were identified as the potential therapeutic targets of DGSNT against SSc.

Construction of the Active ingredients-Drug targets

The screened active ingredients and drug action targets of DGSNT

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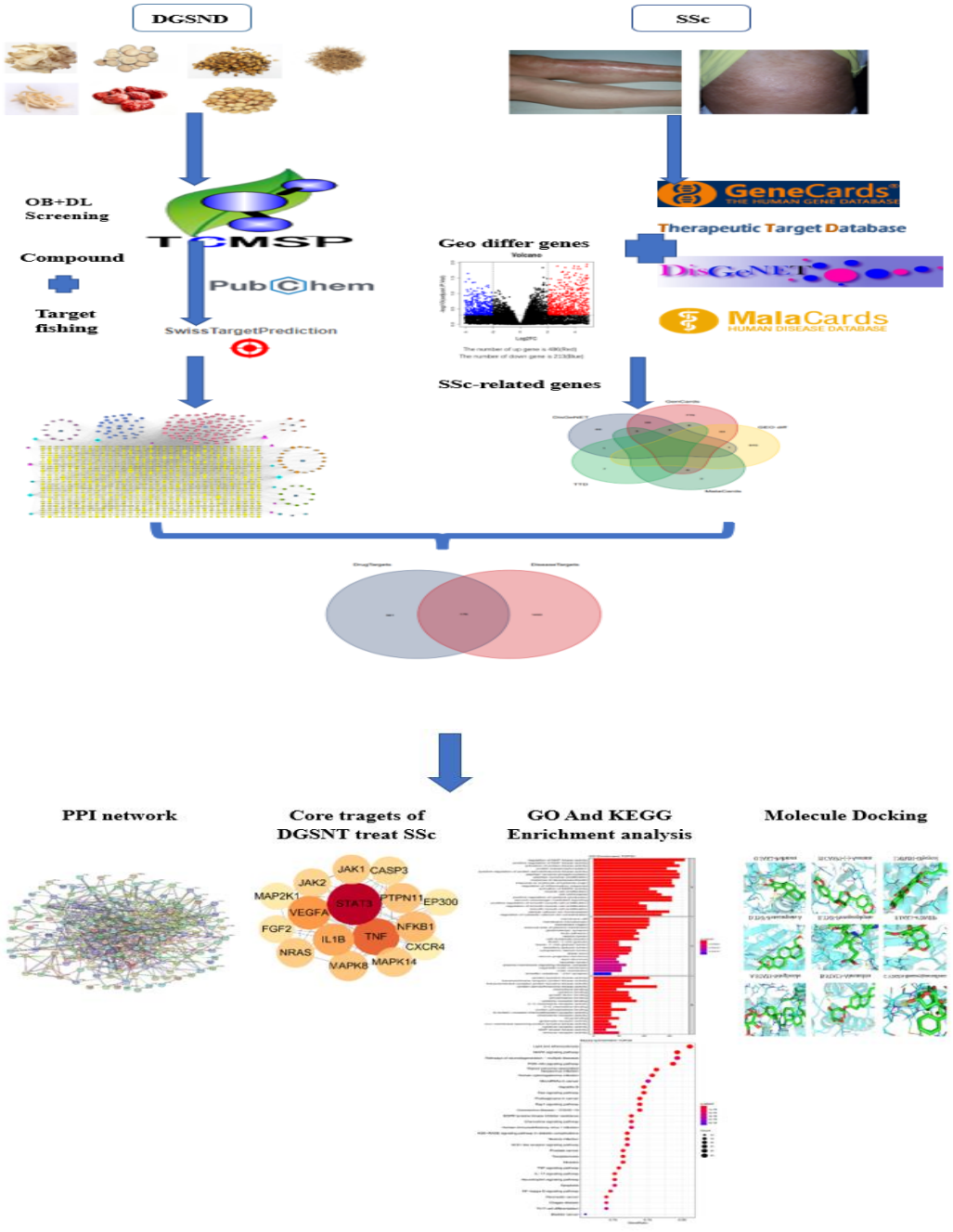


Figure 1 Framework based on an integration strategy of network pharmacology

gained from Methods subsection 2.2 were imported into Cytoscape3.9.0 (https://www.cytoscape.org/) [17] to construct an "Active ingredient-Drug target" of DGSNT visualization network. Moreover, we use the Network Analyzer plug-in to compute the degree and node values for this network. The degree and node betweenness of the nodes are important for this network. The degree represents the number of edges of a node, and the node betweenness represents the number of shortest paths between pairs of nodes that run through the node, and can be used to evaluate the degree of association between different classes of components.

Construction of the Drug targets-Disease targets

Based on the above analysis, the drug action targets genes of DGSND were intersected with SSc-related disease genes to plot a Venn diagram of the intersection of drug action targets and disease targets. The "Venn" (https://bioinformatics.psb.ugent.be/webtools/Venn/) packages of Rmur 4.0.2 were used to plot a Venn diagram.

Construction and visualization of protein interaction networks of core targets

We constructed a protein-protein interaction (PPI) network to explore the interactions of the therapeutic targets of DGSNT against SSc. The PPI analysis was conducted using Search Tool for the Retrieval of Interacting Genes/Proteins platform (STRING, https://string-db.org/) [18], and the criteria were limited in "Homo sapiens" and a high confidence score of 0.700. The networks were constructed by Cytoscape3.9.0, and the cytoNCA plug-in was used to analyze the nodes' topological parameters in the network. There are 3 topological

parameters, Betweenness Centrality (BC) [19], Centrality (DC) [20], and Closeness Centrality (CC) [21], were calculated to measure the importance of the nodes.

Function of GO and KEGG enrichment analysis

The core targets genes data obtained from Methods subsection 2.5 by Bioconductor platform (https://www.bioconductor.org/) to install packages “BiocManager”, “DOSE”, “org.Hs.eg.”, “db.colorspace”, “stringi” and “ggplot” in Rmur 4.0.2, used to conduct Gene ontology (GO) enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis. The GO enrichment analysis was performed from biological process (BP), cellular component (CC) and molecular function (MF). The top 30 entries of each component were analyzed based on P-value. The Common enrichment analysis based on hypergeometric distribution (Fisher's Exact Test), hypergeometric distribution is an important kind of discrete probability distribution, the probability mass function can define like this: suppose finite population consists of N samples, the quality qualified for m, then the rest of the N-m for unqualified samples, if the extract from the finite population N samples, including the probability of k is qualified for:

$$P(X=k)=\frac{C_m^k \times C_{N-m}^{n-k}}{C_N^n}$$

To take genes as an example. For actual KEGG enrichment, the total N is the total number of genes (background genes), and m genes are qualified, that is, there are altogether M genes annotated into a pathway. N samples are selected as target genes (differential genes are usually selected), among which K are qualified, that is, k of target genes are annotated into this pathway. Through this algorithm, a P-value will be obtained, and $P < 0.05$ is generally considered as significant about enrichment [22].

