Regular article

Synergistic action of the Daphnes Cortex and Liquorice Root herb pair in rheumatoid arthritis treatment: A network pharmacology strategy

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Abstract

The combination of Daphnes Cortex (DC) and Liquorice Root (LR), two traditional Chinese medicinal herbs, has shown significant therapeutic effects on rheumatoid arthritis (RA), but its synergistic mechanism of action remains to be elucidated. Employing a network pharmacology and molecular docking approach, this study systematically investigated the synergistic mechanism of the herb pair DC and LR in RA treatment. Active components and their corresponding targets were retrieved from the TCMSP database and relevant literature, and RA-related targets were collected from established disease databases. A total of 73 overlapping targets between DC-LR and RA were identified, among which core targets such as AKT1, TNF, and CASP3 were highlighted. GO and KEGG enrichment analyses revealed that these targets are involved in biological processes such as oxidative stress response and cell migration, and are significantly enriched in key pathways including HIF-1, TNF, and PI3K-Akt signaling pathways. Compatibility analysis further revealed that the combination of DC and LR may enhance therapeutic effects through synergistic regulation of shared targets and complementary modulation of upstream and downstream pathway components. Molecular docking confirmed strong binding affinities between core active components and key targets. This study provides a multi-dimensional "component-target-pathway" perspective on the potential synergistic anti-RA mechanism of the DC-LR herb pair, offering a theoretical basis for further experimental validation and clinical application.

Keywords: Daphnes Cortex; Liquorice Root; rheumatoid arthritis; synergistic action; network pharmacology

1 Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by inflammation of the synovium, joints, and other organ systems [1]. Its primary pathological manifestations include synovitis and symmetric, destructive joint lesions, with a disability rate as high as 60-80%. Epidemiological surveys indicate that the global prevalence of RA is 0.5%-1%, while in mainland China it is 0.42% [2]. Currently, RA

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treatment primarily relies on pharmaceuticals, including Disease-Modifying Anti-Rheumatic Drugs (DMARDs), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), glucocorticoids, biologics, and targeted agents [3]. However, the management of RA faces several significant challenges. Firstly, the pathogenesis of RA is not fully elucidated, hindering the development of curative therapies. Secondly, existing treatments often carry substantial side effects, and long-term use can lead to drug resistance and increased risk of infection, particularly with biologics and immunosuppressants. Moreover, the high cost of targeted biologics imposes a considerable economic burden on patients and healthcare systems. These limitations underscore the urgent need for safer, more effective, and more affordable therapeutic strategies.

In this context, Traditional Chinese Medicine (TCM) has garnered increasing attention for its holistic approach and multi-component, multi-target characteristics, which align well with the complex pathophysiology of RA [5]. In TCM theory, RA is classified as "impediment syndrome" (*Bi Zheng*), attributed to the invasion of external wind, cold, and dampness pathogens that lead to stagnation of *Qi* and blood and deficiency of the liver and kidney. Rather than targeting a single pathway, TCM formulations aim to restore the body's balance by dispelling pathogens, promoting circulation, and tonifying deficiency. A representative example of this approach is the herb pair of Daphnes Cortex (DC) and Liquorice Root (LR). DC, derived from the stem and root bark of *Daphne giraldii* Nitsche, *Daphne genkwa* Sieb et Zucc or *Daphne retusa* Thunb, functions to dispel wind-dampness, alleviate pain, and dissipate blood stasis. As the principal herb in the pair, it undertakes the primary role of combating the pathogenic factors. Modern pharmacological studies have confirmed that its active constituents possess anti-inflammatory, antitumor, and immunomodulatory activities [5-7]. Clinically, DC and its preparations are widely used in the treatment of RA and other conditions, demonstrating significant efficacy [8]. LR, the dried root and rhizome of *Glycyrrhiza uralensis* Fisch, *Glycyrrhiza inflata* Bat, or *Glycyrrhiza glabra* L., acts to tonify the spleen and augment *Qi*, clear heat, and detoxify. Serving as the adjuvant herb, it not only enhances the therapeutic efficacy of DC but also moderates its potential toxicity. Research further indicates that the components of LR exhibit significant anti-inflammatory and antioxidant effects [9-10]. Their combination, documented in the *Ningxia Chinese Herbal Medicine Manual*, is recognized in TCM theory as producing a "Mutual Enhancement" effect. Literature studies also suggest that this combination can enhance the anti-rheumatic arthritis efficacy of DC [11].

Despite the documented clinical efficacy, the modern scientific understanding of the synergistic mechanism between DC and LR remains incomplete. The complex chemical composition of herbal pairs poses a challenge to traditional reductionist research methods. Therefore, this study aims to systematically investigate the synergistic mechanism of the DC-LR herb pair in treating RA using a network pharmacology strategy. Network pharmacology, with its ability to analyze "multi-component, multi-target, multi-pathway" interactions, aligns perfectly with the holistic principles of TCM. The significance of this work lies in bridging TCM theory with modern biomedical sciences, providing a theoretical foundation for the clinical application and further experimental validation of the DC-LR herb pair, and ultimately contributing to the development of novel RA therapeutics inspired by TCM wisdom. The workflow chart of this study is shown in Fig. 1.

