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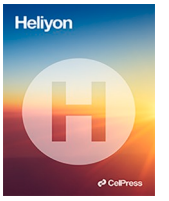

Research article

Evaluation of comparative chemical profiling and bioactivities of medicinal and non-medicinal parts of *Ampelopsis delavayana*

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ABSTRACT

Ampelopsis delavayana, a distinctive Yi medicine, utilized the roots as an essential medicinal substance for trauma treatment of the "Yunnan Hong Yao". *A. delavayana*, however, cannot be cultivated artificially presently, and it has been described with a phenomenon of mixed utilization of roots and stems, impeding pharmaceutical quality control. In response to resource scarcity and standardization issues, the research comprehensively compares the material basis and efficacy of medicinal (roots) and non-medicinal (stems) parts by using chemical profiling and pharmacological methodologies. Chemical disparity between two parts was compared by TLC and HPLC. Analgesia and anti-inflammatory capabilities of both parts were comprehensively evaluated through acetic acid writhing test, hot plate test, and xylene-induced mouse ear swelling test. Additionally, all the extracts were evaluated for anti-inflammatory activities by monitoring regulation of the levels of TNF-α, IL-1β, IL-6, and IgE in ear tissue. Consequently, the findings of TLC and HPLC revealed substantial similarity in the material basis of the medicinal and non-medicinal parts of *A. delavayana*, and pharmacological activities of anti-inflammatory and analgesic between two parts were consistent. Different extracts remarkably reduced the levels of TNF-α, IL-1β, IL-6, and IgE, demonstrating no discernible differences. Collectively, the comprehensive exploitation indicated that the medicinal and non-medicinal parts of *A. delavayana* exhibited identical chemical profiling and bioactivities, providing a theoretical rationale and scientific evidence for using stems as a therapeutic part, thereby holding considerable potential for ameliorating the current status of its medicinal reserves.

1. Introduction

In China, the incorporation of traditional Chinese medicine (TCM) into prescriptions is contingent upon adherence to standards, notably those set out by the Chinese Pharmacopoeia and province/ministerial-level authorities, to maintain drug quality and efficacy [1–3]. In many scenarios, the processing of raw materials used in TCM often produces a large amount of waste containing non-medicinal parts, such as branches or leaves, posing potential pollutant concerns [4–6]. The roots of *Panax notoginseng*, a medicinally recommended herb part, have long been commonly employed for treating various ailments, while its terrestrial section is

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often discarded [7]. Recently, comprehensive systematic exploration revealed that its non-medicinal parts, such as stems, leaves, and flowers, demonstrate significant pharmaceutical efficacies and are subsequently developed and endorsed for medical application, addressing the cultivation difficulty of parallel herbs [8]. A similar case is the leaves of *Eucommia ulmotyuiopides*, once overlooked, have now been proven to possess the same efficacy as the medicinal parts and were subsequently incorporated into the China Pharmacopoeia [9–11]. Making full use of non-medicinal items and transforming wastage into valuable medicinal resources has always been an essential aspect of sustainable resource exploration.

The dried roots of *Ampelopsis delavayana* Planch. ex Franch. (Fig. 1), a member of Vitaceae family, have been recorded in traditional medicine of Yi nationality (one of the ethnic minorities in Yunnan, China) with the effects of dispersing stasis, relieving pain, and reducing inflammation or hemostasis, and have been extensively applied for the treatment of trauma, fractures, rheumatic and arthritic pain [12,13]. Notably, *A. delavayana* is one of the important traumatology preparations of "Yunnan Hong Yao", a globally renowned trauma therapy product demanding substantial amount of the resources annually [14]. *A. delavayana*, however, cannot be cultivated artificially currently, relying mainly on wild resources for industrial utilization, and records indicated that its roots boast medical applications, further stressing the resource limitation [15,16]. Folk medicinal works, such as "Chinese Yi Pharmacy", described the phenomenon of *A. delavayana* mixed use of roots and stems, hindering pharmaceutical quality control [17]. It is worth noting that the scarcity availability of medicinal materials restricts the drug development of *A. delavayana*, despite the mixed use of medicinal and non-medicinal parts eases the phenomenon of resource shortage, comparative research into their material foundations and pharmacodynamic effects is still somewhat limited. Consequently, in this paper, we conducted an extensive comparative analysis of both the medicinal and non-medicinal parts of *A. delavayana* through a blend of chemical and biological methods. The material basis of the medicinal and non-medicinal parts of *A. delavayana* was compared by TLC and HPLC, and the chemical disparity between the two parts was scrutinized from a comprehensive perspective, thereby providing a theoretical basis for establishing the quality standard of the stems of *A. delavayana*. Subsequent pharmacological investigations on mice, including acetic acid writhing test, hot plate test, and xylene-induced ear swelling test, were conducted to assess the analgesic and anti-inflammatory properties of different extracts from both medicinal and non-medicinal parts of *A. delavayana*, with aspirin serving as positive control. Meanwhile, all the extracts evaluated for their anti-inflammatory activities by monitoring regulation of the levels of the inflammatory mediators, such as TNF-α, IL-1β, IL-6 in ear tissue, and IgE in serum. The outcomes HPLC and TLC revealed similar chemical profiles between medicinal and non-medicinal plant parts of *A. delavayana*, albeit with minor variations in component concentration. Animal studies testing their pain relieving and inflammation reducing abilities using the xylene-induced ear swelling model, as well as their impact on pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and immunoglobulin E (IgE), confirmed consistent anti-inflammatory and analgesic effects regardless of part type or origin. To some extent, this study could potentially mitigate the existing excessive exploitation of wild resources, and provide scientific rationale for the mixed utilization of folk medicinal materials.

2. Materials and methods

2.1. Plant material

The root and stem of *A. delavayana* were collected in October 2019 from Yanjin County, Zhaotong City, Yunnan Province, and authenticated by Professor Wen-Hong Tan. The voucher specimens (No: AD20191001) were deposited at the Engineering Research Center of Chinese herbal pieces in Universities of Yunnan Province (Yunnan University of Chinese Medicine).

2.2. Animals

SPF Kunming mice, comprising 200 females and 100 males (weighing 20 ± 2 g), were purchased from Sifu (Beijing) Biology Technology Co., Ltd. [License No. SCXK (jing) -2019-0010]. Mice were housed in a standard laboratory at 24 ± 3 °C, with a 12 h dark/light cycle, and fed with sufficient water and food for three consecutive days. Animal experimentation was conducted in adherence to internationally accepted guidelines for the use and care of laboratory animals, and the stipulations of the Animal Welfare Law of China. All comprehensive experimental procedures involving animals were approved by the Laboratory Animal Ethics Committee of Yunnan University of Chinese Medicine, under protocol code SYXK (DIAN) K2017-0005.

