Regular article

Discovery of quality marker and study on its pharmacodynamic differences in antirheumatoid arthritis in the three original plants of Clematidis Radix et Rhizoma

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Abstract

Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) technology was employed to analyze the chemical composition of three Clematidis Radix et Rhizoma sourced from different origins. Common characteristic components were identified through comprehensive literature review and the integration of fragment ion data. A rheumatoid arthritis (AA) model was induced in Sprague-Dawley (SD) rats using Freund's complete adjuvant, and simultaneous administration of extracts from the three original plants of Clematidis Radix et Rhizoma, along with different doses of magnoflorine, was conducted to evaluate differences in therapeutic efficacy. A common characteristic component was identified, magnoflorine, present in *Clematis chinensis* Osbeck. *Clematis hexapetala* Pall., and *Clematis manshurica* Rupr. Extracts from all three original plants, as well as each dose group of the Rhizomas of *Clematis chinensis* Osbeck. *Clematis hexapetala* Pall., *Clematis manshurica* Rupr., and magnoflorine, demonstrated efficacy in alleviating rheumatoid arthritis symptoms by enhancing cellular infiltration, reducing vascular opacification, and lowering serum levels of inflammatory factors. Magnoflorine has the potential to be used as a quality marker for the three original plants of Clematidis Radix et Rhizoma. Additionally, both the three original plants and magnoflorine exhibit therapeutic potential for rheumatoid arthritis, which provides a solid theoretical and experimental basis for the establishment of more scientifically grounded quality standards of Clematidis Radix et Rhizoma and promotes the rational utilization of medicinal resources.

Keywords: Clematidis Radix et Rhizoma; three fundamentals; quality markers; magnoflorine; rheumatoid arthritis

1 Introduction

Clematidis Radix et Rhizoma refers to the dried roots and rhizomes of the plants *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall., and *Clematis manshurica* Rupr., all of which belong to the Ranunculaceae family. According to *Chinese Pharmacopoeia* (2025 edition) [1], Clematidis Radix et Rhizoma has the ability to dispel wind-dampness and unblock the meridians. Clinical pharmacological studies have demonstrated that it possesses various therapeutic effects, including anti-inflammatory, analgesic, antioxidative,

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cholagogic, anti-malarial properties, and the ability to reduce cholesterol levels. This substance is widely used as traditional Chinese medicinal material. In clinical settings, compound preparations featuring Clematidis Radix et Rhizoma as a primary ingredient are frequently employed to treat conditions such as rheumatoid arthritis and arthralgia syndrome, exemplified by formulations like Lingxian Dieda Tablets and Jingfukang Granules [2].

The current edition of the *Chinese Pharmacopoeia* (2025 edition) [1] designates oleanolic acid as the reference component for determining the content of Clematidis Radix et Rhizoma. However, the original medicinal plant *Clematis hexapetala* Pall. is rarely available in the market because its oleanolic acid content does not meet the standards stipulated in the 2025 edition of the *Chinese Pharmacopoeia* [3,4]. Furthermore, the existence of numerous counterfeit products containing oleanolic acid makes it complicated to distinguish between authentic and fraudulent Clematidis Radix et Rhizoma. To solve these problems and ensure the integrity of medicinal resources, this study systematically analyzed the components of three original plants constituting Clematidis Radix et Rhizoma. The purpose of this study is to identify new quality markers and compare the pharmacodynamics of *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall., *Clematis manshurica* Rupr., and their common characteristic components in the treatment of rheumatoid arthritis. This study provides theoretical and experimental basis for establishing a rational quality standard of Clematidis Radix et Rhizoma and exploring the pharmacodynamic material basis related to rheumatoid arthritis.

2 Materials and methods

2.1 Materials

2.1.1 Instrument and materials

The ACQUITY UPLC H-Class ultra-high performance liquid chromatography system and the Vion QT of high-resolution mass spectrometer were both purchased from Waters Corporation, USA. The ACQUITY UPLC H-Class column (50 mm × 2.1 mm, 1.7 μm) and the TGL-16C table high-speed centrifuge were obtained from Shanghai Anting Electronic Instrument Factory. The standard inspection screen was sourced from Zhejiang Shangyu Huafeng Hardware Instrument Co., Ltd. The Varioskan Flash full-wavelength multifunctional microplate reader was purchased from Thermo Company, USA. The thermostat was obtained from Ningbo Xinzhi Technology Co., Ltd. The vortex mixer was sourced from Shanghai Qite Analytical Instruments Co., Ltd. The RE-2000A rotary evaporation instrument was purchased from Shanghai Yarong Biochemical Instrument Factory.

Acetonitrile, methanol, and formic acid were of chromatographic grade, all of which were purchased from Tianjin Concord Technology Co., Ltd.; Glacial acetic acid was purchased from Chengdu Colon Chemical Co., Ltd.; Magnoflorine (98%) was obtained from Chengdu Pfede Biotechnology Co., Ltd.; Tripterygium Glycoside Tablets were sourced from Shanghai Fudan Fuhua Pharmaceutical Co., Ltd.; Freund's complete adjuvant (F5881) was purchased from Sigma Corporation, USA; TNF-α and IL-1β ELISA kits were obtained from Lianke Biotechnology Co., Ltd.

The medicinal materials of Clematidis Radix et Rhizoma were sourced from the wild and the market.

These materials were identified by Professor Yingni Pan from Shenyang Pharmaceutical University as the dried roots and rhizomes of *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall., and *Clematis manshurica* Rupr. (all belonging to the family Ranunculaceae), batch was illustrated in Table 1.

Table 1 Information of medicinal material samples

No.	Sample name	Origin/Place of collection	Time of collection
S1	*Clematis chinensis* Osbeck.	/	2021.2
S2		YunNan/BoZhou	2021.5
S3	*Clematis hexapetala* Pall.	Guaipo of ShenYang	2020.11
S4		ShenYang	2021.5
S5		ChaoYang	2021.5
S6	*Clematis manshurica* Rupr.	TieLing	2021.4
S7		ShenYang	2021.5
S8		LiaoNing/Da Ling	2021.5
S9		Hu Bei/JiNan	2021.5
S10		Northeast/YueYang	2021.5
S11		JiLin/GuangXi	2021.5
S12		LiaoNing/ChangDe	2021.5
S13		JiLin/HeFei	2021.5
S14		TieLing/XiaMen	2021.5
S15		Northeast/BoZhou	2021.5
S16		LiaoNing/BoZhou	2021.5
S17		LiaoNing/WuHan	2021.5
S18		FuShun	2021.5

2.1.2 Experimental Animals

Male SD rats, weighing 200-250 g, provided by Liaoning Changsheng Biotechnology Co., LTD., production license No. SCXK (Liao) 2020-0001, were raised in a free feeding and drinking environment for one week.