KEGG enrichment. The KEGG rich set analysis was performed to annotate the signaling pathways involved in the targets, and the top 30 entries were selected based on *P*-value, and if there were less than 30 entries, all entries were analyzed.

Molecular Docking of Active ingredients-targets

The core targets of Methods subsection 2.6 were downloaded through the PDB database to obtain the protein crystal structures of the core targets (macromolecular receptors). After obtaining the core target, identified its corresponding core drug component, downloaded the "mol2" format file of the profiled active ingredient from the TCMSP database, and imported it into ChemB+io3D Ultra14.0 software to calculate the minimum free energy. Obtained PDB format files of drug active ingredients (small molecule ligands). The core targets (macromolecular receptors) preferably select a model with ligand binding smaller than 2.5 Å, and then imports the crystal into the Pymol (Version4.5.0) software (https://pymol.org/2/) for dehydration, hydrogenation, adjust charge and separation of ligands [23], etc. Last, the PDB format files of small molecule ligands and large molecule receptors were converted to PDBQT format files using AutoDockTools 1.5.6 to construct the docking grid box and set up for molecular docking [24], and then molecular docking was completed by AutoDock Vina 1.1.2 software [25]. The molecule docking results were visualized with PyMol (Version4.5.0) software.

Results

The Active ingredients-Drug Targets network of DGSNT

The core active ingredients were selected based on the screening criteria of ADME (Absortion, Distribution, Metabolism, Elimination). We choose Oral bioavailability (OB) ≥ 20%, Drug-likeness (DL) ≥ 0.10 to enlist main ingredients of DGSNT. Finally, a total of 223 active ingredients were obtained from TSMSP. Based on the number of Canonical SMILES of core active ingredients, we downloaded the active ingredients SDF file from PubChem and imported it into Swiss Target Prediction. Finally, 7516 targets were asscociated the active ingredients identified in the target fishing and integration obtained from the Swiss Target Prediction. Then, we used Strawberry Perl to integrate the active ingredients and the drug action targets to take the intersection and then imported into Cytoscape3.9.0. (Figure 2)

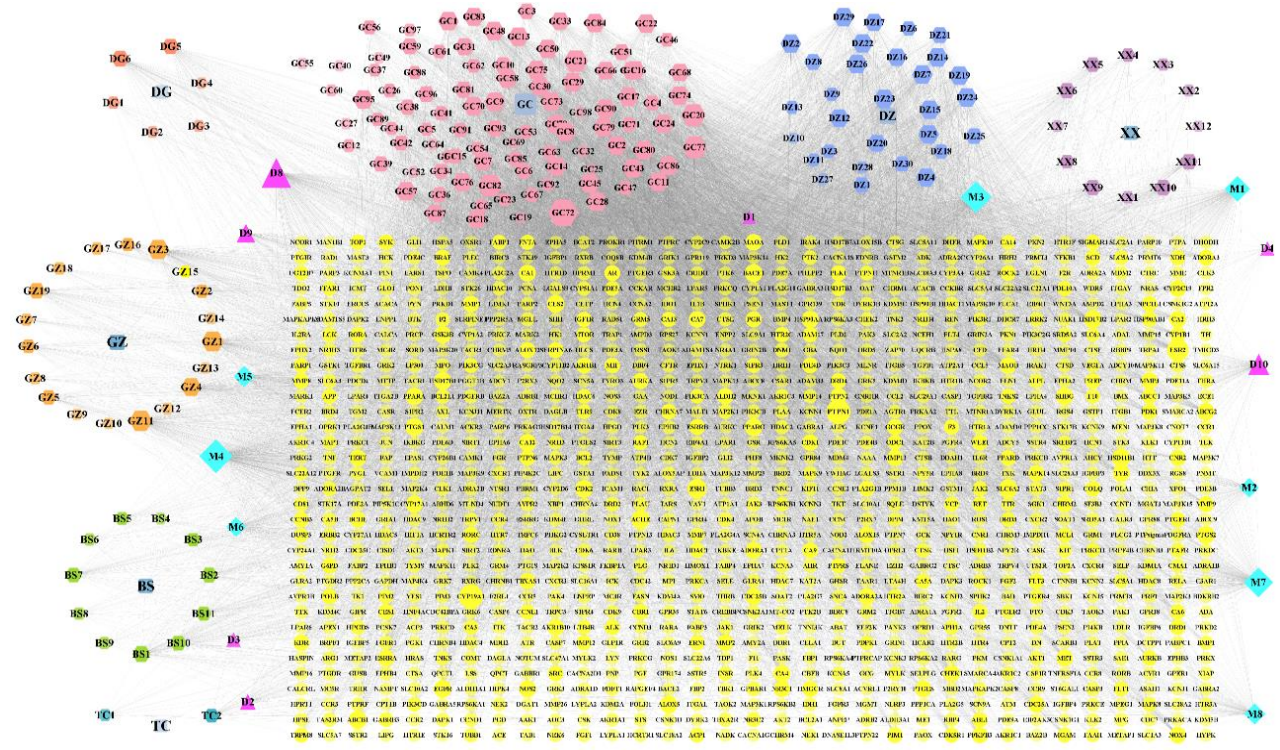


Figure 2 The “Active ingredient-Drug target" interaction network of DGSNT with 1289 Nodes and 11886 Edges

The SSc-related targets obtain and analysis

Conjoint analysis of the GEO microarray GSE34663 and GSE117928 in the GEO database with standard adjusted $P < 0.05$ and log2 (fold change) > 2 or log2 (fold change) < –2 identified 699 differentially expressed genes associated with SSc. Among them, there were 486 up-regulated genes and 213 down-regulated genes which were used to construct volcano maps (Figure 3). In addition, followed the steps in Methods subsection 2.3, we integrated disease targets from GeneCards, DisGeNET, TTD, and MalaCards databases and combined them with GEO microarray results to eliminate duplicates, resulting in the identification of 1629 disease targets (Appendix 1) and the construction of Venn diagrams (Figure 4). The core active ingredient targets of DGSNT were matched with disease targets of SSc.

