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A new approach for preparation of essential oil, followed by chlorogenic acid and hyperoside with microwave-assisted simultaneous distillation and dual extraction (MSDDE) from *Vaccinium uliginosum* leaves

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ABSTRACT

Volatile essential oil, non-volatile chlorogenic acid and hyperoside were effectively extracted and isolated from leaves of *Vaccinium uliginosum* by microwave-assisted simultaneous distillation and dual extraction. After optimization by response surface methodology, satisfactory yields for the three above-mentioned components were obtained under the following optimum conditions: liquid–solid ratio of 20 mL/g, microwave irradiation time of 26 min, and microwave irradiation power of 389 W. Compared with conventional methods, gas chromatography–mass spectrometry analysis of the essential oil showed that this method did not produce substantial changes in its chemical composition. However, the proposed method produced higher yields of volatile essential oil, non-volatile chlorogenic acid and hyperoside in a shorter time than conventional methods. Adsorption and desorption experiments with HPD 100B macroporous resin and ethanol/water solutions of various volume fractions led to optimization of the ethanol volume fraction for effective isolation of chlorogenic acid and hyperoside from the aqueous extract.

1. Introduction

Vaccinium uliginosum, a berry-producing shrub that is closely related to the blueberry, is an important source of wild berries and is widely cultivated in Northeast China (Li et al., 2011). Bioactive compounds found in the berries of *V. uliginosum* include anthocyanins, which are found at high levels therein. The presence of these compounds may indicate antioxidant (Wang et al., 2014), anti-inflammatory (Greenspan et al., 2005) and anticancer activity in these berries (Wang and Stoner, 2008). However, there are few reports on the active ingredients in *V. uliginosum* leaves. The leaves are known to contain essential oil (EO), chlorogenic acid (CA, also known as 3-*O*-caffeoylquinic acid) and hyperoside (HR, also known as quercetin-3-*O*-galactoside) (Wang et al., 2008a, 2015).

EOs from many plant species are used in various applications because of their antibacterial, antioxidant, and antitumor activities (Hancke et al., 1999) and their unique aromas (Angioni et al., 2004). Because of these properties, EOs have been used as seasonings in the food industry, insecticide in agriculture, ingredients in perfumes and cosmetics, and as antioxidants in the pharmaceutical industry. CA is a major component in many edible plant species, including ramie leaves (Tan et al., 2014), coffee (Nardini et al., 2002), tobacco leaves (Kono et al., 1998) and tea (Upadhyay and Mohan Rao, 2013). This compound has been used in the cosmetic, food and healthcare industries (Zhang et al., 2008; Stanojević et al., 2009). In recent years, it has attracted much attention because of its multiple pharmacological actions, including as an antioxidant (Wang et al., 2008b), anti-inflammatory (dos Santos et al., 2006), antibacterial (Memon et al., 2010), antiviral (Daglia, 2012), antiproliferative (Memon et al., 2010), hypoglycemic (Hemmerle et al., 1997) and hepatoprotective agent (Basnet et al., 1996). HR is usually extracted from *Hypericum perforatum* (Zou et al., 2004), and is used to improve heart function and relieve pain (Hu et al., 2009). It has anti-inflammatory (Middleton et al., 2000), antinociceptive (Kalegari et al., 2014), antioxidant (Wu et al., 2012), antiviral (Wu et al., 2007), antithrombotic (Ku et al., 2013) and neuroprotective (Zhang et al., 2010) effects. To investigate the potential pharma-

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cological activities of EO, CA and HR from *V. uliginosum*, these components need to be separated from the plant matrix.

Many methods have been reported for the isolation of volatile EO and non-volatile components of plant species in respective procedure. The most common extraction methods for EOs are Soxhlet extraction (Li et al., 2003), hydrodistillation (Mejri et al., 2010; Stashenko et al., 2004), steam distillation (Sahraoui et al., 2008), supercritical fluid extraction (Arranz et al., 2015; Shao et al., 2014) and simultaneous distillation–extraction (SDE) (Stashenko et al., 2004; Teixeira et al., 2007). Non-volatile CA and HR have been extracted using a number of conventional methods with volatile organic solvents, including maceration extraction, heat reflux extraction (Proestos and Komaitis, 2008), and ultrasound or microwave-assisted extraction with methanol, ethanol, acetone, or a solvent mixture. Unfortunately, due to conventional isolation of both volatile EO and non-volatile components from plant species include two processes, thus conventional methods are time-consuming, difficult and inefficient, they can also cause degradation of the target compound, and they typically result in unsatisfactory recoveries. Therefore, a co-extraction method for EO and non-volatile CA and HR that can overcome these issues is required.

Conventional SDE combines steam distillation with continuous extraction using a solvent or a mixture of solvents (Chaintreau, 2001). It is considered to be superior to other methods for the preparation of EOs. Ferhat et al. (2007) developed a rapid extraction method for volatile compounds from aerial parts of *Zygophyllum album*. Their extraction involved *in situ* evaporation of water within fresh samples for distillation of volatile compounds. The steam containing volatile compounds condensed into drops, and the volatile compounds were extracted into a small volume of an extraction solvent in the phase separation tube of the Clevenger apparatus. While this technique was rapid, it could only be used for liquid–liquid extraction of volatile compounds, and could not be applied to water-soluble non-volatile ingredients such as polysaccharides.

