Network pharmacology analysis of molecular targets and related mechanisms of Guizhi decoction in treating of menopausal syndrome

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Abstract

Compared with hormone therapy, TCM had the advantages of overall adjustment and less side effects in the treatment of menopausal syndrome. But due to the complex pharmacodynamic composition of Guizhi decoction (GZD), the mechanism of TCM treating diseases was not clear. Network pharmacology could analyze drug action pathways through multi-pathway and multi-target, which provide a new direction for TCM mechanism research. The common targets of GZD and menopausal syndrome (MPS) were obtained by TCMSP and DisGeNET databases. And for the common targets, protein-protein interaction networks were established using the STRING database and analyzed by Gene Ontology and the Kyoto Encyclopedia of Genes and Genomes. (Our research does not require ethical approval). One hundred forty-six active ingredients with 283 targets were obtained from GZD by network pharmacological analysis. Besides, 230 target genes were found to have interactions with MPS, 52 of which were common targets between MPS and GZD and were predicted to be potential targets for MPS treatment of GZD. GO enrichment analysis revealed that GZD could affect 51 biological processes, 15 cellular components, and 13 molecular functions. Kyoto Encyclopedia of Genes and Genomes enrichment analysis yielded a total of 223. The pathways that are closely related to the pathogenesis of MPS are MAPK, PI3K-Akt. In this study, the relevant targets and mechanisms of GZD in the treatment of MPS were discussed from the perspective of network pharmacological analysis, reflecting the characteristics of multi-component, multi-target and multiple pathways, and it provides a good theoretical basis for the clinical application of GZD.

Abbreviations: DL = drug similarity, GZD = Guizhi decoction, KEGG = Kyoto Encyclopedia of Genes and Genomes, MPS = menopausal syndrome, OB = oral bioavailability.

Keywords: MPS, network pharmacology, GZD

1. Introduction

Menopausal syndrome (MPS) refers to a series of physical and psychosomatic states caused by fluctuations or decreases in sex hormones in women before and after menopause, which may even cause coronary heart disease, diabetes mellitus, and other highly lethal diseases without timely intervention.[1] In China, about 170,000 women were in perimenopause in 2018 as the aging society intensified,[2] accompanied by a gradually increasing incidence of menopausal syndrome. At this stage, the medical treatment for this disease is mainly hormone replacement therapy (estrogen and progesterin supplementation, hormone replacement therapy). Although hormone replacement therapy can effectively relieve most postmenopausal symptoms,[3] there are adverse effects such as abnormal uterine bleeding, breast tenderness, and atherosclerosis. The search for non-hormonal therapies with low adverse effects has become a significance task in the current efforts to control and alleviate the symptoms of menopausal syndrome.

GZD (Guizhi decoction) is the first formula in the Treatise on Typhoid Fever by Zhang Zhongjing and consists of five herbs, namely Gui Zhi (Cinnamomi Mmulus, GZ), Bai Shao (Paeonia Radix Alba, BS), Zhi Gan Cao (Raxix Glycyrrhizae Preparata, ZGC), Da Zao (Ziziphus jujubaMill, DZ), and Sheng Jiang (Zingiber officinale Roscoe, SJ). GZD is commonly used clinically to treat internal, external, gynecological and pediatric diseases caused by the imbalance of yin and...
yang and disharmony of Ying and Wei. TCM believes that MPS is mainly caused by the imbalance between Yin and Yang of the kidney, which often involves other Zang-fu, especially the heart, liver, and spleen, and can be combined with complex pathogenesis such as qi depression, blood stasis, phlegm and dampness. And GZD fits well with the pathomechanism of imbalance of kidney yin and yang in MPS. In a study, it was found that GZD can be the basis of body temperature, sweat derangement, and bidirectional regulation of heart rate, blood pressure, and large intestine conduction derangement. Among them, the bidirectional regulation of body temperature may be achieved by affecting adenylate cyclase activity in the hypothalamus, and some studies have shown that: the antipyretic effect of Gui Zhi Tang may be related to the reduction of serum IL-1, TNF-α, plasma and hypothalamic PGE2. Besides, animal experiments have shown that GZD can inhibit inflammation and oxidative stress in rats with high-fat myocardial ischemia, exerting cardiovascular protective effects. However, the mechanism of its therapeutic effect on MPS has not been clarified.

