Contents lists available at ScienceDirect

Arabian Journal of Chemistry

journal homepage: www.ksu.edu.sa

Original article

A comprehensive strategy combined chemical spectrum with anti-inflammatory activity for screening combinatorial quality markers of *Valeriana jatamansi* Jones

Chunxiao Liang [a,b], Kunze Du [a,b,1], Shujing Chen [a], Ye Shang [a], Lirong Wang [a], Shuangqi Wang [a], Omachi Daniel Ogaji [a], Jin Li [a,*], Yanxu Chang [a,b,*]

[a] State Key Laboratory of Component-based Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China
[b] Tianjin Key Laboratory of Phytochemistry and Pharmaceutical Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China

ARTICLE INFO

Keywords:
Anti-inflammatory
Quality control
Spectrum-effect relationship
Valeriana jatamansi Jones

ABSTRACT

Valeriana jatamansi Jones (*V. jatamansi*) is an ethnomedicinal herb widely used worldwide for its excellent anti-inflammatory activity. However, the quality markers that were correlated with the anti-inflammatory activity in *V. jatamansi* remained unclear. This study aimed to identify combinatorial quality markers based on the anti-inflammatory effects of *V. jatamansi* for enhancing its quality control. The chemical constituents of *V. jatamansi* were identify by UPLC-Q-TOF-MS, and 36 compounds were identified. The anti-inflammatory mechanism was predicted by network pharmacology, STAT3 and JAK1 were selected as key targets. The anti-inflammatory activity and predicted targets were validated by using lipopolysaccharide (LPS)-stimulated RAW264.7 cells *in vitro*. A combination of bivariate correlation analysis (BCA) and grey correlation analysis (GCA) was applied to evaluate the correlation between the chemical spectrum and the anti-inflammatory activities. Hesperidin, acevaltrate and valtrate were screened as potential quality markers. These three compounds exhibited anti-inflammatory effects by inhibiting the production of inflammatory mediators (NO, IL-6, IL-1β and TNF-α) and suppressing the expression of the two key targets (JAK1 and STAT3). The quantitative analysis results showed that the contents of the combinatorial quality markers were positively correlated with the anti-inflammatory activity of *V. jatamansi*. The contribution rate of the combinatorial quality markers reached 45.0 %. This study provides a comprehensive strategy for the quality control of *V. jatamansi* based on combinatorial quality markers.

1. Introduction

Valeriana jatamansi Jones (*V. jatamansi*) is an ethnomedicinal herb widely used in China. It possessed strong therapeutic potential on gastrointestinal diseases, for example, ulcerative colitis (Wang et al., 2023a), diarrhea (Ma et al., 2022) and irritable bowel syndrome (Yan et al., 2011). The *V. jatamansi* has been shown to be effective anti-inflammatory by reducing carrageenan induced paw oedema (Subhan et al., 2007), and alleviating dextran sodium sulfate induced ulcerative colitis in mice (Wang et al., 2023a). *V. jatamansi* contained many

Abbreviations: BCA, bivariate correlation analysis; CCK8, cell counting kit-8; DMEM, dulbecco's modified eagle's medium; DMSO, dimethyl sulfoxide; EPC, edge percolated component; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GC-MS, gas chromatography-mass spectrometry; GRA, grey relational analysis; HPLC, high performance liquid chromatography; JAK1, Janus kinase 1; KEGG, kyoto encyclopedia of genes and genomes; LOQ, limits of quantifications; LPS, lipopolysaccharide; MCC, maximal clique centrality; MNC, maximum neighborhood component; NO, nitrogen monoxide; PPI, protein-protein interaction; PVDF, performance of polyvinylidene fluoride; Q-marker, quality marker; QRT-PCR, quantitative real-time polymerase chain reaction; RSD, relative standard deviation; S/N, signal-to-noise; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; STAT3, signal transducer and activator of transcription 3; TBST, tris buffered saline with Tween 20; TCMs, traditional Chinese medicines; TLC, thin-layer chromatography; TPEB, total protein extraction buffer; UPLC-Q/TOF-MS, ultra-high performance liquid chromatography-quadrupole time of flight mass spectrometry.

Peer review under responsibility of King Saud University. Production and hosting by Elsevier.

* Corresponding authors at: State Key Laboratory of Component-based Chinese Medicine, Tianjin University of Traditional Chinese Medicine, China.
E-mail addresses: Lijin@tjutcm.edu.cn (J. Li), Tcmcyx@tjutcm.edu.cn (Y. Chang).

[1] Chunxiao Liang and Kunze Du contributed equally to this study.

https://doi.org/10.1016/j.arabjc.2023.105367
Received 7 August 2023; Accepted 14 October 2023
Available online 21 October 2023

chemical compositions, such as iridoids, flavones, phenolic acids and volatile oil (Maurya et al., 2022; Quan et al., 2022). Iridoid is an important class of bioactive compounds in *V. jatamansi* that inhibit influenza virus, anxiety and inflammation (Shi et al., 2023; Wang et al., 2021). Furthermore, valejatadoid E showed the significant inhibitory effects of NO production at $IC_{50} = 3.99$ μM (Liu et al., 2021). Flavones have also been reported to exhibit anti-oxidant, anti-inflammatory and anti-cancer effects (Li *et al.*; Zhen et al., 2017). In addition, linarin was reported to suppress glioma *in vitro* and *in vivo* (Zhen et al., 2017).

The current methods and standards for quality control of *V. jatamansi* are chemically oriented and various analytical methods have been applied to assess its quality. The total flavone contents were measured by UV spectrophotometry (Li et al., 2008); the hesperidin content was determined by TLC (Li et al., 2018); the volatile oil content was analyzed by GC–MS (Hao et al., 2022); the valtrate, acevaltrate, and baldrinal contents were quantified by HPLC (Di et al., 2007). The quality marker (Q-marker) provides a scientific model for establishing a quality control system for traditional Chinese medicines (TCMs) (Yang et al., 2017). Presently, several screening strategies such as bioactive-chemical quality marker (Zhuang et al., 2023), PK markers (Wang et al., 2023b) and combinatorial quality markers (Zhou, R. et al., 2023), were established to reveal Q-markers in TCMs, which offered new angles and references for the probing Q-markers. Combinatorial markers were able to reveal the synergistic effect of the different quality marker. The current quality control methods for *V. jatamansi* are based on the quantification of specific compounds, which neglects the evaluation of the activity-related constituents (Li et al., 2014; Shukla et al., 2021). Therefore, a comprehensive strategy for screening combinatorial quality markers with anti-inflammatory activity is a requisite.

Traditional methods for screening bioactive components of TCMs, such as extraction, purification and bioassays are tedious as well as inefficient. Spectrum-effect relationship has been used to identify active components by correlating the chemical fingerprint and the biopotency of the components using chemometric methods (An et al., 2022). UPLC-Q-TOF-MS, a fast, strong separation ability and high mass accuracy analysis technology, can be employed to identify chemical components of TCMs (Lv et al., 2023; Wang et al., 2023c). Chemometrics revealed the intrinsic connection between chemical composition and biological activity more scientifically and effectively by applying mathematical strategies and statistical methods, such as bivariate correlation analysis (BCA) and grey correlation analysis (GCA). (Du et al., 2022; Han et al., 2022; Liu et al., 2022). The network pharmacology is a comprehensive method to predict the action mechanism of complex TCMs, which needed pharmacological experiments to validate its predictions (Zong et al., 2023).

A comprehensive strategy has been proposed for the discovery of combinatorial quality markers of TCMs with anti-inflammatory activity. Chemical components were rapidly identified by UPLC-Q-TOF-MS, and the targets-pathways of the identified constituents alongside anti-inflammatory activity were predicted by network pharmacology. The potential activity markers were screened by BCA and GCA methods to correlate chemical fingerprints and anti-inflammatory activities. Then, the anti-inflammatory activities and the key targets for quality markers were further confirmed and validated. Conclusively, the contribution of the combinatorial quality markers to NO inhibition rate in *V. jatamansi* was evaluated. This strategy incorporates multiple approaches to enhance the correlation of chemical compounds with the bioactivity; and its contribution to discover the valid and reliable combinatorial quality markers. This comprehensive strategy successfully screen the combinatorial quality markers which was meaningful to improve the quality control of *V. jatamansi* and its clinical applications.

2. Materials and methods

2.1. Materials and chemicals

Acevaltrate, valtrate, and hesperidin, neochlorogenic acid, isochlorogenic acid B, linarin, baldrinal with 98 % purity were supplied by Chengdu Despite Bio Technology (Chengdu, Sichuan, China). HPLC grades of acetonitrile and methanol were supplied by Concord (Tianjin, China). Macrophage RAW264.7 cell was supplied by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). DMEM medium and fetal bovine serum were supplied by Zhongaobio (Tianjin, China). CCK-8 kit and NO kit were supplied by Beyotime (Shanghai, China). LPS was supplied by Sigma-Aldrich (St. Louis, MO, USA). TransZol Up Plus RNA Kit were supplied by transgen (Beijing, China). Antibodies of JAK1 and STAT3 were supplied by Cell Signaling Technologies (Danvers, MA, United States). Antibodies of β-actin were obtained from Abclonal (Wuhan, China).

A total of 24 samples of roots and rhizomes of *V. jatamansi* were collected from pharmacies in different regions. The cultivation area, harvesting time and source were shown in **Table S1 in the Supplementary Material.** They were identified by Prof. Yanxu Chang (Tianjin University of Traditional Chinese Medicine). *V. jatamansi* roots and rhizomes were ground using a grinder and sieved through 50 mesh to obtain *V. jatamansi* dried powder.

2.2. Preparation of sample solutions and standard solutions

The sample solution preparation method was adopted from previous studies (Wang et al., 2023a) 1.0 g of dried powder was accurately suspended in 20 mL methanol. The mixture was ultrasonic extracted for 30 min in water bath. The sample solution was centrifuged at 7000 rpm for 10 min. The supernatant was dried at stable nitrogen and rediscovered with 10 mL water. Afterwards, 10 mL ethyl acetate was added into the extraction and vortexed for 1 min. The ethyl acetate was dried and redissolved by 10 mL methanol. The sample solutions were stored at −20 °C until use. The ethyl acetate was dried and redissolved by 10 mL methanol for UPLC-Q-TOF-MS and HPLC analysis. The ethyl acetate was dried and redissolved by 1 mL DMSO for cell treated.

Appropriate acevaltrate, valtrate, hesperidin, neochlorogenic acid, isochlorogenic acid B, linarin, baldrinal were dissolved in methanol to prepare a standard solution of 1.00 mg/mL. All standard solutions were stored at −20 °C until use.

2.3. UHPLC-Q-TOF-MS conditions

The compound characterization of the medicinal material samples was collected at 1290 UHPLC system tandem 6520 Q-TOF system (Agilent, Santa Clara, CA, USA). Dubhe C18 (4.6 × 250 mm, 5 μm) column was used for gradient elution program. The mobile phase was consisted of acetonitrile (A) and water (B) with a gradient elution program: 0 ~ 5 min, 5 % ~ 23 % A; 5 ~ 10 min, 23 % ~ 27 % A; 10 ~ 15 min, 27 % A; 15 ~ 16 min, 27 %~30 % A; 16 ~ 24 min, 30 %~30 % A; 24 ~ 25 min, 30 % ~ 51 % A; 25 ~ 62 min, 51 % ~ 52 % A; 62 ~ 63 min, 52 % ~ 65 % A; 63 ~ 76 min, 65 % ~ 65 % A; 76 ~ 77 min, 65 % ~ 70 % A; 77 ~ 82 min, 70 % ~ 70 % A. The column temperature was set as 30 °C, the flow rate was set as 1 mL/min, and the injection volume was set as 10 μL. The MS parameters in positive ionization mode were as follows: capillary voltage, 3000 V; collision energy (CE), 20 V; gas temperature, 350 °C; detection range, *m/z* 50–1700.

