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The antinociceptive and anti-inflammatory activities of *Petasites formosanus* Kitamura extract

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ABSTRACT. *Petasites formosanus* Kitamura (Compositae) is native to Taiwan and is used in folk medicine to treat hypertension and asthma. Aqueous methanolic (50%) extracts of leaves of *P. formosanus* (Leaves-MeOH extract) showed the strongest inhibitory effect against NO production by lipopolysaccharide (LPS)-induced RAW 264.7 cells with an IC_{50} of 22.85 μg/mL. In an *in vivo* assay, 200 mg/kg of the extract also significantly decreased the acetic acid-induced writhing response, increased the hot-plate latency, and significantly suppressed carrageenan-induced paw edema. The principle anti-inflammatory constituents of the leaf extract were isolated by column chromatography combined with bioassay-guided fractionation, and 4 compounds, isopetasin, S-isopetasin, S-petasin, and caffeic acid methyl ester, were obtained. S-Ispopetasin and S-petasin more significantly inhibited NO production, inducible nitric oxide synthase, and cyclooxygenase-2 expression in a dose-dependent manner in LPS-induced RAW 264.7 cells, and S-ispopetasin showed stronger potency than S-petasin. S-Isopetasin also showed stronger reductions in the acetic acid-induced writhing response and carrageenan-induced paw edema than S-petasin. Taken together, the results indicate that *P. formosanus* possesses both anti-inflammatory and anti-nociceptive activities, and S-isopetasin was the major constituent mediating those activities and may be used as a lead for new anti-inflammatory drug development.

Keywords: Anti-inflammation; Antinociceptive; Compositae; *Petasites formosanus* Kitamura; S-isopetasin; S-petasin.

INTRODUCTION

Petasites formosanus Kitamura is a *Petasites* species of the Asteraceae (Compositae) indigenous to Taiwan. Interest in this plant arose because in Taiwanese folk medicine, the plant is used to treat asthma and hypertension (Lin et al., 1998). A study by Lin et al. first isolated several compounds, including S-petasin and S-isopetasin, from the aerial parts of *P. formosanus* (Lin et al., 1998). A later study demonstrated significant relaxant effects of S-petasin and S-isopetasin in the isolated guinea pig trachea pretreated with a contractile agent (Ko et al., 2000). The antispasmodic and tracheal relaxation may be due to antimuscarinic activity of the two compounds, supporting the folkloric usage of *P. formosanus* to treat asthma (Ko et al., 2001; Lin et al., 2008). Supporting evidence for the traditional beneficial claims of *P. formosanus* in hypertensive management was also reported. Administration of S-petasin and S-isopetasin dose-dependently reduced the heart rate and blood pressure in anesthetized rats. Mechanistic studies suggested that these two sesquiterpenes have calcium channel-blocking actions in vascular smooth muscle cells (Wang et al., 2001, 2002, 2004). The traditional usage also suggests that this plant may have anti-inflammatory and analgesic activities (Lin et al., 2004). Indeed, in other parts of the world, other species of *Petasites* are used to treat inflammatory disorders (Thomet et al., 2001; Panthong et al., 2003; Fiebich et al., 2005).

Usually the entire plant of *P. formosanus* is used indigenously. However, the part of the plant that is most useful has not been explored, and the active ingredients of *P. formosanus* for anti-inflammation and anti-nociception are as yet unknown. The purpose of this study was to investigate the ability of *P. formosanus* extracts and constituents to in-

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hibit inflammatory and nociceptive responses. The results provide scientific evidence for the clinical use of *P. formosanus* in Taiwanese folk medicine.

MATERIALS AND METHODS

Plant material

Petasites formosanus was identified and cultivated by Dr. Din-Lin Chang of the Taiwan Seed Improvement and Propagation Station, Council of Agriculture, Taichung, Taiwan for 1 year and was harvested in April 2006. Voucher specimens (PF-006) were deposited in the School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan. After harvest, leaves of *P. formosanus* were dried in a 40°C oven before being used.

Drug and Chemicals

Dimethyl sulfoxide (DMSO), MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide], trypan blue, lipopolysaccharide (LPS) (*E. coli* serotype 0127-8B), carrageenan, indomethacin, tramadol and other chemicals were purchased from Sigma Chemical (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), antibiotics, L-glutamine, and trypsin-EDTA were purchased from Gibco BRL (Grand Island, NY, USA). Western blotting was performed using an antibody specific to mouse iNOS (sc-650), anti-COX-2 (sc-1745), anti-GAPDH (sc-32233), anti-rabbit IgG-AP (sc-2007), and anti-mouse IgG-AP (sc-2008), anti-goat IgG-AP (sc-2022) which was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Column chromatography was carried out on silica gel (Merck). All solvents used for column chromatography were of analytical grade.

