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A combined procedure of ultrasound-assisted and supercritical carbon dioxide for extraction and quantitation oleanolic and ursolic acids from *Hedyotis corymbosa*

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ABSTRACT

Hedyotis corymbosa is a good source of oleanolic acid (OA) and ursolic acid (UA), which have many beneficial effects on human health and are two interesting compounds that are widely utilized in medicine and functional food. To isolate OA and UA from the plant, a novel procedure employing ultrasound-assisted supercritical carbon dioxide (USC–CO_2) extraction was developed, and its performance was compared with that of conventional processes. HPLC analysis of the obtained extracts revealed that the highest yields of OA (2.316 mg/g dry plant) and UA (9.284 mg/g dry plant) were achieved by USC–CO_2 extraction using a dried sample with a mean particle size of 0.355 mm at a CO_2 flow rate of 2.1 mL/min, temperature of 53 °C and pressure of 26.5 MPa. The static extraction time was 15 min and the dynamic extraction time was 80 min with 11.0% aqueous ethanol (80% (v/v) ethanol in water) as a cosolvent. The yields obtained with USC–CO_2 extraction were higher than those obtained with conventional SC–CO_2 extraction and heat-reflux extraction for the two discussed compounds. With the newly developed process, these results were obtained faster and at a lower temperature and pressure; additionally, less organic solvent was used. Therefore, USC–CO_2 extraction, as performed under the above-mentioned conditions, is a more effective and selective method for extraction of OA and UA from *H. corymbosa* compared with the other two techniques. USC–CO_2 extraction is efficient and environmentally friendly for using in the pharmaceutical industry.

1. Introduction

Dried whole plants of *Hedyotis corymbosa* (L.) Lam. (also known as *Oldenlandia corymbosa* (L.) Lam., family Rubiaceae) are commonly used in traditional Chinese medicine for the treatment of skin disease, malaria, intestinal abscess, hepatitis and cancer (Sadasivan et al., 2006). Moreover, *H. corymbosa* has a long tradition of use as a healthy tea commonly consumed in China for health maintenance (Zhao et al., 2013). This herb is commonly known to be rich in oleanolic acid (OA, 3β-hydroxy-olea-12-en-28-oic acid) and ursolic acid (UA, 3β-hydroxy-urs-12-en-28-oic acid) (Liang et al., 2008; Sadasivan et al., 2006), both of which are widely applied in medicine and functional foods and have many beneficial effects on human health. OA and UA (Table 1) are known have a wide variety of pharmacological properties, including anticancer, chemopreventive, hepatoprotective, antiviral, antibacterial, antidiabetic, antioxidant, anti-inflammatory and gastroprotective properties (Alvarado et al., 2015; Liese et al., 2015), and they are an integral part of the human diet. Because of their anticancer properties, OA and UA have recently been studied for use in the treatment of hepatocellular carcinoma, prostate carcinoma, colorectal cancer, acute myelogenous leukemia, skin tumorigenesis, cervical carcinoma and lung carcinoma (Liu, 2005; Yang et al., 2012). Therefore, both UA and OA are important anticancer molecules and contribute to the pharmacological efficacy of *H. corymbosa* plants. Due to the above-mentioned findings, these two compounds are attractive to the expanding functional food market for dietary supplements. Hence, it is important to identify reliable methods with which to prepare UA and OA from *H. corymbosa* for use in foods or medicines for human consumption.

Because both OA and UA are slightly polar compounds and are poorly water soluble or insoluble in water, a larger amount of medium-polarity organic solvent is necessary for effective extraction of these analytes from various raw materials. Several publications have described the extraction of OA and UA from various types of biomass (Avila et al., 2007; Vetal et al., 2013; Xia

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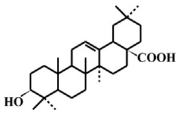
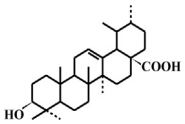
Table 1
Structures of the compounds studied in this work and their physicochemical properties.

Compound	Formula	Structure	M (g/mol)[a]	T_m (°C)[b]	λ_{max} (nm)[c]	D[d]	B[e]	V[f]
OA	$C_{30}H_{48}O_3$		456.7	310	210	1.1	553.5	1.01×10^{-14}
UA	$C_{30}H_{48}O_3$		456.7	283–285	210	1.09	556.9	3.49×10^{-14}

[a] Molar mass (obtained from ChemNet; http://www.chemnet.com).
[b] T_m is the melting point of the compound (obtained from ChemNet).
[c] The wavelength of maximum absorbance (obtained from Yang et al., 2012).
[d] Density of compound (g/cm^3) (obtained from ChemNet).
[e] Boiling point (°C) (obtained from ChemNet).
[f] Vapour pressure (mmHg) at 25 °C (obtained from PubChem; http://pubchem.ncbi.nlm.nih.gov/compound).