2.4. Network pharmacology analysis

2.4.1. Collection of inflammatory and V. Jatamansi related-targets

SwissTargetPrediction database (http://www.swisstargetprediction.ch/), TCMSP database (https://old.tcmsp-.com/tcmsp.php), TCMIP database (http://www.tcmip.cn/ETCM/index.php/Home/Index/index.

html), Bionet database (https://bionet.ncpsb.org.cn/batman-tcm/) was used to predict the targets of compounds of *V. jatamansi*. GeneCards (https://www.genecards.org/) databases were searched for "inflammatory". *V. jatamansi* predicted targets in common with inflammatory targets as potential targets.

2.4.2. Protein-protein interaction (PPI) analysis

The potential targets were imported into the online mapping platform (https://www.bioinformatics.com.cn/) to construct Venn diagrams. The intersection targets were uploaded to the STRING 11.5 platform (https://strin g-db.org/). Select the organism as Homo sapiens. The minimum score for interaction is 0.9.

The topological properties of the PPI network were analyzed using the CytoHubba plug in Cytoscape 3.7.2. CytoHubba analysis including degree, edge percolated component (EPC), maximal clique centrality (MCC), maximum neighborhood component (MNC) calculations.

2.4.3. Pathway analysis

The pathways of the potential targets were enriched and analyzed by the DAVID database (https://david.ncifcrf.gov/tools.jsp). The species was set as Homo sapiens. Signaling pathways were analyzed using Kyoto Encyclopedia of Genomes (KEGG) ($p < 0.01$). Enrichment dot bubble was plotted by https://www.bioinformatics.com.cn.

2.5. HPLC analysis

2.5.1. HPLC-DAD condition

HPLC analysis was performed on a Thermo Scientific™ Dionex™ UltiMate™ 3000 system (Thermo Fisher Scientific, Bremen, Germany). The chromatographic column, gradient elution program, flow rate and temperature conditions were same with *2.4*. The detection wavelength was at 254 nm. The injection volume was set as 10 μL.

2.5.2. Method validation

Precision was verified by evaluating the same sample solution for six consecutive injections. Repeatability was evaluated by six sample solution. The stability of the same samples (0, 2, 4, 6, 8, 10, 12, 17, 19, 21, 24, 24 h) was analyzed by the above method. Relative standard deviation (RSD) was used to calculate the area of each characteristic peak with relative accuracy.

2.5.3. Establishment of fingerprints

The chromatographic fingerprints of 24 batches of *V. jatamansi* were imported into the Similarity Evaluation System (Version 2012A; Beijing, China) according to the literature (Li et al., 2021).

2.5.4. Quantitative analysis of bioactivity markers

The three markers quantification was conducted by a HPLC System. The chromatographic conditions were the same as in *2.5.1*. The linearity, limit of quantitation, repeatability, precision, stability and recovery of the method were verified. The linearity of the method was calculated by plotting the peak area (y) against the concentration (x, μg /mL). The LOD (S/N = 3) and LOQ (*S/N* = 10) were calculated by the concentration of the mixed standard.

2.6. Cell viability assay

The cytotoxicity of different concentrations of sample solution was detected by CCK-8 method (Zhou, S. *et al.*, 2023). RAW264.7 cells (2×10^5 per well) were inoculated in 96-well microplates. After the cells adhered, the sample solution was intervened. After 24 h incubation at 37 °C in a 5 % CO_2 incubator, 10 μL CCK-8 was added to each well and incubated in an incubator at 37 °C for 0.5 h. The absorbance (A_{450}) was measured by microplate reader.

2.7. Nitric oxide assay

RAW264.7 cells (2×10^5 per well) were cultured in 96-well microplates. After the cells adhered, the cells were treated with different concentrations of sample solution and 1 μg/mL LPS for 24 h. The model group was treated with equal volume of DMSO and LPS, and the control group was treated with equal volume of complete medium. According to the instructions, the NO concentration in the supernatant was analyzed by NO kit.

2.8. Quantitative real-time polymerase chain reaction (qRT-PCR) assay

Total RNA was extracted from each group of cells using a Trizol reagent. Total RNA was reverse transcribed into cDNA and then amplified by qRT-PCR. GAPDH was used as the internal reference gene to calculate the relative expression of the target gene. Primer sequences are shown in **Table S2**.

2.9. Western blotting assay

Cells were lysed by total protein extraction buffer with protease inhibitor. After incubation on ice for 30 min, the supernatant was centrifuged at 14,000 × g, 4 °C and 10 min. The proteins were separated by 10 % SDS-PAGE electrophoresis at constant voltage. Then, the proteins were transferred to PVDF membranes at a constant current. The PVDF membranes were treated with blocking buffer (5 % skim milk dissolved in TBST buffer) for 3 h at room temperature, and incubated with the relevant primary antibodies (JAK1, STAT3, β-actin) overnight at 4 °C. Afterwards, the PVDF membranes were incubated with secondary antibody for 2 h at room temperature.

2.10. Spectrum-effect relationship analysis

The spectrum-effect relationship between anti-inflammatory (NO Inhibition rate) and peak area of chromatography was established by gray relational analysis and bivariate correlation analysis.

2.10.1. Gray relational analysis (GRA)

The grey correlation theory was used to calculate the grey correlation coefficient and rank of each common peak of 24 samples. The specific operation was to use the IC_{50} value as the reference sequence and the peak area of the 17 common peaks as the comparison sequence.

2.10.2. Bivariate correlation analysis (BCA)

The Pearson model was used for bivariate correlation analysis (BCA) analysis. The independent variable was the common peak area, and the dependent variable was the IC_{50} value of the anti-inflammatory activity.

2.11. In silico ADME prediction

The physicochemical properties and pharmacokinetic profile of acevaltrate and valtrate were predicted by SwissADME tool (http://www.swissadme.ch/) (Daina et al., 2017).

2.12. Statistical analysis

All biological activity-related data were analyzed using Graph Pad Prism 8.0 software (Graph Pad Software, CA) and presented as the mean ±SD. Western blot densitometric analysis using ImageJ. One-way analysis of variance was carried out for multiple comparisons of the data with statistical significance set at $P < 0.05$. All relational analyses were performed using SPSS (version 26.0, IBM, USA).

3. Results

3.1. Identification of the chemical components

To identify possible combinatorial quality markers for anti-inflammatory effect, the basic chemical constituents of *V. jatamansi* were investigated. The total ion chromatogram of samples in positive ion mode was shown in Fig. 1**A**. 36 chemical components were identified (Table 1), including 32 iridoids, 2 flavones (P7, P8), 2 phenolic acids (P2, P6). For iridoids, the structure of the parent nucleus was mainly cyclopentane-[C]-pyran, which could be divided into three main types according to the number of double bonds, including monoene-type iridoids (P5, P12, P14, P17, P18, P20, P25-P27, P33, P35), diene-type iridoids (P13, P15, P16, P19, P21-P24, P28, P29, P32, P34, P36) and tetraterpene-type iridoids (P4, P9, P30). The structures of the compounds are shown in Fig. 1**B**. The chromatograms, mass spectra and mass fragmentation pathways of the standard compounds are shown in the **supplementary material**.

3.2. Network pharmacology analysis

3.2.1. PPI network construction analysis

A total of 36 compounds identified from *V. jatamans* and out of which 6 compounds could not be assessed from their targets database. 426 cross-targets that were obtained from 478 targets of 30 compounds and 13,697 anti-inflammation targets (Fig. 2**A**). The PPI network was constructed by uploading 426 targets to the string platform (Fig. 2**B**), which was then imported into cytoscape for further visualization. The degree value represents the number of connections of a node, it indicates the importance of a node in the network. The higher degree of nodes connected implies higher centrality and influence of a node. A total of 28 targets were considered potential targets with degree > 20 (Fig. 2**D**). In addition, the key targets were screened from the intersection of the top 20 targets of the four parameters, which are EPC, MCC, MNC and degree parameters in cytoHubba plug (Fig. 2**C**) as well as 13 key targets were identified from 28 targets based on EPC, MCC and MNC parameters (**Table S3** in **Supplementary materials**). The results showed that 13 key targets screened from 28 potential targets were key targets for the anti-inflammatory activity of *V. jatamansi*, including STAT3, LCK, EGFR, JAK2, PTK2, PIK3CA, MAPK1, FYN, JAK1, MAPK14, PIK3R1, SRC, and HRAS (Fig. 2**D**).

3.2.2. Pathway enrichment analysis

To further explore the anti-inflammatory mechanisms of *V. jatamansi*, KEGG enrichment analysis of the potential targets were performed using the DAVID database. Based on "$P < 0.01$"and "signaling pathway", the top 20 pathways were screened (Fig. 2**E**). The name of the signaling pathway is displayed on the Y-axis, while the enrichment factor is displayed on the X-axis. The number of genes in each pathway is proportional to the bubble size, and the bubble color reflects how significant the gene enrichment in that pathway is. The results suggested that *V. jatamansi* may exert anti-inflammatory activity by modulating JAK-STAT signaling pathway, PI3K-Akt signaling pathway, NOD-like receptor signaling pathway, Chemokine signaling pathway, TNF signaling pathway, IL-17 signaling pathway, *etc*.

3.3. Inflammation inhibitory activity of V. Jatamansi

3.3.1. Cell cytotoxicity analysis

The cytotoxicity of *V. jatamansi* extract on RAW264.7 cells was measured by the CCK-8 assay (Fig. 3**A**). It showed that the cell survival rate was reduced significantly by higher concentrations of the extract, and the concentrations below 50 μg/mL had no cytotoxic effect.

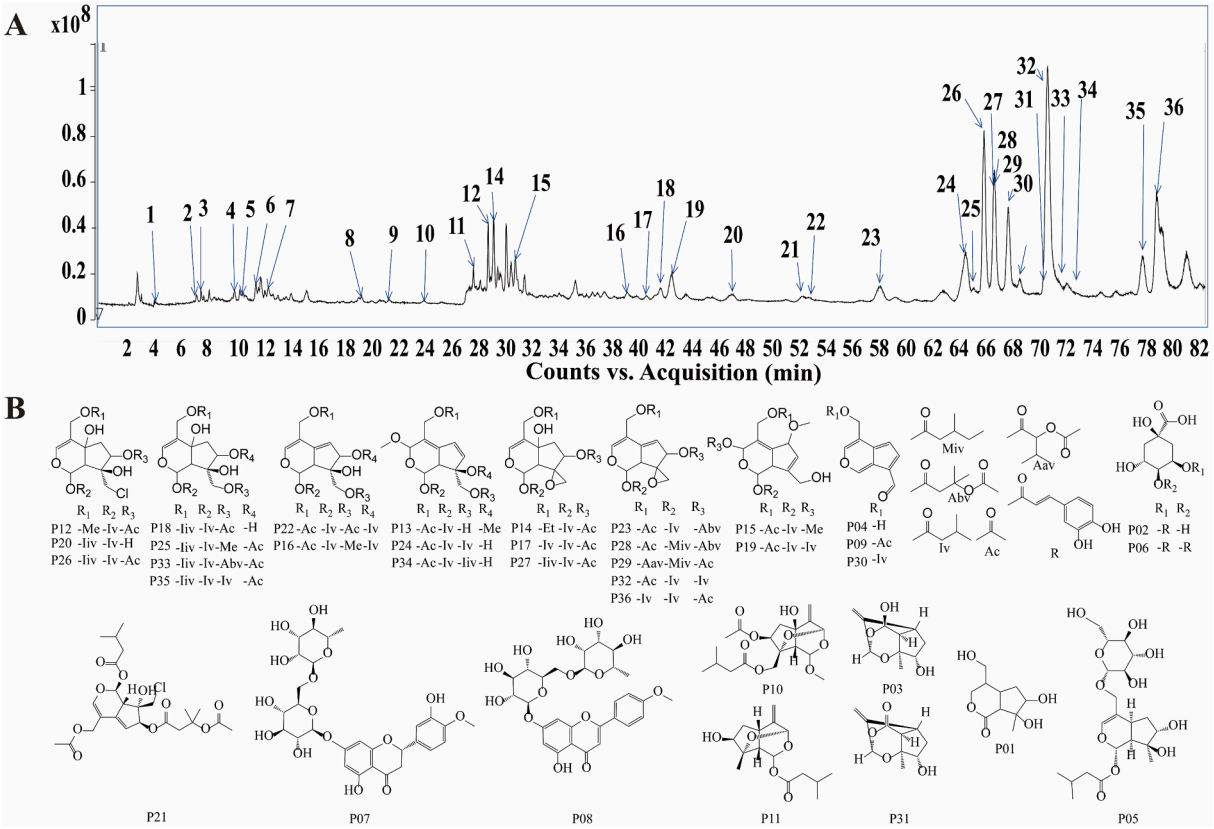

Fig. 1. Identification of the chemical composition. (A) Total ion chromatogram of samples analysed by UPLC-QTOF-MS in positive ion mode. (B) Structures of the compounds from *V. jatamansi* samples.

