DOI: 10.58490/ctjump.2026i11TA.5117

NETWORK PHARMACOLOGY ANALYSIS OF THE ANTI-INFLAMMATORY MECHANISMS OF WENQING BITONG DECOCTION IN RHEUMATOID ARTHRITIS

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Received: 23/5/2026
Reviewed: 02/6/2026
Accepted: 25/6/2026

ABSTRACT

Background: *Rheumatoid arthritis is a chronic autoimmune disease involving complex inflammatory networks and multiple pathogenic pathways. Although Wenqing Bitong Decoction has been used clinically for rheumatoid arthritis, its underlying anti-inflammatory mechanisms have not been systematically elucidated.* ***Objectives:*** *To systematically explore the anti-inflammatory mechanisms of Wenqing Bitong Decoction in rheumatoid arthritis using a network pharmacology approach.* ***Materials and methods:*** *Active compounds of the 14 Wenqing Bitong Decoction herbs were retrieved from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and HERB. TCMSP compounds were screened using OB ≥ 30% and DL ≥ 0.18; HERB compounds used a probability cutoff > 0.1 (SwissTargetPrediction, species Homo sapiens). Duplicates were removed. Rheumatoid arthritis targets from GeneCards (three-time median filtering, cutoff ≥ 5.71) and OMIM were intersected with drug targets. PPI was analyzed via STRING (Homo sapiens, confidence ≥ 0.7). GO/KEGG enrichment used adjusted $p < 0.05$.* ***Results:*** *A total of 183 bioactive compounds, 656 putative targets, and 867 rheumatoid arthritis-related targets were identified, yielding 93 overlapping targets. Network analysis indicated that key active compounds included quercetin, luteolin, kaempferol, wogonin, baicalein, and naringenin. Core targets such as IL6, IL1B, TNF, STAT3, and AKT1 were identified as central nodes in the PPI network. KEGG enrichment analysis revealed that these targets were mainly involved in TNF, IL-17, NF-κB, MAPK, PI3K-Akt, and JAK-STAT signaling pathways, which are closely associated with inflammatory response regulation and immune modulation.* ***Conclusion:*** *Wenqing Bitong Decoction may exert anti-inflammatory effects in rheumatoid arthritis through multi-component, multi-target, and multi-pathway mechanisms, potentially involving cytokine signaling modulation and key inflammatory pathways (NF-κB, MAPK, PI3K-Akt, JAK-STAT). These findings are hypothesis-generating and require experimental and clinical validation, as no validation was performed here.*

Keywords: *Wenqing Bitong Decoction, rheumatoid arthritis, network pharmacology, anti-inflammatory.*

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease driven by cytokines such as TNF-α, IL-1β, IL-6, and IL-17, which activate NF-κB, MAPK, PI3K-Akt, and JAK-STAT pathways, leading to synovial inflammation and joint destruction [1], [2]. Current therapies include DMARDs, biologics, and targeted synthetic drugs. However, their long-term use is limited by adverse effects, high costs, immunosuppression, and incomplete

responses [3]. Thus, identifying approaches that regulate multiple inflammatory targets and pathways remains an important direction in RA research.

From the perspective of traditional Chinese medicine (TCM), RA belongs to the category of "Bi syndrome" [4], [5]. Professor Wang Yue (汪悦), a nationally recognized expert in TCM rheumatology, summarized Wenqing Bitong Decoction (WQBT) based on long-term clinical experience in treating RA with mixed cold-heat syndrome [6]. The prescription consists of Guizhi (GZ, cortex cinnamomi), Fangfeng (FF, saposhnikoviae radix), Qinjiao (QJ, gentianae macrophyllae radix), Tufuling (TFL, smilacis glabrae rhizoma), Fangji (FJ, stephaniae tetrandrae radix), Qingfengteng (QFT, sinomenii caulis), Quanxie (QX, Buthus martensii), Huangqin (HQ, scutellariae radix), Cangzhu (CZ, atractylodis rhizoma), Shengdihuang (SD, rehmanniae radix), Jinyinhua (JYH, lonicerae japonicae flos), Pianjianghuang (PJH, wenyujin rhizoma concisum), Chuanniuxi (CNX, cyathulae radix), and Gancao (GC, glycyrrhizae radix). Clinically, the formula has been used to alleviate joint pain, swelling, and recurrent inflammatory manifestations, suggesting potential therapeutic value in chronic inflammatory regulation [6].