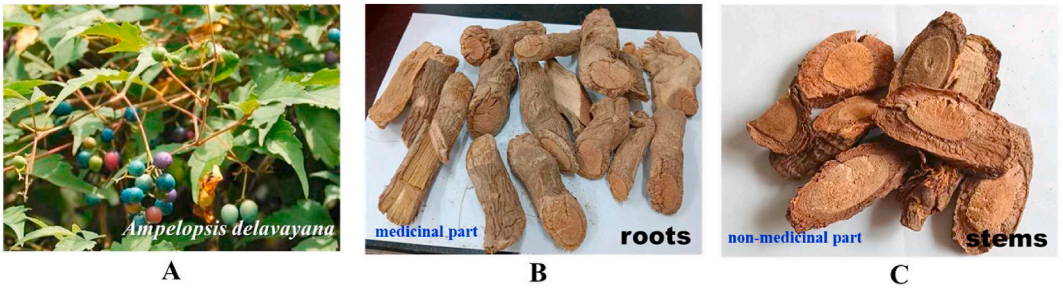

Fig. 1. The pictures of *A. delavayana.* (A) the picture of the plant; (B) the graphic of the roots; (C) the graphic of stems.

2.3. Reagents and materials

Xylene (AR, 99 %) was procured from Tianjin Bodi Chemical Co., Ltd. Glacial acetic acid was purchased from Tianjin Fengchuan Chemical Reagent Co., Ltd Sodium carboxymethylcellulose (CMC-Na) (BR, 99.5 %) was obtained from Tianjin Guangfu Fine Chemical Research Institute. Aspirin (Purity: 99.99 %) was procured from Beijing Solarbio Science Technology Co., Ltd. Vanillin (Purity: 98.0 %) was purchased from Shanghai Macklin Biochemical Co., Ltd. ELISA kits of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), and immunoglobulin E (IgE) were purchased from Jiangsu Meimian Industrial Co., Ltd. Chloroform (AR, 99 %) was purchased from Nanjing Chemical Co., Ltd. Methanol (GR, 99.5 %) was procured from Chengdu Colon Chemical Co., Ltd. Formic acid (AR, 88 %) was obtained from Nanjing Chemical Co., Ltd. Acetonitrile was purchased from Fisher Chemical Co., Ltd. The acetonitrile used was chromatographic grade, and other reagents were analytical grade. Ultrapure water was used.

2.4. Comparative study on material basis

2.4.1. Sample preparation

The identical amounts of the root (medicinal part) and stem (non-medicinal part) of *A. delavayana* were accurately weighed, crushed, extracted through methanol reflux, concentrated and dried to yield respective methanol extracts of roots and stems. For analysis, the specified amounts of the aforementioned samples were prepared in methanol to a concentration of 30 mg/mL for thin layer chromatographic (TLC) experiments and 50 mg/mL (filtered through a 0.22 μm filter membrane) for High Performance Liquid Chromatography (HPLC) experiments.

2.4.2. TLC analysis

Based on the existing literature and 2007 edition of the Yunnan Traditional Chinese Medicinal Herbs Standard (the second volume, Yi medicine) [18], a comparative study on TLC identification of the extracts derived from the medicinal and non-medicinal sections of *A. delavayana* was established Equal volumes of 3 μL aliquots from both medicinal and non-medicinal parts were placed on the identical silica gel G plate. To compare the material basis of both medicinal and non-medicinal parts across varying polarities, two developing solutions were selected: chloroform/methanol/formic acid (7:3:0.5) and chloroform/methanol (7:1). After completion of the chromatography, the plates were removed, vibrated to dry, sprayed with a 5 % vanillin/sulfuric acid/ethanol solution, and exposed to an elevated temperature of 105 °C until distinct spots became visible.

2.4.3. HPLC analysis

The fingerprint of medicinal materials analyzed using an Agilent 1260 Infinity II High Performance Liquid Chromatograph (Agilent Technologies, USA) analysis, utilizing an Agilent 5 TC-C18 column (250 × 4.6 mm i.d.; 5 μm) at 30 °C. A mobile phase of 1.0 % (v/v) aqueous formic acid (A) and acetonitrile (B) was employed with a gradient of 4–30 % B (0 → 71 min), 30 % B (71 → 83 min), and 30−100 % B (83 → 110 min), subsequently followed by an isocratic elution for 10 min, at a flow rate of 1.0 mL/min. An injection volume of 5 μL was utilized for the samples of the medicinal and non-medicinal parts of *A. delavayana*, with the detection wavelength set at 203 nm.

HPLC analysis was performed under the established chromatographic conditions, and the chromatograms of the samples were processed by comparing with the TCM Chromatographic Fingerprint Similarity Assessment System (2012 edition). The medicinal part sample (S1) of *A. delavayana* was selected as the reference peak, with the time window width was delineated to 0.1 min, and the common mode and control fingerprints of S1 and non-medicinal part (S2) were generated using the median method. Additional similarity evaluations were conducted, and the characteristic peaks were calibrated.

2.5. Comparative study on biological basis

2.5.1. Sample preparation

The roots and stems of *A. delavayana* (220 g each) were crushed and extracted with methanol reflux for twice (1 h each duration), subsequently filtered, concentrated, and dried to obtain methanol extracts of roots (30.69 g) and stems (22.99 g). After undergoing

Table 1
Grouping, abbreviation, and drug administrations of experimental mice.

grouping	abbreviation	dosage (mg/kg)
control group (saline water)	C	–
positive drug group (aspirin)	ASP	150
high dose group of methanolic extract of medicinal parts (roots)	GCH	800
low dose group of methanolic extract of medicinal parts (roots)	GCL	200
high dose group of aqueous extract of medicinal parts (roots)	GSH	800
low dose group of aqueous extract of medicinal parts (roots)	GSL	200
high dose group of methanolic extract of non-medicinal parts (stems)	JCH	800
low dose group of methanolic extract of non-medicinal parts (stems)	JCL	200
high dose group of aqueous extract of non-medicinal parts (stems)	JSH	800
low dose group of aqueous extract of non-medicinal parts (stems)	JSL	200

water reflux, ethanol crystallization, filtration, and freeze-drying, the residue was extracted to generate aqueous extracts of roots (18.57 g) and stems (14.98 g), which were subsequently sealed and refrigerated for utilization. Two concentrations of methanolic and aqueous extracts (200 and 800 mg/kg) were administered orally to mice. Aspirin (150 mg/kg) was used as the standard drug, and saline served as the control. The grouping, abbreviation, and dosage of the experimental animals in the biological activities are described in Table 1.

2.5.2. Acetic acid writhing test

The experiment was employed the existing methodology [19,20]. The acetic acid writhing test involves injecting glacial acetic acid into the abdominal cavity of experimental animals to induce pain, leading to peristalsis or contraction of the organism, designated as twisting. In this experiment, Kunming mice, half male and half female, were randomly divided into 10 groups with 10 mice in each group. After one week of administration, 0.2 mL/10 g body weight of the corresponding drug was taken orally, and 0.6 % acetic acid was injected intraperitoneally after 1 h of administration on the 7th day, with the number of twisting mice within 15 min noted for each group.

2.5.3. Hot-plate test

To assess thermal hyperalgesia, animals were placed on a heat plate individually, and the exit latency of each hind paw was measured until an adverse reaction (such as foot licking) appeared [21,22]. The temperature of the thermal plate was set to 55 ± 0.5 °C, and the Kunming mice were placed on the thermal plate, with the normal pain threshold of each mouse ascertained pre-administration. A total of 60 qualified mice were randomly divided into 10 groups, with given continuous intragastric administration for 7 days, once daily, at a dose of 0.2 mL/10 g. The pain threshold was assessed once at 30, 60, 90, 120, and 150 min after to the last administration, and the alterations over a period of 60s in the pain threshold were meticulously monitored and compared across each group.