Table 1
The components of *V. jatamansi*.

Peaks	T_R(min)	Molecular formula	Massnumber	Calculated mass(m/z)	Observed mass(m/z)	Ion Mode	Error (ppm)	ESI-MS/MS data	Peak identification
P01	4.516	$C_{10}H_{16}O_5$	216.0998	217.1071	217.1064	$[M+H]^+$	3.01	135.0805, 109.0638, 153.0902	Longiflorone
P02	7.335	$C_{16}H_{18}O_9$	354.0951	355.1024	355.1026	$[M+H]^+$	−0.68	193.0696, 181.0482, 163.0393	Neochlorogenic acid*
P03	7.604	$C_{10}H_{14}O_4$	198.0892	199.0965	199.0967	$[M+H]^+$	−1.08	177.07, 135.0796, 107.0855, 105.0697	JatamaninD
P04	10.289	$C_{10}H_8O_3$	176.0473	177.0546	177.0551	$[M+H]^+$	−2.72	159.0435, 148.0509, 121.061, 116.9301	Desacylbaldrinal
P05	10.910	$C_{21}H_{34}O_{11}$	462.2101	485.1993	485.1997	$[M+Na]^+$	−379	383.1303, 305.1341, 203.0609, 163.0747	Valerosidate
P06	11.430	$C_{25}H_{24}O_{12}$	516.1268	517.1341	517.1346	$[M+H]^+$	−1.06	499.1148, 355.1087, 337.0914, 319.0813, 163.0356	Isochlorogenic acid B*
P07	12.974	$C_{28}H_{34}O_{15}$	610.1898	611.1970	611.1997	$[M+H]^+$	0.08	465.1375, 303.0850	Hesperidin*
P08	20.023	$C_{28}H_{32}O_{14}$	592.1792	593.1865	593.1892	$[M+H]^+$	−0.03	447.1311,285.0770	Linarin*
P09	21.811	$C_{12}H_{10}O_4$	218.0579	219.0652	219.0658	$[M+H]^+$	−4.19	177.0550, 159.0431, 131.0480	Baldrinal*
P10	25.142	$C_{18}H_{26}O_8$	370.1628	393.1520	393.1526	$[M+Na]^+$	−1.65	260.1025, 177.0504	JatamaninU
P11	28.431	$C_{15}H_{22}O_5$	282.1467	283.1540	283.1542	$[M+H]^+$	−0.71	181.0865, 164.0775, 101.0668	RupesinE
P12	29.153	$C_{18}H_{27}ClO_8$	406.1416	429.1287	429.1290	$[M+Na]^+$	4.58	295.0349, 267.0327, 249.0299, 227.0487, 217.0456	ChlorovaltrateH
P13	29.757	$C_{19}H_{28}O_8$	384.1784	407.1676	407.1698	$[M+Na]^+$	−5.63	223.0965, 193.0861, 191.0703, 163.0751	Jatamanvaltrate N
P14	30.697	$C_{19}H_{28}O_8$	384.1806	407.1676	407.1698	$[M+Na]^+$	−5.63	245.0787, 224.1043, 193.0860	Jatamanvaltrate M
P15	31.385	$C_{19}H_{28}O_8$	384.1784	407.1676	407.1698	$[M+Na]^+$	−5.63	245.0825, 223.0978, 191.0712, 159.0442	JatairidoidA/ Jatairidoid B
P16	39.424	$C_{23}H_{34}O_9$	454.2203	477.2095	477.2115	$[M+Na]^+$	−4.39	273.0741, 219.0627, 213.0535, 191.0713, 159.0463	ValeriandoidF
P17	40.851	$C_{22}H_{32}O_9$	440.2046	463.1939	463.1964	$[M+Na]^+$	−5.72	237.0784, 219.0639, 199.0362, 177.0580, 159.0429	5-Hydroxydidrovaltrate
P18	42.193	$C_{27}H_{42}O_{12}$	558.2676	581.2568	581.2596	$[M+Na]^+$	−4.93	480.1877, 463.1838	ValeriotriateB
P19	43.587	$C_{23}H_{34}O_9$	454.2203	477.2095	477.2115	$[M+Na]^+$	−4.39	375.1442, 293.1403, 273.0735, 261.1107, 251.0927, 159.0412	Jatadoid A
P20	47.373	$C_{25}H_{39}ClO_{10}$	534.2232	552.2570	552.2574	$[M+NH_4]^+$	−0.01	457.1331, 357.0883, 273.0263	ChlorovaltrateE
P21	52.261	$C_{24}H_{33}ClO_{10}$	516.1762	534.2101	534.2109	$[M+NH_4]^+$	0.09	277.0232	ChlorovaltrateM
P22	53.453	$C_{24}H_{34}O_{10}$	482.2152	500.2490	500.2423	$[M+NH_4]^+$	−4.11	321.1392, 301.0737, 279.0839, 219.0674, 177.0539	10-Acetoxyvaltrathydrin
P23	58.738	$C_{24}H_{32}O_{10}$	480.1995	503.1888	503.1884	$[M+Na]^+$	0.77	503.1884, 321.1226, 219.0634, 159.0421	Acevaltrate*
P24	65.050	$C_{23}H_{34}O_9$	454.22.03	472.2541	477.2561	$[M+NH_4]^+$	−4.39	273.0741, 251.0916, 219.0627, 201.0598, 159.0463	ValejatadoidE
P25	65.798	$C_{28}H_{44}O_{12}$	572.2833	595.2725	595.2740	$[M+Na]^+$	−2.62	494.2079, 293.0902	JatamanvaltrateE
P26	66.805	$C_{27}H_{41}ClO_{11}$	576.2337	599.2230	599.2208	$[M+Na]^+$	−5.1	498.162, 398.1055	VolvaltrateB
P27	67.187	$C_{27}H_{40}O_{11}$	540.2571	558.2909	558.2895	$[M+Na]^+$	−5.4	301.1017, 159.0462, 121.0607	IVHD-Valtrate
P28	67.900	$C_{25}H_{34}O_{10}$	494.2152	517.2044	517.2065	$[M+Na]^+$	−4.21	237.0679, 219.0653, 177.0504, 159.0449	1-Homoacevaltrate
P29	68.811	$C_{25}H_{34}O_{10}$	494.2152	517.2044	517.2065	$[M+Na]^+$	−4.21	297.1112, 219.0679, 159.0471	1-Homoisoacevaltrate
P30	69.002	$C_{15}H_{16}O_4$	260.1649	261.1121	261.1136	$[M+H]^+$	−5.63	159.0439, 131.0419, 101.062	Homobaldrinal
P31	70.831	$C_{10}H_{12}O_4$	196.0736	219.0628	219.0614	$[M+Na]^+$	7.04	159.0438, 131.0489, 121.0643	JatamaninB
P32	71.163	$C_{22}H_{30}O_8$	422.1941	445.1833	445.1821	$[M+Na]^+$	3.29	445.1819, 321.1324, 219.0640	Valtrate*
P33	72.643	$C_{34}H_{52}O_{15}$	700.3331	723.3198	723.3240	$[M+Na]^+$	−5.95	521.1982, 461.174, 419.1298, 133.0875	JatamanvaltrateA
P34	73.649	$C_{28}H_{42}O_{11}$	554.2727	572.3065	572.3051	$[M+NH_4]^+$	2.59	462.1805,375.1431,339.12,260.0615	JatamanvaltrateO
P35	78.749	$C_{32}H_{50}O_{13}$	642.3251	665.3144	665.3180	$[M+Na]^+$	−5.66	464.1980,363.1321,339.1527	JatamanvaltrateB
P36	79.224	$C_{22}H_{30}O_8$	422.1941	423.2013	423.2036	$[M+H]^+$	−5.34	219.0651,177.0548,159.0427,149.0594	Isovaltrate

*: comparing with the reference standards.

3.3.2. *Anti-inflammatory effects of V. Jatamansi in vitro*

To induce an inflammation model in macrophages, 1 μg/mL LPS was stimulated for 24 h (Cho et al., 2023). The nitric oxide (NO) levels were measured in the cell supernatant using Griess reagents. The results showed that LPS significantly enhanced the NO production ($P < 0.01$), indicating that the inflammation model was successfully established. In parallel, *V. jatamansi* (0.78 ~ 50 μg/mL) decreased the level of NO production on LPS-induced RAW264.7 cells in a dose-dependent manner (Fig. 3**B**). In inflammatory diseases, pro-inflammatory factors were elevated, including IL-6, IL-1β, TNF-α (Jing et al., 2021). The mRNA expression of IL-6, IL-1β and TNF-α was examined. It was shown that the mRNA levels of IL-6, IL-1β and TNF-α were significantly increased by LPS than control group ($P < 0.01$), while *V. jatamansi* treatment significantly reversed the elevated expression levels caused by LPS. ($P < 0.05$) (Fig. 3**C-E**). This indicated that *V. jatamansi* extracts have good anti-inflammatory activity in our experiment.

3.3.3. *V. Jatamansi regulate key targets in vitro*

The anti-inflammatory activity of *V. jatamansi* may involve the JAK-STAT signaling pathway, according to the KEGG pathway enrichment analysis results. To measure the mRNA and protein levels of JAK1 and STAT3, two key targets of this pathway, qRT-PCR and western blotting analysis were performed. It was found that LPS-treated RAW264.7 cells had higher JAK1 and STAT3 expression than control group ($P < 0.01$). *V. jatamansi* reduced JAK1 and STAT3 expression compared with the model group ($P < 0.05$) (Fig. 3**F-I**). These results indicate that *V. jatamansi* may have anti-inflammatory activity by modulating JAK1 and STAT3 mRNA and protein expression.

3.4. *Spectrum-effect relationship results*

3.4.1. *Similarity analysis*

The HPLC fingerprint chromatogram similarity analysis was used to analyze the variability among different batches. Firstly, good precision and reproducibility of the HPLC instrument and stability of the sample solution within 24 h were shown by the method validation results (**Table S5 in Supplementary materials**). The fingerprint method was suitable for fingerprint analysis.

