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Research Article

Identification and quantification of oleanane triterpenoid saponins and potential analgesic and anti-inflammatory activities from the roots and rhizomes of *Panax stipuleanatus*

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ABSTRACT

Background: *Panax stipuleanatus* represents a folk medicine for treatment of inflammation. However, lack of experimental data does not confirm its function. This article aims to investigate the analgesic and anti-inflammatory activities of triterpenoid saponins isolated from *P. stipuleanatus*.
Methods: The chemical characterization of *P. stipuleanatus* allowed the identification and quantitation of two major compounds. Analgesic effects of triterpenoid saponins were evaluated in two models of thermal- and chemical-stimulated acute pain. Anti-inflammatory effects of triterpenoid saponins were also evaluated using four models of acetic acid–induced vascular permeability, xylene-induced ear edema, carrageenan-induced paw edema, and cotton pellet–induced granuloma in mice.
Results: Two triterpenoid saponins of stipuleanosides R_1 (SP-R_1) and R_2 (SP-R_2) were isolated and identified from *P. stipuleanatus*. The results showed that SP-R_1 and SP-R_2 significantly increased the latency time to thermal pain in the hot plate test and reduced the writhing response in the acetic acid –induced writhing test. SP-R_1 and SP-R_2 caused a significant decrease in vascular permeability, ear edema, paw edema, and granuloma formation in inflammatory models. Further studies showed that the levels of inflammatory mediators, nitric oxide, malondialdehyde, tumor necrosis factor-α, and interleukin 6 in paw tissues were downregulated by SP-R_1 and SP-R_2. In addition, the rational harvest of three- to five-year-old *P. stipuleanatus* was preferable to obtain a higher level of triterpenoid saponins. SP-R_2 showed the highest content in *P. stipuleanatus*, which had potential as a chemical marker for quality control of *P. stipuleanatus*.
Conclusion: This study provides important basic information about utilization of *P. stipuleanatus* resources for production of active triterpenoid saponins.

1. Introduction

Panax stipuleanatus H. T. Tsai et K. M. Feng belongs to an evolutionary group of *Panax* species. It is mainly found in the tropical rain forest, at 1100–1700 m above sea level, in Pingbian, Maguan, and Malipo County, Yunnan Province, China, and its roots and rhizomes can be used as medicinal materials. In traditional Chinese medicine, *P. stipuleanatus* is known to act as an analgesic, being able to remove blood stasis and provide essential nourishment, promoting wound healing and hemostasis. *P. stipuleanatus* remains an underutilized natural resource.

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At present, there are only a few studies on *P. stipuleanatus*, limited to plant classification [1], biological characteristics [2,3], and preliminary chemical composition and biological activity [4]. Identified saponins from *P. stipuleanatus* are mainly oleanane-type triterpenoid saponins, e.g., stipuleanosides R_1 (SP-R_1) and R_2 (SP-R_2) [4]. Liang et al [5] reported that the oleanane-type triterpenoids isolated from *P. stipuleanatus* had cytotoxic activity against HL-60 cells (leukemia) and HCT-116 cell lines (colon cancer). Liang et al [6] also found that the extract had an inhibitory effect on proinflammatory mediators of nuclear factor kappa B (NF-κB). In HepG2 cells (hepatocellular carcinoma), NF-κB activity was inhibited by reducing the concentration of inflammatory factors, using oleanane-type triterpenoids isolated from *P. stipuleanatus*. However, all currently available studies on the anticancer activity of *P. stipuleanatus* were conducted on cell lines, and its traditionally anti-inflammatory and analgesic effects had not been reported.

At present, the clinical use of both steroids and nonsteroidal drugs in the treatment of inflammatory diseases has been associated with numerous adverse reactions. For example, nonsteroidal anti-inflammatory drugs stimulate the gastrointestinal tract and damage the kidneys, selective cyclooxygenase inhibitors increase the risk of cardiovascular diseases, and glucocorticoid drugs may activate or aggravate infections [7,8]. Recent studies have shown that a variety of natural active ingredients such as most terpene compounds have good anti-inflammatory activity [1,8] (Yang et al, 2006; Yang et al, 2012). Anti-inflammatory terpenes can inhibit production of cytokines, growth factors, adhesion molecules, NF-κB, and nitric oxide (NO) and prostaglandin expression [9]. Drugs derived from natural compounds and plant extracts have the advantage of being less toxic than chemical drugs. The development of such new drugs can represent a potential alternative to the treatment of acute and chronic inflammation with chemical drugs [10]. To develop natural active compounds into treatment of inflammatory diseases, more research is urgently needed for the isolation and identification of the compounds and its functional evaluation from *P. stipuleanatus*.

Therefore, in view of the traditional application of *P. stipuleanatus*, chemical composition and anti-inflammatory and analgesic activities were established in this study. The first objective of this study was to isolate oleanane triterpenoid saponins from *P. stipuleanatus* and determine the contents of saponins in *P. stipuleanatus* of different growth years and from different producing areas. The analgesic and anti-inflammatory activities of oleanane triterpenoid saponins in *vivo* were evaluated by two analgesic and four acute inflammation animal models. This study provides data support for the development of anti-inflammatory drugs from natural products or plant extracts.

2. Materials and methods

2.1. Materials and reagents

Xylene, carrageenan, hematoxylin and eosin, acetic acid, phosphoric acid, and acetonitrile [high-performance liquid chromatography (HPLC)—grade] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone was purchased from Zhejiang Xianchen Pharmaceutical Co., Ltd (Xianju, China). Aspirin was purchased from Shenyang Ojina Pharmaceutical Co., Ltd (Shenyang, China). All the other reagents and solvents were of analytical grade.

2.2. Plant material

The roots and rhizomes of *P. stipuleanatus* H.T. Tsai et K.M. Feng were collected in 2017 in Pingbian, Yanshan, and Wenshan County of Yunnan Province, China (detailed in Table 1). This plant was identified by Professor Xiuming Cui (Faculty of Life Science and Technology, Kunming University of Science and Technology) and given the voucher number KMUST017 at Seed Resource Center of Kunming University of Science and Technology.

2.3. Purification and isolation of SP-R_1 and SP-R_2

The medicinal parts of *P. stipuleanatus* are shown in Fig. 1. The root and rhizome powder of *P. stipuleanatus* (500 g) was extracted using 70% methanol three times. After filtration, the filtrate was evaporated to get methanol extract (121.36 g). The methanol extract was dissolved in water and partitioned with n-butanol. After obtaining the n-butanol fraction (16.28 g), it was chromatographed on silica gel and then on octadecylsilyl silica gel (ODS) to yield compounds SP-R_1 (0.64 g) and SP-R_2 (3.67 g). The structures of compounds SP-R_1 and SP-R_2 were determined by spectroscopic analyses. The NMR spectra were recorded on a Bruker AV III-500 spectrometer (Bruker, Berlin, Germany). HRESIMS data were collected on an Agilent 6530 quadrupole time-of-flight mass spectrometer (Agilent Technologies, California, America).

SP-R_1: white powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ: 0.80 (3H, s), 0.83 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 1.04 (3H, s), 1.15 (3H, s); for ^{13}C-NMR (CD_3OD, 125 MHz) data, see Table 2. The structure of compound SP-R_1 is shown in Fig. 2. HRESIMS at *m/z* 949.4540 $[M + Na]^+$ (calcd for $C_{47}H_{74Na}O_{18}$, 949.4773).

SP-R_2: white powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ:0.77 (3H, s), 0.78 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 1.03 (3H, s), 1.14 (3H, s); for ^{13}C-NMR (CD_3OD, 125 MHz) data, see Table 2. The structure of compound SP-R_2 is shown in Fig. 2. HRESIMS at *m/z* 1111.5023 $[M + Na]^+$ (calcd for $C_{53}H_{84}NaO_{23}$, 1111.5301).

2.4. HPLC analysis of SP-R_1 and SP-R_2

The dried and powdered sample (0.25 g) from roots and rhizomes of *P. stipuleanatus* (PS-1 to PS-6 and PS-13 to PS-24) was placed in a tube containing 70% methanol (10 mL). The sample was sonicated for 30 min at room temperature and centrifuged at 3500 rpm for 15 min, and the supernatant was added with the solvent

Table 1
The list of plant material samples.

