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Antinociceptive and anti-inflammatory activities of *Schefflera octophylla* extracts

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ABSTRACT

Ethnopharmacological relevance: *Schefflera octophylla* (Lour.) Harms, a traditional Chinese herb mainly distributed in Southeast Asia, is extensively prescribed to alleviate pain and treat rheumatoid arthritis (RA), influenza, throat swelling, pain, etc. In this paper, the antinociceptive and anti-inflammatory activities of the ethanol extract and its five different polar fractions of this plant were evaluated. Furthermore, the anti-rheumatoid arthritis activity of the ethanol extract and its active fraction ($CHCl_3$ fraction) were evaluated. And the chemical constituents of the $CHCl_3$ active fraction displayed significant antinociceptive and anti-inflammatory activities were investigated.

Materials and methods: Antinociceptive and anti-inflammatory activities were investigated by hot plate test, acetic acid-induced abdominal writhing test and formalin test, xylene-induced ear edema test. The anti-rheumatoid arthritis activity was evaluated through the model of adjuvant-induced arthritis (AA) in rats, paw swelling, pain response, arthritis index and histopathological changes of ankle, the levels of TNF-α, IL-1β, IL-6 and rheumatoid factor (RF) of rats were detected. The chemical constituents of the $CHCl_3$ fraction were isolated using chromatographic techniques. Their structures were elucidated by spectroscopic data analysis.

Results: The results showed that the ethanol extract of *S. octophylla* has significant dose-dependent anti-inflammatory and antinociceptive activities. And its five different polar fractions especially the $CHCl_3$ fraction significantly inhibited the abdominal writhing induced by acetic acid and ear edema induced by xylene, also increased pain threshold in hot plate test in 120 min and reduced ticking times in formalin test. The ethanol extract of *S. octophylla* and the $CHCl_3$ fraction demonstrated an anti-RA effect in a dose-dependent manner. The levels of TNF-α, IL-1β and IL-6 in ethanol extract (600 mg/kg) and $CHCl_3$ fraction (300 mg/kg) groups were significantly lower than those of the model group. The chemical constituents study of the $CHCl_3$ fraction from *S. octophylla* led to six triterpenoids which were identified as taraxerone (**1**), 3-epi-taraxerol (**2**), aleuritolic acid (**3**), 3-oxofriedelan-28-oic acid (**4**), 3β,19α -dihydroxy-urs-12-ene-24,28-dioic acid (**5**) and asiatic acid (**6**). Compounds **1**–**5** were obtained from this plant for the first time.

Conclusion: This study proved the antinociceptive, anti-inflammatory and anti-rheumatoid arthritis activities of *S. octophylla*. Triterpenoids obtained from its $CHCl_3$ fraction may be responsible for those activities. These results could support the fact that *S. octophylla* is used traditionally to cure inflammatory and pain diseases.

1. Introduction

Arthritis is one of the most common chronic diseases in the world. Among all kinds of arthritis, rheumatoid arthritis and osteoarthritis are the two most common types of arthritis. According to reports in the literature (Li et al., 2014), rheumatoid arthritis affects around 0.5–1% of adults in the developed world with between 5 and 50 per 100,000 people newly developing the condition each year, while osteoarthritis affects nearly 3.6% of the global population and nearly 27 million people in the United States. The symptoms of arthritis are very diverse, mainly shown as red, swollen, and dysfunctional joints, with feeling of heat and pain. Thus the conventional medication treatments for arthritis, especially in acute period, are analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). However, those drugs have undesirable side effects on gastrointestinal, kidney, respiratory and cardiovascular system (Wallace and Vong, 2008). As a result, researchers are looking for safer, more effective alternatives to both the traditional analgesics and NSAIDs (Walker-Bone, 2003). Furthermore, inflammation is typically a fundamental and important pathological process in many diseases. Recent research focused on the

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antinociceptive and anti-inflammatory activities of many medicinal plants-derived natural products such as flavonoids, steroids, polyohenols, terpenes, and stearic acid. Those natural compounds have demonstrated a wide range of pharmacological efficacy but lesser side effects (Shah and Alagawadi, 2011;Shukla et al., 2010).

The genus *Schefflera* (Araliaceae) comprises of 200 species distributed in the tropical and some subtropical areas in the world. There are 38 species and 2 varieties in China. *Schefflera octophylla* (Lour.) Harms is distributed mainly in Guangxi, Guangdong and Yunnan provinces in China. It is widely used as a folk medicine for the rheumatic pain, traumatic pain, sore throat, etc. (Editorial Committee of Chinese Materia Medica, 1999). Previous phytochemical investigations have revealed that triterpenoids were the main components. More than 40 triterpenoids were isolated from this plant, including oleanane type (Sung et al., 1991b; Maeda et al., 1994), ursane type (Maeda et al., 1994; Sung et al., 1992) and lupane type (Sung et al., 1991a, 1991b; Kitajima et al., 1990; Adam et al., 1982; Lischewski et al., 1984; Kitajima and Tanaka, 1989; Sung and Adam, 1992), etc. However, in the pharmacological aspects, only anti-virus and antioxidant effects of the extract from *S. octophylla* have been reported previously (Li and Ooi Vincent, 2004; Li et al., 2004; Zheng et al., 2009). In Southeast Asia, *S. octophylla* is extensively prescribed to alleviate pain and treat rheumatoid arthritis (RA).Considering the traditional uses in alleviating pains of this herb, we firstly evaluated the antinociceptive and anti-inflammatory activities of ethanol extract of *S. octophylla* and its five different polar fractions through three antinociceptive tests and one anti-inflammatory test. For further investigation, we evaluated the anti-rheumatoid arthritis activity through the model of adjuvant-induced arthritis (AA) in rats, arthritis index, paw swelling, pain response and histopathological changes of ankle, the levels of TNF-α, IL-1β, IL-6 and rheumatoid factor (RF) of rats were detected. In order to clarify the main active components of *S. octophylla*, we also investigated the chemical constituents of the $CHCl_3$ fraction which displayed significant antinociceptive and anti-inflammatory activities.