Extraction and isolation

Dried leaves of *P. formosanus* (950 g) were refluxed with 50% methanol (10 L × 2) for 1 h. The filtrate was concentrated with a rotary evaporator at 45°C to obtain a syrup-like aqueous methanolic extract (258 g). The aqueous methanolic extract was suspended in water and partitioned with ethyl acetate (EtOAc). The ethyl acetate layer was absorbed by Celite 545 and was chromatographed over a silica gel column (9.6 cm i.d. × 70 cm) with *n*-hexane→ *n*-hexane-EtOAc (10:1 → 5:1 → 1:1 → 1:5 →1:10)→ EtOAc→ EtOAc-acetone (10:1)→ acetone→ MeOH). Among these fractions, *n*-hexane-EtOAc (10:1, 5:1, and 1:1) eluates were further characterized (Figure 1).

The *n*-hexane-EtOAc (10:1) eluate was chromatographed over a VersaPak silica gel column (40 mm i.d. × 150 mm, Supelco, Bellefonte, PA, USA), developed with *n*-hexane-EtOAc (5:1). The major compound was monitored with thin-layer chromatography (TLC) and recrystallized with *n*-hexane and EtOAc to yield isopetasin (**1**) (1.9 mg, 0.0002%) (Figure 2).

The *n*-hexane-EtOAc (5:1) eluate yielded crude S-isopetasin (**2**) and S-petasin (**3**). Each compound was recrystallized with *n*-hexane to yield pure **2** (153.6 mg, 0.0162%) and **3** (142.9 mg, 0.0150%) (Figure 2).

The *n*-hexane-EtOAc (1: 1) eluate was chromatographed over a silica gel column (2.5 cm i.d. × 40 cm) eluted with *n*-hexane-EtOAc (5: 2) to yield caffeic acid methyl ester (**4**) (43.3 mg, 0.0046%) (Figure 2).

Isopetasin (**1**) ESI-MS (*m/z)*: 317 $[M+H]^+$. ^{1}H-NMR (500MHz, $CDCl_3$) δ: 1.01 (3H, *d*, *J* =6.8Hz, H-14), 1.99 (3H, *dd*, *J* =7.0, 1.1Hz, H-4'), 1.05 (3H, *s*, H-15), 1.50 (1H, *m*, H-2a), 1.71 (1H, *m*, H-4),1.86 (3H, *s*, H-12), 1.89 (3H, *s*, H-5'), 2.10 (3H, *d*, *J* =1.7 Hz, H-13), 2.19 (1H, *m*, H-6a), 2.24 (1H, *m*, H-2b), 2.36 (1H, *m*, H-1a), 2.48 (1H, *m*, H-1b), 2.93 (1H, *d*, *J* =13.7Hz, 6b), 4.93 (1H, *td*, *J* =4.3, 11.1Hz, H-3), 5.78 (1H, *d*, *J* =1.58Hz, H-9), 6.07 (1H, *m*, H-3'). ^{13}C-NMR (126MHz, $CDCl_3$) δ: 10.8 (C-14), 15.7 (C-4'), 17.1 (C-15), 20.6 (C-5'), 22.1 (C-12), 30.1 (C-1), 31.7 (C-2), 41.2 (C-6), 42.2 (C-5), 46.2 (C-4), 73.3 (C-3), 126.7 (C-9), 127.1 (C-7), 128.0 (C-2'), 137.9 (C-3'), 143.4 (C-11), 165.1 (C-10), 167.7 (C-1'), 191.6 (C-8).

S-Isopetasin (**2**) ESI-MS (*m/z*): 335 $[M+H]^+$. ^{1}H-NMR (500MHz, $CDCl_3$) δ: 0.99 (3H, *d*, *J* =6.7Hz, H-14), 1.03 (3H, *s*, H-15), 1.49 (1H, *qd*, *J* =1.4, 14.3 Hz, H-2a), 1.66 (1H, *m*, H-4), 1.85 (1H, *s*, H-13), 2.09 (3H, *d*, *J*=1.04Hz, H-12), 2.18 (1H, br*d*, *J* =13.7 Hz, H-6a), 2.22 (1H, *m*, H-2b), 2.34 (1H, *td*, *J* =4.0, 15.0Hz, H-1a), 2.41 (3H, *s*, S-CH_3), 2.45 (1H, *td*, *J*=3.8, 14.5 Hz, H-1b), 2.92 (1H, *m*, H-6b), 4.93 (1H, *td*, *J* =4.3, 12.7Hz, H-3), 5.84 (1H, *d*, *J* =10.1Hz, H-2'), 5.77 (1H, *s*, H-9), 7.08 (3H, *d*, *J* =10.1Hz, H-3'). ^{13}C-NMR (126MHz, $CDCl_3$) δ: 10.7 (C-14), 17.1 (C-15), 19.3 (S-CH_3), 22.1 (C-13), 22.6 (C-12), 30.1 (C-1), 31.8 (C-2), 41.2 (C-6), 42.2 (C-5), 46.2 (C-4), 73.3 (C-3), 113.0 (C-2'), 126.7 (C-9), 127.2 (C-7), 143.3 (C-11), 152.4 (C-3'), 165.2 (C-10), 166.3 (C-1'), 191.6 (C-8).