et al., 2012; Wei and Yang, 2014; Yang and Wei, 2015a). Nevertheless, extraction of OA and UA directly from plants is more widely conducted and is conventionally performed by solvent extraction. This method is inexpensive, but it can induce the degradation of thermo-sensitive compounds; additionally, it is a time-consuming method that often has a low efficiency and requires a large volume of organic solvent. Toxic organic residues can also pose some health-related risks if safety standards are not applied during irradiation. For the aforementioned reasons, these techniques are not recommended for use with food products or cosmetics or in phytochemical research or bioassays of botanicals and therapeutic compounds. Supercritical carbon dioxide (SC–CO_2) extraction is an emerging clean technology that can overcome the previously mentioned disadvantages. Thus, SC–CO_2 extraction is considered to be a more attractive method compared to conventional techniques, such as heat-reflux extraction (HRE), Soxhlet extraction and ultrasound-assisted extraction. In the case of oils and extracts with a high added value, SC–CO_2 extraction is very promising, especially because extraction yields and product quality are very important aspects related to the economic feasibility of the process. In this regard, CO_2 is typically used as a supercritical solvent mainly due to its unique properties, such as its moderate critical constants (T_c = 304.15 K, P_c = 7.38 MPa) and the fact that it is a non-flammable, non-explosive, inexpensive, odorless, colorless, non-toxic and clean solvent. It is also generally accepted as a harmless ingredient in food, cosmetics, nutraceuticals and pharmaceuticals. In addition, owing to its modest critical constants, it is attractive for use in the extraction of heat-sensitive compounds. The low viscosity and high solute diffusibility of SC–CO_2 facilitate mass transfer during extraction. It also leaves no solvent residue or contamination in the extract, and it can be removed by simple depressurization because it is a gas under ambient conditions. Its low surface tension permits the rapid penetration of supercritical solvent into the pores of heterogeneous matrices, therefore enhancing extraction efficiencies. Moreover, the solvent strength of SC–CO_2 can be manipulated by altering the pressure and/or temperature and cosolvent; therefore, remarkably high selectivity may be achieved. Finally, SC–CO_2 extraction has recently been highlighted as an effective and environmentally friendly extraction method for the recovery of various bioactive compounds from a wide range of raw materials, and the quality of the extracts obtained is superior compared with that of extracts obtained by conventional organic solvent extraction methods (Przygoda and Wejnerowska, 2015; Zekovic et al., 2015). However, polar species are sparingly soluble in neat SC–CO_2, most likely due to its nonpolarity and low dielectric constant (*ca.* 1.5), and pure SC–CO_2 frequently failed to efficiently extract many organics from a sample matrix (Girard et al., 2012). This problem is often addressed via the use of modifier fluids or cosolvents. The cosolvents can either increase the solubility of the solute in SC–CO_2 or interact with active sites on the sample matrix, which help SC–CO_2 to efficiently extract the target analyte. For example, Yang et al. (2013b) reported ultrasound-assisted SC–CO_2 extraction of OA and UA from *Scutellaria barbata* D. Don over a temperature range of 35–75 °C and a pressure range of 11.3–30.0 MPa. These authors used aqueous ethanol as a cosolvent to improve the solubilities of OA and UA in SC–CO_2 and to make these processes feasible. Similarly, Domingues et al. (2013) used the SC–CO_2 extraction technique with 5% ethanol as a cosolvent to extract triterpenic acids from *Eucalyptus globulus* bark.

Environmentally friendly extraction techniques are generally associated with conserving resources, which can occur through the optimization of extraction conditions and/or the introduction of other process technologies. For such purposes, combining different techniques based on similarity in their controlling mechanisms or supplementary roles can yield synergistic effects; thus, combination techniques can be a viable option with possible commercial applications. Ultrasound-assisted extraction can be conveniently coupled with other extraction techniques such as SC–CO_2 extraction (Hu et al., 2007; Klejdus et al., 2010) and microwave-assisted extraction (Cheng et al., 2011). The combined extraction process may result in an overall increase in the efficacy of the operation and a significant increase in the extraction yield. Use of SC–CO_2 for rapid and effective extraction of various compounds from natural materials has attracted much attention in the food, pharmaceutical and biotechnology industries due to the relatively mild operating conditions of this extraction technique, which prevent the degradation of thermosensitive materials. However, the high-pressure equipment required for performing this technique is more expensive than that needed for conventional separation processes and would therefore result in an increase in capital and operating costs. Thus, the SC–CO_2 extraction conditions must be optimized to minimize operating costs. Additionally, it is difficult to apply mechanical stirring in the SC–CO_2 extraction process because high pressures are normally used; this results in slow kinetics. Several studies have shown that the use of ultrasonic techniques is likely to be a unique and practical way to produce agitation in SC–CO_2 to enhance SC–CO_2 extraction (Wei et al., 2014). However, almost no report has been conducted to examine the use of a combined procedure involving SC–CO_2 and ultrasound-assisted extraction for the determination of OA and UA from *H. corymbosa*.

The objective of this research was to develop an environmentally friendly and efficient method for extracting OA and UA from