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Effect of different extraction techniques on quantification of oleanolic and ursolic acid in *Lamii albi flos*

Magdalena Wójciak-Kosior[a,*], Ireneusz Sowa[a], Ryszard Kocjan[a], Renata Nowak[b]

[a] Department of Analytical Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland
[b] Department of Pharmaceutical Botany, Medical University, Chodźki 1, 20-093 Lublin, Poland

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ABSTRACT

The classic techniques such as: maceration (ME), Soxhlet (SE) and heat reflux extraction (HRE) were compared with modern techniques: ultrasonic extraction (UE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) for their extraction efficiency of two triterpenic acids from *Lamii albi flos*. Quantifications of oleanolic and ursolic acid in obtained extracts were performed by HPLC method on a RP-18 column with use of mobile phase consisting of acetonitrile–water–1% phosphoric acid (85:15:0.5, v/v/v), the flow rate was 0.8 mL/min and temperature was 10 °C.

MAE in closed system was the most effective technique. The best results for ursolic acid were obtained with use of MAE in closed system for 10 min and 100% of generator power. Oleanolic acid was better extracted with use of milder conditions (30% generator power and 30 min). Ultrasonic assisted extraction proved to be noteworthy, alternative method due to its simplicity, inexpensive equipment and relatively good extraction efficiency.

1. Introduction

Lamium album L., known as "white dead nettle" is a perennial herb with various biological activities. It is widely used in folk and officinal medicine (Bremness, 1995; Weiss, 1988) to treatment of skin inflammation and alimentary or urinary tract diseases (Lutomski, 2002). However, its anti-inflammatory, bacteriostatic, astringent and anti-septic activities are especially utilized in menorrhagia, uterine hemorrhage, vaginal and cervical inflammation and leucorrhoea treatment (Ożarowski and Jaroniewski, 1987); therefore it is a common component of herbal mixtures used in the women's diseases. *Lamii albi flos* also exhibits antioxidant and antiproliferative properties (Trouillas et al., 2003).

These plants belong to the *Lamiaceae* family which is known as a source of large amounts of triterpenes, especially oleanolic and ursolic acids (Janicsák et al., 2006).

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) and its isomer, oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid) are bioactive compounds with confirmed pharmacological properties. In recent years they became the subject of many publications because of their various activities combined with low toxicity. In the literature there are numerous data on their properties which include anti-inflammatory, hepatoprotective, anti-tumor, anti-viral, anti-HIV, anti-microbial, anti-fungal, anti-diabetic, gastroprotective, and anti-hyperlipidemic effects (Liu, 2005; Siqueira et al., 2007; Ma et al., 2005; Ovesná et al., 2006; Sánchez et al., 2006; Yu et al., 2006).

Sample preparation is the primary, essential step in most analytical procedures. The technique applied for the isolation of investigated component should be exhaustive, reproducible, fast, simple, inexpensive and environmentally friendly where possible. There is no universal method for the isolation of various types of compounds from the sample; therefore an important issue is verification of different techniques, both classic and modern for their efficiency of extraction of target component.

Many extraction techniques, both conventional, such as Soxhlet extraction (Büchele et al., 2003; Dominguesa et al., 2011; Janicsák et al., 2006; Kontogianni et al., 2009; Liao et al., 2005; Mehta et al., 2010; Yang et al., 2007), maceration (Banerjee et al., 2006; Gbaguidi et al., 2005; Novotny et al., 2003), reflux extraction (Chen et al., 2003; Zhao et al., 2007) and modern, e.g., ultrasonic extraction (UE) (Banik and Pandey, 2008; Chen et al., 2011; Feng et al., 2008; Lan et al., 2010; Lee et al., 2009; Qi et al., 2006; Wang et al., 2008), accelerated solvent extraction (ASE) (Shen and Shao, 2005) and microwave-assisted extraction (MAE) (Diouf et al., 2009; Pai et al., 2011; Sánchez-Ávila et al., 2009; Xia et al., 2011) have been used to extract different triterpenes and their derivatives from plant material. However, there are no large number of research describing isolation of oleanolic and ursolic acids and, generally, the authors focus on optimizing one specific procedure (Banik and Pandey, 2008; Li et al., 2011; Sánchez-Ávila et al., 2009; Xia et al., 2012;

* Corresponding author. Tel.: +48 815357350.
E-mail address: kosiorma@wp.pl (M. Wójciak-Kosior).

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Fang et al., 2010). Tarvainen et al. (2010) investigated efficiency of supercritical fluid (CO_2) extraction in comparison to Soxhlet and obtained results were better for classic methods.

The present study compares commonly used extraction techniques for their extraction efficiency of oleanolic and ursolic acid from *L. albi flos*. Accelerated solvent extraction (ASE) for the isolation of these compounds was described for the first time.

2. Materials and methods

2.1. Materials and reagents

Oleanolic and ursolic acid standards were purchased from Sigma (St. Louis, MO, USA). HPLC-grade ethyl acetate, methanol, acetonitrile and acetone were from Merck (Darmstadt, Germany). *L. albi flos* (Flos, Mokrsko, Poland; serial number: 2022, the expiry date: 02.2013) was obtained from local market.