Microwave-assisted simultaneous distillation and dual extraction (MSDDE) combines SDE with a microwave oven. This makes the technique less time consuming than conventional SDE, and allows for continuous recycling of extraction solvent. Additionally, the microwave provides instantaneous and homogenous heating of solvents, which enhances the rate and extent of mass transfer. Therefore, both liquid–liquid extraction in the phase separation tube to obtain EO and solid–liquid extraction in the reaction flask to obtain non-volatile CA and HR can occur simultaneously. Combination of SDE with microwave extraction in MSDDE could be useful for simultaneous preparation of volatile EO and non-volatile CA and HR from the leaves of *V. uliginosum*.

In this research, a MSDDE technique was developed for *V. uliginosum* leaves and used to isolate and quantify volatile EO and non-volatile CA and HR. Response surface methodology (RSM) with a Box–Behnken design (BBD) was used to evaluate and optimize the conditions that could influence the yields of these components, including liquid–solid ratio, microwave irradiation time and microwave irradiation power. Additionally, the yields of EO, CA, and HR obtained with the proposed technique were compared with the yields from conventional approaches. The chemical compositions of the EOs were determined by gas chromatography–mass spectrometry (GC–MS) analysis, and compared among the different methods. Stability, repeatability, and recovery experiments were performed to validate the proposed method. Isolation of CA and HR from extract was attempted using macroporous resin dynamic adsorption and desorption experiments with ethanol solutions of various volume fractions (in water). This work highlights the potential for production of EO, CA and HR from the leaves of *V. uliginosum*.

2. Experimental

2.1. Materials and chemicals

Fresh leaves of the *V. uliginosum* were harvested by hand in September, 2014 from the Tahe Forestry (Heilongjiang province, China). All samples were air dried, milled in a blender (Xulang Food Machinery Co., Ltd., Guangzhou China), sieved and stored in closed desiccators at room temperature before use. The sample used in all the following experiments was the same batch of sample powders that was from many plants and was mixed evenly. The particle size of the sample powder used is 40–60 mesh, and the loss on drying of the powder is 8.6%.

Dichloromethane, ethyl ether and petroleum ether (boiling range 30–60 °C) were purchased from Beijing Chemical Reagents Co. (Beijing, China). CA and HR reference standards were obtained from Sigma–Aldrich Inc. (St. Louis, MO). For HPLC analysis, chromatographic grade acetonitrile and sulfuric acid were bought from J&K Chemical Ltd. (Beijing, China). Reverse osmosis Milli-Q water (Millipore, Billerica, MA) was used for preparation of all solutions and for all dilutions. All the solvents were filtered through a 0.45-μm microporous membrane (Guangfu Fine Chemicals Research Institute, Tianjin, China) and degassed by ultrasonication before use in HPLC analysis.

Various macroporous resins used, including ADS7, ADS17, D101, DM130, HPD80, HPD100B, HPD300L, HPD400A, HPD417, HPD600, and HPD750, were purchased from Cangzhou Bon Adsorber Technology Co., Ltd. (Cangzhou, China). During synthesis of the resin, monomers and porogenic agents can become trapped in the pores of the resin, and these compounds can influence the adsorption and desorption processes. To overcome this, the resins were pretreated by: soaking in ethanol for one day at room temperature and then washing with deionized water until no ethanol residue remained (Sovová, 2012).

2.2. Microwave-assisted simultaneous distillation and dual extraction

The MSDDE apparatus (Fig. 1) consisted of a microwave oven, a Likens–Nickerson apparatus, and a heated thermostatic water bath. The microwave extraction unit used was a domestic WP700 microwave oven (Glanz Electrical Appliance Industrial Co., Ltd., Guangdong, China), with an irradiation frequency of 2.45 GHz, continuously adjustable power with a maximum output power of 700 W, and an interior cavity size of 215 mm × 350 mm × 330 mm. In our laboratory, the joint where the Likens–Nickerson apparatus entered the microwave oven was coated with polytetrafluoroethylene to prevent microwave leakage. For distillation and extraction with solvents with lower densities than water (e.g., ethyl ether, petroleum ether), the apparatus was setup as presented in Fig. 1. By contrast, for distillation and extraction with solvents with higher densities than water (e.g., dichloromethane), the apparatus was setup in the reverse manner with transposition of the low-density liquid return arm and high-density liquid return arm.

For MSDDE with ethyl ether (MSDDE-EE) used as the extraction solvent for liquid–liquid extraction, 30 g of a *V. uliginosum* leaf sample was accurately weighed and then mixed with 450 mL of deionized water in a 1000-mL round-bottom flask. This flask was attached to the modified Likens–Nickerson apparatus, and a second 500-mL round-bottom flask containing 150 mL of ethyl ether was attached to the other side of the apparatus. The sample mixture in the 1000-mL round-bottom flask was then irradiated by the microwave oven, and the ethyl ether in the 500-mL round-bottom flask was heated in a thermostat water bath at temperature about five degrees above boiling point of ethyl ether. The volatile components in the sample could be released and evaporated with steam,