Journal of Ethnopharmacology 155 (2014) 1353–1361

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

Inhibition of airway inflammation by the roots of *Angelica decursiva* and its constituent, columbianadin

Hun Jai Lim [a], Ju Hee Lee [a], Jae Sue Choi [b], Sang Kook Lee [c], Yeong Shik Kim [c], Hyun Pyo Kim [a,*]

[a] College of Pharmacy, Kangwon National University, Chunchon 200-701, Republic of Korea
[b] Department of Food Science and Nutrition, Pukyong National University, Busan 608-737, Republic of Korea
[c] College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form
9 June 2014
Accepted 16 July 2014
Available online 25 July 2014

Keywords:
Angelica decursiva
Columbianadin
Coumarin
Lung inflammation

ABSTRACT

Ethnopharmacological relevance: The roots of *Angelica decursiva* Fr. Et Sav (Umbelliferae) have been frequently used in traditional medicine as anti-inflammatory, antitussive, analgesic agents and expectorant, especially for treating cough, asthma, bronchitis and upper respiratory tract infections. To establish the scientific rationale for the clinical use of *Angelica decursiva* and to identify new agents for treating inflammatory lung disorders, pharmacological evaluation of the roots of *Angelica decursiva* and the isolated constituents was performed.

Methods: In vitro study was carried out using two lung cells, lung epithelial cells (A549) and alveolar macrophages (MH-S). The inflammatory markers such as IL-6 and nitric oxide (NO) for each cell line were examined. For *in vivo* study, a mouse model of lipopolysaccharide (LPS)-induced acute lung injury was used and the effects on lung inflammation were established by measuring the cell numbers in bronchoalveolar lavage fluid (BALF) and by histological observation.

Results: Water and 70% ethanol extracts of the roots of *Angelica decursiva* showed considerable inhibitory activity against LPS-induced lung inflammation in mice following oral administration at a dose of 400 mg/kg. Five coumarin derivatives including columbianadin, umbelliferone, umbelliferone 6-carboxylic acid, nodakenin and nodakenetin were isolated. Among the isolated compounds, columbianadin was found to possess strong inhibitory activity against the inflammatory response of IL-1β-treated A549 cells and LPS-treated MH-S cells. Columbianadin was found to inhibit NO production by down-regulation of inducible NO synthase. Moreover, columbianadin was also proved to possess significant inhibitory activity against LPS-induced lung inflammation following oral administration at a dose of 20–60 mg/kg.

Conclusions: The roots of *Angelica decursiva* were proved to be effective in the treatment of lung inflammation. Columbianadin can be a potential new agent for treating inflammatory lung disorders.

1. Introduction

Angelica decursiva Fr. Et Sav (Umbelliferae) is a perennial herb and it is widely distributed in China, Japan and Korea. The roots of this plant have been long used in traditional Korean medicine as antitussive, analgesic, antipyretic, and coughs remedy and in traditional Chinese medicine, it is used as a remedy for thick phlegm, asthma, and upper respiratory tract infections (Kong et al., 1996; Bae, 2000; Lee et al., 2009). The major constituents isolated from this plant are various types of coumarins. They include decursin, nodakenin, umbelliferone, scopoletin, bergapten, imperatorin, *etc.* (Hata and Sano, 1969; Avramenko et al., 1970; Chen et al., 2008).

Some pharmacological activities of this plant material and its constituents were reported previously. For instance, the ethanol extract of the roots demonstrated an anticancer activity against osteosarcoma cells, C6 rat glioma cells and human oral cancer cells *via* an apoptosis-inducing mechanism (Cho et al., 2009; Lee et al., 2009, 2010). The antioxidative and anti-inflammatory activities of this plant extract were also demonstrated (Zhao et al., 2012). Among the coumarin constituents, umbelliferone 6-carboxylic acid was shown to possess anti-inflammatory activity in RAW 264.7 cells and in carrageenan-induced mice paw edema (Islam et al., 2012; Zhao et al., 2012). Umbelliferone and nodakenetin weakly inhibited NO production in RAW 264.7 cells at a high concentration of 125 μg/ml (Zhao et al., 2012). Umbeliferone from a similar species, *Angelica pubescens*, was also reported to show *in vitro* and *in vivo* anti-inflammatory activities (Chen et al., 1995; Kim et al., 2006). Moreover, umbelliferone was found to inhibit an allergic response in

* Corresponding author. Tel.: +82 33 2506915; fax: +82 33 2557865.
E-mail address: hpkim@kangwon.ac.kr (H.P. Kim).

http://dx.doi.org/10.1016/j.jep.2014.07.033
0378-8741/

asthmatic mice by reducing the number of eosinophils and mucus production (Juliana et al., 2009). All of these previous findings indicate that *Angelica decursiva* possesses anti-inflammatory activity, and umbelliferone and umbelliferone 6-carboxylic acid can contribute to the anti-inflammatory effect of this plant.