The Drug Targets-Disease Targets network for SSc treatment by DGSNT

The action target genes of DGSNT (Results subsection 3.1) were intersected with SSc disease gene (Results subsection 3.2) using R and plotted on a Venn diagram (Figure 5); The total number of their intersected target genes was 178.

PPI network construction and visualization analysis

The data obtained from Results subsection 3.3 were imported into STRING database website, 178 intersecting genes were imported into STRING database, species was selected as human (*Homo species*), high confidence score of 0.700 was selected as to construct PPI network. The PPI protein interaction network relationship graph (Figure 6) was obtained and tsv file of this network was exported. The tsv file was imported into Cytoscape 3.9.0 to visualize the PPI network, and 176 nodes (nodes represent disease targets) and 2025 edges (edges represent target-target interactions) were obtained. Also, imported it (176 nodes and 2025 edges) into Rmur 4.0.2 to screen for targets greater than the BC, DC and CC median value. Finally, we acquire final core target (16 nodes and 149 edges) and exported it for next steps. The core target network relationship map was constructed by importing Cytoscape 3.9.0. With the help of cytoCNA plug-in, the topological indicators BC DC and CC values of the visualized network were calculated, and the nodes larger than the median BC, DC and CC to extract and build a new visualized network to obtain the final core target network of DGSNT treat SSc, with 16 nodes and 149 edges. (Figure 7). We methioned Methods subsection 2.6, that Betweenness Centrality (BC) [19], Centrality (DC) [20], and Closeness Centrality (CC) [21], were calculated to measure the importance of the nodes. BC calculates the number of shortest paths through a node. The more the number of shortest paths through a node, the higher its intermediary centrality. DC refers to the number of other nodes associated with a node in the network. The greater the degree centrality, the greater the importance of the node. Closeness centrality defined as the average of the shortest path length from a node to all other nodes, indicating the closeness of a node to the others in the network, the shorter the path from this point to all other points, the point is closer to all other points. According to relevant literature reports, we choose to use results with greater than the median value of BC, DC and CC [26–28].

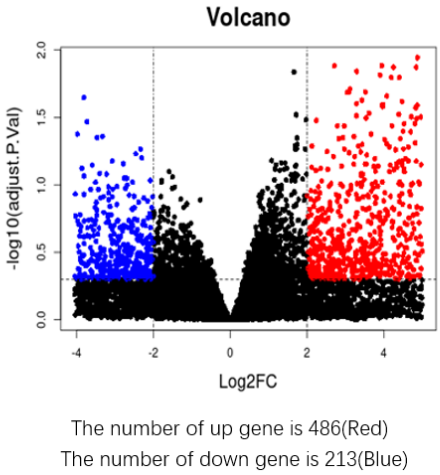


Figure 3 Volcano map of SSc differentially expressed genes in GEO probe matrix

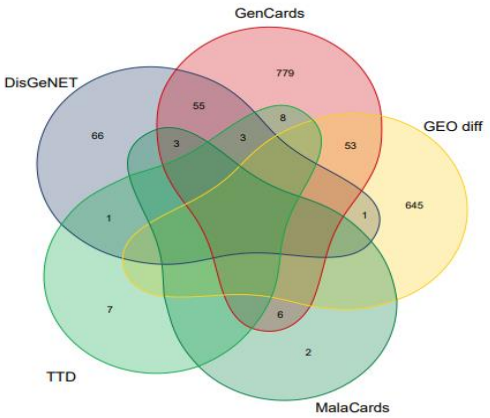


Figure 4 Venn diagram of the targets in SSc-related Disease

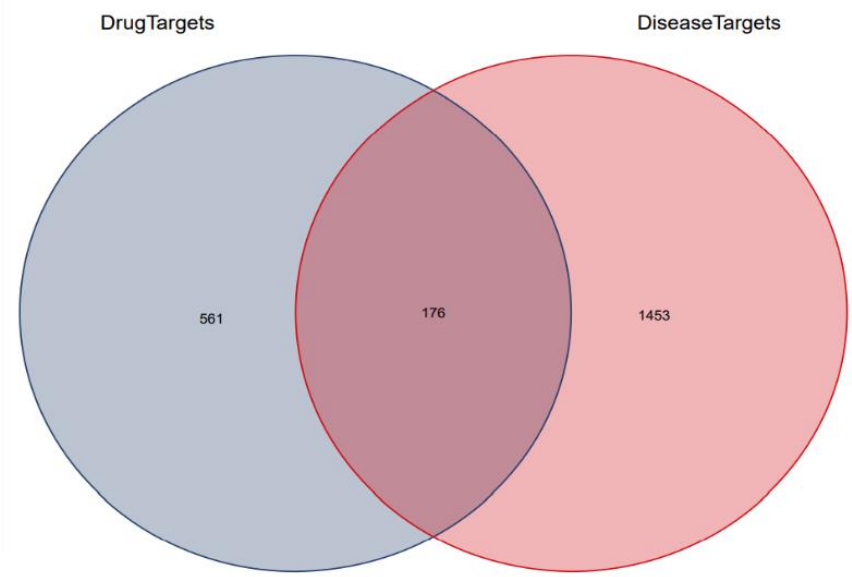


Figure 5 Venn diagram of the targets in DGSNT(Drug) and SSc(Disease)

