

Comparison of the anti-inflammatory and anti-nociceptive effects of three medicinal plants known as "Snow Lotus" herb in traditional Uighur and Tibetan medicines

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3 **Comparison of the Anti-inflammatory and Anti-nociceptive Effects of Three Medicinal Plants**
4 **Known as “Snow Lotus” Herb in Traditional Uighur and Tibetan Medicines**
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3 **ABSTRACT**

4 *Ethnopharmacological relevance:*

5 *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip. (Compositae) has long been used under the herbal name
6 “Snow Lotus” for the treatment of rheumatoid arthritis, stomachache and dysmenorrhea in Uighur folk
7 medicine. In traditional Tibetan medicine, *S. laniceps* Hand.-Mazz. and *S. medusa* Maxim. have also
8 been used under the name “Snow Lotus” and prescribed for the treatment of pain and inflammatory
9 conditions.
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12 *Aim of the study:*

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14 The present study evaluated the pharmacological effects of three species of “Snow Lotus” in
15 experimental inflammation and pain models, and determined the chemical compounds that may
16 correlate with their pharmacological activities.
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19 *Material and methods:*

20 The anti-inflammatory activities of the three herbs were observed by using carrageenan-induced paw
21 edema in rats and xylene-induced ear edema in mice. Investigations on the analgesic effects were
22 conducted, including acetic acid-induced writhing and hot-plate test. An UPLC-MS method was
23 developed to analyse the chemical composition of the three herbs and of plasma samples after herb
24 administration.
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27 *Results:*

28 In rat paw edema model, the peak inhibitory effects of *S. laniceps* and *S. involucrata* (55.1% and 42.2%,
29 respectively) were recorded with the dose of 400 mg/kg at 3 h post carrageenan injection. In mouse ear
30 edema model, oral administration of *S. laniceps*, *S. involucrata* and *S. medusa* extract (400 mg/kg)
31 resulted in a significant inhibition of ear edema by 40.9%, 33.3%, and 9.1%, respectively. In the
32 writhing test, oral administration of *S. laniceps* extract (100, 200 and 400 mg/kg) resulted in a
33 significant inhibition of writhings by 13.5%, 22.3%, and 43.5%, respectively. In the hot plate test, *S.*
34 *laniceps* extract significantly increased the latency of jumping response by 38.2% and 52.7% when
35 treated orally at 200 and 400 mg/kg in mice, respectively. Flavonoids, coumarins and lignins were
36 found to be present in plasma after administration of the extracts and may be on the basis of the
37 observed pharmacological effects.
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42 *Conclusion:*

43 The results clearly demonstrated that *S. laniceps* was most effective; *S. involucrata* exhibited a
44 moderate potency, whereas *S. medusa* possessed little effect against the experimental edema and pains.
45 This study also supported discrimination among the three herbs when using them in folk medicine.
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48 *Keywords:*

49 Snow Lotus; *Saussurea involucrata*; *Saussurea laniceps*; *Saussurea medusa*; Anti-inflammatory;
50 Anti-nociceptive
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3 **1. Introduction**
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5 Because of biodiversity, related medicinal plants from the same family or genus have been and are
6 being used for similar therapeutic purposes in various folk medicines. *Saussurea involucrata* (Kar. et
7 Kir.) Sch.-Bip. (SI, Fig. 1), of the Composite family, grows in the mountains at heights of 4000-4300 m
8 in the Tianshan and A'er Tai areas in China (Chen et al., 1999). With a reputation for diminishing
9 inflammation and facilitating blood circulation, the dried aerial parts of SI have long been used under
10 the name "Snow Lotus" for the treatment of rheumatoid arthritis, stomachache and dysmenorrhea in
11 Uighur folk medicine (Chinese Pharmacopoeia Commission, 2005). Two other species of the same
12 genus, *S. laniceps* Hand.-Mazz. and *S. medusa* Maxim. (SL and SM, Fig. 1), mainly distributed in the
13 Qinghai-Tibet plateau at heights of 3500-5300 m, have also been used under the name "Snow Lotus"
14 and prescribed for the treatment of pain and inflammatory conditions in Tibetan folk medicine
15 (Commission of Chinese Ethnomedicine, 1984).
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26 *Insert Fig. 1 here*
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29 Currently, these three species of *Saussurea* genus are all used as an herbal drug for the treatment of
30 inflammation and pain-related diseases in Chinese medicine (Commission of Chinese Materia Medica,
31 1999). However, whether these related plants can be used interchangeably is still unknown thereby
32 limiting further bioactive screening and clinical popularization. This is particularly important because
33 one of the species, SI, is in danger of extinction due to the pressures of herbal collection (Ministry of
34 Environmental Protection of the People's Republic of China, 1987). Studies on the pharmacological
35 effects of the three medicinal plants have been performed using various models (Zheng et al., 1993;
36 Duan et al., 2002; Gao et al., 2005); however, the existing studies have each focused on the
37 pharmacological investigation of a single herb, and no systematic comparison of the pharmacological
38 potencies related to the clinical effects of these herbs has been reported. Therefore, to encourage
39 reasonable and sustainable use of these related species, a comparison of their chemical composition and
40 pharmacological activities is urgently needed.
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51 In our previous study, we reported the chemical composition of these three herbs used under the
52 name "Snow Lotus" (Yi et al., 2009a; Yi et al., 2009b). In this follow-up study, we compare the
53 suppressive effects of the three herbs in two inflammatory models and two nociceptive models in
54 rodents. In addition we sought to determine the correlation between herb constituents absorbed in the
55 blood stream and pharmacological activity. To do this, the herbal constituents found in blood plasma
56 after herb administration were detected using ultra-performance liquid chromatography-electrospray
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3 ionization mass spectrometry (UPLC-ESI-MS). The results reveal that the pharmacological potencies
4 of the three herbs varied, which would contribute to the different chemical composition and absorbed
5 constituents of the three herbs. As for physiological effects, among the three herbs, SL significantly
6 inhibited inflammatory responses and nociceptive production to a greater degree than either of the other
7 two. Thus, SL is the most likely candidate to become a complementary and alternative drug for the
8 treatment of inflammatory and painful diseases.
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16 **2. Materials and methods**