2.2 Chromatographic and mass spectrometry conditions

1 g of the medicinal powder (sieved through a No. 5 sieve) was accurately weighed and mixed with 10 mL of 70% methanol. Ultrasonic extraction was performed for 30 min, then the solution was cooled and the weight was adjusted accordingly. The solution was filtered through a 0.45 μm filter membrane to obtain the final extract. Chromatography was conducted using an ACQUITY UPLC H-Class column (50 mm × 2.1 mm, 1.7 μm) with a mobile phase consisting of acetonitrile (A) and a 0.1% formic acid aqueous solution (B). The gradient elution procedure is detailed in Table 2. The flow rate was set to 0.3 mL/min with the injection volume of 1 μL. The column temperature was maintained at 30 °C. The electrospray ionization source (ESI) operated in both positive and negative ion modes. The ion source parameters were as follows: cone-hole voltage of 30 V,

capillary voltage of 3.0 kV, cone-hole backblowing gas flow (N2) at 50 L/h, desolvent gas flow at 1000 L/h, ion source temperature at 120 °C, and desolvent temperature at 450 °C. The acquisition spectrum frequency was set to 0.2 s per scan, with a scanning range of m/z 50–2000.

Table 2 Gradient elution procedure

Time	A phase/%	B phase/%
0	5	95
2	15	85
8	26	74
17.5	26	74
18	33	67
21	33	67
23	90	10
27	5	90

2.3 Animal grouping and modeling method

After one week of adaptive feeding (temperature 22-25 °C, humidity 50%-55%), 54 male SD rats were randomly divided into 9 groups, namely normal group, model group, *Clematis chinensis* Osbeck. extract group (magnoflorine concentration at 1.954 mg/kg·d), *Clematis hexapetala* Pall. extract group (magnoflorine concentration at 1.301 mg/kg·d) and *Clematis manshurica* Rupr. extract group (magnoflorine concentration at 0.7603 mg/kg·d). magnoflorine low, medium and high groups (low dose group 0.5 mg/kg, medium dose group 1.0 mg/kg, high dose group 2.0 mg/kg), positive drug group (tripterygium wilfordii polyglycoside tablet 10 mg/kg). In this study, the dosage was determined according to *Chinese Pharmacopoeia* (2025 edition) [1], in which the daily clinical dosage of Clematidis Radix et Rhizoma for an adult is 6 - 9 g crude drug. Therefore, the maximum dosage for an adult (60 kg) is 0.15 g crude drug/kg·d. The experimental dosage was set to 18 times that of clinical adults. The equivalent dose ratio between human and SD rats was calculated based on body surface area [5].

The model of SD rat rheumatoid arthritis induced by Freund's complete adjuvant [6] was established as follows: Except the normal group, 0.1 mL of Freund's complete adjuvant with a concentration of 10 mg/mL was injected intradermal into the left hind foot of rats in other groups. One week after modeling, the test drug with corresponding concentration was given by intragastric administration according to the above dose, and the normal group and the model group were given equal volume of distilled water once a day for 28 d.

2.4 General morphological observation and arthritis index score of model rats

The general conditions of the rats in each group were systematically observed, focusing on parameters such as weight, fur color, level of consciousness, activity, and mortality. The animal experiment spanned 35 d, during which the rats were weighed from the first day and then every 7 d, and their weights were recorded in grams. The occurrence and severity of polyarthritis were assessed using a five-level scoring method before and 7 d after administration. Specific scoring criteria were referenced [7].

2.5 Degree of toe swelling

The swelling volume of toes indicated the index of local inflammatory lesions. The changes of toe swelling before and after medication were measured and compared. By inhibiting the growth of capillaries and fibroblasts, the synthesis of collagen and mucopolysaccharides, and the growth of granulation tissue, the drug prevented tissue adhesion and callus formation.

The thickness of the left posterior toe of the rats was measured every other week by vernier caliper method before and one week after the modeling. Toe swelling = (foot thickness after inflammation - foot thickness before inflammation)/foot thickness before inflammation × 100%.

2.6 Histopathological examination of ankle joint

The left hind ankle joint of the rat was fixed in 4% paraformaldehyde solution and then decalcified. The selected tissue blocks were rinsed with running water, washed, and dehydrated using a gradient of alcohol concentrations. Paraffin-embedded sections were then prepared, followed by hematoxylin and eosin (HE) staining. Observations were conducted under an optical microscope, and photographs were taken to record the pathological features of the synovial tissue.

2.7 Determination of immune organ index

On the 31st d of the experiment, the thymus and spleen of rats in each group were excised after blood was collected from the abdominal aorta. The organs were then rinsed with normal saline, excess adipose tissue was removed, and moisture was absorbed using filter paper before weighing. The thymus and spleen indices were calculated using the formula: organ index = organ weight (mg) / body weight (g), in order to assess the effects of the drugs on the immune organs of the rats.

2.8 The contents of TNF-α and IL-1β in serum of rats were measured by ELISA

After anesthesia, blood samples were collected from the abdominal aorta of SD rats. The upper serum was subsequently isolated by centrifugation at 3500 rpm for 15 min and stored at -80 °C for future analysis. The concentrations of TNF-*α* and IL-1*β* were quantified according to the standard operating procedures outlined in the ELISA kit instruction manual, with absorbance values measured using an enzyme-labeled apparatus at the wavelength of 490 nm.

2.9 Statistical processing

All experimental data were expressed as mean ± SD. SPSS software (version 26.0, SPSS, Inc., US) was used for statistical analysis, and one-way analysis of variance was used to investigate whether there were differences in the contents of various biochemical indicators between groups. $P < 0.05$ was considered statistically significant.

3 Results

3.1 UPLC-MS analysis of three primitive plants of Clematidis Radix et Rhizoma

Fig. 1 showed the total ion flow chromatogram of three primary plant samples, displayed in both positive and negative modes. 34 compounds were identified through the comprehensive analysis of literature and PubChem data. The relative retention times, molecular formulas, and fragment ion information for the identified chemical components were shown in Table 3. Notably, a common characteristic component identified in Clematidis Radix et Rhizoma was magnoflorine, as illustrated in Fig. 2.

