Research Square

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Clinical Efficacy and Mechanistic Investigation of Qingre Huashi Touzhen Decoction in Treating Chikungunya Fever

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Research Article

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Abstract

Background

Chikungunya fever (CF) is a mosquito-borne viral disease causing acute febrile illness and debilitating chronic arthralgia. Qingre Huashi Touzhen Decoction (QHTD), the guideline-preferred first-line Traditional Chinese Medicine (TCM) regimen for CF, urgently warrants further study into its efficacy and underlying mechanisms.

Methods

A multicenter retrospective cohort study was conducted. Clinical data of CF patients diagnosed with acute-phase ShiYu HuaRe Syndrome (SYHRS) from August to October 2025 were collected according to strict inclusion and exclusion criteria. A total of 135 patients in the QHTD group (conventional supportive therapy + QHTD) and 255 patients in the control group (conventional supportive therapy alone) were initially screened. Following 1:1 propensity score matching, 130 patients were finally included in each group. Outcome measures included overall treatment response rate and TCM syndrome scores on Days 3 and 7, resolution rates and resolution times of primary symptoms (fever, arthralgia, and rash), incidence of adverse reactions, and the incidence of arthralgia sequelae at the 3-month follow-up. Mechanistic validation employed network pharmacology, molecular docking, and dynamics to quantify QHTD's components, targets, pathways, binding affinity, and stability.

Results

The QHTD group exhibited statistically superior outcomes across all primary endpoints: significantly higher overall treatment response rate on Day 3, greater mean reductions in TCM syndrome scores on Days 3 and 7(all $P < 0.05$), lower arthralgia sequelae incidence at the 3-month follow-up ($P < 0.05$). Network pharmacology predicted that QHTD's bioactive components target inflammation-related proteins and pathways (TNF signaling), which was further supported by molecular docking and dynamics simulations confirming stable interactions (e.g., kaempferol-MAPK14, baicalein-NOS2, bicuculline-PTGS, bicuculline-RELA and luteolin-TNF).

Conclusion

QHTD demonstrates robust, clinically meaningful efficacy in accelerating symptom resolution and preventing chronic arthralgia in acute-phase CF with SYHRS. Mechanistically, this appears to be mediated by the synergistic regulation of inflammatory cascades via multiple bioactive components and targets.

Trial Registration

ITMCTR2026000351

Introduction

CF is an acute febrile illness caused by the Chikungunya virus (CHIKV), an enveloped, positive-sense single-stranded RNA virus primarily transmitted to humans through the bite of infected *Aedes aegypti* and *Aedes albopictus* mosquitoes. The disease is characterized clinically by the acute onset of fever, rash, myalgia, and often debilitating arthralgia. A distinguishing feature is the propensity for the joint pain to persist, becoming chronic and refractory arthralgia that lasts for months to years[1].This chronic arthralgia represents the primary cause of CF-related morbidity and disability, potentially evolving from a state of persistent inflammation to a diffuse pain syndrome resembling fibromyalgia, thereby reducing health-related quality of life[2, 3].Furthermore, CHIKV infection can, in some cases, involve the central nervous system, leading to impairments in cognitive and motor functions[4].First identified in Tanzania in 1952, the virus has exhibited a expanded beyond endemic regions since 2004. This resurgence is attributed to a confluence of factors, including enhanced viral adaptation to the Ae. albopictus vector, climate change, and increased globalization. A comprehensive study analyzing the decade from 2011 to 2020 reported 18.7 million cases of CF

across 110 countries, contributing to an estimated 1.95 million disability-adjusted life years and imposing direct and indirect economic costs exceeding USD 1.2 billion annually [5].The July 2025 outbreak in Foshan, Guangdong Province, China, triggered by an imported case, following which the virus disseminated rapidly. Critically, no licensed antiviral agents or prophylactic vaccines exist. current management remains purely supportive (e.g., hydration, analgesia, rest), leaving patients vulnerable to long-term sequelae and highlighting an urgent, unmet need for pathogenesis-targeted therapeutics[6].

Within the framework of TCM, CF lacks a classical nosological counterpart. In light of its distinct epidemiological traits and clinical phenotypic features, and in compliance with the Diagnosis and Treatment Protocol for Chikungunya Fever jointly promulgated by the National Health Commission and the National Administration of Traditional Chinese Medicine of China (2025), CF is formally categorized under the TCM domain of epidemic febrile diseases (Yibing: A highly contagious febrile disorder arising from epidemic pathogenic factors that rapidly impair the body's defensive Qi and disrupt Zang-fu organ function). The etiology of CF in TCM is attributed to the invasion of mosquito-borne pathogenic toxins into the human body, with SYHRS (A TCM pattern characterized by dampness stagnation transforming into heat, manifesting as sticky mouth sensation, dark-yellow urine, yellow-greasy tongue coating, and a slippery-rapid pulse — identified as the dominant pathogenic pattern during the acute phase[1]. TCM possesses a millennia-old empirical foundation and a rigorously structured theoretical system for the prevention, containment, and treatment of epidemic diseases. Through centuries of clinical iteration across successive outbreaks, TCM practitioners have forged systematic and comprehensive expertise in the diagnosis and intervention of epidemic disorders, providing both conceptual coherence and pragmatic clinical strategies applicable to emerging and re-emerging infectious threats[1, 7, 8]. The Diagnosis and Treatment Protocol for Chikungunya Fever (2008 Edition)[9] and Technical Guidelines for Prevention and Control of Chikungunya Fever (2012 Edition)[10] established early clinical validation of TCM interventions in CF management. Notably, amid the 2025 CF outbreak in Guangdong Province, China, a full-cycle TCM intervention paradigm encompassing prevention, treatment, and rehabilitation was implemented across regional healthcare networks, achieving an overall TCM utilization rate of 85%. This integrated strategy conferred substantial benefits in rapidly ameliorating clinical manifestations and attenuating disease severity[11].Building on this real-world experience, the present study was designed to rigorously evaluate the clinical efficacy of QHTD in acute CF with SYHRS and to mechanistically characterize its latent mechanistic underpinnings, with the ultimate objective of furnishing rigorous, high-grade evidence-based support for TCM-based strategies in the clinical management of CF.

1. Clinical Study

1.1 Study Design

This multicenter, parallel-group, evaluator-blinded, retrospective observational study was conducted in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine (Approval No. ZE2025-391-01) and was registered with the International Traditional Medicine Clinical Trial Registry on December 24, 2025 (Registration No. ITMCTR2026000351).

1.2 Study Participants

Participants were patients diagnosed with acute-phase CF accompanied by SYHRS who were admitted to four medical institutions between July and October 2025: Guangdong Provincial Hospital of Chinese Medicine, Foshan Lecong Hospital, Foshan Chencun Hospital, and Foshan Beijiao Hospital. Clinical data were collected via electronic medical record systems and/or dedicated case registration databases, including: demographic characteristics, medical history, present illness, vital signs, liver and renal function, complete blood count, cycle threshold (Ct) values of CHIKV nucleic acid, and therapeutic regimens. Additionally, data related to TCM syndrome scores, changes in major symptoms (fever, arthralgia and rash), and the presence of arthralgia during the 3-month follow-up were systematically recorded. Consent to Participate declaration: not applicable.

1.3 Western Diagnostic Criteria

Diagnosis was established in accordance with the *Diagnosis and Treatment Protocol for Chikungunya Fever (2025 Edition)*[1] issued by the National Health Commission of China. Confirmed CF was defined as: (1)History of exposure to an epidemic area or case-related setting within 12 days before onset. 2Presence of typical clinical manifestations including fever, arthralgia, and/or rash. 3 Exclusion of dengue fever and other febrile exanthematous diseases. 4Positive nucleic acid test for CHIKV.

1.4 TCM Diagnostic Criteria for SYHRS

Diagnosis was performed according to the criteria for SYHRS specified in the *Diagnosis and Treatment Protocol for Chikungunya Fever (2025 Edition)*[1]. Typical clinical manifestations included: fever with or without aversion to wind, arthralgia of the extremities with or without swelling, generalized myalgia, diffuse erythema or papules on the trunk and limbs with or without pruritus, thirst, and lassitude. The tongue was commonly pale or pale red with white or yellow greasy coating, and the pulse was soft and slippery. TCM syndrome differentiation was independently conducted by two senior TCM physicians with professional senior titles. Patients with inconsistent judgments were excluded from the study.

1.5 Inclusion and Exclusion Criteria

Inclusion Criteria:

(1)Met both the Western and TCM diagnostic criteria for CF. 2Interval from onset of typical symptoms (fever, arthralgia, etc.) to initial hospital visit < 72 hours. 3Age 18–70 years.4Complete key clinical data, good patient compliance, and ability to complete follow-up.

Exclusion Criteria:

(1)Complicated with other acute infections, severe acute organ dysfunction, or chronic diseases that may cause arthralgia or fever. 2Mental disorders, pregnancy, malignant tumors, or other conditions interfering with tongue and pulse diagnosis. 3Use of other Chinese herbal medicines or proprietary Chinese medicines. 4Incomplete data or loss to follow-up. 5Any other conditions deemed inappropriate for enrollment by the investigators.

1.6 Study Grouping and Propensity Score Matching

Eligible patients were divided into a QHTD group and a control group according to whether QHTD was administered.

To minimize confounding from baseline imbalance, 1:1 nearest-neighbor propensity score matching (PSM) was performed with a caliper width of 0.02. Matching covariates included: age, sex, comorbidities, viral nucleic acid Ct value, liver and renal function, white blood cell (WBC) count, and baseline TCM syndrome score on day 1. After matching, baseline balance between groups was assessed using standardized mean differences (SMD) of all covariates.