17 *2.1 Plant materials and reagents*

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19 *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip. (SI) was collected from the Tianshan areas in the
20 Xinjiang Uighur Autonomous Region of China in 2007. *S. laniceps* Hand.-Mazz. (SL) and *S. medusa*
21 Maxim. (SM) were collected from Lhasa in Tibet of China in 2007 and 2005, respectively. Identity of
22 the herbs was confirmed by Dr. Hubiao Chen (School of Chinese Medicine, Hong Kong Baptist
23 University, Hong Kong) by means of geographical origin and macroscopic character assessment.
24 Voucher specimens were deposited in the Chinese Medicines Center, Hong Kong Baptist University
25 (TS-03 for SI, MT-01 for SL and SM-04 for SM, respectively).
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33 Indomethacin, carrageenan, acetic acid and carboxy methylcellulose were purchased from Sigma
34 Chemical Co. (St. Louis, MO, USA). Rotundine, an analgesic drug was purchased from Zhenang
35 Pharmaceutical Co., Ltd. (Nanjing, China). Acetonitrile and methanol of chromatography grade were
36 purchased from Lab-scan (Bangkok, Thailand). Formic acid of chromatography grade and ethanol of
37 analytical grade were purchased from Merck (Darmstadt, Germany). Water was purified using a
38 Milli-Q water system (Millipore; Bedford, MA, USA).
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45 *2.2 Animals*

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47 ICR mice weighing 18-23 g and SD rats weighing 150-200 g were purchased from the Laboratory
48 Animal Services Center, the Chinese University of Hong Kong, Hong Kong. All animals were fed with
49 a standard chow diet and water. All animals were housed at a room temperature of 23 ± 1 °C with a 12
50 h light/dark cycle. A standard rodent diet and water were provided *ad libitum*. All experimental
51 protocols were approved by the Committee on the Use of Human & Animal Subjects in Teaching and
52 Research of Hong Kong Baptist University, in accordance with the Animals Ordinance (Department of
53 Health, Hong Kong).
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2.3 Preparation of herbal extracts

Plant materials were cut into small pieces and ground into powder. Herbal sample powder (0.5 kg) was extracted with 50% ethanol by means of percolation at room temperature. The operations were repeated until the extract became colorless. The combined extracts were evaporated to remove ethanol at reduced pressure in a rotary evaporator (50 °C), and then were lyophilized with a freeze-dry system. SI extract (129 g, yield 25.8%, w/w), SL extract (86 g, yield 17.2%, w/w) and SM extract (56 g, yield 11.2%, w/w) were thus obtained. The dried extracts were suspended in 1% (w/v) aqueous carboxy methylcellulose for administration to animals.

2.4 Carrageenan-induced paw edema in rat

For the determination of effects on acute inflammation, the carrageenan-induced paw edema model described by Yesilada and Kupeli (Yesilada and Kupeli, 2002) was employed with modifications. SD rats were weighed and randomized into eleven groups of ten animals each, namely a control group (aqueous carboxy methylcellulose-treated), a reference drug group (indomethacin-treated), and three groups each (receiving 100, 200, or 400 mg/kg of drug) for SI treatment, SL treatment and SM treatment. The test agents were orally administered to the rats for 5 consecutive days. One hour after the last delivery, 100 µl of a 1% solution of carrageenan in 0.9% physiological saline was injected subcutaneously into the subplantar region of the right hind paw. The left hind paw received 100 µl of saline solution similarly injected. The difference in footpad volume between the right and left foot was measured with a water displacement plethysmometer (plethysmometer 7150, UGO Basile, Italy) at 1, 3, and 5 h after induction of inflammation. Mean differences of treated groups were compared with the mean difference of the control group and analysed using statistical methods.

