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Characteristic Chromatogram of Yao Medicine *Kadsura longipedunculata* and the Analysis of Its Anwulignan Content and Anti-inflammatory Activity

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Abstract [**Objectives**] To establish a characteristic chromatogram of the traditional Yao medicine *Kadsura longipedunculata*, develop a method for quantifying its primary component, anwulignan, and evaluate the anti-inflammatory activity of anwulignan. [**Methods**] Gradient elution was performed using high-performance liquid chromatography (HPLC) with a mobile phase consisting of acetonitrile and 0.5% phosphoric acid solution. A characteristic chromatogram of *K. longipedunculata* was established, and its similarity was assessed using the *Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine* (*2012 Edition*). Additionally, the content of anwulignan in *K. longipedunculata* was quantified. Lipopolysaccharide-induced RAW264.7 macrophages were employed as an inflammatory cell model to investigate the effects of anwulignan on the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in the cell culture supernatant. [**Results**] The similarity indices of characteristic chromatograms for 10 batches of *K. longipedunculata* ranged from 0.901 to 0.994, with 9 common peaks identified. Among these, three components were characterized, including changnan schisantherin E, kadsulactone A, and anwulignan. The content of anwulignan was quantified as ranging from (0.72 ± 0.05) to (1.21 ± 0.03) mg/g (n = 3). Furthermore, anwulignan at concentrations of 0.125 - 0.5 μg/mL significantly reduced the levels of TNF-α, IL-1β, and IL-6 in the supernatant of inflammatory model cells (P < 0.05 or P < 0.01). [**Conclusions**] HPLC characteristic chromatogram of *K. longipedunculata* and the method for determining the content of anwulignan have been established. Anwulignan may represent the active ingredient responsible for the anti-inflammatory effects observed in *K. longipedunculata*.

Key words Yao medicine *Kadsura longipedunculata*, HPLC, Characteristic chromatogram, Quality control, Anwulignan, Anti-inflammatory action

1 Introduction

Kadsura longipedunculata Finet et Gagnep, a species belonging to the genus *Kadsura* within the Magnoliaceae family, is a constituent of "Shibazuan", a collection of traditional Yao medicinal plants utilized in Laoban Yao medicine for the treatment of rheumatism and circulatory disorders. This plant is predominantly distributed across regions including Yunnan, Sichuan, Hubei, Hunan, Guangdong, Guangxi, and other areas. It is traditionally recognized for its therapeutic properties, such as regulating qi and promoting blood circulation, dispelling wind and unblocking meridians, as well as reducing swelling and alleviating pain. Commonly, it is utilized in folk medicine to address conditions such as postpartum edema, amenorrhea accompanied by abdominal pain, stomachache, rheumatoid arthritis, and related ailments[1]. Contemporary research indicates that the primary chemical constituents of *K. longipedunculata* are lignans and triterpenoids, which exhibit diverse pharmacological properties, including anti-tumor and antioxidant activities[2].

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The current quality standards for *K. longipedunculata* are delineated in the *Quality Standards for Yao Medicinal Materials in the Guangxi Zhuang Autonomous Region* (*Volume I*), encompassing parameters such as source, appearance, inspection, and extract content[3]. Nevertheless, there remains a paucity of systematic research concerning its intrinsic quality, thereby hindering comprehensive and effective quality control of this medicinal material. Previous investigations conducted by our research group have demonstrated that extracts of *K. longipedunculata* exhibit significant anti-inflammatory and analgesic properties, and that anwulignan can be isolated from the medicinal materials of *K. longipedunculata*[4-5]. The literature indicates that anwulignan exhibits pharmacological properties, including anti-inflammatory effects, hepatoprotection, and anti-gastric ulcer activity[6-7]. Accordingly, this study established the characteristic chromatogram of 10 batches of *K. longipedunculata* medicinal materials obtained from various regions in Guangxi and developed a quantification method for anwulignan using high-performance liquid chromatography (HPLC). Furthermore, the anti-inflammatory activity of anwulignan was evaluated. The objective of this research is to provide a scientific basis for the quality control of *K. longipedunculata* medicinal materials used in ethnic medicine in Guangxi.