2.2. Sample preparation

L. albi flos was pulverized, accurately weighted (2.0 g) and extracted with acetone using of various techniques (Soxhlet extraction, heat reflux extraction, maceration, ultrasonic extraction, microwave-assisted extraction and accelerated solvent extraction). The procedures were repeated three times. Plant material was washed twice with small portion (5–10 mL) of acetone after each extraction.

2.2.1. Soxhlet extraction (SE)

Dried plant material was placed in cellulose extraction thimbles and extracted in Soxhlet apparatus with 200 mL of acetone for 20 h. Next, the obtained extract was filtered, concentrated by evaporation of solvent under vacuum and transferred into a 5 mL volumetric flask. Finally, the volume was made up with the extraction solvent.

2.2.2. Heat reflux extraction (HRE)

Dried plant material was mixed with 100 mL of acetone into a round-bottom flask.

The flask was connected with water cooler and heated in a water bath for 30 min at 60 °C. This procedure was conducted three times with fresh portions of solvent. The extracts were combined, filtered, concentrated and transferred into a 5 mL volumetric flask.

2.2.3. Maceration extraction (ME)

Dried plant material was mixed with 100 mL of acetone and shaken for 10 h. The procedure was conducted in three cycles with fresh portions of solvent. The extracts were combined, filtered, concentrated and transferred into a 5 mL volumetric flask.

2.2.4. Ultrasonic extraction (UE)

Ultrasound-assisted extraction was performed with use of ultrasonic bath RU102H (Sonorex, Bandelin). Samples were placed into a conical flask, into which 50 mL of acetone was added and sonicated for 15 min at temperature of 30 °C and 50 °C, respectively. Extraction was carried out three times with fresh portions of solvent in the above conditions. The extracts were combined, concentrated, filtered and transferred into a 5 mL volumetric flask. Finally, the volume was made up to the mark with the extraction solvent.

2.2.5. Accelerated solvent extraction (ASE)

ASE was performed on a Dionex ASE 100 system (Dionex Corp., Sunnyvale, CA, USA). Samples were placed into stainless steel extraction cell and extracted in 2 static cycles, at the pressure of 100 bar at two different temperatures: 40 °C and 120 °C. After extraction, the extraction cell was flushed using 65% of cell volume during 90 seconds purging with N_2. The obtained extract was concentrated and transferred into a 5 mL volumetric flask which was brought up to its volume with the same solvent.

Table 1
Calibration data for determination of triterpenic acids.

Parameters	Oleanolic acid	Ursolic acid
Concentration range (μg/mL)	5–100	5–100
Correlation coefficient (r^2)	0.9998	0.9994
Linear regression equation	$y = 17578x + 12{,}514$	$y = 18485x - 14{,}147$
RSD values of peak area (%)	0.71–1.52	0.63–1.38
LOD (μg/mL)	0.14	0.15
LOQ (μg/mL)	0.45	0.47
Recovery (%)	98.3–100.2	98.6–100.9

2.2.6. Microwave-assisted extraction (MAE)

MAE was carried out using Plazmotronika UniClever (350W) BMZ I (Wrocław, Poland). Plant material was placed into the extraction vessel and extracted with 40 mL of acetone using various generator powers (30%, 65%, 100%) during 10, 20 and 30 min. The obtained extracts were filtered, evaporated, transferred into 5 mL flasks and made up to the mark with acetone.

2.3. HPLC analysis

Quantitative HPLC analysis was performed using a Waters chromatograph (Milford, MA, USA) with binary pump, an online degasser, thermostat, Rheodyne injector (20 μL loop) and Waters 2996 PAD detector. The analytes were separated on LiChrospher 100 (Merck, Darmstadt, Germany) C18 reversed-phase column (25 cm × 4.0 mm i.d., 5 μm particle size) with use of mobile phase consisting of acetonitrile–water–1% phosphoric acid (85:15:0.5, v/v/v). Elution was performed with a 0.8 mL/min flow rate, at 10 °C. The triterpenic acids were detected at 200 nm. All samples were filtered through a 0.45 μm membrane filter before injection.

Stock solution containing 200 μg/mL of ursolic acid and 200 μg/mL of oleanolic acid was prepared by dissolving the standards in acetone and diluted to a series of appropriate concentrations to construct the calibration curve.

3. Results and discussion

3.1. HPLC analysis

Chromatographic conditions to separate oleanolic and ursolic acids in *L. albi flos* were established on the basis of our earlier study and only slightly modified. The mobile phase composed of acetonitrile–water–1% phosphoric acid (85:15:0.5, v/v/v), the flow rate was 0.8 mL/min. The analyses were performed at 10 °C. Oleanolic and ursolic acid are closely related structural isomers and their separation is rather difficult. The lowering of temperature of column improved the resolution of both acids (Wójciak-Kosior and Sowa, 2009). The significant influence of temperature on separation triterpenic compounds was also noted by Apers et al. (1998) and Sánchez-Ávila et al. (2009). Under above conditions oleanolic and ursolic acids were well separated from the other components of the extract. Peaks were identified by comparison of retention times and UV spectra with those of the corresponding standards.

Table 2
The average content of oleanolic and ursolic acid (μg/g dry plant material ± SD) obtained with use of classic techniques ($n = 3$).

Method	Maceration	Soxhlet	HRE
OA	5.4 ± 0.2	6.9 ± 0.2	9.4 ± 0.3
UA	39.1 ± 1.4	55.4 ± 1.8	67.3 ± 1.8