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DATABASE ANALYSIS

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Network Pharmacology-Based Analysis on the Mechanism of Action of Ephedrae Herba-Cinnamomi Ramulus Couplet Medicines in the Treatment for Psoriasis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: This study explored the mechanism of action of Ephedrae Herba-Cinnamomi Ramulus couplet medicine (MGCM) at the pharmacological level in the treatment of psoriasis.

Material/Methods: The active ingredients in MGCM were mined through literature retrieval and the BATMAN-TCM database, and potential targets were predicted. In addition, targets associated with psoriasis were acquired using multiple disease-related databases. Thereafter, an interaction network between candidate MGCM targets and the known psoriasis-associated targets was constructed based on the protein–protein interaction (PPI) data, using the STRING database. Then, the topological parameter degree was determined for mining the core targets for MGCM in the treatment of psoriasis, which also represented the major hubs within the PPI network. In addition, the core networks of targets and ingredients were constructed using Cytoscape software to apply MGCM in the treatment for psoriasis. These core targets were then analyzed for Gene Ontology biological processes and Kyoto Encyclopedia of Genes and Genomes pathway enrichment using OmicShare.

Results: The ingredient-target core network of MGCM for treating psoriasis was constructed; it contained 52 active ingredients and corresponded to 19 core targets. In addition, based on enrichment analysis, these core targets were majorly enriched for several biological processes (immuno-inflammatory responses, leukocyte differentiation, energy metabolism, angiogenesis, and programmed cell death) together with the relevant pathways (Janus kinase-signal transducer and activator of transcription, toll-like receptors, nuclear factor κB, vascular endothelial growth factor, and peroxisome proliferator-activated receptor), thus identifying the possible mechanism of action of MGCM in treating psoriasis.

Conclusions: The present network pharmacology study indicated that MGCM alleviates various pathological factors of psoriasis through multiple compounds, multiple targets, and multiple pathways.

MeSH Keywords: Ethnopharmacology • Medicine, Chinese Traditional • Psoriasis

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Background

Psoriasis, a common skin disorder, is easily diagnosed but refractory to treatment and prone to recurrence [1]. According to an epidemiological survey, psoriasis has an incidence of about 0.5% in the Chinese population [2]. However, the underlying pathogenesis of psoriasis remains incompletely understood. Existing studies suggest that aberrant psoriasis-susceptible gene expression, autoimmune disorders, obesity, and abnormalities in multiple inflammatory signaling pathways contribute to the development of psoriasis [3]. However, the pathogenesis remains largely unclear, which hinders specific treatment. Currently, there is no curative treatment for psoriasis. Traditional Chinese medicine (TCM) has a distinct effect in the treatment of psoriasis, and it has a therapeutic impact via multiple targets and pathways that correspond to the diverse pathways that are dysregulated in psoriasis [4–6]. Yet the underlying mechanism of action of TCM in psoriasis treatment remains unclear, thus restricting its internationalization and standardization in treating psoriasis.

In the TCM clinical treatment of psoriasis, the heat-clearing and blood-cooling method is usually adopted, but no satisfactory effect is consistently achieved [7]. Based on the treatment idea of promoting the expulsion of exogenous pathogenic evils, numerous TCM experts have proposed that the additional application of the sweating method could significantly increase the therapeutic effect of TCM on psoriasis [8]. Ephedrae Herba-Cinnamomi Ramulus couplet medicine (MGCM) contains the 2 most representative traditional Chinese herbal medicines for inducing sweating and dispelling exogenous evils: Ephedrae Herba (Mahuang, MH) and Cinnamomi Ramulus (Guizhi, GZ). This combination represents the empirical couplet medicines adopted in the Department of Dermatology in our hospital to treat psoriasis. Numerous preclinical and clinical studies indicate that these 2 herbal medicines are effective for treating psoriasis [9,10]. In addition, years of clinical practice show that MGCM is effective against psoriasis. MGCM was shown in our prior research to suppress abnormal keratinocyte proliferation and chemokine release and thus to inhibit infiltration of multiple immunocytes [11–13]. Nonetheless, the scientific foundation and exact molecular mechanisms of MGCM remain unknown, so more research is warranted.

In traditional studies that examine the TCM mechanism, the "one drug, one target, one disease" model is adopted, but it cannot reveal the "multiple components, multiple targets, and multiple pathways" of TCM. In the present study, several algorithm- and network-based computational methods were adopted in combination to predict active ingredients, mine various drug targets, and construct core networks of targets and ingredients of MGCM for treating psoriasis. Macroscopic network analysis was then performed to illustrate the possible mechanisms of MGCM and provide a basis for future research.

Material and Methods

Selection of candidate MGCM active ingredients and targets

The BATMAN-TCM database (*http://bionet.ncpsb.org.cn/batman-tcm/*) has been developed as the bioinformatics analytical approach to analyze the active ingredients in TCM [14,15]. To obtain information on MGCM ingredients, "Ephedrae Herba" and "Cinnamomi Ramulus" were used as keywords to search the BATMAN database. A total of 116 compounds were identified, and their names and code numbers are shown in **Table 1**.

The BATMAN-TCM database also predicts the candidate compound targets according to their similarities to known drug-target interactions (Target score cutoff ≥20 and *P* value cutoff <0.05) [16]. In addition, the Traditional Chinese Medicine System Pharmacology Database (TCMSP, *http://lsp.nwu.edu.cn/tcmsp.php*) [17] was used to predict the potential targets of medicinal components.