2 Materials

2.1 Main instruments The primary instruments employed in

this study comprised the Waters E2695 HPLC meter (Waters, USA), the SK5200H ultrasonic cleaner (Shanghai Kedao Ultrasonic Instrument Co., Ltd.), the CP225 electronic analytical balance (Sartorius, Germany), the DZKW-S-6 electric constant temperature water bath (Guangxi Kemirui Laboratory Instrument Equipment Co., Ltd.), and the Infinite F200 Pro multifunctional microplate reader (Tecan, Switzerland).

2.2 Main drugs and reagents The reference substances changnan schisantherin E and kadsulactone A (batch No.: 20220413 and 2021109 respectively, both with purities exceeding 95%) were prepared in-house by the Guangxi Key Laboratory of Medicinal Resource Protection and Genetic Improvement. The reference substance anwulignan (batch No.: M30GB143568, purity ≥98%) was procured from the National Institutes for Food and Drug Control, China. Lipopolysaccharide (LPS) was obtained from Sigma (United States). The MTT kit (batch No.: 917Q051) was obtained from Beijing Solarbio Science & Technology Co., Ltd. Enzyme-linked immunosorbent assay (ELISA) kits for mouse tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), with batch No.: 2411559805, 1321596923, and 1161590923 respectively, were procured from Wuhan Boster Biological Technology Co., Ltd. Acetonitrile and phosphoric acid were of chromatographic grade, ethanol was of analytical grade, and ultrapure water was used throughout the experiments.

Ten batches of *K. longipedunculata* medicinal materials (designated S1 to S10) were collected from various production areas within Guangxi. These samples were authenticated as roots and stems of *K. longipedunculata* Finet et Gagnepain by Associate Researcher Zhao Yimin of the Guangxi Medicinal Plant Garden. The source details are presented in Table 1.

Table 1 Source information of 10 batches of *Kadsura longipedunculata*

No.	Collection site	Collection time
S1	Jiangdi Township, Longsheng County	November, 2022
S2	Dazhang Township, Jinxiu County	November, 2022
S3	Jinxiu County	November, 2022
S4	Liula Village, Jinxiu County	November, 2022
S5	Luoxiang Township, Jinxiu County	February, 2023
S6	Sanjiang Township, Gongcheng County	February, 2023
S7	Changdong Township, Jinxiu County	February, 2023
S8	Sanjiao Township, Jinxiu County	March, 2023
S9	Heping Township, Longsheng County	March, 2023
S10	Botang Town, Cenxi City	March, 2023

2.3 Cells The mouse mononuclear macrophage cell line RAW264.7 was obtained from Shanghai Enzyme Research Biotechnology Co., Ltd.

3 Methods and results

3.1 Establishment of HPLC characteristic chromatogram

3.1.1 Chromatographic conditions. An Elite Hypersil ODS C_{18} chromatographic column (250 mm × 4.6 mm, 5 μm) was employed for the analysis. Gradient elution was conducted using acetonitrile (solvent A) and 0.5% phosphoric acid solution (solvent B) as the mobile phase, with the following program: 0 – 15 min, 30% A→50% A; 15 – 25 min, 50% A→70% A; 25 – 35 min, 70% A→95% A; 35 – 45 min, 95% A→30% A; and 45 – 50 min, 30% A. The flow rate was maintained at 1.0 mL/min, the column temperature was controlled 30 ℃, the detection wavelength was set to 210 nm, and the injection volume was 10 μL.

3.1.2 Preparation of mixed reference solution. Appropriate quantities of the reference substances changnan schisantherin E, kadsulactone A, and anwulignan were accurately weighed and dissolved in methanol to prepare mixed reference solutions with mass concentrations of 21.83, 17.48, and 27.65 μg/mL, respectively.

3.1.3 Preparation of test solution. Approximately 2 g of *K. longipedunculata* powder (sieved through a No. 3 mesh) was accurately weighed and placed into a 250 mL round-bottom flask. Subsequently, 20 mL of 80% ethanol solution was added, and the mixture was subjected to reflux extraction for 2 h. After cooling, the flask was reweighed, and the lost mass was compensated with 80% ethanol, followed by thorough mixing. This extraction procedure was repeated twice more, and the three extracts were combined and filtered through a 0.45 μm microporous membrane filter. The resulting filtrate was used as the test solution.