1.7 Intervention

Control Group

Patients in the control group received conventional symptomatic supportive care during the acute phase of CF, istrictly adhering to the *Diagnosis and Treatment Protocol for Chikungunya Fever (2025 Edition)*[1]: (1)Antipyretic and analgesic therapy: Acetaminophen (0.5 g oral tablet) was administered when body temperature reached ≥ 38.5°C or the Visual Analog Scale score for joint pain was ≥ 4. Dosing was repeated every 4–6 hours as needed, with a maximum of four doses within 24 hours. 2Antihistamine therapy: Loratadine (10 mg oral tablet) was prescribed once daily for rash-associated pruritus refractory to topical measures. 3Fluid and electrolyte management: Intravenous or oral repletion was guided by dehydration status and hepatic/renal function to maintain fluid and electrolyte balance. 4Concomitant medication restrictions: Use of other nonsteroidal anti-inflammatory drugs, corticosteroids, immunosuppressants, or traditional Chinese medicines (including raw herbs, granules, or patent medicines) was prohibited throughout the study period.

QHTD Group

Patients in the QHTD group received QHTD in addition to the above conventional supportive therapy. To ensure reproducibility and pharmacological fidelity, a three-tiered quality assurance framework governed QHTD preparation: (1)Prescription composition and raw material quality control: QHTD comprised11 authenticated herbal ingredients. The herb name, Latin name, English name, supply batch number and dosage for each component are presented in Table 1. 2Herb sourcing and authentication: All herbs were sourced exclusively from the pharmacy departments of the participating centers. Each batch was authenticated and tested for quality by a qualified specialist (associate chief Chinese pharmacist or above) in accordance with the *Chinese Pharmacopoeia (2025 Edition)* and the *Guangdong Province Processing Standards for Chinese Herbal Pieces*, ensuring compliance with established quality criteria.3Standardized QHTD decoction protocol: 1) Soaking of medicinal herbs: Herbs immersed in 200 mL purified water at room temperature for 30 minutes; 2) Decoction parameters: Herbs simmered in approximately 1000 mL water for 20–30 minutes until

final volume reached 100 mL, maintaining a raw material-to-final product concentration of 5:1 (w/v), i.e., 100 mL decoction contained 130 g total crude herb weight; 3) Storage and labeling: Freshly prepared decoction dispensed into sterile, thermally insulated containers labeled with preparation date, batch number, and expiration time (≤ 4 hours at room temperature or ≤ 72 hours refrigerated at 2–8°C). The consistency and quality assurance methods for QHTD preparations included: 1) Medicinal material identification: Conducted via sensory evaluation (appearance, aroma, and texture) according to Chinese Pharmacopoeia standards, confirmed by senior pharmacists; 2) Standardized manufacturing protocol: Precise weighing (± 1% accuracy), strictly control of decoction conditions and concentration (final volume maintained at 100 mL ± 2%), with real-time video surveillance applied throughout the preparation process; 3) Full traceability: Comprehensive documentation was maintained for all prescriptions, batch identifiers, operators, and decoction parameters (e.g., heating time, stirring frequency, cooling rate); 4) Batch-specific sensory evaluation and density checks: Each batch underwent rigorous sensory assessment (color, odor, viscosity, no visible impurities), with density verified using a calibrated hydrometer (acceptable density range: 1.05–1.10 g/mL); 5) Quality assessments: Representative samples were tested for specific gravity via both refractometer and hydrometer (inter-method deviation < 0.005 permitted per batch); microbial contamination was assessed using standardized culture-based methods. These rigorous procedures ensured reliable clinical-grade QHTD production, combining traditional methodology with modern quality control standards. 4The QHTD was administered twice daily for 7 consecutive days under direct supervision and documented administration by attending physicians and nursing staff.

Table 1
The TCM botanical drugs in QHTD

Chinese name	Latin name	English name	Batch number	Dosage (g)
Huo Xiang	*Pogostemon cablin* (Blanco) Benth.	Patchouli	C12511070	10
Ge Gen	*Pueraria lobata* (Willd.) Ohwi	Kudzu root	YP25K01	15
Qiang Huo	*Notopterygium incisum* Ting ex H.T.Chang	Notopterygium root	2507001	10
Hua Shi	*Talcum*	Talc	250701841	20
Chi Shao	*Paeonia lactiflora* Pall.	Red peony root	YPB5K002	10
Dan Dou Chi	*Glycine max* (L.) Merr.	Fermented soybean	2507001	10
Dan Zhu Ye	*Lophatherum gracile* Brongn.	Lophatherum herb	251001	10
Sheng Di Huang	*Rehmannia glutinosa* Libosch.	Rehmannia root	HX25B01	10
Lian Qiao	*Forsythia suspensa* (Thunb.) Vahl	Forsythia fruit	YP25J01	15
Di Fu Zi	*Kochia scoparia* (L.) Schrad.	Kochia fruit	290701	10
Jing Jie	*Schizonepeta tenuifolia* Briq.	Schizonepeta herb	2507001	10

1.8 Outcome

Primary Outcome

Overall treatment response rate.

Secondary Outcomes: (1)TCM syndrome scores on Days 3 and 7 of treatment.2Resolution rates of individual primary symptoms (fever, arthralgia, rash) on Days 3 and 7.(Fever: body temperature returned to normal. Arthralgia: complete absence of joint pain and no sleep disturbance attributable to pain. Rash: sustained normalization of axillary temperature (< 37.3°C) for ≥ 24 hours without new lesions). 3Time to resolution of fever, arthralgia, and rash. 4Incidence of adverse events (allergic reactions, gastrointestinal symptoms, etc.). 5Presence of long-term arthralgia at the 3-month follow-up.

Evaluation Criteria

The criteria for Overall treatment response rate and TCM syndrome score were referenced from *Guiding Principles for Clinical Research of New Traditional Chinese Medicines (2002 Edition)* [12] and *Criteria for Diagnosis and Therapeutic Effects of Traditional Chinese Medicine Diseases and Syndromes (2012 Edition)*[13] issued by the National Administration of Traditional Chinese Medicine.

TCM syndrome scores were assessed using a quantitative grading scale for epidemic febrile diseases[14](Table 2). Symptoms were graded as none, mild, moderate, or severe, with scores of 0, 1, 3, and 5, respectively. Higher scores indicated more severe symptoms. Detailed criteria for overall treatment response rate are presented in Table 3.

Table 2
Quantitative Grading Criteria for Clinical Symptoms and Signs of Epidemic Diseases in TCM

Symptom/Sign	None (0)	Mild (1)	Moderate (3)	Severe (5)
Fever	None	37.5–38.0°C	38.1–39.0°C	> 39.0°C
Sweating	None	Mild sweating	Moderate sweating	Profuse sweating
Headache	None	Mild, intermittent	Persistent, moderate	Severe, incapacitating
Body heaviness, fatigue	None	Fatigue without heaviness	Fatigue with heaviness, mild activity limitation	Marked fatigue and heaviness, unable to move
Muscle/joint pain	None	Mild limb soreness	Moderate limb/joint pain	Severe joint pain with limited movement
Nausea/vomiting	None	Nausea	Vomiting	Frequent vomiting, vomiting immediately after eating
Dry mouth, bitter taste	None	Occasional	Present upon waking	Persistent throughout the day
Poor appetite	None	Slightly reduced	Moderately reduced	Severely reduced
Skin petechiae	None	Scattered on chest/flank	Multiple on limbs, chest/flank	Confluent ecchymosis
Mucosal bleeding	None	Congestion of the hard palate, mild conjunctival congestion; no hemoptysis, hematuria, or melena	Scattered petechiae on the hard palate, marked conjunctival congestion; or pinkish urine; no hemoptysis or melena	Dense petechiae on the hard palate with hemoptysis, or bright red urine, or melena/hematochezia
Altered mental status	None	Somnolence	Stupor	Coma
Cold extremities	None	—	—	Present

Table 3
TCM Syndrome Efficacy Evaluation Criteria

Efficacy outcome	Definition
Clinical remission	TCM syndrome score reduction ≥ 95% after treatment
Marked improvement	TCM syndrome score reduction ≥ 70% after treatment
Improvement	TCM syndrome score reduction ≥ 30% after treatment
No response	TCM syndrome score reduction < 30% after treatment
Note: Total response rate (%) = (number of clinical remission + marked improvement + improvement) / total number of cases × 100%.	
Calculation formula (Nimodipine method):Efficacy rate (%) = [(pre-treatment score–post-treatment score) / pre-treatment score] × 100%.	

1.9 Blinding

Blinding was implemented for investigators responsible for data collection and outcome assessment, who remained unaware of group assignments throughout the study period to minimize potential bias in outcome evaluation.

2. Data Management and Statistical Analysis

Two uniformly trained researchers independently entered data using standardized case report forms and performed cross-validation to establish the study database. Discrepancies between the two datasets were resolved by rechecking the original medical records item by item. After completion of data entry, a random proportion of case data was manually verified to ensure accuracy and consistency with the original source documents.

Statistical analysis was performed using IBM SPSS Statistics 25.0 software. Normality testing was conducted for continuous variables. Data with a normal distribution were presented as the mean±standard deviation ($\bar{x} \pm s$), and between-group comparisons were performed using the independent-samples t-test. Non-normally distributed data were expressed as the median (interquartile range), M (P_{25}, P_{75}), and compared using the Wilcoxon rank-sum test. Categorical variables were described as counts and percentages (%), and between-group differences were analyzed using the chi-square test or Fisher's exact test. All statistical tests were two-sided, and a P value < 0.05 was considered statistically significant.