2.5 Xylene-induced ear edema in mice

The xylene-induced ear edema test with modifications was performed as previously described (Luo et al., 2008). Oral administrations with vehicle, reference drug and herbal extracts before inducing ear edema were conducted for 5 consecutive days. Thirty minutes after the last administration of tested drugs, a total of 20 µl of xylene was applied to the inner and outer surface of the right ear of each mouse. The left ear remained untreated. Control animals received the irritant and an equal volume of aqueous carboxy methylcellulose, while indomethacin (10 mg/kg) served as the reference. Fifteen minutes later, the mice were sacrificed by cervical dislocation and the plugs were removed with a cork borer (6.5 mm in diameter) from both the right and left ear. The difference in weight between the two plugs was taken as a measure of edematous response.

2.6 Writhing test in mice

Writhing test was performed as the chemical pain model (Arihan et al., 2009). Briefly, vehicle, rotundine (50 mg/kg), and test solution (100, 200 and 400 mg/kg) were orally administered for five consecutive days. Half hour after the last delivery, 0.2 ml of acetic acid (0.7% w/v) in saline was injected intraperitoneally. The numbers of abdominal writhing movements were recorded for 15 min starting 5 min after *i.p.* injection in each animal.

2.7 Hot plate test in mice

The hot plate test was used as the thermal pain model (Suzuki et al., 2009). Briefly, vehicle, rotundine (50 mg/kg), and test solution (100, 200 and 400 mg/kg) were orally administered for five consecutive days. Half hour after the last delivery, each mouse was placed on a hot-plate surface (IITC Model 39, Woodland Park, CA) maintained at $55 \pm 0.2^\circ\text{C}$. The reaction time from hot-plate placement to hind-paw lick was recorded. The cut-off time was set at 60 second to prevent tissue injury. Before the experiments, all animals were tested for heat stimulation latency, and those with response time < 5 second or > 30 second were excluded.

2.8 UPLC-MS fingerprint analysis of herb extracts and animal plasma

A Waters AcquityTM ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, USA) consisting of a binary pump, autosampler, thermostated column compartment and diode array detector (DAD), was used for fingerprint analysis. For mass spectrometric determination, the UPLC system was hyphenated to a Bruker MicroTOFQ system by an electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany).

The chromatographic separation was carried out on a Waters BEH C₁₈ column (1.7 μm , 2.1 \times 100 mm, Waters Corp.) with a VanGuardTM pre-column (BEH, C₁₈, 1.7 μm , 2.1 \times 5 mm). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) using a gradient program of 3% (B) in 0-2 min, 3-15% in 2-14 min, 15-20% (B) in 14-22 min and 20-45% (B) in 22-30 min. The solvent flow rate was 0.3 ml/min, the column temperature was set to 40 $^\circ\text{C}$ and the detection wavelength was 280 nm. The conditions of MS analysis in the positive ion mode were as follows: drying gas (nitrogen), flow rate, 8 l/min; gas temperature, 200 $^\circ\text{C}$; scan range, 50-1000 *m/z*; end plate offset voltage, -500 V; capillary voltage, 4500 V; nebulizer press, 1.5 Bar.

For analysis of herb extracts, three extracts of 0.1 g each were dissolved in 50 ml of methanol, and then centrifuged at 12000 rpm for 10 min to obtain sample solution. For the analysis of animal plasma

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3 after herb administration, the three herbal extracts (400 mg/kg) were orally administered to three
4 groups of two rats each for 5 consecutive days. Three hours after the last delivery, blood samples (ca.
5 0.6 ml) were withdrawn from the fossa orbitalis into heparin-stabilized test-tubes and immediately
6 centrifuged at 3000 rpm for 5 min to obtained plasma. One milliliter methanol was added into 0.2 ml
7 plasma and vortex-mixed for 5 s, then centrifuged at 12000 rpm for 10 min to precipitate the protein
8 and extracted analytes. The supernatant was transferred into another tube and dried under a stream of
9 nitrogen. The residue was reconstituted with 0.1ml methanol and then centrifuged at 12000 rpm for 10
10 min. To obtain mouse plasma after drug administration, the three herbal extracts (400 mg/kg) were
11 orally administered to three groups of five mice each for 5 consecutive days, respectively. One hour
12 after the last delivery, the mice were sacrificed by decapitation, and the blood was collected and
13 handled using the procedures described above. An aliquot of 3 μ l for the supernatant of all the test
14 solutions was analyzed by UPLC–MS.
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26 *2.9 Statistical analysis*