3.1.4 Precision test. *K. longipedunculata* test solution (No. S3) was injected six times consecutively under the chromatographic conditions described in Section **3.1.1**. The peak corresponding to anwulignan was selected as the reference peak due to its excellent resolution, large and stable peak area, and well-defined peak shape. Using this reference, the relative retention time and relative peak area of each common peak were calculated. The results demonstrated that the relative standard deviation (*RSD*) of the relative retention time for all common peaks was less than 1% ($n=6$), and the *RSD* of the relative peak area was less than 2% ($n=6$), indicating that the method exhibited good precision.

3.1.5 Stability test. *K. longipedunculata* test solution (No. S3) was stored at room temperature for 0, 4, 8, 12, 16, 20, and 24 h, respectively, and analyzed according to the chromatographic conditions described in Section **3.1.1**. The peak of anwulignan was used as the reference peak, and the relative retention time and relative peak area of each common peak were calculated. The results indicated that the *RSD* of the relative retention time for each common peak was less than 1% ($n=7$), and the *RSD* of the relative peak area was less than 3% ($n=7$), demonstrating that the test solution remained stable after being stored at room temperature for 24 h.

3.1.6 Repeatability test. *K. longipedunculata* powder (No. S3) was prepared into six test solution samples following the procedure outlined in Section **3.1.3** and analyzed under the chromatographic conditions specified in Section **3.1.1**. The peak corresponding to anwulignan was employed as the reference peak, and the relative retention time and relative peak area of each common peak were calculated. The results demonstrated that the *RSD* of the relative retention time for each common peak was less than 1% ($n=6$),

while the *RSD* of the relative peak areas was below 3% ($n=6$), indicating that the method exhibited good repeatability.

3.1.7 Establishment of characteristic chromatogram and evaluation of similarity. An appropriate amount of *K. longipedunculata* powder from 10 batches of different origins was collected and prepared into test solutions following the procedure outlined in Section **3.1.3**. These solutions were subsequently injected and analyzed under the chromatographic conditions specified in Section **3.1.1**, and the resulting chromatograms were recorded. The obtained chromatograms were imported in AIA file format into the *Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine* (*2012 Edition*) for analysis and comparison. Using the chromatogram of sample S1 as the reference, multi-point correction and automatic matching were performed to generate a superimposed chromatogram. The median method was employed to produce the control fingerprint chromatogram R, which served as the basis for similarity evaluation. The results presented in Fig. 1 indicate that a total of 9 common peaks were identified across the 10 batches of samples. Further comparison with the chromatograms of the mixed reference solution (Fig. 2) allowed for the identification of three components: peak No. 2 as changnan schisantherin E, peak No. 5 as kadsulactone A, and peak No. 6 as anwulignan. The similarity indices between the 10 batches of samples and the control fingerprint chromatogram R were 0.993, 0.928, 0.984, 0.938, 0.982, 0.994, 0.990, 0.901, 0.970, and 0.982, respectively, all exceeding 0.9. These results indicate a high degree of similarity among the 10 batches, suggesting consistent chemical composition.

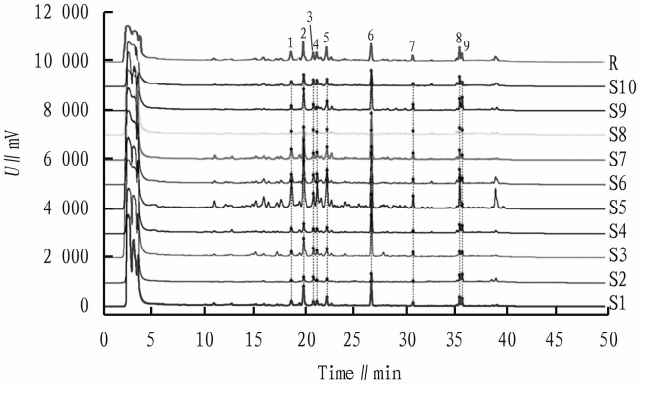
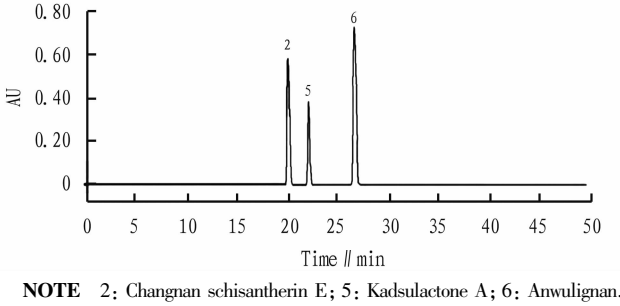


Fig. 1 Superimposed chromatograms and control fingerprint chromatogram of 10 batches of *Kadsura longipedunculata* medicinal materials



NOTE 2: Changnan schisantherin E; 5: Kadsulactone A; 6: Anwulignan.