Parts	Period of growth	Producing area	Code
Root	One year	Wenshan,Yunnan province	PS-1
Root	Three years	Wenshan,Yunnan province	PS-2
Rhizome	Three years	Wenshan,Yunnan province	PS-3
Rhizome	Five years	Wenshan,Yunnan province	PS-4
Rhizome	Ten years	Wenshan,Yunnan province	PS-5
Rhizome	Fifteen years	Wenshan,Yunnan province	PS-6
Root	One year	Wenshan,Yunnan province	PS-7[1)]
Root	Three years	Wenshan,Yunnan province	PS-8[1)]
Rhizome	Three years	Wenshan,Yunnan province	PS-9[1)]
Rhizome	Five years	Wenshan,Yunnan province	PS-10[1)]
Rhizome	Ten years	Wenshan,Yunnan province	PS-11[1)]
Rhizome	Fifteen years	Wenshan,Yunnan province	PS-12[1)]
Root	One year	Yanshan,Yunnan province	PS-13
Root	Three years	Yanshan,Yunnan province	PS-14
Rhizome	Three years	Yanshan,Yunnan province	PS-15
Rhizome	Five years	Yanshan,Yunnan province	PS-16
Rhizome	Ten years	Yanshan,Yunnan province	PS-17
Rhizome	Fifteen years	Yanshan,Yunnan province	PS-18
Root and rhizome	Three to five years	Yanshan,Yunnan province	PS-19
Root and rhizome	Three to five years	Yanshan,Yunnan province	PS-20
Root and rhizome	Three to five years	Pinbian,Yunnan province	PS-21
Root and rhizome	Three to five years	Pinbian,Yunnan province	PS-22
Root and rhizome	Three to five years	Wenshan,Yunnan province	PS-23
Root and rhizome	Three to five years	Wenshan,Yunnan province	PS-24

[1)] The samples PS-7 to PS-12 are fresh roots or rhizomes of *P. stipuleanatus*, and others are dried samples.

Fig. 1. (A) The medicinal parts of three-year-old *P. stipuleanatus*. (B) The medicinal parts of fifteen-year-old *P. stipuleanatus*.

and made up to 10 mL. The fresh sample (1 g) from roots and rhizomes of *P. stipuleanatus* (PS-7 to PS-12) was chopped and ultrasoniclly extracted using 70% methanol (20 mL) for 30 min. The sample was centrifuged, and the supernatant was added with the solvent and made up to 20 mL. Each sample solution of the dried and fresh products was filtered through a 0.45-μm filter and prepared for HPLC analysis.

Quantitative analysis of SP-R_1 and SP-R_2 in *P. stipuleanatus* from different growing areas and harvesting dates was determined by HPLC (Fig. 3). The HPLC data were recorded on a Shimadzu Analytical Instrument (Shimadzu, Kyoto, Japan), equipped with a solvent degasser (DGU-20A3R(C)), a binary pump (LC-20AB), an auto sampler (SIL-20A), and a UV detector (SPD-20A). The separation was performed on a YMC-Pack ODS-A column (250 mm × 4.6 mm, 5μm; YMC Co., Ltd, Kyoto, Japan) at the flow rate of 1.0 mL/min. The mobile phase consisted of 0.05% (v/v) phosphoric acid water (Solvent A) and acetonitrile (Solvent B), and the following gradient program was used: 0–15 min, 30–35% B; 15–30 min, 35–80% B; 30–35 min, 80–85% B. The detection wavelength and the column temperature were at 203 nm and 35 °C, respectively. Reference compounds of SP-R_1 and SP-R_2 were isolated from roots and rhizomes of *P. stipuleanatus* and identified by NMR and high resolution mass spectrometry (HRMS). The calibration curves for SP-R_1 and SP-R_2 were $y = 7\times10^6x - 12851$ ($R^2 = 0.9996$) and $y = 4\times10^6x + 22414$ ($R^2 = 0.9997$) in the range of 0.2–2 mg/mL, respectively. The samples were analyzed by HPLC three times.

2.5. Animals

Kunming mice of clean-grade mice (18–22 g) of both sexes, aged 6–8 weeks, were obtained from Changsha Tianqin Biotechnology Co., China. The qualified number is SCXK 2014-0011. The mice were kept at 25 ± 2 °C and under 12-h dark–light cycle conditions. The animals were fed with normal diet and water. All experimental protocols were approved by the Animal Ethical Committee of Laboratory Animals of Kunming University of Science and Technology.

2.6. Hot plate test in mice

The hot plate test was used according to the method described by Sun et al [11]. The latency period of pain response was counted when the mice were placed on a hot plate at 55 °C until the mice licked their hind paw or jumped. Mice with latency longer than 30 s or less than 5 s were removed. The other mice were randomly divided into the negative control group (0.9% saline, same volume), positive group (aspirin, 400 mg/kg), SP-R_1 group (1 and 10 mg/kg), and SP-R_2 group (1 and 10 mg/kg), with ten mice in each group. The mice were administered orally for 7 days. The hot plate reaction time of 30, 60, and 90 min after administration was determined.

2.7. Acetic acid writhing test in mice

The acetic acid writhing test was carried out in mice as described by Meymandi and Keyhanfar [12]. Sixty mice were

Table 2
C-NMR data of compounds SP-R_1 and SP-R_2 in CD_3OD.

Carbon no.	Aglycone		Carbon no.	Sugar	
	SP-R_2 [1)]	SP-R_1 [1)]		SP-R_2 [1)]	SP-R_1 [1)]
1	39.8	39.7	*3-O-GlcUA* [2)]		
2	26.9	26.9	1′	106.4	106.5
3	91.0	91.2	2′	76.4	76.3
4	40.2	40.2	3′	79.5	81.2
5	57.0	57.0	4′	79.5	79.4
6	19.3	19.3	5′	78.1	77.9
7	33.9	34.0	6′	176.5	172.7
8	40.7	40.6	*Glc-I* [3)] *(1→3)GlcUA*		
9	49.1	49.1	1″	104.4	104.4
10	37.9	37.9	2″	75.6	75.5
11	24.5	24.5	3″	78.2	78.2
12	123.8	123.67	4″	71.0	71.0
13	144.6	145.2	5″	78.3	78.2
14	42.9	42.9	6″	63.2	63.0
15	28.9	28.8	*Ara(f)* [4)] *(1→4)GlcUA*		
16	24.0	24.0	1‴	107.9	108.2
17	48.0	47.6	2‴	82.0	82.3
18	42.6	42.7	3‴	74.8	74.5
19	47.2	47.2	4‴	87.1	87.0
20	31.5	31.6	5‴	62.4	62.2
21	34.9	34.9	*28-O-Glc-II* [3)]		
22	33.1	33.7	1⁗	95.7	
23	28.5	28.4	2⁗	73.9	
24	17.0	17.0	3⁗	78.0	
25	16.0	16.0	4⁗	71.0	
26	17.7	17.7	5⁗	78.7	
27	26.3	26.4	6⁗	62.2	
28	178.0	181.82			
29	33.5	33.8			
30	24.0	24.0			

SP-R_1, stipuleanoside R_1; SP-R_2, stipuleanoside R_2.
1) Recorded at 125 MHz.
2) GlcUA: β-D-glucuronopyranosyl.
3) Glc: β-D-glucopyranosyl.
4) Ara(f): α-L-arabinofuranosyl.

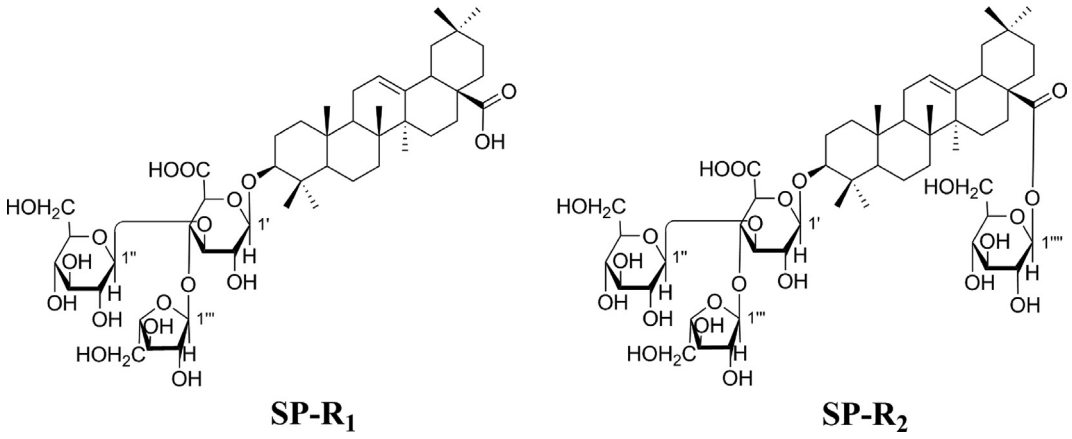

Fig. 2. Two compounds identified from *P. stipuleanatus*.
SP-R_1, stipuleanoside R_1; SP-R_2, stipuleanoside R_2.

randomly divided into six groups, with ten mice in each group. The negative control group (0.9% saline, same volume), positive group (aspirin, 400 mg/kg), SP-R_1 group (1 and 10 mg/kg), and SP-R_2 group (1 and 10 mg/kg) were orally administered, once a day for seven days. After 1 hour of administration, 0.6% acetic acid (v/v) was injected intraperitoneally (10 ml/kg of body weight) into each mouse. The numbers of writhing in mice were counted for 20 min.