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28 Values obtained from experiments were expressed as mean \pm S.E.M and further analyzed using
29 one-way ANOVA followed by Dunnett test for multiple comparisons, with the level of significance
30 chosen as $P < 0.05$.
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34 **3. Results and discussion**

35 *3.1 Suppressive effects in inflammatory models*

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38 Carrageenan-induced rat paw edema and xylene-induced mouse ear edema are the typical animal
39 models used to evaluate the anti-inflammatory effect of natural products; they have a good reputation
40 for screening anti-inflammatory agents. Inflammation induced by carrageenan is an acute and highly
41 reproducible inflammatory model. Cardinal signs of inflammation develop immediately following
42 subcutaneous injection, resulting from action of proinflammatory agents. The inflammatory response is
43 usually quantified by increase in paw size (edema) and is also modulated by inhibitors within the
44 inflammatory cascade (Winyard and Willoughby, 2003).
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53 In our study, the suppressive effects of the three herbal extracts on carrageenan-induced paw edema
54 in rat are shown in Table 1. The extracts of the three herbs exhibited varying degrees of
55 anti-inflammatory activity. SL extract administered orally at doses of 100-400mg/kg showed significant
56 dose-dependent reduction of the carrageenan-induced paw edema, SI extract showed a moderate
57 inhibition of edema formation, whereas SM extract apparently had little effect on edema. The peak
58 inhibitory effects of SL and SI (55.1% and 42.2%, respectively) were recorded with the dose of 400
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3 mg/kg at 3 h post-carrageenan injection ($p < 0.01$), compared with the indomethacin-treated group (10
4 mg/kg, 75.5%).

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7 *Insert Table 1 here*

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9 Xylene-induced mouse ear edema leads to an acute inflammatory response characterized by fluid
10 accumulation and edema in the *in vivo* model. Suppression of this response is taken as an indication of
11 antiphlogistic effect (Atta and Alkofahi, 1998). The suppressive effects of the three herbal extracts on
12 xylene-induced mouse ear edema are shown in Figure 2. In our study, SL extract administered orally at
13 a dose of 200-400mg/kg and SI extract at a dose of 400mg/kg significantly reduced ear edema, and the
14 mean weight of ear edema in SL-treated group (3.9 mg) of 400 mg/kg was lower than that of the
15 SI-treated group (4.4 mg) as well as the SM-treated group (6.0 mg) at equivalent doses. Compared with
16 the control group (6.6 mg), oral administration of SL, SI and SM extract (400 mg/kg) resulted in a
17 significant inhibition of ear edema by 40.9%, 33.3%, and 9.1%, respectively.

18 Hence, SL extract appeared to be the most effective of the three herbs in reducing inflammation,
19 implying that SL possesses anti-inflammatory components.

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31 *Insert Fig. 2. here*

32 33 3.2 *Suppressive effects in analgesic models*

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35 Animal pain tests have been developed primarily for the screening of potential analgesic drugs.
36 Various tests have different profiles of sensitivity; thus, while no single laboratory test procedure can
37 give a comprehensive evaluation of analgesics, a combination of several tests provides a good
38 substitute (Boulton et al., 1989). Therefore, the methods of both peripherally and centrally mediated
39 effects were selected for investigating anti-nociceptive effect. The acetic acid-induced abdominal
40 constriction and hot-plate methods are the animal models typically used to elucidate peripheral and
41 central activity, respectively (Verma et al., 2005).

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43 In the acetic acid-induced abdominal writhing test (Table 2), oral administration of SI (100, 200 and
44 400 mg/kg) resulted in a significant inhibition of writhing by 12.4%, 29.5%, and 35.2%, respectively,
45 and oral administration of SL (100, 200 and 400 mg/kg) resulted in a significant inhibition of writhings
46 by 13.5%, 22.3%, and 43.5%, respectively. The SI and SL extract at a dose of 400 mg/kg inhibited the
47 acetic acid-induced pain with a potency comparable to that of the reference drug, rotundine ($p > 0.05$).
48 Acetic acid itself may cause pain; at the same time, it can also stimulate the tissue to produce several
49 mediators such as histamine, serotonin, cytokines, and eicosanoids with an increase in peritoneal fluid
50 levels of these mediators (Bentley et al., 1983, Rinaldi et al., 2009). Therefore, anti-nociceptive activity

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3 of SI and SL may be related to the reduction in the liberation of those inflammatory mediators or by
4 direct blockage of receptors resulting in peripheral anti-nociceptive effects.

