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A metabolomics approach to identify factors influencing their activity relative to oleanolic acid contents in Korean mistletoe types

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ABSTRACT

The metabolite changes in Korean mistletoe, as functional food, and their effects on its biological activity were monitored. The Korean mistletoe species were successfully distinguished by hierarchical clustering analysis (HCA) in atmospheric-pressure chemical ionization (APCI) and electrospray ionization (ESI) mode. As shown by the PCA scatter plot and loading plot, the Santalaceae family exhibited significant markers derived from oleanolic acid, whereas the Loranthaceae family exhibited tentative markers derived from glycosidic derivatives of quercetin and kaempferol. The mistletoe extracts also inhibited plasminogen activator inhibitor 1 (PAI-1) differently depending on the mistletoe family. The Santalaceae family was shown to be a potent inhibitor of PAI-1, with an IC_{50} value of around 40.0 μg/mL. In contrast, the Loranthaceae family extracts did not inhibit PAI-1. Importantly, discrimination was shown across the chemotaxonomy of the plant, which correlated strongly with the oleanolic acid contents in Korean mistletoe.

1. Introduction

Mistletoes are evergreen, hemi-parasitic plants, normally found growing on a variety of trees. They are commonly known to number approximately 1400 species in the four families of Loranthaceae, Misodendraceae, Santalaceae and Viscaceae, belonging to Santalales, of which Loranthaceae is the largest family, with 900 or more species (Reid, 1989). Mistletoe extract has been used as functional food and traditional medicine for several therapeutic purposes such as such as hypertension, elevated blood lipids, immune modulation, diabetes mellitus, arthritis, and rheumatism and to treat many ailments in Europe and Asia (Fletcher-Hyde, 1990; Stein & Berg, 1998; Wichtl, 2004). In Korea, there are five taxa of four genera in two families of mistletoe: *Viscum coloratum* (Komarov) Nakai f. *coloratum* (VC), *Viscum coloratum* (Komarov) Nakai f. *rubroaurantiacum* (Makino) Kitagawa (VCr) and *Korthalsella japonica* (Thunb.) Engl. (KJ) in

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Chemical compounds: Vicenin-2 (PubChem CID: 442664); Peltatoside (PubChem CID: 5484066); Rhamnetin-3-*O*-rhamnoside (PubChem CID: 5491382); Oleanolic acid (PubChem CID: 10494).

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the Santalaceae family, along with *Loranthus tanakae* Franch. et Sav. (LT) and *Taxillus yadoriki* (Sieb. ex Maxim.) Danser (TY) in the Loranthaceae family (Kim, 2007a; 2007b; Kim, Kim, Sun, & Yi, 2013). Korean mistletoe species have been used extensively in functional food, botanical, traditional medicine for several therapeutic purposes.

Ethnopharmacological studies in Korea report the use of several mistletoe species for therapeutic purposes. Many mistletoe species have been used extensively in functional food and traditional medicine for several therapeutic purposes. The varieties of mistletoe are reported to be very similar, morphologically and chemically, and the main applications are for the treatment of cancer therapy and for immunostimulatory purposes. It is also reported to be used in the form of injectable extract, infusion, tincture, and fluid extract in various cultures in almost every continent to treat or manage various health problems, including hypertension, diabetes mellitus, inflammatory conditions, irregular menstruations, menopause, epilepsy, arthritis, and cancer (Adesina, Illoh, Johnny, & Jacobs, 2013). According to some authors, these effects are usually more evident for the crude extracts from dried plant than for purified mistletoe lectins and viscotoxins alone (Eggenschwiler et al., 2007). Such extracts are mainly composed of phenolic compounds, namely quercetin and its glycosides, and the triterpene acid, oleanolic acid, as demonstrated by Jäger et al. (2007; 2009) and in previous research on a water extract from mistletoe (Fukunaga et al., 1988; Leu & Chuang, 2010; Nhiem et al., 2013). Mistletoe is rich in metabolites with a broad spectrum of biological activities, including anti-tumour (Ovesna, Vachalkova, Horvathova, & Tothova, 2004), anti-inflammation (Safayhi & Sailer, 1997), anti-HIV (Mengoni et al., 2002), and immunomodulatory activities (Weissenstein, Toffol-Schmidt, Baumgartner, & Urech, 2011). Although some reports have arisen regarding the pharmacological inhibitory activities of mistletoe extracts, none of these have been able to disclose the fundamental connection of activity with respect to the potential metabolite. The importance is underlined because plasminogen activator inhibitor-1 (PAI-1) was key factor linking clinical conditions in pharmacological activities that consists of broad biological activities (Cesari, Pahor, & Incalzi, 2010).