Due to the complex pharmacodynamic composition of GZD, the molecular mechanism of its therapeutic effect on MPS is still not well understood. In this study, the mechanism of action of GZD was analyzed by using network pharmacology to systematically elucidate and explore the mechanism of the therapeutic effect of GZD on MPS, which provides a theoretical basis for further research on the mechanism of the therapeutic effect of GZD on MPS.

2. Materials and Methods

2.1. Database building and active compound screening

The active ingredients of GZD (GZ, BS, ZGC, DZ, SJ) were collected from the TCMSP database (http://lsp.nwu.edu.cn/tcmsp.php). TCMSP is a Chinese medicine pharmacology database containing information about the herbs used in TCM, and absorption, distribution, metabolism and excretion (ADME) characteristics of the individual compounds, their targets, related diseases, and pathways. Pharmacokinetics parameters such as oral bioavailability (OB) and drug likeness (DL) were investigated. OB is commonly used to measure whether oral drugs can be through obstacles as well as be transported into the systemic blood circulation. DL is mainly used to predict exactly how “drug like” an ingredient is, which helps to assist pharmacokinetic and pharmaceutical properties, for example, solubility and chemical stability. OB≥30% and DL≥0.18 were used as screening criteria to screen out the possible active ingredients in GZD.

2.2. Identification of drug targets

After screening out the active ingredients in GZD, the target proteins retrieved from the TCMSP database were entered into Uniprot (https://www.uniprot.org/) and the corresponding gene names were extracted by Uniprot to standardize the target proteins corresponding to each herbal ingredient. The species was selected as “Homo sapiens.” A GZD active ingredient-disease-target network interplay map was constructed using Cytoscape 3.7.2 software, and the screened GZD active ingredients were correlated with MPS-related potential targets to clarify the interaction between their active ingredients and potential targets.

2.3. Screening of potential targets for MPS

The search was conducted from the DisGeNET database (https://www.disgenet.org/) and Genecard database (https://www.genecards) using “menopause syndrome” as the keyword (The results of the two databases were combined and duplicate targets were removed to obtain the disease targets of MPS. The specific target information corresponding to the active ingredient was entered into the Uniprot database to obtain the standard gene names of the targets of action.

2.4. PPI network of compound-disease targets PPI network

The GZD and MPS common targets were entered into the STRING database (https://string-db.org/), the study species was selected as human (Homo sapiens), the minimum required interaction score was selected >0.9, and other The protein interaction network (PPI) was obtained by selecting human (Homo sapiens) as the study species, selecting the minimum required interaction score >0.9, and keeping other parameters as default settings.

2.5. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

To understand the relational role of GZD active ingredients and disease corresponding common targets in gene function and pathways, GO enrichment analysis was performed on the common targets of GZD active ingredients and diseases using David (HTTPS://David.ncifcrf.gov/) and KEGG databases (species set to human, P<0.05 for effective pathway) and KEGG pathway enrichment analysis. The GO enrichment analysis included three parts: cellular component, biological process, and molecular function. The results of the obtained data were presented in the form of histograms.

2.6. Construction of active ingredient-target-pathway network

Cytoscape v3.7.2 was used to construct a network of common targets and signaling pathways of active ingredients and MPS in Chinese medicine. The “nodes” in different colors represent the targets and signaling pathways respectively. The “edges” refer to the interactions between common targets and signaling pathways.

2.7. Molecular docking

To further validate the interaction effect of GZD acting on MPS, molecular docking was applied for key compounds and kernel targets. The plug-in CytoHubba of Cytoscape software was used to screen top 10 hub genes (Table 1). Subsequently, Akt1 was molecular docking with key active ingredients, and Autodock Vina was used to predict the accuracy of key ingredients and predicted targets. Choose the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and protein data bank (http://www.rcsb.org/) to download MOL2 format of ligand and protein PDB format. Protein crystals were introduced into the PyMol software (https://pymol.org/2/; Version 2.4.1) for the dehydration and separation of ligands. The crystal is then introduced into AutoDockTools to build the target’s docking grid framework. Autodock Vina was used to realize molecular docking. Low Vina score is one of the results of molecular docking, representing a more stable binding affinity between protein and ligand. Finally, PyMol software was used to visualize the protein and compound complexes.