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6 To check for possible central anti-nociceptive activity of the herbs, the hot plate test was performed,
7 and the results are shown in Table 2. When mice were treated with extracts of SL (100, 200 and 400
8 mg/kg), there was a dose-dependent increase in response to thermal stimulation compared with control
9 mice. These results clearly indicate that SL significantly increased the latency of jumping response by
10 38.2% and 52.7% when treated at 200 and 400 mg/kg, respectively, suggesting that SL has central
11 analgesic properties.
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18 19 20 *3.3 Phytochemicals and absorbed constituents after herb administration*

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22 Ultra-performance liquid chromatography (UPLC), a new category of analytical separation science,
23 retains the practicality and principles of high-performance liquid chromatography (HPLC) while
24 increasing the overall interlaced attributes of speed, sensitivity, and resolution. In the present study, the
25 extracts of the three herbs and plasma samples after herb administration were analyzed using
26 UPLC-MS, and the typical chromatograms are shown in Figure 3 to Figure 5. Based on our previous
27 study (Yi et al., 2009a; Yi et al., 2009b), the main constituents of the three herbs are found to be:
28 organic acids and flavonoids in SI, coumarins and their glucosides in SL, and lignins in SM.
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35 For the analysis of absorbed constituents in the SI-treated group (Figure 3), chlorogenic acid, one of
36 the main constituents in SI extract, was not detected, neither in mouse nor rat plasma samples. In
37 accordance with the literature, chlorogenic acid possesses antioxidant as well as antiviral effects with
38 lower bioavailability by oral administration (Olthof et al., 2001; Gonthier et al., 2003). In contrast, rutin,
39 another main constituent of the SI extract, was detected in mouse plasma. It has been reported that rutin
40 posses anti-inflammatory and anti-nociceptive effects (Jia et al., 2005). Therefore, the moderate
41 inhibition of SI on the inflammatory and nociceptive models in this study would contribute to the
42 presence of rutin in SI herb.
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52 For the analysis of absorbed constituents in the SL-treated group, umbelliferone and scopoletin
53 were detected both in mouse and rat plasma samples. The typical UPLC chromatogram is shown in
54 Figure 4. The results indicate that umbelliferone and scopoletin are absorbed by animals after SL
55 extract oral administration. In recent years, it has been reported that umbelliferone and scopoletin are
56 easy to transport through the small intestine mucosal barrier directly, and their bioavailability compared
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3 to inderal, an easily absorbed reference drug (Yang et al., 2008). On the other hand, umbelliferone and
4 scopoletin showed cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) dual inhibitory activity
5 (Kim et al., 2006), which suggests that the two compounds produce anti-inflammatory activity in part
6 via the inhibition of the generation of eicosanoids. It has also been reported that umbelliferone and
7 scopoletin exhibit significant and dose-related antinociceptive effects against acetic acid-induced
8 visceral pain in mice (Tanaka et al., 1977; Meotti et al., 2006). Moreover, scopoletin is a commonly
9 used anti-asthmatic in modern medicine, and it is mainly prescribed for the treatment of acute and
10 chronic bronchitis. Therefore, the anti-inflammatory and analgesic effect of SL extract in the present
11 study would contribute to the relatively greater bioavailability as well as the pharmacological activities
12 of umbelliferone and scopoletin.
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24 For the analysis of the absorbed constituents in the SM-treated group (Fig. 5), arctigenin, an
25 aglycone and the main metabolite of arctiin (Zheng et al., 2005), was detected in rat plasma samples.
26 However, the pharmacological activities of arctigenin are mainly anti-cancer and anti-viral (Awale et
27 al., 2006; Wang et al., 2006); they are not reported to have strong anti-inflammatory activity. Thus,
28 these features are probably related to the small suppressive effects of SM on inflammation and
29 nociception in the models in the present study. It is speculated that SM and SL have been and are being
30 interchangeably used in Tibetan folk medicine because of their taxonomic similarity rather than
31 equivalence in pharmacological effectiveness.
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41 **4. Conclusion**

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43 The present study investigated the pharmacological effects of three species traditionally used under
44 the herbal drug name “Snow Lotus” against experimental inflammation and pain. Four animal models,
45 i.e., carrageenan-induced rat paw edema and xylene-induced mouse ear edema models, were used to
46 study anti-inflammatory activity, and acetic acid-induced mice abdominal writhing and mouse hot-plate
47 models were used to study the anti-nociceptive effect. All animal models clearly demonstrated that
48 *Saussurea laniceps* was most effective; *S. involucrata* exhibited a moderate potency, whereas *S.*
49 *medusa* possessed little effect against the experimental edema and pains.
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57 Comparative analysis of the three herbs and of blood plasma after herb administration was used to
58 elucidate correlation between compounds and pharmacological activity. The results revealed that the
59 chemical composition, absorbed constituents and pharmacological activities of the three herbs were
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different, which supported discrimination among the three herbs when using them in folk medicine. The results demonstrate that *S. laniceps* is the most potent of the three, and suggested that it is the best species to be used in traditional medicine as an anti-inflammatory and analgesic drug instead *S. involucrata* or *S. medusa*. The results also indicate that *S. involucrata* is less potent than *S. laniceps*; if this information was disseminated among herb collectors and drug dealers it could help reduce collection pressures on this rare and endangered species.