S-Petasin (**3**) ESI-MS (*m/z*): 335 $[M+H]^+$. ^{1}H-NMR (500MHz, $CDCl_3$) δ: 0.96 (3H, *d*, *J* =6.8Hz, H-15), 1.23 (3H, *s*, H-14), 1.50 (1H, *m*, H-2a), 1.62 (1H, *m*, H-4), 1.74 (3H, *s*, H-13), 1.90 (1H, *td*, *J* =13.9Hz, H-6a), 2.03 (1H, *dd*, *J* =4.4, 13.1Hz, H-6b), 2.24 (1H, *m*, H-2b), 2.36 (1H,

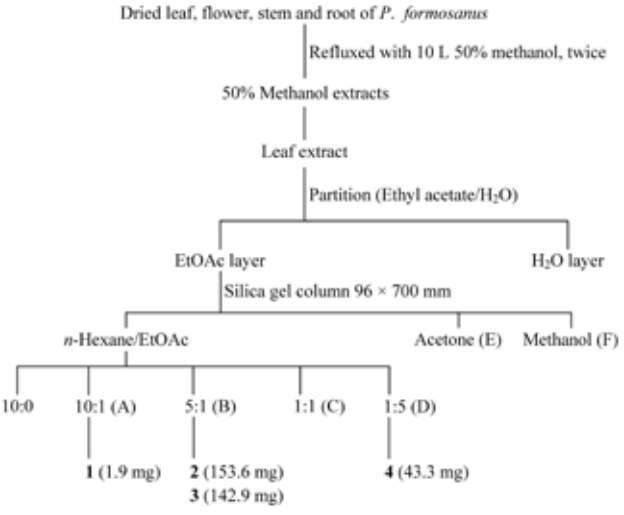

Figure 1. Fractionation and isolation procedures from the dried leaves of *P. formosanus*.

m, H-1a), 2.41 (3H, *s*, S-CH_3), 2.52 (1H, *m*, H-1b), 3.11 (1H, *dd*, *J* =4.5, 14.4Hz, H-7), 4.82 (1H, *s*, H-12a), 4.95 (1H, *dd*, *J* =4.4, 15.5, H-3), 4.98 (1H, *d*, *J* =1.5 Hz, H-12b), 5.78 (1H, *d*, *J* =1.5Hz, H-9), 5.83 (1H, *d*, *J* =10.1Hz, H- 2'), 7.09 (1H, *d*, *J* =10.1Hz, H-3'). ^{13}C-NMR (126MHz, $CDCl_3$) δ: 10.4 (C-15), 17.1 (C-14), 19.3 (S-CH_3), 20.0 (C-13), 30.6 (C-1), 31.7 (C-2), 40.0 (C-5), 41.7 (C-6), 47.3 (C-4), 50.3 (C-7), 73.0 (C-3), 112.9 (C-2'), 114.4 (C-12), 124.6 (C-9), 143.3 (C-11), 152.5 (C-3'), 166.3 (C-1'), 166.8 (C-10), 198.5 (C-8).

Caffeic acid methyl ester (**4**) ESI-MS (*m/z*): 195 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.74 (3H, *s*, H-10), 6.25 (1H, *d*, *J* =16.0Hz, H-8), 6.77 (1H, *d*, *J* =8.2Hz, H-3), 6.93 (1H, *dd*, *J* =16.0, 8.2 Hz, H-2), 7.02 (1H, *d*, *J* =1.8Hz, H-6), 7.53 (1H, *d*, *J* =16.0Hz, H-7). ^{13}C-NMR (126MHz, CD_3OD) δ: 52.0 (C-10), 114.8 (C-8), 115.1 (C-2), 116.5 (C-5), 122.9 (C-6), 127.7 (C-1), 146.8 (C-3), 146.9 (C-7), 149.6 (C-4), 169.8 (C-9).