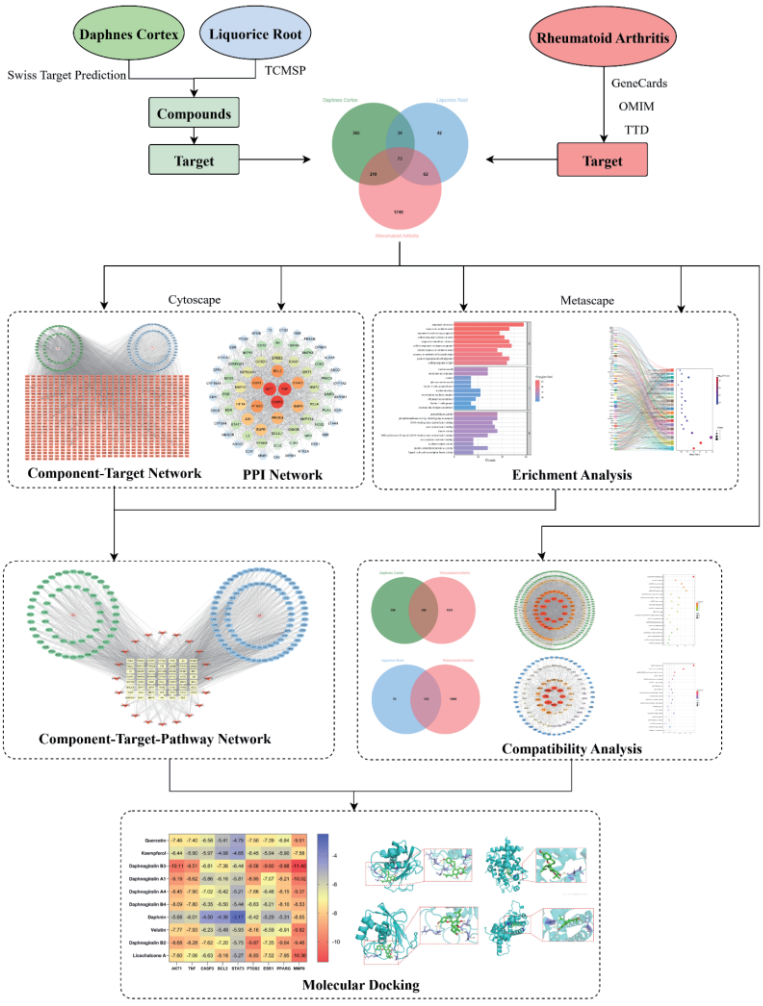

Fig. 1 The workflow chart of network pharmacology study on DC-LR for rheumatoid arthritis treatment

2 Methods

2.1 Acquisition of active components and targets of DC and LR

Active components and targets of LR were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/tcmsp.php) [12], using the keyword "Licorice" and screening criteria of Oral Bioavailability (OB) ≥ 30% and Drug-Likeness (DL) ≥ 0.18. The resulting gene names were standardized using the UniProt database (https://www.uniprot.org/) [13].

DC active components were identified through a literature search in the PubMed database. The SMILES structures of these components were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [14]. Target genes corresponding to each component were predicted using the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) [15]. After removing duplicate targets, the resulting target set was used as the target set for DC's active components for subsequent analysis. Finally, the screened and organized components and targets were imported into Cytoscape 3.9.1 to construct a DC-LR component-target network diagram [16].

2.2 Acquisition of rheumatoid arthritis targets

Using "rheumatoid arthritis" as the keyword, RA-related targets were retrieved from three databases, namely GeneCards (https://www.genecards.org/), the Online Mendelian Inheritance in Man database (OMIM, https://omim.org/), and the Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/) [17-19]. To ensure the quality and relevance of the disease targets, a screening criterion was applied. For the GeneCards database, targets with a Relevance Score greater than 2.68 were selected. All targets obtained from the OMIM and TTD databases were included due to their curated nature and relatively limited dataset sizes. The search results from the three databases were then merged, and duplicate entries were removed to obtain a final set of disease-related target genes.

2.3 Drug-Disease intersection targets and PPI analysis

The targets of DC active components, LR active components and RA targets were imported into the Venny 2.1 online tool to generate Venn diagrams and identify intersection targets. These intersection targets were then imported into the STRING database (https://string-db.org [20], with the species set to "Homo sapiens". The PPI network parameters were set as follows: a minimum interaction score of "medium confidence (0.400)", and active interaction sources limited to "Experiments", "Databases", "Co-expression", "Neighborhood", "Gene Fusion", and "Co-occurrence", while "Textmining" was excluded. This configuration was used to obtain interaction relationships among the active component targets related to RA treatment. The results were exported in TSV format and further analyzed using Cytoscape 3.9.1 to generate a Protein-Protein Interaction (PPI) network. The PPI graph was visualized based on Degree values.

2.4 GO function and KEGG pathway enrichment analysis

The Metascape platform (http://metascape.org/) was utilized to perform GO functional enrichment and KEGG pathway enrichment analyses on the 73 intersection targets of the DC-LR combination against R [21]. The species was selected as "Homo sapiens". GO terms and KEGG pathways with $P < 0.01$ were selected, and visualization was performed using the Microbioinfo online platform.