2.5.4. Xylene-induced ear swelling test

Ear swelling, a recognized inflammatory, was induced by applying xylene to the auricle of experimental animals, with observation the degree of ear edema, and was often employed to assess the effects of anti-inflammatory drugs [23,24]. In this research, Kunming mice, half male and half female, were randomly divided into 10 groups (10 mice per group) and administered the corresponding drugs for one week (0.2 mL/10 g body weight). After the final administration, 50 μL of xylene was injected into both sides of the right ear, with the left ear serving as a control. 30 min later, the mice were sacrificed by taking the eye blood, and a circular section was taken at the same position of bilateral ears by punching a diameter of 8 mm along the baseline of the auricle. The weight of the ear was measured utilizing an electronic analysis balance, and the edema degree (mg) of the ears was indicated by the weight disparity between the left ear weight and right ear.

After the swelling experiment, the right ear of each group was enclosed within the EP tubing, frozen in liquid nitrogen, and preserved in a refrigerator at −80 °C. Eye blood samples of mice for ear swelling test were taken, placed in 1.5 mL EP tube for 30 min, and centrifuged with a frozen centrifuge at 3000 r/min for 15 min, with the supernatant was procured by pipette and stored in the EP tube at −80 °C. ELISA kits were used to detect the levels of TNF-α, IL-6, and IL-1β in the ear tissue [25], as well as the level of IgE in serum according to the manufacturer's instructions. The absorbance was measured and calibrated using a microplate reader at 450 and 570 nm. To ascertain the validity of the experimental data, the experiment was repetitively executed thrice.

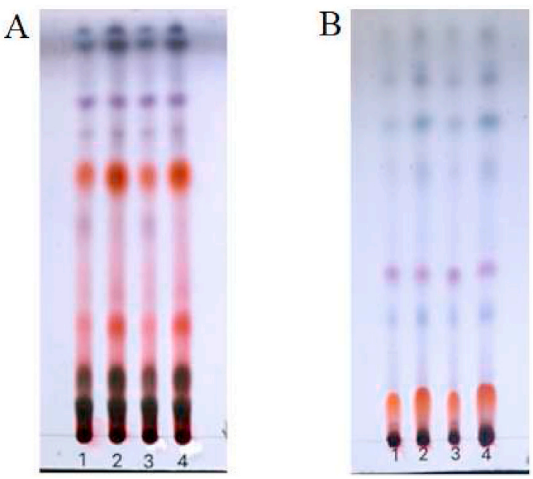

Fig. 2. TLC of the medicinal and non-medicinal parts. (**A**) Developing solvent: chloroform/methanol/formic acid (7:3:0.5); (**B**) Developing solvent: chloroform/methanol (7:1). Medicinal spots numbered 1 and 3; non-medicinal spots numbered 2 and 4.

2.5.5. Statistic analysis

All experiments were repeated three times, using blind or randomization, with all experimental data were presented as mean ± SD, and statistical analyses were performed using GraphPad Prism (8.4.0.671) software. Data were evaluated by analyzing the mean of each column and control column by multiple comparisons in one-way ANOVA, where a *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Chemical experiment research

3.1.1. TLC analysis

The TLC analysis of samples under varying polarities were delineated in Fig. 2. Briefly, the TLC analysis showed that the samples of the medicinal and non-medicinal parts of *A. delavayana* exhibited the identical corresponding spots across distinct solvent systems, suggesting that the predominant chemical constituents between the medicinal and non-medicinal parts were identical. Despite equivalent spotting quantity, the spots of the non-medicinal parts were more pronounced, whereas those in the medicinal parts in the medicinal parts showed more numerous spots with consistent corresponding major points, indicating that the material basis was similar.

3.1.2. HPLC analysis

HPLC fingerprints and reference fingerprints of the medicinal and non-medicinal parts of *A. delavayana* were generated. As shown in Fig. 3, samples of the medicinal and non-medicinal constituents displayed similar HPLC fingerprints, both of which encompassed identical primary compounds, further validated the experimental results of TLC.

The HPLC fingerprint and control fingerprint (R) of medicinal and non-medicinal parts of *A. delavayana.* were evaluated using the Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 edition), and the similarity assessment outcomes ranged from 0.986 to 0.987 (Table 2), indicating that the medicinal and non-medicinal parts of *A. delavayana* process exhibit a high degree of similarity (see Table 3).

The chromatogram was processed by the Similarity Evaluation System of Traditional Chinese Medicine Chromatographic Fingerprint (2012 edition). The chromatogram diagram of S1 sample was set as the reference peak, the time window width was set at 0.1 min, and the common pattern fingerprint and control fingerprint (R) under the medicinal and non-medicinal parts of *A. delavayana.* were generated by the median method. A total of 18 common peaks were calibrated, the retention time deviation RSD% was 0, and all the calibrated peaks corresponded one by one. However, the difference in the area of some calibrated peaks was relatively large, such as peaks **5**, **7**, **9**–**13**, and the RSD% of the sub-area was greater than 50, indicating that the content of these components was significantly different in the two parts.

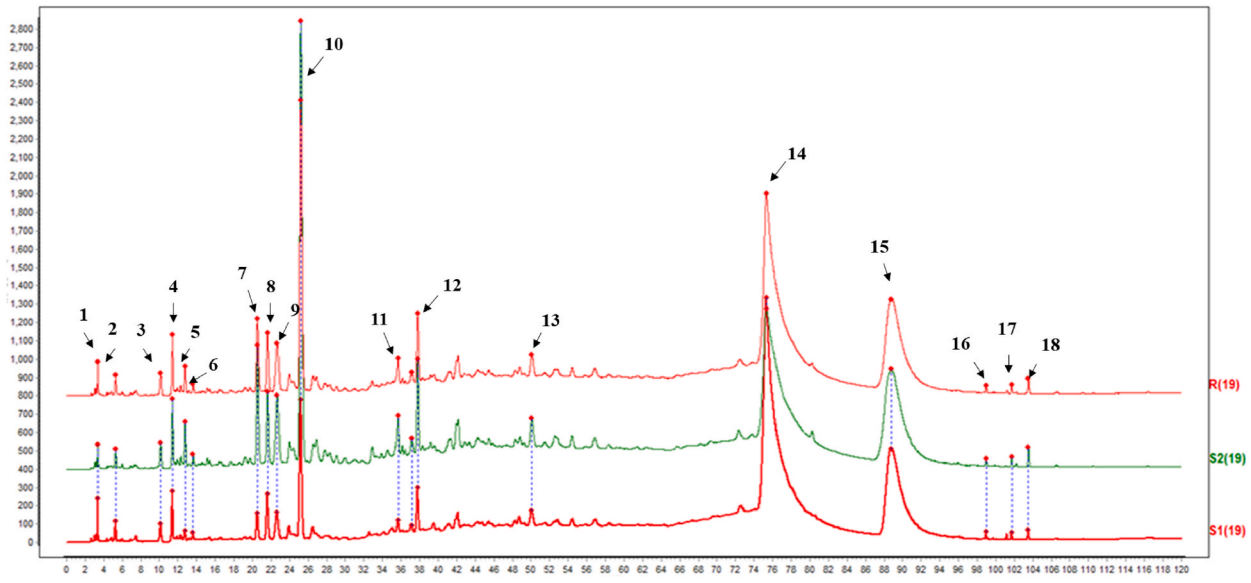

Fig. 3. HPLC and reference fingerprints of medicinal and non-medicinal parts of *A. delavayana*. R: the control fingerprint; S1: medicinal part; S2: non-medicinal part.