Screening of the known psoriasis-associated targets

To obtain the known targets related to psoriasis, "psoriasis" was used as the keyword in searches of the DisGeNet platform [18], MalaCards database [19], DrugBank database [20], and Therapeutic Target Database [21]. The DisGeNet database results were classified according to the disease specificity index (DSI), and targets with a DSI value lower than the median value of genes known to be related to psoriasis were removed. In addition, relevant targets were removed if the drugs had aberrant status in DrugBank and the Therapeutic Target Database. **Supplementary Table 1** summarizes more details of the known targets related to psoriasis following redundancy deletion.

Mining of core targets of MGCM in the treatment of psoriasis and establishment of core active ingredient-target network

First, *Homo sapiens* was selected as the species to standardize targets that were acquired in the aforementioned 2 steps (including candidate MGCM targets and the known targets related to psoriasis) based on the UniProt database [22], to obtain the names of individual universal genes. Thereafter, the candidate MGCM targets and known targets related to psoriasis were imported into the Wayne diagram online tool (*http://bioinformatics.psb.ugent.be/webtools/Venn/*) for mapping. In other words, targets obtained via the 2 sets were intersected for acquiring potential MGCM targets in psoriasis treatment.

For every interaction, the STRING server [23] produced a "combined score" of 0–1, with a higher score indicating more confidence that an interaction exists. In STRING, the interactions

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Table 1. All the candidate ingredients of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM).

Compound No.	Compound name	ID from TCMID database
GZ01	Procurcumenol	ID: 17861 from TCMID database
GZ02	Tetradecanal	ID: 24042 from TCMID database
GZ03	Cinnamaldehyde	ID: 3693 from TCMID database
GZ04	5-Cinnamoyl-9-O-Acetylphototaxicin I	ID: 3697 from TCMID database
GZ05	Anethole	ID: 1186 from TCMID database
GZ06	Protocatechuic Acid	ID: 23246 from TCMID database
GZ07	Coumarinic Acid	ID: 30820 from TCMID database
GZ08	Gamma-Sitosterol	ID: 29509 from TCMID database
GZ09	Camphor	ID: 3048 from TCMID database
GZ10	Proanthocyanidin B2	ID: 17855 from TCMID database
GZ11	Melilotocarpan A	ID: 13672 from TCMID database
GZ12	Farnesol	ID: 7733 from TCMID database
GZ13	Nerolidol	ID: 23421 from TCMID database
GZ14	Trans-Cinnamic Acid	ID: 23114 from TCMID database
MH01	Alpha-Linolenic Acid	ID: 23145 from TCMID database
MH02	Dimethyl Phthalate	ID: 6397 from TCMID database
MH03	Ethanol	ID: 23458 from TCMID database
MH04	Carvacrol	ID: 3231 from TCMID database
MH05	2,4-Decadienal	ID: 23260 from TCMID database
MH06	Nor-Rubrofusarin	ID: 15782 from TCMID database
MH07	-Epiafzelechin	ID: 25807 from TCMID database
MH08	Octanol	ID: 15967 from TCMID database
MH09	Cis-P-2-Menthen-1-Ol	ID: 13763 from TCMID database
MH10	Gamma-Terpinene	ID: 23910 from TCMID database
MH11	Pseudoephedrine	ID: 24296 from TCMID database
MH12	D-Norpseudoephedrine	ID: 15780 from TCMID database
MH13	P-Cymene	ID: 4549 from TCMID database
MH14	Lauric Acid	ID: 23228 from TCMID database
MH15	Tetradecanoic Acid	ID: 23983 from TCMID database
MH16	O-Xylene	ID: 23233 from TCMID database
MH17	D-Pseudoephedrine	ID: 18010 from TCMID database
MH18	Apigenin	ID: 1476 from TCMID database
MH19	Methyl Acetate	ID: 24578 from TCMID database
MH20	Guaiazulene	ID: 9037 from TCMID database
MH21	Safranal	ID: 19105 from TCMID database
MH22	1,8-Cineole	ID: 3689 from TCMID database
MH23	Methyl Benzoate	ID: 24934 from TCMID database
MH24	Alpha-Terpineol	ID: 23119 from TCMID database
MH25	1,4-Cineole	ID: 3688 from TCMID database

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Table 1 continued. All the candidate ingredients of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM).

Compound No.	Compound name	ID from TCMID database
MH26	Ephedrine	ID: 6814 from TCMID database
MH27	1-Octen-3-Ol	ID: 15973 from TCMID database
MH28	M-Xylene	ID: 23212 from TCMID database
MH29	Decanoic Acid	ID: 23454 from TCMID database
MH30	11-Methoxyhumantenine	ID: 13941 from TCMID database
MH31	Beta-Eudesmol	ID: 23867 from TCMID database
MH32	Phenanthrene	ID: 23852 from TCMID database
MH33	2,3,5,6-Tetramethyl-Pyrazine	ID: 24520 from TCMID database
MH34	2-Methyl-2-Butenal	ID: 24323 from TCMID database
MH35	Kaempferol	ID: 12017 from TCMID database
MH36	Geraniol	ID: 8311 from TCMID database
MH37	Dibutyl Phthalate	ID: 5403 from TCMID database
MH38	Maragenin Ii	ID: 13539 from TCMID database
MH39	Limonene	ID: 23184 from TCMID database
MH40	Alpha-Pinene	ID: 23880 from TCMID database
MH41	Terpinen-4-Ol	ID: 20976 from TCMID database
MH42	Delta-Terpineol	ID: 25205 from TCMID database
MH43	Naphthalene	ID: 15244 from TCMID database
MH44	Beta-Pinene	ID: 23545 from TCMID database
MH45	6-Methyl-2-Heptanone	ID: 23701 from TCMID database
MH46	Hexadecanoic Acid	ID: 24748 from TCMID database
MH47	Xylene	ID: 24148 from TCMID database
MH48	O-Methylptelefolonium	ID: 14697 from TCMID database
MH49	Camphor	ID: 3048 from TCMID database
MH50	Citronellol	ID: 3768 from TCMID database
MH51	Heptanoic Acid	ID: 23191 from TCMID database
MH52	7-Demethylsuberosin	ID: 5097 from TCMID database
MH53	Methylpseudoephedrine	ID: 24866 from TCMID database
MH54	N-Triacontanol	ID: 21525 from TCMID database
MH55	Beta-Cyclocitral	ID: 24417 from TCMID database
MH56	Linalool	ID: 12843 from TCMID database
MH57	Nerolidol	ID: 23421 from TCMID database
MH58	Norpseudoephedrine	ID: 23736 from TCMID database
MH59	Myrcene	ID: 15138 from TCMID database
MH60	Cibarian	ID: 3634 from TCMID database
MH61	Methyl-7-Epiganoderate	ID: 14390 from TCMID database
MH62	Cuminyl Alcohol	ID: 24396 from TCMID database
MH63	Thymol	ID: 21344 from TCMID database
MH64	Menthyl Acetate	ID: 13772 from TCMID database