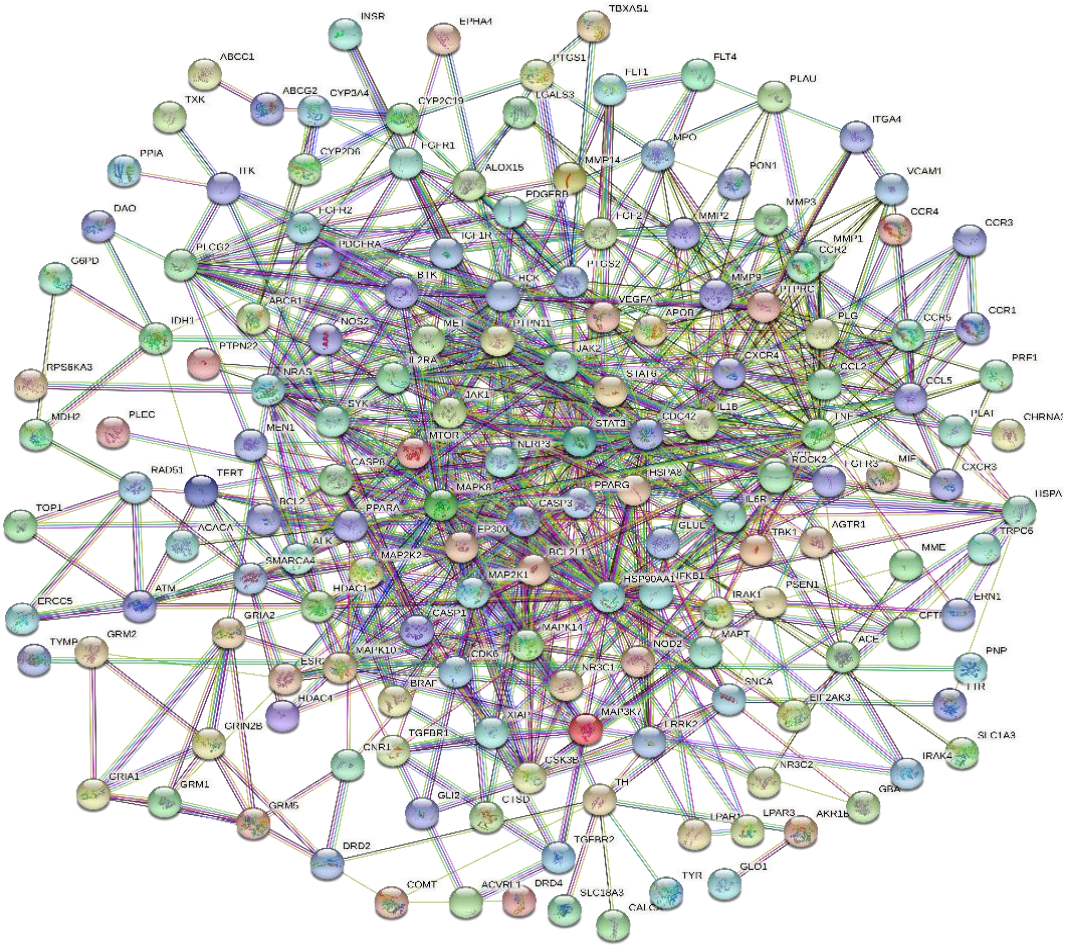


Figure 6 PPI network construction and visualization analysis of 178 and nodes2025 edges

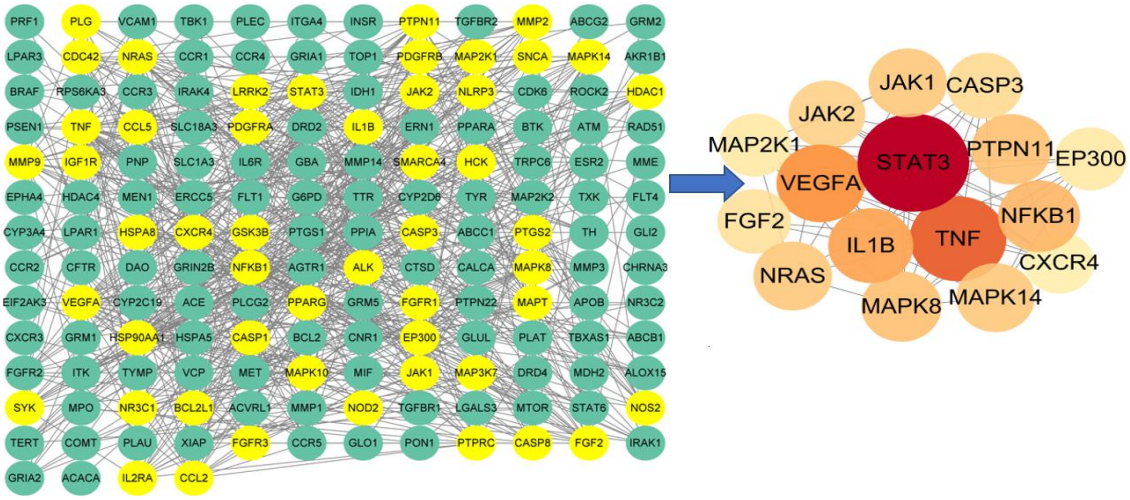


Figure 7 The network diagram of core tragets of DGSNT treat SSc with 16 nodes and 149 edges

GO and KEGG enrichment analysis

Using Rmur 4.0.2 to enrich 178 intersectional targets and setting $P < 0.05$, a total of 1) 2814 GO functional enrichment analysis activity fee biological function entries were obtained, including 2537 (Biological Process, BP) entries related to SSc involving regulation of MAP kinase activity, positive regulation of MAP kinase activity, response to lipopolysaccharide, positive regulation of cytokine, production cellular calcium ion homeostasis, etc.; 94 CC (Cellular Components, CC) entries, SSc-related involving membrane raft, cell-substrate junction, external side of plasma membrane, membrane microdomain, etc.;183MF (Molecular Function, MF) entries, related to protein serine/threonine kinase activity, protein serine, threonine kinase activity and cytokine receptor binding, etc. The top 30 biological function entries were listed according to P value (Figure 8); 2) KEGG pathway enrichment analysis among 157 KEGG pathway enrichments involved MAPK signaling pathway, PI3K-Akt signaling pathway, Lipid and atherosclerosis, EGFR tyrosine kinase inhibitor resistance, Rap1 signaling pathway, Ras signaling pathway, etc. The top 30 related pathways are listed according to their P values (Figure 9).

Molecule Docking

Based on the review of literature and current research hotspots, 11 targets among the obtained core targets, STAT3, VEGFA, TNF, MAPK8, MAPK14, PTPN11, IL1β, JAK1, JAK2, NFκB1 and CASP3, and downloaed crystal structures of those core target: 5E1E (STAT3), 1FLT (VEGFA), 6M95 (TNF), 4AWI (MAPK8), 3HUC (MAPK14), 3O5X (PTPN11), 1T4O (IL1β), 6M88 (JAK1), 4O7E (JAK2), 1NFI (NFkB1) and 3KJF (CASP3) from PDB (https://www.rcsb.org/) protein database. Then, some of the core ingredients of the drug corresponding to the core targets to perform molecularly docking. Their drug core ingredients was downloaded the PDB format from PubChem and perform energy minimization of the core components using ChemBioUltra14.0. Finally, the AutoDockTools1.5.6 was used to perform semi-flexible molecular docking to obtain their binding energies (Affnity), with smaller values representing stronger binding energies (Table 1).