3. Network Pharmacology Analysis

3.1 Screening of Active Components and Target Prediction of QHTD

The chemical components of each herb in QHTD were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://www.tcmsp-e.com/tcmsp.php). Potential active components were screened using the thresholds of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. Putative therapeutic targets of the major active components were predicted using the SwissTarget Prediction web server (http: //www.swisstargetprediction.ch/). All target names were standardized to official gene symbols using the UniProt database (https://www.uniprot.org/). The GSE176361 dataset was downloaded from the Gene Expression Omnibus (GEO) database（https://www.ncbi.nlm.nih.gov/geo/). Differentially expressed genes (DEGs) were analyzed using the ggplot2 package in R software (version 4.3.0), and volcano plots and heatmaps were generated. Concurrently, CF-related disease gene targets were retrieved from the GeneCards (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/) databases. Genes obtained from these three sources were combined and duplicates removed to establish a comprehensive set of CF-associated disease targets.

3.3 Identification and Enrichment Analysis of Overlapping Targets

Venn diagrams were constructed using an online bioinformatics platform(https://www.bioinformatics.com.cn)to identify overlapping genes between QHTD active component targets and CF-related targets, representing the potential therapeutic targets of QHTD against CF. Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the DAVID database (http://david.ncifcrf.gov/) and Metascape platform (http://metascape.org/).

3.4 Network Construction and Core Target Identification

The overlapping targets were imported into the STRING databas (https://cn.string-db.org/) for protein–protein interaction (PPI) analysis. A multidimensional QHTD-Active components-CF-Targets network was established and visualized using Cytoscape software (version 3.9.1). Based on topological parameters including degree, betweenness centrality (BC), and closeness centrality (CC), the top 10 active components and top 5 core hub genes were screened. Furthermore, pharmacokinetic properties of the candidate components were evaluated using the SwissADME database (https://www.swissadme.ch/). Components were defined as core active ingredients if they met the following criteria: high gastrointestinal (GI) absorption, and at least three of the five drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge) were satisfied.

4. Molecular Docking and Molecular Dynamics Simulation

4.1 Molecular Docking

Molecular docking was performed to validate the binding affinity between the core active components of QHTD and the corresponding hub targets. The crystal structures of the core target proteins were retrieved from the Protein Data Bank (PDB, https://www.rcsb.org/). Water molecules and the original bound ligands were removed, and the protein structures were isolated using PyMOL software. Hydrogen atoms were subsequently added, and Gasteiger charges were calculated using AutoDock software; the processed protein structures were saved in PDBQT format. Molecular docking simulations were conducted using

AutoDock Vina. A binding energy of < − 5.0 kcal·mol⁻¹ was adopted as the cutoff criterion to define stable binding between the ligand and the receptor. The docking results were visualized and analyzed using PyMOL.

4.2 Molecular Dynamics Simulation

Molecular dynamics simulations were performed using the GROMACS 2020 software package to further evaluate the binding stability and dynamic conformational behavior of the representative core receptor–ligand complexes.

5. Results

5.1 Study Population and Baseline Characteristics

A total of 390 eligible patients were finally enrolled across four participating centers, including 135 cases in the QHTD group (67 from Guangdong Provincial Hospital of Chinese Medicine, 33 from Foshan Lecong Hospital, 20 from Foshan Chencun Hospital, and 15 from Foshan Beijiao Hospital) and 255 cases in the control group (110 from Guangdong Provincial Hospital of Chinese Medicine, 60 from Foshan Lecong Hospital, 53 from Foshan Chencun Hospital, and 32 from Foshan Beijiao Hospital). After 1:1 propensity score matching, 130 patients were retained in each group (Fig. 1. Flow chart of the study). No statistically significant differences were observed between matched groups in demographic, clinical, or TCM-relevant baseline characteristics (Table 4).

Table 4
Clinical baseline data of enrolled patients

	Before PSM					After PSM				
	QHTD group(n = 135)	**Control group(n = 255)**	**Statistical value**	**SMD**	***P***	**QHTD group(n = 130)**	**Control group (n = 130)**	**Statistical value**	**SMD**	***P***
Gender [n (%)]			$\chi^2 = 3.125$	> 0.1	0.077			$\chi^2 = 0.065$	< 0.1	0.799
Man	53(39.26)	124(48.63)				51(39.23)	49(37.69)			
Woman	82(60.74)	131(51.37)				79(60.77)	81(62.31)			
Age(years)	41.04 ± 15.92	45.55 ± 17.04	t=-2.541	> 0.1	0.011	41.22 ± 15.96	41.79 ± 16.36	t=-0.288	< 0.1	0.774
Underlying diseases [n (%)]										
hypertension	22(16.30)	46(18.04)	$\chi^2 = 0.156$	< 0.1	0.666	18(13.85)	13(10.00)	$\chi^2 = 0.916$	< 0.1	0.339
diabetes	6(4.44)	10(3.92)	$\chi^2 = 0.711$	< 0.1	0.399	17(13.08)	11(8.46)	$\chi^2 = 1.441$	< 0.1	0.230
Rheumatic diseases	3(2.22)	9(3.53)	$\chi^2 = 0.506$	< 0.1	0.477	1(0.39)	1(0.39)	$\chi^2 = 0.000$	< 0.1	1
Gout	11(8.15)	25(9.80)	$\chi^2 = 0.289$	< 0.1	0.591	10(3.92)	7(5.38)	$\chi^2 = 0.566$	< 0.1	0.452
Chronic kidney disease	4(2.96)	11(4.31)	$\chi^2 = 0.435$	< 0.1	0.509	4(3.08)	3(2.31)	$\chi^2 = 0.147$	< 0.1	0.702
Nucleic acid CT value	25.46 ± 6.66	27.27 ± 6.62	t=-2.499	> 0.1	0.015	25.77 ± 6.66	26.07 ± 6.74	t=-0.366	< 0.1	0.714
White blood cell(10^9/L)	6.09 ± 2.32	6.54 ± 2.05	t=-1.556	< 0.1	0.121	6.08 ± 2.03	5.99 ± 2.03	t=-0.340	< 0.1	0.734
Creatinine (μmol/L)	82.59 ± 19.06	84.79 ± 18.73	t=-1.098	> 0.1	0.279	82.20 ± 19.27	82.80 ± 19.85	t=-0.250	< 0.1	0.803
AST(μmol/L)	23.54 ± 8.02	28.35 ± 16.64	t=-3.187	> 0.1	0.003	23.66 ± 7.84	23.61 ± 9.10	t=-0.044	< 0.1	0.965
TCM syndrome score on day 1	15.59 ± 9.06	17.28 ± 10.32	t=-1.627	> 0.1	0.105	15.13 ± 9.18	15.28 ± 8.67	t=-0.132	< 0.1	0.895

5.2 TCM Syndrome Scores and Overall Treatment Response Rate

On Day 3 and Day 7, mean TCM syndrome scores were significantly lower in the QHTD group versus the control group (Day 3: 5.85 ± 1.45 vs 7.42 ± 1.31, Day 7: 2.08 ± 1.67 vs 2.49 ± 1.54, respectively, both $P < 0.05$). The overall treatment response rate was markedly higher in the QHTD group than in the control group at Day 3 (92.31% vs 83.85%, $P < 0.05$). Although the overall treatment response rate remained numerically higher in the QHTD group at Day 7, the difference did not reach statistical significance (Table 5, **Fig. 2**). These results indicate that adjunctive QHTD therapy produces more rapid and pronounced improvements in TCM-defined symptom burden among patients with acute CF, with maximal benefit evident within the first 72 hours.

Table 5
Comparative Analysis of TCM Syndrome Scores and Overall treatment response rate

TCM syndrome scores on day 3	QHTD group(n = 130)	Control group(n = 130)	Statistical value	*P*
	5.85 ± 1.45	**7.42 ± 1.31**	**t=-9.107**	**< 0.0001**
TCM syndrome scores on day 7	2.08 ± 1.67	2.49 ± 1.54	t=-2.042	0.042
Overall treatment response rate on day 3(%)	120/130(92.31)	109/130(83.85)	$\chi^2 = 4.432$	0.035
Overall treatment response rate on day 7(%)	126/130(96.92)	121/130(93.08)	$\chi^2 = 2.024$	0.155

5.3 Comparison of Primary Symptom Resolution Rates

On Day 3 of treatment, the resolution rates of primary symptom resolution rates in the QHTD group were all significantly higher than those in the control group (fever: 78.46% vs 66.15%, arthralgia: 61.54% vs 49.23%, skin rash: 69.23% vs 56.92%, respectively, all $P < 0.05$). By Day 7, resolution rates approached ceiling levels in both groups (> 95% for all symptoms), and no statistically significant differences persisted (Table 6). This pattern supports the role of QHTD in accelerating early symptomatic recovery—particularly during the critical acute phase of CF.

Table 6
Comparative Analysis of Primary Symptom Remission Rates

Fever Resolution Rateson day 3(%)	QHTD group(n = 130)	Control group(n = 130)	Statistical value	*P*
	102/130(78.46)	**86/130(66.15)**	**$\chi^2 = 4.917$**	**0.027**
Fever Resolution Rateson day 7(%)	124/130(95.38)	120/130(92.31)	$\chi^2 = 1.065$	0.302
Arthralgia Resolution Rateson day 3(%)	80/130(61.54)	64/130(49.23)	$\chi^2 = 3.985$	0.046
Arthralgia Resolution Rateson day 7(%)	100/130(76.92)	92/130(70.77)	$\chi^2 = 1.275$	0.259
Skin Rash Resolution Rateson day 3(%)	90/130(69.23)	74/130(56.92)	$\chi^2 = 4.228$	0.040
Skin RashResolution Rateson day 7(%)	122/130(93.85)	119/130(91.54)	$\chi^2 = 0.511$	0.475

5.4 Time to Resolution of Primary Symptoms

Median time-to-defervescence was significantly shorter in the QHTD group, and similarly, time-to-rash-resolution was significantly reduced (fever: 2.62 ± 1.38d vs 2.98 ± 1.25d, rash: 2.79 ± 1.51d vs 3.21 ± 1.67d, respectively, both $P < 0.05$). No significant difference was identified between the two groups regarding time to resolution of arthralgia (Table 7, Fig. 3). These results suggest that conventional supportive therapy + QHTD effectively shortens the duration of fever and rash in patients with CF.

