Industrial Crops and Products 84 (2016) 72–79

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Novel approaches to optimize extraction processes of ursolic, oleanolic and rosmarinic acids from *Rosmarinus officinalis* leaves

Jurga Bernatoniene[a,*], Ugne Cizauskaite[a], Liudas Ivanauskas[b], Valdas Jakstas[c], Zenona Kalveniene[a], Dalia M. Kopustinskiene[a]

[a] Department of Drugs Technology and Social Pharmacy, Lithuanian University of Health Sciences, Medical academy, Kaunas LT-50161, Lithuania
[b] Department of Analytical and Toxicological Chemistry, Lithuanian University of Health Sciences, Medical academy, Kaunas LT-50161, Lithuania
[c] Department of Pharmacognosy, Lithuanian University of Health Sciences, Medical academy, Kaunas LT-50161, Lithuania

ARTICLE INFO

Article history:
Received 2 September 2015
Accepted 18 January 2016
Available online 8 February 2016

Keywords:
Triterpenoid acid
Phenolic acid
Antioxidant activity
Extraction conditions
Rosemary

ABSTRACT

The leaves of rosemary [*Rosmarinus officinalis* L. (Lamiaceae)] are extensively used as a remedy in medicine and as a condiment in food industry. The beneficial biological effects of rosemary are admitted to its triterpenoid and fenolic acids. However, the optimal extraction conditions of these acids remain unknown. The aim of this study was to evaluate influence of novel extraction methods, their conditions and solvents on the extract yield of ursolic (UA), oleanoic (OA) and rosmarinic acids (RA) from rosmarine leaves, to determine relationship between content of phenolic compounds in obtained extracts and their antioxidant activities. The highest yield of UA (15.8 ± 0.2 mg/g), RA (15.4 ± 0.1 mg/g), and OA (12.2 ± 0.1 mg/g) from rosemary leaves was obtained by ultrasound-assisted extraction with 90% ethanol, with 70% ethanol or water (at pH 9) and by maceration, respectively. Individual extraction conditions are required to increase the yield of OA, UA and RA.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Rosemary [*Rosmarinus officinalis* L. (Lamiaceae)] is common in the regions of Mediterranean climate and widespread in Asia and Africa (Mulas and Mulas, 2005). The plant contains essential oils, phenolic and triterpene acids, flavonoids and other componds (Cuvelier et al., 1996). In this study, we investigated ursolic (UA), oleanolic (OA) and rosmarinic (RA) acids from the leaves of rosemary. Triterpenoid acids – ursolic and oleanolic – have been reported to have a number of biological activities, such as anti-inflammatory, hepatoprotective, gastroprotective, antiulcer, etc., (Kashiwada et al., 2000; Vetal et al., 2013). Our previous results (Bernatoniene et al., 2014) showed that ursolic acid in a dose-dependent manner induced uncoupling of oxidative phosphorylation in the heart mitochondria and significantly suppressed the H_2O_2 production in isolated mitochondria, implying that ursolic acid could be beneficial in cardioprotection. Also triterpenoids have been reported to exhibit cytotoxicity against different cancer cells and antitumor efficacy in preclinical animal models of cancer (Bishayee et al., 2011).

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Petersen and Simmonds, 2003). Due to antialergic, antioxidative and antiinflammatory activities, extracts of plants accumulating rosmarinic acid are widely included in the commercial pharmacological preparations used for internal and external applications (Osakabe et al., 2004). Results of experimental investigations revealed that the antioxidative activity of rosmarinic acid was higher than that of vitamin E or trolox (Lin et al., 2002; Lu and Foo, 2002). Furthermore, the antioxidative activity was shown to depend mostly on the amount of phenolic compounds (Wang et al., 2003). Other investigations demonstrated that the antioxidant activity directly depended on the content of phenolic compounds in the extracts (Tsao et al., 2003). However, the influence of different extraction methods on the yield of phenolic compounds and antioxidant activity has not been investigated yet to our knowledge.

Since different compounds are extracted with different solvents, it is rather complicated to prepare the extract with high content of ursolic, rosmarinic and oleanolic acids. Alcohols (methanol, ethanol) or alcohol–water mixtures are usually used for extraction of phenolic acids (rosmarinic, caffeic acid). Commonly used alcohol concentrations are around 40–60% v/v (Angelov et al., 2007; Wu et al., 2008). Organic extraction solvents—methanol, ethanol, acetonitrile and acetone are used for extraction of triterpene saponins (for example, ursolic, oleic, betulinic acid). In this case, solvent

* Corresponding author.
E-mail addresses: jurgabernatoniene@yahoo.com, jurga.bernatoniene@lsmuni.lt (J. Bernatoniene).

http://dx.doi.org/10.1016/j.indcrop.2016.01.031
0926-6690/© 2016 Elsevier B.V. All rights reserved.

concentration is around 90–100% v/v (Vetal et al., 2012). Fu et al. (2014) has shown that ethanol is the most effective solvent from *n*-butanol, acetone, chloroform, ethyl acetate and water for the extraction yield of UA and OA from *Punica granatum* L. (Lythraceae) (Fu et al., 2014). Most organic solvents (methanol chloroform and acetone) are toxic: therefore for the extraction of ursolic, rosmarinic and oleanolic acids we have chosen ethanol-water mixture and water in the presence of surfactant Tween 20. There is no data to our knowledge that this surfactant could influence the extraction yield of constituents of rosemary leaves and their antioxidant activity. However, in generally, the extraction method could influence biological activity of extracted substances. Ultrasound-assisted extraction and two-phase extraction were used as extraction methods in this study and their efficiency compared with traditional extraction methods percolation and maceration. It was demonstrated that optimal extraction temperature is 40 °C for extraction of UA from pomegranate flowers (Fu et al., 2014). However, other studies showed that maximum yield of ursolic acid from *Ocimum sanctum* L. (Lamiaceae) leaves (16.47 mg UA/g plant material) was obtained at 45 °C (Vetal et al., 2012, 2013). Thus, the aim of this study was to evaluate influence of novel extraction methods, conditions and solvents on the extract yield of ursolic, oleanoic and rosmarinic acids from rosmarine leaves, to determine relationship between content of phenolic compounds in obtained extracts and their antioxidant activities.