Animals

Adult male Wistar rats weighing about 250 ± 10 g and mice weighing about 25 ± 2 g were purchased from the BioLASCO Taiwan Co., Ltd. and kept on a 12:12-h, day-night cycle. Animals were maintained in polycarbonate cages at 21 ± 2°C and provided food and water ad libitum. All experimental procedures involving animals followed the ethical regulations of Taipei Medical University.

Acetic acid-induced writhing test in mice

Acetic acid (0.6%, 0.1 ml) was injected into the peritoneal cavity of a mouse. Oral treatment with the vehicle, indomethacin (100 mg/kg), or *P. formosanus* (100~400 mg/kg) was given 1 h prior to an acetic acid injection (6 mice per group). The total number of writhes that occurred within 30 min were observed and counted after the animal was placed in a plastic cage. The writhing response consisted of a contraction of the abdominal muscles together with a stretching of the limbs. The anti-nociceptive activity was expressed as a writhing number over a period of 30 min (Ojewole, 2005).

Hot-plate latent pain response test in rats

For thermal hyperalgesia, rats were individually placed in a hot plate instrument (Ugo Basile, Comerio, VA, Italy). Oral treatment with the vehicle, tramadol (10 mg/kg), or *P. formosanus* (100, 200, or 400 mg/kg) was given 1 or 2 h prior to the hot plate test (3 rats per group). A radiant heat source was applied underneath the glass floor. The time between placement of the rats on the platform and licking of the paws was recorded as the hot-plate latency (Marrassini et al., 2010).

Carrageenan-induced paw edema in mice

Edema in the left hind paw of mice was induced by an injection of 50 µl of 1% (w/v) carrageenan from Sigma (St. Louis, MO) in saline into the subplantar region. *P. formosanus* at different doses (100~400 mg/kg) and indomethacin (100 mg/kg, as a reference substance) were given orally 1 h before the injection. The control group was given the vehicle (0.1 ml/10 g). Another group of mice received a subplantar injection of 0.9% saline and vehicle, and was designated the blank group. Each group consisted of six animals. The paw volume of the animals was measured 1 h before and 1~6 h after the injection using a plyethysometer (Ugo Basile, Comerio, VA, Italy) (Tseng et al., 2006).

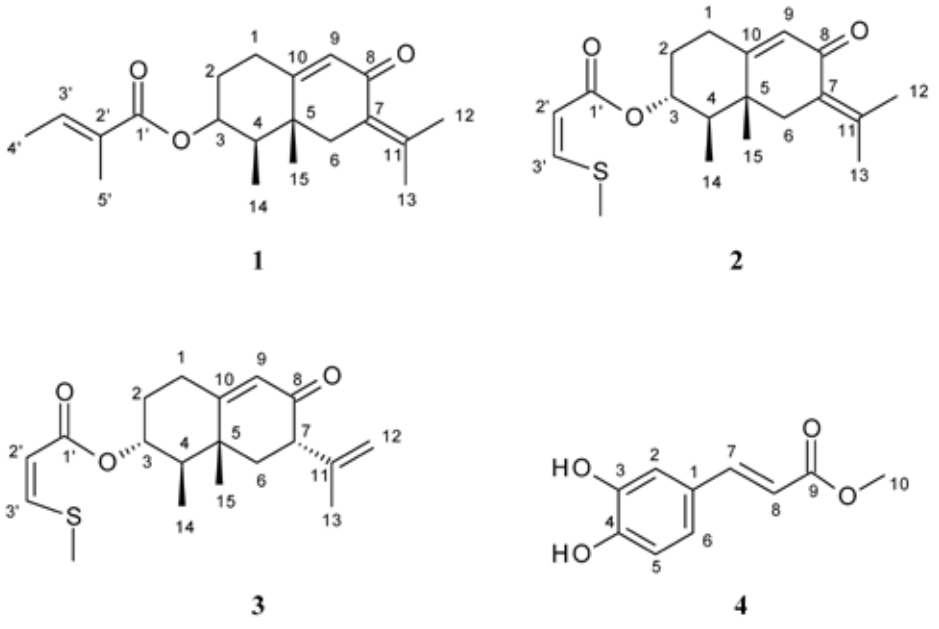

Figure 2. The chemical structures of isopetasin (1), S-isopetasin (2), S-petasin (3), and caffeic acid methyl ester (4) isolated from the dried leaves of *P. formosanus*.