Table 2
HPLC fingerprint similarity evaluation results of medicinal parts and non-medicinal parts of *A. delavayana*.

	S1 (medicinal part)	S2 (non-medicinal part)	R (Control fingerprint)
S1	1	0.948	0.987
S2	0.948	1	0.986
R	0.987	0.986	1

Table 3
Statistical analysis of prevalent peaks within the medicinal and non-medicinal parts of *A. delavayana*.

No.	retention time (min)	peak area (S1)	peak area (S2)	peak area (R)	rt. RSD (%)	peak area RSD (%)	matches (No.)
1	3.366	54507.965	40915.254	47711.609	0	20.14	2
2	5.294	53795.887	49691.164	51743.525	0	5.61	2
3	10.15	79478.914	115411.359	97445.137	0	26.07	2
4	11.413	136778.25	194753.703	165765.977	0	24.73	2
5	12.761	32286.557	148362.891	90324.724	0	90.87	2
6	13.653	23770.523	35412.672	29591.598	0	27.82	2
7	20.545	129107.094	581210.313	355158.703	0	90.01	2
8	21.613	218367.625	356792.844	287580.234	0	34.04	2
9	22.677	195691.234	531914.188	363802.711	0	65.35	2
10	25.201	749980.063	2535942.25	1642961.156	0	76.87	2
11	35.67	57111.199	137364.328	97237.764	0	58.36	2
12	37.133	35006.563	87726.039	61366.301	0	60.75	2
13	37.791	192521.922	416471.656	304496.789	0	52.01	2
14	50.088	108356.32	204982.406	156669.363	0	43.61	2
15	75.307	6185440.5	5074902.5	5630171.5	0	13.95	2
16	88.779	3132926.75	3535836.5	3334381.625	0	8.54	2
17	98.989	19654.227	18821.602	19237.914	0	3.06	2
18	103.511	28849.338	57284.141	43066.739	0	46.69	2

3.2. Pharmacological experimental research

3.2.1. Acetic acid writhing test

As shown in Fig. 4, in comparison with the control group, diverse extracts derived from both medicinal and non-medicinal parts of *A. delavayana* objectively reduced the count of writhing reactions instigated by 0.6 % acetic acid in a dose-dependent manner. Aqueous extract of medicinal parts significantly decreased the writhing responses at the dose of 800 mg/kg, surpassing the control group. For the non-medicinal or the medicinal parts, at identical dosages, compared with methanolic extract, aqueous extract exhibited enhanced capability to reduce the frequency of writhing responses, albeit insignificantly. Upon administration of equivalent dosage, the aqueous or methanolic extracts of the medicinal part significantly diminished the number of torsion reactions compared to the extract of non-medicinal part, nevertheless, the analgesic efficacy of the non-medicinal extracts was comparable to that of the positive control, ASP, at a high dose of 800 mg/kg. Collectively, these findings suggested that the medicinal and non-medicinal parts exhibit nearly identical analgesic effect at high dose, with minimal gender differences.

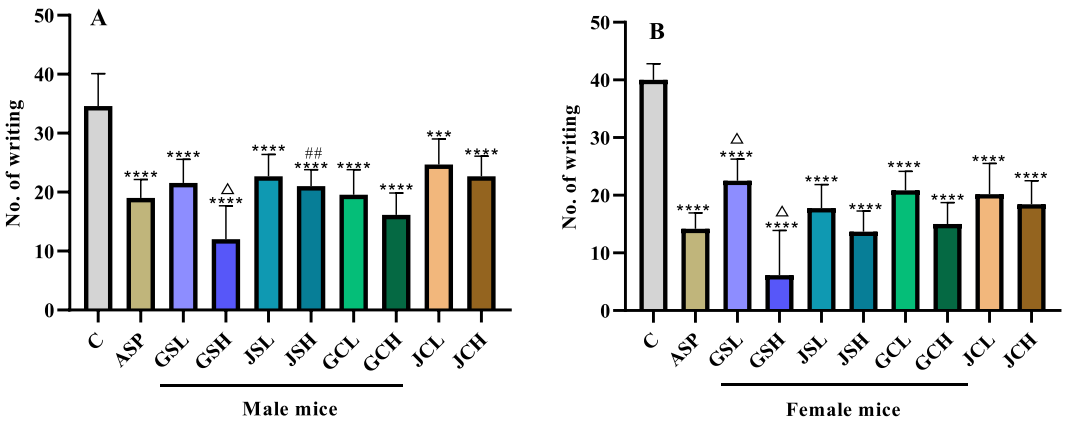

Fig. 4. Effects of medicinal and non-medicinal parts on writhing number of male and female mice induced by acetic acid. ***$p < 0.001$, ****$p < 0.0001$ vs. control group; Δ$p < 0.05$ vs. positive control group; ##$p < 0.01$ vs. the medicinal and non-medicinal parts.

3.2.2. Hot-plate test

The hot plate assay has been widely utilized to assess the effectiveness of analgesic interventions. The pain thresholds of methanolic extracts of medical and non-medical parts, as well as the aqueous extracts of the medicinal and non-medical parts in aspirin-treated mice were evaluated for a span of 150 min. As shown in Fig. 5, the effects of the extracts on pain induced by heat stimulation in mice were observed, and there was no significant change in the pain threshold of the control group mice at each measurement time point. Either extracts of methanolic or aqueous, the medicinal and non-medicinal parts (200 and 800 mg/kg) manifested the analgesic activity that lasted until 150 min after administration. In contrast to the control group, both methanolic and aqueous extracts of the two parts exhibited promising analgesic effects spanning 60–120 min. Regarding the methanolic extracts (Fig. 5A), the non-medicinal parts (200 or 800 mg/kg) further augmented the pain threshold beyond that of the positive control, ASP. Similarly, the aqueous extracts (Fig. 5B), both of medicinal and non-medicinal parts of *A. delavayana* hold enhanced analgesic effects when compared with the positive control, extending from 60 to 150 min. The aforementioned findings demonstrated that there existed no differentiation between the medicinal and non-medicinal parts of *A. delavayana*, with the analgesic efficacy of the aqueous extract of both sections being superior to that of ASP.

3.2.3. Xylene-induced ear swelling test

The anti-inflammatory effects of medicinal (GCL, GCH, GSL, and GSH) and non-medicinal (JCL, JCH, JSL, and JSH) parts were evaluated utilizing the xylene-induced mouse ear swelling model. As shown in Fig. 6, subsequent to administration, both the positive control group and the test group, comprising both medicinal and non-medicinal parts, effectively mitigated ear edema in mice. As depicted in Fig. 6A, the inhibitory efficacy of both GCH and JCH on ear swelling is comparable. At a dose of 800 mg/kg, the suppression effect of GCH was superior that of any test group, substantially outperforming the positive control, ASP ($P < 0.0001$), while JCH demonstrated an equivalent suppression as ASP ($P < 0.01$). Notably, at the dosage of 200 mg/kg, methanolic extracts of the medicinal (GCL) and non-medicinal (JCL) parts successfully reduced ear swelling in male mice, with enhanced efficacy compared to the positive control group. For female mice (Fig. 6B), all experimental groups, including the positive control, exhibited a comparable effect in mitigating ear inflammation when compared to control, demonstrating a dose-dependent relationship. The aforementioned findings suggested that there was no difference in the efficacy of the medicinal and non-medicinal parts within the test, suggesting that they may share the identical anti-inflammatory properties as the high-dose treatment groups that equated to ASP.