Then, the fingerprint chromatograms of different batches were established (Fig. 4**)**. The common peaks with relatively high intensity that appeared in each sample were selected as the characteristic peaks of *V. jatamansi*. The 17 characteristic peaks were determined by peak alignment. The similarity values among the 24 batches varied from 0.705 to 0.997, suggesting that their chemical characteristics were

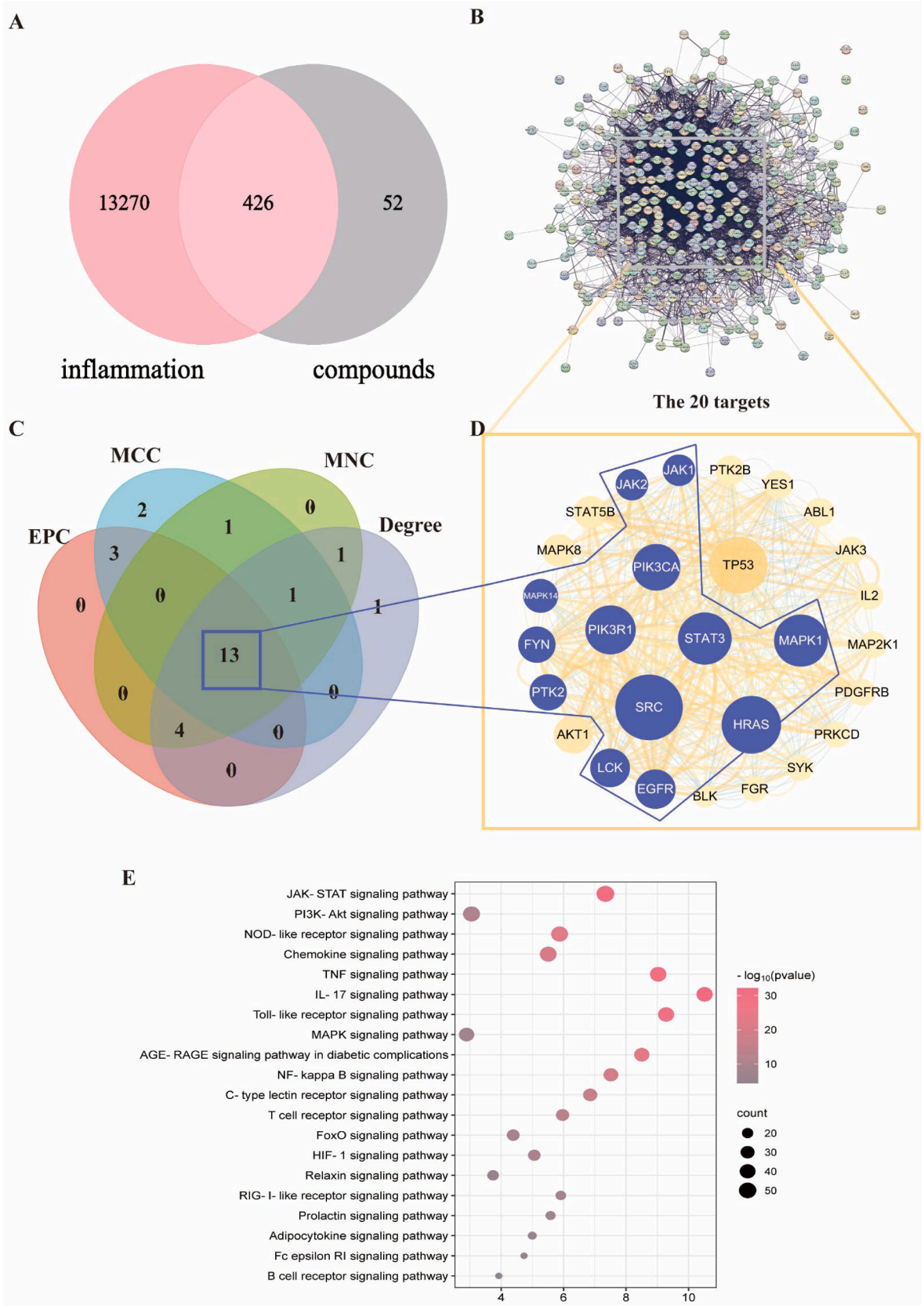

Fig. 2. Network pharmacology analysis. (A) Veen diagram. (B) PPI network. (C) Four parameters (degree, EPC, MCC, MNC) were used to calculate the intersection analysis of the top 20 targets (D) Core target network. The size of a node in the network indicates how important the node is in the network. The yellow circles refer to the potential targets (degree > 20), and the purple circles refer to the core targets (four parameter intersection targets). (E) KEGG pathway enrichment analysis.

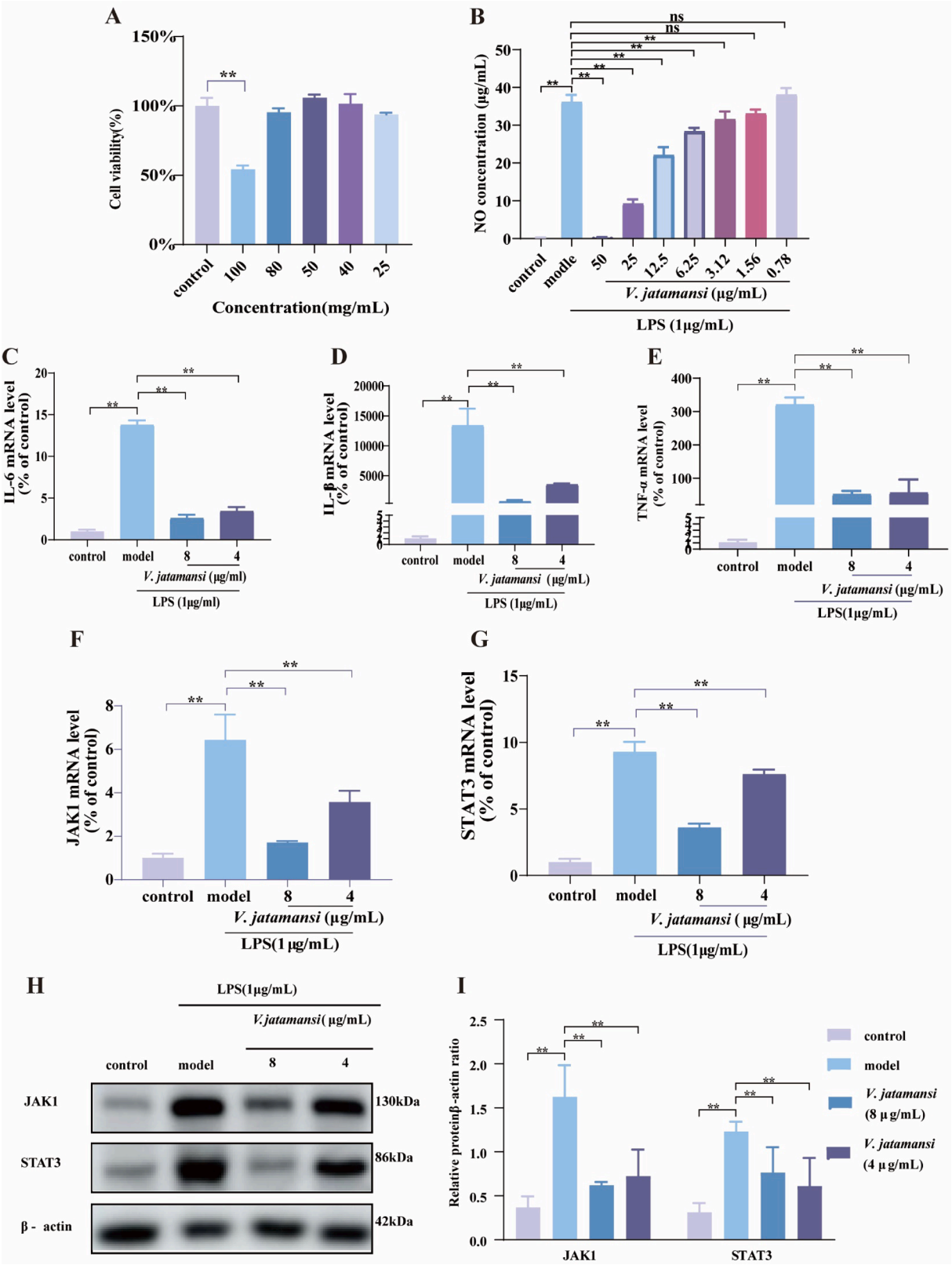

Fig. 3. Anti-inflammatory effects of *V. jatamansi* against RAW264.7 cells. (A) RAW264.6 cells were treated with different concentrations of *V. jatamansi* for up to 24 h to examine the cell viability. (B) NO concentration in cell supernatant. (C-G) The mRNA levels of IL-6, IL-1β, TNF-α, JAK1, STAT3 in cells. (H-I) The protein expression of JAK1, STAT3 in cells; (H)Western blot image and (I) densitometric analysis. Data are shown as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ and ns, no significant.

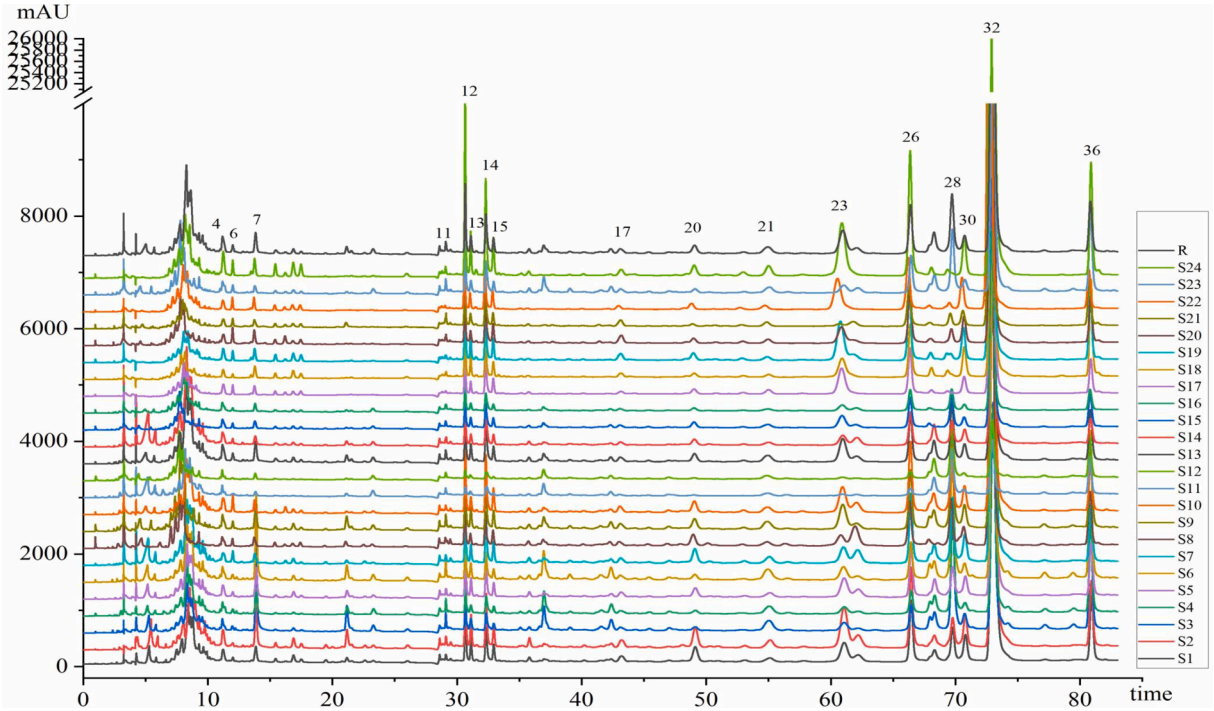

Fig. 4. HPLC fingerprints of 24 batches of *V. jatamansi* extracts.

highly similar and chemical fingerprints alone were insufficient to differentiate the quality (**Table S6 in Supplementary materials**).