2. Materials and methods

2.1. Chemicals and equipment

Dried *Rosmarinus officinalis* leaves were purchased from UAB "Sirdazole", Lithuania. 0.2–1 mm *R. officinalis* leaves were used for the experimentation. Voucher specimen (No. L170712) has been deposited at the Herbarium of the Department of drug technology and social pharmacy, Lithuanian university of health sciences, Lithuania.

Folin–Ciocalteu reagent, 90% DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate anhydrous, Tween ®20, ursolic acid (>97%), oleanolic acid (>97%) and HPLC standards were purchased from Sigma–Aldrich (St. Louis, MO). Rosmarinic acid (>98%) HPLC standard was purchased from ChromaDex (Santa Ana, TX USA). Trolox (>97%) ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and rosmarinic acid (>96%) was purchased from Sigma–Aldrich (St. Louis, MO). Methanol for HPLC analysis was of HPLC grade and purchased from Carl Roth GmbH (Karlsruhe, Germany). Distilled water was used throughout the experiment. AUY922 (>99%) was obtained from Selleckchem. Compounds diluted in DMSO were stored at −20 °C in the dark.

An ultrasonic bath (Bandelin electronic GmbH & Co. KG, Berlin, Germany), vacuum rotatory evaporator Heidolph Laborata 4000 efficient (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), Memmert WNB7 waterbath (Memmert GmbH & Co. KG, Schwabach, Germany), Waters 2695 chromatography system (Waters, Milford, USA), electronic balance HF-200GD (A&D engineering, San Chose, CA, USA), UV–vis spectrophotometer Spectronic Genesys 2 (Thermo Scientific,Waltham, MA, USA) were used.

2.2. Ultrasound-assisted extraction (UAE)

UAE has been carried of internal dimensions 500 mm × 140 mm × 100 mm and tank capacity 6 L approximately, with an ultrasonic peak output of 200 W, equipped with digital temperature controller/indicator. The experiment was performed by adding 1 g of powdered plant material into 5–20 mL of solvent in a flask. The optimization of the extraction parameters was conducted with different experimental conditions. The extract passed through a 0.2 μm membrane filter for HPLC analysis.

2.3. Percolation

90% ethanol (v/v) was used as a solvent. The duration of percolation process was 48 h at room temperature. Powdered plant material was moistened with solvent (ratio 1:1) and allowed to swell for 4 h. The swollen material was transferred to the percolator, appropriate amount of solvent was added and macerated for 48 h. Then the first concentrated extract was collected (85% of the finished product). Extraction of plant material was continued until complete exhaustion of the constituents. After the percolation, the second volume of extract was vaporized using vacuum rotatory evaporator Heidolph Laborata 4000 efficient (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) to remove the solvent until the required volume. Both extracts were mixed thoroughly and the obtained extract passed through a 0.2 μm membrane filter for HPLC analysis.

2.4. Maceration

Dried powdered plant material (5 g) was macerated using 25 mL of 90% ethanol (v/v) at room temperature for 48 h. The extract passed through a 0.2 μm membrane filter for HPLC analysis.

2.5. Two-phase extraction (TPE)

The extraction was performed in Memmert WNB7 waterbath (Memmert GmbH & Co. KG, Schwabach, Germany). 1 g of powdered plant was added into 15 mL of distilled water or water/surfactant mixture. For the water/surfactant mixtures the concentration of the used surfactant was 7 wt-%. Experiments were performed in fixed time intervals: 90, 180, 300 min. The extract passed through a 0.2 μm membrane filter for HPLC analysis.

2.6. HPLC conditions for determination of RA

HPLC analyses were carried out using a model Waters 2695 chromatography system (Waters, Milford, USA), equipped with Waters 996 PDA detector. Data were collected and analyzed using personal computer and the Empower 2 chromatographic manager system (Waters Corporation, Milford, USA).

For determination of RA the ACE 5C18 250 × 4.6 mm (Advanced Chromatography Technologies, Aberdeen, Scotland) column was used. The mobile phase consisted of solvent A (methanol) and solvent B (0.5% (v/v) acetic acid in water). The linear gradient elution profile was as follows: 95%A/5%B 0 min, 40%A/60%B 40 min, 10%A/90%B 41–55 min, 95%A/5%B 56 min. The flow rate was 1 mL/min and injection volume was 10 μL. Absorption was measured at 329 nm. Quantification was carried out by the external standard method. The linear calibration curve was made (R^2 = 0.999918), peak areas were used for quantification.

2.7. HPLC conditions for determination of UA and OA

The separations of UA and OA were carried out using the ACE 5C18 250 × 4.6 mm (Advanced Chromatography Technologies, Aberdeen, Scotland) column. The mobile phase was composed of methanol and water (90/10, v/v). The flow rate was 0.6 mL/min and injection volume was 10 μL. Absorption was measured at 203 nm. Quantification was carried out by the external standard method and calibration curves were obtained (OA R^2 = 0.999383, UA R^2 = 0.998872).

Download English Version:

https://daneshyari.com/en/article/4512384

Download Persian Version:

https://daneshyari.com/article/4512384

Daneshyari.com