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Analytical Methods

Optimisation of microwave-assisted enzymatic extraction of corilagin and geraniin from *Geranium sibiricum* Linne and evaluation of antioxidant activity

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ABSTRACT

Plant polyphenols exhibit effective bio-activities, particularly antioxidant activities. Previous studies showed that *Geranium sibiricum* Linne contains high levels of polyphenolic compounds such as corilagin (CG) and geraniin (GE). In this study, a microwave-assisted enzymatic extraction (MAEE) method was evaluated for the simultaneous extraction of CG and GE only with deionised water. The optimal extraction conditions were as follows: irradiation power 500 W, ratio of solvent to material 40 ml/g, irradiation temperature 33 °C, pH 5.2, amount of cellulase 3600 U/g and irradiation time 9 min. Under these conditions, the extraction yields of CG and GE achieved were 6.79 and 19.82 mg/g, which increased by 64.01% and 72.95%, respectively, as compared with the control ones. Furthermore, the antioxidant activities of crude extracts were 2.61 mmol $FeSO_4$/g DW and 0.118 mg/ml (IC_{50}), according to the FRAP and DPPH assays, which indicated *G. sibiricum* Linne possesses good potential for natural antioxidants.

1. Introduction

Polyphenolic compounds, which are known to possess numerous biological activities, such as potential candidates for usage of AIDS, heart ailments and bacterial infection (Handique & Baruah, 2002; Pompeu, Silva, & Rogez, 2009; Yu et al., 2002), are found in all parts of higher plant, such as roots, barks, stems, leaves, fruits, and flowers (Schwarz et al., 2009). In recent years, considerable attention has been paid to polyphenols which have been found to be strong antioxidants for human health benefits (Kim, Tsao, Yang, & Cui, 2006; Schwarz et al., 2009). In particular, regular consumption of fruit and vegetables is associated with a lower risk of some chronic diseases and certain cancers. Although there is still uncertainty about the relationship between polyphenols and ailments, the health-promoting potential of these foods may be attributed to the phytochemical compounds present in the plants (Rangkadilok, Worasuttayangkurn, Bennett, & Satayavivad, 2005).

Geranium sibiricum Linne is always consumed as an additive in distilled spirit for drink and green feed additives for animal feed stuff (Xiang, Tang, Chen, & Shi, 2001). Recently, it can be treated as nutritional and sanitarian wild vegetation for folk foods (Huang, Wu, & Wu, 1999). It is also widely used for the therapy of dysentery and enteritis in traditional Chinese medicine (TCM) (Guo, Wang, Li, & Zhu, 1987). In addition, *G. sibiricum* Linne has been used for treating diarrhea and intestinal inflammation in traditional Korean folk medicine (Guo et al., 2007). Shim and Lim (2008) found that it had anti-proliferative character. Research indicated that it is rich in polyphenolic compounds (Ivancheva, Manolova, Serkedjieva, Dimov, & Ivanovska, 1992; Shim et al., 2008), which have been considered as being responsible for the beneficial efficacies on human health (Li et al., 2008). Among the polyphenolic compounds in *G. sibiricum* Linne, corilagin and geraniin (Fig. 1) are the main substances with better pharmacological activities including antiviral, hepatoprotective, and antihypertensive activities (Cheng, Lin, & Hsu, 1995; Kinoshita, Inoue, Nakama, Ichiba, & Aniya, 2007).

In higher plant cell, phenols may be classified as cell-wall (CW) phenols, which are bound to polysaccharides, and non-cell-wall phenols associated with vacuoles and the cell nucleus (Pinelo, Arnous, & Meyer, 2006). The retention of phenols in the CW depends on compositional and structural characteristics. Furthermore, physical traits of the CW can also influence the eventual aggregation between conformational CW polysaccharides and phenolic substances (Pinelo et al., 2006). Anyway, cross-linking of CW polysaccharides is the main barrier for the release of intracellular substances. Therefore, degradation of cell-wall polysaccharides is one of the key steps in releasing phenols from CW. Hydrolytic enzymes can hydrolyse and degrade the plant CW constituents and improve

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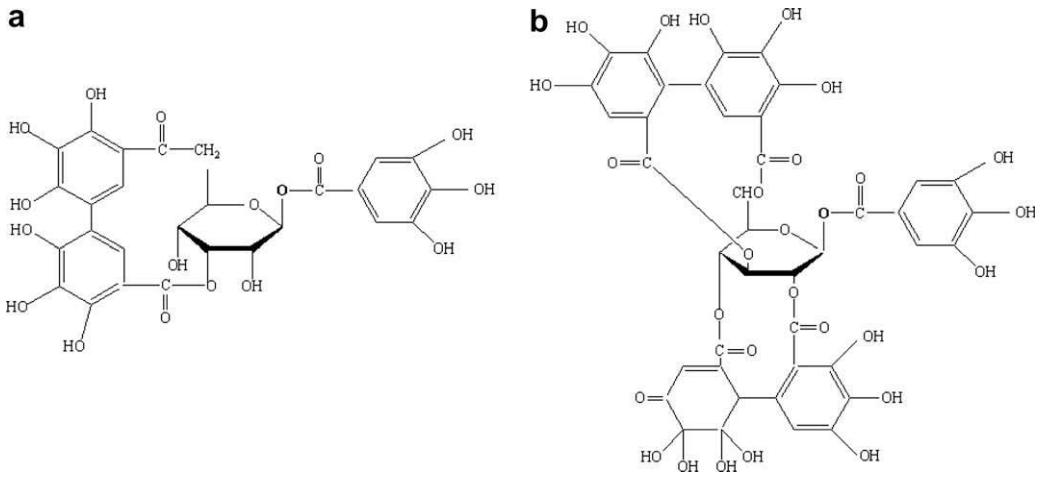