The affnity of best mode STAT3-Sitogluside and STAT3-Alexandrin are –9.3 kcal/mol and –7.1 kcal/mol. The results of Sitogluside-STAT3 molecular docking showed an arginine residues (ARG-875), and STAT3-Alexandrin molecular docking had a threonine residues (THR-1036) and glutamic acid residues (GLU-1029). In the process of docking with the affnity of best mode VEGFA-Benzoylpaeoniflorin are -8.1kcal/mol and it had a cysteine amino acid residues (CYS-61) and an aspartic acid residues (ASP-63). The Affnity of TNF-Fatsicarpain A and TNF-Protoporphyrin are –7.9 kcal/mol and –7.8kcal/mol. The results of TNF-Fatsicarpain A had an aspartic acid residues (ASP-88), two arginine residues (ARG-5) and an arginine residues (ARG-23). Then, the molecular docking model on TNF-Protoporphyrin showed in a phenylalanine amino acid residue (PHE-348), a threonine amino acid residue resulted in a phenylalanine amino acid residue, a threonine amino acid residue and a valine residue (THR-9139) and a valine residue (VAL-89), Also, the affnity of JAK1-4'DMEP (4'-Demethylepipodophyllotoxin) and JAK2-Palbinon are –7.5kcal/mol and –7.1 kcal/mol. The result showed an aspartic acid residues (ASP-132) and glutamic acid residues (GLU-67) in molecularly docking on JAK1-4'DMEP. And the result showed an asparagine amino acid residues (ASN-117), a valine residue (THR-60), a glutamine amino acid residues (GLN-64), and a leucine residue (LEU-114) With regards to the molecularly docking of JAK2-Palbinon. Lastly, the affnity of best mode CASP3- (-) Asarinin are –7.8 kcal/mol which showed the molecularly docking results to a histidine residue (HIS-121) and an arginine residues (ARG-207). The affnity of best mode NFκB1-Glycyrol are –7.6kcal/mol which showed a serine amino acid residues (SER-329) a leucine residue (LEU-328) and a glutamine amino acid residues (GLN-320) in the molecularly docking results (Figure 10).

Discussion

Network pharmacology can consistently observe interactions between active ingredients, proteins, genes and diseases at a molecular level. In this study, we constructed the "Active ingredients -Drug targets" network of DGSND by the network pharmacology method. The DGSND has the effect of warming meridian and dispersing cold, nourishing blood and connecting veins. Danggui (Angelica sinensis), and Baishao (Radices paeoniae alba) can Yi Qi Bu Xue (benefiting Qi for activating Blood circulation for relieving pain); Guizhi (Cmnamomi mmulus) and Xixin (Asarum sieboldii) Wen Jing San Han (warming channel for dispelling cold); Tongcao (Medulla tetrapanacis) can Tong Mai Huo Xue (prompting circulation of Blood); DaZao (Fructus Jujubae) and Gancao (Licorice) can Huan Zhong Bu Xu (benefiting all organs). In the circulatory system, the DGSND can dilate peripheral blood vessels, increase effective blood perfusion and increase circulating blood volume, and also anticoagulate and antiplatelet aggregation. Huang Fang [29] found that the oral administration of Angelica Sinensis Si-reverse Tang to mice significantly prolonged the clotting time and prothrombin time, indicating that the formula has anticoagulant effects. Wang Wenda [30] randomly divided 96 patients with Raynaud's disease into 2 groups of 48 cases each. The control group was treated with nifedipine and the observation group was treated with DGSNT. The changes of peak systolic finger artery blood flow were observed before and after the treatment in the two groups. The results showed that the DGSNT group was effective.