To scrutinize further the accurate drug basis of both medicinal and non-medicinal parts, all extracts derived from the methanolic (GCL, GCH, JCL, and JCH) and aqueous (GSL, GSH, JSL, and JSH) were evaluated for their inhibitory effects on inflammatory mediators (TNF-α, IL-6, and IL-1β) within the ear tissue and IgE in serum, respectively. As shown in Fig. 7, compared with the control group, extracts derived from both medicinal and non-medicinal parts significantly reduced the levels of TNF-α, IL-1β, IL-6, and IgE in a dose-dependent manner, whereas there was minimal distinction between the methanolic and aqueous extracts. Dosing at 800 mg/kg, all parts exhibited identical anti-inflammatory activity, capable of effectively reducing the levels of IL-6, IL-1β, TNF-α, and IgE to those of positive control, ASP. It is noteworthy that GSH, JSH, GCH, and JCH with 800 mg/kg showed a remarkable decreasing trend in IL-6 and IgE production (Fig. 7B and D), aligning closely with ASP. Taken together, all of these data indicated that consistent with it were suppressed by administration of medicinal or non-medicinal extracts, demonstrating an evident influence on pro-inflammatory cytokine proliferation between the control group and the high-dose test group.

4. Discussion

A. delavayana primarily inhabits Yunnan, Sichuan, Fujian, and other Chinese provinces [15], with its roots have been documented in both the 1977 iteration of the Chinese Pharmacopoeia and the 2005 edition of the Yunnan Chinese Medicinal Materials Standard [26]. As a folk medicine within Yunnan, *A. delavayana* boasts a century-long history as a common local remedy utilized in the treatment of traumatic injuries, as well as benefiting from spinal fractures, by effectively reducing inflammation and pain, while promoting fracture repair [27]. The roots of *A. delavayana* serve as a vital raw material for the Jin Pin series brands of Yunnan Baiyao Group Co., Ltd and Yunnan Hong Yao, along with other products [28]. Presently, the sourcing of medicinal materials mainly involves wild collection, with an escalating demand for roots each year, resulting in the waste of non-medicinal parts, such as stems [14]. Folk medicinal works, such as “Chinese Yi Pharmacy”, described the phenomenon of *A. delavayana* mixed use of roots and stems, impeding

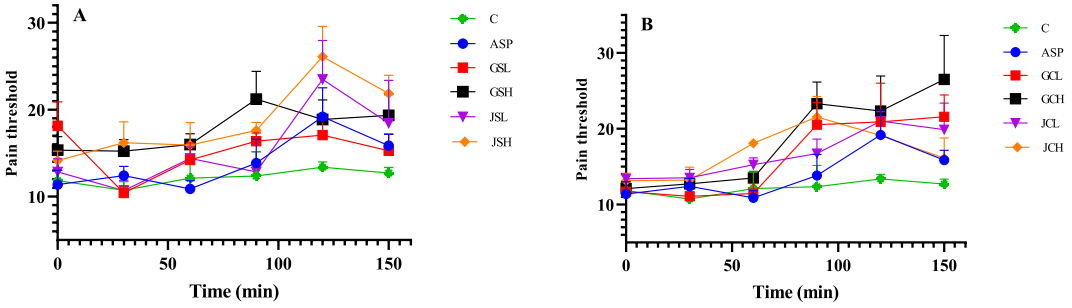

Fig. 5. Effects of medical and non-medical parts on thermal pain in mice. (**A**) Methanolic extract pain threshold; (**B**) Aqueous extract pain threshold.

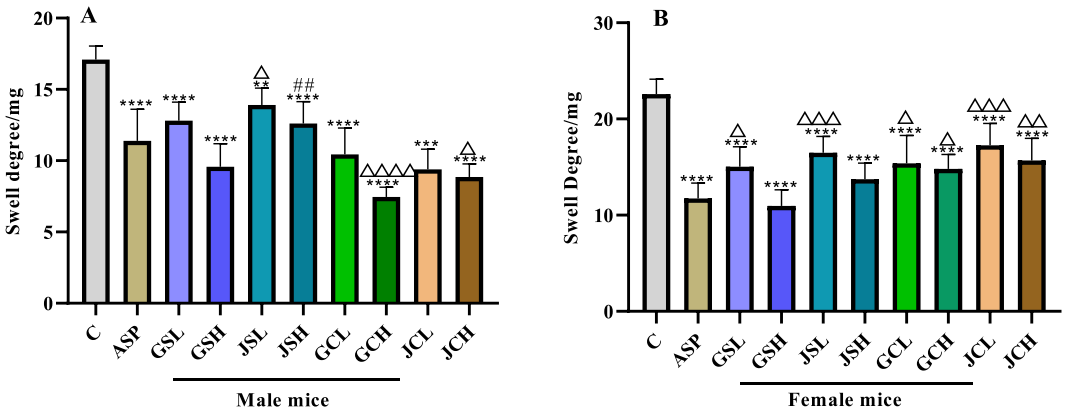

Fig. 6. Impact of medicinal and non-medicinal parts on xylene-induced mouse ear edema. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ vs. control group; $\Delta p < 0.05$, $\Delta\Delta p < 0.01$, $\Delta\Delta\Delta p < 0.001$, $\Delta\Delta\Delta\Delta p < 0.0001$ vs. positive control group; $\#\#p < 0.01$ vs. the medicinal and non-medicinal parts.