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Table 1 continued. All the candidate ingredients of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM).

Compound No.	Compound name	ID from TCMID database
MH65	Methyl Palmitate	ID: 23038 from TCMID database
MH66	N-Methylephedrine	ID: 14388 from TCMID database
MH67	Linolenic Acid	ID: 23046 from TCMID database
MH68	Octanoic Acid	ID: 23059 from TCMID database
MH69	Dihydro-Beta-Ionone	ID: 24357 from TCMID database
MH70	3,4-Dimethyl-5-Phenyloxazolidine	ID: 6395 from TCMID database
MH71	Acetophenone	ID: 115 from TCMID database
MH72	1-Phenyl-1,2-Propanedione	ID: 24685 from TCMID database
MH73	Hexahydrofarnesylacetone	ID: 23775 from TCMID database
MH74	Alpha-Terpinolene	ID: 24431 from TCMID database
MH75	Pseudoginsenoside F11	ID: 18011 from TCMID database
MH76	1,5-Dimethyl-Naphthalene	ID: 24177 from TCMID database
MH77	Dodecanoic Acid	ID: 24924 from TCMID database
MH78	Norephedrine	ID: 15736 from TCMID database
MH79	Maokonine	ID: 13536 from TCMID database
MH80	Nonanal	ID: 24697 from TCMID database
MH81	6-Methyl-5-Hepten-2-One	ID: 23462 from TCMID database
MH82	1-Octanol	ID: 23425 from TCMID database
MH83	Chuanxiongzine	ID: 3633 from TCMID database
MH84	Tetramethylpyrazine	ID: 23142 from TCMID database
MH85	Linoleic Acid	ID: 24136 from TCMID database
MH86	Beta-Ionone	ID: 23950 from TCMID database
MH87	Octadecanoic Acid	ID: 23678 from TCMID database
MH88	2,3,4-Trimethyl-5-Phenyloxazolidine	ID: 21957 from TCMID database
MH89	Pentadecanoic Acid	ID: 23379 from TCMID database
MH90	Phenethylamine	ID: 17069 from TCMID database
MH91	Trans-2-Nonenal	ID: 23241 from TCMID database
MH92	Isobutyl Benzoate	ID: 24655 from TCMID database
MH93	Leucodelphinidin	ID: 12711 from TCMID database
MH94	Alpha-Ionone	ID: 24480 from TCMID database
MH95	Phytol	ID: 17251 from TCMID database
MH96	Myricadiol	ID: 15146 from TCMID database
MH97	Leucopelargonidin	ID: 12712 from TCMID database
MH98	Nonanoic Acid	ID: 23252 from TCMID database
MH99	Hexanol	ID: 9513 from TCMID database
MH100	16-Triacontanol	ID: 21524 from TCMID database
MH101	2-Pentadecanone	ID: 24695 from TCMID database
MH102	Sabinene	ID: 19084 from TCMID database
MH103	Hexanoic Acid	ID: 23052 from TCMID database
MH104	Piperitone	ID: 17442 from TCMID database

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Figure 1. Construction of the network of Ephedrae Herba-Cinnamomi Ramulus couplet medicine (MGCM) compounds and their potential targets. The active compounds (compounds ID) collected from diverse herbal medicines were linked with corresponding potential targets to construct the compound-target network, with a node indicating an active compound (the diverse colors of circle stand for diverse herbal medicines) and the target (green square).

>0.4 and >0.7 indicate medium and high confidence, respectively. The potential targets were uploaded to the STRING database to obtain PPIs, with a minimum interaction score of 0.4 and *Homo sapiens* as the species. For every target (node) within the network, the topological factor degree, which means the number of edges shared with other nodes, was determined through the plug-in cytoHubba [24]. Then, twice the median of degrees for all targets served as the screening criterion. Nodes in which the degree values were greater than twice the median were selected to be the pivotal hubs within that PPI network; that is, they were the core MGCM targets for treating psoriasis. Finally, the core active ingredient-target network was constructed using Cytoscape.

Core target enrichment analysis

Core targets were assessed in Gene Ontology (GO) biological process (BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses using OmicShare software [25] with the species of *Homo*. The adjusted *P* value of ≤0.01 was utilized as the criterion in enrichment analysis.