2.8. Acetic acid–induced vascular permeability test

The acetic acid–induced vascular permeability test was carried out as described by Yesilada et al [13]. Sixty mice were divided into six groups, with ten mice in each group. The model group (0.9% saline, same volume), positive group (dexamethasone, 10 mg/kg), SP-R_1 group (1 and 10 mg/kg), and SP-R_2 group (1 and 10 mg/kg) were orally administered, once a day for seven days. One hour after

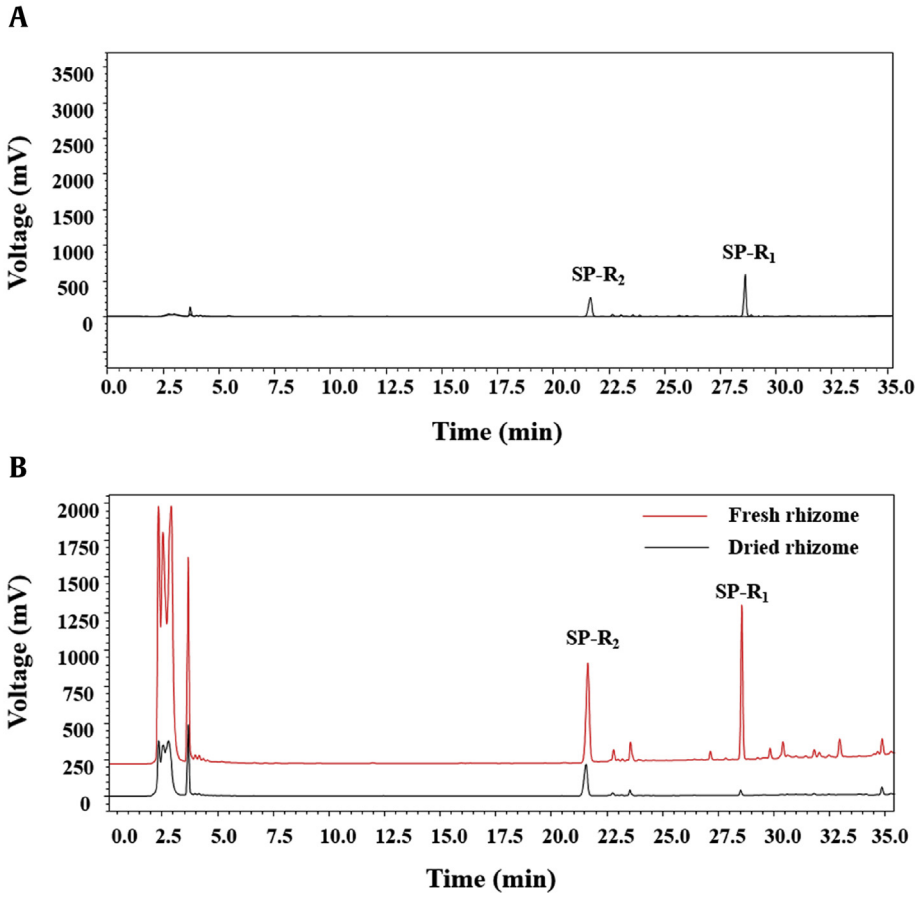

Fig. 3. (A) HPLC chromatograms of standards from *P. stipuleanatus*. (B) HPLC chromatograms of 70% methanol extract from *P. stipuleanatus.* Peaks: SP-R_1, stipuleanoside R_1; SP-R_2, stipuleanoside R_2. HPLC chromatogram of the fresh rhizome of three-year-old *P. stipuleanatus* from Wenshan County of Yunnan Province is shown as the red line (top) and that of the dried rhizome of *P. stipuleanatus* of the same age and from the same place is shown as the black line (bottom).
HPLC, high-performance liquid chromatography.

the last administration, each mouse was intravenously injected 2% Evans blue in saline (0.1 mL/10 g) and then immediately injected 0.6% acetic acid (10 mL/kg). After 20 min, the mice were killed by removing the cervical vertebra, and peritoneal exudates were collected after the abdominal cavity was washed several times with saline (8 mL) and centrifuged at 3000 rpm for 10 min. The optical density (OD) value of supernatant was measured at a wavelength of 590 nm using a SHIMADZU UV2600 UV−VIS spectrophotometer (Shimadzu, Tokyo, Japan). The inhibition rate was presented according to the following formula:

$$\text{Inhibition rate}\,(\%) = (\text{mean OD value}\,(\text{model group}) - \text{mean OD value}\,(\text{drug experimental group}))/\text{mean OD value}\,(\text{model group}) \times 100\%.$$

2.9. Cotton pellet−induced granuloma test

The cotton pellet−induced granuloma model was carried out by the method of Wu et al [14]. Sixty mice were divided into six groups, with ten mice in each group. Under ether anesthesia, sterile cotton pellets (10 ± 0.5 mg) were inserted subcutaneously, one in each axilla. The same volume of sterile 0.9% saline (model group), dexamethasone (positive group, 10 mg/kg), SP-R_1 (1 and 10 mg/kg), and SP-R_2 (1 and 10 mg/kg) were given orally to mice for seven days. The mice were put to death by cervical vertebra dislocation on the 8th day, and the granuloma tissue was removed and dried at 60 °C to a constant weight. The granuloma weight was calculated according to the following formula: Granuloma weight (mg) = (W_2 − W_1), where W_1 and W_2 were dry weight of the pellets before and after implantation, respectively. The inhibition rate was presented according to the following formula:

$$\text{Inhibition rate}\,(\%) = (\text{mean granuloma weight}\,(\text{model group}) - \text{mean granuloma weight}\,(\text{drug experimental group}))/\text{mean granuloma weight}\,(\text{model group}) \times 100\%.$$

2.10. Xylene-induced ear edema test

Xylene-induced ear edema in the mouse model was carried out as described by Ergene et al [15]. Sixty mice were divided into six groups, with ten mice in each group. The model group (sterile 0.9% saline, same volume), positive group (dexamethasone, 10 mg/kg), SP-R_1 group (1 and 10 mg/kg), and SP-R_2 group (1 and 10 mg/kg) were orally administered for seven days. After the last administration, each mouse was applied 30 μL of xylene to the anterior and posterior surface of the right ear. The left ear was considered as a negative control, applied with the same volume of acetone. One hour later, the mice were killed by cervical dislocation. Both ears were taken out through the perforator (6 mm in diameter) and weighed. The degree of swelling was calculated according to the following formula: Ear edema (mg) = (W_2 − W_1), where W_1 and W_2 were the left and right ear weights, respectively. The inhibition rate was presented according to the following formula:

$$\text{Inhibition rate}\,(\%) = (\text{mean ear edema}\,(\text{model group}) - \text{mean ear edema}\,(\text{drug experimental group}))/\text{mean ear edema}\,(\text{model group}) \times 100\%.$$

2.11. Carrageenan-induced paw edema test

The carrageenan-induced paw edema test was carried out as described by Kassuya et al [16]. Seventy mice were divided into seven groups, with ten mice in each group. The control group (sterile 0.9% saline, same volume), model group (sterile 0.9% saline, same volume), positive group (dexamethasone, 10 mg/kg), SP-R_1 group (1 and 10 mg/kg), and SP-R_2 group (1 and 10 mg/kg) were orally administered for seven days. Except for the negative control group, each mouse in all groups was given a subcutaneous intraplantar injection with 1% carrageenan suspended in sterile 0.9% saline (30 μL) into the right hind paw. The thickness of paw edema was measured using a digital micrometer (03000002; Guilin Guanglu Measuring Instrument Co., Ltd, Guilin, China) 1 h before any treatment and at intervals of 1, 2, 3, 4, 5, and 6 h after the injection of carrageenan. Paw edema degree was presented according to the following formula: Paw edema degree (%) = (T_t − T_0)/T_0 × 100%, where T_0 and T_t were the thickness of paw edema before and after injection, respectively. The inhibition rate was presented according to the following formula:

$$\text{Inhibition rate}\,(\%) = (\text{mean paw edema degree}\,(\text{model group}) - \text{mean paw edema degree}\,(\text{drug experimental group}))/\text{mean paw edema degree}\,(\text{model group}) \times 100\%.$$

2.12. Histopathological analysis

Ear and paw tissue samples obtained in ear and paw edema assays were preserved in 10% formaldehyde. After decalcification, dehydration, paraffin embedding, and sectioning (5 μm), the tissues were stained with hematoxylin and eosin. The pathological changes were observed using a Nikon Eclipse E100 microscope (Nikon, Tokyo, Japan). Every paw tissue slice was randomly chosen from each group. The images were captured using a Nikon DS-U3 (Nikon, Tokyo, Japan).