2.5 Construction of "Drug Core Component-Target-Pathway" network

The top 20 pathways identified from the KEGG enrichment analysis were selected. Their associated targets and the active components of DC-LR were organized and imported into Cytoscape 3.9.1 software to establish a "Drug Core Component-Target-Pathway" network diagram. The network topology parameters were analyzed using built-in tools, and the top 10 active components ranked by Degree value were selected as core components.

2.6 Compatibility analysis

The targets of DC active components and LR active components were separately imported into Venny 2.1 along with RA targets to generate Venn diagrams for DC vs. RA and LR vs. RA, yielding their respective intersection targets. Subsequently, PPI analyses and KEGG pathway enrichment analyses were performed separately on the DC-RA intersection targets and the LR-RA intersection targets using the STRING database and the Metascape platform. The PPI graph was visualized based on Degree values using Cytoscape 3.9.1 software. KEGG pathways with $P < 0.01$ were selected and visualized via the Microbioinfo online platform.

2.7 Molecular docking

The top 9 potential target proteins (by Degree value) from section 2.3 and the top 9 active components (by Degree value) from section 2.5 were selected. Small molecule ligands and protein receptors were obtained from PubChem and RCSB PDB (https://www.rcsb.org/). Small molecule ligands were preprocessed using AutoDockTools. Water molecules and small molecule ligands were removed from protein receptors using PyMOL. Hydrogen atoms were added, charges were calculated, and molecules were set as rigid using AutoDockTools. Docking boxes were set using AutoDockTools, and docking was performed using AutoDock 4 to calculate binding energies. Visualization was performed using PyMOL software.

3 Results

3.1 Acquisition of active components and targets of DC and LR

Seventy-one active components in DC were identified through literature revie [22-23]. Prediction using the Swiss Target Prediction database yielded corresponding 691 potential targets of these DC components. After searching the TCMSP database for LR and filtering components with OB ≥ 30% and DL ≥ 0.18, 88 active components and 211 targets were obtained. Kaempferol was the only shared component of both DC and LR. Finally, the component-target network diagram for DC and LR was constructed using Cytoscape 3.9.1, as shown in Fig. 2.

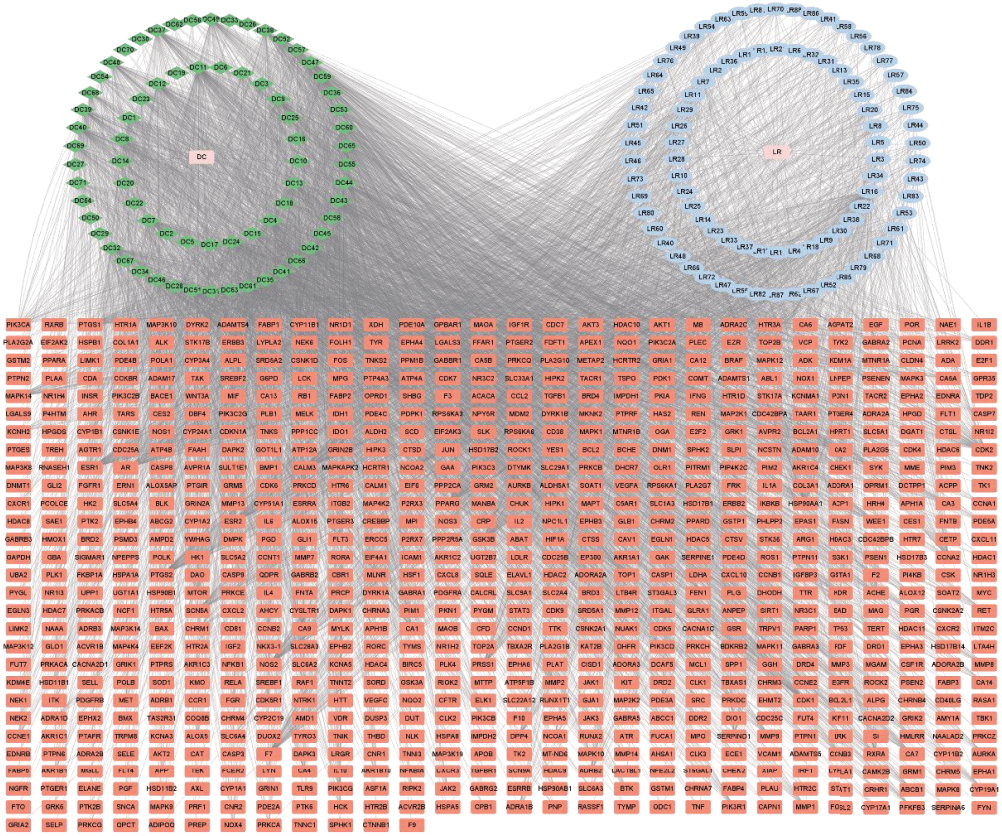

Fig. 2 Component-Target Network. Green circles in the top-left region represent active components in DC; blue circles in the top-right region represent active components in LR; red squares in the bottom region represent targets of the components

3.2 Acquisition of rheumatoid arthritis targets

Using "rheumatoid arthritis" as the keyword, 1998, 46 and 147 disease-related targets were obtaind in the GeneCards, OMIM, and TTD databases, respectively. After removing duplicates, a total of 2103 targets were obtained. The 691 DC targets, 211 LR targets, and 2103 RA targets were imported into the Venny 2.1 online tool, resulting in 73 common intersection targets, as depicted in Fig. 3.

