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Anti-oxidant and anti-cancer activities of *Angelica dahurica* extract via induction of apoptosis in colon cancer cells

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ABSTRACT

Introduction: *Angelica dahurica Radix* is the common herbal medicine with anti-cancer activities. However, details of its anti-cancer activities are lacking.
Materials and methods: We investigated the anti-cancer effects of *Angelica dahurica* extract in HT-29 colon cancer cell line. Cell viability, apoptotic and necrotic activities and the mechanism of actions of the active fraction were measured.
Results and discussion: The organic extract of *Angelica dahurica Radi* decreased significantly the gene expression of p53, Bcl, Bax and induced apoptosis via caspase cascade and cell cycle arrest. The ethanol-ethyl acetate fraction showed anti-cancer activities in HT-29 cancer cells. A HPLC-DAD analysis of the fraction indicated the presence of Imperatorin and isoimperatorin, which are the major coumarins in the active fraction that contribute to the anti-cancer activities.
Conclusions: This study has evaluated the ant-cancer activity of the organic extract of *Angelica dahurica Radix* against colon cancer cells and provided a basis of further development of the herbal extract for treatment of colon cancer.

Introduction

Chemotherapy of cancer remains to be improved due to its side effects. Many anti-cancer drugs commonly used are developed from potent herbal phytochemicals. Thus, medicinal plants became the good source of anti-cancer agents. Herb-based drugs would be developed after systematic evaluation and the chemical modification.

Angelica dahurica Radix is the commonly used traditional herbal medicine in combination with other herbs for various antigens such as inflammation, liver dysfunction, infection and urinary disorders in China. A recent study reported the anti-oxidant effect of imperatorin, an active compound of *Angelica dahurica Radix* in hypertension by inhibiting NADPH oxidase and MAPK pathway (Cao et al., 2014; Li et al., 2015). Imperatorin and isoimperatorin were reported to be active components from roots of *Angelica dahurica* (Chen et al., 2012; Liang et al., 2015; Jeong et al., 2015). The anti-cancer effects of imperatorin were shown to induce apoptosis in HepG2 cell line (Luo et al., 2011; Zhao et al., 2014). The study suggested that imperatorin can inhibit cancer growth through cell death receptor. Imperatorin was also shown to be responsible for mediation of vasodilation (Wei & Ito, 2008; Nie et al., 2013; Zhu et al., 2013). The vasodilation effects of imperatorin were via inhibition of nitric oxide synthase. The protective activity of imperatorin in cultured neural cells was also reported (Wang et al., 2013). Though the medicinal properties of *Angelica dahurica Radix* is recorded in Chinese Pharmacopeia, details of its anti-cancer activities are lacking.

Materials and methods

Cells, chemicals and reagents

HT-29 cell line was obtained from ATCC (Manassas, VA, USA). Acetonitrile (ACN) (E. Merck, Germany), Methanol, trifluoroacetic acid and other reagents were of analytical grade purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA). All antibodies were purchased from Cell signaling (Danvers, MA, USA), except anti-caspase-8, Bax, Bcl-2, p21 and MDM2 which were obtained from Santa Cruz (Dallas, TX, USA).

Preparation of Extract of Angelica dahurica

Angelicae dahuricae Radix (Sichuan, China) used was purchased from a local vendor. *Angelicae dahuricae Radix* (100 g) was boiled in

Abbreviations: (EAD), Ethyl acetate extract of Angelicae dahuricae Radix; (MTT), 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; (LDH), Lactate dehydrogenase activity; (ROS), reactive oxygen species; (HPLC-DAD), High-Performance Liquid Chromatography - Diode-Array Detection.

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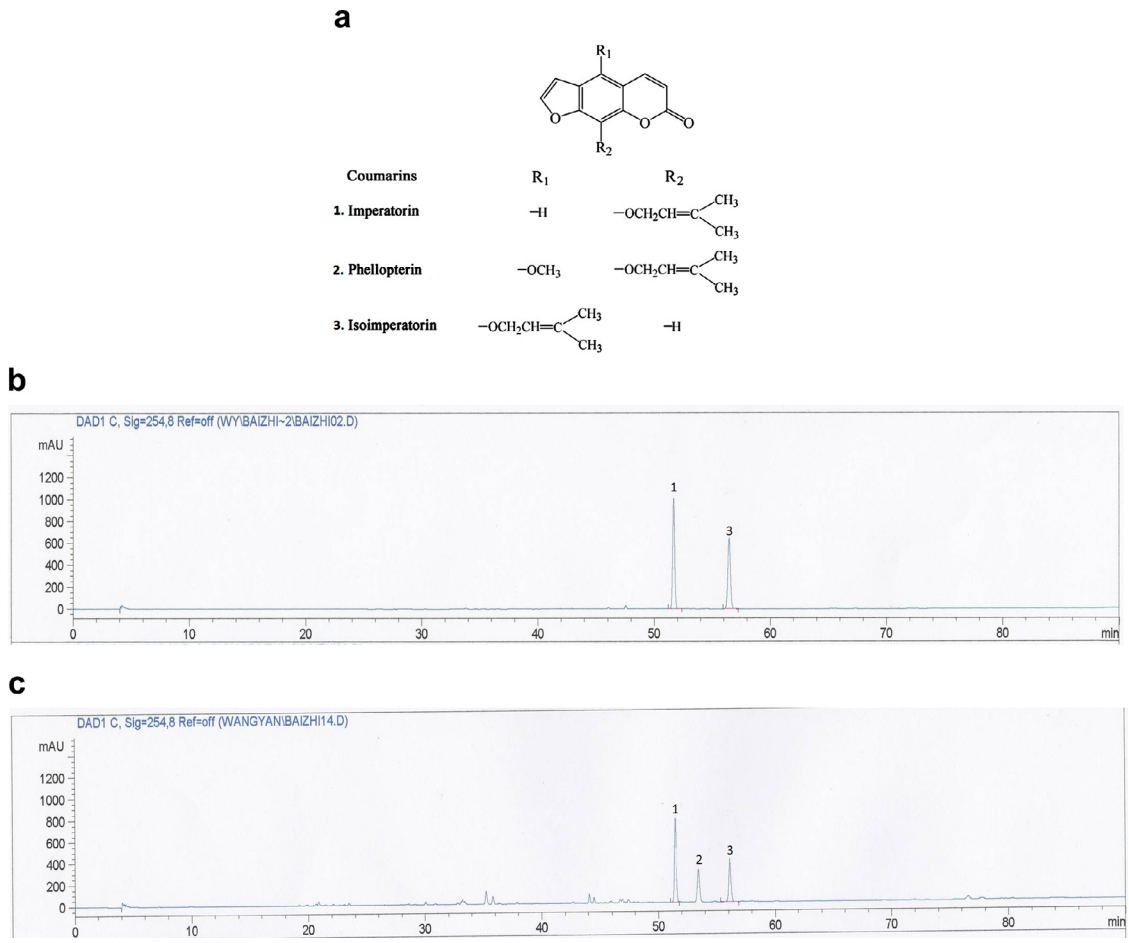