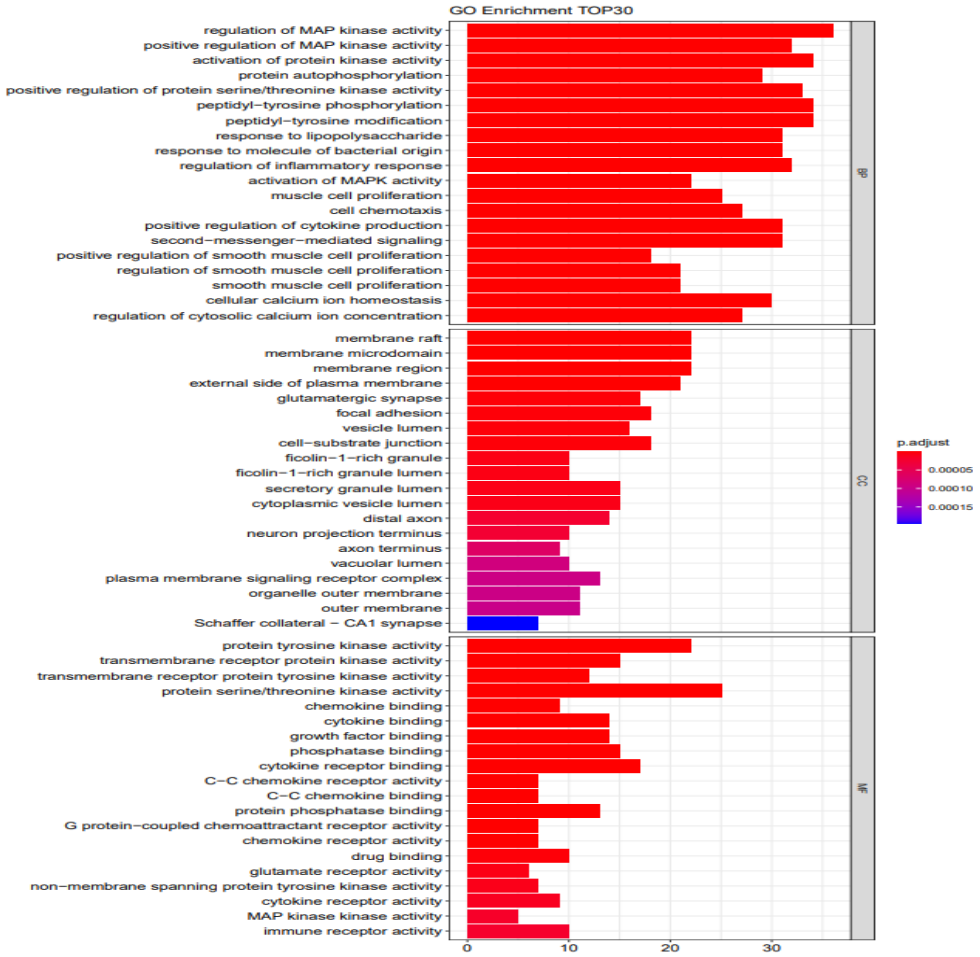


Figure 8 Bar chart of GO enrichment analysis core nodes

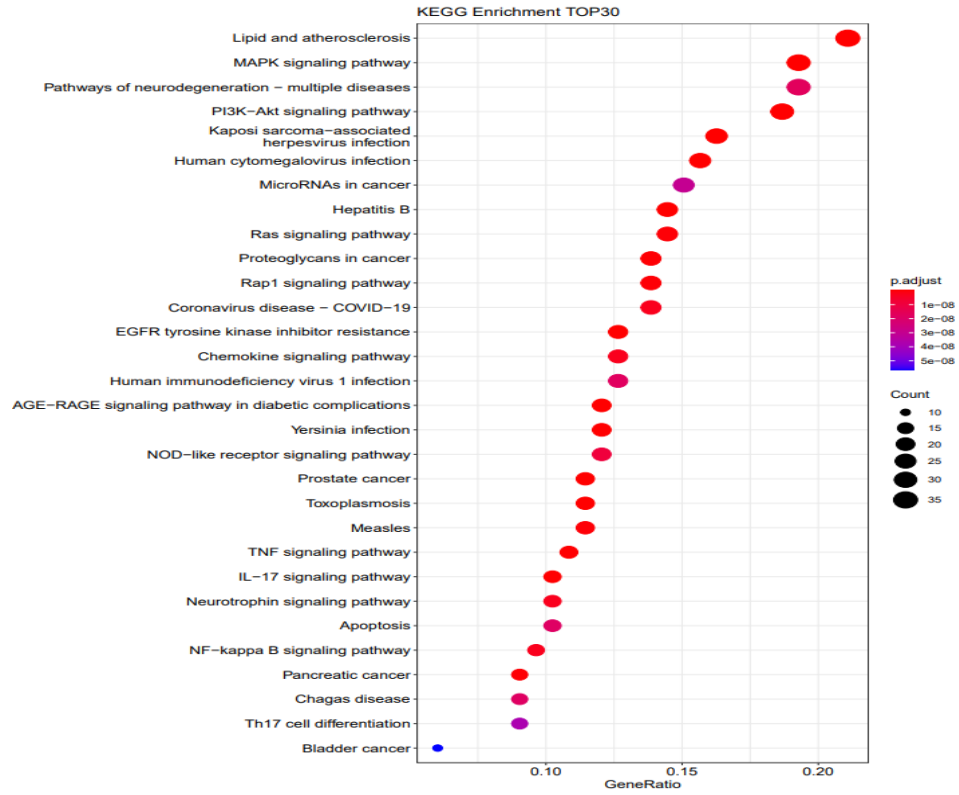


Figure 9 Bubble diagram of KEGG pathway enrichment analysis

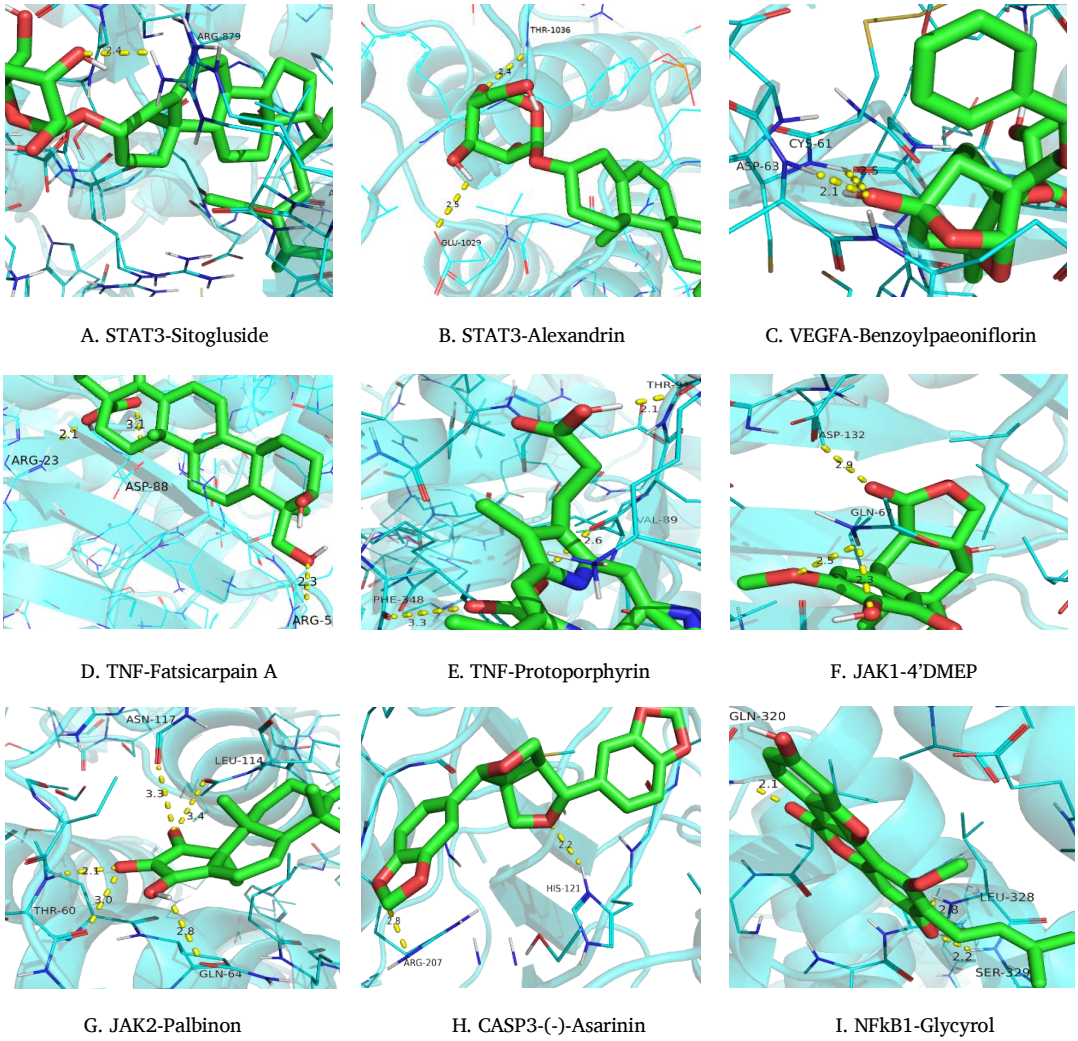
Table 1 Binding energy (Affnity) of the core targets and the core ingredients