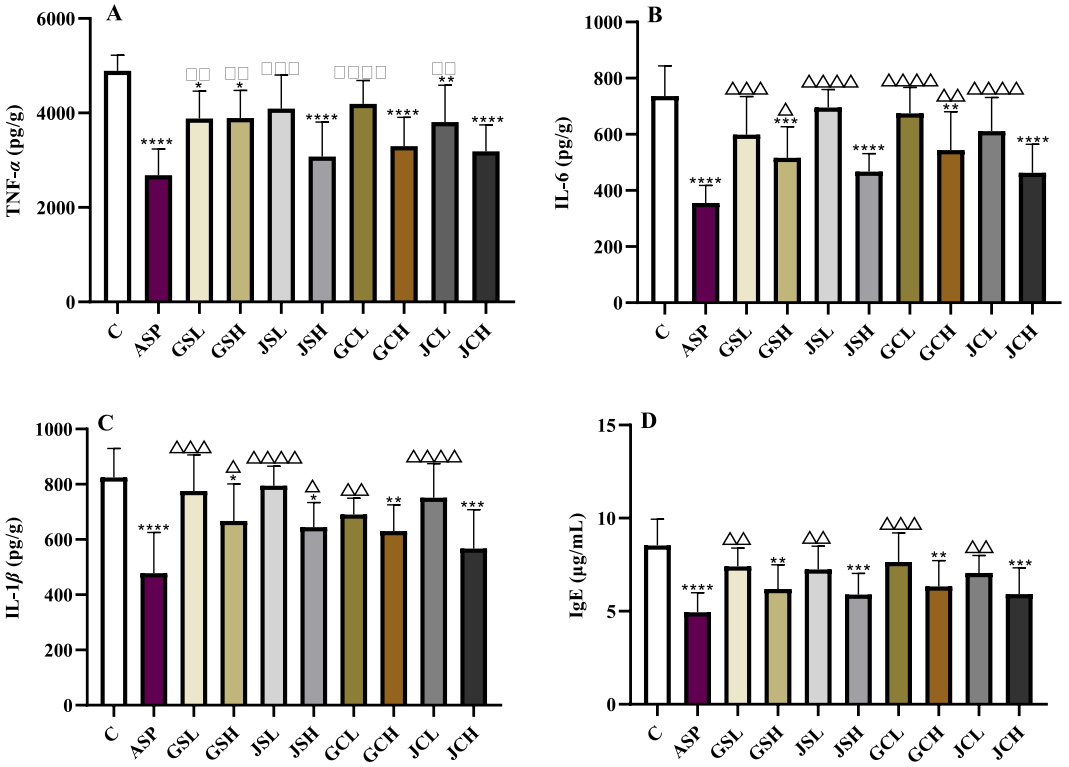

Fig. 7. Effects of medicinal and non-medicinal parts of on inflammatory mediators. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ vs. control group; $\Delta p < 0.05$, $\Delta\Delta p < 0.01$, $\Delta\Delta\Delta p < 0.001$, $\Delta\Delta\Delta\Delta p < 0.0001$ vs. positive control group.

pharmaceutical quality control [17]. Relatively limited comparative investigations into the material basis and pharmacological effects of its non-medicinal and medicinal parts. Therefore, we compared the material basis and pharmacological effects of the roots and stems, aiming to establish a theoretical basis for the utilization of stems as a therapeutic part, which holds considerable potential for ameliorating the prevailing status of its medicinal reserves. If the pharmacological effects of roots and stems are similar, the stems can become new medicinal parts instead of being discarded as waste, and this will significantly reduce environmental pollution.

TLC and HPLC serve as pivotal methodologies for material-based research and quality control of medicinal materials. In the TLC system, the sample solution was loaded onto a thin layer plate, followed the solution expansion in an expansion container utilizing a developer to fractionate the components encapsulated within the sample according to their polarity. This methodology is readily operational and extensively employed in the pharmaceutical industry, typically for qualitative analysis [29]. The HPLC fingerprint of TMC reflects the comprehensive information of the chemical composition of medicinal substances, and is frequently employed to

identify the authenticity of TCM and its preparations, as well as to assess the uniformity and stability of product quality. Owing to its intuitive operation and superior repeatability, TCM fingerprinting has emerged as the primary analytical technique for TCM formulations [30]. Previously, investigations have identified differences between transversal sections of the roots and stems of *A. delavayana*, whilst TLC experiments showcased that spots and color bands were identical for both roots and stems, suggesting that the primary chemical constituents of the two were substantially analogous [17]. In this research, the material foundation of the medical and non-medical parts of *A. delavayana* was compared by TLC and HPLC analysis, which scrutinized the chemical composition discrepancy between the medicinal and non-medicinal parts from a more comprehensive perspective, and both experimental outcomes validate that the two parts exhibited high resemblance. Simultaneously, it also provides a theoretical rationale for establishing the quality standard of *A. delavayana* stem.

The roots of *A. delavayana* are typically employed for the management of enteritis, injuries, and external treatment of burns and scalds, with a demonstrated analgesic and anti-inflammatory effect [31]. Nonetheless, limited pharmacological research is carried out on its stems. We therefore investigated whether the medicinal and non-medicinal parts exhibit the identical pharmacological potency utilized *in vivo* for acetic acid writhing test, hot-plate test, and xylene-induced ear swelling test. Concurrently, by monitoring the regulation on the levels of TNF-α, IL-1β, IL-6, and IgE, we further elucidate the rationale of their anti-inflammatory and analgesic effects.

Acetic acid-induced writhing models are widely used as classic anti-nociceptive paradigms to evaluate analgesic efficacy [32]. During the test, the writhe response is attributed to stimulation of nociception by excess proinflammatory mediators within the peripheral tissues, with the predominant cytokines implicated in acetic acid-induced nociception being TNF-α, IL-1β, and IL-8, which are released by resident peritoneal macrophages and mast cells [25,33]. The outcomes detailed herein demonstrated that under the control of a single variable, the frequency of written responses was substantially inhibited by a variety of dose-sensitive extracts from the medicinal and non-medicinal parts of *A. delavayana*. Within the same extract and dosage, when compared to the non-medicinal part, the medicinal part showed enhanced potency for reducing the frequency of twisting reactions. Nevertheless, collectively, the medicinal and non-medicinal parts of *A. delavayana* displayed nearly identical analgesic effects, with essentially no difference observed between the two in the experiment, suggesting that diverse extracts from both the medicinal and non-medicinal parts of *A. delavayana* exerted peripheral antinociceptive activity.

Hot-plate method serves as an effective model for evaluating the therapeutic efficacy of centrally acting analgesic agents and is recognized as the classical experimental analgesic model [23]. The examination involves placing the animals on a heated platform, subsequently causing the animals to lick their paws to relieve discomfort after a prescribed duration. Given that the paw licking response necessitates central nervous system coordination to indicate animal behavior, it is frequently served to evaluate the effectiveness of potent central analgesic drugs [34]. The results obtained by hot plate method indicated that different extracts from both medicinal and non-medicinal parts could substantially prolong the latency period of paw licking, persisting up to 150 min post-administration, suggesting the presence of central analgesic action in these various extracts.

Xylene has been reported to provoke acute edema by inducing the release of inflammatory mediators, making the xylene-induced inflammatory animal model a classical paradigm for preliminary evaluation of potential anti-inflammatory drugs [35]. The results of the xylene-induced ear swelling test in this study demonstrated that under the condition of a single variable, different extracts derived from the medicinal and non-medicinal parts could substantially reduce the degree of ear edema in a dose-dependent manner, with no difference in the efficacy of these sections. To assess its anti-inflammatory activity, the levels of IL-1β, TNF-α, IL-6, and IgE, representative factors associated with inflammation, were determined within mouse ear tissue or serum [36,37]. TNF-α is an early inflammatory factor, and IgE plays as a pivotal role in type I hypersensitivity, while IL-1β serves as a pleiotropic pro-inflammatory factor that constitutes the central vehicle for immune and inflammatory responses within the body, and can stimulate production of downstream inflammatory cytokines such as IL-6 and COX_2. In this present study, different extracts of medicinal and non-medicinal parts of *A. delavayana* remarkably diminished the levels of TNF-α, IL-1β, IL-6 in ear tissue and IgE in serum, without any appreciable difference between them. This indicated that different extracts derived from the medicinal and non-medicinal parts may exhibit identical anti-inflammatory and analgesic activities by inhibiting the release of the inflammatory mediators mentioned above.

