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Original Article

Characterization of metabolite profiles from the leaves of green perilla (*Perilla frutescens*) by ultra high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry and screening for their antioxidant properties

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ABSTRACT

The objective of this research was to access the determination of metabolite profiles and antioxidant properties in the leaves of green perilla (*Perilla frutescens*), where these are considered functional and nutraceutical substances in Korea. A total of 25 compositions were confirmed as six phenolic acids, two triterpenoids, eight flavonoids, seven fatty acids, and two glucosides using an ultra high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q-TOF-MS/MS) technique from the methanol extract of this species. The individual and total compositions exhibited significant differences, especially rosmarinic acid (10), and linolenic acids (22 and 23) were detected as the predominant metabolites. Interestingly, rosmarinic acid (10) was observed to have considerable differences with various concentrations in three samples (Doryong, 6.38 μg/g; Sinseong, 317.60 μg/g; Bongmyeong, 903.53 μg/g) by UPLC analysis at 330 nm. The scavenging properties against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals also showed potent effects with remarkable differences at a concentration of 100 μg/mL, and their abilities were as follows: Sinseong (DPPH, 86%; ABTS, 90%) > Bongmyeong (71% and 84%, respectively) > Doryong (63% and 73%, respectively). Our results

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suggest that the antioxidant activities of green perilla leaves are correlated with metabolite contents, especially the five major compositions 10 and 22–25. Moreover, this study may be useful in evaluating the relationship between metabolite composition and antioxidant activity.

1. Introduction

In recent years, metabolites including phenolic compounds and triterpenoids have been of great interest in food and medical industries because of their beneficial effects on human health [1–5]. Phenolic compounds are distributed in crops, fruits, vegetables, and edible natural plants [6–8] and are associated with a wide range of health beneficial effects including antioxidant, antidiabetic, anti-inflammatory, and anticancer agents [1,3,7,9]. Triterpenoids have also been reported to play essential roles in preventing human diseases because of their anticancer, antioxidant, antibacterial, and antiatherosclerotic properties [2,4,10]. Moreover, many studies have reported that fatty acids have beneficial properties on lipoprotein profile, blood cholesterol level, and basal metabolism of humans [11,12]. For these reasons, several researchers have focused on natural sources with high phytochemical contents for the manufacture of supplements with preventive and therapeutic capacities. In our continuing survey of bioactive substances, an investigation of the metabolite profile in the leaves of green perilla was carried out.

Perilla [*Perilla frutescens* (L.) Britt], which belongs to the Labiatae family, is a widely cultivated edible and medicinal plant in Asian countries such as China, Japan, India, and South Korea [13]. Moreover, this plant has long been used as an important traditional medicine for treating diseases such as tumor, cough, allergy, and intoxication [14,15]. Numerous researches have described that the health beneficial capacities of perilla are related to its metabolite contents (phenolic acids, monoterpenes, flavonoids, and triterpenoids) [2,6,16,17]. In particular, the leaves, seeds, and stems of this species have shown to have antipyretic and antibiotic effects for treating intestinal disorders [18]. Perilla leaves are used as food and medicinal materials for their antimicrobial, antioxidant, anticancer, antidiabetic, antitumor, and antiallergic effects owing to the presence of phenolic compounds, monoterpenes, and triterpenoids [18–21]. Perilla seeds are an important source of fatty acids (α-linolenic acid, linoleic acid, oleic acid, and palmitic acid) and possess health benefits, such as lowering risk of colon cancer as well as plasma lipid levels, reducing the cholesterol level, and reducing the triglyceride level [22–24]. Commonly, human health benefits are associated with many antioxidant metabolites. Among these various biological benefits, perilla plant has demonstrated potent antioxidant properties by *in vitro* method. For example, studies on purple (red) perilla have revealed that the high antioxidant activities are attributable to several anthocyanins [25]. Also, green perilla is known to be associated with phenolic compounds except anthocyanins [26]. We recently reported the optimal harvest time for the phytochemical contents in the leaves of purple perilla [27]. Moreover, we evaluated information concerning the antioxidant effects and inhibitory activities against α-glucosidase as well as aldose reductase of perilla seeds [28]. Several studies have also established the metabolite contents and their biological activities in perilla plant [18–24]. Even though many reports have evaluated the beneficial health effects of perilla regarding metabolites, the exact chemical components in the leaves of green perilla have still not been fully characterized. In addition, the antioxidant capacities of natural plants are considered to have higher synergistic activities in metabolite extracts compared to the effects of a single phytochemical in recent years [1,3,6]. For these reasons, in order to evaluate the antioxidant abilities of perilla leaves, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical methods, based on an electron transfer and involving the reduction of a colored oxidant (DPPH: purple; ABTS: blue/green), have been measured by the spectrophotometric assay. Therefore, our work was designed to investigate the metabolite profiles and antioxidant capacities from the methanol extract of a widely used green perilla leaves.

The purpose of the present research was to characterize the metabolite profiles in the leaves of Korean green perilla using ultra high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q-TOF-MS/MS) system. Furthermore, this study is the first to investigate the changes in metabolite compositions from three methanol extracts of this species. We also determined the antioxidant properties of the scavenging abilities on DPPH and ABTS radicals in view of simplicity, easy control, and cost-effectiveness aspects.

2. Materials and methods

2.1. Plant material and chemicals

The leaves of green perilla were obtained from local markets (Daejeon) in Korea. Three samples (Doryong, Sinseong, Bongmyeong) of perilla leaves were air-dried for 3 days at room temperature to remove the moisture. The collected perilla leaves were immediately freeze-dried at −40°C until analysis. Caffeic acid and rosmarinic acid standards were isolated by chromatographic techniques using silica gel column chromatography (230–400 mesh silica gel, kieselgal 60; Merck, Darmstadt, Germany) based on the data reported in a previous study [6]. Silica gel column chromatography was