Table 7
Comparison of Time to Resolution of Primary Symptoms

Time to Resolution of Fever	QHTD group(n = 130)	Control group(n = 130)	Statistical value	*P*
	2.62 ± 1.38	**2.98 ± 1.25**	**t=-2.224**	**0.027**
Time to Resolution of Arthralgia	2.97 ± 1.89	3.30 ± 1.89	t=-1.375	0.170
Time to Resolution of Skin Rash	2.79 ± 1.51	3.21 ± 1.67	t=-2.118	0.035

5.5 Incidence of Adverse Events

During the entire treatment period, adverse events occurred in 3 patients in the QHTD group (2 cases of diarrhea and 1 case of vomiting) and 2 patients in the control group (all vomiting). The overall incidence of adverse events did not differ significantly between groups, and no serious adverse events or treatment discontinuations were reported ($P > 0.05$).

5.6 Arthralgia at the 3-Month Follow-Up

At the 3-month follow-up assessment, the incidence of arthralgia was significantly lower in the QHTD group than in the control group (10.77% vs 20.00%, $P < 0.05$, Table 8). This suggests that early adjunctive QHTD therapy may confer durable protective effects against residual joint morbidity in convalescent CF patients.

Table 8
Comparison of The Incidence of Arthralgia at the 3-Month Follow-Up

	QHTD group(n = 130)	Control group(n = 130)	Statistical value	*P*
The Incidence of Arthralgia at the 3-Month Follow-Up(%)	14/130(10.77)	26/130(20.00)	$\chi^2 = 4.255$	0.039

5.7 Results of Network Pharmacology Analysis

A total of 38 active components of QHTD were screened from the TCMSP database. Using the SwissTarget Prediction platform for target prediction and subsequent calibration via the UniProt database, 237 potential targets were ultimately identified. For CF-related genes, a comprehensive search was conducted across the GeneCards and DisGeNET databases, combined with DEGs of the GEO dataset GSE176361 (Fig. 4A, Volcano plot of DEGs), resulting in the identification of 2,498 CF-associated genes. Intersection of the drug targets and disease targets yielded 98 potential therapeutic targets of QHTD for CF treatment (Fig. 4B, Venn diagram). Subsequently, the QHTD-Active components-CF-Targets interaction network (Fig. 4C) and the PPI network (Fig. 4D) were constructed. The results revealed that QHTD exerts its therapeutic effects through a synergistic mechanism involving multi-component and multi-target regulation. GO functional enrichment analysis demonstrated that the intersection targets were primarily enriched in biological processes such as positive regulation of gene expression, protein complex binding and enzyme binding (Fig. 5A). KEGG pathway enrichment analysis indicated significant enrichment of these targets in pathways including the TNF signaling pathway, IL-17 signaling pathway and C-type lectin receptor signaling pathway (Figs. 5B, 5C). All datasets of the Network Pharmacology Analysis are available in **Supplementary Table S1**. Based on the network topological parameters analyzed by Cytoscape software, the top 10 core active components and the top 5 core targets (closely associated with infection, inflammatory immunity and pain) are summarized in Table 9 and Table 10.

Table 9
The Top 10 Main Compounds of QHTD in Network Pharmacology

No.	Mol ID	Compound	Degree
1	MOL000098	quercetin	64
2	MOL000006	luteolin	32
3	MOL000173	wogonin	24
4	MOL000422	kaempferol	22
5	MOL005916	irisolidone	13
6	MOL002714	baicalein	13
7	MOL000392	formononetin	10
8	MOL001002	ellagic acid	9
9	MOL008400	glycitein	9
10	MOL000791	bicuculline	8

Table 10
The Top 5 Main Target Genes in Network Pharmacology

No.	Hub genes	Degree	Betweenness Centrality	Closeness Centrality
1	PTGS2	32	0.10737350189748307	0.5609756097560976
2	NOS2	10	0.00964418317364149	0.4618473895582329
3	RELA	8	0.00467766956334502	0.4545454545454543
4	TNF	7	0.00217502148757075	0.4474708171206226
5	MAPK14	7	0.00431336328814699	0.4509803921568627

5.8 Results of Molecular Docking and Molecular Dynamics Simulation

Molecular docking was performed between the 10 screened core active components and the 5 core target proteins. The heatmap of docking binding energies demonstrated that the binding energies of all pairs were lower than − 5 kcal·mol^{-1}, indicating favorable binding affinity between each active component and the corresponding target protein (Fig. 6A). The 5 combinations with the optimal binding energies were selected for visualization (Fig. 6B: kaempferol–MAPK14, Fig. 6C: baicalein–NOS2, Fig. 6D: bicuculline–PTGS2, Fig. 6E: bicuculline–RELA, Fig. 6F: luteolin–TNF). Furthermore, molecular dynamics simulations were carried out on these five complexes (Fig. 7A-D: kaempferol–MAPK14, Fig. 7E-H: baicalein–NOS2, Fig. 7I-L: bicuculline–PTGS2, Fig. 7M-P: bicuculline–RELA, Fig. 7Q-T: luteolin–TNF). The results revealed that all complexes maintained stable conformations throughout the simulation period. Parameters including Root Mean Square Deviation(RMSD), Solvent Accessible Surface Area(SASA), Radius of gyration (Rg) and Hydrogen Bond Occupancy Heatmap(Hbond) confirmed that the systems reached equilibrium and that the small molecules bound stably to the receptor proteins. These molecular dynamics simulation results further validated the strong binding affinity and high structural stability between the core constituents of QHTD and the key therapeutic targets.

Discussion

Following transmission via the bite of an Aedes mosquito, CHIKV establishes systemic infection through a well-characterized viremic phase. The first 1-2 days post-onset represent the high viremia phase, followed by a progressive decline from days 3 to 4, with most patients achieving complete viral clearance by days 5-7. Through its envelope E1/E2 proteins, CHIKV recognizes and binds to specific receptors on the surfaces of macrophages, epithelial cells, endothelial cells, and fibroblasts. Viral entry proceeds via endocytosis to initiate replication[15]. Viral replication not only directly damages target cells but also triggers cascades of pro-inflammatory cytokines (e.g., IL-6, IL-1β, TNF-α), dysregulating coagulation and complement pathways, and promoting endothelial activation, collectively driving systemic inflammation and multi-organ involvement. Infection of macrophages and fibroblasts in synovial joints by CHIKV can lead to tissue damage and excessive production of pro-inflammatory factors; persistent viral replication-driven inflammation and sustained T-cell activation may contribute to chronic arthritis[16]. Clinically, CHIKV infection progresses through three temporally defined phases: the acute phase (≤3 weeks; median symptom duration 5–10 days), the post-acute phase (3–12 weeks), and the chronic phase (symptoms persisting >3 months)[17].T Acute manifestations are characterized by abrupt-onset high fever, severe symmetric polyarthralgia or arthritis and constitutional symptoms. Approximately 80% of patients develop macular, papular, or purpuric rashes on the trunk, extensor surfaces of the limbs, palms, and soles 2–5 days after onset, often accompanied by mild desquamation[18].

Located in the Lingnan region, Guangdong Province of China exhibits a subtropical monsoon climate marked by prolonged summer periods dominated by “summer-heat” and “dampness” pathogenic factors in TCM theory. Epidemiological data indicate that the peak incidence of chikungunya fever (CF) in this region coincides precisely with this climatic window, during which CF cases frequently evolve into the syndrome of SYHRS[7]. A cross-sectional study of TCM syndromes among 255 CF patients in Foshan City, Guangdong Province, China, confirmed SYHRS as the predominant pattern[19]. Based on the above pathogenic insights, QHTD, the first-line regimen recommended by guidelines, is widely used in CF patients with SYHRS in the acute phase. In this formula, Pogostemon cablin (Huoxiang), a genuine Lingnan herb, contains volatile oils (e.g., pogostone), flavonoids, and phenolic acids, conferring documented anti-inflammatory, antimicrobial, antioxidant, and gastrointestinal motility–modulating activities[20]. Pueraria lobata (Gegen), another commonly used herb in Lingnan, has anti-inflammatory, anti-oxidative, and vascular protective effects[21].Notopterygium incisum (Qianghuo) demonstrates broad-spectrum antiviral activity against RNA viruses, suppresses

COX-2 and iNOS expression, and alleviates thermal hyperalgesia via TRPV1 modulation[22].Forsythia suspensa (Lianqiao) possesses anti-inflammatory and antiviral properties[23],while fermented soybean (Dandouchi) exerts antipyretic effects through hypothalamic thermoregulatory modulation and promotes rash resolution via histamine receptor antagonism[24]. The pairing of Talcum with Lophatherum gracile (Danzhuye) alleviates summer-heat, and promotes diuresis without depleting yin. The combination of Paeonia lactiflora var. rubra (Chishao) and Rehmannia glutinosa (Shengdi) exerts analgesic and heat-clearing effects. And the use of Kochia scoparia (Difuzi) with Schizonepeta tenuifolia (Jingjie) alleviates rash and pruritus, thereby clearing heat. Collectively, QHTD embodies a rational, multi-component, multi-target therapeutic strategy grounded in both TCM syndrome differentiation and modern pharmacological evidence, enabling coordinated modulation of viral replication, inflammatory signaling, vascular integrity, and neuroimmune crosstalk in acute CF.