5. Conclusions

Taken together, HPLC and TLC results confirmed that although there were some differences in the content of some components, yet the chemical composition types of medicinal parts and non-medicinal parts had high similarity. The combined HPLC and TLC data indicated some variations in component content, analgesia and anti-inflammatory abilities observed in animal models mentioned above, particularly those of the xylene-induced ear swelling test, demonstrated that different extracts of medicinal and non-medicinal parts of *A. delavayan* exert consistent anti-inflammatory and analgesic activities, unaffected by plant source. Moreover, both parts exhibited properties of analgesia and anti-inflammation by reducing inflammatory mediators such as TNF-α, IL-1β, IL-6, and IgE. Since no extensive investigations have been carried out on the material basis and pharmacological research of the non-medicinal and medicinal parts of *A. delavayana*, this study proposes that the non-medicinal parts could serve as a potential medicinal part for analgesia and anti-inflammation, providing a theoretical rationale and scientific evidence for the comprehensive exploitation and utilization of stem resources. Additionally, integrating non-medicinal substances into medication could lower overall market standards of medicinal materials, significantly decrease waste, and contribute to environmental conservation.

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Ethics approval and consent to participate

Animal experiments were conducted in accordance with globally recognized guidelines for the utilization and care of laboratory animals and the precepts of China's Animal Welfare Law. Detailed experimental procedures involving animals were approved by the Laboratory Animal Ethics Committee of Yunnan University of Chinese Medicine (protocol code SYXK (DIAN) K2017-0005).

Data availability

We authors confirmed that the data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Jin Qiong: Writing – original draft, Investigation, Formal analysis, Data curation. **Haiqin Yang:** Methodology, Investigation, Formal analysis, Data curation. **Yanqing Xie:** Formal analysis, Data curation. **Peifeng Zhu:** Methodology, Formal analysis. **Gong Chen:** Methodology. **Qixiu Zhou:** Investigation. **Zhuya Yang:** Writing – review & editing, Validation, Supervision. **Wenhong Tan:** Writing – review & editing, Validation, Supervision, Resources, Project administration. **Lu Liu:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology.

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.

References

[1] M. Wang, P.F. Yao, P.Y. Sun, W. Liang, X.J. Chen, Key quality factors for Chinese herbal medicines entering the EU market, Chin. Med. 17 (2022) 29, https://doi.org/10.1186/s13020-022-00583-x. PMCID: PMC8861989.
[2] M. Fu, Drug discovery from traditional Chinese herbal medicine using high content imaging technology, J. Tradit. Chinese Medical Sci. 8 (2021) 198–204, https://doi.org/10.1016/j.jtcms.2021.07.005.
[3] X. Wu, S.P. Wang, J.R. Lu, Y. Jing, M.X. Li, J.L. Cao, L.B. Bai, C.J. Hu, Seeing the unseen of Chinese herbal medicine processing (*Paozhi*): advances in new perspectives, Chin. Med. 17 (2018) 4, https://doi.org/10.1186/s13020-018-0163-3.
[4] H.W. Li, P. Liu, H.Q. Zhang, W.M. Feng, H. Yan, S. Guo, D.W. Qian, J.A. Duan, Determination of bioactive compounds in the nonmedicinal parts of *Scrophularia ningpoensis* using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry and chemometric analysis, J. sep. sci. 43 (2020) 4191–4201, https://doi.org/10.1002/jssc.202000723. PMID: 32975375.
[5] F. Yang, X. Qi, W. Liu, J. Li, D.J. Wang, L. Fang, Y.Q. Zhang, Separation of five flavonoids from aerial parts of *Salvia Miltiorrhiza* Bunge using HSCCC and their antioxidant activities, Molecules 24 (2019), https://doi.org/10.3390/molecules24193448, 3448−3448. PMCID: PMC6804221.
[6] Z.T. Li, F.X. Zhang, C.L. Fan, M.N. Ye, W.W. Chen, Z.H. Yao, X.S. Yao, Y. Dai, Discovery of potential Q-marker of traditional Chinese medicine based on plant metabolomics and network pharmacology: *periplocae Cortex* as an example, Phytomedicine 85 (2021) 153535, https://doi.org/10.1016/j.phymed.2021.153535. PMID: 33819766.
[7] Y. Qu, Y. Liu, L.Q. Huang, L.P. Guo, X.M. Cui, Analysis and evaluation of nutritive elements in aerial part of *Panax notoginseng*, China J, Chin. Mater. Med. 39 (2014) 601–605. PMID: 25204129.
[8] L. Liu, F.R. Xu, Y.Z. Wang, Traditional uses, chemical diversity and biological activities of *Panax* L. (Araliaceae): a review, J. Ethnopharmacol. 263 (2020) 112792, https://doi.org/10.1016/j.jep.2020.112792. PMID: 32311488.
[9] M.M. Bai, W. Shi, J.M. Tian, M. Lei, J.H. Kim, Y.N. Sun, Y.H. Kim, J.M. Gao, Soluble epoxide hydrolase inhibitory and anti-inflammatory components from the leaves of *Eucommia ulmoides* Oliver (duzhong), J. Agric. Food Chem. 63 (2015) 2198–2205, https://doi.org/10.1021/acs.jafc.5b00055.
[10] Y.Y. Xing, J.Y. Wang, K. Wang, Y. Zhang, K. Liu, X.Y. Chen, Y. Yuan, Inhibition of rheumatoid arthritis using bark, leaf, and male flower extracts of *Eucommia ulmoides*, Evid. Based Complement Alternat, Méd. 13 (2020) 3260278, https://doi.org/10.1155/2020/3260278. PMID: 32855647, PMCID: PMC7443016.
[11] Q. Huang, F. Zhang, S. Liu, Y. Jiang, D. Ouyang, Systematic investigation of the pharmacological mechanism for renal protection by the leaves of *Eucommia ulmoides* Oliver using UPLC-Q-TOF/MS combined with network pharmacology analysis, Biomed. Pharmacother. 140 (2021) 111735, https://doi.org/10.1016/j.biopha.2021.111735. PMID: 34020251.
[12] S.X. Mei, X.H. Li, L.G. Yang, X.H. Li, J.Y. Xie, Q. Yao, S. Mei, C. Tao, J.K. Wang, Z.Y. Zhu, Chemical constituents from the roots of *Ampelopsis delavayana* and their antibacterial activities, Nat. Prod. Res. 31 (2017) 190–195, https://doi.org/10.1080/14786419.2016.1224875. PMID: 27615738.
[13] Z.Q. Zhang, S.L. He, R. Zhao, X.X. Shan, Resource investigation and biological characteristics of the roots of *Ampelopsis delavayana*, J. Tradit. Chin. Med. 35 (2014) 72–74. LK, https://qikan.cqvip.com/Qikan/Article/Detail?id=50278099.
[14] J.R. Yang, N. Li, H. Peng, Clinical research of Yunnanhongyao capsule on fundus hemorrhage diseases, Chinese Journal of Medicinal Guide 17 (2015) 1263–1264. LK, http://qikan.cqvip.com/Qikan/Article/Detail?id=667741274.
[15] Z.H. Lu, J. Zhang, W.H. Tan, Preliminary study on crude drugs from roots and stems of *Ampelopsis delavayana*, J. Tradit. Chin. Med. 36 (2013) 32–33. LK, https://qikan.cqvip.com/Qikan/Article/Detail?id=47180032.
[16] Y.F. Ren, D.H. Fu, X.F. Li, Study on pharmacognosy of roots of *Ampelopsis delavayana*, Yunnan Journal of Traditional Chinese Medicine and Materia Medica 32 (2011) 52–54. LK, http://qikan.cqvip.com/Qikan/Article/Detail?id=37818351.