Results

Selection of candidate active ingredients as well as targets of MGCM

The BATMAN-TCM database was retrieved comprehensively, and altogether 116 MGCM compounds were identified. Of these compounds, 104 and 14 were the active ingredients of MH and GZ, respectively. Several compounds were extensively distributed in these 2 herbs, including camphor and nerolidol. **Table 1** presents the basic MGCM ingredients.

Thereafter, the potential targets of MGCM components were identified, and altogether 1338 targets were identified (**Supplementary Table 2**). There were 1257 and 505 potential targets in MH and GZ, respectively, and there were several targets overlaps between the 2 herbs, regardless of the different numbers of targets related to each herb in MGCM. Such results suggested that the diverse MGCM ingredients exerted antergic or congenerous roles through regulation of similar targets.

To acquire comprehensive understanding of the network of candidate ingredients and targets of MGCM, we established a network map with the Cytoscape software, which included 1384 nodes and 8464 edges (**Figure 1**). Specifically, the node degree indicated the number of targets or edges correlated with the node based on topological analysis. Altogether, 58 ingredients with the degree of ≥33 were discovered in our established network, including piperitone, ephedrine, and cinnamic acid, which acted on 266, 219, and 56 targets, respectively. These compounds have been proven to have a wide range of pharmacological activities (e.g., anti-inflammatory, antioxidant, immune regulation) [26,27], which were subsequently considered to be the active ingredients of MGCM.

Mining of MGCM core targets in the treatment of psoriasis

Psoriasis is considered a polygenic disease. Investigating the association of genes with the environment might contribute to revealing the pathogenesis of psoriasis. Targets that had an aberrant status from DrugBank and Therapeutic Target Database

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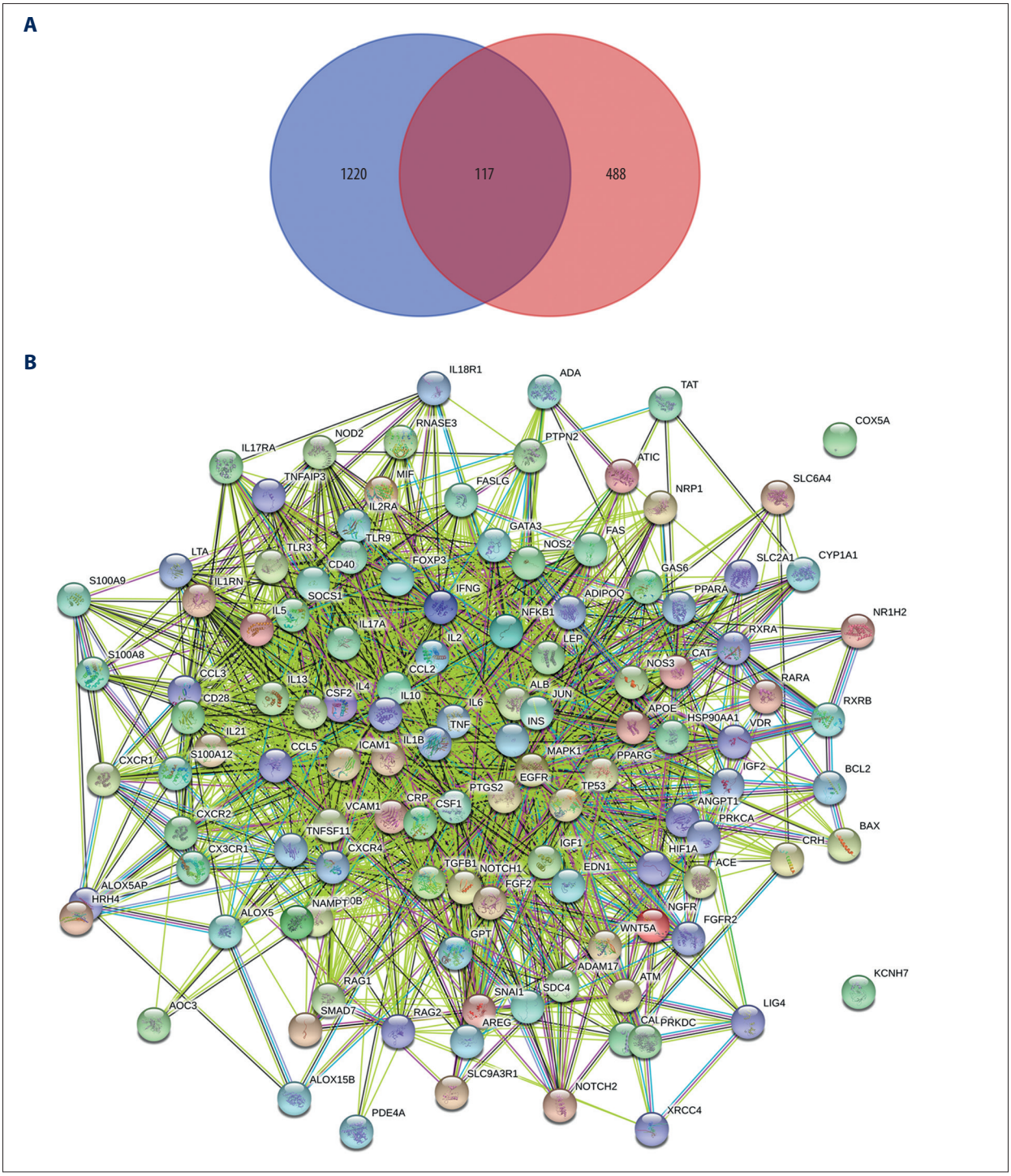
or with the median DSI of <0.535 based on the DisGeNet database were removed, and a total of 605 psoriasis-related targets (**Supplementary Table 1**) were obtained from those 4 sources. In addition, 117 recognized candidate MGCM targets were also targets related to psoriasis (or therapeutics) (**Supplementary Table 3, Figure 2A**) and were identified as potential MGCM targets in psoriasis treatment.