2.13. Malondialdehyde and NO assay

The malondialdehyde (MDA) and NO contents of paw tissue samples were determined by thiobarbituric acid reactive substances using the MDA assay kit (Jiancheng Institute of Biotechnology engineering, Nanjing, China) and NO assay kit (Beyotime Biotechnology, Shanghai, China), respectively.

2.14. Tumor necrosis factor-α and interleukin 6 assay

The contents of tumor necrosis factor (TNF)-α and interleukin 6 (IL-6) in paw tissue samples were determined using the enzyme-linked immunosorbent assay kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

2.15. Statistical analysis

Single-factor analysis of variance was used to compare between groups. Statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation. The values of $p < 0.05$, $p < 0.01$, and $p < 0.001$ were considered to be statistically significant.

3. Results and discussion

3.1. Identification of SP-R_1 and SP-R_2 from P. stipuleanatus

To confirm the structures, the compounds SP-R_1 and SP-R_2 have been isolated from *P. stipuleanatus* and identified using NMR and MS data by comparing with the spectroscopic data reported in the literature [4,5] (Fig. 2).

The molecular formula of the compound SP-R_2 was determined to be $C_{53}H_{84}O_{23}$ by positive-mode ESI-MS data at *m/z* 1111.5023

$[M + Na]^+$. The NMR data contained signals for the presence of seven tertiary methyl groups [δ_H 0.77 (3H, s), 0.78 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 1.03 (3H, s), and 1.14 (3H, s)], two olefinic carbons (δ_C 123.8, 144.6), one carboxyl group (δ_C 178.0), and four anomeric carbons (δ_C 95.7, 104.4, 106.4, 107.9) (Table 2). The structure of the compound SP-R_2 was identified as 3-*O*-[β-D-glucopyranosyl-(1→3)] [α-L-arabinofuranosyl-(1→4)]-β-D-glucuronopyranoside-28-*O*-β-D-glucopyranosyl oleanolic acid and named SP-R_2 by comparing the NMR and MS data with the previously reported data [5].

The molecular formula of the compound SP-R_1 was shown to be $C_{47}H_{74}O_{18}$ based on the (+)-HRESIMS ion peak at *m/z* 949.4540 $[M + Na]^+$. Comparison of the NMR data of SP-R_1 (Table 2) with those of SP-R_2 suggested that their structures were similar and they differed only in the number of sugar moieties. The sugar moieties was established by the presence of three anomeric carbons at δ_C 104.4, 106.5, and 108.2. The structure of the compound SP-R_1 was identified as oleanolic acid 3-*O*-[β-D-glucopyranosyl-(1→3)] [α-L-arabinofuranosyl-(1→4)]-β-D-glucuronopyranoside and named SP-R_1 by comparing the NMR and MS data with the previously reported data [4].

3.2. Analgesic effect of SP-R_1 and SP-R_2 from *P. stipuleanatus*

The hot plate and acetic acid–induced writhing tests in mice were two classic models of thermal-and chemical-stimulated acute pain, which were used to evaluate the central and peripheral analgesic effects, respectively [11,12]. To observe the analgesic activity of SP-R_1 and SP-R_2 on thermal- and chemical-stimulated acute pain, the mice were treated with isotonic saline, aspirin, and two doses of SP-R_1 and SP-R_2. As shown in Table 3, SP-R_1 and SP-R_2 (1 and 10 mg/kg) in the hot plate test significantly increased the latency time to thermal pain in the reaction times 30, 60, and 90 min, compared with the saline group. SP-R_1 at the dose of 10 mg/kg was found to exhibit stronger analgesic activity, with the latency time of 20.70 ± 4.22 and 20.60 ± 5.36 s in the reaction times 30 and 60 min, than aspirin (400 mg/kg), with the latency time of 18.90 ± 4.01 and 20.10 ± 3.25 s, respectively.

Compared with the isotonic saline group, aspirin, SP-R_1 and SP-R_2 at the dose of 10 mg/kg significantly reduced the number of acetic acid–induced writhing responses ($p < 0.05$), whereas SP-R_1 and SP-R_2 (1 mg/kg) had no inhibition effect (Table 4). The result indicated that SP-R_1 and SP-R_2 relieved chemical-stimulated acute pain.

It is reported that triterpenoid saponins have excellent analgesic properties owing to their safety and efficacy. For example, total saponins of *Psammosilene tunicoids* inhibited algesia threshold and effectively decreased the contents of MDA, IL-1β, and TNF-α in the inflammatory tissue soak of rats with adjuvant-induced arthritis [17,18]. The triterpenoid saponins from *Stauntonia chinensis* enhanced the threshold of thermal- and chemical-stimulated acute pain, which directly increased the inhibitory synaptic response in mouse cortex neurons at the resting state [19]. *P. stipuleanatus* was traditionally used to treat pain-related diseases. In this report, we investigated its antinociceptive properties. Two thermal and chemical nociception models were used to elucidate the analgesic effect of the isolated pure triterpenoid saponins.

Table 3
Effects of stipuleanosides R_1 and R_2 on hot plate reaction time in mice.

Group	Dose (mg/kg)	Hot plate reaction time (s)		
		30 min	60 min	90 min
Control	0	12.50 ± 1.84	12.80 ± 4.64	12.50 ± 3.06
Aspirin	400	18.90 ± 4.01***	20.10 ± 3.25**	19.40 ± 2.95***
SP-R_1	1	17.40 ± 2.84***	19.20 ± 2.74**	16.10 ± 2.96*
	10	20.70 ± 4.22***	20.60 ± 5.36**	18.70 ± 3.47***
SP-R_2	1	17.70 ± 5.06**	16.60 ± 3.53	16.70 ± 5.36*
	10	18.50 ± 4.93**	18.10 ± 3.70*	18.10 ± 4.20**

*$p < 0.05$, compared with the negative control group.
**$p < 0.01$, compared with the negative control group.
***$p < 0.001$, compared with the negative control group.
SD, standard deviation; SP-R_1: stipuleanoside R_1; SP-R_2: stipuleanoside R_2.
Values are mean ± SD ($n = 10$).

Table 4
Effects of stipuleanosides R_1 and R_2 on writhing induced by acetic acid in mice.

Group	Dose (mg/kg)	Number of writhing
Control	0	56.78 ± 4.78
Aspirin	400	40.85 ± 5.48*
SP-R_1	1	50.47 ± 2.89
	10	41.14 ± 2.19*
SP-R_2	1	54.49 ± 5.26
	10	40.16 ± 3.28*

*$p < 0.05$, compared with the negative control group.
SD, standard deviation; SP-R_1: stipuleanoside R_1; SP-R_2: stipuleanoside R_2.
Values are mean ± SD ($n = 10$).

3.3. Anti-inflammatory effects of SP-R_1 and SP-R_2 from *P. stipuleanatus*

The anti-inflammatory effects of triterpenoid saponins SP-R_1 and SP-R_2 from *P. stipuleanatus* were evaluated by a series of acute and chronic inflammatory models *in vivo*. In the early stage of inflammation, the main manifestations are telangiectasia, increased permeability, inflammatory exudation, and tissue edema. Acetic acid–induced vascular permeability, xylene-induced ear edema, and carrageenan-induced paw edema in mice are commonly used animal models of acute inflammation, suitable for screening of anti-inflammatory drugs [20,21]. In the acetic acid–induced vascular permeability model, the permeability of abdomen capillaries was significantly decreased by the treatment with SP-R_1 and SP-R_2 ($p < 0.01$) (Table 5). SP-R_1 (10 mg/kg) exhibited stronger inhibitory activity with an inhibition rate of 62.83%, than dexamethasone (10 mg/kg). In the xylene-induced ear edema model, the inhibition effect of ear edema was observed in the SP-R_2 (10 mg/kg) treatment group, and the inhibition rate was 33.33% ($p < 0.05$), while the dexamethasone group (10 mg/kg) showed significant inhibition of ear edema, with an inhibition rate of 45.42% ($p < 0.001$) (Fig. 4A). However, SP-R_1 had no obvious inhibitory effect on ear edema. Xylene is a commonly used chemical inflammatory agent that can induce the release of proinflammatory mediators (such as histamine and serotonin), thereby promoting vasodilation and leukocyte infiltration [11]. It was suggested that SP-R_2 might show inhibitory effect on ear edema by inhibiting vasodilatation and leukocyte infiltration. Further histopathological analysis of ear tissue sections showed that SP-R_2 at the dose of 10

Table 5
Effect of stipuleanosides R_1 and R_2 on vascular permeability induced by acetic acid in mice.