Fig. 1. Chemical structures of (a) corilagin (CG) and (b) geraniin (GE).

the release of intracellular contents. Nowadays, enzyme assisted extraction has been reported for the extraction of various kinds of compounds. Cellulase is a multicomponent enzyme system and all these enzyme components act effectively in a synergistic function while it breaks cellulose chains into glucose, beta-glucosidase cleaves beta-1, 4 linkages in cellulose and thus cleaves polysaccharide cross-link with polyphenols. Hence, it has been employed for the extraction of polyphenols from *Pinus taiwanensis* and *Pinus morrisonicola* and apple pulp, luteolin and apigenin form pigeonpea (Fu et al., 2008; Lin, Chang, & Deng, 2009; Missang, Massiot, Baron, & Drilleau, 1993). Enzymatic hydrolysis presents some advantages over conventional extraction procedures, such as mild conditions of temperature and pH. Another research showed good promise for the release of phenols from CW (Pinelo et al., 2006).

Microwave-assisted extraction (MAE), which combines microwave and traditional solvent extraction, has gained wide acceptance as a powerful tool for sample preparation of solid matrices. In contrast with traditional extraction techniques, it possesses more advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost and lower decomposing of the target species (Proestos & Komaitis, 2007). Up to our best knowledge, combinational usage of enzymes and MAE has not been previously reported in plant materials extraction.

The aims of the present work were to evaluate the feasibility of microwave-assisted enzymatic extraction (MAEE) for simultaneous extraction of CG and GE from *G. sibiricum* Linne. The important parameters involved in the MAEE process were optimised by single-factor experiments, and then the critical parameters were investigated by a 2^3 full factorial central composite design (CCD) design to obtain maximum extraction yields. Mass transfer characteristics of MAEE were described and optimal irradiation time of MAEE was obtained through a pseudo first-order equation.

2. Materials and methods

2.1. Chemicals and reagents

CG and GE were brought from Delta Co. Ltd. (Anhui province, China). Cellulase (Celluclast 1.5 l, P1000 U/mg), 1,1-diphenyl-2-picrylhydrazyl (DPPH), acetonitrile of HPLC grade, FRAP regent and Folin–Ciocalteu reagent were purchased from Sigma–Aldrich (Steinheim, Germany). All other reagents were of analytical grade. Deionised water was purified by a Milli Q Water Purification system from Millipore (Bedford, MA, USA). Appropriate amount of reference compounds were dissolved in acetonitrile–water (15:85, v/v) to obtain the stock solutions at concentrations of 0.5 mg/ml for CG and GE, respectively. All solutions prepared for HPLC were filtered through 0.45 μm nylon membranes prior to use.

2.2. Plant material

G. sibiricum Linne were collected in May from the arboretum of the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin, PR China, and was authenticated by Prof. Shaoquan Nie from the same laboratory. Voucher specimens were deposited in the herbarium of this Key Laboratory. The whole plant was air dried and then pulverised into a homogeneous size by a disintegrator (HX-200A, Yongkang Hardware and Medical Instrument Plant, China) and then sieved (30–40 mesh).

2.3. Analytical methods

HPLC analysis was performed using a Jasco LC system (Jasco Company, Japan) equipped with a Jasco PU-1580 intelligent HPLC pump, a Jasco UV-1575 intelligent UV IS Detector as well as Millennium 32 system software. Chromatographic separation was performed on a HiQ Sil C18 V reversed-phase column (250 mm × 4.6 mm i.d., 5 μm, Kya Tech, Hachioji City, Japan) equipped with an Analytical KJ0-4282 C18 guard cartridge system (Phenomenex, Torrance, CA, USA).

The mobile phase consisted of acetonitrile (A) and water (B). Baseline separation of CG and GE was achieved with an elution program as follows: 15% A held until 25 min. The UV detector was set at the wavelength of 220 nm. The flow rate was 1 ml/min, injection volume was 10 μl, column temperature was maintained at 25 °C, and the retention time for CG and GE were 10.14 and 12.54 min, respectively. The working calibration curves based on reference compounds of CG and GE showed good linearity over the range of 1.54–292.32 and 1.61–295.14 μg/ml, respectively. The regression lines were $Y = 41084491X - 139264$ ($R^2 = 0.9987$, $n = 5$) and $Y = 38811257X - 380265$ ($R^2 = 0.9994$, $n = 5$), where Y is the peak area of analyte, and X is the concentration of reference compound (μg/ml).

2.4. MAEE procedure

The microwave extractor, which includes a time controller and a temperature controller, was manufactured by Shanghai Sineo microwave Products Company (Shanghai, China). In the preliminary MAE experiments (data not shown), 1 g material in deionised