3. Results

3.1. Active ingredient and target screening for each herbal medicine in GZD

The chemical active ingredients of each herbal medicine in GZD were retrieved from TCMSP, and after the screening, 7 active
Table 1
Basic information of active compounds of GZD.

| Herb | Mol ID   | Molecule name                                                                 | OB (%) | DL     |
|------|----------|-------------------------------------------------------------------------------|--------|--------|
| GZ   | MOL001736| (-)-Taxifolin                                                                 | 60.51  | 0.27   |
| GZ   | MOL000492| (+)-Catechin                                                                  | 54.83  | 0.24   |
| GZ   | MOL000358| Beta-sitosterol                                                                | 36.91  | 0.75   |
| GZ   | MOL000073| Ent-Epicatechin                                                               | 49.96  | 0.24   |
| GZ   | MOL011169| Peroxyergosterol                                                              | 44.39  | 0.82   |
| GZ   | MOL000359| Sitosterol                                                                    | 36.91  | 0.75   |
| GZ   | MOL004576| Taxifolin                                                                     | 57.84  | 0.27   |
| BS   | MOL001910| 11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide  | 64.77  | 0.38   |
| BS   | MOL001918| Paeonifloropergone                                                            | 87.59  | 0.37   |
| BS   | MOL001919| (3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione | 43.56  | 0.53   |
| BS   | MOL001921| Lactiflorin                                                                   | 49.12  | 0.8    |
| BS   | MOL001924| Paeoniflorin                                                                  | 53.87  | 0.79   |
| BS   | MOL001925| Paeoniflorin_qt                                                                | 68.18  | 0.4    |
| BS   | MOL001928| Albitiflorin_qt                                                                | 66.64  | 0.33   |
| BS   | MOL001930| Benzyol_paeoniflorin                                                          | 31.27  | 0.75   |
| BS   | MOL000211| Mairin                                                                        | 55.38  | 0.78   |
| BS   | MOL000358| Beta-sitosterol                                                                | 36.91  | 0.75   |
| BS   | MOL000359| Sitosterol                                                                     | 36.91  | 0.75   |
| BS   | MOL000422| Kaempferol                                                                    | 41.88  | 0.24   |
| BS   | MOL000492| (+)-Catechin                                                                   | 54.83  | 0.24   |
| GC   | MOL001484| Inermine                                                                       | 75.18  | 0.54   |
| GC   | MOL001792| DFV                                                                            | 32.76  | 0.18   |
| GC   | MOL000211| Mairin                                                                        | 55.38  | 0.78   |
| GC   | MOL002311| Glycyrol                                                                       | 90.78  | 0.67   |
| GC   | MOL000239| Jaranol                                                                        | 50.83  | 0.29   |
| GC   | MOL002565| Medicarpin                                                                    | 49.22  | 0.34   |
| GC   | MOL000354| Isorhamnetin                                                                   | 49.6   | 0.31   |
| GC   | MOL000359| Sitosterol                                                                     | 36.91  | 0.75   |
| GC   | MOL003656| Lupienlightone                                                                 | 51.64  | 0.37   |
| GC   | MOL003896| 7-Methoxy-2-methyl Isoflavone                                                  | 42.56  | 0.2    |
| GC   | MOL000392| Formomonetin                                                                   | 69.67  | 0.21   |
| GC   | MOL000417| Calycosin                                                                      | 47.75  | 0.24   |
| GC   | MOL000422| Kaempferol                                                                     | 41.88  | 0.24   |
| GC   | MOL004328| Naringenin                                                                     | 59.29  | 0.21   |
| GC   | MOL004805| (2S)-2-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropropyran[2,3-f]Chromen-4-one | 31.79  | 0.72   |
| GC   | MOL004806| Euchrenone                                                                     | 30.29  | 0.57   |
| GC   | MOL004808| Glyasperin B                                                                   | 65.22  | 0.44   |
| GC   | MOL004810| Glyasperin F                                                                   | 75.84  | 0.54   |
| GC   | MOL004811| Glyasperin C                                                                   | 45.56  | 0.4    |
| GC   | MOL004814| Isotriflolid                                                                   | 31.94  | 0.42   |
| GC   | MOL004815| (β)-1-(2,4-dihydroxyphenyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one        | 39.62  | 0.35   |
| GC   | MOL004820| Kanzononis W                                                                   | 50.43  | 0.52   |
| GC   | MOL004824| (2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropan-2-yl)-4-methoxy-2,3-dihydrofururo[3,2-g]chromen-7-one | 60.25  | 0.63   |
| GC   | MOL004827| Semilicisoflavone                                                             | 48.78  | 0.55   |
| GC   | MOL004828| Glepidotin A                                                                   | 44.72  | 0.35   |
| GC   | MOL004829| Glepidotin B                                                                   | 64.46  | 0.34   |
| GC   | MOL004833| Phaseolinosiflavon                                                            | 32.01  | 0.45   |
| GC   | MOL004835| Glypallichalcone                                                               | 61.6   | 0.19   |
| GC   | MOL004838| 8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol                          | 58.44  | 0.38   |
| GC   | MOL004841| Licochalcone B                                                                 | 76.76  | 0.19   |
| GC   | MOL004846| Licochalcone G                                                                  | 49.25  | 0.32   |
| GC   | MOL004849| 3-(2,4-dihydroxyphenyl)-8-(1,1-dimethylprop-2-enyl)-7-hydroxy-5-methoxy-coumarin | 59.62  | 0.43   |
| GC   | MOL004855| Licorone                                                                       | 63.58  | 0.47   |
| GC   | MOL004856| Gancanolin A                                                                   | 51.08  | 0.4    |
| GC   | MOL004857| Gancanolin B                                                                   | 48.79  | 0.45   |