3.4.2. NO inhibitory activity of V. Jatamansi

NO inhibition rate and IC_{50} value were used to evaluate the anti-inflammatory activity of 24 batches of *V. jatamansi*. Celecoxib has the effect of inhibiting NO as a reference for this experiment (Bekkouch et al., 2023; Cui et al., 2023). In this work, 24 batches of *V. jatamansi* extracts showed good inhibition of NO production (IC_{50} = 5.57–60.19 μg/mL) (Table 2). Surprisingly, S18, S20-S22, S24 batches showed excellent inhibition (IC_{50} = 5.57–7.625 μg/mL) than the positive control celecoxib (IC_{50} = 20.9 μM = 7.97 μg/mL). The value of IC_{50} indicating that there were significant differences in activity among different batches.

3.4.3. Bivariate correlation analysis

The area of 17 common peaks and the IC_{50} value of the anti-inflammatory activity were analyzed by BCA to determine their correlation degree and direction. The Pearson's model correlation coefficient showed that 13 common peaks had a negative correlation with the IC_{50} value, indicating that the 13 common peaks may possess anti-inflammatory activity. As shown in Table 3, among 9 common peaks, P6, P7, P12, P14, P17, P23, P26, P30 and P32 were showed significant correlation between peaks area and IC_{50} value ($P < 0.05$), suggesting that 9 compounds may have anti-inflammatory activity.

3.4.4. Grey relational analysis

To screen the potential anti-inflammatory components, the spectrum-effect relationship between IC_{50} and the area value of 17 common peaks were studied by the GRA method. As can be seen from Table 3, the correlation values between 17 common peaks and inhibition of NO production were 0.6883 ~ 0.8549. The results indicated that 16 common peaks may be highly correlated with anti-inflammatory activity (with GRA > 0.7). To prevent bias caused by a single screening method, we combined the methods of GRA and BCA in a comprehensive analysis, and the results showed that P6, P7, P12, P14, P17, P23, P26, P30 and P32 were considered as potentially bioactive compounds with anti-inflammatory activity.

Table 2
Anti-inflammatory activity of 24 batches of *V. jatamansi*.

Sample	IC_{50} (μg/mL)	Sample	IC_{50} (μg/mL)
S1	30.82	S14	40.33
S2	26.79	S15	15.92
S3	17.88	S16	11.70
S4	32.29	S17	48.13
S5	25.23	S18	7.625
S6	60.19	S19	8.686
S7	20.51	S20	7.321
S8	22.60	S21	6.888
S9	27.24	S22	7.226
S10	13.24	S23	24.32
S11	12.94	S24	5.572
S12	43.89	celecoxib	20.90 (μM)
S13	43.33		

Table 3
The results of GRA and BCA of *V. jatamansi* for anti-inflammatory activity.

Peaks	Case	GRA		BCA	
		Correlations	Rank	Correlations	Significantly
P04	24	0.811	10	−0.383	0.065
P06	24	0.855	1	−0.524	0.009
P07	24	0.726	16	−0.475	0.019
P11	24	0.761	13	0.029	0.893
P12	24	0.838	3	−0.602	0.002
P13	24	0.824	7	−0.382	0.065
P14	24	0.847	2	−0.649	0.001
P15	24	0.753	14	0.008	0.970
P17	24	0.812	9	−0.554	0.005
P20	24	0.769	12	−0.095	0.659
P21	24	0.750	15	0.058	0.788
P23	24	0.832	5	−0.510	0.011
P26	24	0.833	4	−0.549	0.005
P28	24	0.688	17	0.349	0.095
P30	24	0.830	6	−0.537	0.007
P32	24	0.823	8	−0.473	0.020
P36	24	0.771	11	−0.055	0.798

Based on principle of measurability, easily acquired standard and specificity, we predict that P6 (isochlorogenic acid B), P7 (hesperidin), P23 (acevaltrate) and P32 (valtrate) may be potential combinatorial quality markers for the anti-inflammatory activity of *V. jatamansi*.

3.5. The verification of the combinatorial quality markers

3.5.1. In silico ADME prediction

The pharmacokinetic parameters of the potential combinatorial quality markers were investigated to assess their bioavailability and absorption. Previous studies showed that hesperidin has good absorption properties *in vivo* (Li et al., 2023), but acevaltrate and valtrate have not been reported. The pharmacokinetic and physicochemical parameters of acevaltrate and valtrate were predicted using SwissADME website. The prediction results indicated that both compounds had favorable lipophilicity (WLogP = 2.35, 2.66) and water solubility (class: soluble) (**Table S7 in Supplementary materials**). According to different reporting filters provided, acevaltrate complied with the Lipinski, Egan and Muegge rules, while valtrate satisfied the Lipinski, Veber, Egan, and Muegge rules. The abbott bioavailability score for both compounds were 0.55, suggesting moderate bioavailability. Both compounds also exhibited high gastrointestinal absorption properties and good central nervous system safety profiles (no BBB permeability). Moreover, neither compound was predicted to be a substrate for CYP1A2, CYP2C19, CYP2C9 and CYP3A4, which could reduce the possibility of drug-drug interactions. The potential combinatorial quality markers exhibited favorable pharmacokinetic properties.

3.5.2. Anti-inflammatory effects of potential quality markers in vitro

CCK-8 assay was used to detect the cytotoxic effects of potential quality markers at different concentrations on RAW264.7 (Fig. 5**A-C;** **Fig. S4 in Supplementary materials**). The cell viability of 800 μM isochlorogenic acid B, 300 μM hesperidin, 6 μM valtrate, and 6 μM acevaltrate were used as experimental concentrations for subsequent experiments. NO, IL-6, IL-1β, and TNF-α are important inflammatory mediators involved in the inflammatory response. Firstly, the IC_{50} of hesperidin, acevaltrate, and valtrate were, 131.6 μM, 4.33 μM, and 2.58 μM, respectively (Fig. 5**D-F**). Unfortunately, isochlorogenic acid B with weak inhibition of NO production (IC_{50} = 637.9 μM) (**Fig. S4 in Supplementary materials**). A compound is considered weakly activity if its IC_{50} value is more than an order of magnitude higher than that of a positive control. Thus, isochlorogenic acid B was excluded as a quality marker. Subsequently, the mRNA expression of IL-6, IL-1β and TNF-α were decreased in three compounds treated with model group ($P < 0.05$) (Fig. 5**G-O**). In conclusion, these compounds may exert anti-inflammatory effects in LPS-stimulated macrophages by inhibiting NO, IL-6, IL-1β and TNF-α.

3.5.3. Potential quality markers regulate key targets

Under inflammatory conditions, the inhibitory effects of the three compounds on JAK1 and STAT3 expression were equally intriguing. Surprisingly, compared with the model group, the three compounds were able to significantly reduce the mRNA level of JAK1 and STAT3 ($P < 0.05$) (Fig. 6**A-F**), and also significantly reduce the protein expression levels of JAK1 and STAT3 ($P < 0.05$) (Fig. 6**G-L**). It was further confirmed that the three compounds (hesperidin, acevaltrate and valtrate) were shown to modulate the same anti-inflammatory targets as *V. jatamansi*.

3.5.4. Quantitative analysis of potential quality markers

In order to verify the potential quality markers of *V. jatamansi*, these three compounds (hesperidin, acevaltrate and valtrate) were analyzed quantitatively. The HPLC results of *V. jatamansi* samples and the three compounds showed good separation (Fig. 7**A and 7B**). The verification method showed that the RSD of each compound precision, repeatability, 24 h stability, and recovery were all less than 3.84 %, indicating that the method was feasible. The linear results showed that the three compounds all met the expected linear requirements within the target concentration range (**Table S8 for Supplementary materials**). The results showed that the contents of the three potential quality markers in different batches were different (Table 4). There was a positive correlation between the total content of the three compounds in the batches and their anti-inflammatory activity as measured by the IC_{50} values, indicating that the batches with lower total concentration of the three compounds had lower potency in inhibiting inflammation. (Fig. 7**C**).

3.5.5. Contribution of combinatorial quality markers

To evaluate the contribution rate of the combinatorial quality markers, we compared the NO inhibition rate of a certain batch of *V. jatamansi* extracts (S2) and a mixture of three combinatorial quality markers, with 1644.31 μg/g of hesperidin, 680.93 μg/g acevaltrate, and 1406.20 μg/g valtrate. Contribution rate = $AUC_{\text{combinatorial quality markers inhibition rate}} / AUC_{\text{V. jatamansi extracts inhibition rate}}$ * 100 %. The results showed that the inhibition trend of the combinatorial quality markers and the *V. jatamansi* extracts was consistent at different dilution factors, and the contribution of the combinatorial quality markers reached 45.0 % of the *V. jatamansi* (Fig. 7**D**). This suggests that the combinatorial quality markers are important in evaluating the anti-inflammatory activity of *V. jatamansi*.

4. Discussion

V. jatamansi is a traditional Chinese medicine with good anti-inflammatory activity for soothing ulcerative colitis and diarrhea. In the 2020 edition of the Chinese Pharmacopoeia, it is recorded that acevaltrate and valtrate are used as the thin-layer identification items of *V. jatamansi*, and the quantitative analysis is lacking. Chen *et al.* proposed hesperidin as a marker for the HPLC quantification of *V. jatamansi* from different sources in Guizhou province (Chen et al., 2010). Li *et al.* selected 3-*O*-caffeoylquinic acid, hesperidin, and 4,5-*O*-dicaffeoylquinic acid as the quality markers for *V. jatamansi*, considering their high content, good stability and broad pharmacological effects (Li et al., 2014). These chemical compositions of quality markers cannot be directly related to their activities, which significantly reduces the value of quality control. Therefore, this study aimed to develop a comprehensive strategy for screening combinatorial quality markers in *V. jatamansi* based on their anti-inflammatory activity for quality control.

The identification of chemical components is a crucial step for the quality control of TCMs. Previous studies showed that the ethyl acetate fraction of *V. jatamansi* has a mitigating effect on ulcerative colitis (Wang et al., 2023a). Therefore, the sample solution preparation method was based on and enhanced from previous studies in this study. Afterwards, the chemical components of *V. jatamansi* Jones were analyzed by UHPLC-Q-TOF-MS, which offers high sensitivity, accuracy and mass resolution for the identification and quantification of compounds. This study identified 36 components from *V. jatamansi* Jones, including 32 iridoids, 2 flavones and 2 organic acids. It revealed the composition of *V. jatamansi* Jones and provided a basis for further screening of anti-inflammatory active ingredients.

M1-type macrophages, which differ from lipopolysaccharide (LPS)-stimulated macrophages, secrete pro-inflammatory cytokines and mediators, such as IL-6, IL-1β and TNF-α. The anti-inflammatory effect of *V. jatamansi* was verified using LPS-induced macrophage inflammation model. In this study, the anti-inflammatory effects exerted by *V. jatamansi* was found through down-regulation of NO, IL-6, IL-1β and TNF-α levels.

