Available online at www.sciencedirect.com
ScienceDirect
Journal of Ethnopharmacology 116 (2008) 138–143
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of *Sedum sarmentosum* extract

Hyun-Joo Jung [a,1], Hyun-Jung Kang [b,1], Yun Seon Song [a], Eun-Hee Park [a], Young-Myeong Kim [c,d], Chang-Jin Lim [b,*]

[a] *College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Republic of Korea*
[b] *Division of Life Sciences and Research Institute of Life Sciences, Kangwon National University, Chuncheon 200-701, Republic of Korea*
[c] *Department of Molecular and Cellular Biochemistry, School of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea*
[d] *Vascular System Research Center, Kangwon National University, Chuncheon 200-701, Republic of Korea*

Received 1 August 2007; received in revised form 23 October 2007; accepted 14 November 2007
Available online 21 November 2007

Abstract

This study aimed to assess some novel pharmacological activities of *Sedum sarmentosum* Bunge, a perennial herb widely distributed on the mountain slopes in Oriental countries and traditionally used for the treatment of certain inflammatory diseases. *Sedum sarmentosum* was extracted with absolute methanol to generate the methanol extract (SS). SS exhibited a significant inhibitory activity in the chick embryo chorioallantoic membrane (CAM) angiogenesis in a dose-dependent manner (IC_{50} = 2.29 μg/egg). The anti-nociceptive activity of SS was demonstrated using acetic acid-induced writhing model in mice. SS reduced the levels of anti-inflammatory markers, such as volume of exudates, number of polymorphonuclear leukocytes and nitrite content, in the air pouch model. It dose-dependently exhibited an inhibitory activity in the acetic acid-induced vascular permeability in mice. It suppressed production of nitric oxide in the lipopolysaccharide (LPS)-activated RAW264.7 macrophages. Additionally, it suppressed induction of inducible nitric oxide synthase (iNOS) in the activated macrophages. In brief, the results provide some pharmacological basis for the therapeutic use of *Sedum sarmentosum*.

Keywords: Anti-nociceptive; Anti-angiogenic; Anti-inflammatory; Inducible nitric oxide synthase; Nitric oxide; *Sedum sarmentosum* Bunge

1. Introduction

Among the genus Sedum (Crassulaceae), *Sedum sarmentosum* Bunge has been frequently used for the treatment of chronic inflammatory diseases in Oriental countries, such as Korea and China (Wu, 1996; Kang et al., 2000). A few research reports were documented on pharmacological activities of *Sedum sarmentosum*. The crude alkaloid fraction of *Sedum sarmentosum* caused a dose-dependent inhibition of cell proliferation of murine and human hepatoma cell lines without necrosis or apoptosis, which were associated with an increase in the number of cells in the G1 phase of cell cycle (Kang et al., 2000). The ethyl acetate extract of *Sedum sarmentosum* was found to exhibit distinct angiotensin converting enzyme (ACE) inhibitory activity, and a few flavonoids were identified to be responsible for the ACE inhibitory activity (Oh et al., 2004). Since *Sedum sarmentosum* also contained the estrogenic activities, it was suggested to improve the quality of life in menopausal women (Kim et al., 2004). Recently, six new megastigmane glycoside, sedumosides E_1, E_2, E_3, F_1, F_2 and G, were puri-

Abbreviations: CAM, chorioallantoic membrane; COX-2, cyclooxygenase-2; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NO, nitric oxide; ROS, reactive oxygen species.

* Corresponding author at: Division of Life Sciences, College of Natural Sciences, Kangwon National University, 192-1 Hyoja-2-dong, Chuncheon 200-701, Republic of Korea. Tel.: +82 33 250 8514; fax: +82 33 242 0459.
E-mail address: cjlim@kangwon.ac.kr (C.-J. Lim).
[1] These authors equally contributed to this work.

0378-8741/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2007.11.014

fied from the whole plant of *Sedum sarmentosum* (Morikawa et al., 2007). In this article, it is demonstrated that *Sedum sarmentosum* contains anti-inflammatory and related activities.

2. Materials and methods

2.1. Chemicals

Evans blue, sodium dodecyl sulfate (SDS), leupeptin, pepstatin, phenylmethanosulfonyl fluoride (PMSF), indomethacin, dexamethasone, retinoic acid, *Escherichia coli* lipopolysaccharide (LPS), HEPES, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), and Griess reagent were purchased from Sigma Chemical Co. (St. Louis, MI, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin and trypsin-EDTA were from Gibco-BRL (Gaithersburg, MD, USA). All other chemicals used were of reagent grade or better. All experiments, performed in this work, were independently repeated at least three times.

