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Microwave-assisted method for simultaneous hydrolysis and extraction in obtaining ellagic acid, gallic acid and essential oil from *Eucalyptus globulus* leaves using Brönsted acidic ionic liquid $[HO_3S(CH_2)_4mim]HSO_4$

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ABSTRACT

Brönsted acidic ionic liquid and microwave energy are introduced for catalytic hydrolysis of hydrolysable tannins into ellagic acid and gallic acid and simultaneous extraction bioactive substances in the present study. Brönsted acidic ionic liquid-based microwave-assisted simultaneous hydrolysis and extraction (BMSHE) method was employed in obtaining ellagic acid, gallic acid and essential oil from *Eucalyptus globulus* leaves. $[HO_3S(CH_2)_4mim]HSO_4$ was used as a dual hydrolysis catalyst and extraction solvent in the proposal approach. The optimized parameters were obtained as follows: $[HO_3S(CH_2)_4mim]HSO_4$ concentration of 1.0 M, liquid–solid ratio of 30 mL/g, microwave irradiation time of 20 min and microwave irradiation power of 385 W. Yields of ellagic acid, gallic acid and essential oil under these conditions are 3.32 ± 0.08 mg/g, 3.83 ± 0.11 mg/g and 4.82 ± 0.18 mg/g, respectively. Compared with the reference solvent, $[HO_3S(CH_2)_4mim]HSO_4$ shows excellent solvent effect and catalytic activity. Comparison between BMSHE and hydrodistillation indicated the proposed approach is a high efficient, time-saving, low energy consumption and eco-benign methodology. Method validation studies also evidenced that BMSHE is credible for the preparation of ellagic acid and gallic acid. It is reasonable to assume that this method would become a new potential candidate for the preparation of some other kinds of bioactive substances from plant materials.

1. Introduction

Eucalyptus globulus, a member of Myrtaceae family, is a magnificent, tall, and evergreen tree which originates from Australia and has been extensively cultivated in subtropical and Mediterranean regions. In the southern provinces of China, it even has been widely cultivated as a major economic crop. Due to its high quality cellulosic fibres and medicinally important essential oil (EO), *E. globulus* is widely used in paper-making, pharmaceutical, sanitary, agricultural, cosmetic and food industries (Pirralho et al., 2014; Pavela, 2015). *E. globulus* also contains some other high value-added bioactive components, including ellagic acid (EA), gallic acid (GA) and their derivatives (González et al., 2004; Amakura et al., 2009) and so on.

E. globulus leaves EO has a therapeutic application in therapy of pulmonary tuberculosis (Sherryl and Warnke, 2004), analgesic and anti-inflammatory effects (Silva et al., 2003), antibiotic-susceptible and antibiotic-resistant pathogens (Mulyaningsih et al., 2010). Traditionally, EO used to be extracted by hydrodistillation (HD) (Bachir and Benali, 2012), steam distillation (Aazza et al., 2011), supercritical fluid extraction or Soxhlet extraction with methylene chloride (Pavlić et al., 2015), and ultrasonic extraction with ethyl alcohol (Oniszczuk and Podgórski, 2015). These extraction methods are inefficient, time and energy consuming, and even result in losses of some volatile components, low extraction yield, and toxic solvent residue. Disadvantages motioned above impel to the consideration of using new “green” technology for the extraction of EO.

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According to literatures, EA and GA have important influences on human nutrition and are endowed with numerous biological properties, such as antioxidant (González et al., 2004; Amakura et al., 2009), anti-inflammatory and anticancer (Albrecht et al., 2004), and antibacterial (Aviram et al., 2004) activity. As an important natural polyphenols, EA is widely contained in various mushy fruits such as blackberry, raspberry, blueberry and pomegranate. Due to its strong antioxidant activity, it has been used to scavenge cancer-causing toxins from the human body and improve immunity. In Japan, GA has even been processed into a series of antioxidant products (such as G1000 antioxidants, EG-5 emulsion and oily products) with other auxiliaries in the 1980s. It was also employed as preservative for maintaining the quality of the aquatic products (Song, 1988). Nowadays, the extracts or products of EA or GA have been put into use in food industry, disease prevention and treatment and cosmetic production in several countries (Soong and Barlow, 2006; Amakura et al., 2009; Fyhrquist et al., 2014; Gottschalck and Bailey, 2008). In the natural world, the presence of these two compounds is different with tree species. Flores et al. (2013) has reported pomegranate contains free EA; Pardeshi et al. (2014) has successfully separated free GA from *Emblica officinalis*. However, many reports indicated EA and GA exist as derivates (gallotannins and ellagitannins) more often in plant materials (Mueller-Harvey, 2001). Previous studies for the preparation of EA and GA usually adopt water boiling extraction method, volatile organic solvents (methanol and ethanol and so on) reflux extraction method, or acid hydrolysis method with HCl or H_2SO_4 as catalyst (Mueller-Harvey, 2001). Hence, steps were usually carried out for the preparation of EA and GA as follows: (1) extraction of their derives with appropriate concentration of organic solvent; (2) isolation of crude extracts (contain EA or GA derivates) from plant materials; (3) removing extraction solvent from crude extracts; (4) adding HCl or H_2SO_4 solution to the crude extracts and hydrolyzing their derives to EA and GA under the appropriate conditions. Above scheme have limitations, such as requirement of tedious procedure, long hydrolysis time, high energy consumption, and harsh hydrolysis conditions (Cruz et al., 2005; Häkkinen et al., 2000). Therefore, development of simple, high-yielding, efficient, and environmental friendly approaches using new dual solvent-catalysts for the hydrolysis of these two derivatives is an important task.