Fig. 2 Chromatogram of mixed reference solution

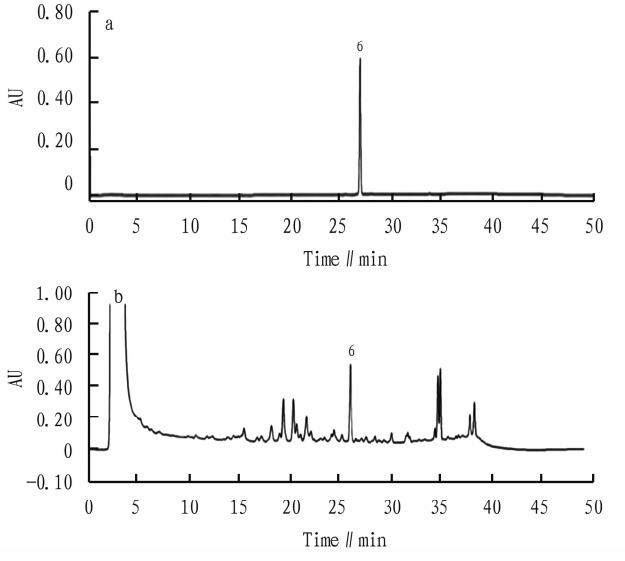
3.2 Determination of anwulignan content

3.2.1 Chromatographic conditions. The chromatographic conditions employed were identical to those described in Section **3.1.1**.

3.2.2 Preparation of reference solution. An appropriate amount of anwulignan reference substance was accurately weighed and transferred into a 50 mL volumetric flask. Methanol was added to dissolve the substance, and the volume was adjusted to the mark. The solution was then thoroughly mixed to prepare a reference solution with a mass concentration of 40.10 μg/mL.

3.2.3 Preparation of test solution. The preparation method for the test solution was consistent with the procedure described in Section **3.1.3**.

3.2.4 System suitability test. 10 μL each of the reference solution (Section **3.2.2**) and the test solution (Section **3.2.3**) were injected and analyzed in accordance with the chromatographic conditions specified in Section **3.2.1**. The results indicated that the resolution between the chromatographic peak of anwulignan and its adjacent peaks exceeded 1.5, and the theoretical plate number for anwulignan was at least 5 000. The corresponding chromatogram is presented in Fig. 3.



NOTE a. Reference solution; b. Test solution (No. S3); 6: Anwulignan.

Fig. 3 Chromatograms of the reference solution and the test solution

3.2.5 Investigation of linear relationship. The reference solution described in Section **3.2.2** was precisely aspirated and injected at volumes of 4, 6, 8, 10, 12, and 14 μL, respectively. Chromatographic analysis was conducted in accordance with the conditions outlined in Section **3.2.1**, and the corresponding peak areas were recorded. A standard curve was constructed by plotting the peak area of the anwulignan reference substance on the vertical axis (y) against its mass concentration on the horizontal axis (x). The resulting regression equation was $y = 939\ 321.41x + 360\ 886.00$, with a correlation coefficient (r) of 0.999 8. These results demonstrate a strong linear relationship between the mass concentra-

tion and peak area of the anwulignan reference substance within the concentration range of 160.40 to 561.40 μg/mL.

3.2.6 Precision test. The test solution (No. S3) was precisely aspirated and subjected to six consecutive injections and measurements under the chromatographic conditions outlined in Section **3.2.1**, with the peak area recorded each time. The results demonstrated that the *RSD* of the anwulignan peak area was 0.32% ($n=6$), indicating that the method exhibited good precision.

3.2.7 Stability test. The test solution (No. S3) was accurately aspirated and maintained at room temperature for 0, 2, 8, 16, 20, and 24 h, respectively. Subsequently, the samples were injected and analyzed under the chromatographic conditions described in Section **3.2.1**, and the peak areas were recorded. The results demonstrated that the *RSD* of the anwulignan peak area was 1.16% ($n=6$), indicating that the test solution remained stable when stored at room temperature for up to 24 h.

