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

Shun Guo*  
Jin-Yong Zhou*  
Cheng Tan  
Le Shi  
Yue Shi  
Jianxin Shi

* Shun Guo and Jin-yong Zhou contributed equally to the study

Corresponding Author: Shun Guo, e-mail: 260873@njucm.edu.cn

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

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/927421
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/](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](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/](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...
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-Acetylphotoracicin I | ID: 3697 from TCMID database     |
| GZ05         | Anethole                    | ID: 1186 from TCMID database        |
| GZ06         | Procatechuic 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: 3048 from TCMID database        |
| GZ11         | Mellilotocarpan A           | ID: 13672 from TCMID database       |
| GZ12         | Farnesol                    | ID: 7733 from TCMID database        |
| GZ13         | Nerolidol                   | ID: 21621 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-OI        | 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         | 1,4-Cineole                 | ID: 23233 from TCMID database       |
| MH16         | O-Xylene                    | ID: 18010 from TCMID database       |
| MH17         | D-Pseudoephedrine           | ID: 1476 from TCMID database        |
| MH18         | Apigenin                    | ID: 24578 from TCMID database       |
| MH19         | Methyl Acetate              | ID: 9037 from TCMID database        |
| MH20         | Gualazulene                 | ID: 19105 from TCMID database       |
| MH21         | Safranal                    | ID: 36889 from TCMID database       |
| MH22         | 1,8-Cineole                 | ID: 24934 from TCMID database       |
| MH23         | Methyl Benzoate             | ID: 23119 from TCMID database       |
| MH24         | Alpha-Terpinol              | ID: 3688 from TCMID database        |
| MH25         | 1,4-Cineole                 | ID: 3688 from TCMID database        |
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        | Dipropyl Phthalate         | ID: 5403 from TCMID database         |
| MH38        | M-Argentin 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-Dimethylsuberosin        | ID: 5097 from TCMID database         |
| MH53        | Methylpseudoephedrine      | ID: 24866 from TCMID database        |
| MH54        | N-Triacontanol             | ID: 21525 from TCMID database        |
| MH55        | Beta-Cyclocitrall          | 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        | Methyl Acetate             | ID: 13772 from TCMID database        |
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-Phenylloxazolidine | ID: 6395 from TCMID database |
| MH71 | Acetophenone | ID: 115 from TCMID database |
| MH72 | 1-Phenyl-1,2-Propanedione | ID: 24685 from TCMID database |
| MH73 | Hexahydropfarnesylacetone | 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 | Norphedrine | 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 | Tetramethylpyrazine | ID: 23142 from TCMID database |
| MH84 | Linoleic Acid | ID: 24136 from TCMID database |
| MH85 | Beta-Ionone | ID: 23950 from TCMID database |
| MH86 | Octadecanoic Acid | ID: 23678 from TCMID database |
| MH87 | 2,3,4-Trimethyl-5-Phenylloxazolidine | ID: 21957 from TCMID database |
| MH88 | Pentadecanoic Acid | ID: 23379 from TCMID database |
| MH89 | Phenethylamine | ID: 23379 from TCMID database |
| MH90 | Trans-2-Nonenal | ID: 23241 from TCMID database |
| MH91 | Isobutyl Benzoate | ID: 24655 from TCMID database |
| MH92 | Leucodelphinidin | ID: 12711 from TCMID database |
| MH93 | Nonanoic Acid | ID: 24480 from TCMID database |
| MH94 | Myricadiol | ID: 17251 from TCMID database |
| MH95 | Linolenic Acid | ID: 17251 from TCMID database |
| MH96 | Leucodaphenidin | ID: 12712 from TCMID database |
| MH97 | Palmitic Acid | ID: 17251 from TCMID database |
| MH98 | Myristic Acid | ID: 12712 from TCMID database |
| MH99 | Myristoleic Acid | ID: 12712 from TCMID database |
| MH100 | Hexanoic Acid | ID: 24480 from TCMID database |
| MH101 | Myristoleic Acid | ID: 17251 from TCMID database |
| MH102 | Sabinene | ID: 15736 from TCMID database |
| MH103 | Hexanoic Acid | ID: 12712 from TCMID database |
| MH104 | Piperitone | ID: 17442 from TCMID database |
>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 anter- 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...
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
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, pseudoe Ephedrine, 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
### Table 2. Degree values of core targets for Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) against psoriasis.