In our network pharmacology study, QHTD's anti-CF mechanism converges on key inflammatory and immune-regulatory pathways, such as the TNF signaling pathway. The core target genes including PTGS2, NOS2, RELA, TNF and MAPK14. PTGS2 encodes cyclooxygenase-2 (COX-2), a key regulator of inflammatory responses. Under pathogen stimulation, it can rapidly be induced and catalyze the production of prostaglandins and other pro-inflammatory mediators, which are involved in pain, edema, and the recruitment of immune cells, and are the core link in severe joint pain and persistent inflammation after CF infection[25].NOS2 encodes inducible nitric oxide synthase (iNOS), which produces nitric oxide (NO) under immune stimulation, participating in pathogen clearance, immune cell activation, and tissue damage regulation, and is a critical mediator of innate immunity and inflammatory responses[26].RELA, a transcription factor that can regulate the expression of pro-inflammatory cytokines, chemokines, and immune-related genes, playing an essential role in innate immune activation, inflammation, and cell survival, and serving as a key regulatory node in the host's immune response to pathogenic infection[27]. TNF encodes tumor necrosis factor-alpha (TNF-α), a pivotal pro-inflammatory cytokine involved in immune cell activation, vascular permeability modulation, and tissue damage repair, and plays a central role in the inflammatory response and immune defense during infection[28].MAPK14 is a core component of the MAPK signaling pathway, modulating cell proliferation, differentiation, apoptosis, and inflammation by phosphorylating downstream transcription factors. It plays an important role in the cellular response to CF infection, stress, and immune regulation[29].It is worth noting that the TNF signaling pathway has been identified as a key regulatory axis in cystic fibrosis infection[30].In the early stage of infection, recognition of the virus by macrophages and monocytes triggers rapid activation of this pathway, leading to TNF-α release, which subsequently induces PTGS2 expression via RELA, promoting prostaglandin synthesis and enhancing pro-inflammatory cytokine transcription through the MAPK14 pathway, thereby initiating an inflammatory cascade[31].Excessive TNF-αinduces vascular endothelial cell apoptosis and increases vascular permeability via TNFR1, causing joint edema, rash, and even possible hemorrhagic injury. In joint tissues, TNF-α directly stimulates synoviocytes to secrete matrix metalloproteinases (MMPs), which synergize with NOS2-derived NO to accelerate cartilage damage and bone resorption. Moreover, the TNF signaling pathway is involved in the adaptive immune response to CF: moderate levels of TNF-α promote dendritic cell maturation and T/B cell activation, whereas excessive levels suppress B cell antibody production and, together with NOS2, impair adaptive immunity, which may help with the persistence of the virus[32]. The key targets predicted by our network pharmacology study align closely with the pathophysiological mechanisms underlying CF, providing a scientific rationale for the clinical application of QHTD.

Clinically, adjunctive treatment with QHTD significantly reduced the TCM syndrome scores during the acute phase of CF (Days 3-7), accelerated fever resolution, and enhanced rash clearance, indicating its potent suppression of early systemic inflammation by modulating the TNF signaling pathway and its downstream inflammatory mediators. Although QHTD did not significantly shorten the duration of arthralgia, it markedly reduced the incidence of chronic arthralgia sequelae at the 3-month follow-up, underscoring QHTD's disease-modifying potential. Mechanistically, these outcomes align with QHTD's capacity to concurrently dampen upstream TNF-α production, thereby blocking the transition from the acute to the chronic phase. Collectively, QHTD exerts a coordinated, systems-level intervention across the TNF signaling axis: it modulates in the TNF signaling pathway and its downstream key molecules (e.g., PTGS2, NOS2, RELA and MAPK14), thereby alleviating acute symptoms and improving long-term joint outcomes in CF(**Figure 8**).

Conclusion

Our study demonstrates that adjunctive treatment with QHTD in patients with acute-stage CF characterized by SYHRS improves clinical symptoms and shortens the duration of primary symptoms, while also reducing the incidence of post-recovery arthralgia.

These findings provide a foundation for further investigation into its mechanisms of action and underscore the need for additional studies to validate its therapeutic efficacy and underlying mechanisms.

Abbreviations

QHTD: Qingre Huashi Touzhen Decoction, CF: Chikungunya fever, TCM: Traditional Chinese Medicine, CHIKV: Chikungunya virus , PSM: propensity score matching, WBC: white blood cell, SMD: standardized mean differences, TCMSP: Traditional Chinese Medicine Systems Pharmacology, OB: oral bioavailability, DL: drug-likeness, GEO: Gene Expression Omnibus, DEGs:Differentially expressed genes, GO:Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, COX-2: cyclooxygenase-2, iNOS: inducible nitric oxide synthase, TNF-α: tumor necrosis factor-alpha, MMPs: matrix metalloproteinases.

Declarations

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Credit Authorship Statement

Zhuang Jieqin: Conceptualization, Data curation, Formal analysis, Funding acquisition,Investigation,Methodology, Project administration, Resources,Software, Supervision,Validation, Visualization, Writing-original draft, Writing-review & editing.

Ma Qixin:Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing-review & editing.

Fan Qiyan: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-review & editing;

Nie Hao: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-review & editing;

Ye Ye: Project administration, Resources, Software,Supervision.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

QHTD: Qingre Huashi Touzhen Decoction, CF: Chikungunya fever, TCM: Traditional Chinese Medicine, CHIKV: Chikungunya virus , PSM: propensity score matching, WBC: white blood cell, SMD: standardized mean differences, TCMSP: Traditional Chinese Medicine Systems Pharmacology, OB: oral bioavailability, DL: drug-likeness, GEO: Gene Expression Omnibus, DEGs:Differentially expressed genes, GO:Gene Ontology, KEGG: Kyoto Encyclopedia of Genes and Genomes, COX-2: cyclooxygenase-2, iNOS: inducible nitric oxide synthase, TNF-α: tumor necrosis factor-alpha, MMPs: matrix metalloproteinases.

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Figures

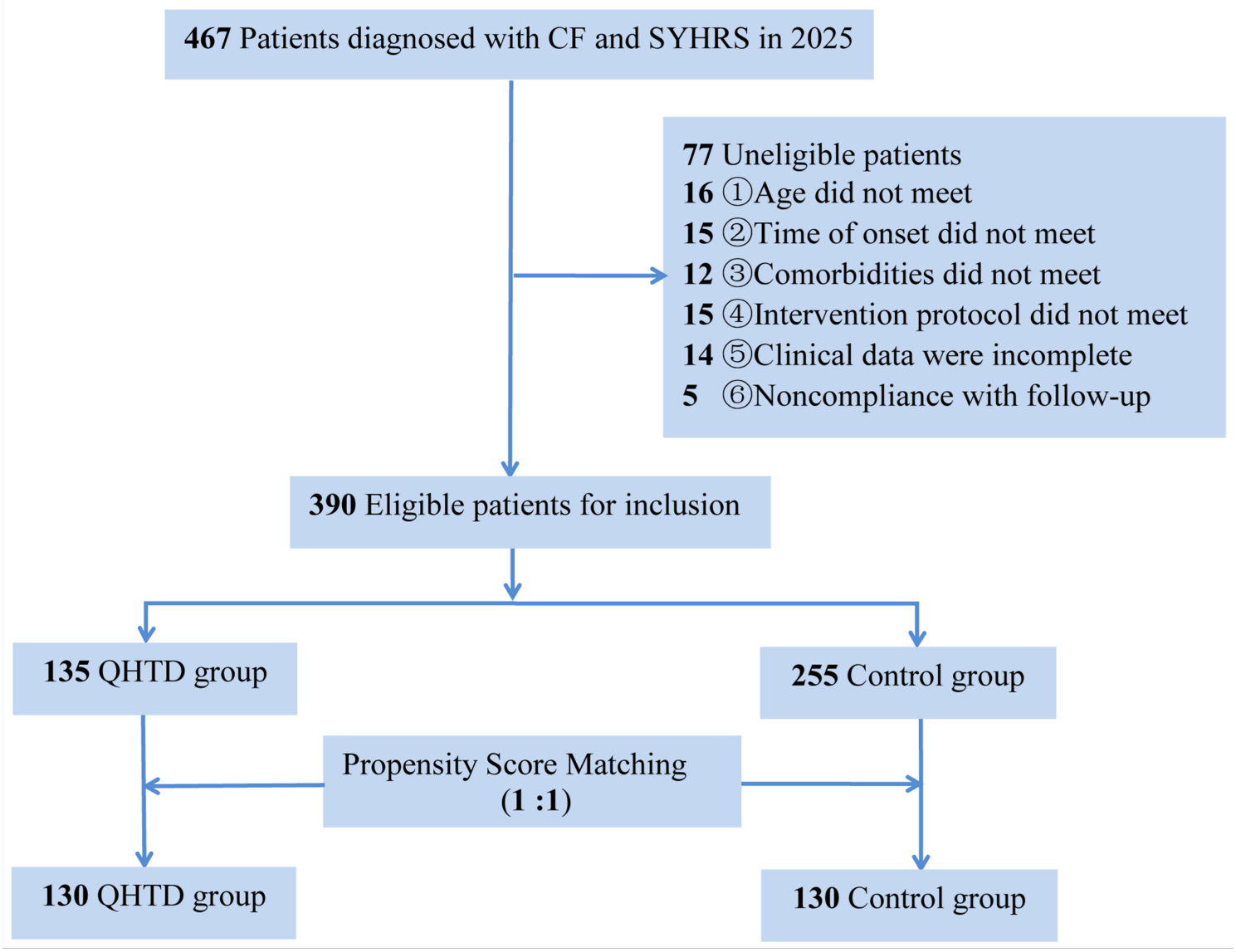


Figure 1

Study flowchart.