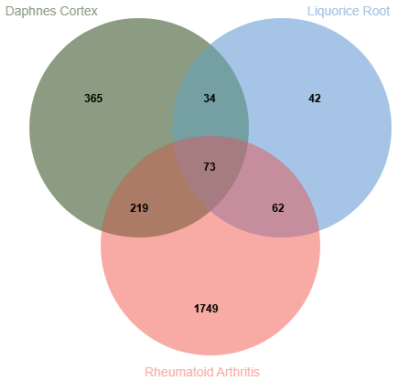

Fig. 3 Venn diagram of DC targets, LR targets, and rheumatoid arthritis targets. The top-left green circle represents DC targets; the top-right blue circle represents LR targets; the bottom red circle represents rheumatoid arthritis targets. The intersection of all three circles represents the targets for DC-LR combination therapy for rheumatoid arthritis

3.3 PPI analysis

The 73 intersection targets were imported into the STRING online database (species: "Homo sapiens"). The results were exported in TSV format and further processed using Cytoscape 3.9.1 to generate a Protein-Protein Interaction (PPI) network comprising 73 nodes and 1105 interaction edges, as shown in Fig. 4. Ranked by Degree value, 9 core targets were identified: AKT1, TNF, CASP3, BCL2, STAT3, PTGS2, ESR1, PPARG, and MMP9. This indicates that these 9 core targets play significant roles in the mechanism of RA treatment by DC-LR.

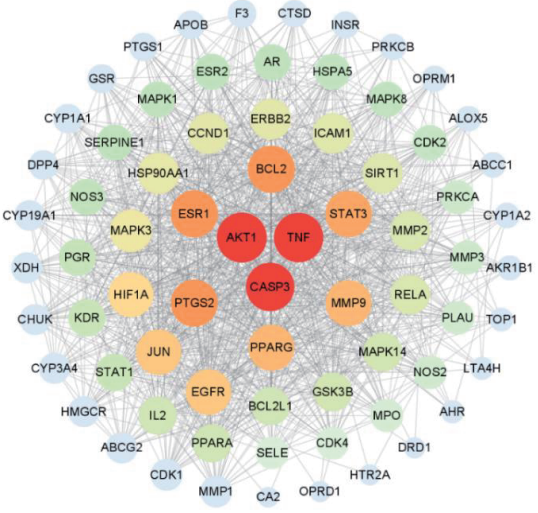

Fig. 4 PPI network of DC-LR combination therapy for rheumatoid arthritis. Nodes represent potential therapeutic targets of the DC-LR herb pair for RA. Larger and darker nodes correspond to targets with higher Degree values, indicating more connections with other nodes. Node size and color display a gradient from the center outward

3.4 GO function and KEGG pathway enrichment analysis

To analyze the biological characteristics of the candidate targets, GO annotation analysis was performed on the 73 intersection targets using the Metascape platform. The results indicated that these 73 targets were closely associated with various biological processes (BP), cellular components (CC), and molecular functions (MF), yielding a total of 1309 entries: 1127 for GO-BP, 72 for GO-CC, and 110 for GO-MF. The top 10 entries in each category are visualized in Fig. 5A.

The results of GO annotation analysis showed that the biological processes involving these potential targets include response to hormone, response to oxidative stress, response to reactive oxygen species, cellular response to chemical stress, response to xenobiotic stimulus, cellular response to nitrogen compound, response to molecule of bacterial origin, positive regulation of cell migration, and cellular response to lipid. Cellular components primarily involve membrane raft, membrane microdomain, caveola, ficolin-1-rich granule lumen, and nuclear envelope. Molecular functions are mainly manifested in protein kinase activity, phosphotransferase activity, nuclear receptor activity, protein serine/threonine kinase activity, and ligand-activated transcription factor activity.

To analyze the mechanism of DC-LR in treating RA, KEGG pathway enrichment analysis was performed on the 73 intersection targets using Metascape. KEGG enrichment results showed that the potential synergistic anti-RA targets of DC-LR were enriched in 184 pathways. The top 20 pathways were selected, and the visualization results are shown in Fig. 5B. The key pathways related to the anti-RA effect of DC-LR include the HIF-1 signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, IL-17 signaling pathway, AGE-RAGE signaling pathway in diabetic complications, and lipid and atherosclerosis. The HIF-1 signaling pathway regulates immune responses and inflammatory processes under hypoxic conditions, impacting immune cell function, cytokine production and other inflammatory processe [24]. The TNF signaling pathway plays crucial roles in various physiological and pathological processes, including cell proliferation, differentiation, apoptosis, immune response regulation and inflammation induction [25]. Abnormal activation of the PI3K-Akt signaling pathway can regulate various immune cells and inflammatory factors and is closely associated with the pathological processes of many disease [26]. These results suggested that the active components of the DC-LR combination primarily exert their synergistic effects against RA through these pathways.