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Legends for Tables

Table 1

Anti-inflammatory effect of three herbal extracts on carrageenan-induced paw edema in rats.

Table 2

Antinociceptive effect of three herbal extracts on acetic acid-induced writhing and hot plate test in mice.

Table 1

Anti-inflammatory effect of three herbal extracts on carrageenan-induced paw edema in rats

Groups	Dose (mg/kg)	Edema volume (EV, ml) and edema inhibition (EI, %) after injection (h)					
		1 h		3 h		5 h	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
Control	-	0.645 ± 0.042	-	0.787 ± 0.048	-	0.756 ± 0.065	-
SI	100	0.528 ± 0.040	18.1	0.636 ± 0.042	19.2	0.625 ± 0.026	17.3
	200	0.572 ± 0.043	11.3	0.604 ± 0.052	23.2	0.555 ± 0.071*	26.6
	400	0.518 ± 0.072	19.7	0.455 ± 0.047**	42.2	0.491 ± 0.035**	35.1
SL	100	0.463 ± 0.032*	28.2	0.541 ± 0.065*	31.3	0.628 ± 0.057	16.9
	200	0.509 ± 0.040	21.1	0.405 ± 0.047**	48.5	0.440 ± 0.041**	41.8
	400	0.469 ± 0.026*	27.3	0.353 ± 0.057**	55.1	0.372 ± 0.040**	50.8
SM	100	0.627 ± 0.044	2.8	0.726 ± 0.065	7.8	0.688 ± 0.048	9.0
	200	0.611 ± 0.043	5.3	0.672 ± 0.053	14.6	0.698 ± 0.025	7.7
	400	0.505 ± 0.060	21.7	0.543 ± 0.061*	31.0	0.630 ± 0.063	16.7
Indomethacin	10	0.226 ± 0.026**	65.0	0.193 ± 0.029**	75.5	0.214 ± 0.040**	71.7

SI: *Saussurea involucrata*; SL: *Saussurea laniceps*; SM: *Saussurea medusa*.

Values of edema shown are mean ± S.E.M. (n=10).

* $p < 0.05$; ** $p < 0.01$ vs. control group.

Table 2**Antinociceptive effect of three herbal extracts on acetic acid-induced writhing and hot plate test in mice**

Groups	Dose (mg/kg)	Frequency (Counts/15 min)	Inhibition (%)	Latency time (sec)	Increase (%)
Control	-	19.3 ± 1.2	-	16.5 ± 1.2	-
SI	100	16.9 ± 2.6	12.4	17.4 ± 1.5	5.5
	200	13.6 ± 1.4	29.5	19.2 ± 1.8	16.4
	400	12.5 ± 1.2*	35.2	18.8 ± 1.3	13.9
SL	100	16.7 ± 1.9	13.5	18.3 ± 2.1	10.9
	200	15.0 ± 2.4	22.3	22.8 ± 1.2*	38.2
	400	10.9 ± 1.3**	43.5	25.2 ± 1.5**	52.7
SM	100	18.2 ± 1.3	5.7	17.1 ± 0.9	3.6
	200	19.9 ± 2.1	-3.1	17.9 ± 1.9	8.5
	400	15.4 ± 1.8	20.2	20.1 ± 1.3	21.8
Rotundine	50	8.1 ± 0.8**	58.0	27.7 ± 1.6	67.9

SI: *Saussurea involucrata*; SL: *Saussurea laniceps*; SM: *Saussurea medusa*.

Values of edema shown are mean ± S.E.M. (n=10).

* $p < 0.05$; ** $p < 0.01$ vs. control group.

Legends for Figures

Fig. 1. Photos of *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip. (SI), *S. laniceps* Hand.-Mazz. (SL), *S. medusa* Maxim. (SM) plants and their medicinal materials.

Fig. 2. Anti-inflammatory effect of three herbal extracts on xylene-induced ear edema in mice.

Fig. 3. UPLC chromatogram of a sample of SI extract (SI-E), a plasma sample of SI-treated rat (SI-R) and a plasma sample of SI-treated mouse (SI-M). Peak 1 to peak 3 were identified as chlorogenic acid, rutin and 1,5-dicaffeoylquinic acid, respectively.

Fig. 4. UPLC chromatogram of a sample of SL extract (SL-E), a plasma sample of SL-treated rat (SL-R) and a plasma sample of SL-treated mouse (SL-M). Peak 1 to peak 7 were identified as umbelliferone 7-*O*- β -D-glucoside, chlorogenic acid, scopoletin 7-*O*- β -D-glucoside, syringoside, umbelliferone, scopoletin and 1,5-dicaffeoylquinic acid, respectively.

Fig. 5. UPLC chromatogram of a sample of SM extract (SM-E), a plasma sample of SM-treated rat (SM-R) and a plasma sample of SM-treated mouse (SM-M). Peak 1 to peak 3 were identified as chlorogenic acid, arctiin and arctigenin, respectively.