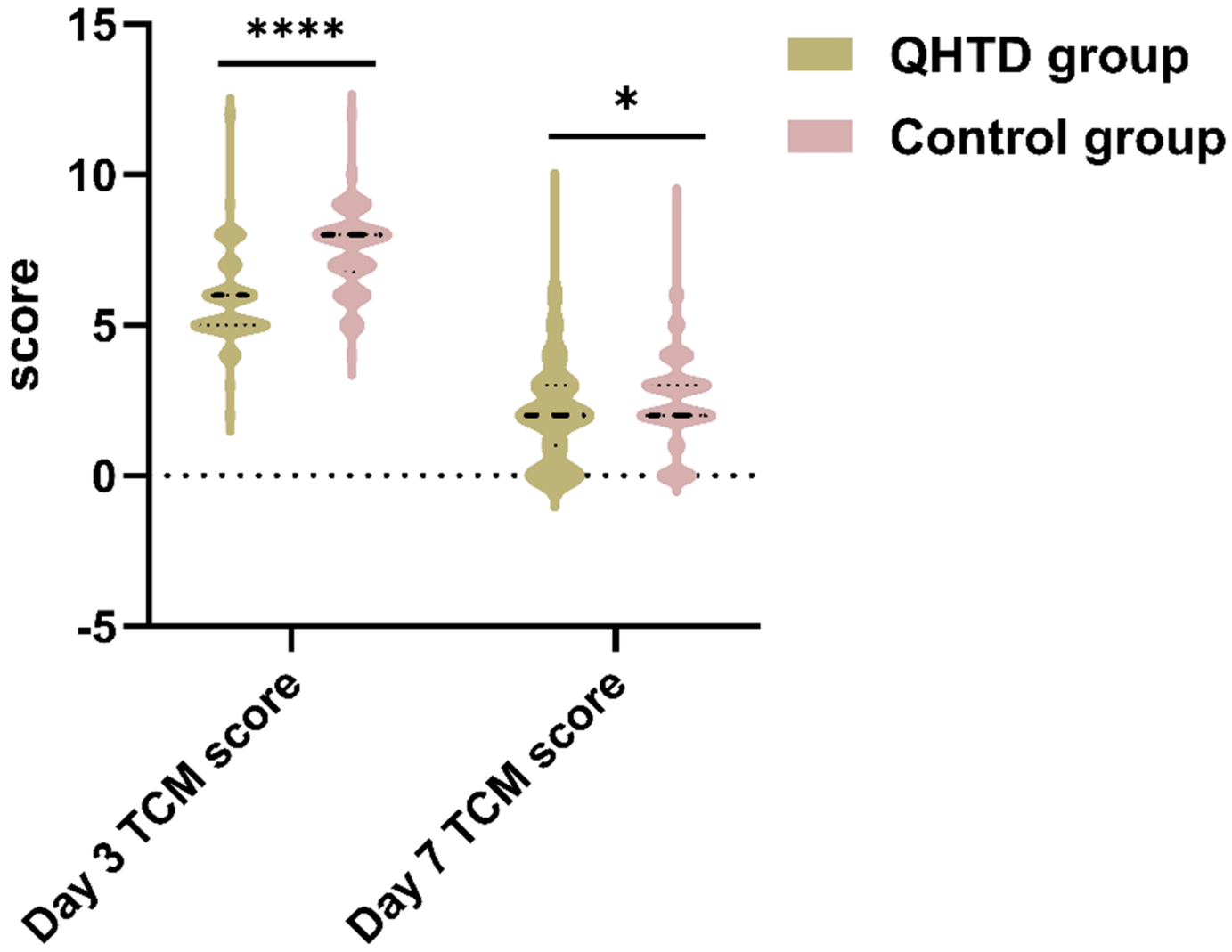


Figure 2

TCM Syndrome Scores on Days 3 and 7. *$P < 0.05$, ****$P < 0.0001$.

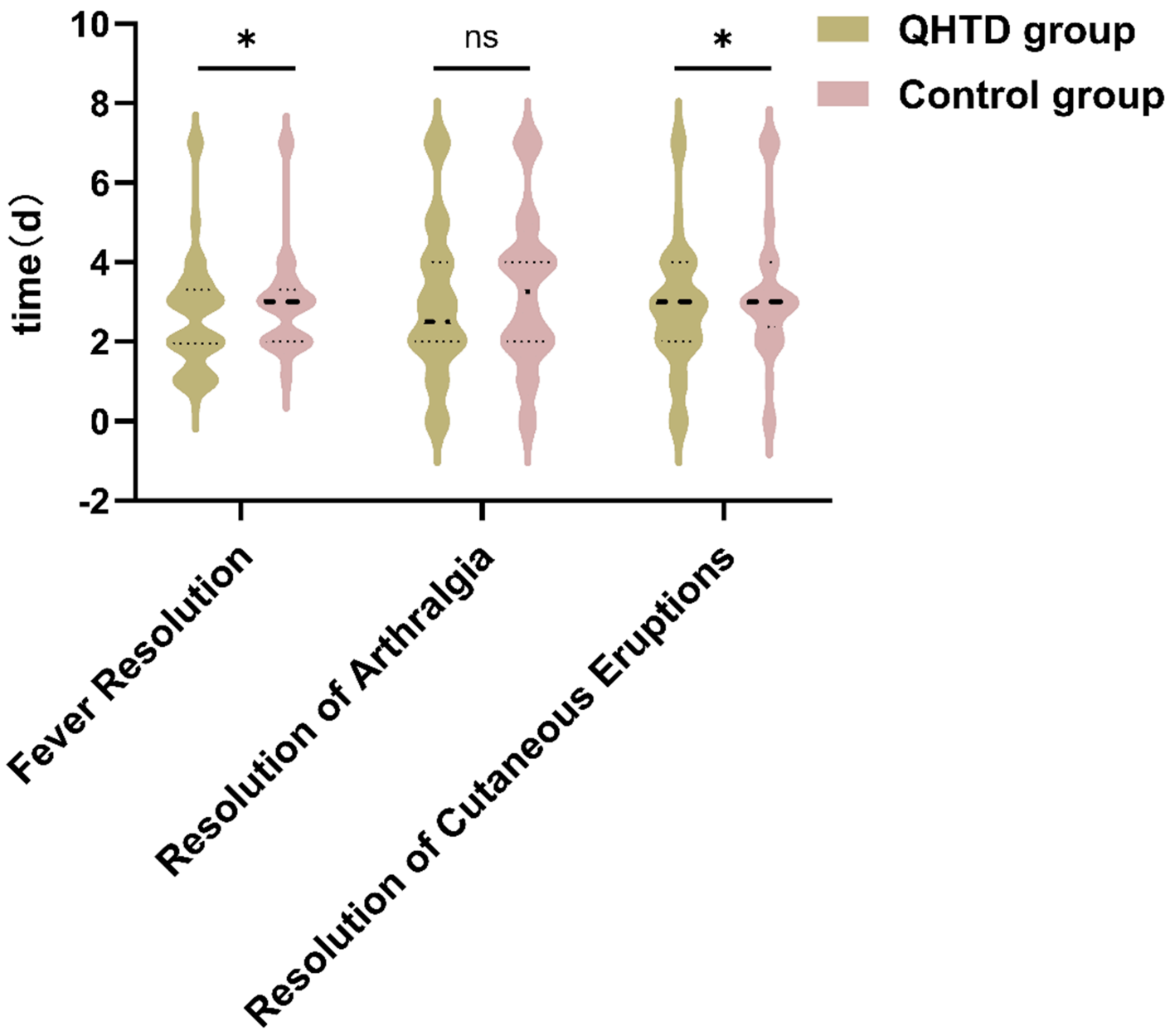


Figure 3

Time to Resolution of Primary Symptom Between the Two Groups . *$P < 0.05$, ns$P > 0.05$.