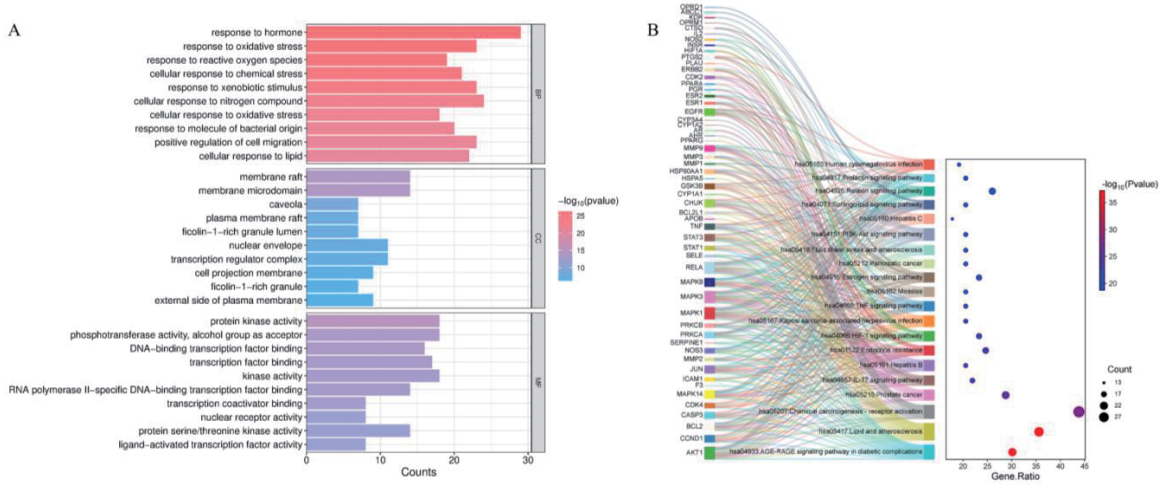

Fig. 5 GO function and KEGG pathway enrichment analysis on the screened targets. A: GO enrichment analysis of intersection targets, including GO BP, GO CC, GO MF. Color gradient represents the significance of gene enrichment; B: KEGG enrichment analysis of intersection targets. Bubble size indicates the number of genes; color gradient represents the significance of gene enrichment

3.5 Construction of active component-disease target-pathway network

The DC-LR active component-RA target-pathway network was constructed using Cytoscape 3.9.1, resulting in 219 nodes and 1684 interaction edges, as shown in Fig. 6. Degree value analysis identified Quercetin, Kaempferol, Daphnegiralin B3, Daphnegiralin A1, Daphnegiralin A4, Daphnegiralin B4, Daphnin, Velutin, Daphnegiralin B2 and Licochalcone A as the primary active components responsible for the anti-RA effect of DC-LR. Furthermore, the figure shows that all 9 core targets predicted by the PPI network in section 3.3 are closely related to the top 20 pathways.

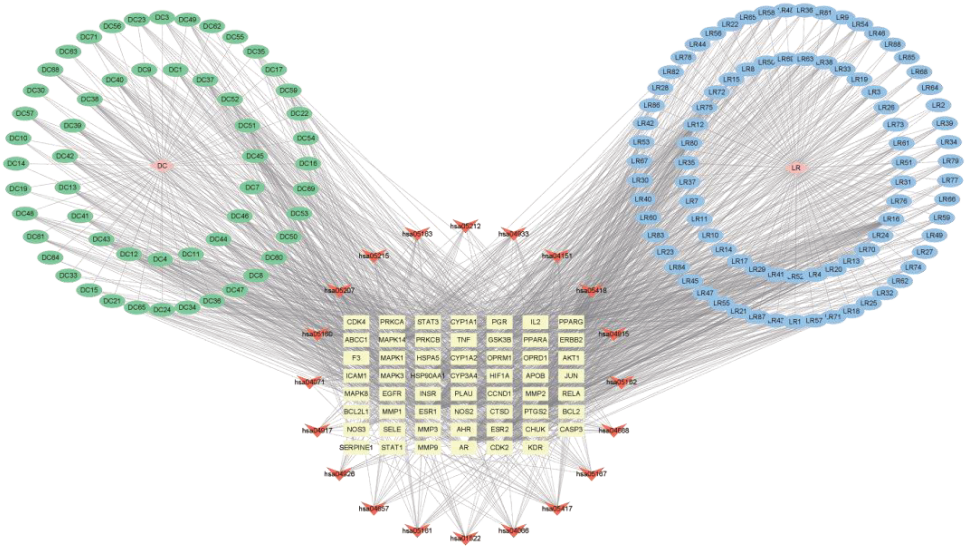

Fig. 6 Active component-target-pathway network. Pink circles located centrally in the top-left and top-right regions represent the two drugs; green circles clustered in the left region represent active components in DC; blue circles clustered in the right region represent active components in LR; red triangles represent the top 20 pathways; yellow squares represent targets associated with the top 20 pathways

3.6 Analysis of the "Mutual Enhancement" compatibility between DC and LR

To explore the synergistic mechanism of the DC-LR combination in treating RA in greater detail, a compatibility analysis was conducted. First, Venn diagrams were generated for the intersection targets of DC vs. RA and LR vs. RA, as shown in Fig. 7A, B.