Previous studies have shown anti-rheumatic or anti-inflammatory activities for some individual herbs in WQBT, including Fangfeng, Guizhi, Qinjiao, and Tufuling [7], [8], [9], [10]. However, no study has investigated the integrated formula as a whole. We therefore hypothesize that WQBT may exert coordinated anti-inflammatory effects in RA through its multi-component and multi-target network. Network pharmacology is a systems-level approach suitable for exploring such multi-compound mechanisms [11]. Thus, this study adopted network pharmacology to systematically explore this hypothesis and provide a theoretical basis for potential clinical application.

II. MATERIALS AND METHODS

2.1. Materials

WQBT was subjected to network pharmacology analysis to elucidate its potential mechanisms in RA. The prescription consists of 14 herbs, including Guizhi, Fangfeng, Qinjiao, Tufuling, Fangji, Qingfengteng, Quanxie, Huangqin, Cangzhu, Shengdihuang, Jinyinhua, Pianjianghuang, Chuanniuxi, and Gancao.

2.2. Research methods

Active compounds from TCMSP herbs were screened using OB ≥ 30% and DL ≥ 0.18 (https://www.91tcmsp.com/, accessed January 2025). For herbs not available in TCMSP (Quanxie, Shengdihuang, Pianjianghuang), compounds were retrieved from the HERB database (http://herb.ac.cn/, accessed January 2025), and targets were predicted using SwissTargetPrediction (http://www.swisstargetprediction.ch/index.php, species: *Homo sapiens*, probability > 0.1). Compounds without valid SMILES were manually verified or excluded. All targets were standardized using UniProt (https://www.uniprot.org/), restricted to reviewed *Homo sapiens* entries. Each compound was assigned a unique identifier based on its herb of origin (e.g., FF1, FF2 for Fangfeng; TFL1, TFL2 for Tufuling).

RA-related targets were collected from GeneCards and OMIM using "rheumatoid arthritis" as the keyword (https://www.genecards.org/ and http://www.omim.org/, accessed January 2025, species: *Homo sapiens*). GeneCards targets were filtered using a three-time median filtering strategy (final cutoff: relevance score ≥ 5.71). After merging with OMIM targets and removing duplicates, the drug-disease intersection was identified using a Venn

diagram. A "herb-compound-target" network was constructed using Cytoscape 3.8.2, and degree centrality was calculated.

Protein-protein interaction (PPI) analysis was conducted using the STRING database (https://cn.string-db.org/) with the organism set to *Homo sapiens* and a confidence score threshold ≥ 0.7. Disconnected nodes were removed, and the network was visualized in Cytoscape. Key hub genes were screened based on network topology. Functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), were performed using Metascape (https://metascape.org/). A threshold of adjusted $p < 0.05$ was considered statistically significant.

III. RESULTS

3.1. Screening of active compounds and target prediction

A total of 213 chemical constituents were initially identified from the 14 herbs of WQBT, including GZ (6), FF (18), FJ (2), QJ (2), QFT (6), CNX (4), TFL (14), JYH (17), HQ (32), CZ (4), GC (88), PJH (3), QX (3), and SD (14).

After removing duplicate entries, 183 unique active compounds were retained, including 13 shared compounds across multiple herbs (Table 1).

Table 1. Shared compounds among multiple herbs in WQBT

Compound	Herbs	Mark	Compound	Herbs	Mark
beta-sitosterol	GZ, FF, FJ, QJ, QFT, CNX, JYH, HQ, TFL, SD	A	wogonin	FF, HQ, CZ	F
sitosterol	GZ, FF, QJ, HQ, TFL, GC	B	Stigmasterol	JYH, HQ, TFL	G
ent-Epicatechin	GZ, HQ	C	quercetin	CNX, TFL, JYH, GC	H
(-)-taxifolin	GZ, TFL	D1	Eriodyctiol (flavanone)	JYH, HQ	I
taxifolin	GZ, TFL	D2	naringenin	TFL, GC, PJH	J
Mandenol	FF, JYH	E	kaempferol	JYH, GC	K

Target prediction and normalization from active compounds yielded 656 putative drug targets (pre-intersection), forming the basis for subsequent network analysis.

3.2. Identification of RA-related targets and intersection analysis

A total of 6704 rheumatoid arthritis-related genes were retrieved from GeneCards. After filtering (final cutoff: relevance score ≥ 5.71), 834 genes were retained. Additionally, 48 targets were obtained from OMIM. After integration and deduplication, a total of 867 disease-related targets were identified.