Group	Dose (mg/kg)	OD value	Inhibition (%)
Model	-	0.452 ± 0.992	-
Dexamethasone	10	0.182 ± 0.135**	59.73
SP-R_1	1	0.235 ± 0.048**	48.01
	10	0.168 ± 0.044**	62.83
SP-R_2	1	0.286 ± 0.084**	36.73
	10	0.254 ± 0.014**	43.81

**$p < 0.01$, compared with the model group.
SD, standard deviation; SP-R_1: stipuleanoside R_1; SP-R_2: stipuleanoside R_2; OD: optical density.
Values are mean ± SD ($n = 10$).

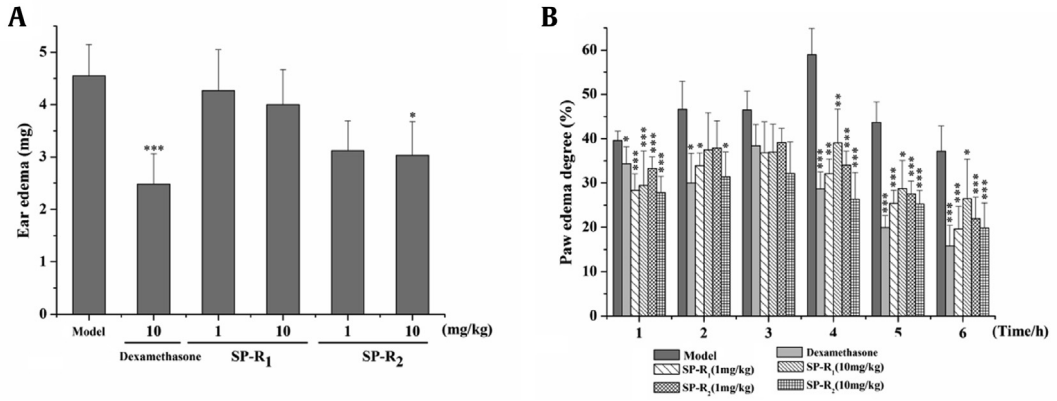

Fig. 4. (A) Effect of SP-R_1 and SP-R_2 on xylene-induced ear edema in mice. (B) Effect of SP-R_1 and SP-R2 on carrageenan-induced paw edema in mice. The mice were treated with SP-R_1 (1 and 10 mg/kg), SP-R_2 (1 and 10 mg/kg), dexamethasone (10 mg/kg), or model (sterile 0.9% saline, same volume) orally for seven days. (A) After the last administration, 30 μL of xylene was applied to the anterior and posterior surface of the ear. The bars show the effect of different doses of SP-R_1 and SP-R_2 on ear edema (mg) 1 h after xylene stimulation. (B) After the last administration, the mice were given an intraplantar injection of 1% carrageenan (30 μL/paw). The bars show the effect of different doses of SP-R_1 and SP-R_2 on paw edema degree (%) 1–6 h after carrageenan injection. Values are mean ± SD ($n = 10$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with the model group. SD, standard deviation; SP-R_1, stipuleanoside R_1; SP-R_2, stipuleanoside R_2.

mg/kg and dexamethasone significantly reduced xylene-induced edema and inflammatory cell infiltration in the ear tissue (Fig. 5).

As shown in Fig. 4B, at 1 h after injection of carrageenan, acute inflammation of the right hind paw was observed from 1 h to 6 h, and paw edema peaked at 4 h. SP-R_1 and SP-R_2 at the dose of 1 and 10 mg/kg showed inhibition of paw edema in both early and late phases of the experiment. Compared with the model group, SP-R_1 (10 mg/kg) and SP-R_2 (10 mg/kg) showed inhibition rates of 25.70% and 29.76% at the 1rd h, which increased to 33.76% and 55.40% at the 4th h and decreased to 28.92% and 46.61% at the 6th h in the late phase, respectively. The inhibition rate of SP-R_2 (10 mg/kg) at 4 h was 55.40% ($p < 0.001$) and was better than dexamethasone (51.38%, 10 mg/kg). Pathological analysis of paw tissue sections showed that compared with the negative group, the connective tissue of paw tissue in the model group was significantly damaged. The paw tissue showed swelling, and the connective tissue had obvious inflammatory cell infiltration. SP-R_1 (1 and 10 mg/kg), SP-R_2 (1 and 10 mg/kg), and dexamethasone significantly reduced carrageenan-induced edema and inflammatory cell infiltration in the connective tissue (Fig. 6).

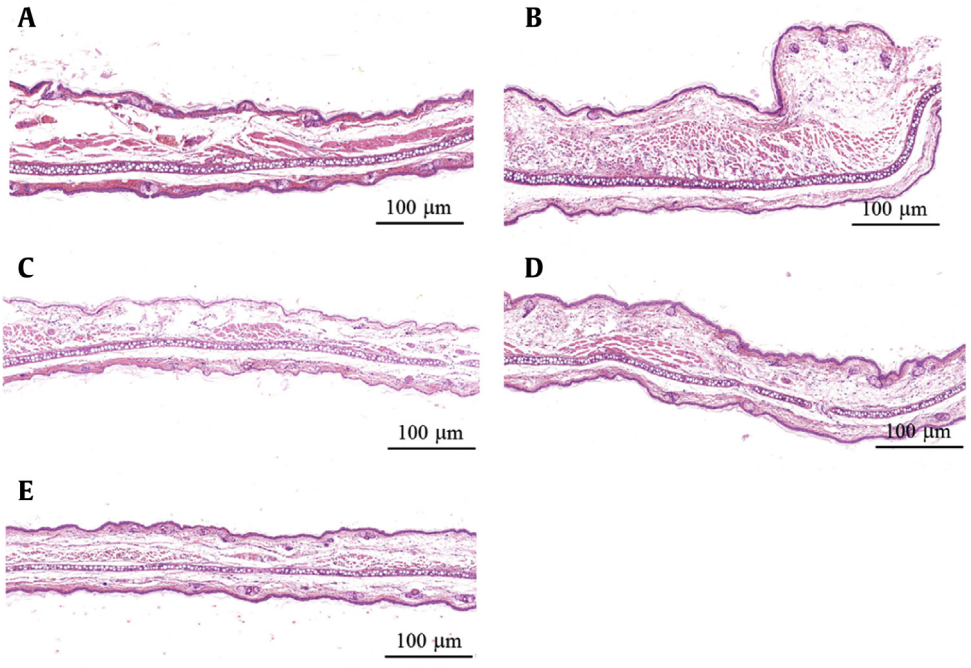

Fig. 5. Histopathological examinations of xylene-induced mouse ear tissue. (A) Control group: noninflamed ear that received only acetone; (B) model group: inflamed ear that received 30 μL of xylene; (C) positive control group: inflamed ear that was treated with dexamethasone (10 mg/kg); (D) inflamed ear that was treated with SP-R_2 (1 mg/kg); (E) Inflamed ear that was treated with SP-R_2 (10 mg/kg). The tissue sections were observed under a microscope at 200× magnification.
SP-R_2, stipuleanoside R_2.

Chronic inflammation is caused by the persistent effects of inflammatory factors, characterized by proliferation of small vessels and connective tissue. As a chronic inflammatory model, cotton pellet–induced granuloma in mice was previously used to investigate the effects of drugs on the formation of cellular and inflammatory edema [22,23]. As shown in Table 6, SP-R_1 and SP-R_2 significantly inhibited granuloma formation. After treatment with SP-R_1 and SP-R_2 (10 mg/kg), SP-R_1 and SP-R_2 showed inhibition rates of 25.71% and 24.59%, respectively, and dexamethasone treatment showed an inhibition rate of 48.47%. The two saponins inhibited granuloma formation, and it is possible to inhibit synthesis of collagen and mucopolysaccharide during the formation of granuloma tissue.

Table 6
Effects of stipuleanosides R_1 and R_2 on cotton pellet–induced granuloma in mice.

Group	Dose (mg/kg)	Granuloma weight (mg)	Inhibition (%)
Model	-	9.80 ± 1.61	-
Dexamethasone	10	5.05 ± 0.93**	48.47
SP-R_1	1	8.15 ± 1.26*	16.84
	10	7.28 ± 1.58**	25.71
SP-R_2	1	7.95 ± 1.98*	18.88
	10	7.39 ± 1.46**	24.59

*$p < 0.05$, compared with the model group.
**$p < 0.01$, compared with the model group.
SD, standard deviation; SP-R_1: stipuleanoside R_1; SP-R_2: stipuleanoside R_2.
Values are mean ± SD ($n = 10$).