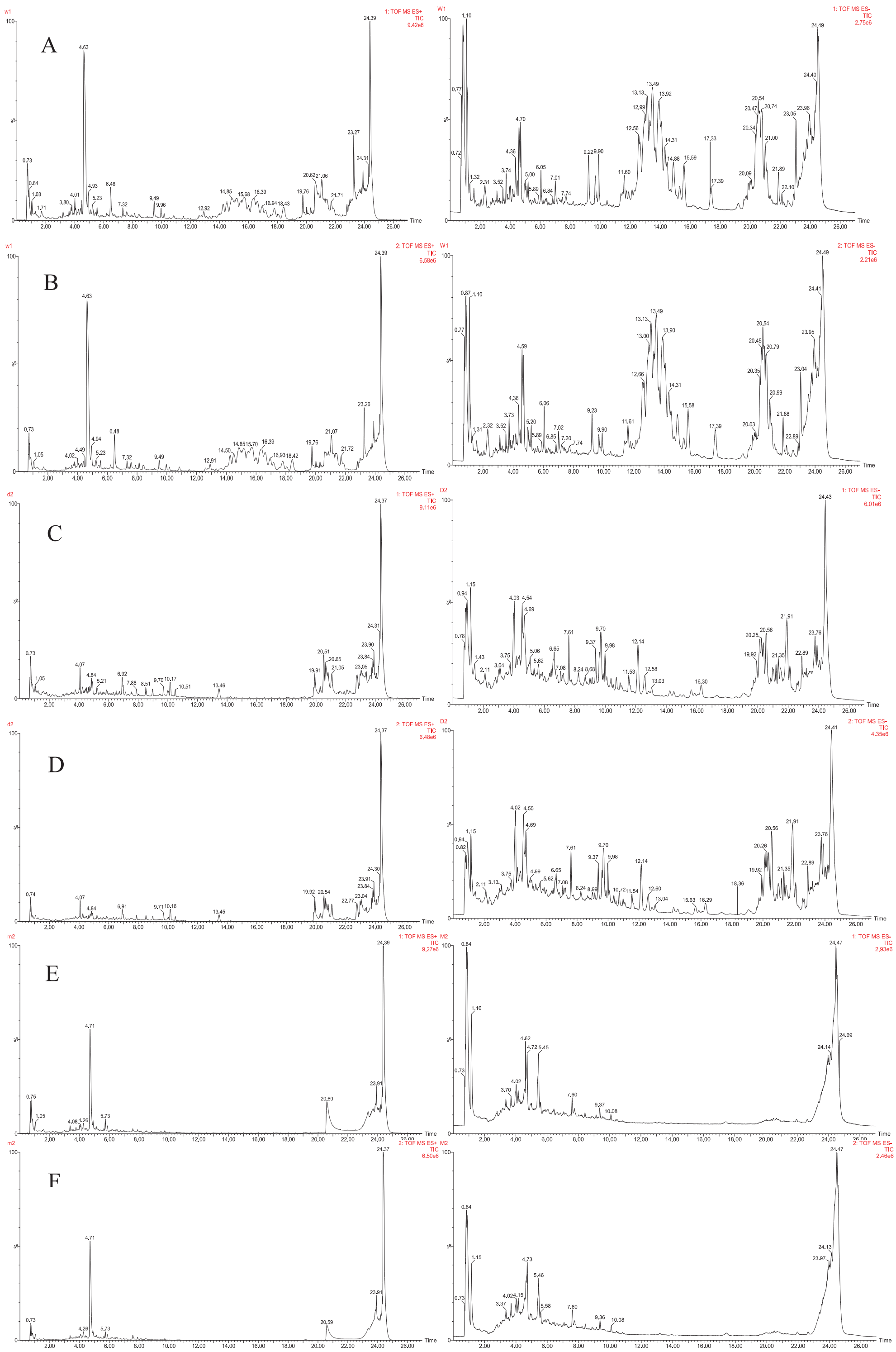

Fig. 1 Total ion flow chromatograms of positive and negative ion modes of 3 original plants. A: Positive Ion Mode of *Clematis chinensis* Osbeck; B: Negative Ion Mode of *Clematis chinensis* Osbeck; C: Positive Ion Mode of *Clematis hexapetala* Pall.; D: Negative Ion Mode of *Clematis hexapetala* Pall.; E: Positive Ion Mode of *Clematis manshurica* Rupr; F: Negative Ion Mode of *Clematis manshurica* Rupr.

Table 3 Chemical constituents identification of 3 original plants

No.	tR /time	Formula	Found	Caculated	Error /ppm	Excimer ion	Fragment ion /(m/z)	Compound	Basigen	Reference
1	0.73	$C_{19}H_{34}O_7$	375.2377	375.2372	-1.33	$[M+H]^+$	375.1284	myrsinionoside A	①	[8]
2	0.77	$C_{30}H_{36}O_{18}$	683.1818	683.1842	3.51	$[M-H]^-$	683.3578	clemochinenoside A	②	[9]
3	0.84	$C_{28}H_{32}O_{16}$	623.1007	623.1060	8.51	$[M-H]^-$	624.1815 623.1775 312.0772 311.0721	clemahexapetoside B	①	[9]
4	1.32	$C_{35}H_{56}O_8$	603.3891	603.3882	-1.49	$[M-H]^-$	605.168	kizuta saponin K3	①	[10]
5	3.00	$C_{29}H_{34}O_{17}$	653.1712	653.1766	8.27	$[M-H]^-$	655.1492	clemochinenoside B	②	[11]
6	3.52	$C_{28}H_{36}O_{13}$	579.2072	579.2123	8.81	$[M-H]^-$	579.2013 581.2114 580.2090	(-)-syringaresinol-4-O-*β*-D-glucopyranoside	①	[12]
7	4.01	$C_{20}H_{22}O_6$	359.1489	359.1456	-9.19	$[M+H]^+$	357.1527 356.1496 314.1746	(+)-pinoresinol/ (+)-epipinoresinol	①	[13]
8	4.06	$C_{27}H_{34}O_{12}$	551.2123	551.2150	4.90	$[M+H]^+$	550.2679	isoeucommin A	③	[12]
9	4.69	$C_{22}H_{30}O_{13}$	501.1603	501.1626	4.59	$[M-H]^-$	501.1422 502.1449	clemomandshuricoside B	②	[11]
10	4.73	$C_{20}H_{24}NO_4$	343.1778	343.1775	-0.87	$[M+H]^+$	342.1813 297.1218 265.0955 237.0996	magnoflorine	①②③	[14]
11	5.26	$C_{30}H_{48}O_4$	473.3625	473.3653	5.92	$[M+H]^+$	470.0948 471.0985 473.1038	corosolic acid	②	[11]
12	5.40	$C_{30}H_{48}O_5$	489.3575	489.3532	-8.79	$[M+H]^+$	489.2486 488.2452 343.1857 342.1813 265.0934	cimigenol	③	[12]
13	5.45	$C_{35}H_{56}O_9$	619.3841	619.3824	-2.74	$[M-H]^-$	620.2333	cimigoside	③	[12]
14	5.62	$C_{26}H_{34}O_{11}$	521.2017	521.2068	9.79	$[M-H]^-$	523.1959 522.1897 521.1845	(+)-lariciresinol-4'-*O*-*β*-D-glucopyranoside	②	[12]
15	5.73	$C_{28}H_{32}O_{14}$	593.1865	593.1892	4.55	$[M+H]^+$	597.2399 593.2045	linarin	③	[12]
16	6.48	$C_{16}H_{20}O_9$	357.1180	357.1172	-2.24	$[M+H]^+$	357.1910 356.1878	1-feruloyl-*β*-D-glucopyranoside	①	[8]
17	6.65	$C_{27}H_{36}O_{12}$	551.2123	551.2133	1.81	$[M-H]^-$	554.0815 553.0729 552.1193	(+)-lariciresinol-4-*O*-*β*-D-glucopyranoside	①③	[12]
18	6.92	$C_{30}H_{48}O_5$	489.3575	489.3552	-4.70	$[M+H]^+$	488.1837	tormentic acid	②	[11]