Ionic liquids, a kind of organic salt which comprises organic cations and inorganic or organic anions, exist as liquids near room temperature (Quijano et al., 2010). Much attention has been paid to extensive application of ionic liquids as green solvent in various applications, including separation technology (Ma et al., 2011b; Yang et al., 2011a; Yang et al., 2011b; Wang et al., 2011), catalysis (Ren et al., 2015), and organic synthesis (Ren et al., 2013). This is due to their excellent properties compared to traditional solvent (lower steam pressure, higher thermal stability, wider liquid scope, more tunable viscosity, and better solubility). Furthermore, microwave energy can be efficiently absorbed and transferred by ionic liquids (Poole, 2004). Recently, ionic liquid based microwave assisted extraction method have been employed in several studies for the extraction of EOs (Liu et al., 2011b; Ma et al., 2011b). Meanwhile, microwave assisted extraction method also presents higher efficiencies compared to traditional techniques for the hydrolysis of polyphenols (Theocharis and Andlauer, 2013). The properties of low acidities or acid-free limit the use of conventional ionic liquids in hydrolysis reactions as catalysts for the preparation of polyphenols (Wilkes, 2002). Cole et al. (2002) firstly reported SO_3H-functionalized ionic liquids with strong Brönsted acidity have huge application prospects in replacement of traditional acidic catalysts and could be used as dual solvents-catalysts. They are flexible, nonvolatile, reusable, and immiscible with many organic solvents. To our best knowledge, there is no research in the literature on the extraction of EO and simultaneous hydrolysis of EA and GA derivatives for the preparation of EA and GA using Brönsted acidic ionic liquids.

In this study, Brönsted acidic ionic liquid-based microwave-assisted simultaneous hydrolysis and extraction (BMSHE) method was developed in obtaining EA, GA and EO from *E. globulus* leaves. A Brönsted acidic ionic liquids, 3-methyl-1-(4-sulfonylbutyl) imidazolium hydrogensulfate $[HO_3S(CH_2)_4mim]HSO_4$ was used as a dual solvent-catalyst in the processes of BMHSE and compared to reference solvent. The hydrolysis reaction is given in Fig. 1. Various parameters of BMSHE were studied including concentration of $[HO_3S(CH_2)_4mim]HSO_4$, liquid–solid ratio, microwave irradiation power and time. Comparisons have been made between BMSHE and HD in terms of treatment time, yields, mass transfer parameters, EOs composition, energy consumption and environmental impact.

2. Experimental

2.1. Reagents and materials

Reference standards of EA and GA (Purity ≥95%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). $[HO_3S(CH_2)_4mim]HSO_4$ and 1-butyl-3-methylimidazolium bromide $[C_4mim]Br$ were obtained from Chengjie Chemical Co. Ltd., (Shanghai, China) and put into use without purification. Methanol and acetic acid of chromatographic grade for HPLC analysis were bought from J&K Chemical Ltd., (Beijing, China); other chemicals of analytical grade were acquired from Beijing Chemical Reagents Co., (Beijing, China). Deionized water was depurated by a Milli-Q Water Purification system (Millipore, Waltham, MA, USA). For HPLC analysis, both samples and solutions were prepared by filtering through a 0.45 μm nylon membrane (Guangfu Chemical Reagents Co., Tianjin, China).

E. globulus leaves obtained from Yaan city (Sichuan, China) were identified by Prof. Kailin Mo from Sichuan Academy of Forestry, China. All the samples were dried in the shade at room temperature for seven days, then powdered into a suitable size (40–60 meshes) and stored in closed desiccators at 4 °C until use.

2.2. Instruments

Apparatus employed for BMSHE process consists of a domestic microwave oven (WP800SL23, Galanz Company, China), a multimode reactor, and a temperature IR sensor and operates at a frequency of 2450 MHz for simultaneous obtaining EA, GA and EO from leaves of *E. globulus*. The microwave oven for laboratory applications was modified by the addition of a Clevenger condenser on the top as described in previous study (Bayramoglu et al., 2008). The interior size of the oven cavity was 215 × 350 × 330 mm. A round bottom flask (2 L) was placed in the oven and connected to the condenser through a hole. The hole around the neck of the round bottom flask was covered with polytetrafluoroethylene to avoid microwave leakage (Bayramoglu et al., 2008; Gavahian et al., 2015). Five levels of nominal power (700 W, 540 W, 385 W, 230 W and 120 W) are marked on the power feedback/control by microwave oven manufacturers. It was observed that the real power delivered inside the cavity was equivalent to 71.6% of maximum nominal power in Bizzi's study (Bizzi et al., 2011). This result indicated the real powers delivered inside the cavity have differences with the nominal power. In the present study, a constant solution volume of 900 mL was used in each experiment to make the efficiencies of microwave energy conversion approximately equal at different nominal powers. Microwave irradiation powers are expressed by nominal powers which could be obtained by adjusting the power feedback/control in BMSHE processes during experiments