(Continued)
| Herb     | Mol ID      | Molecule name                                                                 | OB (%) | DL |
|----------|-------------|-------------------------------------------------------------------------------|--------|----|
| GC       | MOL004860   | licorice glycoside E                                                           | 32.89  | 0.27 |
| GC       | MOL004863   | 3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl) chromone           | 66.37  | 0.41 |
| GC       | MOL004864   | 5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl) chromone              | 30.49  | 0.41 |
| GC       | MOL004866   | 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl) chromone          | 44.15  | 0.41 |
| GC       | MOL004879   | Glycyrrhizin                                                                  | 52.61  | 0.47 |
| GC       | MOL004882   | Licocoumarone                                                                 | 53.21  | 0.47 |
| GC       | MOL004883   | Licoisoflavone                                                                | 41.61  | 0.42 |
| GC       | MOL004884   | Licoisoflavone B                                                              | 38.93  | 0.55 |
| GC       | MOL004885   | Licoisoflavone ane                                                            | 52.47  | 0.54 |
| GC       | MOL004891   | Shinpterocarpin                                                               | 80.3   | 0.73 |
| GC       | MOL004898   | (E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one | 46.27  | 0.31 |
| GC       | MOL004903   | Liquiritin                                                                    | 65.69  | 0.74 |
| GC       | MOL004904   | Licopyranocoumarin                                                            | 80.36  | 0.66 |
| GC       | MOL004905   | 3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxy-29-oic acid          | 34.32  | 0.55 |
| GC       | MOL004907   | Glycyrrhizinuretinin                                                          | 61.07  | 0.35 |
| GC       | MOL004908   | Glabridin                                                                     | 53.25  | 0.47 |
| GC       | MOL004910   | Glabranin                                                                     | 52.9   | 0.31 |
| GC       | MOL004911   | Glabrene                                                                       | 46.27  | 0.44 |
| GC       | MOL004912   | Glabrone                                                                       | 52.51  | 0.5  |
| GC       | MOL004913   | 1,3-dihydroxy-9-methoxy-6-benzofuran[3,2-c]chromenone                          | 48.14  | 0.43 |
| GC       | MOL004914   | 1,3-dihydroxy-8,9-dimethoxy-6-benzofuran[3,2-c] chromenone                     | 62.9   | 0.53 |
| GC       | MOL004915   | Eurycarpin A                                                                   | 43.28  | 0.37 |
| GC       | MOL004917   | glycyroside                                                                    | 37.25  | 0.79 |
| GC       | MOL004924   | (-)-Medicarpin                                                                | 40.99  | 0.95 |
| GC       | MOL004935   | Sigmoidin-B                                                                   | 34.88  | 0.41 |
| GC       | MOL004941   | (2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one                                | 71.12  | 0.18 |
| GC       | MOL004945   | (2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl) chroman-4-one        | 36.57  | 0.32 |
| GC       | MOL004948   | Isoglycyroside                                                                 | 44.7   | 0.84 |
| GC       | MOL004949   | Isolicoisoflavonol                                                            | 45.17  | 0.42 |