Targets	Ingredients	Source	Affnity (kcal/mol)
STAT3	Sitogluside	DG、DZ、BS、GZ	-9.3
STAT3	Cryptopin	XX	-6.7
STAT3	Alexandrin	TC	-7.1
TNF	Protoporphyrin	DZ	-7.8
TNF	Licoagrocarpin	GC	-9.0
TNF	Fatsicarpain A	BS	-7.9
VEGFA	Lactiflorin	BS	-7.2
VEGFA	Paeoniflorin	BS	-7.3
VEGFA	Albiflorin	BS	-7.1
VEGFA	Benzoylpaeoniflorin	BS	-8.1
VEGFA	Oxypaeoniflorin	BS	-7.4
MAPK8	7-Acetoxy-2-methylisoflavone	GZ、GZ	-5.9
MAPK8	4'DMEP	GZ	-8.4
MAPK8	Peroxyergosterol	XX	-9.5
MAPK8	Cryptopin	XX	-6.9
MAPK8	Nornuciferine	DZ	-8.2
JAK1	4'DMEP	GZ	-7.5
JAK1	Caribine	XX	-9.1
JAK1	Palbinone	BS	-8.3
JAK2	Methyl linolelaidate	BS	-4.7
JAK2	Palbinone	BS	-7.1
JAK2	3'-Hydroxy-4'-O-Methylglabridin	XX	-8.5
JAK2	4'DMEP	GZ	-7.4
MAPK14	2-Methyl-5-Decanone	DG	-5.9
MAPK14	DBP	BS、GC、GZ	-7.0
MAPK14	Methyl linolelaidate	BS	-5.4
PTPN11	Mairin	BS、GC、DZ	-6.9
PTPN11	Oleic acid	GZ、DZ	-3.6
CASP3	Vestitol	GC、GZ	-7.8
CASP3	4'DMEP	GZ	-8.0
CASP3	(-)-Asarinin	GZ	-7.8
IL1β	20-Hexadecanoylingenol	DZ	-6.2
NFκB1	Glycyrol	GC	-7.6
NFκB1	Glyasperin F	GC	-6.8
NFκB1	Gancaonin G	GC	-7.0
NFκB1	Coumarin	GC	-5.8

Sources: DG: Danggui (Angelica sinensis); BS: Baishao (Radices paeoniae alba); GZ: Guizhi (Cmnamomi mmulus); XX: Xixin (Asarum sieboldii); TC: Tongcao (Medulla tetrapanacis); DZ: DaZao (Fructus Jujubae); GC: Gancao (Licorice).

DGSNT is clinically effective, and we hope to explore the relevant targets and mechanisms through network pharmacology to deeply explore its efficacy, continuously expand the scope of application, and realize the great potential of Chinese traditional medicine for SSc. We hope explore its efficacy can provides a reference direction for further research and new ideas for drug development to meet the needs of clinical treatment of SSc.

GO analysis showed that the relevant drug targets were mainly enriched in intracellular calcium homeostasis dysregulation, smooth muscle cell proliferation, lipopolysaccharide response, and cell chemotaxis. Studies have shown that dysregulation of calcium homeostasis is a pathogenic driver of neurodegeneration and is associated with a variety of neurodegenerative diseases [31]. Studies have reported the progression of SSc involving an abnormal nervous system, particularly the peripheral and autonomic nervous systems [32]. The results of KEGG analysis indicate that the balance of Lipid and atherosclerosis metabolism is essential for the maintenance of cellular and organismal life activities, and that abnormal cholesterol metabolism is closely related to the development of neurodegenerative diseases, in addition to cardiovascular and cerebrovascular diseases and tumors [33]. Intracerebral lipids are the main components of nerve cells that perform structural functions and



A. STAT3-Sitogluside B. STAT3-Alexandrin C. VEGFA-Benzoylpaeoniflorin

D. TNF-Fatsicarpain A E. TNF-Protoporphyrin F. JAK1-4'DMEP

G. JAK2-Palbinon H. CASP3-(-)-Asarinin I. NFkB1-Glycyrol

Figure 10 The Molecular docking of core targets (receptor) and core active ingredients (ligand)

physiological roles [34]. While the PI3K/Akt signaling pathway is protective of the nervous system, activation of this pathway has the potential to benefit the treatment of neurodegenerative diseases [35]. Akt has been reported to regulate neuronal toxicity through its various substrates like FOXos, GSK3β, and caspase-9 etc. PI3k/Akt is also involved with PI3K in signaling pathway to mediate neuronal survival [36]. Current research suggests that mitogen-activated protein kinase (MAPK), a serine/threonine (Ser/Thr) kinase, is widely expressed in the central nervous system and can participate in the pathological processes of various neurodegenerative diseases by regulating important processes such as cell proliferation, differentiation, growth and apoptosis in response to stimulation by various extracellular factors [37, 38].

A literature report has shown that Wei Huiling et al. found that the Chinese herbal medicine compound in DGSND could effectively inhibit the expression of inflammatory factors TNF-α, IL-1 and IL-17 in the skin tissues of rats with scleroderma, and regulate the expression of HMGB1 protein, which could protect the skin tissues of rats with scleroderma [39]. Park Yong-soo et al. found that the therapeutic effect of DGSND on SSc was more advantageous than D-penicillamine in the treatment of SSc patients with ANA and anti-Scl-70 antibodies, and that the combination of the two (DGSND and D-penicillamine) was more effective [40]. It indicates that DGSND has a role in the treatment of SSc.

STAT (signal transducer and activator of transcription), a unique family of proteins that can bind to DNA. It usually responds to various extracellular cytokine and growth factor signals and is a class of SH2 signaling molecules containing phosphorylated tyrosine that can bind to it. STAT3 is one of the intranuclear transcription factors, and when activated, STAT3 molecules form dimers that enter the nucleus [41]. STAT3 has an important regulatory role in angiogenesis in different pathological settings, in several physiopathological processes such as cell proliferation and differentiation [42]. Activation of STAT3 in normal tissues is subject to strict positive and negative regulation. It was found that in many pathological states, activated STAT3 can alter the cell growth cycle by regulating cell proliferation factor c-ymc, cyclin D1, etc [43]. For the purpose of promoting abnormal cell proliferation and differentiation leading to occurrence of SSc. Moreover, VEGF is the most important and direct factor for vascular growth. It was found that the STAT3 protein binding site exists on the VEGF promoter, and activated STAT3 can directly bind to VEGF to induce the expression of VEGFA transcripts and activate VEGF to mediate vascular endothelial cell migration and angiogenesis [44].