Fig. 1. (A) Chemical structures of the three coumarins in EAD, (B) HPLC-DAD chromatogram of the standard compunds detected at 254 nm. The standard compounds are: peak 1, imperatorin; peak 3, isoimperatorin. (C) HPLC profile of the EAD: peak 1, imperatorin; peak 2, phellopterin; peak 3, isoimperatorin. On the basis of peak areas, EAD contained 3.39 ± 0.12% imperatorin and 2.52 ± 0.08% isoimperatorin.

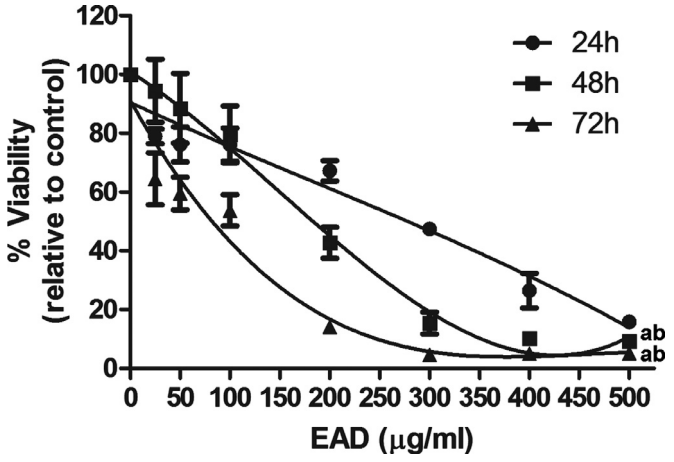

Fig. 2. Inhibitory effects of the EAD on HT-29 cell growth. HT-29 cell lines were treated with various concentrations of the EAD for 24, 48 and 72 h. The cells in solvent control group were treated with 0.5% DMSO. Cell viability was determined by MTT assay. Data were presented as mean ± SD, (n = 6). The superscript letters indicated significant difference between groups ($p < 0.05$): [a] compared to control group; [b] compared to 24 h group.

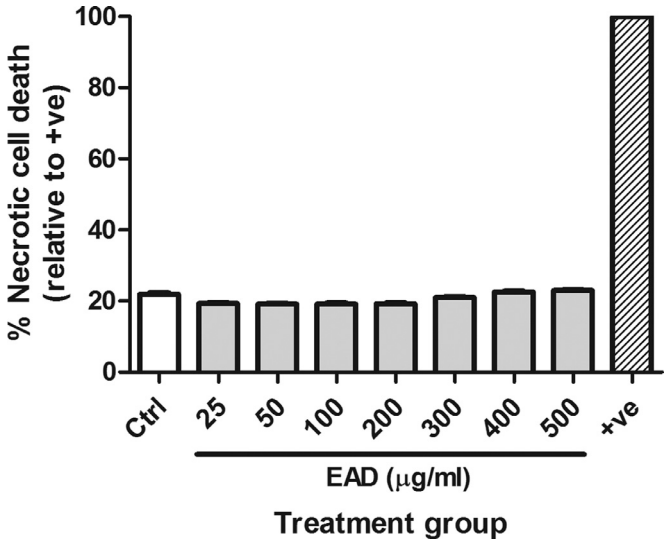

Fig. 3. The percentage of necrotic HT-29 cells after treatment of EAD. HT-29 cell lines were treated with various concentrations of EAD for 48 h. The cells in solvent control (Ctrl)/positive control (+ve) groups were treated with 0.5% DMSO/lysis buffer. The percentage of necrotic cells was determined by LDH assay. Data were presented as mean ± SD (n = 3). Significant difference between treatment and control groups is at $p < 0.05$.

1.5 l of 95% ethanol for 1.5 h and the residue was boiled likewise again. The ethanol extract of *Angelicae dahuricae Radix* was collected and centrifuged at 10,000 × g for 15 min at 18 °C. The supernatant was condensed to 45 ml in a rotary evaporator system (R-210, BUCHI, Switzerland) at 120 mbar pressure at 60 °C. The condensed solution was partitioned with 50 ml ethyl acetate twice to yield the ethyl acetate extract of *Angelicae dahuricae Radix*. The extract was dried by lyophilization overnight. The extract of *Angelicae dahuricae Radix* (abbreviated as EAD hereafter) was stored at −20 °C prior to use.