| Targets name | Degree |
|--------------|--------|
| ALB          | 84     |
| TNF          | 91     |
| IL6          | 93     |
| TPS3         | 75     |
| MAPK1        | 69     |
| INS          | 78     |
| IL10         | 82     |
| EGFR         | 69     |
| 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         | Procurcumol           | MH49         | Camphor               |
| GZ05         | Anethole              | MH52         | 7-Demethylsuberosin    |
| GZ06         | Protocatechuic Acid   | MH54         | N-Triacontanol        |
| GZ07         | Coumarinic Acid       | MH55         | Beta-Cyclocitrinal    |
| 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         | Acetophenone          |
| MH10         | Gamma-Terpinene       | MH68         | Octanoic Acid         |
| MH100        | 16-Triacontanol       | MH70         | 3,4-Dimethyl-5-Phenylxazolidine |
| MH102        | Hexanoic Acid         | MH71         | Acetophenone          |
| MH11         | Pseudoephedrine       | MH72         | 1-Phenyl-1,2-Propanedione |
| MH12         | D-Norpseudoephedrine  | MH73         | Hexahydrofarnesylacetone |
| MH14         | Lauric Acid           | MH74         | Pseudoginsenoside F11 |
| MH15         | Decanoic Acid         | MH75         | Pseudoginsenoside F11 |
| MH17         | D-Pseudoephedrine     | MH78         | Norephedrine          |
| MH23         | Methyl Benzoate       | MH79         | Maokonine             |
| MH26         | Ephedrine             | MH80         | Nonanal               |
| MH29         | Decanoic Acid         | MH82         | 1-Octanol             |
| MH31         | Beta-Eudesmol         | MH83         | Chuanxiangzine        |
| MH40         | Alpha-Pinene          | MH86         | Beta-Ionone           |
| MH41         | Terpin-4-Oi           | MH87         | Octadecanoic Acid     |
| MH44         | Beta-Pinene           | MH88         | 3,4-Trimethyl-5-Phenylxazolidine |
| MH45         | 6-Methyl-2-Heptanone  | MH90         | Phenethylamine        |
| MH47         | Xylene                | MH95         | Phytol                |
|              |                       | MH99         | Hexanol               |
**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.
were based on multiple network pharmacological approaches, ingredients for the treatment of psoriasis. The identifications "holistic view" of patient care [32]. As a result, applying the ease, multiple targets, multiple drugs" model for the develop-lished "disease, single target, single drug" model to the "dis-

[31] in 2007, this concept has led to a change from the estab-
cology and systemic biology. Initially put forward by Hopkins 
that is based on rapidly developing multidirectional pharma-
gogy represents a novel drug design and development approach 
and further clinical application of MGCM. Network pharma-
remain largely unknown, which has blocked the development 
treating psoriasis; however, its core targets and active ingredients 
pital. MGCM has achieved significant clinical efficacy in treat-
treat psoriasis in the Department of Dermatology in our hos-

Discussion

MGCM has been developed as an empirical prescription to treat psoriasis in the Department of Dermatology in our hos-
pital. MGCM has achieved significant clinical efficacy in treat-
ing 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 "dis-
eease, multiple targets, multiple drugs" model for the develop-
ment 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 ingredi-
ets 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 histo-pathological 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 infiltra-
tion. These targets modulate lymphocyte chemotaxis and differ-
entiation, generate cytokines, and control immunological inflam-matory responses in the epidermis and dermis [29,37–39]. Second, MAPKs, TP53, and JUN exhibit abnormal kerati-
nocyte biological behaviors in the context of psoriasis [40–42]. Third, abnormalities in insulin and albumin metabolism usu-
ally occur in patients with psoriasis [43,44]. Finally, intercel-
lar adhesion molecule 1 is tightly correlated with the pro-
iferation, 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 IAK-
STAT, TLRS, NF-κB, VEGF, and PPAR. These 5 signal transduc-
tion pathways had cross-talk effects within this network. The VEGF signaling pathway is suggested to cause pathological
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.

**Acknowledgments**

The authors thank members of their laboratory and their collaborators for their research work.

**Conflicts of interest**

None.
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 of candidate targets for Ephedrae Herba-Cinnamomi Ramulus couplet medicines (MGCM) against psoriasis.