TCMs are characterized by multi-component, multi-target and synergistic effects. Thus, the emerging field of network pharmacology analysis is based on widely available databases, which allows preliminary prediction of bioactive components and action mechanisms of TCMs. The KEGG results showed that gene enrichment factors and gene

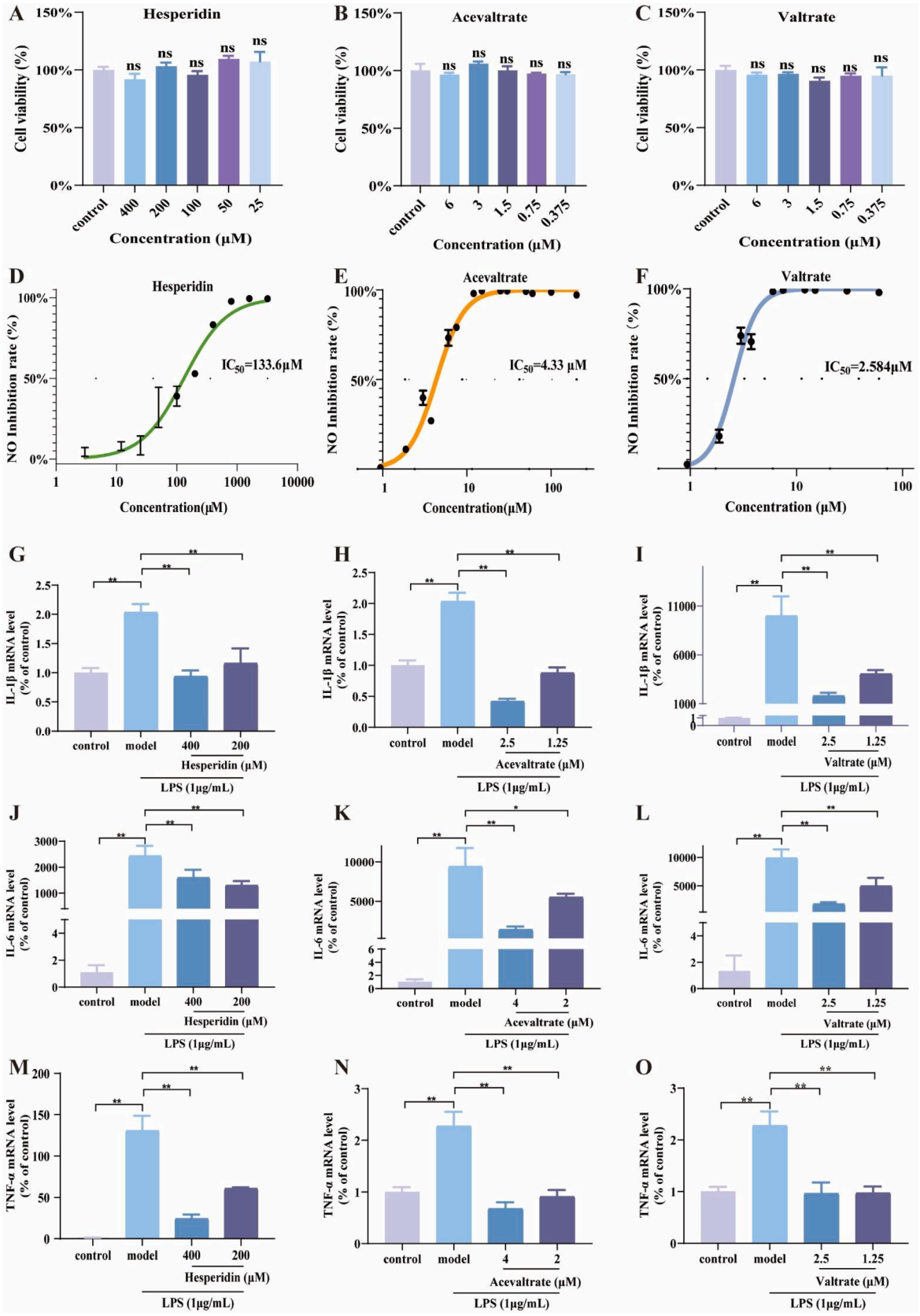

Fig. 5. Anti-inflammatory effects of potential quality markers on RAW264.7 cells. (A-C) Cell viability of hesperidin, valtrate and acevaltrate. (D-F) NO concentration in cell supernatant. (G-O) The mRNA levels of IL-6, IL-1β and TNF-α in cells. Data are shown as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ and ns, no significant.

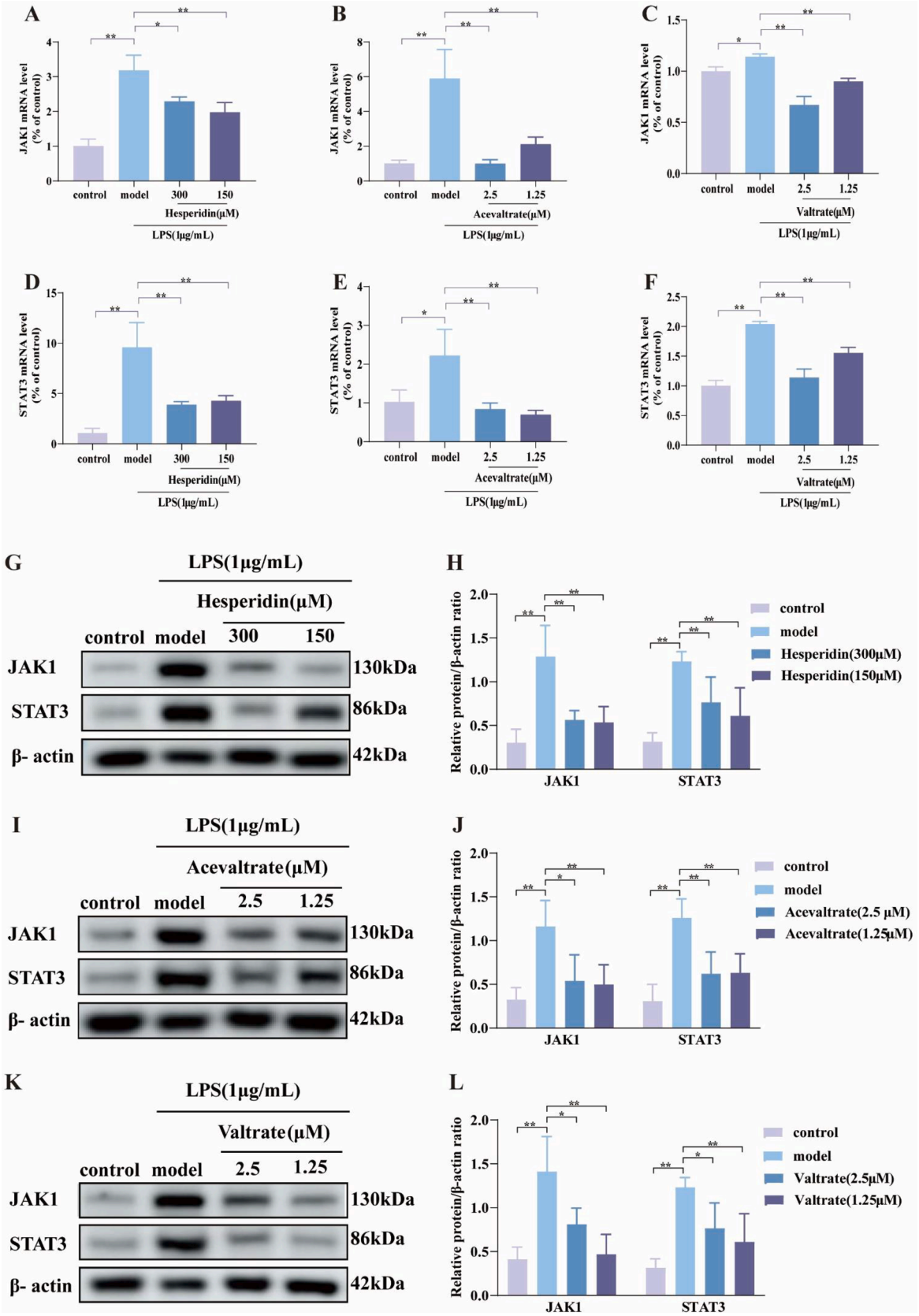

Fig. 6. *V. jatamansi* potential quality markers regulate the key targets. (A-F) The mRNA levels of JAK1 and STAT3 in cells. (G-L) The protein expression of JAK1, STAT3 in cells; (G-I) Western blot image and (J-H) densitometric analysis. Data are shown as mean ± SD. *$p < 0.05$, **$p < 0.01$ and ns, no significant.

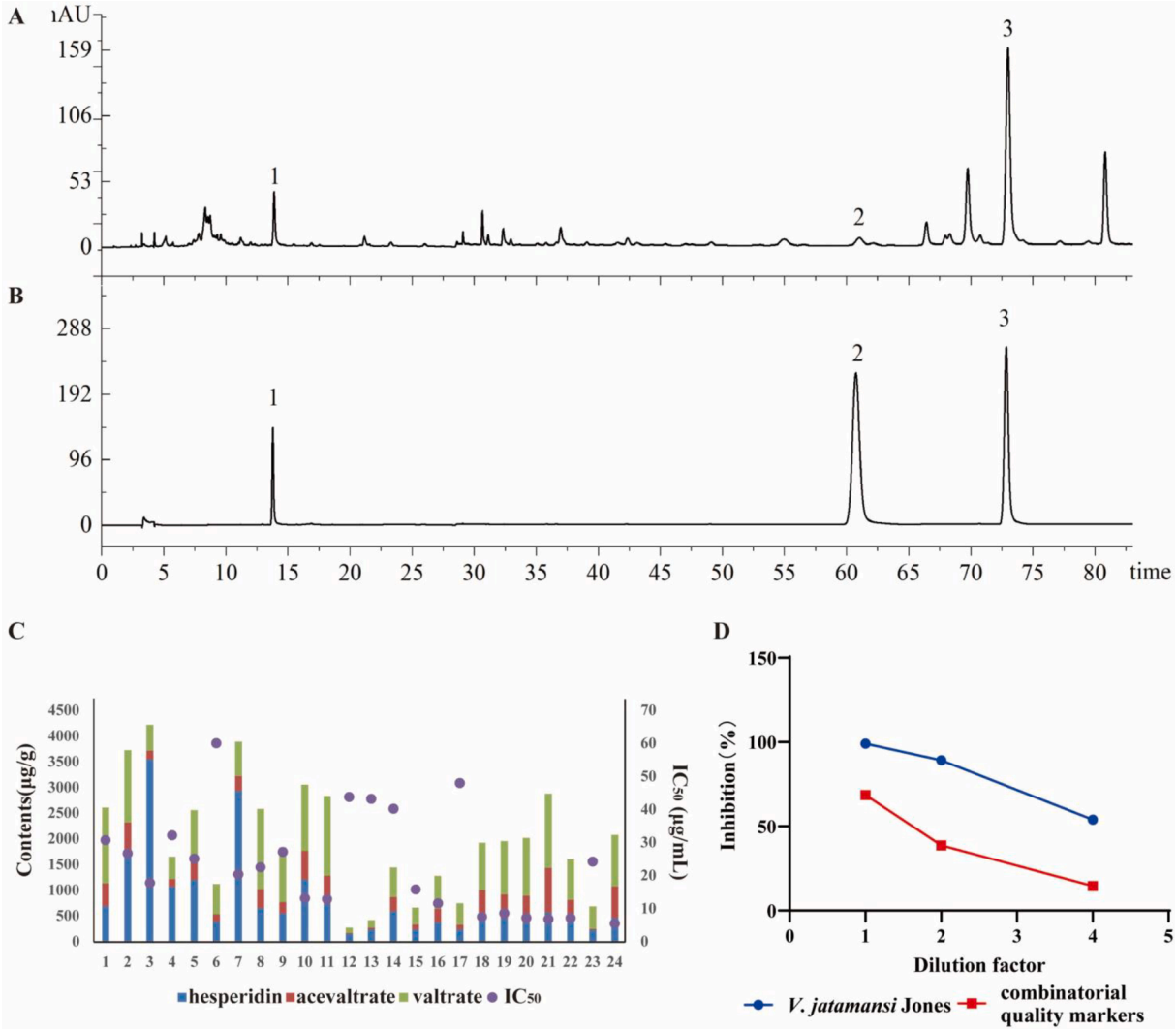

Fig. 7. Quantitative analysis and contribution of *V. jatamansi*. (A) HPLC chromatogram of *V. jatamansi*. 1: hesperidin; 2: acevaltrate 3: valtrate. (B) HPLC of chromatogram mixed standard solution. 1: hesperidin; 2: acevaltrate 3: valtrate. (C) Correlation of anti-inflammatory activity and combinatorial quality markers content of different batches of *V. jatamansi*. (D) Inhibition rates of anti-inflammatory activity of *V. jatamansi* and combinatorial quality markers at different dilutions.