PAI-1 is one of the most attractive targets in medicinal chemistry, as this serine-protease inhibitor (or serpin) is involved in the pathophysiology of diverse diseases through the regulation of fibrinolysis and the extracellular matrix. Many of the most common human diseases, including atherosclerosis and cardiovascular disease (Lupu et al., 1995; Raghunath et al., 1995; Robbie, Booth, Brown, & Bennett, 1996; Stoop, Lupu, & Pannekoek, 2000), pulmonary and renal disorders (Eddy, 2000; Ma & Fogo, 2009), cancer progression (Andreasen, 2007; Binder & Mihaly, 2008) and recently obesity and metabolic syndrome (Alessi & Juhan-Vague, 2006; Iwaki, Urano, & Umemura, 2012; Jankun, Al-Senaidy, & Skrzypczak-Jankun, 2012), express PAI-1 and require active PAI-1 for stimulation.

The aim of this study was to attempt to classify and identify Korean mistletoe types according to the differences and similarities in their chemical compositions. We show that the chemotaxonomic properties of the Korean mistletoe are due to four metabolite markers. Furthermore, lipophilic extracts including secondary metabolites from five Korean mistletoe types were investigated to determine their PAI-1 inhibitory activities.

2. Materials and methods

2.1. Chemicals and materials

HPLC-grade acetonitrile and methanol were obtained from SK Chemicals Corp, Korea. The aqueous solutions were prepared using ultrapure water from a Milli-Q system (18.2 MΩ, Millipore, Bedford, MA, USA). Leucine–enkephalin and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Forty-one mistletoe samples were verified and provided by the Warm-temperate Subtropical Forest Research Center, National Institute of Forest Science (Kim et al., 2013).

2.2. Sample preparation

Korean mistletoe lists collection dates, sample codes and voucher identifiers (Supplementary data). To assess chemical composition of plants, metabolites were extracted from whole plant of the mistletoe type: root, bark, twig, and leaf. The mistletoe samples were dried and homogenized to powder using a mill and passed through a 40-mesh sieve. The fine mistletoe powder was weighed (0.1 g) and poured into 3 mL of 100% methanol to generate the extract after sonication in an ultrasonic water bath for 60 min. The extract was filtered through a syringe filter (0.22 μm) and injected directly into the UPLC system.

2.3. UPLC-QTOF MS analysis

Mistletoe metabolite profiling was performed using an ACQUITY UPLC™ system (Waters Corporation, Milford, MA, USA) equipped with a binary solvent delivery manager and a sample manager coupled to a Micromass Q-TOF Premier™ mass spectrometer (Waters Corporation) with an atmospheric-pressure chemical ionization (APCI) and electrospray ionization (ESI) interface. Chromatographic separation was performed using an ACQUITY $BEHC_{18}$ chromatography column (2.1 × 100 mm, 1.7 μm). The column temperature was maintained at 35 °C, and the mobile phases A and B were water with 0.1% formic acid and acetonitrile with 0.1% formic acid, respectively. The gradient elution programme was as follows: 0 min, 10% B; 0–7 min, 10–33% B; 7–14 min, 33–56% B; 14–21 min, 56–100% B; wash for 23.5 min with 100% B; and a 1.5 min recycle time. The injection volumes were 1.0 μL, and the flow rate was 0.4 mL/min. N_2 was used as the desolvation gas. The conditions of the APCI positive ion mode were as follows: the desolvation temperature was 600 °C, the flow rate was 800 L/h, and the source temperature was 110 °C. The capillary and cone currents were 4.5 and 27 A, respectively. The conditions of the ESI negative ion were as follows: the desolvation temperature was set to 350 °C at 400 L/h with a source temperature of 110 °C. The capillary and cone voltages were set to 2350 and 50 V, respectively. Leucine–enkephalin was used as the reference compound (*m*/*z* 556.2771 in the positive mode and *m*/*z* 554.2615 in the negative mode).

2.4. Chemometric data analysis

The raw mass data were analysed using the MarkerLynx applications manager version 4.1 (Waters, Manchester, UK). The