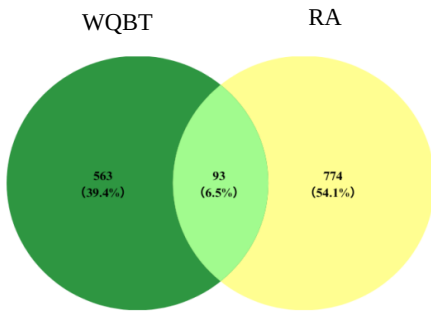

Figure 1. Venn diagram of overlapping targets between WQBT and RA-related genes

Intersection analysis between drug targets and disease targets identified 93 overlapping genes (93/1440 = 6.5% of total targets), as visualized in the Venn diagram (Figure 1), leaving 563 WQBT-only and 784 RA-only targets.

3.3. Construction of herb-compound-target network

The herb-compound-target interaction network consisted of 853 nodes (14 herbs, 183 compounds, and 656 targets) and 3438 edges, demonstrating a complex multi-component and multi-target interaction pattern (Figure 2A). To further identify the core active constituents associated with rheumatoid arthritis, the 93 intersecting targets between WQBT and RA were imported into Cytoscape to construct a compound-target interaction network (Figure 2B).

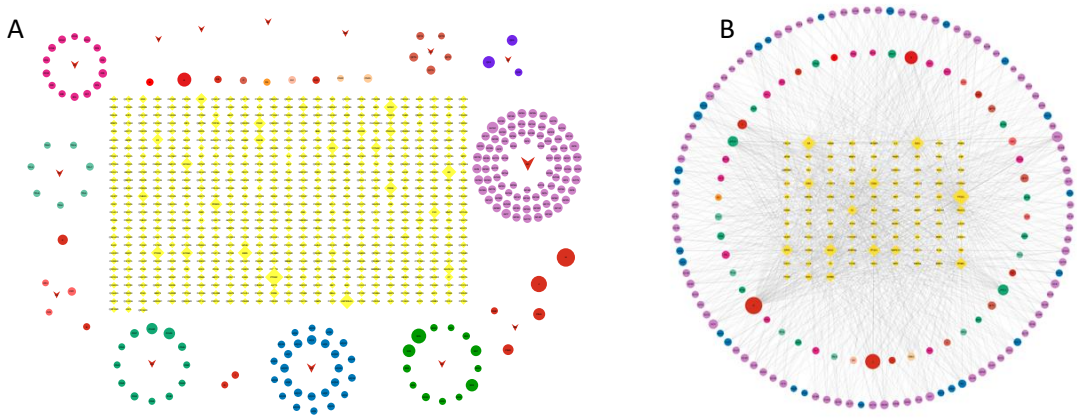

Figure 2. Network pharmacology analysis of Wenqing Bitong Decoction

(A) Herb-compound-target network. (B) Compound-target network based on RA-intersecting targets. Targets: yellow diamonds (center). Compounds: circles (periphery), colors indicate source herbs. Node size = degree centrality (larger = more interactions). Gray edges = compound-target interactions.

Table 2. Top 10 active compounds of WQBT for RA based on network topology analysis

No	Symbol	Compound name	Degree	Betweenness	Closeness
1	H	quercetin	66	0.257	0.529
2	JYH12	luteolin	33	0.078	0.462
3	K	kaempferol	28	0.077	0.462
4	F	wogonin	23	0.034	0.447
5	HQ1	acacetin	16	0.019	0.434
6	J	naringenin	16	0.032	0.431
7	HQ3	baicalein	15	0.013	0.43
8	GC7	isorhamnetin	15	0.004	0.431
9	GC64	licochalcone a	15	0.014	0.434
10	GC9	7-Methoxy-2-methyl isoflavone	14	0.003	0.431

Topological analysis of the network identified hub compounds based on degree centrality (top 10), with betweenness and closeness centrality also reported for additional characterization (Table 2).