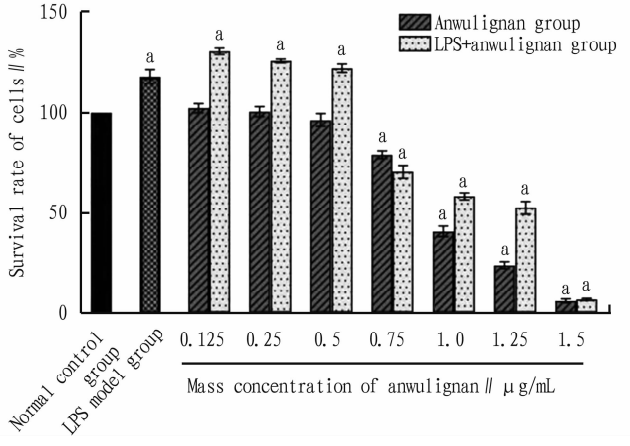
3.2.8 Repeatability test. *K. longipedunculata* powder (No. S3) was prepared into six test solution samples following the procedure described in Section **3.2.3**. These samples were subsequently injected and analyzed under the chromatographic conditions specified in Section **3.2.1**. The peak areas were recorded, and the content of anwulignan was quantified using the external standard method. The results demonstrated that the *RSD* of anwulignan content was 1.28% ($n=6$), indicating that the method exhibited good repeatability.

3.2.9 Spiked recovery rate. A total of six samples, each consisting of 1.0 g of *K. longipedunculata* powder from the same batch (No. S3) with a known content, were prepared. An appropriate volume of reference solution was added to each sample based on the content of the target component. The test solutions were prepared following the procedure described in Section **3.2.3**, and subsequently analyzed under the chromatographic conditions specified in Section **3.2.1**. The peak areas were recorded, and the average spiked recovery rate was calculated. The results demonstrated that the average spiked recovery rate of anwulignan was 98.68%, with a *RSD* of 1.34% ($n=6$), indicating that the method exhibited good accuracy.

3.2.10 Determination of sample content. Ten batches of *K. longipedunculata* medicinal materials from various origins were collected. Test solutions were prepared according to the procedure described in Section **3.2.3**. Subsequently, each sample was injected and analyzed under the chromatographic conditions specified in Section **3.2.1**, with three parallel determinations performed for each. Peak areas were recorded, and the anwulignan content was quantified using the external standard method. The results indicated that the anwulignan contents in samples S1 to S10 were (0.81 ± 0.01), (0.76 ± 0.02), (0.91 ± 0.03), (0.85 ± 0.06), (1.21 ± 0.03), (0.79 ± 0.03), (1.03 ± 0.02), (0.72 ± 0.05), (0.83 ± 0.04), and (0.78 ± 0.02) mg/g, respectively ($n=3$).

3.3 Anti-inflammatory activity of anwulignan

3.3.1 Effects on the growth of LPS-induced RAW264.7 cells. The MTT assay was employed for detection. RAW264.7 cells were cultured in an incubator maintained at 37 ℃ with 5% CO_2, using DMEM incomplete high-glucose medium supplemented with 10% fetal bovine serum until reaching the logarithmic growth phase. The experiment was organized into five groups: blank control, normal control, LPS model, anwulignan, and LPS + anwulignan groups. Except for the blank control group, which received only the culture medium, cells in the other groups were seeded into 96-well culture plates at a density of 8×10^4 cells/mL, with 100 μL per well. After the cells reached 80% confluence, the normal control group received no treatment. The LPS model group was stimulated with 1 μg/mL LPS for 1 h. The anwulignan group was treated with varying concentrations of anwulignan (0.125, 0.5, 0.75, 1.0, 1.25, and 1.5 μg/mL) based on preliminary experiments. The LPS + anwulignan group was first stimulated with LPS (1 μg/mL) for 1 h, followed by treatment with the aforementioned concentrations of anwulignan. Five replicate wells were prepared for each group. After 24 h of incubation, 20 μL of 5 mg/mL MTT solution was added to each well, followed by incubation in the dark for 4 h. Subsequently, the supernatant was discarded, and 150 μL of dimethyl sulfoxide was added to each well. The plates were then shaken for 10 min, and the optical density (*OD*) was measured at 490 nm using an ELISA reader. The cell survival rate was calculated using the following formula: Cell survival rate (%) = [(*OD* value of experimental group − *OD* value of blank control group)/(*OD* value of normal control group − *OD* value of blank control group)] × 100%. The results are presented in Fig. 4.