[17] Yunnan Yi Medicine Institute, Chinese Yi Pharmacy, Yunnan Ethnic Publishing House, China, Yunnan, 2004, p. 525.
[18] Y. Wang, T.T. Zhao, Y.L. Dai, R.C. Yang, S.J. Li, Study on analgesic and sedative effects of Tianmu Jiangya Tablets in mouse model, Chin. J. Integr. Med. 17 (2019) 364–367, https://doi.org/10.12102/i.issn.1672-1349.2019.03.011.
[19] D. Dong, N.N. Zhou, R.X. Liu, J.W. Xiong, H. Pan, S.Q. Sun, L. Ma, R. Wang, Sarsasapogenin-AA13 inhibits LPS-induced inflammatory responses in macrophage cells *in vitro* and relieves dimethylbenzene-induced ear edema in mice, Acta Pharmacol. Sin. 38 (2017) 699–709, https://doi.org/10.1038/aps.2016.180. PMID: 28239159, PMCID: PMC5457694.
[20] C.L. Li, N. Liu, Expression of interleukin-1β, interleukin-4, interferon-γ and tumour necrosis factor α in different tissue in a dinitrochlorobenzene-induced ear swelling test in mice, Skin Res. Technol. 29 (2022) e13255, https://doi.org/10.1111/srt.13255.
[21] T. Numata, S. Takahagi, K. Ishii, S. Morioke, T. Kan, H. Mizuno, Y. Yanase, T. Kawaguchi, A. Tanaka, M. Hide, Immunological changes of basophil hyperreactivity to sweat in patients with well-controlled atopic dermatitis, Front. Immunol. 13 (2022) 883605, https://doi.org/10.3389/fimmu.2022.883605.
[22] Medical Products Administration of Yunnan Province, the Chinese Herbal Medicine Standard Volume in Yunnan II•Yi Medicine, Yunnan Science and Technology, Publishing House, China, Yunnan, 2007, pp. 45–46.
[23] M.R. Hossain, R. Alam, H.J. Chung, T.A. Eva, M.F. Kabir, H. Mamurat, S.T. Hong, M.A. Hafiz, S.M.M. Hossen, *In vivo, in vitro* and in silico study of *Cucurbita moschata* flower extract: a promising source of natural analgesic, anti-inflammatory, and antibacterial agents, Molecules 28 (2023) 6573, https://doi.org/10.3390/molecules28186573. PMID: 37764349, PMCID: PMC10536299.
[24] Q. Jin, Y.L. Zhao, Y.P. Liu, R.S. Zhang, P.F. Zhu, L.Q. Zhao, X.J. Qin, X.D. Luo, Anti-inflammatory and analgesic monoterpenoid indole alkaloids of *Kopsia officinalis*, J. Ethnopharmacol. 285 (2022) 114848, https://doi.org/10.1016/j.jep.2021.114848. PMID: 34798159.
[25] D. Wang, L. Liu, K. Li, H. Cao, M. Liu, Q. Chen, Y. Wu, Y. Zhang, T. Wang, Eurycoma longifolia alkaloid components ameliorate hyperuricemic nephropathy via regulating serum uric acid level and relieving inflammatory reaction, J. Nat. Med. 77 (2023) 867–879, https://doi.org/10.1007/s11418-023-01729-3. PMID: 37433989.
[26] N. Arya, O.V. Prakash, A.K. Pant, Central and peripheral analgesic activity of *Turmeric Rhizome* collected from Uttarakhand, India, Asian J. org. chem. 28 (2016) 1024–1026, https://doi.org/10.14233/ajchem.2016.19571.
[27] M. Sajid, M.R. Khan, M.U. Ijaz, H. Ismail, M.Z. Bhatti, S.A. Shah, S. Ali, M.U. Tareen, S.S. Alotaibi, S.M. Albogami, G.E. Batiha, Evaluation of phytochemistry and pharmacological properties of *Alnus nitida*, Molecules 27 (2022) 4582, https://doi.org/10.3390/molecules27144582. PMID: 35889458, PMCID: PMC9320741.
[28] Z.G. Tian, Experimental study on effect of Tongfuning granule on xylene-induced ear swelling model in mice, Liaoning, J. Tradit. Chin. Med. 34 (2007) 1494. LK, https://qikan.cqvip.com/Qikan/Article/Detail?id=25537672.
[29] Y.A. Choi, J.H. Yu, H.D. Jung, S. Lee, P.H. Park, H.S. Lee, T.K. Kwon, T.Y. Shin, S.W. Lee, M.C. Rh, Y.H. Jang, S.H. Kim, Inhibitory effect of ethanol extract of *Ampelopsis brevipedunculata* rhizomes on atopic dermatitis-like skin inflammation, J. Ethnopharmacol. 238 (2019) 111850, https://doi.org/10.1016/j.jep.2019.111850. PMID: 30953820.
[30] Y. Li, B. Qiu, L. Zheng, Study on seed testing rules of *Ampelopsis delavayana*, Chin. Med. J. Res. Prac. 31 (2017) 5–8, https://doi.org/10.13728/j.1673-6427.2017.03.002.
[31] X.B. Liu, X.Z. Liu, C.F. Yan, M.H. Yang, J.B. Li, M.H. Zhao, Study on anoxia tolerance function of *Ampelopsis Delavayand* Planah root, J. Tradit. Chin. Med. 28 (2007) 39–40. LK, https://qikan.cqvip.com/Qikan/Article/Detail?id=1000105027.
[32] J. Xiao, J.Z. Liu, S.L. Liu, T.G. Xiao, G.M. Kuang, Yunnan Hongyao capsules for treating hemorrhage of internal hemorrhoids in 60 cases, Chinese Pharm 25 (2016) 95–96, https://doi.org/10.3969/j.issn.1006-4931.2016.24.034.
[33] I. Ulea, H. Stavri, Thin layer chromatography characterization of ELISA specific glycolipids antigens immunomagnetically purified from environmental mycobacteria, Roum, Arch. Microbiol. Immunol. 72 (2013) 105–120. PMID: 24187809.
[34] Q. Zhou, J. Ma, Q. Liu, C. Wu, Z. Yang, T. Yang, Q. Chen, Y. Yue, J. Shang, Traditional Chinese Medicine formula, Sanwujiao granule, attenuates ischemic stroke by promoting angiogenesis through early administration, J. Ethnopharmacol. 321 (2023) 117418, https://doi.org/10.1016/j.jep.2023.117418. PMID: 37979814.
[35] J. Zhang, X. You, X.Y. Duan, M.B. Zhou, Pharmacological research on anti-inflammatory and analgesic effects of *Ampelopsis delavayana* (Franch.) planch, J. Dalian Medical Univ. 5 (2022) 22–24. Corpus ID: 75381935.
[36] N.T. Katole, J.S. Kale, H.V. Salankar, Evaluation of the antinociceptive action of simvastatin in mice, Cureus J. med. Science 14 (2022) e26910, https://doi.org/10.7759/cureus.26910. PMID: 35983393, PMCID: PMC9376206.
[37] S.A. Onasanwo, Q.T. Oyebanjo, A.M. Ajayi, M.A. Olubori, Anti-nociceptive and anti-inflammatory potentials of *Vernonia amygdalina* leaf extract via reductions of leucocyte migration and lipid peroxidation, J. Intercult. Ethnopharmacol. 6 (2017) 192–198, https://doi.org/10.5455/jice.20170330010610. PMID: 28512601, PMCID: PMC5429079.