| GC       | MOL004957   | HM-O                                                                          | 38.37  | 0.21 |
| GC       | MOL004959   | 1-Methoxyphaseollidin                                                          | 69.98  | 0.64 |
| GC       | MOL004961   | Quercetin dier                                                                 | 46.45  | 0.33 |
| GC       | MOL004966   | 3'-Hydroxy-4'-0-Methylglabridin                                                | 43.71  | 0.57 |
| GC       | MOL004977   | icocochaconol a                                                               | 40.79  | 0.29 |
| GC       | MOL004974   | 3'-Methoxyglabridin                                                           | 46.16  | 0.57 |
| GC       | MOL004978   | 2-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyranol[6,5-f] chromen-3-yl]-5-methoxyphenol | 36.21  | 0.52 |
| GC       | MOL004980   | Inflacoumarin A                                                                | 39.71  | 0.33 |
| GC       | MOL004985   | lico-5-enolic acid                                                             | 30.7   | 0.2  |
| GC       | MOL004988   | Kanzonol                                                                       | 32.47  | 0.89 |
| GC       | MOL004989   | 6-prenylated eriodictyol                                                       | 39.22  | 0.41 |
| GC       | MOL004990   | 7',2',4'-trihydroxy-5-methoxy-3-arylcoumarin                                    | 83.71  | 0.27 |
| GC       | MOL004991   | 7-Acetoxy-2-methylisoflavone                                                    | 38.92  | 0.26 |
| GC       | MOL004993   | 8-prenylated eriodictyol                                                       | 53.79  | 0.4  |
| GC       | MOL004996   | Gadelaidic acid                                                                | 30.7   | 0.2  |
| GC       | MOL005000   | Vestitol                                                                       | 74.66  | 0.21 |
| GC       | MOL005000   | Gancaonin G                                                                    | 60.44  | 0.39 |
| GC       | MOL005001   | Gancaonin H                                                                    | 50.1   | 0.78 |
| GC       | MOL005003   | Licoagrocarpin                                                                | 58.81  | 0.58 |
| GC       | MOL005007   | Glyasperins M                                                                  | 72.67  | 0.59 |
| GC       | MOL005008   | Glycyrrhiza flavonol A                                                          | 41.28  | 0.6  |
| GC       | MOL005012   | Licoagrolisoflavone                                                           | 57.28  | 0.49 |
| GC       | MOL005013   | 18a-hydroxyglucyrhetic acid                                                    | 41.16  | 0.71 |
| GC       | MOL005016   | Odoratin                                                                       | 49.95  | 0.3  |
| GC       | MOL005017   | Phaseol                                                                        | 78.77  | 0.58 |
ingredients of GZ, 13 active ingredients of BS, 92 active ingredients of ZGC, 5 active ingredients of SJ, and 29 active ingredients of DZ were obtained, totaling 146 active ingredients. Information about the active ingredients of GZD is shown in Table 1. The active ingredient action targets of GZ, 111 potential targets of BS, 222 potential targets of ZGC, 53 potential targets of SJ, and 209 potential targets of DZ were obtained. After protein targets were entered into Uniprot and duplicate entries were removed, a total of 282 gene targets were obtained for further study.