NOTE Compared to normal control group, $^aP<0.01$.

Fig. 4 Effects of anwulignan on the growth of LPS-induced RAW264.7 cells ($\bar{x}\pm s$, $n=5$)

As illustrated in Fig. 4, compared to the normal control group, the cell survival rate in the LPS model group was significantly increased ($P<0.01$), indicating that LPS stimulated the growth of RAW264.7 cells. When the mass concentration of anwulignan was no more than 0.5 μg/mL, no significant change in cell survival rate was observed in the anwulignan group relative to the normal control group ($P>0.05$). Conversely, the cell survival rates in the LPS + anwulignan group were significantly increased compared to the LPS model group ($P<0.01$). However, at an-

wulignan concentrations no less than 0.75 μg/mL, cell survival rates in both the anwulignan and LPS + anwulignan groups were significantly decreased ($P < 0.01$). These findings suggest that anwulignan at concentrations no more than 0.5 μg/mL does not exhibit cytotoxicity toward RAW264.7 cells, either alone or in combination with LPS. Consequently, concentrations of 0.125, 0.25, and 0.5 μg/mL were selected for subsequent experiments.

3.3.2 Effects on the levels of TNF-α, IL-1β and IL-6 in LPS-induced RAW264.7 cells. Detection was performed using the ELISA assay. Cells in the logarithmic growth phase were seeded into 96-well plates at a density of 8×10^4 cells/mL, with 100 μL per well. The experiment comprised three groups: a normal control group, an LPS model group, and an LPS + anwulignan group, each containing five replicate wells. Except for the normal control group, which received an equivalent volume of culture medium, the other groups were stimulated with 1 μg/mL LPS for 1 h. Subsequently, the LPS + anwulignan group was treated with anwulignan at concentrations of 0.5, 0.25, and 0.125 μg/mL, respectively. After 24 h of incubation, the samples were centrifuged at 800 rpm for 3 min, and the supernatant was collected. The levels of TNF-α, IL-1β, and IL-6 in the supernatant were measured according to the manufacturer's instructions. Statistical analyses were conducted using GraphPad Prism 8.0 software. Data were presented as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance (ANOVA) was employed for comparisons among multiple groups, followed by the *LSD-t* test for pair-wise comparisons, with a significance level of $\alpha = 0.05$. The results are presented in Table 2.

Table 2 Comparison of TNF-α, IL-1β and IL-6 levels in the supernatants of cells in each group ($\bar{x} \pm s$, $n = 5$, pg/mL)

Group	Mass concentration of anwulignan // μg/mL	TNF-α	IL-1β	IL-6
Normal control		177.34 ± 6.38	2.76 ± 0.47	15.29 ± 0.96
LPS model		370.35 ± 8.71 a	7.65 ± 0.69 a	554.79 ± 12.42 a
LPS + anwulignan	0.125	350.62 ± 6.43 b	6.24 ± 0.25 b	292.37 ± 7.74 c
	0.250	291.21 ± 7.69 c	5.11 ± 0.38 c	156.12 ± 6.59 c
	0.500	283.64 ± 5.17 c	3.03 ± 0.53 c	100.47 ± 3.12

NOTE Compared to the normal control group, $^aP < 0.01$; compared to the LPS model group, $^bP < 0.05$; and compared to the LPS model group, $^cP < 0.01$.

As presented in Table 2, the levels of TNF-α, IL-1β, and IL-6 in the cell supernatant were significantly elevated in the LPS model group compared to the normal control group ($P < 0.01$). Furthermore, treatment with anwulignan at concentrations of 0.125, 0.25, and 0.5 μg/mL in the LPS + anwulignan group resulted in a significant reduction of TNF-α, IL-1β, and IL-6 levels in the cell supernatants compared to the LPS model group ($P < 0.05$ or $P < 0.01$).