3.4. Protein-Protein Interaction (PPI) network analysis

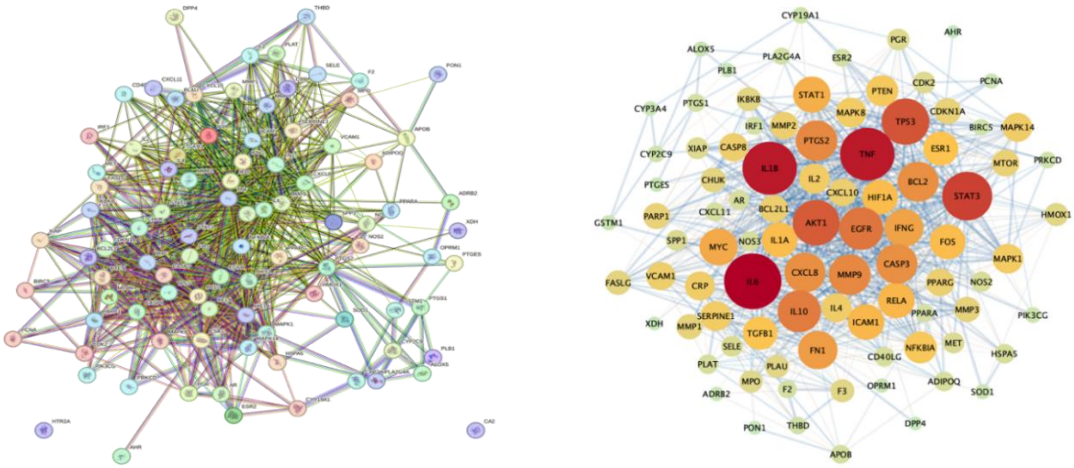

Figure 3. Protein-protein interaction network of 93 intersecting targets

(A) PPI network generated using STRING database. (B) Visualization of PPI network in Cytoscape. Nodes represent proteins. Node size and color intensity increase with degree centrality, indicating greater functional importance in the network. Edges represent protein-protein interactions.

The PPI network constructed from 93 intersecting targets contained 93 nodes and 889 edges. Visualization of the network is shown in Figure 3A (STRING output) and Figure 3B (Cytoscape visualization). The top 20 hub genes identified by degree centrality are listed in Table 3.

Table 3. Top 20 hub genes from PPI network based on topological analysis

No	Name	Degree	Betweenness	Closeness
1	IL6	60	0.0945	0.7438
2	IL1B	56	0.0687	0.7143
3	TNF	56	0.0754	0.7087
4	STAT3	50	0.0454	0.6716
5	TP53	47	0.0475	0.6522
6	AKT1	46	0.0479	0.6569
7	EGFR	42	0.0288	0.6383
8	IL10	41	0.0185	0.6338
9	PTGS2	39	0.1026	0.6250
10	MMP9	39	0.0216	0.6338
11	CXCL8	38	0.0350	0.6250
12	CASP3	38	0.0187	0.6081
13	BCL2	38	0.0199	0.6122
14	FN1	36	0.0447	0.6000
15	IFNG	35	0.0141	0.6122
16	MYC	34	0.0162	0.6000
17	STAT1	33	0.0109	0.5769
18	ICAM1	32	0.0100	0.5960
19	RELA	32	0.0071	0.5882
20	FOS	31	0.0079	0.5882

3.5. GO functional enrichment analysis

GO enrichment analysis yielded 1716 significantly enriched terms, including: 1505 biological processes (BP), 63 cellular components (CC), and 148 molecular functions (MF).

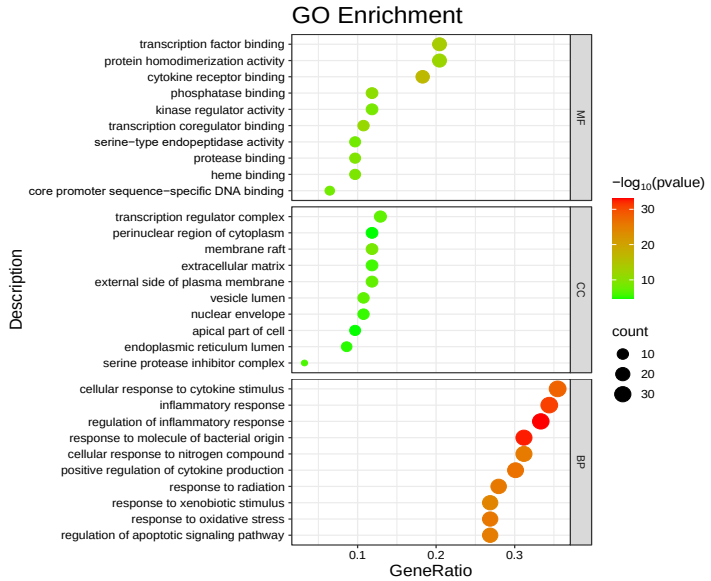

Figure 4. Gene Ontology enrichment analysis of intersecting targets

The top enriched GO terms are presented in Figure 4 (bubble plot), illustrating functional distribution and enrichment significance.