2.2. Experimental animals

Male ICR mice (about 25 g) or male Sprague–Dawley rats (130–150 g) were obtained from Samtaco Animal Farm, Osan, Korea. The animal room was maintained at 23 ± 2 °C with a 12-h light/dark cycle. Food and tap water were supplied *ad libitum*. The ethical guidelines, described in the NIH Guide for Care and Use of Laboratory Animals, were followed throughout the experiments. Fertilized brown Leghorn eggs were obtained from Pulmuone Food Co., Seoul, Korea.

2.3. Cell culture

The RAW264.7 cells, a murine macrophage cell line, were obtained from American Type Culture Collection (Manassas, VA, USA). The mammalian cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES (pH 7.5), 100 U/ml penicillin and 100 μg/ml streptomycin. The RAW264.7 cells were plated at a density of 1×10^6 and preincubated for 24 h at 37 °C, and maintained in a humidified atmosphere containing 5% CO_2. For all experiments, the cells were grown to 80–90% confluence, and subjected to no more than 20 cell passages.

2.4. Plant material

The fresh aerial parts of *Sedum sarmentosum* were purchased at a local market, Seoul, Korea in March 2006, and authenticated by Prof. Ki-Oug Yoo, Division of Life Sciences, Kangwon National University, Chuncheon, Korea. The voucher specimen of the plant material was deposited in the herbarium of the Division of Life Sciences, College of Natural Sciences, Kangwon National University under the acquisition number KWNU56519.

2.5. Preparation of methanol extract (SS)

The whole plants were ground under liquid nitrogen and extracted for 1 week with absolute methanol at room temperature. The methanol extract (SS) was evaporated *in vacuo*. The yield was measured to be 1.7%. For animal experiments, SS was dissolved in 2% Tween 80 in saline, while, for other experiments, it was dissolved in absolute methanol.

2.6. Chorioallantoic membrane (CAM) assay

Anti-angiogenic activity was measured using CAM assay as previously described (Song et al., 2003). The fertilized chicken eggs used in this work were kept in a humidified egg incubator at 37 °C. After 3.5-day incubation, about 2 ml of albumin was aspirated from the eggs through the small hole drilled at the narrow end of the eggs, allowing the small chorioallantoic membrane and yolk sac to drop away from the shell membrane. The shell covering the air sac was punched out and removed by forceps, and the shell membrane on the floor of the air sac was peeled away. In the 4.5-day-old chick embryo, a SS-loaded Thermanox® coverslip was applied to the CAM surface. Two days after returning the chick embryo to the incubator, an appropriate volume of a 10% fat emulsion (Intralipose, 10%) was injected into a 6.5-day-old embryo chorioallantois. The eggs were then evaluated using a score system under a microscope.

2.7. Acetic acid-induced vascular permeability

According to a modification of the method of Whittle (1964), acetic acid-induced vascular permeability test was performed. One hour after oral administration of vehicle (2% Tween 80 in saline), SS (10, 30 or 100 mg/kg) or indomethacin (IND, 5 mg/kg) as a positive control, 0.1 ml/10 g body weight of 1% Evans blue solution was injected intravenously in each mouse. Thirty minutes later, 0.1 ml/10 g body weight of 0.7% acetic acid in saline was intraperitoneally injected. Thirty minutes after the administration of acetic acid, the mice were killed by cervical dislocation. After 10 ml of saline was injected into the peritoneal cavity, the washing solutions were collected in test tubes. Concentrations of Evans blue leaked into the peritoneal cavities were measured by the absorbance at 590 nm of the collected washing solutions. The vascular permeability was represented in terms of the absorbance (A_{590}).

2.8. Acetic acid-induced writhing response

Anti-nociceptive activity of SS was detected as previously described (Olajide et al., 2000). The response to an intraperitoneal injection of acetic acid solution, manifesting as a contraction of the abdominal muscles and stretching of hind limbs, was measured. Nociception was induced by intraperitoneal injection of 0.7% acetic acid solution at the dose of 0.1 ml/10 g body weight. Each experimental group of mice was treated orally with vehicle (2% Tween 80 in saline), SS (10, 30 or 100 mg/kg) or indomethacin (IND, 5 mg/kg) as a positive