number of JAK-STAT signaling pathway were significant, suggesting that *V. jatamansi* may exert anti-inflammatory effects through regulating these signaling pathways. The JAK-STAT signaling pathways transmit cytokine signals and regulates systemic inflammatory response (Lee et al., 2023) The network pharmacology analysis results were confirmed by experimentally testing two key targets (JAK1 and STAT3) that were highly related to *V. jatamansi* anti-inflammatory effect. The results showed that *V. jatamansi* and bioactivity quality markers inhibited JAK1 and STAT3 expression, which was consistent with the network pharmacology prediction. However, further validation of the anti-inflammatory effects of *V. jatamansi* and its active components using cellular and other animal models is needed for further studies.

The active compounds were initially explored using the spectrum-effect relationship combined with the chemometric strategy, based on the clarification of chemical compounds and anti-inflammatory activity. The correlation coefficients and significance of 17 common peaks in chemical fingerprint with the anti-inflammatory activity were obtained based on the application of the BCA mathematical model. The IC_{50} values were negatively correlated with 13 common peaks, suggesting that these compounds may enhance the anti-inflammatory activity of *V. jatamansi* with their increased content. Among them, 9 common peaks, including compounds P6 (isochlorogenic acid B), P7 (hesperidin), P12 (chlorovaltrateH), P14 (jatamanvaltrate M), P17 (5-hydroxydidrovaltrate), P23(acevaltrate), P26 (volvaltrateB), P30 (homobaldrinal) and P32 (valtrate), which had significant correlation by GCA analysis. According to the principle of measurability, easily acquired standard and specificity (Chu et al., 2022), four compounds were selected as potential quality markers, including P6 (isochlorogenic acid B), P7 (hesperidin), P23 (acevaltrate) and P32 (valtrate). Then the inhibitory ability of NO production of four compounds were evaluated, and three compounds (hesperidin, acevaltrate and valtrate) were finally considered as potential combinatorial quality markers exhibiting potential anti-inflammatory activity. Isochlorogenic acid B was excluded out from the quality marker with weak inhibition of NO production. While hesperidin has been reported to have good absorption properties *in vivo* (Li et al., 2023), the pharmacokinetic properties of acevaltrate and valtrate remains unknown. The quality markers (acevaltrate and valtrate) exhibited favorable pharmacokinetic properties, such as high lipophilicity, high gastrointestinal absorption and good central nervous system safety, and complied with several drug-likeness prediction models (e.g., Lipinski, Egan, and Muegge), which suggested their potential for absorption and efficacy *in vivo*.

The anti-inflammatory activity and molecular targets of combinatorial quality markers need to be validated *in vitro*. This is essential for the assessment of their reliability as quality markers of anti-inflammatory activity in *V. jatamansi*. These three compounds showed

Table 4
The content of 3 compounds in 24 batch**es of samples. (μg/g, mean ± SD, n = 3).**

Sample	Hesperidin(μg/g)	Acevaltrate(μg/g)	Valtrate(μg/g)
S1	692.72 ± 1.83	448.07 ± 7.73	1470.72 ± 10.09
S2	1644.31 ± 2.86	680.93 ± 7.64	1406.20 ± 0.64
S3	3550.42 ± 25.3	172.36 ± 5.45	502.69 ± 3.35
S4	1073.98 ± 10.76	149.39 ± 7.48	433.76 ± 3.72
S5	1206.99 ± 34.07	377.28 ± 1.42	978.86 ± 3.76
S6	392.53 ± 7.35	146.46 ± 2.69	585.79 ± 4.16
S7	2940.91 ± 7.55	287.8 ± 12.75	669.19 ± 2.08
S8	658.95 ± 1.03	367.51 ± 4.95	1559.9 ± 17.82
S9	554.29 ± 3.73	223.79 ± 2.02	1060.35 ± 0.07
S10	1215.37 ± 12.78	556.95 ± 7.82	1286.13 ± 5.34
S11	733.97 ± 15.58	553.6 ± 2.71	1551.39 ± 3.92
S12	152.9 ± 7.60	20.19 ± 1.01	103.87 ± 1.45
S13	241.74 ± 1.93	36.29 ± 1.61	144.14 ± 2.71
S14	595.9 ± 5.36	276.32 ± 10.36	575.77 ± 3.54
S15	241.03 ± 11.95	95.16 ± 1.39	329.05 ± 3.80
S16	379.61 ± 5.72	264.91 ± 3.77	641.96 ± 1.27
S17	249.85 ± 2.88	91.30 ± 2.75	418.01 ± 2.91
S18	446.98 ± 1.39	562.79 ± 7.71	918.11 ± 5.65
S19	458.38 ± 13.94	466.66 ± 18.21	1037.94 ± 1.76
S20	364.75 ± 4.34	533.65 ± 22.31	1124.24 ± 4.94
S21	570.00 ± 0.96	868.66 ± 8.35	1444.40 ± 3.07
S22	516.82 ± 2.03	301.26 ± 9.35	792.25 ± 4.40
S23	230.94 ± 7.04	18.86 ± 0.91	438.16 ± 4.61
S24	474.65 ± 9.33	611.28 ± 6.88	1002.71 ± 1.42

anti-inflammatory effects in RAW264.7 cells by inhibiting the production of inflammatory mediators (NO, IL-6, IL-1β and TNF-α) (Fig. 5**G-O**) and suppressing the expression of key targets (JAK1 and STAT3) (Fig. 6**A-L**). It was suggested that they play an important anti-inflammatory role. In order to investigate the composition of the combinatorial quality markers, the content of three compounds in 24 batches of *V. jatamansi* were analyzed using HPLC. HPLC has been widely used to determine the content of *V. jatamansi* with better analytical parameters, indicating the reliability of this method (**Table S9 in Supplementary materials**). However, Shukla *et al.* developed a method to measure the contents of nine compounds (quinic acid, protocatechuic acid, caffeic acid, orientin, kaempferol-3-O-rutinoside, luteolin, eugenol, isoeugenol and valerenic acid) in *V. jatamansi* without considering its activity. Therefore, activity-oriented methods were developed for the determination of combinatorial quality markers (hesperidin, acevaltrate and valtrate) with lower limits of quantification in the range of 0.80–2.00 μg/mL. Interestingly, the content of three compounds was positively correlated with anti-inflammatory activity (Fig. 7**C**), suggesting that three compounds have the potential to predict the quality marker of *V. jatamansi*. Furthermore, the contribution of the combinatorial quality markers was assessed, which accounted for 45.0 % of *V. jatamansi*. It was concluded that the combinatorial quality markers are critical in evaluating the anti-inflammatory activity. Thus, the combinatorial quality markers (hesperidin, acevaltrate and valtrate) that were selected have the following characteristics: 1) they are effortless to acquire and quantify; 2) they have anti-inflammatory activities; 3) they have good druglikeness and may suitable accumulation in the herb and absorption in body to take effect.

5. Conclusions

The activity-oriented combinatorial quality markers of *V. jatamansi* based on its anti-inflammatory effects were screened by an integrated process combining spectral-effect relationships and activity validation. Hesperidin, acevaltrate and valtrate could be considered as combinatorial quality markers which contributed 45.0 % for the quality control. The relationships between components and anti-inflammatory activity were revealed. Furthermore, this strategy is an effective approach to find Q-markers and provides a valuable perspective for quality control of herbal medicines. This strategy also offers a useful way to identify Q-markers and a valuable insight for quality control of TCMs.

Funding

This work was supported by National Natural Science Foundation of China (81973704), National Key R&D Program of China (2019YFC1711000) and Scientific research Project of Tianjin Education Commission (2019KJ078), Tianjin Graduate Research Innovation Project (2022BKY183), TUTCM Graduate Research Innovation Project (YJSKC-20221001).

CRediT authorship contribution statement

Chunxiao Liang: Methodology, Investigation, Writing – original draft. **Kunze Du:** Writing – review & editing. **Shujing Chen:** Software, Validation.**Ye Shang:** Formal analysis. **Lirong Wang:** Data curation. **Shuangqi Wang:** Data curation, Investigation. **Omachi Daniel Ogaji:** Writing - review & editing. **Jin Li:** Conceptualization, Resources, Supervision, Writing - original draft. **Yanxu Chang:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2023.105367.

References

An, Q., Wang, L., Ding, X.-Y., Shen, Y.-J., Hao, S.-H., Li, W.-J., Li, H.-Y., Wang, T., Zhan, Z.-L., Zheng, Y.-G., Guo, L., Zhang, D., 2022. Validation of Sennae Folium specification grade classification based on UPLC-Q-TOF/MS spectrum-effect relationship. Arab. J. Chem. 15 (11), 104223 https://doi.org/10.1016/j.arabjc.2022.104223.

Bekkouch, O.-A.-O., Zengin, G., Harnafi, M., Touiss, I., Khoulati, A., Saalaoui, E., Harnafi, H., Abdellattif, M.-A.-O., Amrani, S., 2023. Anti-Inflammatory Study and Phytochemical Characterization of Zingiber officinale Roscoe and Citrus limon L. Juices and Their Formulation. ACS Omega 8 (30), 26715–26724. https://doi.org/10.1021/acsomega.2c04263.

Chen, L., Bao, J.K., Xu, H., Xiong, H.L., Chen, W.L., 2010. Quality analysis for Valeriana jatamansi Jones from different habitats in Guizhou. China Mod Med. 17 (13), 46–47.

Cho, S.-H., Jeong, H., Park, S., Shin, H.-T., Lee, H.-M., Kim, K.-N., 2023. Anti-inflammatory activity of Echinosophora koreensis nakai root extract in lipopolysaccharides-stimulated RAW 264.7 cells and carrageenan-induced mouse paw edema model. J. Ethnopharmacol. 302, 115940 https://doi.org/10.1016/j.jep.2022.115940.

Chu, Y.-J., Wang, M.-L., Wang, X.-B., Zhang, X.-Y., Liu, L.-W., Shi, Y.-Y., Zuo, L.-H., Du, S.-Z., Kang, J., Li, B., Cheng, W.-B., Sun, Z., Zhang, X.-J., 2022. Identifying quality markers of Mailuoshutong pill against thromboangiitis obliterans based on chinmedomics strategy. Phytomedicine 104, 154313. https://doi.org/10.1016/j.phymed.2022.154313.

Cui, E., Qian, S., Li, J., Jiang, X., Wang, H., Du, S., Du, L., 2023. Discovery of Coixol Derivatives as Potent Anti-inflammatory Agents. J. Nat. Prod. 86 (8), 1950–1959. https://doi.org/10.1021/acs.jnatprod.3c00309.