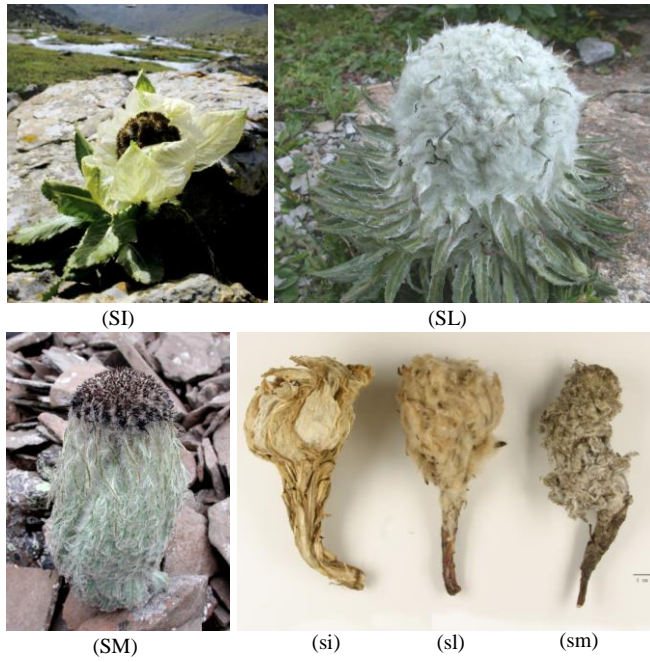


Fig. 1. Photos of *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip. (SI), *S. laniceps* Hand.-Mazz. (SL), *S. medusa* Maxim. (SM) plants and their medicinal materials.

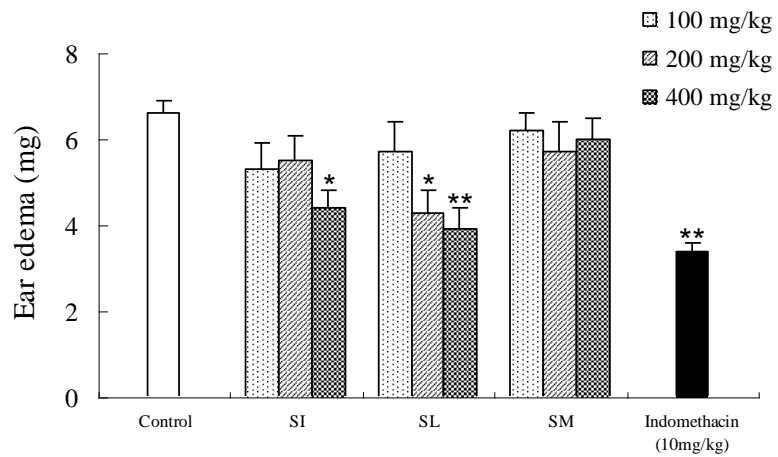


Fig. 2. Anti-inflammatory effect of three herbal extracts on xylene-induced ear edema in mice. SI: *Saussurea involucrata*; SL: *Saussurea laniceps*; SM: *Saussurea medusa*. Values of edema shown are mean \pm S.E.M. ($n=10$).
* $p < 0.05$; ** $p < 0.01$ vs. control group.

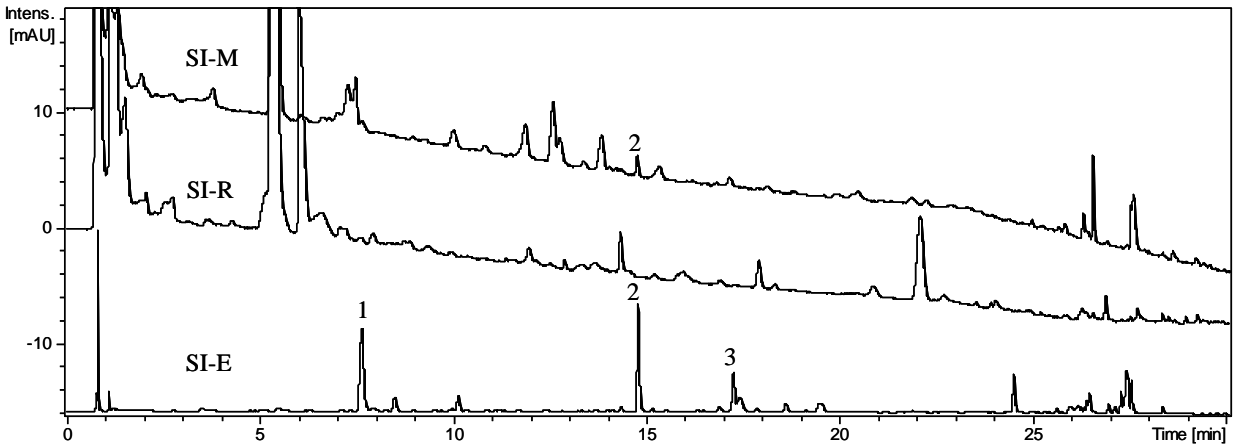


Fig. 3. UPLC chromatogram of a sample of SI extract (SI-E), a plasma sample of SI-treated rat (SI-R) and a plasma sample of SI-treated mouse (SI-M). Peak 1 to peak 3 were identified as chlorogenic acid, rutin and 1,5-dicaffeoylquinic acid, respectively.