Later, to further select the MGCM core targets in psoriasis treatment, the PPI network was established based on the above-mentioned targets using the STRING database (**Figure 2B**). Then, topological parameters (Degree) for all nodes within the network were calculated by the plug-in cytoHubba (**Supplementary Table 4**). Afterwards, twice the median number of degrees for all targets was utilized as the selection criteria. Any node in



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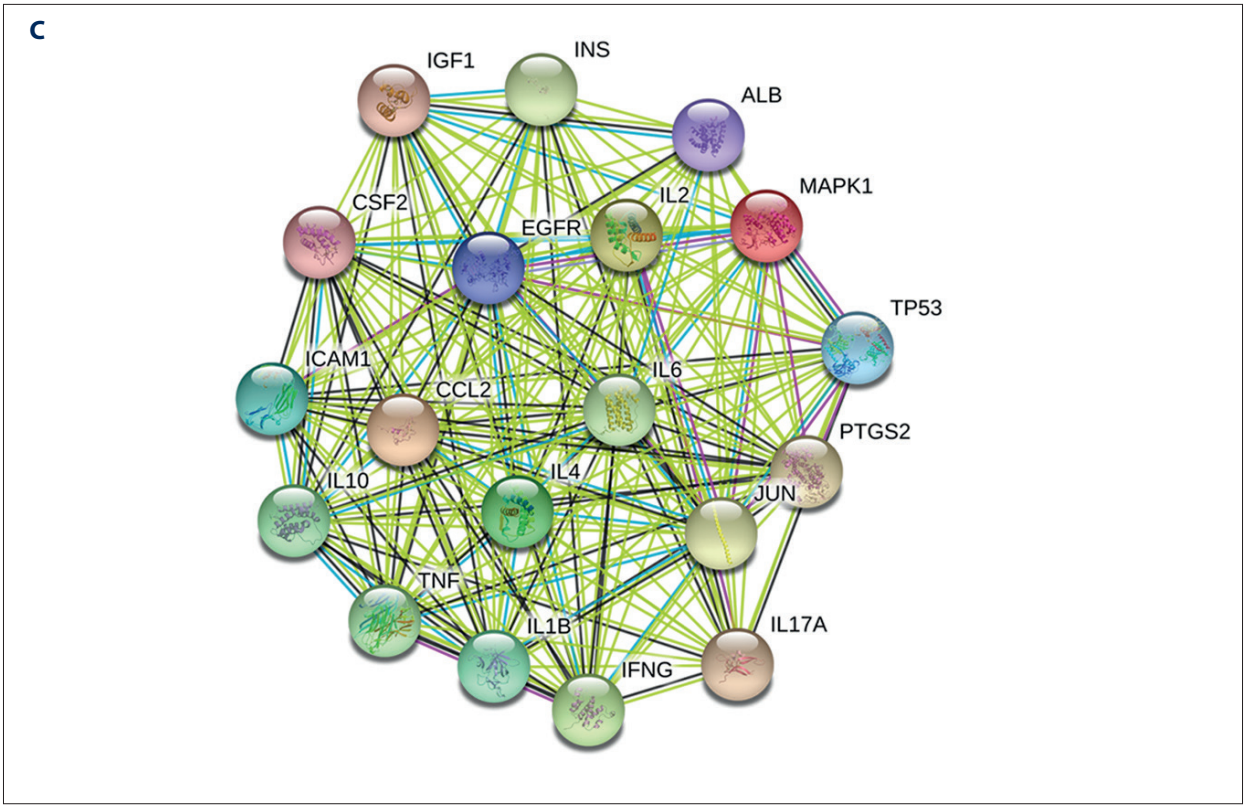

Figure 2. Identification of the core targets of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) in treating psoriasis. (**A**) The Venn diagram shows that MGCM shared 117 potential targets with known components of the pathological course related to psoriasis. (**B**) The protein–protein interaction (PPI) network of all 117 candidate targets of MGCM in treating psoriasis. (**C**) The PPI network of the core targets of MGCM in treating psoriasis.

which the degree values were greater than twice the median (=29) was identified as a pivotal hub that played a vital part within the PPI network. Consequently, 19 targets (**Table 2**) were selected according to the topological parameter values (**Figure 2C**), and they were selected as the MGCM core targets for the treatment of psoriasis.

Establishment of the core network of active ingredients-targets for MGCM in the treatment of psoriasis

To better understanding the "multiple target and multiple ingredient" mechanism of MGCM in the treatment of psoriasis, the candidate MGCM ingredients affecting 19 core targets were identified according to the association of ingredients with corresponding targets (**Table 3**). Thereafter, a core network was established regarding the active ingredients and targets (**Figure 3A**) by Cytoscape, and degree value of each node within the network was analyzed statistically. As shown in **Figure 3B**, the degree values of active ingredients within the core network were between 1 and 11, and the median was 2, which suggested that over half of the compounds had at least 2 targets. In addition, the degree value of targets was between 1 and 23 (**Figure 3C**), and the median was 9. The top 3 active ingredients with the highest degrees were ephedrine, pseudoephedrine, and coumarinic acid. The top 3 targets with the highest degrees were tumor necrosis factor (TNF), interleukin (IL)-10, and IL-1B, which all play vital roles in psoriasis pathogenesis and are involved in activities such as aberrant keratinocyte differentiation, inflammatory reactions, and immune cell infiltration [28–30].