The JAK/STAT signaling pathway is a common pathway for signaling of numerous cytokines, which is widely involved in cell proliferation, differentiation, apoptosis inflammation and immune regulation, and can interact with other signaling pathways through negative regulators, and the activation of this pathway may be related to the ability to promote the occurrence and development of SSc. When extracellular signaling factors such as interleukin (IL), interferon (INF) cause receptor dimerization upon binding to the corresponding receptors [45], JAK1/2 kinase is activated to catalyze the phosphorylation of complex residues in the receptor, which recruits STAT3 downstream, and then JAK1/2 kinase phosphorylates

STAT3, which forms a dimer that enters the nucleus and binds to the target gene to regulate its transcription [46]. JAK kinase can further activate the RAS/RAF signaling pathway by activating SHP2. RAS can be activated by directly binding to c-RAF (RAF1), which activates downstream MEK1/2. Activated MEK1/2 phosphorylates ERK1/2, and activated ERK1/2 directly enters the nucleus to phosphorylate STAT3 to regulate transcription. The RAS/RAF signaling pathway is the classical pathway of MAPK signaling pathway, which also regulates a variety of important cellular physiological and pathological processes such as cell growth, differentiation, stress adaptation, and inflammatory response. Both JAK/STAT pathway and MAPK pathway can directly activate PI3K/AKT signaling pathway to regulate protein synthesis, apoptosis, cell autophagy, glycogen synthesis and fatty acid synthesis.

Excessive activation of NFκB (nuclear factor kappa-B) transcription factors may also be associated with the development of systemic sclerosis [47]. Signaling factors inside and outside the cell bind to the receptor to activate IκB (inhibitor of nuclear factor kappa-B kinase), which activates IκB kinase (inhibit NF-κB). The activated IκB kinase phosphorylates and ubiquitinates IκB protein to degrade and release NFκB dimer, and NFκB dimer protein acts as NFκB dimer protein enters the nucleus as a transcription factor to promote the transcription of target genes and participates in the inflammatory response, immune response, apoptosis and stress response of the organism. In systemic sclerosis, vascular endothelial cell damage and activation are caused by the activation of intra- and extracellular signaling factors due to disturbances in the body's internal environment, which in turn stimulates abnormalities in the synthesis of collagen by fibroblasts, leads to fibrosis of the vessel wall and tissue. For example, TNF-α and IL1β can activate the NFκB pathway. Among them, TNF-α can induce direct apoptosis by binding FADD and TRADD to Caspase-8 after TNF-α binds to TNFR1, which in turn can indirectly activate Caspase-3 and Caspase-7 to promote inflammation and apoptosis [48].

TRAF2 and RIP activate NFκB signaling pathway via NIK (NF-κB inducing kinase) [49]; After IL-1β binds to ILR, it forms a membrane-penetrating complex with the help of receptor-associated protein IL1RAP (interleukin-1 receptor-accessory protein) to recruit IRAK1 (interleukin-1 receptor-associated kinase), which further activates NF-κB transcription factor [50]. It was found that TNF-α levels were elevated in the serum of SSc patients and favored the development of pulmonary fibrosis and pulmonary hypertension. TNF-α is involved in vascular endothelial activation, regulation of immune response and connective tissue metabolism by regulating fibroproliferative function [51]. Thus, TNF-α is considered to be a major factor in the progression of SSc. TNF-α also promotes myofibroblast differentiation of pulmonary mesenchymal stem cells by activating the NF-κB signaling pathway, and these results suggest that the TNF-α-rich proinflammatory microenvironment may play an important role in the development of interstitial inflammation and fibrosis by activating NF-κB signaling [52]. Elevated TNF-α serum levels may influence VEGF to promote endothelial dysfunction and promote the development and progression of endothelial dysfunction and pulmonary arterial hypertension [53]. It has been shown that soluble factors such as IL-1α, IL-1β, IL-6, IL-13, IL-17 and IL-18 secreted by immune system cells may be involved in the regulation of SSc fibrosis or cause vascular damage, among which IL-1β is a member of the IL-1 family of cytokines. Previous studies have pointed to a potential association between the IL-1 family and SSc. The expression of cytokines is regulated at their genetic level, and single nucleotide polymorphisms in IL-1 family cytokine genes may be associated with the pathogenesis of SSc [54]. According to the literature, the IL-17 pathway is also associated with SSc development, promoting proliferation and activation of fibroblasts and accelerating the progression of fibrosis [55] In addition, AKT can also phosphorylate Iκ to activate IκB kinase and activate NF-κB transcription factor.

Based on this literature reports , there are main active ingredients of DGSND for SSc: Sitogluside inhibits keloid fibroblast proliferation, apoptosis and collagen synthesis [56];Since JAK/ERK/STAT mediates VEGFA vascular endothelial cell migration and angiogenesis [57], Benzoylpaeoniflorin binds better to VEGFA target genes, suggesting that it may be associated with mediating VEGFA vascular endothelial cell migration and angiogenesis, and could potentially be a new therapeutic tool against VEGFA hyperproliferation, but the impact of the clinical effectiveness of SSc still needs to be further explored. In addition to inhibiting NFkB expression, (-)-Asarinin can also induce apoptosis through the expression of Caspase3, Caspase8 and Caspase9 [58, 59]; 4'DMEP has good anti-cancer activity, inhibits tumor cell proliferation and promotes tumor cell apoptosis [60, 61], but there are fewer reports on SSc treatment. Palbinone, as a novel terpenoid from Paeonia albiflora,inhibits the activity of the inflammatory vesicle NLRP3 (nucleotide binding oligomerization domain-like receptor protein 3) [62], which may reduce the inflammatory response associated with reduced SSc. Glycyrol is a unique bioactive component of Glycyrrhiza glabra root, which is one of the Glycyrrhiza glabra coumarins, with antibacterial, anti-inflammatory, immunosuppressive, CYP450 enzyme inhibiting activity, anti-tumor and other biological activities [63].

This study investigated the involvement of inflammatory response, abnormal cell proliferation and immune regulation that can be derived from the inhibition of STAT3, TNF, NFkB, VEGFA, MAPK, IL-1 family, IL-17, etc. in Danggui-Sini Deoction. It is also associated with the activation of PI3K/AKT signaling for neuronal cell repair. This may become a new treatment for SSc, but the literature on the treatment of SSc with Danggui-Sini Deoction is still limited to laboratory studies, and its clinical efficacy in blocking the progression of SSc must be further explored. Due to the complex composition of the Chinese herbal compound, it is complicated to study its therapeutic pathways. This study has derived possible therapeutic targets and pathways through the available data, which is expected to provide a theoretical basis for subsequent studies.

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