3.6. KEGG pathway enrichment analysis

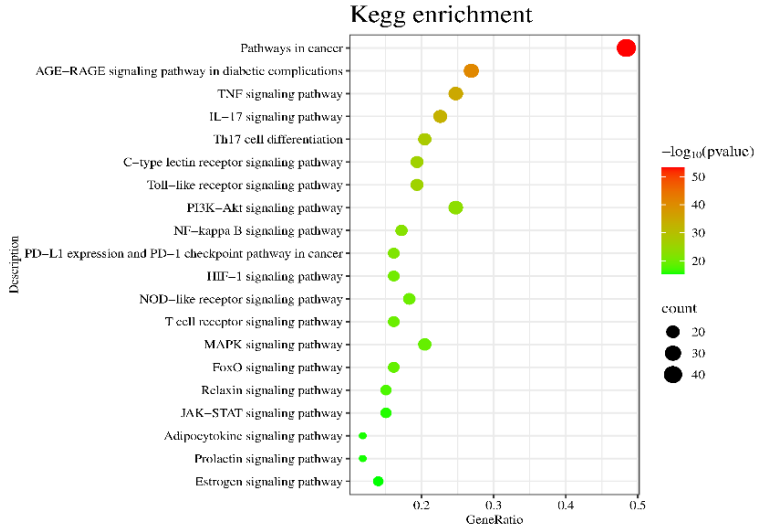

Figure 5. Bubble plot of KEGG pathway enrichment analysis of intersecting targets (top 20 pathways)

A total of 246 KEGG pathways were significantly enriched (adjusted $p < 0.05$). The top 20 by enrichment ratio are shown in Figure 5. Based on the anti-inflammatory focus, 10 RA-relevant pathways were selected and visualized using a Sankey diagram and bubble plot (Figure 6).

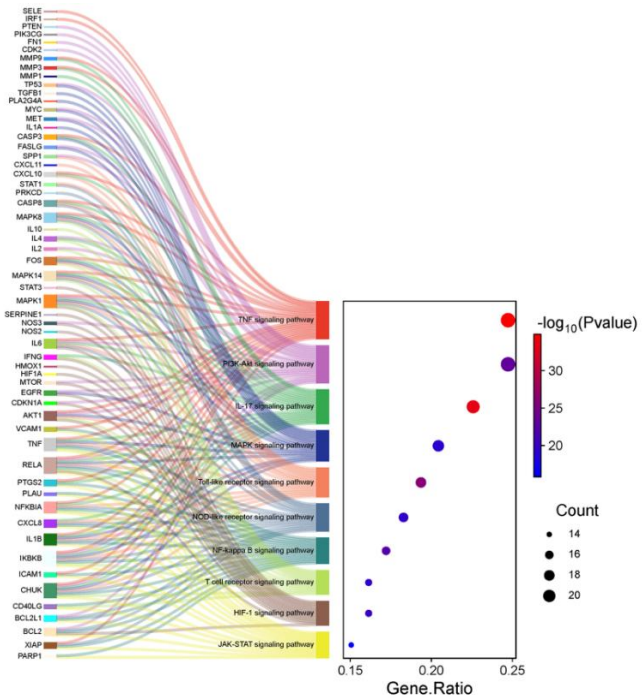

Figure 6. Sankey diagram and bubble plot of KEGG enrichment of RA related targets involved in inflammation

Sankey diagram (left): 10 inflammation-related KEGG pathways connected to their targets. Bubble plot (right): enrichment of the same pathways (size = gene count; color = adjusted p).

IV. DISCUSSION

The present study suggests that WQBT may exert anti-inflammatory effects in RA through coordinated regulation of a cytokine-centered inflammatory network rather than isolated inhibition of individual signaling pathways. The interaction pattern among active compounds, hub targets, and enriched pathways suggests the prescription may interfere with inflammatory amplification and persistence.