MGCM core target enrichment analysis in the treatment of psoriasis

The multiple-target and multiple-pathway mechanism of MGCM in the treatment of psoriasis was further explored through performing GO-BP and KEGG enrichment analyses of the core targets using the OmicShare platform. In addition, MGCM-regulated BPs and related signal transduction pathways in psoriasis treatment were mined. The above-mentioned 19 core targets participated in some BPs, which mainly included the cell responses to multiple stimuli (such as nutrient substances and oxidative stress), immuno-inflammatory responses, leukocyte differentiation, cellular energy metabolism, angiogenesis, and programmed cell death (**Figure 4A**). In addition, the 5 most significant signaling pathways, namely, the JAK-STAT

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Table 2. Degree values of core targets for Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) against psoriasis.

Targets name	Degree	Targets name	Degree
ALB	84	JUN	66
TNF	91	IL4	69
IL6	93	IGF1	59
TP53	75	IL2	67
MAPK1	69	IFNG	67
INS	78	CCL2	69
IL10	82	ICAM1	64
IL1B	78	IL17A	63
EGFR	69	CSF2	61
PTGS2	71		

Table 3. Fifty-two core pharmacologically active ingredients of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) in the treatment of psoriasis.

Compound No.	Compound name	Compound No.	Compound name
GZ01	Procurcumenol	MH49	Camphor
GZ05	Anethole	MH52	7-Demethylsuberosin
GZ06	Protocatechuic Acid	MH54	N-Triacontanol
GZ07	Coumarinic Acid	MH55	Beta-Cyclocitral
GZ12	Farnesol	MH59	Myrcene
GZ14	Trans-Cinnamic Acid	MH60	Cibarian
MH01	Alpha-Linolenic Acid	MH61	Methyl-7-Epiganoderate
MH03	Ethanol	MH64	Menthyl Acetate
MH04	Carvacrol	MH67	Linolenic Acid
MH10	Gamma-Terpinene	MH68	Octanoic Acid
MH100	16-Triacontanol	MH70	3,4-Dimethyl-5-Phenyloxazolidine
MH103	Hexanoic Acid	MH71	Acetophenone
MH11	Pseudoephedrine	MH72	1-Phenyl-1,2-Propanedione
MH12	D-Norpseudoephedrine	MH73	Hexahydrofarnesylacetone
MH14	Lauric Acid	MH75	Pseudoginsenoside F11
MH15	Tetradecanoic Acid	MH78	Norephedrine
MH17	D-Pseudoephedrine	MH79	Maokonine
MH23	Methyl Benzoate	MH80	Nonanal
MH26	Ephedrine	MH82	1-Octanol
MH29	Decanoic Acid	MH83	Chuanxiongzine
MH31	Beta-Eudesmol	MH86	Beta-Ionone
MH40	Alpha-Pinene	MH87	Octadecanoic Acid
MH41	Terpinen-4-Ol	MH88	2,3,4-Trimethyl-5-Phenyloxazolidine
MH44	Beta-Pinene	MH90	Phenethylamine
MH45	6-Methyl-2-Heptanone	MH95	Phytol
MH47	Xylene	MH99	Hexanol

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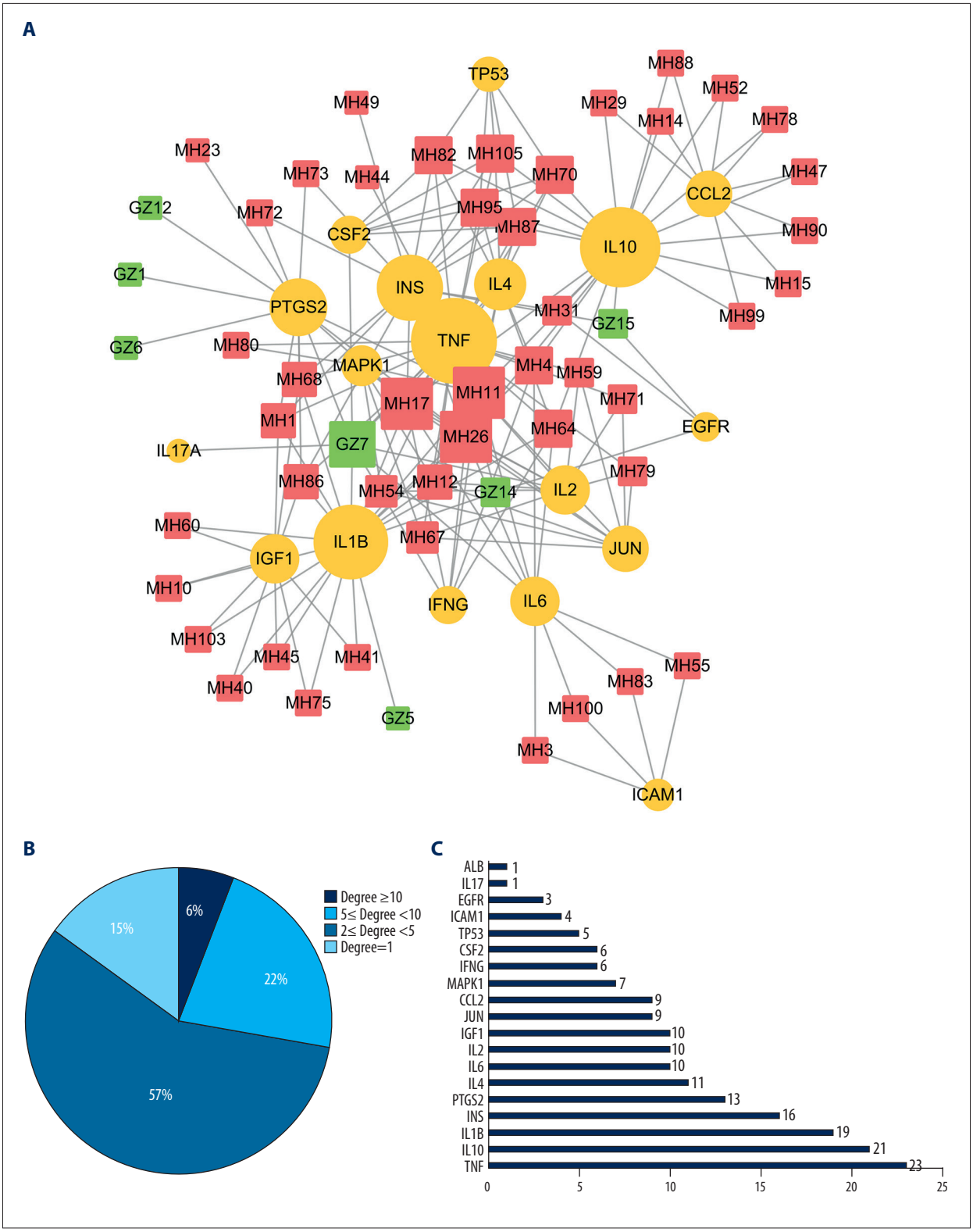