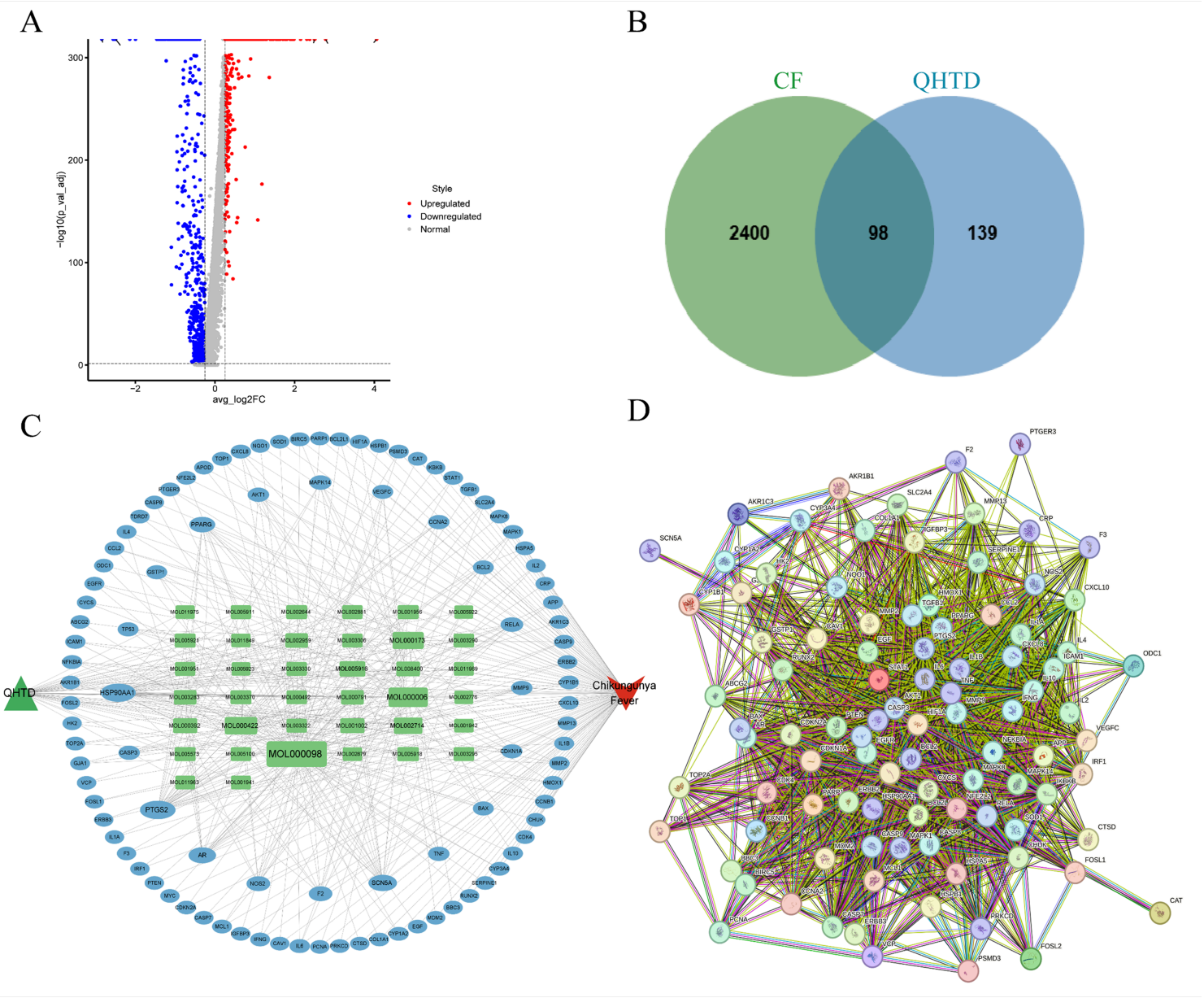


Figure 4

Network pharmacology of QHTD for CF. (A) Volcano plot of DEGs (B)Venn diagram of targets shared by QHTD and CF. (C) QHTD-Active components-CF-Targets interaction network. (D) The PPI network of intersection targets.

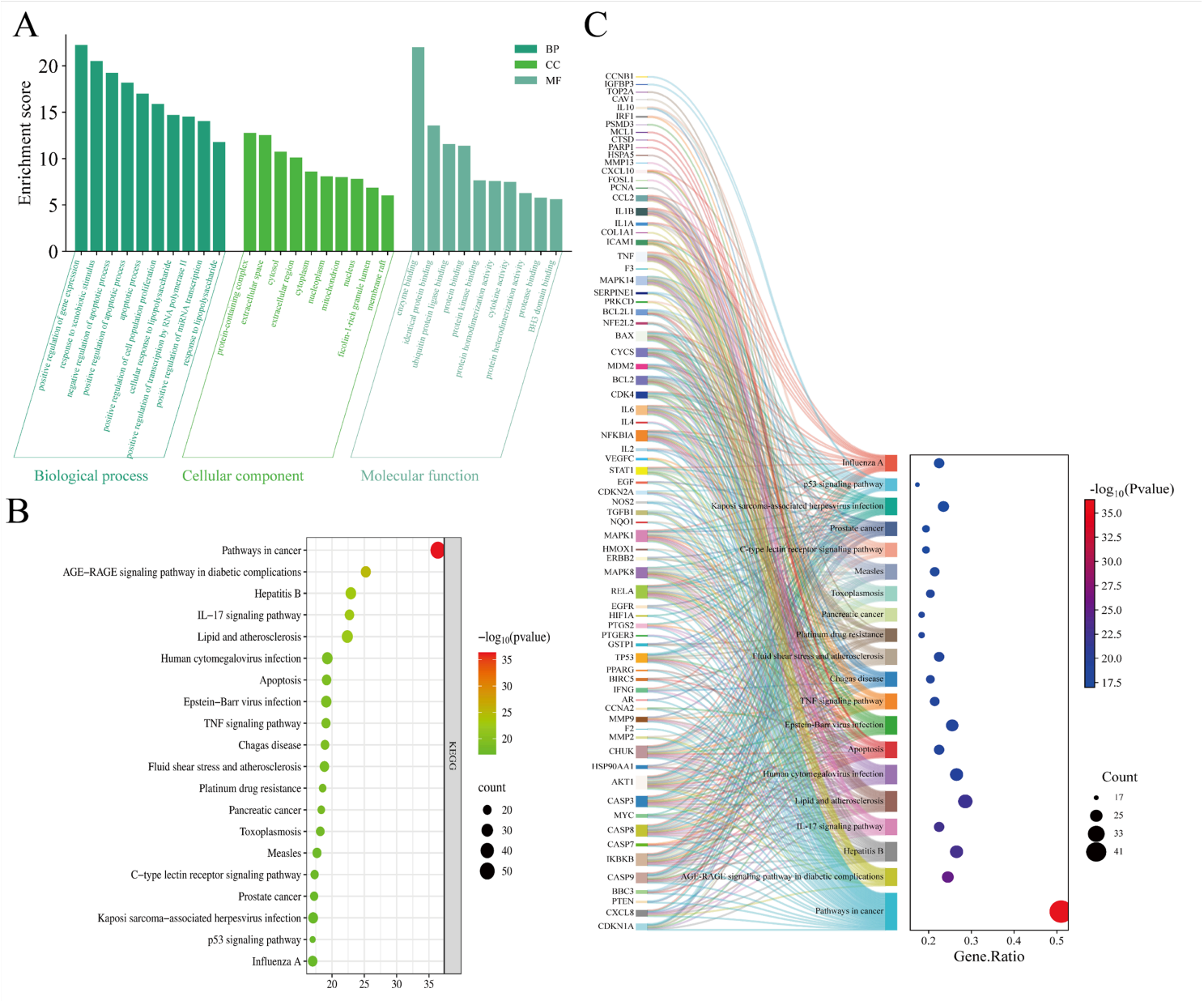


Figure 5

GO functional enrichment analysis and KEGG pathway enrichment analysis. (A) GO-enrichment analysis of common targets. (B-C) KEGG pathway analyses of the hub genes

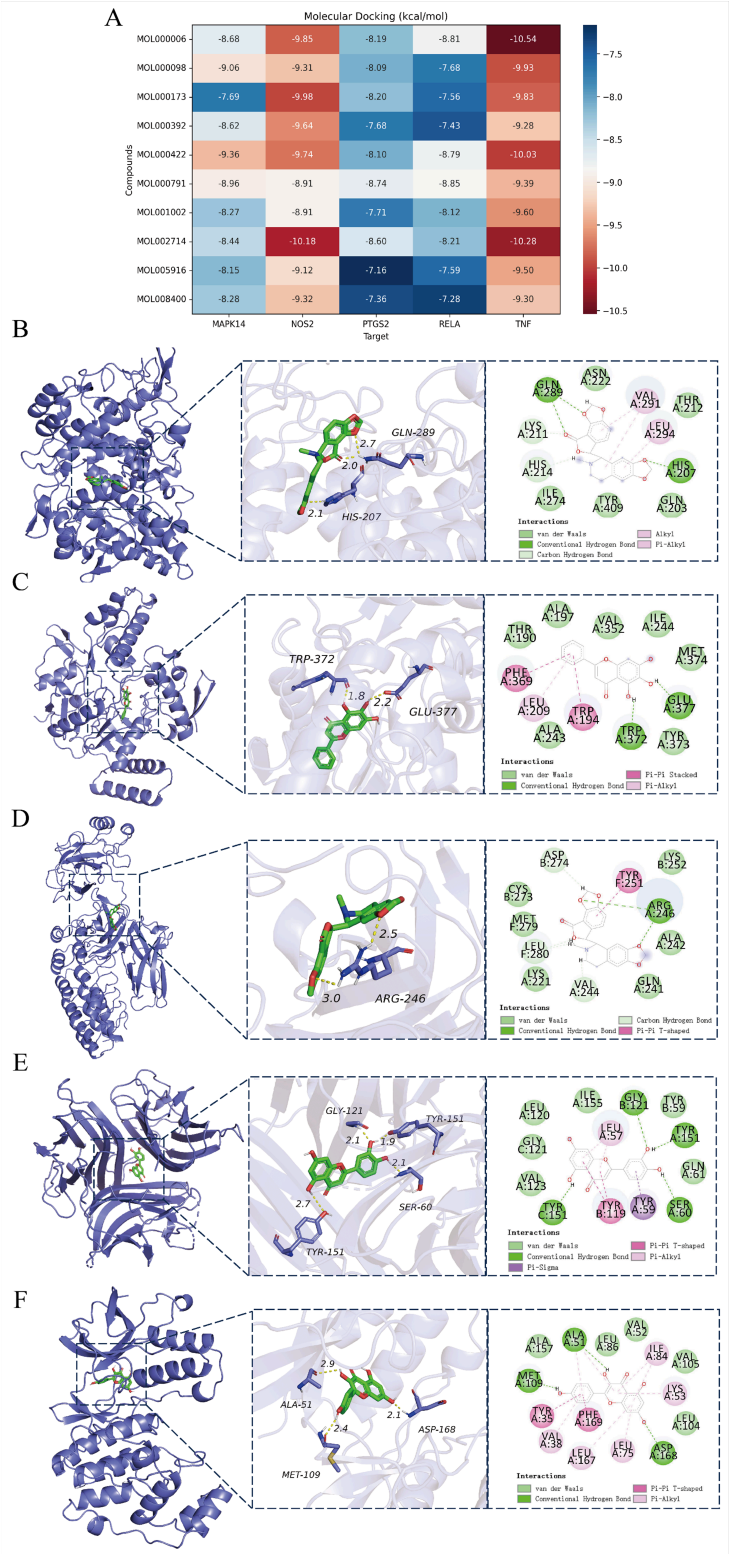


Figure 6

Molecular docking results. (A) The heatmap of docking scores of 10 drug bioactive compounds. combined with 5 key gene targets. (B-F) The representative docking complex of key targets and compounds. (B: kaempferol–MAPK14, C: baicalein–NOS2, D: bicuculline–PTGS2, E: bicuculline–RELA, F: luteolin–TNF).