In vitro anti-inflammatory assay

The anti-inflammatory activity was presented in terms of inhibited production of nitric oxide (NO), inducible NO synthase (iNOS), and inducible cyclooxygenase (COX)-2 by LPS-stimulated RAW 264.7 cells. NO was measured as nitrite production in the medium after 24 h of incubation with or without the extracts and/or LPS (500 ng/ml). Briefly, nitrate in the medium was converted to nitrite and measured spectrophotometrically after the Griess reaction (Tseng et al., 2006). Expressions of iNOS and COX-2 by LPS-stimulated RAW 264.7 cells were investigated by a Western blot analysis. RAW 264.7 cells exposed to extracts for 24 h were collected into tubes and then washed with phosphate-buffered saline (PBS). Protein samples were prepared according to our previous paper (Tseng et al., 2006). Total protein (25 μg) was used for the Western blot analysis. Proteins were transferred to nitrocellulose membranes. Membranes were probed using antibodies specific to COX-2, iNOS, and GAPDH and visualized using a BCIP/NBT kit from Gibco BRL (Grand Island, NY, USA) according to the manufacturer's instructions.

Data analysis

Each experiment was performed at least in triplicate. Results are expressed as Mean ± SD. One way analysis of variance (ANOVA, SPSS 12.0) was used to analyze the data of the animal model. Results were considered statistically significant at $p < 0.05$.

RESULTS

Antinociceptive and anti-inflammatory activities of extracts from *P. formosanus*

After being dried, the different parts (flowers, leaves, stems, and roots) of *P. formosanus* were separately extracted with 50% methanol, and their anti-inflammatory activities were evaluated using LPS-induced RAW 264.7 cells. Aqueous methanolic leaf extract of *P. formosanus* (leaf extract) showed the most potent inhibitory activity against NO production (Table 1). Therefore, the anti-inflammatory and analgesic activities of the Leaves-MeOH extract were further investigated.

Oral administration of the Leaves-MeOH extracts (200 or 400 mg/kg) to rats 1 or 2 h prior to a hot plate test caused a significant delay in the production of the first foot-licking episode induced by radiant heat (Table 2). The Leaves-MeOH extract dose-dependently and significantly inhibited iNOS expression in LPS-induced RAW 264.7 cells (Figure 3A). The *in vivo* anti-inflammatory effect of the Leaves-MeOH extract was then examined with a carrageenan-induced acute inflammatory model in ICR mice. Oral administration of the Leaves-MeOH extract 1 h before the injection exerted a significant dose-dependent inhibitory effect on the development of paw swelling (Figure 3B). Oral administration of the Leaves-MeOH extracts (100, 200, or 400 mg/kg) to mice 1 h before the injection significantly reduced the number of writhing episodes in-

Table 1. Effects of the 50 % methanolic extracts of different amounts of *P. formosanus* parts on NO production and cell viability of LPS-treated RAW 264.7 cells.

50% MeOH extract of:	Inhibited NO effects		Cytotoxicity index (%)[a]
	Inhibition (%)[a]	IC_{50} (μg/mL)	
Leaves	70.02 ± 3.77	22.85	4.09 ± 3.75
Flowers	33.61 ± 5.12	-	0.51 ± 0.74
Stems	3.09 ± 2.72	-	1.81 ± 2.16
Roots	10.31 ± 0.93	-	0.00 ± 0.00

[a]The concentration of tested sample was 200 μg/mL. There were three independent experiments. Results are expressed as Mean±SD.

Table 2. Inhibitory effect of Leaves-MeOH extract on heat-induced foot licking episodes in rats.

	Time (h)		
	0	1	2
Control	7.54 ± 0.42	7.74 ± 0.99	7.32 ± 0.79
Tramal (10 mg/kg)	7.94 ± 0.87	10.29 ± 0.32*	9.39 ± 0.30*
Leaves-MeOH extract (mg/kg)			
100	7.41 ± 0.42	9.30 ± 0.58	8.52 ± 0.91
200	7.39 ± 0.79	10.50 ± 0.33*	10.44 ± 0.77*
400	8.52 ± 0.53	10.75 ± 1.62*	10.58 ± 0.94*

Leaves-MeOH extract was aqueous methanolic leaf extract of *P. formosanus*. Results are expressed as Mean±SD for the foot licking time (second)
*Compared with control group, $p < 0.05$.

Table 3. IC_{50} values of different fractions and natural compounds obtained from the aqueous methanolic leaf extract of *P. formosanus* on NO production in LPS-induced RAW 264.7 cells.

Fraction	IC_{50} (μg/mL)
EtOAc layer	12.68
H_2O layer	> 100
A	10.07
B	9.10
C	11.14
D	12.80
E	38.90
F	34.34
Natural compounds	μg/mL (μM)
S-isopetasin	1.46 (4.24)
S-petasin	3.54 (10.60)
Caffeic acid methyl ester	8.08 (41.43)

There were three independent experiments.

duced by acetic acid compared to the control group (Figure 3C). In according the antinociceptive and anti-inflammatory screening test, we suggested the Leaves-MeOH extracts contains bioactive components.