Figure 3. (**A**) Construction of the core network of active ingredients of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) and their targets in treating psoriasis, and the statistical analysis of the degree of each (**B**) ingredient and (**C**) target in the network. All nodes were sorted and calculated according to the degree of freedom, and the node size in the network was associated with the degree.

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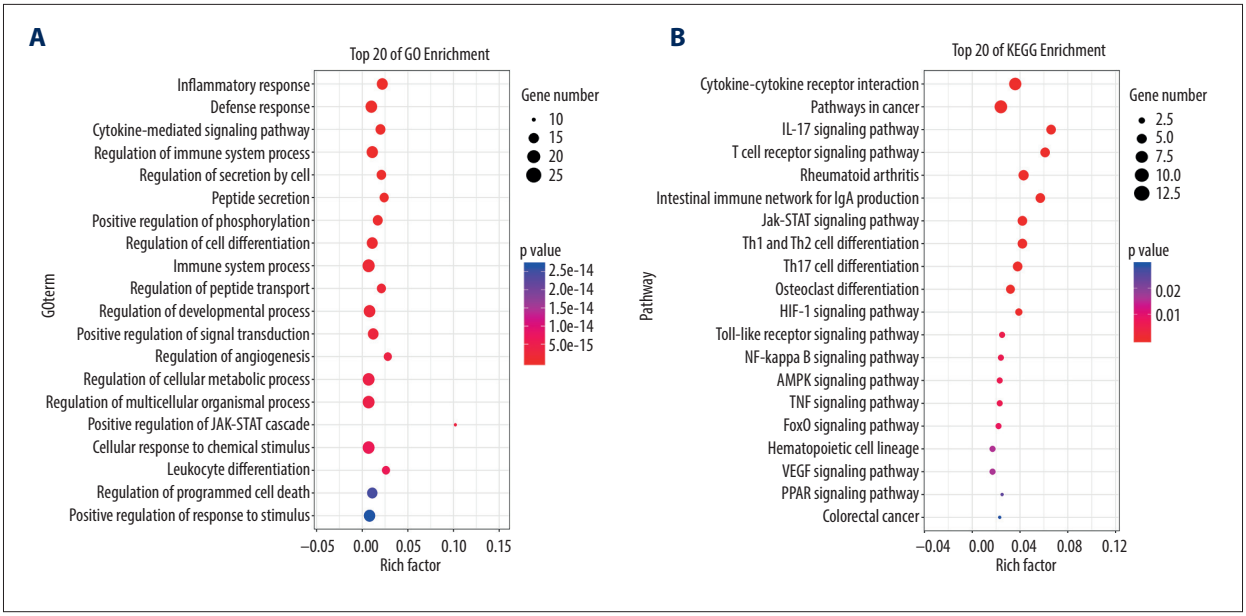

Figure 4. Enrichment analysis of core targets of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) in treating psoriasis based on OmicShare. We considered a *P* value cutoff of ≤0.05 as significant and applied hypergeometric tests to identify (**A**) enriched Gene Ontology biological processes (GO-BP) and (**B**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The chart shows an overview of the analysis with up to 20 significantly enriched processes and pathways.

pathway and pathways involving toll-like receptors (TLRs), nuclear factor (NF)-κB, vascular endothelial growth factor (VEGF), and peroxisome proliferator-activated receptor (PPAR), were selected based on the *P* values of the enriched pathways as well as the corresponding psoriasis correlations (**Figure 4B**).

Discussion

MGCM has been developed as an empirical prescription to treat psoriasis in the Department of Dermatology in our hospital. MGCM has achieved significant clinical efficacy in treating psoriasis; however, its core targets and active ingredients remain largely unknown, which has blocked the development and further clinical application of MGCM. Network pharmacology represents a novel drug design and development approach that is based on rapidly developing multidirectional pharmacology and systemic biology. Initially put forward by Hopkins [31] in 2007, this concept has led to a change from the established "disease, single target, single drug" model to the "disease, multiple targets, multiple drugs" model for the development of new drugs. This concept coincides with the TCM "holistic view" of patient care [32]. As a result, applying the network pharmacology approach provides some insight into the MGCM mechanism in the treatment of psoriasis.