2. Materials and methods

2.1. General

Column chromatography: silica gel (100–200 and 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (Amersham Biosciences; Sweden). TLC: precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). NMR Spectra: Bruker AVANCE III 500 spectrometer with Me_4Si as internal standard. ESI-TOF-MS: Acquity UPLC-Q-TOF Micro instrument; in m/z.

2.2. Plant material

The root bark of *S. octophylla* was collected in April 2010 from Guangzhou, Guangdong province, PR China. The specimen was identified by Associate Professor Jizhu Liu, School of Traditional Chinese Medicine, Guangdong Pharmaceutical University. A voucher specimen has been deposited at the lab 435 of School of Traditional Chinese Medicine, Guangdong Pharmaceutical University. (Accession number: TCMS002).

2.3. Extraction

The air-dried and powdered plant materials of *S. octophylla* (23 kg) were refluxed with 95% ethanol (300 L) for 3 times (2 h per time). After evaporation of the alcohol under reduced pressure, 920 g of viscous residue was obtained with a yield of 4.0%. The ethanol extract (730 g) was suspended in H_2O, and extracted successively with petroleum ether, chloroform ($CHCl_3$), ethyl acetate (EtOAc) and n-butyl alcohol (n-BuOH) for three times. The volume for the above extracted organic solvent was 2 L each time. Each partition was evaporated to dryness and resulting fractions of petroleum ether, $CHCl_3$, EtOAc and water. These fractions and the ethanol extract of *S. octophylla* (EES) were evaluated for activity tests.

2.4. Isolation of the $CHCl_3$ fraction

The $CHCl_3$ fraction (365 g) was separated by silica gel chromatography (100–200 mesh, petroleum ether–EtOAc 100:0–7:3 and $CHCl_3$–MeOH 9:1–7:3) to yield four fractions (F1-4). F1 was differentiated with silica gel chromatography again(200–300 mesh) using gradient of petroleum ether–EtOAc (97:3–8:2) to afford compound **1** (70 mg). F2 was separated by silica gel chromatography (200–300 mesh, petroleum ether–EtOAc 95:5–7:3) to yield three sub-fractions (F2a-2c). F2b was purified by Sephadex LH-20 chromatography using MeOH to yield compound **2** (10 mg), **3** (8 mg) and **4** (10 mg). F4 was run with silica gel chromatography again (200–300 mesh, $CHCl_3$–MeOH 9:1–7:3) to generate compound **5** (3 g) and **6** (12 mg).

2.5. Structure identification of compound ***1–6***

The structures of compound **1–6** were identified by comparing the ^1H NMR and ^{13}C NMR spectral data with previous literature reports.

2.6. Biological activity assays

2.6.1. Drugs and reagents

Acetic acid was purchased from Guangdong Chemical Reagent Factory (China). Formaldehyde was purchased from Luoyang Chemical Reagent Factory (China). Xylene was purchased from Guangdong Guanghua Chemical Reagent Co., Ltd. (China). Indometacin was purchased from Southern China Pharmaceutical Group Co., Ltd. (China). Rotundine was purchased from Sichuan Kangfulai Pharmaceutical Group Co., Ltd. (China); Complete Freund's adjuvants was purchased from Sigma (St. Louis, MO, USA). Leflunomide was purchased from Suzhou Changzheng Xinkai Pharmaceutical Co., Ltd. (China). CMC-Na was purchased from Guangdong Device of Medical Equipment Co., Ltd. (China).

2.6.2. Experimental animals

Male or female NIH mice (18–22 g) and male Sprague–Dawley rats(150–180 g) were obtained from the Medical Experimental Animal Center of Guangdong Province (China). They were kept in plastic cages at 22 ± 2 °C with free access to pellet food and water. The experiment procedures were conducted in compliance with the National Institutes of Health Guide for care and use of the laboratory animals, and were approved by the Committee of Experimental Animal Administration of the university (Document No. SCXK2008-0002). All experimental protocols were approved by the Institutional Animal Ethics Committee.

2.6.3. Hot plate test

Experiments were carried out according to the previously described method (Shinde et al., 1999). During test of antinociceptive and anti-inflammatory activities in mice, EES was administrated to the animals at doses of 300, 600 and 1200 mg(extract weight)/kg(body weight) that equivalent to 7.5, 15, 30 g(herb weight)/kg(body weight) in herb dose. And petroleum ether, $CHCl_3$, EtOAc, n-BuOH, water fractions were administrated at doses of 6 and 600, 32, 55, 100 mg/kg respectively (each dose was equivalent to 30 g/kg in herb dose). The control group received only vehicle (0.5% CMC-Na, 20 ml/kg), and the reference