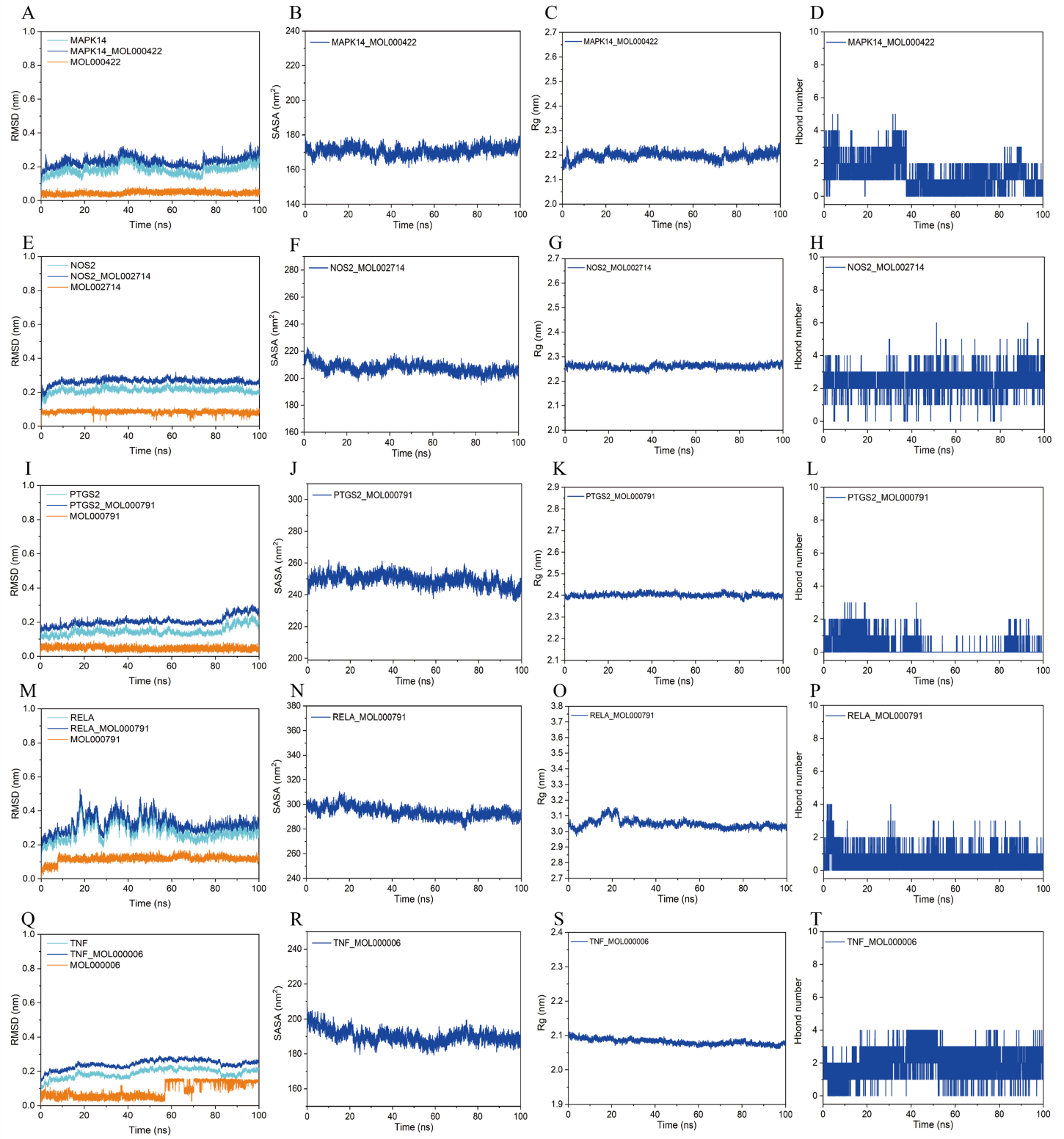
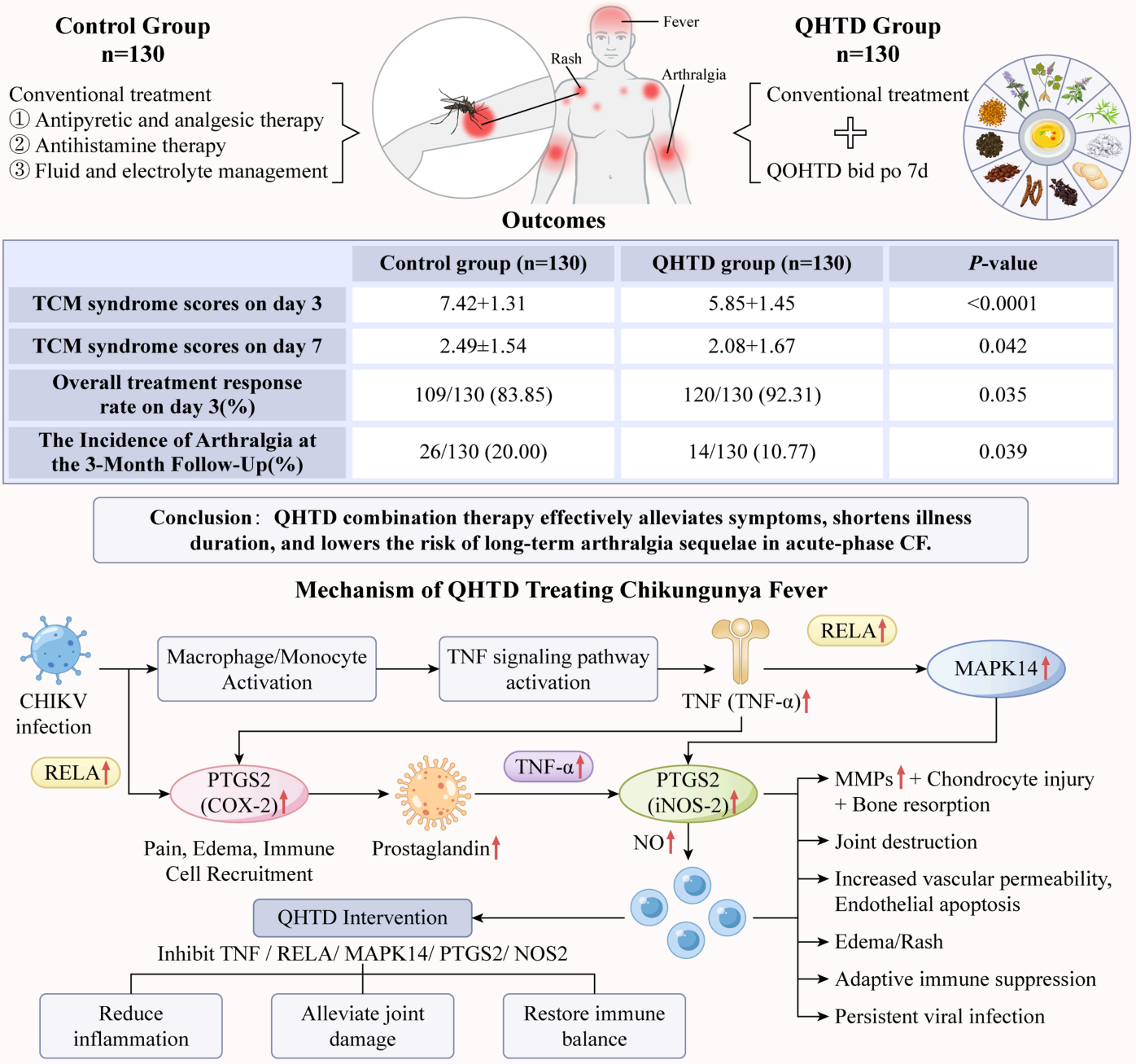


Figure 7

Molecular dynamics simulation of kaempferol–MAPK14（A-D), baicalein–NOS2（E-H）,bicuculline–PTGS2(I-L), bicuculline–RELA(M-P), luteolin–TNF(Q-T),including RMSD Curve, SASA curve, Rg curve and Hydrogen bonds.



	Control group (n=130)	QHTD group (n=130)	*P*-value
TCM syndrome scores on day 3	7.42+1.31	5.85+1.45	<0.0001
TCM syndrome scores on day 7	2.49±1.54	2.08+1.67	0.042
Overall treatment response rate on day 3(%)	109/130 (83.85)	120/130 (92.31)	0.035
The Incidence of Arthralgia at the 3-Month Follow-Up(%)	26/130 (20.00)	14/130 (10.77)	0.039

Figure 8

The therapeutic efficacy and underlying mechanisms of QHTD for CF.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1ResultsofNetworkPharmacologyAnalysis.xlsx