PPI analysis of the 292 intersection targets between DC and RA was performed using the STRING database. Core targets, including TNF, AKT1, STAT3, SRC, CASP3, BCL2, and NFKB1, were screened based on degree values, and the visualization results are presented in Fig. 7C. KEGG analysis yielded 218 related pathways, with the top 20 pathways selected and analyzed, as shown in Fig. 7D. The results indicate that the 292 DC-RA intersection targets were primarily enriched in pathways such as the PI3K-AKT signaling pathway, Ras signaling pathway, MAPK signaling pathway, Rap1 signaling pathway, HIF-1 signaling pathway, endocrine resistance, and FoxO signaling pathway. These findings suggest that DC plays a significant role in treating RA.

Subsequently, PPI analysis was conducted on the 135 intersection targets between LR and RA. The screened core targets included STAT3, AKT1, EGFR, MAPK1, MAPK3, ESR1, TP53, and RELA, as depicted in Fig. 7E. KEGG analysis identified 205 related pathways, revealing that these 135

intersection targets were mainly enriched in the IL-17 signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis, endocrine resistance, and Th17 cell differentiation, as shown in Fig. 7F.

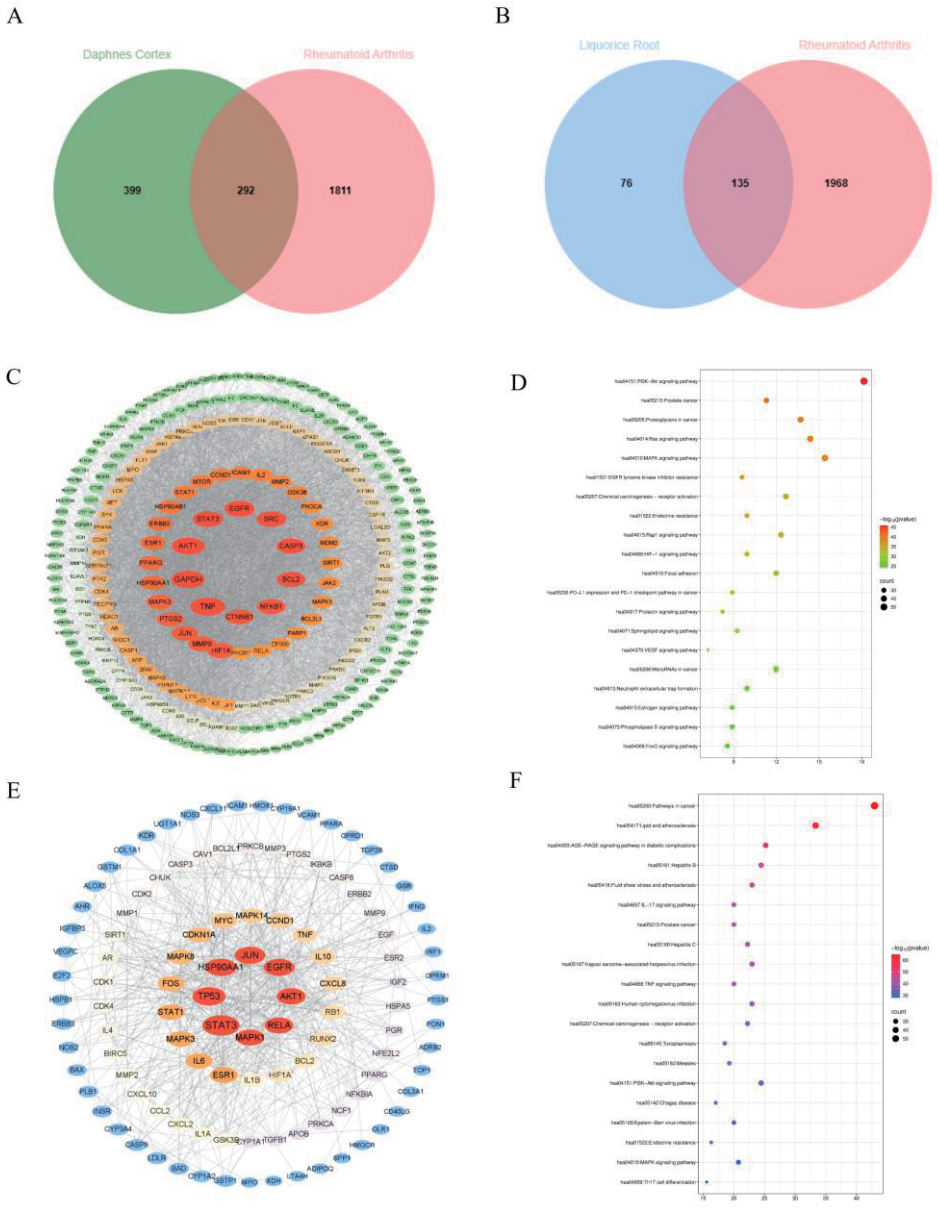

Fig. 7 Analysis of individual herb effects and their synergistic potential. A: Venn diagram of DC-RA targets; B: Venn diagram of LR-RA targets; C: PPI network of the intersection targets between DC and RA; D: Top 20 KEGG pathways for DC-RA targets; E: PPI network of the intersection targets between LR and RA; F: Top 20 KEGG pathways for LR-RA targets