However, the effects of the roots of *Angelica decursiva* as well as those of their coumarin derivatives on airway inflammation have not been demonstrated despite their frequent clinical use in these disorders. Hence, the present investigation was carried out for establishing the therapeutic role of the roots of *Angelica decursiva* and the coumarin derivatives in airway inflammation to support the scientific rationale for their clinical use, and for identifying the potential new agents for the treatment of inflammatory lung disorders.

2. Materials and methods

2.1. Chemicals

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dexamethasone, IL-1β and lipopolysaccharide (LPS, *Escherichia coli* 0127:B8) were purchased from Sigma Chem. (St. Louis, MO). 2-Amino-5,6-dihuydro-6-methyl-4H-1,3-thiazine hydrochloride (AMT) was obtained from Tocris Cookson Ltd. (UK). MEM and other cell culture reagents including FBS were products of Gibco BRL (Grand Island, NY). Protein assay kit was purchased from Bio-Rad Lab. (Hercules, CA).

2.2. Animals

Male ICR mice (male, 18–22 g, specific pathogen-free) were obtained from Nara Biotech. Ltd. (Korea). Animals were fed with standard laboratory chow and water *ad libitum*. The animals were maintained in the animal facility (KNU) at 20–22 °C under 40–60% relative humidity and a 12 h/12 h (light/dark) cycle for at least 7 days prior to the experiment. The experimental design using the animals was approved by the local committee for animal experimentation, KNU (KIACUC-13-0003). In addition, the ethical guideline described in the Korean Food and Drug Administration guide for the care and use of laboratory animals was followed throughout the experiments.

2.3. Plant materials

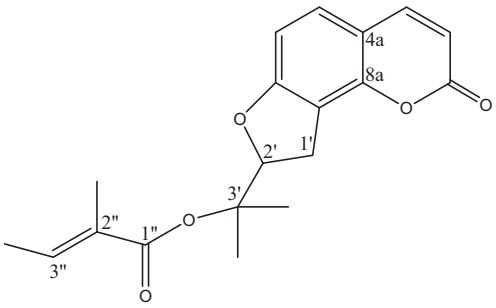
The roots of *Angelica decursiva* were authenticated by Dr. J. H. Lee (Dongguk University, Gyoungju, Korea) after purchase from a local retailer. The voucher specimen (No. 20120220) was deposited in the laboratory of one of the authors (J. S. Choi).

2.4. Preparation of water and 70% ethanol extracts

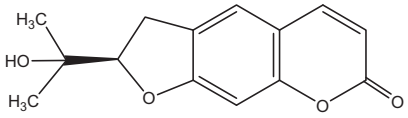
The roots were dried and ground into a powder for solvent extraction. The dried powder (each 50.0 g) was separately refluxed with 70% (v/v) aqueous ethanol and water for 3 h (2 × 0.5 L) at 95 °C. The total filtrate was then concentrated to dryness *in vacuo* at 40 °C to yield the 70% ethanol extract (ADE, 17.1 g, yield: 34.2%) and the water extract (ADW, 15.5 g, yield: 31.0%), respectively.

2.5. Isolation of the constituents

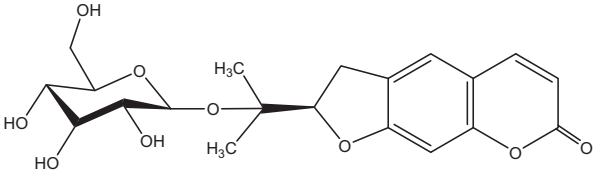
The powder roots of *Angelica decursiva* (2.7 kg) was refluxed with methanol (MeOH) for 3 h (3 × 10 L). The total filtrate was then concentrated to dryness *in vacuo* at 40 °C in order to render the



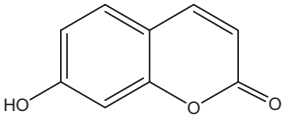
Columbianadin



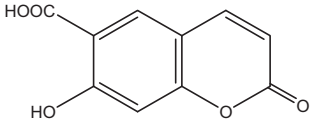
Nodakenetin



Nodakenin



Umbelliferone



Umbelliferone 6-carboxylic acid

Fig. 1. The chemical structures of the isolated coumarins from the roots of *Angelica decursiva*.