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Original Article

Composition of *Asarum heterotropoides* var. *mandshuricum* radix oil from different extraction methods and activities against human body odor-producing bacteria

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ABSTRACT

In this study, oils from *Asarum heterotropoides* were extracted by traditional solvent extraction and supercritical CO_2 (SC-CO_2) extraction methods and their antioxidant activities along with antimicrobial and inhibitory activities against five human body odor-producing bacteria (*Staphylococcus epidermidis*, *Propionibacterium freudenreichii*, *Micrococcus luteus*, *Corynebacterium jeikeium*, and *Corynebacterium xerosis*) were evaluated. The oil was found to contain 15 components, among which the most abundant component was methyl eugenol (37.6%), which was identified at every condition studied in different extraction methods. The oil extracted with *n*-hexane and ethanol mixture exhibited a strong antioxidant activity (92% ± 2%) and the highest ABTS and 2,2-diphenyl-1-picrylhydrazyl scavenging activities (89% ± 0.2%). The highest amounts of total phenolic content and total flavonoid content were 23.1 ± 0.4 mg/g and 4.9 ± 0.1 mg/g, respectively, in the traditional method. In the SC-CO_2 method performed at 200 bar/50°C using ethanol as an entrainer, the highest inhibition zone was recorded against all the aforementioned bacteria. In particular, strong antibacterial activity (38 ± 2 mm) was found against *M. luteus*. The minimum inhibitory concentration (MIC) for the oil against bacteria ranged from 10.1 ± 0.1 μg/mL to 46 ± 2 μg/mL. The lowest MIC was found against *M. luteus*. Methyl eugenol was found to be one of the major compounds working against human body odor-producing bacteria.

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1. Introduction

Chinese people have used different herbs to treat diseases for more than two millenniums. The therapeutic effects of these drugs are attributed to the synergistic effect of their multiple pharmacologically active constituents. *Asarum heterotropoides* var. *mandshuricum*, a perennial herb endemic to China, is a traditional Chinese medicine, which is locally known as *Xixin* [1]. Previous studies have investigated the various components in the essential oil of this herb [2,3], and so far, 82 components have been identified. Among these, methyl eugenol was found to be the most abundant compound [3,4]. Methyl eugenol isolated from *A. heterotropoides* var. *mandshuricum* is not only abundant in this herb but also widely distributed in other aromatic plants. Methyl eugenol possesses a wide spectrum of activities against microorganisms ranging from bacteria to fungi [5]. However, some phytochemical and pharmacological studies on *A. heterotropoides* have reported several types of secondary metabolites, including oils, which display remarkable antioxidant, antimicrobial, antitumor, anti-inflammatory, and larvicidal properties [6–10]. Despite their medicinal importance and availability, only limited knowledge exists about the biological activities of *A. heterotropoides*, which is insufficient to evaluate its pharmacological effects. In addition, there is currently no information about the potential of *A. heterotropoides* root-derived materials to modulate human body odor-producing bacteria.

Body odor is the unpleasant smell caused by the mixing of perspiration (sweat) and bacteria on the skin. Human body odor is thought to occur due to bacterial activities on dead skin cells and secretions. The most dominant phyla responsible for producing human body odor are Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes [11,12]. Sebaceous areas of the skin, such as the forehead and the upper back, are the least diverse habitats and are predominantly colonized by *Propionibacterium* spp. and *Staphylococcus* spp. [13]. Moist skin sites including the groin region and the axilla were commonly found to be dominated by *Corynebacterium* spp., and *Staphylococcus* spp. was reported to be intermediate in diversity compared with other skin-inhabiting organisms [14].

Oxidative stress may be induced by increasing the generation of reactive oxygen species and other free radicals. A previous study [15] reported the role of oxidative stress in the pathogenesis of skin disorders. It is known that scavengers or inhibitors of reactive oxygen species such as antioxidants may reduce hyperpigmentation [16].

In general, for extraction of organic compounds from different sources, organic solvents are used. However, conventional methods have some limitations. Because of the problems associated with traditional solvent extraction methods, there has been a growing interest in developing simpler, faster, and more efficient methods for extraction of organic compounds. In recent years, the use of supercritical (SC) fluid extraction for extracting organic compounds from different liquid and solid matrices has attracted much attention. Being eco-friendly and nontoxic, SC carbon dioxide (SC-CO_2) is a promising process for the extraction and fractionation of edible lipids from different sources [17–19]. Because this extraction process uses a closed chamber, outside air cannot penetrate the vessels. Therefore, the compound recovered and the residues remaining inside experience very less oxidation compared with the traditional solvent extraction method [20].

The objective of our work was therefore to evaluate the SC fluid extraction conditions and compare its results with other solvent extraction methods for obtaining high-quality methyl eugenol from oils in *A. heterotropoides*. In addition, we also evaluated the antioxidant and bacteriostatic activity of these oils using five bacterial strains responsible for producing human body odor.

2. Materials and methods

2.1. Experimental materials and chemicals

Asarum heterotropoides var. *mandshuricum* roots were purchased from Dongeui Hanjae Company (Gyeonggi-do, Korea). Pure carbon dioxide (99%) used in extraction was supplied by KOSEM (Korea). Standard reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used in this study were of analytical or high-performance liquid chromatography grade.

2.2. Sample preparation

Dried roots were crushed using a mechanical blender (PN, SMKA-4000; Ansan, Korea), sieved through a sieve (710-μm mesh size), and then stored at −30°C until their further use in SC-CO_2 and organic solvent extraction.

2.3. SC-CO_2 extraction

A laboratory-scale SC fluid extraction system was used, and the extraction was performed as follows: 180 g of root powder sample was loaded into the stainless steel extraction vessel (volume: 500 mL). A thin layer of cotton was placed at the bottom of the extraction vessel prior to loading. Before plugging the vessel with a cap, another layer of cotton was added to the top of the sample to prevent the enter of root powder into the line. CO_2 was pumped at a constant pressure into the extraction vessel using a high-pressure pump (MILROYAL, Milton Roy, USA) up to the desired pressure. Ethanol as a cosolvent was pumped using a Lab Alliance Series II isocratic pump (Scientific System Inc., State College, PA, USA). The flow rate of the cosolvent was 1 mL/min. A back-pressure regulator was used to control the pressure of CO_2. The extraction temperature was maintained by connecting the extraction vessel with water bath. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter (Shinagawa, Tokyo, Japan). After SC-CO_2 extraction, the remaining root residues and oil were stored at −30°C until further use and analysis. *A. heterotropoides* var. *mandshuricum* root powder was extracted at a temperature of 45–55°C and pressure ranging from 200 bar to 300 bar for 2 hours using a SC-CO_2 apparatus. The flow rate of CO_2 was kept constant at 27 g/min for all extraction conditions.