(to be continued)

Continued Table 3

No.	tR /time	Formula	Found	Caculated	Error /ppm	Excimer ion	Fragment ion /(m/z)	Compound	Basigen	Reference
19	6.97	$C_{15}H_{18}O_9$	343.1024	343.1020	-1.17	$[M+H]^+$	343.1833 342.1813	1-*O*-caffeoyl-*β*-D-glucopyranoside	③	[8]
20	8.25	$C_{27}H_{30}O_{11}$	529.1704	529.1738	6.43	$[M-H]^-$	530.1434 529.1393	3″-*O*-(2‴-methylbutyryl) isoswertisin	②	[13]
21	9.37	$C_{26}H_{34}O_{11}$	521.2017	521.2068	9.79	$[M-H]^-$	524.1855 523.1813	(+)-lariciresinol-4’-*O*-*β*-D-glucopyranoside	②③	[12]
22	9.70	$C_{82}H_{134}O_{44}$	1821.8164	1821.8037	-6.97	$[M-H]^-$	1824.8386 1823.8364 1822.8400	clematomandshurica saponin F	②	[11]
23	9.90	$C_{28}H_{36}O_{13}$	579.2072	579.2046	-4.49	$[M-H]^-$	579.1830 581.1900 580.1845	(-)-syringaresinol-4’-*O*-*β*-D-glucopyranoside	①③	[12]
24	10.08	$C_{29}H_{34}O_{17}$	653.1712	653.1766	8.27	$[M-H]^-$	655.2823	clemahexapetoside A	③	[9]
25	10.51	$C_{74}H_{112}O_{32}$	1513.7209	1513.7107	-6.74	$[M+H]^+$	1515.7847	clematomandshurica saponin J	②	[11]
26	11.85	$C_{87}H_{142}O_{48}$	1953.8587	1953.8540	-2.41	$[M+H]^+$	1957.4532 456.3655 455.3614	clematoside B	②	[15]
27	12.20	$C_{68}H_{102}O_{27}$	1349.6525	1349.6639	8.45	$[M-H]^-$	1352.6523 1351.6544	clematomandshurica saponin K	②	[11]
28	13.00	$C_{70}H_{114}O_{35}$	1513.7057	1513.7141	5.55	$[M-H]^-$	1516.7039 1515.7064 1514.6992 1043.5256	clematichinenoside B	①	[16]
29	15.58	$C_{64}H_{104}O_{30}$	1353.6685	1353.6612	-5.39	$[M+H]^+$	1375 1351.6404	huzhangoside D	①	[17]
30	20.12	$C_{86}H_{132}O_{43}$	1851.8059	1851.8142	4.48	$[M-H]^-$	1853.8409 1852.8363	clematochinenoside D	②	[10]
31	22.14	$C_{92}H_{142}O_{46}$	1983.8845	1983.8772	-3.68	$[M-H]^-$	1982.8984 1981.8876	clematomandshurica saponin B	③	[16]
32	23.89	$C_{64}H_{104}O_{29}$	1335.6580	1335.6663	6.21	$[M-H]^-$	1337.6665 1336.6587 1335.6575	huzhangoside B	①②	[15]
33	23.97	$C_{22}H_{32}O_9$	439.1963	439.2006	9.79	$[M-H]^-$	464.2537 463.2486	11-epi-hexapetol A 3-*O*-*β*-D-glucopyranoside	②	[12]
34	24.37	$C_{15}H_{10}O_5$	271.0601	271.0598	-1.11	$[M+H]^+$	271.3217 270.3182	genistein	③	[18]

Note: ① *Clematis chinensis* Osbeck; ② *Clematis manshurica* Rupr; ③ *Clematis hexapetala* Pall.

