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Chemical composition, *in vitro* anti-tumor activities and related mechanisms of the essential oil from the roots of *Potentilla discolor*

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A R T I C L E I N F O

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A B S T R A C T

Potentilla discolor has been used as the Traditional Chinese Medicine ingredient in prescription for over 400 years, and scientific evidences have confirmed its effectiveness as an anticancer agent. The present study is undertaken to assess, for the first time, the chemical composition, potential *in vitro* anti-tumor activities and related mechanisms of the essential oil, which was extracted from *Potentilla discolor* fresh roots by supercritical CO_2 fluid extraction technology. Twenty-nine compositions were identified by gas chromatography-mass spectrometry. The anti-tumor activity of the essential oil was screened against four cancer cell lines and the lowest IC_{50} value was 19.02 μg/mL on T24 cell line. Then, T24 was selected as a representative cell line for the related mechanism research. As a result, cell cycle assay confirmed that the cell growth was inhibited by *Potentilla discolor* essential oil and cell cycle was arrested in DNA synthesis phase. A series of apoptosis analysis indicated that *Potentilla discolor* essential oil induced apoptosis through mitochondrion-mediated intrinsic pathway. This study revealed that *Potentilla discolor* essential oil has significant anti-tumor activity. It should be useful in the search for new potential anti-tumor agents and pharmaceutical industries.

1. Introduction

Essential oils, produced by plants as secondary metabolites, are generally volatile with complex compounds characterized by a strong aromatic odor. About 300 essential oils, which produced by plant species, are important in agriculture, food, cosmetic and health industries (Raut and Karuppayil, 2014). In recent years, the Traditional Chinese Medicines (TCMs) have attracted increasing interests as potential sources of novel drugs (Vickers, 2002), and the essential oil usually become a significant indicator to evaluate the efficacy of TCMs (Zhang and Wang, 2009). Generally, essential oils have been used with a wide range of biological and pharmacological, including anticancer, antioxidant, bactericidal, and fungicidal (Edris, 2007; Bakkali et al., 2008). In the recent decades, cancer has become one of leading causes of unnatural death globally. There is general call for new drugs that are highly effective, possess low toxicity and have minor environmental impact. Novel natural products offer opportunities for innovation in drug discovery, in fact, natural essential oil constituents play an important role in cancer prevention and treatment. It is reported that various types of malignancies was lowered after treatment with plant essential oils, hence, the exploration of the anti-tumor properties of essential oil as immediate area of research focus should receive the same interest as synthetic anticancer agents (Kaefer and Milner, 2008; Hamid et al., 2011; Shao et al., 2012; Song and Sun, 2016).

As one of the species of *Potentilla*, *Potentilla discolor* Bunge (*P. discolor*) is a perennial herb which abundantly distributes in most of the provinces in China. The description of this kind of plant was included both in *Flora of China* and *Chinese Pharmacopoeia*, which show that it broadly grows in hills, meadows, valleys, and ravines. Besides, it is about 15–40 cm tall with fusiform robust roots, erect flowering stems, pinnate leaves, and yellow cyme (Editorial Committee of Chinese Flora, 1985; Chinese Pharmacopoeia Commission, 2015). As a TCM in prescriptions for over 400 years, *P. discolor* had been recorded in several ancient Chinese medical books like "Compendium of Materia Medica" and "Jiu Huang Ben Cao", which have been used to treat diarrhea, hepatitis, functional uterine hemorrhage and traumatic hemorrhage (Jiangsu New Medical College, 1975; Li et al., 2013). Being proved by modern research, *P. discolor* also helps to cure type 2 diabetes (Meng

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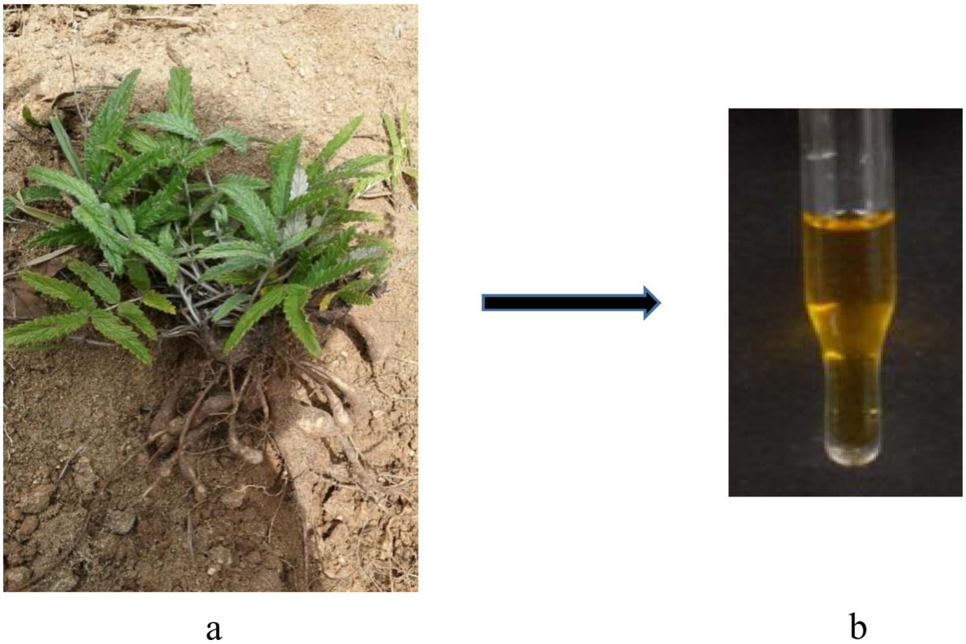