Bioactive sesquiterpenes isolated from leaves of *P. formosanus*

The Leaves-MeOH extract of *P. formosanus* was partitioned with EtOAc and distilled H_2O and chromatographed as described. Among these fractions, *n*-hexane, *n*-hexane-EtOAc (10: 1, 5: 1, and 1: 1), acetone, and methanol elutes were further characterized. The inhibitory effect of each fraction (A~F) on NO production in LPS-induced RAW 264.7 cells was evaluated with IC_{50} values shown in Table 3. The NO inhibitory effect of the EtOAc layer was stronger than that of the H_2O layer, and the A to D fractions isolated from the EtOAc layer also showed strong efficacy. Further isolation of fractions A, B, and D yielded 1.9 mg isopetasin (**1**) from fraction A (yield, 0.0002%); 153.6 mg of S-isopetasin (**2**) and 142.9 mg of S-petasin (**3**) from fraction B (yields, 0.0162% and 0.0150%, respectively); and 43.3 mg of caffeic acid methyl ester (**4**) from fraction D (yield, 0.0046%). However, the yield from fraction C was very little, and the amount of isopetasin also was insufficient for a bio-analysis.

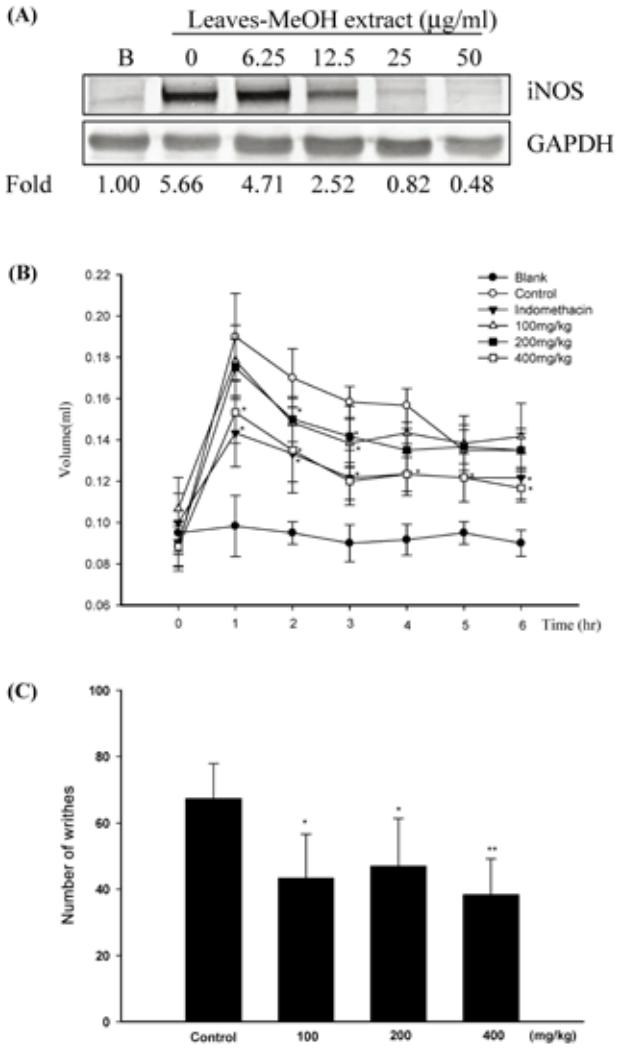

Figure 3. Inhibitory effects of aqueous methanolic leaf extract of *P. formosanus* (Leaves-MeOH extract). (A) Analysis iNOS expression in LPS-induced RAW 264.7 cells, there were three independent experiments; (B) The assay was carrageenan-induced paw edema in mice and per one group were 6 mice; (C) The assay was acetic-acid induce writhing response in mice and per one group were 6 mice. Results are expressed as Mean±SD. *$p < 0.05$, **$p < 0.005$.