3.7 Molecular docking

Based on prior analysis, the top 9 active components from the DC-LR combination and the top 9 potential target proteins were selected for molecular docking using AutoDock 4 software (Fig. 8). Lower binding energy indicates greater stability between the ligand and receptor, suggesting a higher likelihood of interaction. The results showed that Daphnegiralin B3, Licochalcone A, Daphnegiralin A1, Velutin, Daphnegiralin B2, and Quercetin exhibited good binding ability with the disease targets; AKT1, TNF, PTGS2, PPARG, and MMP9 were the disease targets that interacted well with the active components. Notably, Daphnegiralin B3 and MMP9 showed the strongest binding energy, -11.4 kcal/mol.

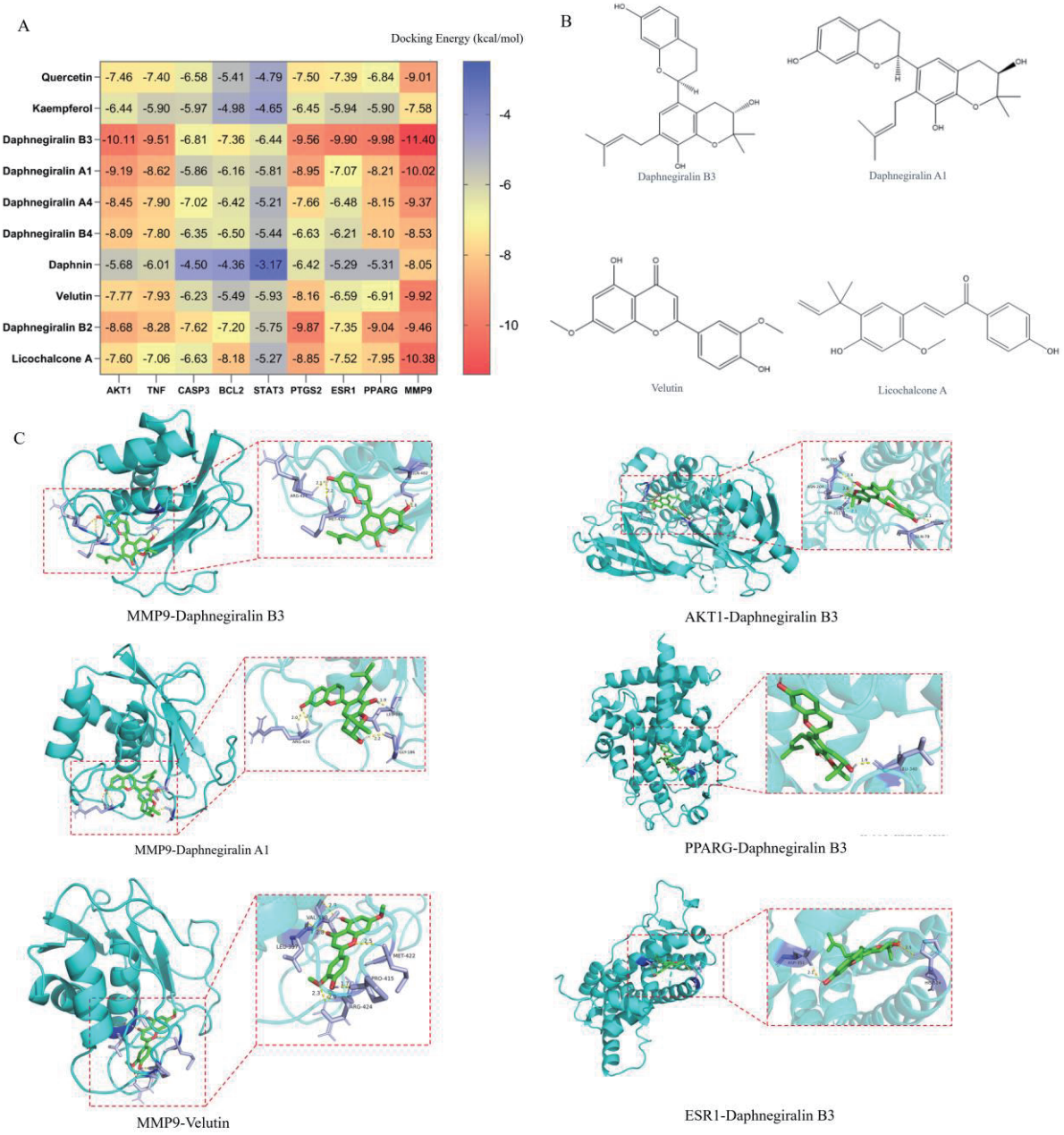

Fig. 8 Validation of core component-target interactions by molecular docking. A: Heatmap of binding energies; B: The structure of core components; C: Representative binding poses of core components with core targets

