



Deep eutectic solvent-based microwave-assisted extraction of genistin, genistein and apigenin from pigeon pea roots



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ABSTRACT

The purpose of the study was to develop a new deep eutectic solvent-based microwave-assisted extraction (DES-MAE) method for extracting the three major active compounds genistin, genistein and apigenin from pigeon pea roots. Optimal conditions for DES-MAE, proposed by the single factor and the Box-Behnken design (BBD) tests, were 30% of water in 1,6-hexanediol/ChCl (7:1, mol/mol) as the extraction solvent, microwave power 600 W, liquid/solid ratio 14 mL/g, temperature 80 °C and time 11 min. The yields of genistin, genistein and apigenin reached 0.449, 0.617 and 0.221 mg/g DW, respectively. The kinetics experiment showed the prominent advantages of DES-MAE such as higher extraction efficiency than other extraction methods. The present results showed that DES could be a kind of green solvent for fast and efficient extraction of the active ingredients from plant materials.

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1. Introduction

In recent years, people pay much attention to green solvent, which is one of the most important aspects of green chemistry. Nowadays, ionic liquids (ILs) and deep eutectic solvents (DESs) have taken place of volatile organic solvents, and have been applied in many chemical processes [1]. Despite ILs have received increasing attention due to their excellent characteristics, such as negligible volatility at room temperature and adjustable physico-chemical properties that have been used in many fields. However, the toxicity of some components, stimulating properties and higher cost have limited ILs utilization as extraction solvents in the pharmaceutical industry [2]. DESs are emerging rapidly as a new type of green solvents instead of traditional solvents and ionic liquids (ILs). DESs are typically formed by mixing two or three kinds of organic components together. Hydrogen bond donors (HBD) such as urea, carboxylic acids and polyols can be mixed with hydrogen bond acceptors (HBA) to form DES in a mixture [3,4]. Choline chloride (ChCl) is one of the most widely used HBAs in DESs. The DESs possess much lower melting points than any of its individual components, maybe due to the effect of

intermolecular hydrogen bonds [5]. Compared with ILs, DESs have more advantages such as low cost, biodegradability, easy preparation, pharmaceutical acceptable toxicity and environmental friendliness [6–9]. They have been used for catalysis, organic synthesis, dissolution and electrochemical material [10]. So, it is a rather interesting approach to employ DESs as extraction solvent in MAE to extract active components from plant matrix samples instead of using traditional organic solvents.

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is a kind of annual or perennial woody grain legume crops that mainly distribute in tropical and subtropical regions of the world [11]. It is cultivated as edible beans and an important source of protein [12]. Furthermore, pigeon pea has been widely used as a traditional folk medicinal plant in many developing countries. It has been used for the treatment of hepatitis, measles, varicella and superficial infection, and for stabilizing the menstrual period [13–17]. However, most of the studies have paid attention to the pigeon pea leaves because of its significant pharmacological properties, including notable anti-inflammatory, anti-bacterial and abirritative properties [18]. According to the previous chemical researches and pharmacological studies on the pigeon pea roots, it was found that genistin, genistein and apigenin were the major beneficial compounds responsible for the bioactivities of this plant [19,20]. As shown in Fig. 1, genistein (4',5,7-trihydroxyisoflavone) is a main isoflavone and plant estrogen with anti-oxidant, anti-inflammatory,

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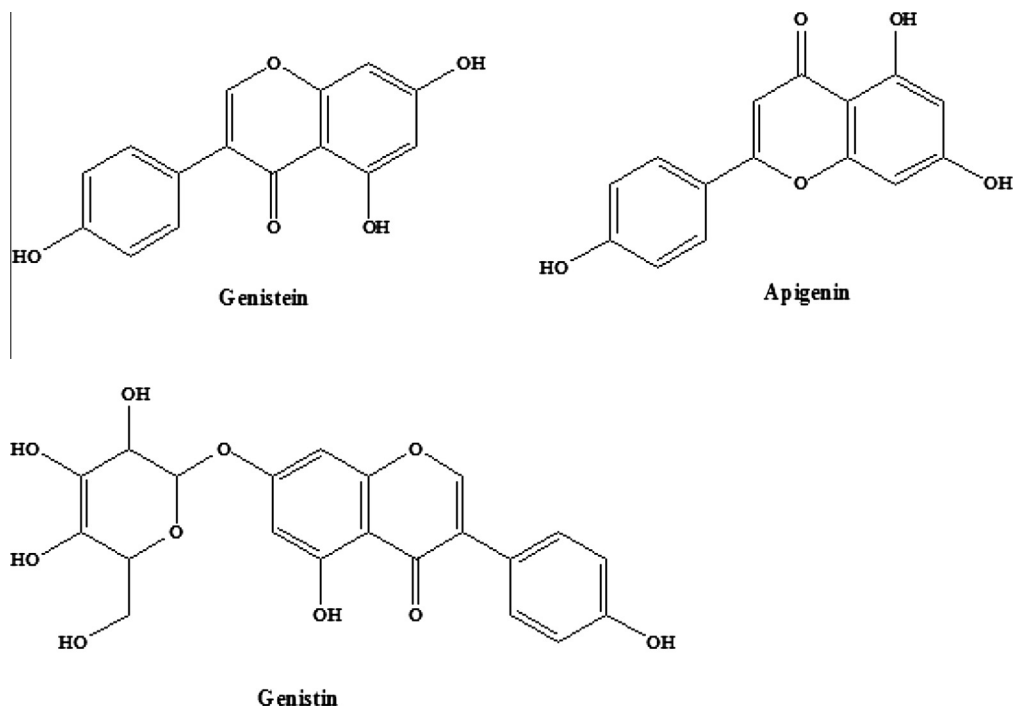