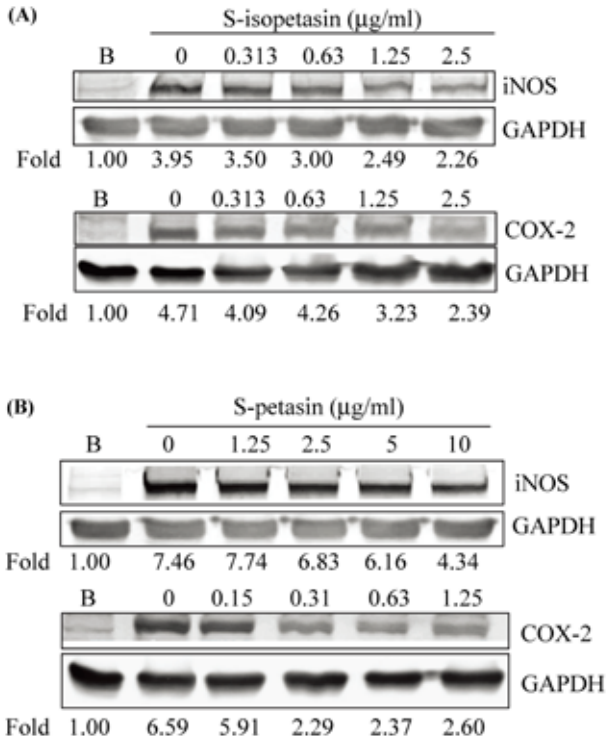

Figure 4. Dose-dependent inhibitory effects of S-isopetasin and S-petasin on iNOS and COX-2 production by LPS-induced RAW 264.7 cells. There were three independent experiments.

Antinociceptive and anti-inflammatory activities of S-isopetasin and S-petasin

The inhibitory effects of S-isopetasin, S-petasin, and caffeic acid methyl against NO were measured in LPS-induced RAW 264.7 cells. S-isopetasin and S-petasin showed the most potent NO inhibitory effect, with respective IC_{50} values of 1.46 and 3.54 μg/mL (Table 3). Both compounds also significantly inhibited iNOS and COX-2 expressions in LPS-induced RAW 264.7 cells (Figure 4), and S-ispopetasin was stronger than S-petasin in a dose-dependent manner. Both compounds also significantly reduced the number of writhing episodes induced by acetic acid compared to the control (Figure 5). However, S-petasin compared to S-ispopetasin at 1.25 mg/kg that S-ispopetasin was a significant anti-nociceptive effect, but not S-petasin (Figure 5). Moreover, only S-isopetasin

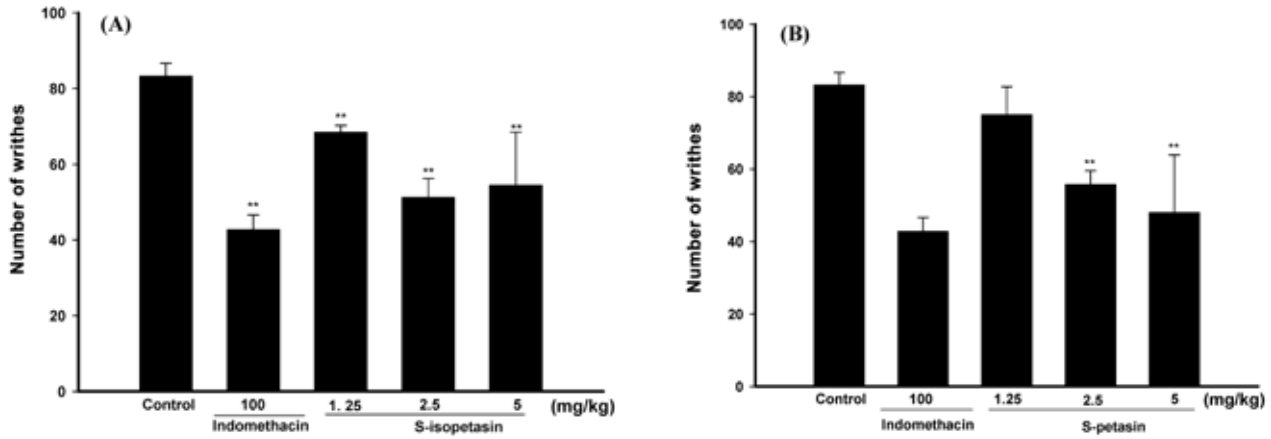

Figure 5. Inhibitory effects of S-isopetasin and S-petasin on the acetic acid-induced abdominal writhing test in mice. Each group was 6 mice. Results are expressed as Mean±SD. *$p < 0.05$, **$p < 0.005$.

exerted significant inhibitory activity on carrageenan-induced paw edema in mice (Figure 6). These results suggest that s-isopetasin is the major anti-inflammatory and anti-nociceptive constituent of leaves of *P. formosanus*.