4 Conclusions and discussion

4.1 Optimization of the extraction method This study examined the effects of three extraction techniques—immersion, ultrasonic, and reflux methods—on the extraction efficiency of *K. longipedunculata*. The findings indicated that the reflux method yielded a more comprehensive extraction of the active components. Subsequently, the study evaluated the impact of six solvents—petroleum ether, ethyl acetate, chloroform, acetone, n-butanol, and ethanol—on the extraction efficiency of *K. longipedunculata*, with ethanol demonstrating the highest efficacy. Finally, the influence of ethanol volume fraction (65%, 70%, 75%, 80%, 85%, 90%, 95%), solid-to-liquid ratio (1 ∶ 10, 1 ∶ 20, 1 ∶ 30, 1 ∶ 40 g/mL), reflux duration (1, 2, 3 h), and reflux frequency (1, 2, 3, 4 cycles) on extraction efficiency of *K. longipedunculata* was investigated. Based on these results, the optimal extraction conditions were established as refluxing 2 g of *K. longipedunculata* with 20 mL of 80% ethanol for 2 h, repeated three times.

4.2 Selection of chromatographic conditions In this study, the number of peaks and resolution were employed as key evaluation indicators. Various chromatographic columns, including Elite Hypersil ODS C_{18} (250 mm × 4.6 mm, 5 μm) and Diamonsil C_{18} (250 mm × 4.6 mm), different mobile phase systems (methanol-water, acetonitrile-water, and acetonitrile-0.5% phosphoric acid solution), and detection wavelengths (210, 254, 280, and 290 nm) were systematically examined. The results indicated that under the chromatographic conditions utilizing the Elite Hypersil ODS C_{18} column, an acetonitrile-0.5% phosphoric acid solution as the mobile phase, and a detection wavelength of 210 nm, the chromatographic chromatogram of *K. longipedunculata* exhibited superior resolution, distinct characteristics, and a greater number of peaks.

4.3 Analysis of characteristic chromatogram and content results In this study, 9 common peaks were identified in the established HPLC characteristic chromatogram of *K. longipedunculata* medicinal materials, and three compounds were characterized: changnan schisantherin E, kadsulactone A, and anwulignan. The similarity indices of the characteristic chromatograms from 10 batches of *K. longipedunculata* samples collected from different regions in Guangxi were relatively high, all exceeding 0.9. The content of anwulignan varied between (0.72 ± 0.05) and (1.21 ± 0.03) mg/g, indicating good chemical composition consistency among the samples from different origins. However, variations in anwulignan content were observed, which may be attributed to environmental factors such as soil composition and water quality in the respective production areas.

4.4 Analysis of anti-inflammatory activity of anwulignan LPS is a critical component of the cell wall of Gram-negative bacteria and is known to stimulate cells to release substantial amounts of inflammatory cytokines, thereby exacerbating the inflammatory re-

sponse. Consequently, LPS is frequently employed in the development of various inflammatory models[8]. Macrophages, as primary immune cells, play an essential role in immune defense by phagocytosing and eliminating foreign pathogens and maintaining immune homeostasis. However, under pathological conditions, hyperactivation of macrophages can amplify inflammatory responses, leading to tissue damage and contributing significantly to the pathogenesis of inflammatory diseases such as pneumonia, arthritis, and pancreatitis[9]. Under LPS stimulation, macrophages exhibit a significant increase in the release of inflammatory cytokines, including TNF-α, IL-1β, and IL-6, thereby initiating an inflammatory response[10]. The present study demonstrated that the concentrations of TNF-α, IL-1β, and IL-6 in the supernatant of RAW264.7 cells were markedly elevated following LPS induction. Treatment with anwulignan effectively inhibited the release of these inflammatory cytokines in LPS-stimulated RAW264.7 cells. These findings suggest that anwulignan can attenuate the inflammatory response induced by LPS. Furthermore, the observed anti-inflammatory activity of anwulignan indicates that it may represent a key bioactive component of *K. longipedunculata* responsible for its anti-inflammatory properties and potential therapeutic effects in rheumatoid arthritis. Future studies will aim to validate its efficacy in animal models.

In summary, this study established the characteristic HPLC chromatogram of *K. longipedunculata* medicinal materials and developed a method for determining the content of anwulignan. Anwulignan may represent the active component of *K. longipedunculata* responsible for its anti-inflammatory effects. This research offers a more comprehensive reference for the quality evaluation of *K. longipedunculata* medicinal materials.

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(From page 25)

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