References:

1. Miceli G, Verzi AE, Gullifrida G et al: Inverse psoriasis: From diagnosis to current treatment options. Clin Cosmet Investig Dermatol, 2019; 12: 953–59
2. Luo Y, Yu Y, Sun X et al: Characteristics of psoriasis vulgaris in China: A prospective cohort study protocol. Ann Transl Med, 2019; 7: 694
3. Li J, Yu M, Wang Y et al: Prevalence of psoriasis and associated risk factors in China: a protocol of a nationwide, population-based, cross-sectional study. BMJ Open, 2019; 9: e027685
4. Meng S, Lin Z, Wang Y et al: Psoriasis therapy by Chinese medicine and modern agents. Chin Med, 2018; 13: 16
5. Chang CC, Cheng WJ, Lin CY et al: Kan-Lu-Hsiao-Tu-Tan, a traditional Chinese medicine formula, inhibits human neutrophil activation and ameliorates imiquimod-induced psoriasis-like skin inflammation. J Ethnopharmacol, 2020; 246: 112246
6. Coyle ME, Yu JJ, Zhang AL et al: Patient experiences of using Chinese herbal medicine for psoriasis vulgaris and chronic urticaria: a qualitative study. J Dermatol Treat, 2020; 31(4): 352–58
7. Lin W, Yu Q, Qin Y et al: To explore the clinical efficacy of Traditional Chinese Medicine bath in the treatment of psoriasis vulgaris with blood-heat syndrome and its effect on related cytokines based on different temperature and different concentration. Medicine (Baltimore), 2020; 99: e20172
8. Xu P: [Treating psoriasis of the Xuere syndrome of the ‘winter severe-summer mild’ type by the Han theory.] Clin J Chin Med, 2019; 11: 112–14 [in Chinese]
9. Li Z, Li J, Guo M et al: [Clinical observation of Mahuang Zimei decoction on treating psoriasis.] Chin Arch Trad Chin Med, 2019; 31: 51–52 [in Chinese]
10. Cui L, Liu A: [Experience of professor LIU Ai-min in treatment for ‘the yang deficiency combined with exogenous cold’ type of psoriasis by Mahuang Fuzi Xiong Decoction.] Chin Arch Trad Chin Med, 2019; 25: 93–96 [in Chinese]
11. Zou Y, Guo S, Jiang Y et al: [Effect of Ephedrae Herba-Cinnamomi Ramulus herb pair on proliferation and apoptosis of interleukin-22-mediated HaCaT cells.] Pract Med, 2016; 32: 3986–89 [in Chinese]
12. Guo S, Zou X, Chen J et al: [Effect of [2-AR/CAMP pathway by “drug pair” of ephedra cassis twig on IL-22 induced HaCaT cells.] Lishizhen Medicine and Materia Medica Research, 2019; 11: 2600–2 [in Chinese]
13. Jiang Y, Guo S, Zou X et al: [Effect of ephedrine on CCL20 secretion of IL-17-induced immortalized human keratinocytes (HaCaT).] Shandong Med J, 2017; 10: 24–27 [in Chinese]
14. Liu Z, Guo F, Wang Y et al: BATMAN-TMCA: a Bioinformatics Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine. Sci Rep, 2016; 6: 21146
15. Zhang R, Zhu X, Bai H, Ning K: Network pharmacology databases for traditional Chinese medicine: Review and assessment. Front Pharmacol, 2019; 10: 123
16. Zhang G, Jiang X, Liu Y et al: Therapeutic efficiency of an external Chinese herbal formula of mammary precancerous lesions by BATMAN-TMCA online bioinformatics analysis tool and experimental validation. Evid Based Complement Alternat Med, 2019; 2019: 2795010
17. Ru J, Li P, Wang J et al: TCMSp: A database of systems pharmacology for drug discovery from herbal medicines. J Cheminform, 2014; 6: 13
18. Piñeiro J, Ramirez-Anguita JM, Saúch-Pitarch J et al: The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res, 2020; 48: D845–55
19. Rappaport N, Twik M, Plaschkes I et al: MalaCards: An amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. Nucleic Acids Res, 2017; 45: D877–87
20. Wishart DS, Feunang YD, Guo AC et al: DrugBank 5.0: A major update to the DrugBank database for 2017. Nucleic Acids Res, 2018; 46: D1074–82
21. Wang Y, Zhang S, Li F et al: Therapeutic Target Database 2020: Enriched resource for facilitating research and early development of targeted therapeutics. Nucleic Acids Res, 2020; 48: D1031–41
22. The UniProt Consortium: UniProt: The universal protein knowledgebase. Nucleic Acids Res, 2017; 45: D158–69
23. Szlarczyk D, Morris JH, Cook H et al: The STRING database in 2017: Quality-controlled protein–protein association networks, made broadly accessible. Nucleic Acids Res, 2017; 45: D362–68