### 3.2. GZD component-target network graph analysis

To better show the relationship between the active ingredient and MPS-related targets in GZD, a component-disease-target network diagram was constructed by Cytoscape 3.7.0 software (Fig. 1). The degree indicates the number of routers connected to the node by other nodes in the network, such as each oval node represents the number of targets owned by the active ingredient. The active ingredients with a high number of associated targets can be found as follows: quercetin (131), kaempferol (63), ß-sitosterol (43), stepharine (27), naringenin (23), and beta-carotene (20).

### 3.3. Prediction of target genes associated with MPS in each component

The disease targets obtained from the DisGeNET and Genecard databases were 1412, and after the screening, the disease targets were uploaded to the UniProt database for correction, and 1148 were obtained after removing duplicates. Active ingredient and disease targets were obtained through the Veeny 2.1 website for 52 generic targets (Fig. 2). These targets are common targets for GZD and diseases.

### 3.4. Construction of PPI GZD and MPS protein interaction network

The 52 potential genes were recorded into the STRING database for analysis to obtain the protein-protein interaction relationships, and then the target protein PPI network graph was drawn using Cytoscape 3.7.0, as shown in Figure 3.
The network graph contains a total of 51 nodes and 334 edges with an average node degree value of 12.8 and a PPI enrichment P value of less than 1.0×10⁻¹⁶, where the nodes represent proteins and each edge represents the protein-protein interaction relationship, with more lines indicating a greater association, as shown in Figure 3. By CytoNCA (an application in Cytoscape) after performing visual topology analysis, 10 core targets were obtained (Table 2). Among the 14 core targets, AKT1 had the highest degree (37), followed by ALB (36), TP53 (29), and MYC (27).

3.5. GO enrichment analysis

The 52 common targets obtained were analyzed by David for GO enrichment (Fig. 4). Cellular component terms mainly included nucleus (16%), cytoplasm (15%) and plasma membrane (12%); molecular function terms mainly included DNA binding (16%), transcription factor activity, sequence-specific DNA binding (8%), and identical protein binding (5%); biological process terms mainly included positive regulation of transcription from RNA polymerase II promoter (9%), positive regulation of transcription, DNA-templated (7%), transcription, DNA-templated (6%), negative regulation of apoptotic process (6%) and positive regulation of cell proliferation (6%).

3.6. KEGG pathway enrichment analysis

KEGG was a database that systematically analyzed the metabolic pathways of gene products in cells and the functions of these gene products. 52 common targets were input into the KEGG database, and 223 signaling pathways were obtained. The main pathways were: Pathways in cancer, Proteoglycans in cancer, Hepatitis C, MAPK signaling pathway, Network Human cytomegalovirus infection, PI3K-Akt signaling pathway, Hepatocellular carcinoma, Hepatitis B etc (Fig. 5).
In these pathways, the ones most closely related to the treatment of MPs were: MAPK signaling pathway (Fig. 6), PI3K-Akt signaling pathway (Fig. 7). MAPK signaling pathway played an important role by regulating: EGF-EGFR-RAS-ERK/JNK, IL1-IL1R-JNK/p38, RAC/CDC42-PAK-ER etc; PI3K-Akt signaling pathway mainly regulated EGF-EGFR-RAS-PI3K, PTEN-PIP3-AKT, EGF-EGFR-PI3K-NFKB.

3.7. Targets-pathways network
Targets-pathways network (Fig. 8), there were 8 main pathways and 27 related targets. As can be seen from the figure, Pathways in cancer, MAPK, and PI3K-Akt were highly enriched, which suggested that these Pathways may play an important role in the treatment of MPS. AKT1, TP53, RAF1, MYC, and other targets had more interaction lines with pathways, which suggested that they may play a major role in the process of various biological pathways.

3.8. Molecular docking results
We carried out molecular docking of the core target protein and active compound involved. Molecular docking between top 1 target proteins (AKT1) and key active compounds (quercetin, kaempferol, β-sitosterol, stepharine) was carried out using AutoDock Vina. The binding energy between AKT1 and the active compounds was approximately between 8.7 and 10.8 kcal/mol. AKT1, ALB, MAPK3, JUN, CASP3, and EGFR have stronger docking energy. Finally, the top four target protein macromolecules and small compound molecules with the best docking affinity were selected and visualized with PyMoL (Fig. 9).