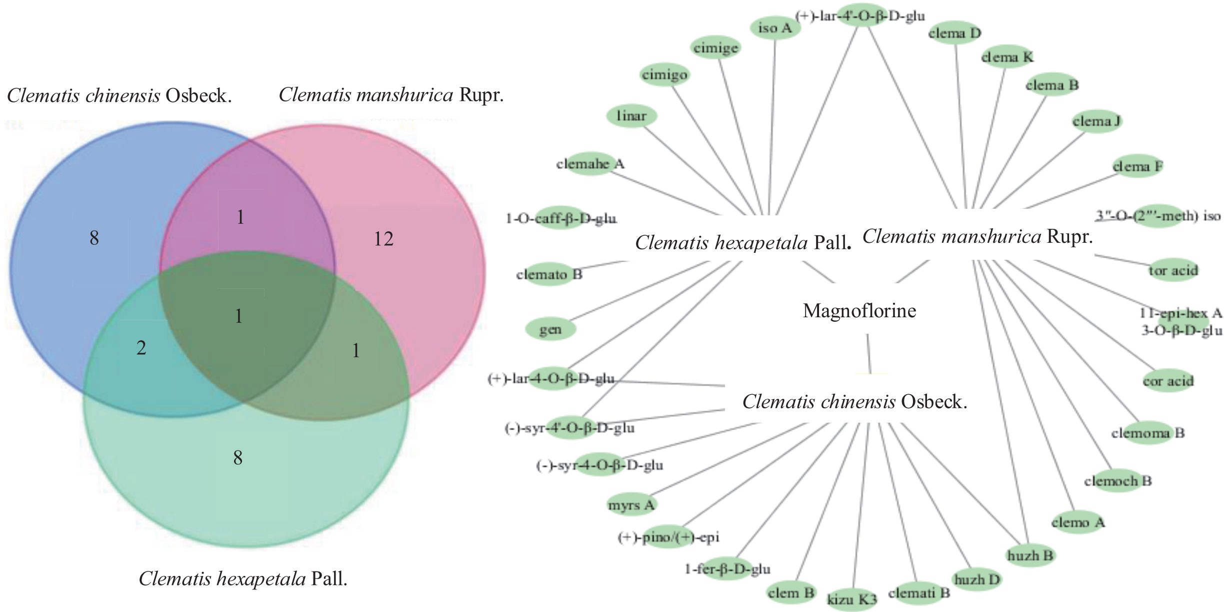

Fig. 2 Analyse of common chemical components in three original plants

3.2 Study on mechanism of action of magnoflorine against rheumatoid arthritis

3.2.1 General morphological observation

During the experiment, the rats in the normal control group exhibited good health, characterized by smooth and glossy fur, normal food and water intake, regular defecation, unrestricted joint movement, and a steady increase in weight and mass. In contrast, after immune modeling, the rats in the model group displayed signs of irritability, frequently scratching each other and licking the paw on the side of the modeling. Their fur appeared dry, their activity levels decreased, and they exhibited abnormal walking behaviors, such as dragging and lifting their limbs. However, no abnormalities were observed in their bowel movements. Compared with the model group, the tripterygium polyside tablet group and the groups receiving crude extracts of *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall. and *Clematis manshurica* Rupr. showed improved activity levels and increased food and water intake after two weeks of administration. Additionally, the redness and swelling of the left hind toe in the rats were improved, with the color of most toe areas returning to light red hue and no hardening upon touch. As illustrated in Table 4 and Fig. 3, the body weight of rats in the model group increased gradually, with a statistically significant difference in body weight observed between the model group and the blank group ($P \leq 0.05$). On the contrary, the body weight of rats in each drug administration group also increased compared to the model group; however, this difference did not reach statistical significance ($P \geq 0.05$).

Table 4 Different treatment groups of body weight changes of rats/g ($\bar{x} \pm s$, $n = 6$).

Group	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35
Blank	197.7 ± 4.6	224.8 ± 5.6	237.9 ± 3.4	248.6 ± 9.0	265.3 ± 8.0	280.8 ± 4.6
Model	193.4 ± 3.3	207.6 ± 3.7	209.6 ± 3.5	210.7 ± 3.0	216.5 ± 3.5	224.6 ± 1.5*
Tripterygium glycosides tablet	194.1 ± 11.7	211.5 ± 17.4	217.0 ± 18.6	224.3 ± 18.9	232.8 ± 15.6	240.5 ± 10.7
Extract of *Clematis chinensis* Osbeck	194.4 ± 2.9	213.6 ± 9.2	229.2 ± 16.9	238.8 ± 11.8	242.2 ± 10.8	248.5 ± 12.9
Extract of *Clematis hexapetala* Pall	196.5 ± 1.9	210.8 ± 2.8	227.1 ± 6.8	236.4 ± 7.6	245.9 ± 3.2	260.9 ± 3.7
Extract of *Clematis manshurica* Rupr	197.3 ± 2.7	213.5 ± 15.8	225.0 ± 18.1	235.7±19.6	245.0 ± 13.7	256.1 ± 13.5
Low dose of Magnoflorine	195.9 ± 3.2	210.6 ± 6.9	217.5 ± 13.8	225.1±17.9	232.0 ± 11.6	241.4 ± 9.4
Medium dose of Magnoflorine	196.5 ± 1.8	211.1 ± 5.9	221.1 ± 8.4	228.0±14.1	234.7 ± 9.7	246.0 ± 11.9
High dose of Magnoflorine	194.4 ± 0.9	216.5 ± 9.0	228.2 ± 12.0	236.2±12.1	241.7 ± 2.7	254.3 ± 4.6

Note: Compared with the blank group, $^{*}P \leq 0.05$

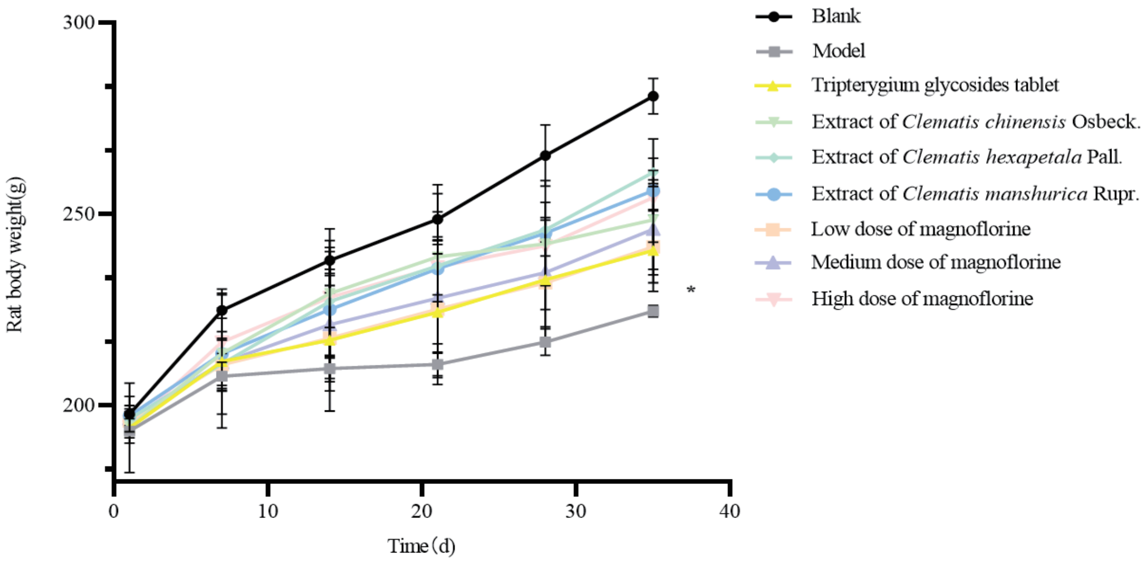

Fig. 3 Different treatment groups of body weight change curves of rats/g ($\bar{x} \pm s$, $n = 6$). Compared with the blank group, $^{*}P \leq 0.05$

3.2.2 Arthritis index score of rats

As illustrated in Table 5 and Fig. 4, the arthritis index score of all treatment groups were significantly elevated compared with the blank group. Before treatment, there was no statistically significant difference in the arthritis index scores between the model group and the drug administration groups ($P \geq 0.05$), confirming the success of the modeling process. Notably, the model group exhibited a smooth trend in the arthritis index compared to other groups, indicating stability of the model. Between the second week and at the end of the fourth week of administration, the arthritis index in both the crude extract group of the three basic plant alkaloids and the magnoflorine dose group showed a significant reduction compared to the model group ($P \leq 0.01$).