24. Chin CH, Chen SH, Wu HH et al: cytoHubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol, 2014; 8(Suppl. 4): S11
25. Su M, Guo C, Liu M, Liang X, Yang B: Therapeutic targets of vitamin C on liver injury and associated biological mechanisms. A study of network pharmacology. Int Immunopharmacol, 2019; 66: 383–87
26. Pontiki E, Hadjipavlou-Litina D: Multi-target cinnamic acids for oxidative stress and inflammation: Design, synthesis, biological evaluation and modeling studies. Molecules, 2018; 24(1): 12
27. Zheng Y, Guo Z, He W et al: Ephedrine hydrochloride protects mice from LPS challenge by promoting IL-10 secretion and inhibiting proinflammatory cytokines. Int Immunopharmacol, 2012; 12: 46–53
28. Furue K, Ito T, Tsuji G et al: Psoriasis and the TNF/NFκB axis. G Ital Dermatol Venereol, 2019; 154: 418–24
29. Chimia M, Lebwohl M: TNF inhibitors for psoriasis. Semin Cutan Med Surg, 2018; 37: 134–42
30. Szabó K, Tata-Csörgő Z, Dallos A et al: Regulatory networks contributing to psoriasis susceptibility. Acta Derm Venereol, 2014; 94: 380–85
31. Hopkins AL: Network pharmacology. Nat Biotechnol, 2007; 25: 1110–11
32. Zhou Z, Chen B, Chen S et al: Applications of network pharmacology in traditional Chinese medicine research. Evid Based Complement Alternat Med, 2020; 2020: 1646905
33. NI X, Lai Y: Keratinocyte: A trigger or an executor of psoriasis? J Lab Clin Med, 2020; 108(2): 485–91
34. Ogawa E, Sato Y, Minagawa A, Okuyama R: Pathogenesis of psoriasis and development of treatment. J Dermatol, 2018; 45: 264–72
35. Sankar L, Arumugam D, Boj S, Pradeep P: Expression of angiogenic factors in psoriasis vulgaris. J Clin Diagn Res, 2017; 11: EC23–27
36. Hiebert P, Wiemer S: Targeting metabolism to treat psoriasis. Nat Med, 2018; 24: 537–39
37. Domala A, Bale S, Godugu C: Protective effects of nanoceria in imiquimod-induced psoriasis by inhibiting the inflammatory responses. Nanomedicine (Lond), 2020; 15: 5–22
38. Grán F, Kerstan A, Serfling E et al: Current developments in the immunology of psoriasis. Yale J Biol Med, 2020; 93: 97–110
39. Behfar S, Hassanshahi G, Nasari A, Khorraramdelazad H: A brief look at the role of monocyte chemoattractant protein-1 (CCL2) in the pathophysiology of psoriasis. Cytokine, 2018; 110: 226–31
40. Zenz R, Wagner EF: Jun signalling in the epidermis: From developmental defects to psoriasis and skin tumors. Int J Biochem Cell Biol, 2006; 38: 1043–49
41. Haase I, Hobbs RM, Romero MR et al: A role for mitogen-activated protein kinase activation by integrins in the pathogenesis of psoriasis. J Clin Invest, 2001; 108: 527–36
42. Raho G, Vena GA, Bizzoca A et al: Influence of infliximab on keratinocyte apoptosis in psoriasis patients. Immunopharmacol Immunotoxicol, 2011; 33: 227–31
43. Işik S, Kılıç S, Öğretmen Z et al: The correlation between the psoriasis area severity index and ischemia-modified albumin, mean platelet volume levels in patients with psoriasis. Postepy Dermatol Allergol, 2016; 33: 290–93
44. Polic MV, Miskulin M, Smolic M et al: Psoriasis severity – a risk factor of insulin resistance independent of metabolic syndrome. Int J Environ Res Public Health, 2018; 15(7): 1486
45. Wen J, Pei H, Wang X et al: Gambogenic acid exhibits anti-psoriatic efficacy through inhibition of angiogenesis and inflammation. J Dermatol Sci, 2014; 74: 242–50
46. Marina ME, Roman II, Constantin A-M et al: VEGF involvement in psoriasis. Clujul Med, 2015; 88: 247–52
47. Picciani BLS, Domingos TA, Teixeira-Souza T et al: Evaluation of the Th17 pathway in psoriasis and geographic tongue. An Bras Dermatol, 2019; 94: 677–83
48. Shi Y, Chen Z, Zhao Z et al: IL-21 induces an imbalance of Th17/Treg cells in moderate-to-severe plaque psoriasis patients. Front Immunol, 2019; 10: 1865
49. Furiati SC, Catarino JS, Silva MV et al: Th1, Th17, and Treg responses are differently modulated by TNF-alpha inhibitors and methotrexate in psoriasis patients. Sci Rep, 2019; 9: 7526