DISCUSSION

The results of our study suggest that the foliar parts of *P. formosanus* have both anti-inflammatory and anti-nociceptive activities, with S-petasin and S-isopetasin being the active compounds for the above-described biological activities. Our data also suggest that both compounds modulate inflammation and nociception at least through their capability to inhibit iNOS and COX-2 expressions. iNOS and COX-2 are well-known mediators of inflammatory responses. In addition, an inhibitor of NOS or a natural compound that can modulate NO was shown to affect the nociceptive response to chemically induced nociception as well (Moore et al., 1991; Perimal et al., 2011). S-Petasin and S-isopetasin exert a calcium channel-blocking action in vascular smooth muscle cells (Wang et al., 2001, 2004). S-Petasin also inhibits calcium channel excitation in neurons (Wu et al., 2003). Blocking calcium channel excitation has the potential to play a valuable role in diseases that require modulation of muscle contractions or neuron excitation, such as hypertension, arrhythmia, and chronic pain. In fact, clinical studies showed the ability of calcium channel blockers to lower pain scores when a noxious pressure was applied to a patient's sternum (Del Giaccio and Eblen-Zajjur, 2010). The calcium channel-blocking activity of S-petasin may also contribute to its analgesic effect.

Studying traditional folk medicine is useful for discovering new natural products with potential medicinal properties. In the last century, natural products played a significant role in drug discovery and development processes (Newman and Cragg, 2007). The results of previous research provided scientific evidence and explained the mechanism of the action of *P. formosanus* as a remedy for

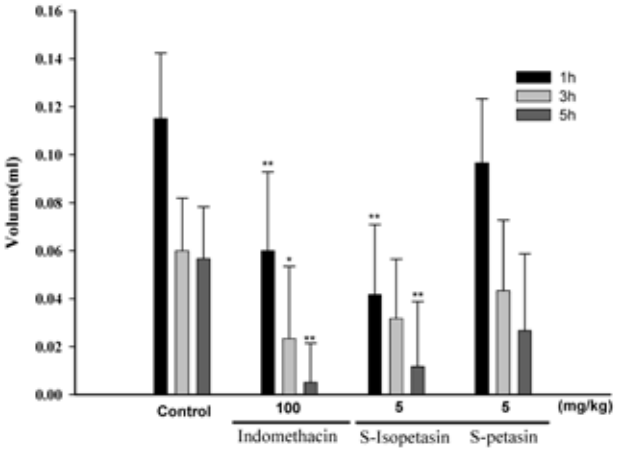

Figure 6. Inhibitory effects of S-isopetasin and S-petasin on carrageenan-induced paw swelling in mice. Each group was 6 mice. Results are expressed as Mean±SD. *$p < 0.05$, **$p < 0.005$.

asthma and hypertension. This study is the first to demonstrate potent anti-inflammatory and anti-nociceptive activities of the methanolic extract of *P. formosanus* leaves. Also, for the first time, the two major sesquiterpene constituents of *P. formosanus* leaves, S-isopetasin and S-petasin, were shown to possess strong anti-inflammatory and anti-nociceptive abilities. Taken together, the results suggest an interesting therapeutic potential for this species in pain and inflammatory diseases. The results also suggest that S-isopetasin is the major constituent mediating the anti-inflammatory and analgesic activities of *P. formosanus* and may be used as a lead compound for new anti-inflammatory drug development.

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臺灣款冬之抗發炎與止痛主成分

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臺灣款冬（菊科）是一種臺灣原生種的植物，且為臺灣民間用來治療高血壓與氣喘的草藥。臺灣款冬的葉子以 50% 甲醇萃取（葉萃取物）顯示具有極強抑制脂多醣誘導 RAW 264.7 巨噬細胞引起的一氧化氮釋放作用，其 50% 的抑制濃度為 22.85 μg/mL。繼而利用體內試驗檢測其功效，結果顯示管餵老鼠 200 mg/kg 萃取物可以有意義的降低醋酸誘導的扭體反應、熱板引起的疼痛刺激及鹿角菜膠誘導的足掌浮腫。因此利用管柱層析配合生物活性分析追蹤分離臺灣款冬葉萃取物中的抗發炎活性成分，結果得到 4 個化合物，分別是 isopetasin、S-isopetasin、S-petasin 及 caffeic acid methyl ester。其中，S-isopetasin 和 S-petasin 於抑制脂多醣誘導 RAW 264.7 巨噬細胞試驗中具較強的抑制一氧化氮釋放、誘導型一氧化氮合成酶及環氧化酶作用，且顯示劑量依存性，而 S-isopetasin 的作用又比 S-petasin 強。綜合而言，臺灣款冬具有抗發炎及止痛作用，其主要的活性成分為 S-isopetasin，且其葉部具發展成抗發炎先驅藥之潛力。

關鍵詞：臺灣款冬；菊科；S-isopetasin；S-petasin；止痛；抗發炎作用。