Fig. 1. The structures of target compounds.

anticancer and protein kinase inhibitory activities [21,22]. Genistin (4',5,7-trihydroxyisoflavone-7-glucoside) is a potent glycoside of genistein, which shows extraordinary anticancer, antiviral, anti-oxidant, anti-inflammatory and free radical scavenging activities. Apigenin (4',5,7-trihydroxyflavone) is a kind of naturally occurring flavonoids, which shows anti-inflammatory, decreasing blood pressure, anti-arteriosclerosis, anti-anxiety, anti-microbial, antiviral, anti-oxidant and free radical scavenging activities [23]. Considering the excellent properties of the three active compounds, it is an attempt of practical significance to develop a rapid and green technology to extract them from pigeon pea roots.

Extraction is the first step for the recovery and purification of bioactive phytochemicals from plant materials. In the past few years, a plenty of traditional extraction methods have been employed, such as heat-refluxing extraction, soxhlet extraction and ultrasound-assisted extraction, etc. Microwave-assisted extraction (MAE) is a rapid and efficient sample extraction method that has been used as an alternative to conventional methods in the extraction of natural products [24–27]. Compared to conventional methods, MAE has shown some advantages such as shorter extraction time, lower consumption of solvent, and higher extraction yield [28]. Therefore, in the present study, it was the first time to propose and apply for DES-MAE to extract the major active compounds from pigeon pea roots instead of the traditional organic solvent. And the extraction procedure was optimized by single factor experiments and response surface method (RSM). Response surface methodology is a collection of statistical and mathematical technique for developing, improving and optimizing process [29,30]. The effects of the three main operating parameters (liquid/solid ratio, extraction temperature and microwave irradiation time) on the extraction yields were investigated by BBD test. The extraction efficiency of three bioactive components with deep eutectic solvent-based microwave-assisted extraction (DES-MAE) was compared with those obtained by deep eutectic solvent-based ultrasound-assisted extraction (DES-UAE) and deep eutectic solvent-based heat-refluxing extraction (DES-HRE). In order to study the process kinetics, extraction time was further studied by first order kinetic model.

2. Materials and methods

2.1. Plant materials

Pigeon pea roots were collected in autumn 2012 from Hainan province, China, and authenticated by Professor Shao-Quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, PR China. Voucher specimens (No. 052056001001001) were deposited in the herbarium of the same laboratory. The material was dried to constant weight in the shade, powdered by a disintegrator, and then collected the sample (20–80 mesh) with sieve (Harbin Ouerfu Filter Material Co., Ltd., China). Then the crushed material was kept in the dark and dry conditions at room temperature prior to use.

2.2. Chemicals and reagent

Sucrose, glucose, L-proline, 1,2-propanediol (>99.0%), malic acid (>99.0%), lactic acid (85.0–90.0%), sorbitol (>98.0%), glycol (>99.5%), glycerol (>99.0%), 1,3-butanediol (>99.0%), 1,4-butanediol (>99.0%), 1,6-hexanediol (>97.0%) and choline chloride (>98.0%) were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Genistin (4',5,7-trihydroxyisoflavone-7-glucoside, $\geq 98\%$) and genistein (4',5,7-trihydroxyisoflavone, $\geq 98\%$) were purchased from Fluka (Buchs, Switzerland). Apigenin (4',5,7-trihydroxyflavone, $\geq 95\%$) was purchased from Aldrich (Milwaukee, WI, USA). Methanol and formic acid for HPLC analysis were of chromatographic grade and obtained from J&K Chemicals Ltd. (Beijing, China). Deionized water was purified by a Milli-Q water-purification system from Millipore (Bedford, MA, USA). All solutions prepared for HPLC were filtered through 0.45 μm nylon membranes (Millipore, USA) prior to use.

2.3. Preparation of DESs

In this study, heating method was used to preparing DESs. Firstly, hydrogen bond donors (HBDs) mixed with hydrogen bond acceptors (HBAs) according to scheduled proportion. Secondly,

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