4 Discussion

RA is a long-term, chronic inflammatory disease characterized by high prevalence, significant harm and challenging treatment. Within the framework of TCM theory, RA is categorized as "impediment syndrome", typically caused by external pathogenic invasion, stagnation of *Qi* and blood, and deficiency of the liver and kidney [27]. To address this pathogenesis, the combination of DC and LR follows the "Mutual Enhancement" principle, where the two herbs work synergistically to comprehensively improve the circulation of *Qi* and blood and dispel pathogenic factors. However, the modern scientific basis underlying this synergistic effect is not yet fully understood. This network pharmacology study confirms that the DC-LR herb pair exerts its therapeutic effects through a complex network characterized by multi-component synergy and multi-target complementarity. The combined regulatory effect is significantly greater than the sum of their individual effects, thereby validating the scientific principle of "Mutual Enhancement" at a molecular level.

Our network pharmacology strategy identified several key active components from both herbs that have documented anti-arthritic activities in the literature. Notably, daphnetin from DC inhibits MAPK and NF-κB signaling pathways while enhancing autophagy protein expression [28]. Its significance is further amplified by licorice flavonoids, which improve the bioavailability of daphnetin, providing a pharmacokinetic basis for their synergistic action [11]. Similarly, kaempferol, a component shared by both herbs, demonstrates potent anti-inflammatory effects through modulation of multiple signaling pathways [29], while luteolin from DC significantly reduces pro-inflammatory mediators including TNF-α, PGE2, and MMP-9 in articular cartilage [30]. Genkwanin from DC exerts anti-RA effects by inhibiting the activation of JAK/STAT and NF-κB signaling pathways [31]. The LR-derived components also contribute substantially to the therapeutic potential. Among them, licochalcone A achieves anti-inflammatory effects by regulating NF-κB pathway [32], liquiritin demonstrates efficacy in suppressing synovial inflammation and angiogenesis in RA models [33], and quercetin exerts anti-inflammatory effects by reducing the production of pro-inflammatory cytokines and other inflammatory molecules [34].

Beyond individual component activities, our network analysis revealed the molecular basis of the "Mutual Enhancement" compatibility between DC and LR.The essence of "Mutual Enhancement" compatibility lies in the adjuvant herb (LR) enhancing the therapeutic effect of the principal herb (DC). On one hand, the two herbs achieve synergistic inhibition of core pathways by acting on common key targets. Both DC and LR core target sets include AKT1 and STAT3. AKT1 is a central hub of the PI3K-Akt signaling pathway, and STAT3 is a key transcription factor in the JAK-STAT pathway. Both critically regulate synovial cell proliferation, inflammatory factor release, cartilage erosionand and Th17 cell differentiation [35-36]. This synergistic regulation of common core nodes enables LR to directly enhance the efficacy of DC, consistent with the findings in sections 3.3 and 3.4. On the other hand, the two herbs form a complementary and enhanced inhibitory pattern by covering upstream and downstream segments of pathways or related biological processes. This complementarity is particularly evident in the MAPK signaling pathway: LR regulates the downstream part of the pathway by acting on MAPK1 and MAPK3, whereas DC regulates the MAPK pathway by targeting SRC. During the pathological process of RA, MAPKs not only regulate the production of pro-inflammatory cytokines and matrix-degrading enzymes but also play a crucial role in downstream signaling cascades of cytokine receptors [37]. Regarding the regulation

of cellular inflammation and apoptosis, core targets of DC such as TNF, CASP3, and NFKB1 showed significant KEGG enrichment in the HIF-1 signaling pathway, FoxO signaling pathway, and Ras/MAPK pathway, indicating its role as the principal herb in directly and potently regulating the core engine of inflammation, adapting to the hypoxic synovial microenvironment, and inducing synovial cell apoptosis [38]. In contrast, LR enhances these effects through targets like TP53, ESR1, and HSP90AA1 from a broader perspective of cellular homeostasis regulation. For instance, LR-mediated activation of TP53 can synergize with the pro-apoptotic effects of DC, and inhibition of HSP90AA1 can lead to the degradation of various inflammation-related kinases, indirectly potentiating the efficacy of DC.

5 Conclusion

This study demonstrates that the DC-LR herb pair exerts anti-RA effects through a multi-component, multi-target, and multi-pathway mechanism. Network pharmacology revealed that the active components of DC and LR synergistically modulate core targets such as AKT1, TNF, and STAT3, primarily involved in inflammatory and immune-related processes. These targets are significantly enriched in key pathways including HIF-1, TNF, PI3K-Akt, and IL-17 signaling pathways. Compatibility analysis further elucidated the “Mutual Enhancement” mechanism, characterized by synergistic inhibition of shared targets and complementary regulation of upstream and downstream pathway components. Molecular docking validated stable binding between core components and targets, supporting the predicted interactions. These findings provide a theoretical foundation for the clinical application of DC-LR in RA treatment and offer insights for further experimental research.

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