Table 5 Different treatment groups of arthritis index score changes of rats/g ($\bar{x} \pm s$, $n = 6$)

Group	Day 1	Day 7	Day 14	Day 21	Day 28
Blank	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Model	$3.43 \pm 0.12^{**}$	$3.75 \pm 0.14^{**}$	$3.63 \pm 0.15^{**}$	$3.51 \pm 0.15^{**}$	$3.35 \pm 0.19^{**}$
Tripterygium glycosides tablet	3.41 ± 0.15	3.55 ± 0.15	$2.80 \pm 0.063^{\#\#}$	$2.30 \pm 0.14^{\#\#}$	$1.72 \pm 0.13^{\#\#}$
Extract of *Clematis chinensis* Osbeck	3.40 ± 0.20	3.58 ± 0.12	$3.00 \pm 0.17^{\#\#}$	$2.65 \pm 0.14^{\#\#}$	$2.33 \pm 0.16^{\#\#}$
Extract of *Clematis hexapetala* Pall	3.40 ± 0.15	3.57 ± 0.18	$2.92 \pm 0.19^{\#\#}$	$2.53 \pm 0.10^{\#\#}$	$2.25 \pm 0.10^{\#\#}$
Extract of *Clematis manshurica* Rupr	3.41 ± 0.19	3.72 ± 0.28	$3.15 \pm 0.14^{\#\#}$	$2.90 \pm 0.13^{\#\#}$	$2.57 \pm 0.12^{\#\#}$
Low dose of Magnoflorine	3.47 ± 0.16	3.72 ± 0.23	$3.28 \pm 0.17^{\#\#}$	$3.07 \pm 0.12^{\#\#}$	$2.85 \pm 0.12^{\#\#}$
Medium dose of Magnoflorine	3.43 ± 0.12	3.68 ± 0.07	$3.13 \pm 0.10^{\#\#}$	$2.90 \pm 0.13^{\#\#}$	$2.68 \pm 0.12^{\#\#}$
High dose of Magnoflorine	3.40 ± 0.17	3.53 ± 0.28	$2.77 \pm 0.16^{\#\#}$	$2.43 \pm 0.10^{\#\#}$	$2.15 \pm 0.10^{\#\#}$

Note: Compared with the blank group, $^{**}P \le 0.01$; Compared with the model group, $^{\#\#}P \le 0.01$

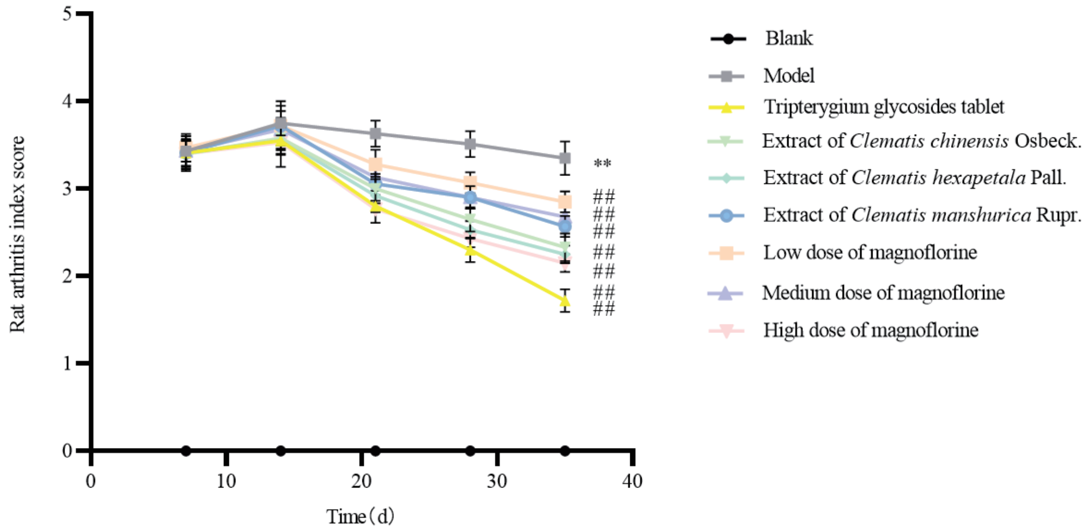

Fig. 4 Different treatment groups of arthritis index score change curves of rats/g ($\bar{x} \pm s$, $n = 6$). Compared with the blank group, $^{**}P \le 0.01$; Compared with the model group, $^{\#\#}P \le 0.01$

3.2.3 Ankle joint histopathology

The left hind ankle joint of the rat was fixed in 4% paraformaldehyde solution and then decalcified. The selected tissue blocks were rinsed with running water, washed, and dehydrated using an alcohol gradient of different concentrations. Paraffin-embedded sections were then obtained, and hematoxylin and eosin (HE) staining was performed. Observations were conducted under an optical microscope, and photographs were taken. As illustrated in Fig. 5, the joint tissue structure of rats in the normal control group remained intact, and there was no sign of injury. Bone cells were neatly arranged. There were no inflammatory cells, no evidence of pannus or other inflammatory symptoms, and no damage in the joint cavity. On the contrary, in the model group, there were obvious infiltration of inflammatory cells, cell swelling, synovial cell proliferation, and disordered arrangement within the joint tissue. In severe cases, the articular cartilage layer was damaged. Pathological conditions of the ankle joint in the AA model rats treated with the positive drug group, *Clematis chinensis* Osbeck., *Clematis hexapetala* Pall., *Clematis manshurica* Rupr. crude extract group, and magnoflorine dose group showed a decrease in severity compared to the model group.