4. Discussion
TCM herbs usually act synergistically on multiple diseases with multiple components, targets, and pathways. Network pharmacology has made great progress in exploring the application of active ingredients, targets, and systems in TCM. In this study, we applied a network pharmacology approach to investigate the relevant targets and pathways of GZD for the treatment of MPS, thus elucidating the synergistic mechanism of GZD on MPS. In menopausal syndrome, the decline in ovarian function, fluctuations, and decreases in estrogen, leading to menstrual irregularities, in addition to mediating the development of chronic diseases such as cardiovascular disease (CVD), obesity, hyperlipidemia, and fatty liver.[17]

In the study, after screening the OB and DL of drug components, 146 active ingredients were identified, the most potent of which was quercetin (131), kaempferol (63), β-sitosterol (43), stepharine (27), naringenin (23), and beta-carotene(20), and their potential value for the treatment of MPS was shown by network and functional analysis.

Quercetin is a naturally occurring flavonoid with strong biological activity that exerts antioxidant, antitumor, anti-inflammatory, antibacterial, and cardiovascular protective pharmacological effects by reducing oxidative stress, interfering with the renin-angiotensin-aldosterone system, and downregulating reactive oxygen species-mediated downstream signaling pathways.[18] The experimental results of Wang et al. indicated that quercetin increased the mRNA and protein expression levels of oxidative stress-related genes SOD-1, CAT, and GSS in postmenopausal rats, suggesting that quercetin improves the antioxidant capacity of the ovary by upregulating the expression of some oxidative stress-related genes.[19] An experiment by Huang et al. found that intake of quercetin significantly reduced blood pressure in humans, and besides, in a parallel design trial, participants who took quercetin for 8 weeks or longer showed significant changes in HDL cholesterol and triglyceride levels.[20]

The dietary flavonoid kaempferol has antioxidant, anti-inflammatory, anti-apoptotic, anti-cancer, estrogenic, and anti-estrogenic activities.[21] The results of an experiment by Touillaud MS et al. showed that low intake of kaempferol, the trace element boron, and β-sitosterol reduced the risk of estrogen...
A trial by Sriramani Sandhiya et al. found that β-sitosterol had similar effects on cell proliferation rates as standard 17β-estradiol, suggesting that β-sitosterol could be a safe alternative to estrogen replacement therapy. A trial by Yu et al. showed that long-term supplementation with 3% naringenin resulted in significant accumulation of naringenin in the plasma and tissues of obese de-ovulated mice and reduced metabolic disorders and muscle loss. A randomized, double-blind trial showed that regular administration of pentenyl naringin affected alleviating mild vasodilatory symptoms in menopausal women.

The results of GO and KEGG pathways indicated that the MAPK and PI3K-Akt were most closely related to the pathogenesis of MPS. These pathways were mainly linked to the core genes including AKT1, TP53, RAF1, MYC e in our research. A lots of studies could confirm these results.

Naringenin is a dietary flavonoid found in a variety of products including citrus fruits. The substance has several pharmacological activities such as anti-inflammatory and antioxidant. A trial by Yu et al. showed that long-term supplementation with 3% naringenin resulted in significant accumulation of naringenin in the plasma and tissues of obese de-ovulated mice and reduced metabolic disorders and muscle loss. A randomized, double-blind trial showed that regular administration of pentenyl naringin affected alleviating mild vasodilatory symptoms in menopausal women.