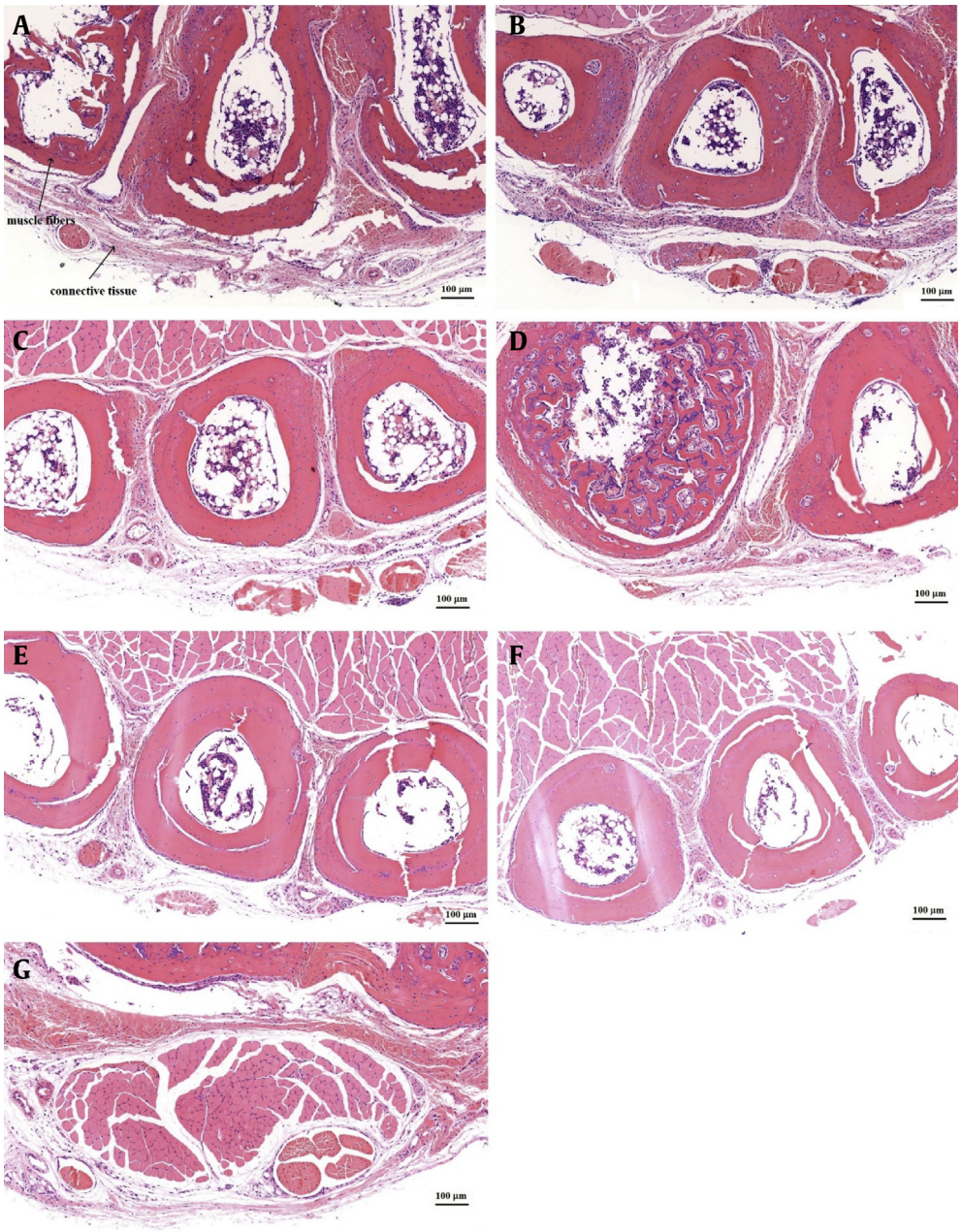

Fig. 6. Histopathological examinations of carrageenan-induced paw tissue swelling and inflammatory cell infiltration. (A) Control group: noninflamed paw that received only sterile 0.9% saline; (B) model group: inflamed paw that received 30 μL of 1% carrageenan; (C) positive control group: inflamed paw that was treated with dexamethasone (10 mg/kg); (D) inflamed paw that was treated with SP-R_1 (1 mg/kg); (E) inflamed paw that was treated with SP-R_1 (10 mg/kg); (F) inflamed paw that was treated with SP-R_2 (1 mg/kg); (G) inflamed paw that was treated with SP-R_2 (10 mg/kg). The tissue sections were observed under a microscope at 100× magnification.
SP-R_1, stipuleanoside R_1.

3.4. SP-R_1 and SP-R_2 suppressed the production of NO, MDA, TNF-α, and IL-6 in mice with paw edema

To assess the anti-inflammatory effects of SP-R_1 and SP-R_2, the production of inflammatory mediators (NO, MDA, TNF-α, and IL-6) in the carrageenan-induced paw edema model was determined by NO and MDA assay and TNF-α, and IL-6 enzyme-linked immunosorbent assay methods. As shown in Fig. 7, compared with the control group, the levels of NO (Fig. 7A), MDA (Fig. 7B), TNF-α (Fig. 7C), and IL-6 (Fig. 7D) increased significantly in paw tissues after injection of carrageenan ($p < 0.05$). SP-R_1 at the dose of 1 and 10 mg/kg significantly and dose dependently decreased the level of NO, MDA, and IL-6 in paw tissues in comparison with the model group ($p < 0.05$, $p < 0.01$ or $p < 0.001$). The inhibitory effect of SP-R_1 on TNF-α was similar at the concentrations of 1 and 10 mg/kg. At the same time, SP-R_2 (1 and 10 mg/kg) significantly decreased NO, MDA, and TNF-α levels in paw tissues with edema ($p < 0.05$, $p < 0.01$ or $p < 0.001$). But SP-R_2 had no inhibitory effect on IL-6 production.

This proinflammatory cytokines are produced by inflammatory cells after stimulation, as it occurs with injury, infection, and inflammation. TNF-α is an important mediator involved in the inflammatory response. It can activate the cytokine cascade reaction and further induce the release of IL-6 and NO in acute or chronic inflammation [24]. The inhibition of TNF-α, IL-6, and NO production can effectively alleviate the inflammatory response [25]. In our study, the contents of TNF-α, IL-6, and NO in paw tissues were significantly decreased with SP-R_1 treatment. SP-R_2 significantly reduced the levels of NO and TNF-α in paw tissues with edema, but it had no inhibitory effect on the production of IL-6. IL-6 is a multifunctional cytokine that plays both proinflammatory and anti-inflammatory roles [26]. The anti-inflammatory activity of IL-6 is mediated by classical signaling, and its cell activation is through the combination of IL-6 and the membrane-bound IL-6 receptor. However, the proinflammatory activity of IL-6 is mediated by trans-signaling, and its cell activation expresses the membrane glycoprotein gp130 via the IL-6–soluble IL-6 receptor complex. In our study, SP-R_1 and SP-R_2 selectively inhibit IL-6 production, possibly owing to the activation of different IL-6 signaling pathways. Accumulation of oxygen metabolites *in vivo* can damage tissues and lead to inflammatory and pathological changes [27]. The increase of MDA and decrease of superoxide dismutase (SOD) activity indicate the aggravation of inflammation [28]. SP-R_1 and SP-R_2 can reduce the content of MDA in paw tissues, which alleviates the excessive damage of