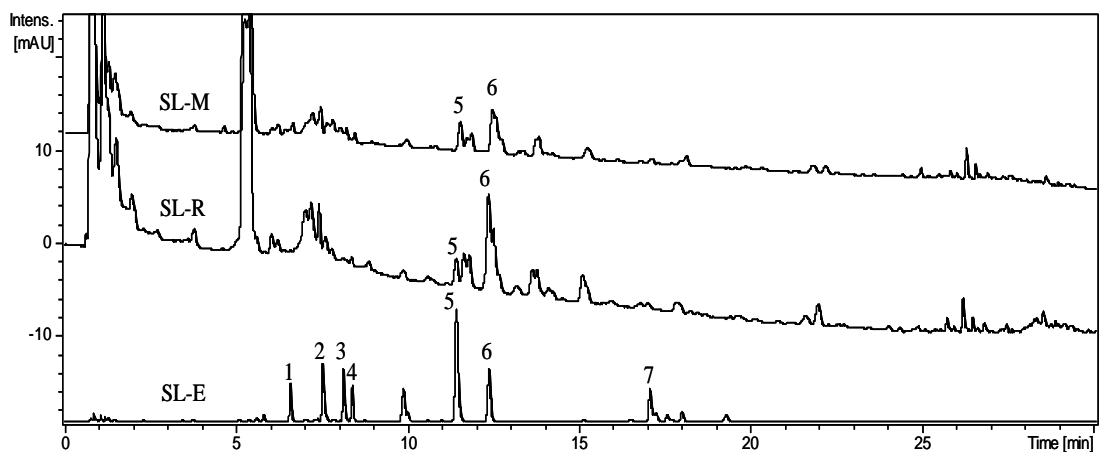


Fig. 4. UPLC chromatogram of a sample of SL extract (SL-E), a plasma sample of SL-treated rat (SL-R) and a plasma sample of SL-treated mouse (SL-M).

Peak 1 to peak 7 were identified as umbelliferone 7-*O*- β -D-glucoside, chlorogenic acid, scopoletin 7-*O*- β -D-glucoside, syringoside, umbelliferone, scopoletin and 1,5-dicaffeoylquinic acid, respectively.

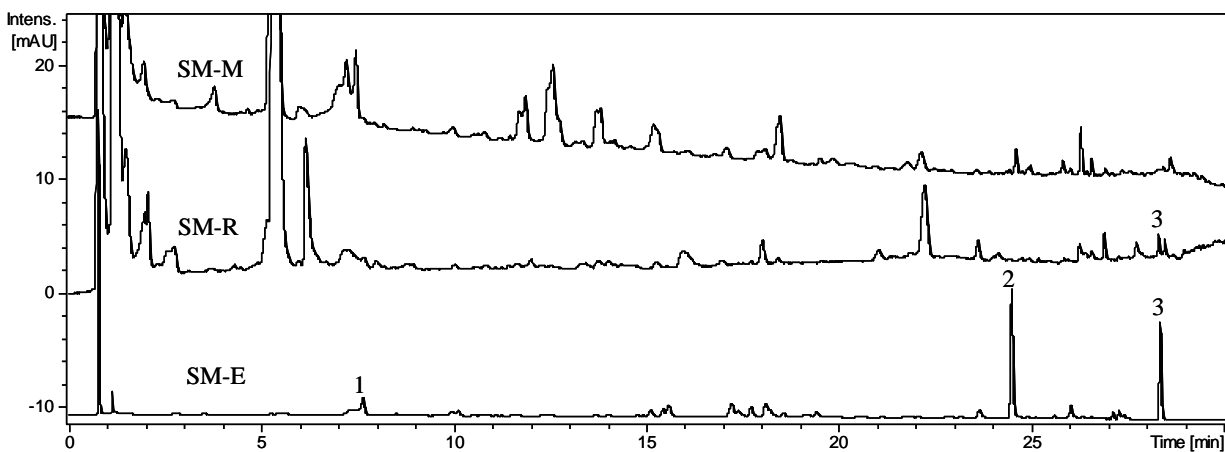


Fig. 5. UPLC chromatogram of a sample of SM extract (SM-E), a plasma sample of SM-treated rat (SM-R) and a plasma sample of SM-treated mouse (SM-M). Peak 1 to peak 3 were identified as chlorogenic acid, arctiin and arctigenin, respectively.