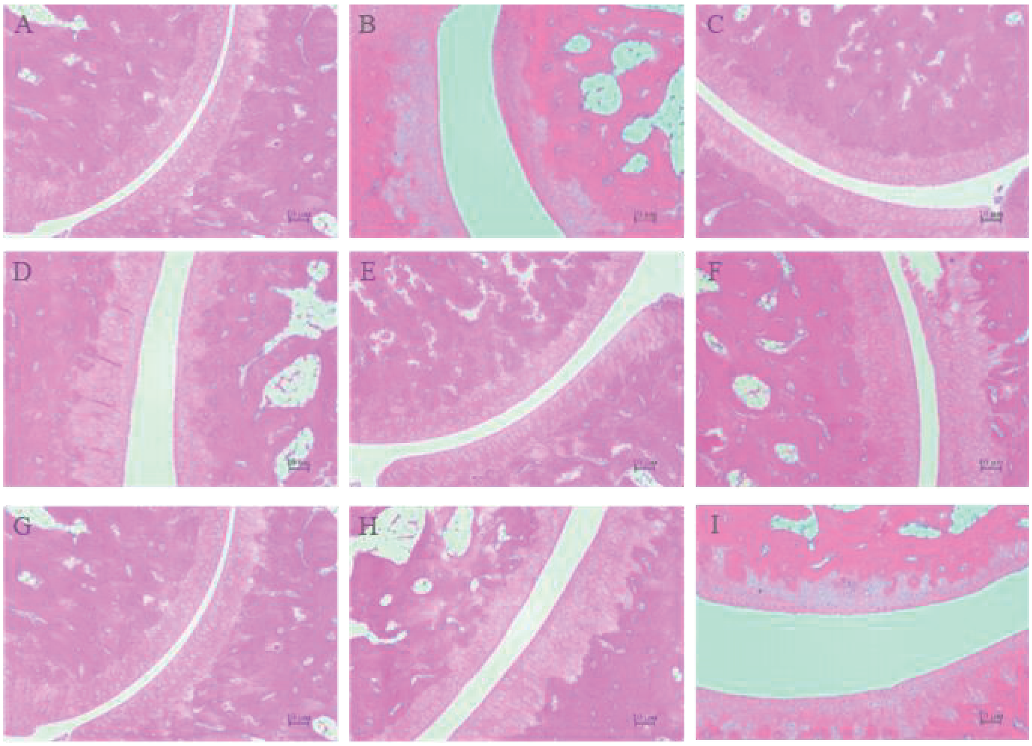

Fig. 5 Different treatment groups of ankle joint histopathologic changes of rats (100×); A: Blank; B: Model; C: Tripterygium glycosides tablet; D: Extract of *Clematis chinensis* Osbeck.; E: Extract of *Clematis hexapetala* Pall.; F: Extract of *Clematis manshurica* Rupr.; G: Low dose of magnoflorine; H: Medium dose of magnoflorine; I: High dose of magnoflorine

3.2.4 Immune organ index

As shown in Fig. 6, compared to the blank group, the spleen and thymus indices in the model group were significantly elevated ($P \leq 0.001$), confirming the success of the modeling process. All drug administration groups exhibited different degrees of decrease in immune organ indices compared to the model group. Notably, the spleen indices in the tripterygium glycoside tablet group, as well as the extract groups of *Clematis chinensis* Osbeck. and *Clematis hexapetala* Pall., showed significant decrease compared to the model group ($P \leq 0.01$ or $P \leq 0.05$). Although the indices of the *Clematis manshurica* Rupr. extract group also decreased, this change was not statistically significant. Additionally, the thymus index in the tripterygium multiside tablet group was significantly reduced compared to the model group ($P \leq 0.001$). The extract groups of *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall., and *Clematis manshurica* Rupr., along with the high-dose and medium-dose magnoflorine groups, also demonstrated significant decrease in thymus indices ($P \leq 0.01$ or $P \leq 0.05$), while the low-dose magnoflorine group did not show statistically significant changes.

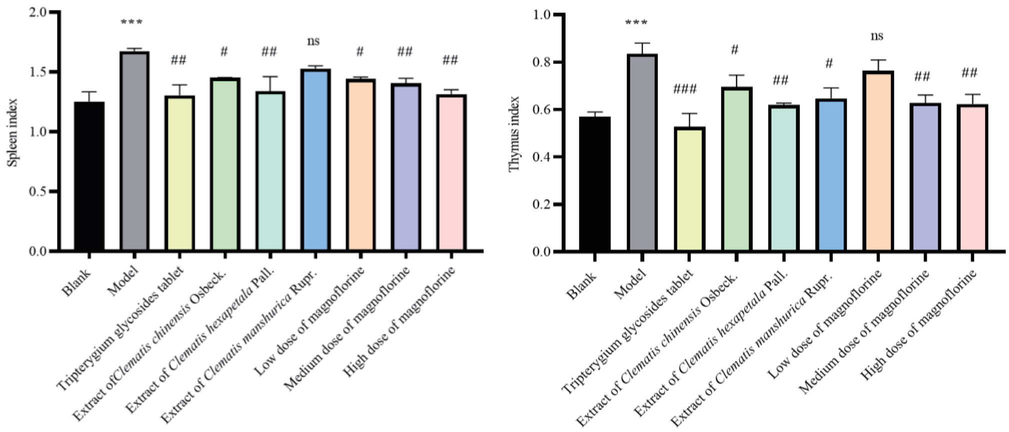

Fig. 6 Spleen index and thymus index of rats in different treatment groups ($\bar{x} \pm s$, $n = 6$). Compared with the blank group, $^{***}P \leq 0.001$; Compared with the model group, $^{###}P \leq 0.001$, $^{\#}P \leq 0.05$, $^{##}P \leq 0.01$

3.2.5 The levels of TNF-α and IL-1β

The concentrations of TNF-*α* and IL-1*β* were measured according to the standard operating procedures outlined in the ELISA kit instruction manual. As shown in Fig. 7, the model group exhibited a significant increase in TNF-*α* and IL-1*β* levels compared with the blank group ($P \leq 0.001$), confirming the success of the modeling process. In contrast, the tripterygium glycosides tablet group, as well as the *Clematis chinensis* Osbeck. and *Clematis hexapetala* Pall. extract groups, showed a significant decrease in TNF-*α* levels compared to the model group ($P \leq 0.001$). Additionally, the *Clematis manshurica* Rupr. extract group and the low-dose magnoflorine group demonstrated a significant decrease in TNF-*α* levels ($P \leq 0.01$). All administration groups exhibited a significant decrease in IL-1*β* levels compared to the model group ($P \leq 0.001$). These findings indicated that the extracts from three fundamental plants of Clematidis Radix et Rhizoma, along with the magnoflorine dosage groups, had significant anti-inflammatory effects in AA model rats. They inhibited the expression levels of pro-inflammatory factors TNF-*α* and IL-1*β* in a dose-dependent manner. The anti-inflammatory effect improved with the increase of magnoflorine dosage.

