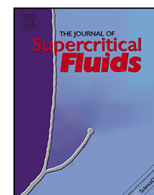
J. of Supercritical Fluids 95 (2014) 318–324

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu



Phenolic and flavonoid content and antioxidants capacity of pressurized liquid extraction and perculation method from roots of *Scutellaria pinnatifida* A. Hamilt. subsp *alpina* (Bornm) Rech. f.

E. Golmakani [a], A. Mohammadi [b], T. Ahmadzadeh Sani [c], H. Kamali [b,*]

[a] Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Research Center of Natural Products Health, North Khorasan University of Medical Sciences, Bojnurd, Iran
[c] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 12 July 2014
Received in revised form
15 September 2014
Accepted 15 September 2014
Available online 28 September 2014

Keywords:
Scutellaria pinnatifida
Pressurized liquid extraction
Phenolic
Flavonoid
Ferric reducing antioxidant power
DPPH scavenging activity

ABSTRACT

The extract was separated from roots of *Scutellaria pinnatifida* using perculation and pressurized liquid extraction (PLE). A circumscribed central composite (CCC) was used to optimize the effective extraction variables. For achieving maximum extraction yield *via* PLE the temperature, pressure, static time, dynamic time, and the solvent flow rate were adjusted 65.8 °C, 39.2 bar, 12.9 min, 18.9 min, and 0.76 mL/min, respectively. Ferric reducing antioxidant power (FRAP) (mmol/g) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (mg/mL) were evaluated and the highest antioxidant activity was observed from the PLE extract. The total phenolic and flavonoid content was calculated and a good correlation founded between phenolic content and antioxidant activity. The results indicated the root of this plant is a potential source of natural antioxidants and flavonoids. The PLE method is quicker and it has more extraction yield than perculation.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Pressurized liquid extraction (PLE, Dionex trade name of accelerated solvent extraction (ASE)) is an efficient, rapid, selective, and reliable extraction method [1]. PLE technology and its applications have been developed and used by various scientists [2–6]. Moreover, it has been applied for the quantitative extraction of different samples of environmental organic compounds from soils and lipids, and also for the analysis of food and biological samples [6]. The possibility of changing several extraction variables such as temperature, pressure, and volume of solvent is a promising characteristic of available automated PLE instruments [7]. Using a modified supercritical fluid extraction (SFE) apparatus, we have developed a PLE method, mainly as an analytical tool, to determine oil content or as a sample preparation method for the oil quality measurements with full capability of automation when the workload is high [8]. Special emphasis is placed on obtaining a rapid, selective, efficient, and reliable extraction process.

The genus of Scutellaria belong to the Lamiaceae family contains more than 300 species spread throughout the world [9,10] and has 20 species and two hybrids in Iran [11] 10 species and two hybrids are endemic in this country [12]. One of the Iranian species of Scutellaria is *Scutellaria pinnatifida* that has two subspecies: *Scutellaria pinnatifida* A. Hamilt. ssp. *pinnatifida* and *Scutellaria pinnatifida* A. Hamilt. ssp. *alpina* [10,13] and the Persian name of this plant is Boshghabi [13]. This genus is well known for using in treatment of hypertension, arteriosclerosis, inflammatory, hepatitis, allergy, cancer, anxiety and sleeplessness [13], this genus also is a sedative, tonic, and anti-spasmodic agent [14–16]. The dried roots of this genus administered in Chinese traditional herbal medicine [17] and they have antioxidant [18], anti-inflammatory [19], sedative [20], antiviral [21] and antithrombotic [22] activity. Flavonoids are the main components of this genus [23]. The major bioactive components in roots of *Scutellaria baicalensis* were baicalein, baicalin, wogonin [24]. Baicalin has anti-inflammatory, anti-allergic, antioxidant and hepatoprotective activities [25]. The flavonoids such as baicalin, baicalein and wogonin extracted from the genus of Scutellaria [23] have been shown to possess antilipoperoxidant and antiinflammatory activities [26,27] and demonstrated cytotoxic to various human tumor cell lines *in vitro* and *in vivo* [28]. In last years, flavonoids in Scutellaria have been widely studied for

* Corresponding author. Tel.: +98 584 2248004; fax: +98 584 2247202.
E-mail addresses: h.kamali@nkums.ac.ir, h.kamali62@yahoo.com (H. Kamali).

http://dx.doi.org/10.1016/j.supflu.2014.09.020
0896-8446/© 2014 Elsevier B.V. All rights reserved.

their chemical properties and biological activities [29]. The hydro-distilled essential oil from dried aerial parts of *S. pinnatifida* A. Hamilt. ssp. *alpina* grown in Khorassan province was analyzed and the major components of the oil were germacrene-D and beta-caryophyllene [30]. Free-radical-scavenging activity, antibacterial activity and brine shrimp toxicity of different extracts of aerial parts of *S. pinnatifida* were assessed and the dichloromethane and methanol extracts exhibited free-radical-scavenging activity [31].

Utilization of effective antioxidants with natural origin is desired and some chemical compounds are safe antioxidants. There are no reports about phytochemical and biological studies carried out on roots of *S. pinnatifida* A. Hamilt. ssp. *alpina* native from Iran and the aim of the present study focuses on determination total phenolic and flavonoids from roots of *S. pinnatifida* A. Hamilt. ssp. *alpina* and investigation of their antioxidant activities *via* PLE and perculation method. The antioxidant properties were investigated using two tests: ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging. A circumscribed central composite method (CCC) was used to guide experimentation of extraction and a mathematical model was constructed to investigate the relationship between the extraction yield, total phenolic, total flavonoid, and antioxidants activity and the variables influencing extraction such as temperature, pressure, static time, dynamic time, and flow rate. The validity of the model was evaluated through the analysis of variance (ANOVA) technique by using Minitab software [32,33].