Fig. 1. Morphological observation of *Potentilla discolor* from Mountain Tai (a) and essential oil extracted by supercritical CO_2 extraction (b).

et al., 2004; Xue et al., 2006; Tomczyk and Latté, 2009). In addition, it has been confirmed that this plant is a promising approach to the treatment of cancer (Jin et al., 2011).

Flavonoids and triterpenoids were reported as typical chemical constituents of *P. discolor* in previous phytochemical analysis, and which have been considered responsible for the pharmacological activities of this plant (Xue et al., 2005; Jang et al., 2006; Jang et al., 2007; Yang et al., 2008). However, all these researches were based on the *P. discolor* ethanolic extract and water decoction. In previous study, several remarkable anticancer compounds had been found from *P. discolor* ethanolic fraction (Zhang et al., 2017), thus, the objective of this study, as part of attempt to analyze the constitution of this plant comprehensively, is to explore the chemical composition of *P. discolor* essential oil, also with its potential anti-tumor activities and related mechanisms.

2. Experimental

2.1. Plant materials

Whole plants of *P. discolor* (Fig. 1a) were collected in a private mountain land of Mountain Tai (36°16′N; 117°6′E; 1532.7 m a.s.l.), Shandong, China, in August 2016. The species was identified by Dr. Cheng-Gang Shan, Institute of Agro-Food Science and Technology, Shandong Academy of Agriculture Sciences, Jinan, China. A voucher specimen (No. 16–08–29) was deposited at nature medicine laboratory of Southeast University.

2.2. Oil extraction by supercritical fluid extraction

The fresh roots (100 g) of *P. discolor* were cut from the plants. After rinsed, the collections were sliced and dried by vacuum freeze dryer (Biocool, Lab series) at 7 MP and −50 °C for 24 h, which designed specially for drying of thermo-sensitive or decompounded and oxidative easily materials. Then, the dry root flakes were ground into powder through a 40-mesh screen (450 μm) and extracted by supercritical fluid extraction using supercritical CO_2 as the solvent (SFE-CO_2), which is an optimization way for essential oil extraction compared with traditional methods. Extraction method was used as described by Hu et al. (2007) and Meng et al. (2016) with minor modifications. SFE was performed using a Spe-ed SFE-2 apparatus (Applied Separations, USA) with a 650 mL vessel (8 cm i.d. × 13 cm i.l.). The optimum extraction condition is at a pressure of 25 MPa and a temperature of 35 °C, and the essential oil (Fig. 1b) was obtained as a light yellow liquid after 30 min static extraction following 30 min dynamic extraction (the CO_2 flow rates were kept at 5.0 L/min). Then, the *P. discolor* oil was kept at −20 °C in the dark for the following analysis.

2.3. Chemical composition

2.3.1. Gas chromatography-mass spectrometry analysis of Potentilla discolor oil

Gas chromatography-mass spectrometry was carried out on an Agilent 6890B GC coupled with a 5977A mass selective detector (MSD). Chromatographic separation was performed on HP-5MS (30 m × 0.25 mm i.d., film thickness 0.25 μm, Agilent), with the following temperature programme: initial temperature 40 °C, subsequently 6 °C/min up to 200 °C, then 4 °C/min up to 300 °C, held for 20 min, for a total run of 71.67 min C_7-C_{40} *n*-alkanes standards were analyzed under the same condition by GC–MS.

2.3.2. Identification of individual components

As described by Li et al. (2014a), identification of the individual components was based on: (i) comparison of calculated experiment GC retention indices (determined with reference to homologous series of *n*-alkanes C_7-C_{40} under identical experimental condition) with the GC retention indices reported in National Institute of Standards and Technology (NIST) Standard Reference Database (NIST Chemistry WebBook, 2014), (ii) match of their mass spectra with those recorded in the NIST 08 database and published data. The percentage composition of individual components was computed by the normalization method from the GC peak areas, assuming an identical mass response factor for all compounds.

2.4. In vitro anti-tumors assay

2.4.1. Cytotoxicity test

The cytotoxic effects of *P. discolor* oil were estimated *in vitro* against Hela, NCI-H460, HepG2, T24 and HL-7702 cell lines (obtained from the Institute of Biochemistry and Cell Biology, China Academy of Sciences) by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay (Plumb et al., 1989; Hou et al., 2007). The commercial

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