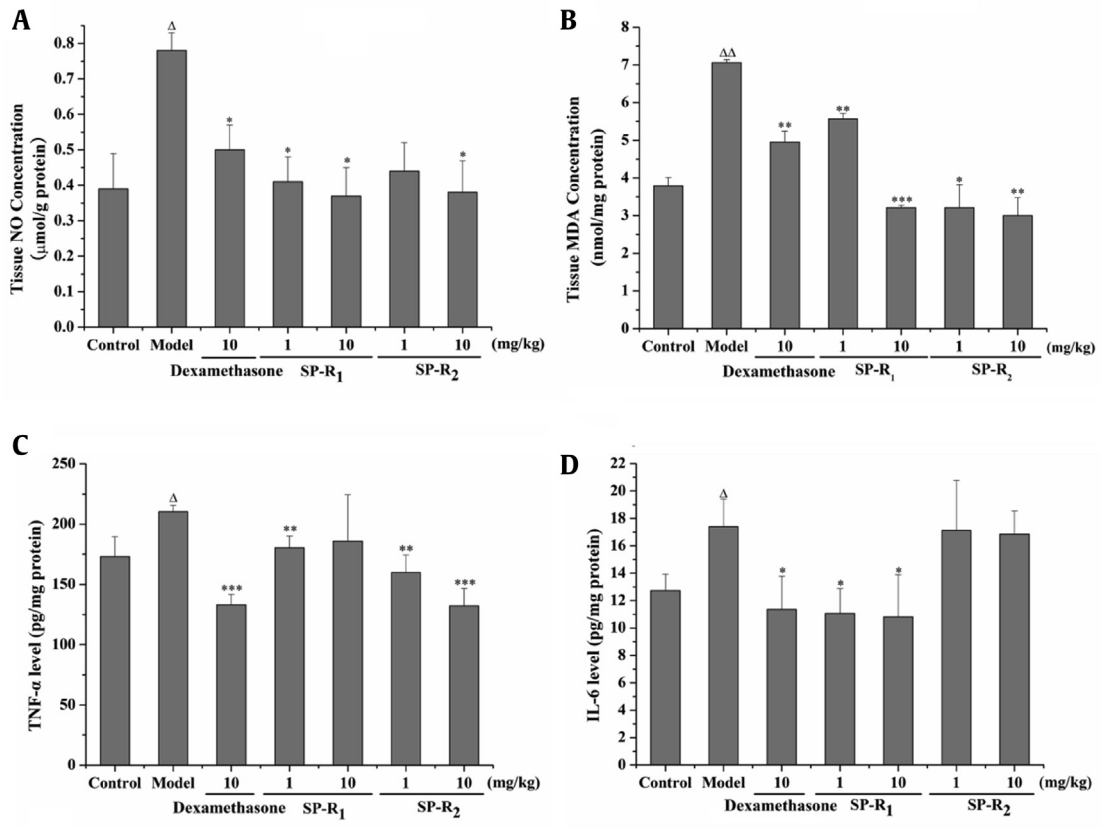

Fig. 7. (A) Effect of SP-R_1 and SP-R_2 on NO levels of paw tissues in the carrageenan-induced paw edema model. (B) Effect of SP-R_1 and SP-R_2 on MDA levels of paw tissues in the carrageenan-induced paw edema model. (C) Effect of SP-R_1 and SP-R_2 on TNF-α levels of paw tissues in the carrageenan-induced paw edema model. (D) Effect of SP-R_1 and SP-R_2 on IL-6 levels of paw tissues in the carrageenan-induced paw edema model. The mice were orally administered with sterile 0.9% saline, dexamethasone (10 mg/kg), SP-R_1 (1 or 10 mg/kg), and SP-R_2 (1 or 10 mg/kg) for seven days. Except for the control group, each mouse in the other groups was injected with 1% carrageenan in the right paw. Six hours later, paw tissue samples were collected for analysis of NO, MDA, TNF-α, and IL-6. Values are mean ± SD ($n = 10$). $^{\triangle}p < 0.05$, $^{\triangle\triangle}p < 0.01$, compared with the control group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with the model group.

IL-6, interleukin 6; MDA, malondialdehyde; NO, nitric oxide; SD, standard deviation; SP-R1, stipuleanoside R1; SP-R^2, stipuleanoside R2; TNF-α, .

Table 7
Contents of stipuleanosides R_1 and R_2 in *P. stipuleanatus* of different growth years and from different producing areas.

No.	SP-R_1 (%)	SP-R_2 (%)	SP-R_1:SP-R_2
PS-1	0.3005 ± 0.0243	2.9251 ± 0.2074	1:9.73
PS-2	0.1268 ± 0.0125	2.2376 ± 1.2568	1:17.65
PS-3	0.1933 ± 0.0471	3.7787 ± 1.5452	1:19.55
PS-4	0.1827 ± 0.0108	3.4522 ± 0.6115	1:18.89
PS-5	0.2106 ± 0.0351	1.5325 ± 1.4259	1:7.28
PS-6	0.2443 ± 0.0042	1.1283 ± 0.7652	1:4.62
PS-7[1)]	0.3127 ± 0.0357	0.6285 ± 0.2054	1:2.00
PS-8[1)]	0.4104 ± 0.0508	0.7672 ± 0.1836	1:1.87
PS-9[1)]	0.2698 ± 0.0652	1.0863 ± 0.2211	1:4.03
PS-10[1)]	0.2547 ± 0.0056	0.7449 ± 0.0187	1:2.92
PS-11[1)]	0.1394 ± 0.0030	0.2708 ± 0.0180	1:1.94
PS-12[1)]	0.1112 ± 0.0054	0.3352 ± 0.0092	1:3.01
PS-13	0.3128 ± 0.0381	2.9239 ± 0.4518	1:9.35
PS-14	0.1305 ± 0.0062	1.0542 ± 0.0674	1:8.08
PS-15	0.1900 ± 0.0146	2.2408 ± 0.3042	1:11.79
PS-16	0.1975 ± 0.0159	3.7259 ± 0.2518	1:18.86
PS-17	0.2347 ± 0.0212	1.2838 ± 0.7489	1:5.46
PS-18	0.2420 ± 0.0025	0.7661 ± 0.1001	1:3.17
PS-19	0.3398 ± 0.0069	3.9409 ± 0.1046	1:11.59
PS-20	0.2677 ± 0.0100	3.4499 ± 0.0116	1:12.89
PS-21	0.4136 ± 0.0523	5.1256 ± 0.4802	1:12.39
PS-22	0.4650 ± 0.0570	5.9492 ± 0.0755	1:12.79
PS-23	0.3526 ± 0.0082	3.3890 ± 0.0224	1:9.61
PS-24	0.3482 ± 0.0016	3.5073 ± 0.0100	1:10.07

SD, standard deviation; SP-R_1, stipuleanoside R1; SP-R2, stipuleanoside R2.
Values are mean ± SD ($n = 3$).
[1)] The samples PS-7 to PS-12 are fresh roots or rhizomes of *P. stipuleanatus*, and others are dried samples.

oxygen-free radicals to the cells and result in an anti-inflammatory effect.

3.5. Comparison of contents of SP-R_1 and SP-R_2 in P. stipuleanatus of different growth years and from different producing areas

At present, more than 10 saponins had been isolated from the roots of *P. stipuleanatus*, but quantitative analysis of saponins in *P. stipuleanatus* has not been reported. Quantitative analysis of the two major compounds (SP-R_1 and SP-R_2) in *P. stipuleanatus* of different growth years and from different producing areas was achieved by HPLC (Table 7). The results showed that the major constituent SP-R_2 from dried *P. stipuleanatus* was in very high concentration, the content of which was approximately 3- to 20-fold higher than that of SP-R_1.

The medicinal parts of *P. stipuleanatus* are roots and rhizomes (Fig. 1A), and the root degenerates gradually with the increase in growth years, leaving only the part of the rhizome, as shown in Fig. 1B. The contents of SP-R_1 and SP-R_2 in one- to fifteen-year-old *P. stipuleanatus* were determined, which indicated the accumulation of saponins in *P. stipuleanatus* with growth years. The results showed that the content of SP-R_1 in *P. stipuleanatus* from Wenshan (PS-1 to PS-6) and Yanshan (PS-13 to PS-18) increased slowly with the growth period, and the SP-R_1 content in fifteen-year-old *P. stipuleanatus* was the highest (0.2443%). However, the content of SP-R_2 first increased and then decreased. The SP-R_2 content in three- to five-year-old *P. stipuleanatus* was higher, ranging from 2.2408% to 3.7877%. Comparing the contents of SP-R_1 and SP-R_2 in roots and rhizomes of three-year-old *P. stipuleanatus* (PS-2, 14 and PS-3, 15), it was found that the contents of the two compounds in the rhizomes were significantly higher than those in the roots.

To further investigate the effect of different geographical locations on the contents of saponins in *P. stipuleanatus*, the roots and rhizomes of three- to five-year-old *P. stipuleanatus* with high saponin content from different main producing areas (PS-19 to PS-24) were selected for analysis (Table 7). We found that there were significant differences in the contents of the principal components in *P. stipuleanatus* from different producing areas. Among them, the contents of SP-R_1 and SP-R_2 in *P. stipuleanatus* from Pingbian County of Yunnan Province (PS-22) were the highest, 0.4650% and 5.9492%, respectively.