2. Materials and methods

2.1. Chemicals and reagents

Methanol (CHROMASOLV, ≥99.9%, Sigma–Aldrich), Folin-Ciocalteau reagent (F9252, Sigma–Aldrich), Na_2CO_3 (451614, anhydrous powder, 99.999%, Sigma–Aldrich), Gallic acid (91215, Fluka), Aluminum Chloride (563919, anhydrous powder, 99.999%, Sigma–Aldrich), 2,2-diphenyl-1-picrylhydrazyl (257621, Sigma–Aldrich), Quercetin (Q4951, ≥95%, Sigma–Aldrich), ascorbic acid (A1300000, European Pharmacopoeia (EP) Reference Standard, Fluka), Butylated Hydroxy Toluene (BHT) (W218405 ≤ 99, Sigma–Aldrich), 2,4,6-tripyridyl-s-triazine (TPTZ) (T1253 for spectrophotometric, ≥98%, Sigma), $FeSO_4.7H_2O$ (21542, ≥99.0%, Sigma–Aldrich)

2.2. Preparation of Scutellaria pinnatifida

The roots of *S. pinnatifida* A. Hamilt. ssp. *alpina* were collected from Tighbal mountain (2700 m) in Darkesh valley in Bojnurd, North Khorasan province of Iran in Jun 2013. The plant was identified from the Ferdowsi University of Mashhad Herbarium. Voucher specimen (No. 11868) was deposited in the Herbarium of School of Pharmacy, Mashhad University of Medical Sciences. The roots were dried at 40 °C for a period of 3 h prior to extraction. In the end of the normal drying process of roots, the water residue was around 10.2%. Following the extraction procedures flowers were finely grinded using laboratory equipments [33]. Since extraction kinetics in this study was controlled by the kernel particle size, an important sieving step was carried out to achieve reproducible extraction yield in which the samples were passed through a sieve with mesh sizes between 20 and 30 (particle diameters ranging over 0.60–0.85 mm). The dried samples were kept within sealed bag in the cold and dry place until they were used.

2.3. Perculation extraction

The dried roots (100 g) were perculated with 1.0 L of methanol (MeOH) at room temperature. The whole extract was filtered and solvent was evaporated under vacuum at 40 °C to afford 10 g extract (yield 10%) [34].

2.4. PLE procedure

The equipment used for PLE is very similar to that used in supercritical fluid extraction. Since CO_2 must be liquefied by using cooler circulator device prior to its pumping. It would be easier to operate with methanol than CO_2. Most published studies described a similar arrangement for the PLE equipment [32,33]. For this research, the apparatus was modified using a switching valve after the pumps to enable pumping of the liquid solvent and CO_2, alternatively into the extraction vessel, and the back pressure regulator [33]. The apparatus used for PLE is shown in Fig. 1. The prepared *S. pinnatifida* sample (∼30 g) was loaded into the 100 mL cylindrical stainless steel cell. Commonly used PLE and SFE methods Cotton wool was packed at the exit end of the cell to prevent transfer of solid samples to the tubing and clogging of the system [32]. The PLE method was performed dynamically by passing methanol at different flow rates, temperatures, pressures, and times through the extraction cell. The methanol residue in the cell, tubing, and back pressure regulator was removed with purging the PLE system with CO_2 at the end of each extraction. To reach the mentioned goal, firstly the CO_2 should be converted into the supercritical state and after that, it will dissolve any contamination remaining in PLE system. Methanol was pumped into the system to wash the tubing, every time the system tubing was clogged [33].

2.5. DPPH radical scavenging assay

The antioxidant activity of the extracts was described by 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging capacity of the extracts [35]. DPPH radical scavenging ability of the extracts was evaluated by the method of Singh et al. [36]. DPPH was freshly prepared in methanol to 0.1 mM. Extracts were dissolved in methanol to several concentrations (8, 4, 2, 1, 0.5, 0.25 mg/mL) and 0.1 mL of the solution and 3.9 mL of DPPH solution were mixed and were kept in darkness for 30 min and the absorbance was read at 517 nm. The experiment was carried out in triplicate. The percentage of radical scavenging activity was calculated from this equation: % DPPH radical scavenging = [(Absorbance of control − Absorbance of Sample)/(Absorbance of control)] × 100. Methanol was used as blank, ascorbic acid and butylated hydroxy toluene (BHT) and Quercetin used as positive controls. Absorbance Inhibitor (AI) was calculated as IC_{50} values were calculated using GraphPad Prism_version 5.01 software [37].

2.6. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) reagent contained 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3{\cdot}6H_2O$ and 0.3 M acetate buffer with pH 3.6 [38]. Three mL freshly prepared FRAP reagent mixed with 100 μL of each sample was incubated at 37 °C for 10 min in a water bath. After incubation, the absorbance was measured at 593 nm. Aqueous solutions of known Fe(II) concentration, in the range of 0–1 mM ($FeSO_4{\cdot}7H_2O$), were used for calibration. One milliliter of several concentrations of $FeSO_4$ plus 1 mL of 10 mM TPTZ and 10 mL of 300 mM acetate buffer (pH 3.6) were used for a calibration curve. FRAP values were expressed as mean ± standard error (SE) mmol Fe(II) per gram.

2.7. Determination of total phenolic content

The total phenolic content in the extract from roots of *S. pinnatifida* was determined using Folin-Ciocalteu phenol reagent method [39]. Briefly, 100 μL extract (1000 mg/L) was mixed with diluted

Download English Version:

https://daneshyari.com/en/article/6671129

Download Persian Version:

https://daneshyari.com/article/6671129

Daneshyari.com