The current study identified 116 candidate MGCM active ingredients for the treatment of psoriasis. The identifications were based on multiple network pharmacological approaches, and the active ingredients corresponded to 19 core targets. Psoriasis is currently associated with 4 well-recognized histopathological characteristics, including inflammatory infiltration in the epidermis and dermis, aberrant keratinocyte biological behaviors (apoptosis, hyperproliferation and differentiation), metabolic dysregulation, and the tortuously elevated formation of dermal capillaries and blood vessels [33–36]. First of all, a majority of the 19 core targets, including ILs, prostaglandin-endoperoxide synthase 2, TNF, C-C motif chemokine ligand 2, epidermal growth factor receptor, and interferon γ, were identified as participating in the aberrant inflammatory infiltration. These targets modulate lymphocyte chemotaxis and differentiation, generate cytokines, and control immunological inflammatory responses in the epidermis and dermis [29,37–39]. Second, MAPKs, TP53, and JUN exhibit abnormal keratinocyte biological behaviors in the context of psoriasis [40–42]. Third, abnormalities in insulin and albumin metabolism usually occur in patients with psoriasis [43,44]. Finally, intercellular adhesion molecule 1 is tightly correlated with the proliferation, adhesion, and migration of endothelial cells, which are correlated with the tortuously elevated dermal capillaries and blood vessels [45].

According to results of GO-BP and KEGG enrichment analyses of core targets, MGCM intervened with psoriasis via several BPs and some signal transduction pathways, including JAK-STAT, TLRs, NF-κB, VEGF, and PPAR. These 5 signal transduction pathways had cross-talk effects within this network. The VEGF signaling pathway is suggested to cause pathological

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angiogenesis within psoriatic lesions through modulating endothelial cell differentiation and proliferation. It induces inflammatory response through enhancing the vascular permeability, which promotes the infiltration of inflammatory cells [46]. Additionally, psoriasis represents a T-lymphocyte-mediated inflammatory disorder, in which aberrant differentiation of T lymphocytes (particularly Th1 and Th17 cells) and excessive secretion of pro-inflammatory factors (e.g., ILs) are closely correlated with disease progression [47–49]. Findings in the present study indicated that some key signal transduction pathways were correlated with the MGCM-mediated differentiation of T lymphocytes and the production of pro-inflammatory factors.

In this study, our network pharmacological analysis supports that the ephedrine alkaloids in MGCM (including ephedrine and pseudoephedrine) may be the core pharmacodynamic active compounds exerting the most critical effects against psoriasis. The ephedrine alkaloids have been verified in previous research to activate the α and β receptors, which can directly activate the adrenergic receptor in the body and indirectly promote the release of noradrenaline neurotransmitter to excite the sympathetic nerve, thus promoting perspiration and dispelling the internal pathogenic evils. In GZ, the cinnamic acid and cinnamaldehyde can dilate blood vessels, promote blood circulation, accelerate blood flow to the body surface, and reinforce the perspiration caused by MH. Both MH and GZ represent drugs for inducing sweat. The combined application of these 2 drugs facilitates expulsion of the internal pathogenic evils, thus producing a therapeutic effect [11]. Moreover, our previous research suggests that ephedrine and pseudoephedrine can suppress the β-adrenergic receptor on the keratinocyte membrane surface, induce the intracellular cAMP level, and regulate cell proliferation. In addition, previous research also indicated that ephedrine and pseudoephedrine can regulate the immune inflammatory response in the body and suppress the release of inflammatory factors at lesion sites [12,13].

According to our previous study, using MGCM to treat psoriasis is safe and effective based on clinical observations. Findings in the present work revealed that MGCM exerts a non-unilateral regulatory effect on the treatment of psoriasis; instead, it has indirect or direct effects on the integrated treatment of those 4 main pathological parameters via several signal transduction pathways related to metabolism, immune and inflammatory responses, and aberrant angiogenesis. Nonetheless, certain limitations should be noted despite the significant findings. First of all, several compounds in MGCM herbal medicines were not taken into account due to insufficient laboratory results or available data. Second, we have already treated the quality control components (such as ephedrine, pseudoephedrine, and cinnamaldehyde) in MH and GZ in the current standard from Chinese pharmacopoeia or the components with relatively high contents as the candidate pharmacodynamic compounds for research. Meanwhile, these components have also been predicted and screened as the core active ingredients in MGCM against psoriasis in this study. However, this study may treat all components equally to some extent and thus ignore the influence of the absolute content of each compound in MGCM and the serum and skin tissue distribution concentrations. Third, this study only predicts the drug-target interactions through network pharmacological means; it does not illustrate the type of effect on targets (e.g., activation or suppression, upregulation or downregulation). As a result, in future research, we aim to extensively examine (1) the enrichment degrees and contents of screened core active ingredients in the blood or skin tissues of experimental animals or patients through UPLC-MS to further confirm the core active ingredients in MGCM against psoriasis; (2) the regulatory effect of MGCM and the core active ingredients on the screened core targets and signaling pathways in patients, animal models, and *in vitro* experiments using molecular biological technology; and (3) the upstream and downstream mechanisms of MGCM in the regulation of the screened targets and signaling pathways.

Conclusions

In this work, we successfully systematically illuminated the possible "multiple compounds, multiple targets" therapeutic action of MGCM on psoriasis, and predicted, screened, and analyzed the genes, proteins, and pathways that might play a vital role in the biological process. However, because this study was based on data mining and data analysis, further studies should be undertaken to validate the findings.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Conflicts of interest

None.

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Supplementary Data

Supplementary Table 1. Known psoriasis-related targets.

Supplementary Table 2. All the potential targets of Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM).

Supplementary Table 3. Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) shared 117 potential targets with known psoriasis-related targets.

Supplementary Table 4. Degree values of candidate targets for Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) against psoriasis.

Supplementary Tables available from the corresponding author on request.

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