Several compounds (quercetin, luteolin, kaempferol, wogonin, baicalein, naringenin) converged onto shared inflammatory targets. Quercetin has been reported to suppress production of TNF-α, IL-1β, and IL-6 through inhibition of NF-κB and JAK/STAT signaling in RA-related inflammatory models [12], [13]. Luteolin, kaempferol, and wogonin all exhibit anti-inflammatory effects in RA-related pathways, including inhibition of NF-κB and MAPK signaling, thereby suppressing inflammatory cytokine production [14], [15], [16]. In addition, baicalein has been shown to attenuate inflammatory injury through modulation of PI3K/Akt-related pathways [17]. This convergence suggests that WQBT's anti-inflammatory activity may involve coordinated network modulation rather than independent actions.

This network convergence became more evident in the PPI and pathway analyses. IL6, IL1B, TNF, STAT3, AKT1, RELA, and PTGS2 occupied central positions within the interaction network and were simultaneously enriched in TNF, IL-17, NF-κB, MAPK, PI3K-Akt, and JAK-STAT pathways. These pathways did not function independently but formed a highly interconnected inflammatory system centered on cytokine amplification. TNF-α

appears to function as an upstream inflammatory driver capable of activating NF-κB and MAPK signaling, thereby promoting transcription of IL-1β, IL-6, CXCL8, PTGS2, and other inflammatory mediators [1]. IL-17 signaling further reinforces this process through synergistic interaction with TNF-α, resulting in persistent inflammatory activation and osteoclastogenesis [1]. Consequently, activation of one inflammatory pathway may continuously enhance the others, forming a self-sustaining inflammatory circuit within synovial tissue.

Within this inflammatory network, NF-κB signaling appeared as a central convergence node. Our analysis predicted that the NF-κB pathway interacts with multiple targets, such as TNF, RELA, NFKB1, IKBKB, PARP1, and others. This centrality may explain the overlap between NF-κB and both hub targets and major compounds in this study. In particular, quercetin, luteolin, kaempferol and wogonin have been widely reported to inhibit NF-κB activation and downstream inflammatory mediator production [12], [13], [14], [15], [16]. Thus, the anti-inflammatory effect of WQBT may be attributed to coordinated regulation of a convergent inflammatory transcriptional network rather than inhibition of a single upstream cytokine or isolated signaling pathway.

In addition to inflammatory initiation, the enriched pathways also reflected mechanisms associated with chronic inflammatory persistence. STAT3 and AKT1 simultaneously participated in multiple pathways linking cytokine signaling with synoviocyte proliferation, angiogenesis, metabolic adaptation, and resistance to apoptosis. Persistent activation of PI3K/Akt signaling contributes to synovial hyperplasia and pathological angiogenesis in RA, whereas abnormal STAT3 activation promotes chronic inflammatory-cell differentiation and synoviocyte proliferation [2], [12], [17]. This suggests that WQBT may regulate not only acute inflammatory responses but also biological processes responsible for sustaining chronic synovitis.

From the perspective of TCM, mixed cold-heat syndrome reflects coexistence of different pathological characteristics during chronic disease progression. We hypothesize that this syndrome may correspond to simultaneous involvement of inflammatory activation and chronic inflammatory persistence pathways, as predicted by our network analysis. While this interpretation is speculative without clinical validation, it is grounded in TCM theory and our network findings, providing a testable hypothesis for future syndrome-stratified studies.

Overall, the present findings suggest that WQBT exerts anti-inflammatory effects mainly through coordinated suppression of cytokine amplification, inhibition of NF-κB-centered inflammatory transcription, and regulation of signaling pathways associated with chronic inflammatory persistence. These results provide mechanistic support for the clinical application of WQBT in RA with mixed cold-heat syndrome and offer a modern biological interpretation for its multi-component therapeutic characteristics.

VI. CONCLUSION

This study predicts that WQBT may exert anti-inflammatory effects in RA through multi-component, multi-target, and multi-pathway mechanisms, involving compounds such as quercetin, luteolin, and kaempferol targeting IL6, TNF, STAT3, and RELA via TNF, NF-κB, and JAK-STAT pathways. These findings are hypothesis-generating and provide a theoretical basis for future research, but clinical application requires experimental and clinical validation.

Limitations: This study has several limitations. The results are based on database predictions, which are subject to database bias and incomplete compound data. No experimental validation or clinical outcomes were assessed, and the analysis was not independently replicated. Therefore, all findings are hypothesis-generating and require further in vitro, in vivo, and clinical validation.

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