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MAPK was a group of serine/threonine protein kinases. As one of the main signaling pathways for intracellular information transmission, MAPK played a key role in many biological processes and could regulate cell growth, differentiation, apoptosis, inflammation, and other biological processes.
Over-activation or reduced expression of MAPK would lead to the occurrence and development of various diseases.\textsuperscript{[28–30]} In menopausal women, due to the decrease of estrogen levels, some women began to experience rapid bone loss, mainly manifested as bone loss, and in severe cases, further development of osteoporosis. This pathway could affect the differentiation and proliferation of osteoblasts and osteoclasts by regulating downstream factors.\textsuperscript{[31]} In our study, MAPK mainly included ERK, JNK, p38 kinase and ERK5 activated kinase 4 subfamilies. Two ERK subtypes and three JNK subtypes were associated with proliferation of osteoclast precursor cells and apoptosis of osteoclasts.\textsuperscript{[32]} In addition, four subtypes of p38 was highly expressed in both osteoclast precursor cells and mature osteoclasts, and played a key role in osteoclast differentiation and bone resorption.\textsuperscript{[33]} In vitro experiments showed that osteoclast formation could be inhibited by regulating MAPK signaling pathway to prevent bone loss in rats.\textsuperscript{[34]} Meanwhile, MAPK was also a positive

![Figure 4. Functional analysis. (A) Cellular component (CC). (B) Molecular function (MF). (C) Biological process (BP).](image_url)

![Figure 5. KEGG pathway. The length of each bar represents the number of enriched genes. KEGG = Kyoto Encyclopedia of Genes and Genomes.](image_url)
regulator of osteoblastic differentiation and bone formation. Research has shown that collagen peptide promoted osteogenesis by activating the p38MAPK and the levels of osteogenic biomarkers such as collagen, alkaline phosphatase and osteocalcin were significantly increased. And, a clinical study had shown that polymorphism of MAPK-1 signaling pathway protein gene was associated with the severity of coronary heart disease and coronary artery disease in menopausal women.

Women with MPS began to lose their ovarian function and menstrual cycle due to hormonal fluctuations. PI3K-Akt was a pathway closely related to cell proliferation and differentiation, and the PI3K-Akt signaling pathway existed in oocytes and granulocytes, which regulated the growth, development, maturation, and periodic ovulation of follicles through mutual coordination. AKT, also known as protein kinase B, was an important downstream molecule of PI3K. AKT activation was an important prerequisite for its function. Activated AKT became Phosphorylated AKT(PAKT) through phosphorylation, and activated or inhibited its downstream target protein to inhibit the apoptosis of granulosa cells, thereby inhibiting the atresia of follicles to some extent. Animal experiments have found that compared with the control group, the fertility of the gene knockout mice decreased, and the number of mature follicles in the ovary decreased, and the number of apoptotic follicles increased. At the same time, the ovarian response to E2 was also very low, which did not cause the growth of follicles and granulosa cell proliferation. Besides, women during this time due to low estrogen caused by hot flashes, night sweats, joint pain or life stress, and many other factors, the quality of sleep significantly decreased. Studies have shown that the PI3K-Akt signaling pathway played a certain role in sleep regulation by regulating its downstream effector molecules, such as immunoregulatory factors such as interleukin 1-β (IL-1β) and tumor necrosis factor-α (TNF-α). In conclusion, MAPK and PI3K-Akt could improve the symptoms of MPS in multiple ways.

5. Conclusion

Based on network pharmacological analysis, this study demonstrated that GZD treated MPS through multi-compounds, multi-targets, and multi-pathways, and preliminarily clarified the related potential mechanism of GZD in the treatment of MPS. Through KEGG pathway enrichment analysis, it was found that GZD played an important role in the treatment of MPS, including MAPK and PI3K-Akt, which might play a role in regulating cell growth, differentiation, and apoptosis. This study provided a potential biological basis for the further study of GZD in the treatment of MPS, but further experimental studies were needed.
Figure 7. PI3K-Akt signaling pathway. Core targets are marked in red.

Figure 8. Targets-pathways network. The pink diamond nodes stand for major biological pathways; The blue ellipse nodes stand for the active component of GZD and the common target of MPS. The gray lines stand for the relationship between the target and the pathway.
Author contributions
Data curation: Jingtao Liang, Qian Zhang
Formal analysis: Jingtao Liang
Methodology: Qian Zhang, Ying Zhou
Supervision: Ying Zhou
Writing – original draft: Qian Zhang

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Figure 9. Molecular docking results of “bioactive compound-hub gene.” (A) Quercetin to AKT1; (B) Kaempferol to AKT1; (C) β-sitosterol to AKT1; (D) Stepharine to AKT1.