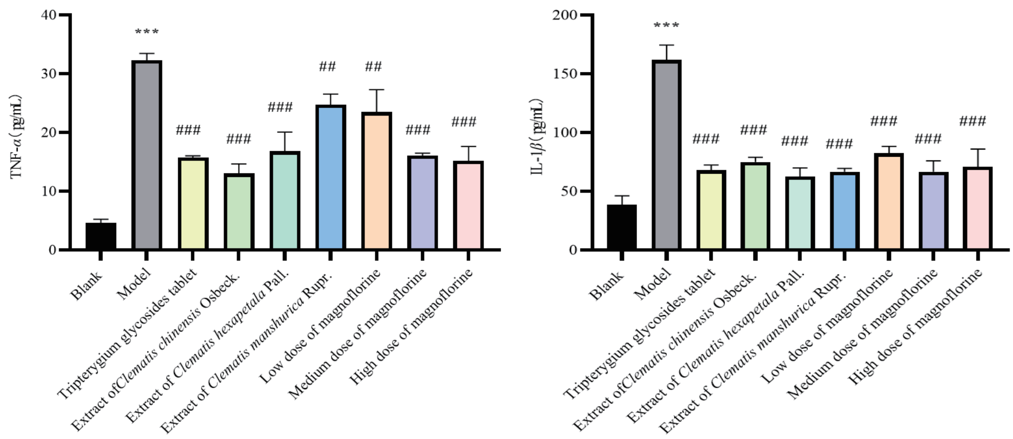

Fig. 7 TNF-*α* and IL-1*β* change in serum of rats in different treatment groups ($\bar{x} \pm s$, $n = 6$). Compared with the blank group, $^{***}P \leq 0.001$; Compared with the model group, $^{###}P \leq 0.001$, $^{##}P \leq 0.01$

4 Discussion

Rheumatoid arthritis is a chronic autoimmune disease characterized by persistent joint pain, swelling and stiffness. In severe cases, it could lead to cardiovascular, respiratory, and other complications, significantly impacting patients' daily lives and work [19-21]. Traditional Chinese medicine attributes the pathogenesis of rheumatoid arthritis to deficiency of healthy qi, which makes the body vulnerable to external factors such as wind, cold and humidity. This invasion of 'evil qi' is thought to hinder the flow of meridians and blood, leading to phlegm accumulation and blood stasis, ultimately resulting in arthralgia. In contrast, modern medicine recognizes that the etiology of rheumatoid arthritis is multifactorial, encompassing infections, immune disorders, environmental influences, genetic predispositions, and psychological factors, and symmetry and destructiveness are the key features.

Clematis chinensis Osbeck as a commonly utilized Chinese medicine known for its efficacy in dispelling wind-dampness and alleviating arthralgia pain. As the earliest medicinal plant derived from Clematidis Radix et Rhizoma, *Clematis hexapetala* Pall. boasts significant curative effect, a long history of medicinal use, and abundant reserves in Chinese northern area. However, the content determination of *Clematis hexapetala* Pall. does not meet the *Chinese Pharmacopoeia* (2025 edition) requirements for oleanolic acid content, resulting in limited market circulation [3,4]. Currently, oleanolic acid, which is prevalent in numerous medicinal materials, is used as the index component for content determination in *pharmacopoeia*. However, using oleanolic acid content as a measure is not specific enough to evaluate the quality of medicinal materials. In this study, the chemical constituents of three species of Clematidis Radix et Rhizoma were characterized using UPLC-MS, leading to the discovery of a common characteristic component, magnoflorine. Magnoflorine is classified as an aporphine alkaloid [22]. Studies showed that magnoflorine, a characteristic component of Clematidis Radix et Rhizoma, was undetectable in *Ardisia japonica* (Thunb) Blume [23-24], which is a counterfeit of Clematidis Radix et Rhizoma, highlighting its specificity. Magnoflorine shows significant concentration-dependent anti-inflammatory activity, with better effects at high concentrations. It can suppress pro-inflammatory cytokines like IL-1β, IL-18, and TNF-α to alleviate inflammation. In a study on rats with ulcerative colitis, high-dose magnoflorine reduced these cytokines and eased inflammation. Moreover, it inhibited NLRP3 inflammatory activity and related protein expression, further boosting its anti-inflammatory effect [25]. These findings suggested high-concentration magnoflorine had great potential in anti-inflammatory treatment, providing a new idea for the treatment of related diseases. Pharmacological evaluation, including arthritis index, toe swelling degree, immune organ index, pathological sections of the ankle joint, and inflammatory factors such as TNF-α and IL-1β, demonstrated that magnoflorine has therapeutic effects on rheumatoid arthritis. Further screening of relevant targets through network pharmacology revealed that magnoflorine can be considered as one of the key active ingredients in the treatment of rheumatoid arthritis. Therefore, magnoflorine can be used as a qualitative and quantitative identification index, which provides a robust theoretical and empirical basis for the quality standards stipulated in the current *pharmacopoeia*.

In this study, the ACQUITY UPLC H-Class ultra-high performance liquid chromatography system and the Vion QT of high-resolution mass spectrometer were employed to analyze the chemical constituents of multiple batches of Clematidis Radix et Rhizoma, focusing primarily on alkaloids. Notably, ivy saponin was not detected in *Clematis manshurica* Rupr., and oleanolic acid was absent in

Clematis hexapetala Pall [26]. This absence may be attributed to the low concentration of these common characteristic components in the tested herbs, and may be caused by the change of growth environment and insufficient maturity.

5 Conclusion

The primary chemical constituents of three Clematidis Radix et Rhizoma were identified through UPLC-MS analysis and literature review, resulting in the identification of 34 distinct components. Notably, magnoflorine was found to be a common characteristic component among *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall., and *Clematis manshurica* Rupr. Furthermore, pharmacodynamic studies demonstrated that both the crude extract containing the three principal alkaloids and the individual dose groups of magnoflorine exhibited significant therapeutic effects in the rat model of rheumatoid arthritis induced by AA. These treatments resulted in the reduction of toe swelling, improvement in arthritis index score, decreased immune organ indices, and lowered serum levels of inflammatory factors. Additionally, three Clematidis Radix et Rhizoma alleviated the histopathological conditions of the ankle joints and delayed the progression of joint destruction in the rats. This study provides a theoretical and experimental basis for the innovation and rational utilization of medicinal material resources.

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