3.6. Comparison of contents of SP-R_1 and SP-R_2 in fresh and dried P. stipuleanatus

Traditionally, both fresh and dried *P. stipuleanatus* were used for anti-inflammatory treatment. When saponin-containing medicinal materials are processed from fresh products into dried products, saponin components are easily degraded [29]. For the sake of making rational use of fresh and dried *P. stipuleanatus*, the contents of SP-R_1 and SP-R_2 in fresh and dried *P. stipuleanatus* of different growth years were determined (Fig. 3 and Table 7). The results showed that the content of SP-R_1 in fresh products (PS-7 to PS-12) was significantly lower than that in dried products (PS-1 to PS-6). As shown in Table 7, the content of SP-R_2 in the dried rhizome of three-year-old *P. stipuleanatus* (PS-3) was 20 times higher than that of SP-R_1, but only 4 times higher in fresh products (PS-9). At the same time, the change in this ratio was found in other growth years of fresh *P. stipuleanatus*, which may be caused by the drastic decrease of SP-R_1 content during the drying process.

4. Conclusions

In summary, the ethanol extract was subjected to chromatography to reveal two oleanane triterpenoid saponins, SP-R_1 and SP-R_2. SP-R_1 and SP-R_2 possessed potent analgesic and anti-inflammatory activity *in vivo*, and their main role was suppression of inflammatory mediator production and regulation of antioxidant systems. The contents of triterpenoid saponins from *P. stipuleanatus* collected at different growth years and from different producing areas were also investigated, indicating that the rational harvest of three- to five-year-old *P. stipuleanatus* was preferable to obtain a higher level of triterpenoid saponins. SP-R_2 content was the highest in *P. stipuleanatus*, which had potential as a chemical marker for quality control of *P. stipuleanatus*. In this study, the triterpenoid saponins in *P. stipuleanatus* have potential anti-inflammatory effects, which provide a basis for the development and utilization of *P. stipuleanatus*.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2020.05.002.

References

[1] Yan XL, Xiao FH, Wen GS, Li X, Duan CL. Investigation and collection of wild Panax species in Yunnan province. J Yunnan Agric Univ 2006;21:419–23.

[2] Chen ZJ, Huang TW, Sun YQ, Cui XM, Zhou SL, Wei ML. Characteristics of F fructification and seed ripening of *Panax stipuleanatus*. Chin J Chin Mater Med 2010;35:673–5.

[3] Sun YQ, Chen ZJ, Zhou SL, Wei ML, Huang TW. Flowering biological characteristics of Panax stipuleanatus. Chin J Chin Mater Med 2009;34:673–5.

[4] Yang CR, Jiang ZD, Zhou J, Kasai R, Tanaka O. Two new oleanolic acid-type saponins from *Panax stipuleanatus*. Acta Bot Yunnanica 1985;1:103–8.

[5] Liang C, Ding Y, Nguyen HT, Kim JA, Boo HJ, Kang HK, Nguyen MC, Kim YH. Oleanane-type triterpenoids from *Panax stipuleanatus* and their anticancer activities. Bioorg Med Chem Lett 2010;20:7110–5.

[6] Liang C, Ding Y, Song SB, Kim JA, Cuong NM, Ma JY, Kim YH. Oleanane-triterpenoids from *Panax stipuleanatus* inhibit NF-kappa B. J Ginseng Res 2013;37:74–9.

[7] Fitzgerald GA. Coxibs and cardiovascular disease. N Engl J Med 2004;351:1709–11.

[8] Yang XL, Guo TK, Wang YH, Huang YH, Liu X, Wang XX, Li W, Zhao X, Wang LP, Yan S, et al. Ginsenoside Rd attenuates the inflammatory response via modulating p38 and JNK signaling pathways in rats with TNBS-induced relapsing colitis. Int. Immunopharamcol. 2012;12:408–14.

[9] Uva L, Miguel D, Pinheiro C, Antunes J, Cruz D, Ferreira J, Filipe P. Mechanisms of action of topical corticosteroids in psoriasis. Int J Endocrinol 2012;2012:1–16.

[10] Babu NP, Pandikumar P, Ignacimuthu S. Anti-inflammatory activity of Albizia lebbeck Benth, an ethnomedicinal plant, in acute and chronic animal models of inflammation. J Ethnopharmcol 2009;125:356–60.

[11] Sun JY, You CY, Dong K, You HS, Xing JF. Anti-inflammatory, analgesic and antioxidant activities of 3,4-oxo-isopropylidene-shikimic acid. Pharm Biol 2016;54:2282–7.

[12] Meymandi MS, Keyhanfar F. Assessment of the antinociceptive effects of pregabalin alone or in combination with morphine during acetic acid-induced writhing in mice. Pharmacol Biochem Be 2013;110:249–54.

[13] Yesilada E, Tanaka S, Sezik E, Tabatam M. Isolationofananti-inflammatory principle from the fruit juice of Ecballium elaterium. J Nat Prod 1988;51:504–8.

[14] Wu YH, Zhou CX, Li XP, Song LY, Wu XM, Lin WY, Chen HY, Bai H, Zhao J, Zhang RP, et al. Evaluation of antiinflammatory activity of the total flavonoids of Laggera pterodonta on acute and chronic inflammation models. Phytother Res 2006;20:585–90.

[15] Ergene ÖB, Saltan İG, Küpeli AE, Süntar İ, Keleş H, Bahadır AÖ. Wound healing and anti-inflammatory activity of some ononis taxons. Biomed Pharmacother 2017;91:1096–105.

[16] Kassuya CAL, Cremoneze A, Barros LFL, Simas AS, Lapa FDR, Mello-Silva R, Stefanello MEA, Zampronio AR. Antipyretic and anti-inflammatory properties of the ethanolic extract, dichloromethane fraction and costunolide from Magnolia ovata (Magnoliaceae). J Ethnopharmacol 2009;124:369–76.

[17] Wang XY, Xu JY, Qiu DW, Huang LQ. Study on the anti-inflammatory effects and possible mechanism of total saponins of Psammosilene tunicoids. Chin J Exp Traditional Med Formulae 2006;12:56–8.

[18] Wang XY, Zhang Y, Xu JY, Qiu DW, Huang LQ. Effects and mechanism of total saponins of Psammosilene tunicoids against rheumatoid arthritis. China J Chin Mater Med 2006;31:419–21.

[19] Chen S, Rong Y, Liu MX, Cheng S, Liu XM, Li XH, Yu Y, Yang GZ, Yang XF. Analgesic effects of triterpenoid saponins from stauntonia chinensis via selective increase in inhibitory synaptic response in mouse cortical neurons. Front Pharmacol 2018;9:1302.

[20] Denny C, Melo PS, Franchin M, Massarioli AP, Bergamaschi KB, Alencar SM, Rosalen PL. Guava pomace: a new source of anti-inflammatory and analgesic bioactives. BMC Complem Altern M 2013;13:235–42.

[21] Huang MH, Wang BS, Chiu CS, Amagaya S, Hsieh WT, Huang SS, Shie PH, Huang GJ. Antioxidant, antinociceptive, and anti-inflammatory activities of Xanthii Fructus extract. J. Ethnopharmacol. 2011;135:545–52.

[22] Gao Y, Wang ZB, Zuo ZP, Guo YD, Su B, Songs CC. Research of the inflammation model of the rat gasbag synovitis. Pharmacol Clin Chin Mater Med 2013;2:189–92.

[23] Talat Z, Tursun X, Cheng L, Mijiti A, Aisa HA. Anti-arthritic and antiinflammatory effects of the traditional Uighur formula Kursi caper in vivo. Phytother Res 2015;29:1901–9.

[24] Schuerwegh AJ, Dombrecht EJ, Stevens WJ, Van JF, Bridts CH, De Clerck LS. Influence of pro-inflammatory (IL-1α, IL-6, TNF-α, IFN-γ) and anti-inflammatory (IL-4) cytokines on chondrocyte function. Osteoarthr Cartilage 2003;11:681–7.

[25] Dey D, Chaskar S, Athavale N, Chitre D. Inhibition of LPS-Induced TNF-α and NO production in mouse macrophage and inflammatory response in rat animal models by a novel ayurvedic formulation, BV-9238. Phytother Res 2014;28:1479–85.

[26] Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro-and anti-inflammatory properties of the cytokine interleukin-6. BBA-mol Cell Res 2011;1813:878–88.

[27] Ding Y, Zhao L, Mei H, Zhang SL, Huang ZH, Duan YY, Ye P. Exploration of Emodin to treat alpha-naphthylisothiocyanate-induced cholestatic hepatitis via anti-inflammatory pathway. Eur J Pharmacol 2008;590:377–86.

[28] Ma C, Long H, Yang C, Cai W, Zhang T, Zhao W. Anti-inflammatory role of pilose antler peptide in LPS-induced lung injury. Inflamm 2017;40:904–12.

[29] Gao MJ, Ma N, Wang C, Cui XM. Comparison of saponins contents of fresh Panax notoginseng, dried Panax notoginseng and active Panax notoginseng. Ginseng Res 2003;2:25–6.