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7 (1), 42717. https://doi.org/10.1038/srep42717.

Di, H.Y., Shi, J.L., Yan, X.L., Zhao, R., Liu, Y., Xiao, P.G., 2007. Determination of valtrate, acevaltrate, and their degradation product-baldrinal in Valeriana walichi by HPLC analysis. Zhong Cao Yao(12), 1892-1894.

Du, K.Z., Cui, Y., Chen, S., Yang, R., Shang, Y., Wang, C., Yan, Y., Li, J., Chang, Y.X., 2022. An integration strategy combined progressive multivariate statistics with anticoagulant activity evaluation for screening anticoagulant quality markers in Chinese patent medicine. J. Ethnopharmacol. 114964 https://doi.org/10.1016/j.jep.2021.114964.

Han, J., Xu, K., Yan, Q.X., Sui, W.W., Zhang, H.T., Wang, S.J., Zhang, Z., Wei, Z.Y., Han, F., 2022. Qualitative and quantitative evaluation of Flos Puerariae by using chemical fingerprint in combination with chemometrics method. J. Pharm. Anal. 12 (3), 489–499. https://doi.org/10.1016/j.jpha.2021.09.003.

Hao, J.X., Yang, L.J., Fan, X., Zha, L.C., Cui, L.L., Cheng, B.D., Li, Y.Z., Ma, Y.S., 2022. Establishment of GC-MS fingerprint and quantitative analysis of multi-components of volatile oil from Compound Zhizhuxiang. Zhong Cao Yao 53 (22), 7048–7057.

Jing, W., Dong, S., Luo, X., Liu, J., Wei, B., Du, W., Yang, L., Luo, H., Wang, Y., Wang, S., Lu, H., 2021. Berberine improves colitis by triggering AhR activation by microbial tryptophan catabolites. Pharmacol. Res. 164, 105358 https://doi.org/10.1016/j.phrs.2020.105358.

Lee, H.-S., Kwon, Y.-J., Seo, E.-B., Kim, S.-K., Lee, H., Lee, J.-T., Chang, P.-S., Choi, Y.J., Lee, S.-H., Ye, S.-K., 2023. Anti-inflammatory effects of Allium cepa L. peel extracts via inhibition of JAK-STAT pathway in LPS-stimulated RAW264.7 cells. J. Ethnopharmacol. 317, 116851 https://doi.org/10.1016/j.jep.2023.116851.

Li, R., Li, X.P., Wu, Y., 2008. Quantitative Determination of Total flavones in Valeriana jatamansi Jones by UV Spectrophotometry. J. Liaoning Univ. Tradit. Chin. Med. 10 (12), 149–150. https://doi.org/10.13194/j.jlunivtcm.2008.12.151.lir.081.

Li, Y.Y., Li, F.T., Jin, L.Q., Chen, Y., Ji, Y.P., Wu, Y., Yang, H.F., Qiao, Q.P., Bu, S.J., Li, Y. J., 2018. TLC ldentification and HPLC Determination of Hesperidin in Valeriana Jatamansi Capsule. Prog Veterinary Med 39 (12), 96–99. https://doi.org/10.16437/j.cnki.1007-5038.2018.12.019.

Li, J., Liu, J., Qiao, L., Wang, S.L., Zheng, L., Dong, L., Li, Y.J., 2014. Simultaneous Determination of Three Components in Valeriana jatamansi by HPLC. Chin. Pharm. J. 49 (20), 1840–1844.

Li, G., Wang, X., Luo, L., Zhang, H., Song, X., Zhang, J., Liu, D., 2023. Identification of chemical constituents of Qingjin Yiqi granules and comparative study on pharmacokinetics of 23 main bioactive components in normal and Lung-Qi deficiency rats by UPLC-MS/MS method. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1226, 123802 https://doi.org/10.1016/j.jchromb.2023.123802.

Li, Z., Zhang, X., Liao, J., Fan, X., Cheng, Y., 2021. An ultra-robust fingerprinting method for quality assessment of traditional Chinese medicine using multiple reaction monitoring mass spectrometry. J. Pharm. Anal. 11 (1), 88–95. https://doi.org/10.1016/j.jpha.2020.01.003.

Liu, H., Liu, D., Jiang, M.Y., Zhao, X.D., Li, R.T., Li, H.M., 2021. Iridoids from Valeriana jatamansi with anti-inflammatory and antiproliferative properties. Phytochemistry 184, 112681. https://doi.org/10.1016/j.phytochem.2021.112681.

Liu, Z., Qu, J., Ke, F., Zhang, H., Zhang, Y., Zhang, Q., Li, Q., Bi, K., Xu, H., 2022. Material basis elucidation and quantification of dandelion through spectrum-effect relationship study between UHPLC fingerprint and antioxidant activity via multivariate statistical analysis. Molecules 27 (9), 2632. https://doi.org/10.3390/molecules27092632.

Lv, Y., Wu, H., Hong, Z., Wei, F., Zhao, M., Tang, R., Li, Y., Ge, W., Li, C., Du, W., 2023. Exploring active ingredients of anti-osteoarthritis in raw and wine-processed Dipsaci Radix based on spectrum-effect relationship combined with chemometrics. J. Ethnopharmacol. 309, 116281 https://doi.org/10.1016/j.jep.2023.116281.

Ma, Y.L., Wu, Z.M., Liu, X., Lan, J.E., Zai, W.J., Jin, X., Xie, H., Mu, Q., Liu, H.R., 2022. Antidiarrheal activity of the extracts of Valeriana jatamansi Jones on castor oil-induced diarrhea mouse by regulating multiple signal pathways. J. Ethnopharmacol. 298, 115560 https://doi.org/10.1016/j.jep.2022.115560.

Maurya, A.K., Kumar, A., Agnihotri, V.K., 2022. New iridoids from the roots of Valeriana jatamansi Jones. Nat. Prod. Res. 36 (13), 3360–3367. https://doi.org/10.1080/14786419.2020.1858412.

Quan, L.Q., Wang, Y., Tang, J.X., Zhou, Y., Liao, C.C., Liu, D., Zhao, X.D., Li, R.T., Li, H. M., 2022. Valeridoids G - Q, eleven seco-iridoids from Valeriana jatamansi and their bioactivites. Chem. Biodivers. 19 (9), e202200609.

Shi, D.Q., Liu, J.J., Feng, Y.M., Zhou, Y., Liao, C.C., Liu, D., Li, R.T., Li, H.M., 2023. Iridoids and sesquiterpenoids from Valeriana officinalis and their bioactivities. Phytochemistry 205, 113478. https://doi.org/10.1016/j.phytochem.2022.113478.

Shukla, V., Singh, P., kumar, D., Konwar, R., Singh, B., Kumar, B., 2021. Phytochemical analysis of high value medicinal plant Valeriana jatamansi using LC-MS and it's in-vitro anti-proliferative screening. Phytomed Plus 1(2), 100025. https://doi.org/10.1016/j.phyplu.2021.100025.

Subhan, F., Karim, N., Ibrar, M., 2007. Anti-Inflammatory Activity of Methanolic and Aqueous Extracts of Valeriana Wallichii DC Rhizome. Pak J Plant Sci 13, 103–108.

Wang, Q., Chen, G., Chen, X., Liu, Y., Qin, Z., Lin, P., Shang, H., Ye, M., He, L., Yao, Z., 2023b. Development of a three-step-based novel strategy integrating DMPK with network pharmacology and bioactivity evaluation for the discovery of Q-markers of traditional Chinese medicine prescriptions: Danlou tablet as an example. Phytomedicine 108, 154511. https://doi.org/10.1016/j.phymed.2022.154511.

Wang, Q., Li, H., You, J., Yan, B., Jin, W., Shen, M., Sheng, Y., He, B., Wang, X., Meng, X.-A.-O., Qin, L., 2023c. An integrated strategy of spectrum-effect relationship and near-infrared spectroscopy rapid evaluation based on back propagation neural network for quality control of Paeoniae Radix Alba. Anal. Sci. 39, 1233–1247. https://doi.org/10.1007/s44211-023-00334-4.

Wang, S.N., Yao, Z.W., Zhao, C.B., Ding, Y.S., Jing, L., Bian, L.H., Li, Q.Y., Wang, X.M., Shi, J.L., Guo, J.Y., Wang, C.G., 2021. Discovery and proteomics analysis of effective compounds in Valeriana jatamansi jones for the treatment of anxiety. J. Ethnopharmacol. 265, 113452 https://doi.org/10.1016/j.jep.2020.113452.

Wang, L., Zhang, H., Tang, F., Yan, H., Feng, W., Liu, J., Wang, Y.-A.-O., Tan, Y.-A.-O.-X., Chen, H., 2023a. Therapeutic Effects of Valeriana jatamansi on Ulcerative Colitis: Insights into Mechanisms of Action through Metabolomics and Microbiome Analysis. J. Proteome Res. 22, 2669–2682.

Yan, X., Hong, Y., Shi, J., Qin, Y., Zhang, J., Lin, Q., Chen, Z., Zhao, R., Cui, X., Gao, X., 2011. Influence of iridoid from Valeriana jatamansi on 5-HT and 5-HIAA in rats with irritable bowel syndrome. Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi =. China Journal of Chinese Materia Medica 36 (9), 1235–1238.

Yang, W., Zhang, Y., Wu, W., Huang, L., Guo, D., Liu, C., 2017. Approaches to establish Q-markers for the quality standards of traditional Chinese medicines. Acta Pharm. Sin. B 7 (4), 439–446. https://doi.org/10.1016/j.apsb.2017.04.012.

Zhen, Z.G., Ren, S.H., Ji, H.M., Ma, J.H., Ding, X.M., Feng, F.Q., Chen, S.L., Zou, P., Ren, J.R., Jia, L., 2017. Linarin suppresses glioma through inhibition of NF-κB/p65 and up-regulating p53 expression in vitro and in vivo. Biomed. Pharmacother. 95, 363–374. https://doi.org/10.1016/j.biopha.2017.08.023.

Zhou, R., Wang, L., Liu, W., Li, J., He, J.-A.-O., Yao, Y., Yuan, H., Chang, Y.-A.-O.-X., 2023a. In-capillary screening and quantifying the antioxidants of Yangxinshi Tablet using field-enhanced sample injection-micellar electrokinetic chromatography. J. Sep. Sci. 46 (13), e2300054.

Zhou, S., Zhuang, C., Liu, R., 2023b. KPNA2 promotes osteosarcoma growth and metastasis in a c-Myc-dependent manner via the hedgehog /GLI1 signaling pathway. Arab. J. Chem. 16 (7), 104805 https://doi.org/10.1016/j.arabjc.2023.104805.

Zhuang, G.D., Gu, W.T., Xu, S.H., Cao, D.M., Deng, S.M., Chen, Y.S., Wang, S.M., Tang, D., 2023. Rapid screening of antioxidant from natural products by AAPH-Incubating HPLC-DAD-HR MS/MS method: A case study of Gardenia jasminoides fruit. Food Chem. 401, 134091 https://doi.org/10.1016/j.foodchem.2022.134091.

Zong, K., Xu, K., Zhang, X., Wang, P., Wang, Z., Yang, S., Li, H., Ke, H., He, S., Hu, Y., Go, Y., Chan, X.H.F., Wu, J., Huang, Q., 2023. Exploring the mechanism of Huangqin Tang against skin lipid accumulation through network pharmacology and experimental validation. J. Ethnopharmacol. 313, 116581 https://doi.org/10.1016/j.jep.2023.116581.