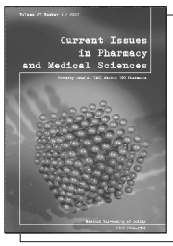

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Determination of oleanolic and ursolic acid in bilberries (*Vaccinium myrtillus* L.)

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ABSTRACT

Vaccinium myrtillus (*Ericaceae*) is used in therapy of various illnesses. Oleanolic and ursolic acids are bioactive triterpenes, which can influence the biological activity of this plant. High-performance thin layer chromatography combined with densitometry was used to determine the content of these compounds in ethanolic extracts from the leaves and fruits of *Vaccinum myrtillus L.* The samples of extracts were treated of 1% iodine solution in chloroform and next separated on HPTLC Si 60 F_{254} plates using mixture of toluene-petroleum ether-diethyl ether 15:15:10 (v/v) as a mobile phase. After drying, the plates were sprayed with 10% (v/v) H_2SO_4 in ethanol and heated for 2-3 minutes at the temperature of 120°C. The quantification was carried out by densitometric scanning in flurescence/reflectance mode at ë = 400 nm. The determined contents of oleanolic and ursolic acid were 0.18 mg/g and 0.33 mg/g of dry herb in fruits and 1.16 mg/g and 0.28 mg/g in leaves of *Vaccinium myrtillus*, respectively.

Keywords: *Vaccinium myrtillus*, oleanolic acid, ursolic acid, HPTLC with densitometry

INTRODUCTION

Bilberries (*Vaccinium myrtillus* L.), popularly known as European blueberry, huckleberry or whortleberry, belong to the *Ericaceae* family. It is a shrubby, perennial plant which can be found in Central and Northern Europe, Asia and North America [1,8].

There is a strong scientific evidence of the positive effect of dietary intake of products from bilberries on human health. These plants possess many important biological and pharmacological properties including antioxidant, astringent and antiseptic, ability to decrease the permeability and fragility of capillaries, inhibition of platelet aggregation, inhibition of urinary tract infection [9,7]. Bilberry leaves are an effective treatment for diarrhea and enteritis, particularly beneficial in therapy of young children and infants. Extracts of *V. myrtillus* can be used against the threadworms [8]. In addition, bilberry leaves contain a substance lowering blood sugar levels. Therefore, it can be used in the treatment of diabetes, especially in its earlier and milder forms [9]. Recent research shows that *V. myrtillus* has a positive effect in the inhibition of cancer cell growth and visual disorders [3,4].

The wide spectrum of therapeutic applications of billberies is related to presence of active constituents, such as flavonoids, anthocyanins, vitamins, sugars, pectins, catechins, tannins, iridoids, and phenolic acids [1,2].

This paper presents the results of determination of oleanolic and ursolic acid in leaves and fruits of *Vaccinium myrtillus*. These triterpenes are commonly known owing to their various biological activity such as anti-inflammatory, antiallergic, antiviral, hepatoprotective, gastroprotective, immunoregulatory effects [5,6] and they can influence pharmacological properties of this plant .

MATERIALS AND METHODS

Chemicals and standard solutions

All solvents and reagents were at least pro analysis grade from Polish Reagents (POCh, Gliwice, Poland). Oleanolic and ursolic acids were purchased from Chromadex (Santa Ana, CA,USA). Stock solutions of both compounds at concentration 0.5 mg/mL were prepared in methanol. To quantification, standard solutions were prepared by dilution of the stock solution with methanol. Leaves and fruits of *Vaccinium myrtillus* L. (Herbapol, Lublin, Poland) were obtained in local market.

Sample preparation

The fruits and leaves of *Vaccinium myrtillus* were powdered and accurately weighted. Each sample (10.1 g) was twice extracted with ethanol (2x100 mL) within 12 hours at room temperature and next four times in an ultrasonic bath with use of 50 mL of ethanol (4x15 min). The obtained ex-

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tracts were combined and evaporated to dryness. The residue was dissolved in methanol, filtered and finally made up to 25 mL for leaves and 50 mL for fruits in volumetric flasks.

TLC conditions

HPTLC was performed on 20 cm × 10 cm silica gel 60 F_{254} plates from Merck (Darmstadt, Germany). Five µL of standard solutions and 3 µL (leaves) or 6 µL (fruits) of extracts were applied as 8 mm bands by means of a CAMAG (Muttenz, Switzerland) ATS4 automatic TLC sampler. Pre-derivatization with 1% iodine solution in chloroform, according to procedure proposed by Wójciak-Kosior [10] was used to improve the separation of the analyzed compounds. Next the plates were developed with mixture of toluene-petroleum ether-diethyl ether 15:15:10 (v/v) on a distance of 70 mm in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) previously saturated with vapours of the mobile phase. After drying, the plates were sprayed with 10% (v/v) H_2SO_4 in ethanol and heated for 2-3 minutes at the temperature of 120°C. The quantification was carried out by densitometric scanning (Desaga CD-60, Heidelberg, Germany) in flurescence/reflectance mode at λ = 400 nm (slit dimension: 4mm×1 mm). The source of radiation was mercury lamp. A cut-off filter at λ = 420 nm was used.

RESULTS AND DISCUSSION

Ursolic and oleanolic acid are natural pentacyclic triterpenes with confirmed pharmacological activity [5,6]. They are common constituents of many medicinal herbs and plants and can strongly influence their therapeutic properties. In presented publication, the chromatographic conditions to quantification of oleanolic and ursolic acids in *Vaccinium myrtillus* are described.

The investigated compounds are isomers that differ only in the position of methyl group and their analysis by TLC is difficult; therefore, the derivatization process was necessary for the separation of both acids. Derivatization with iodine solution is simple, fast and can be used directly on the chromatographic plates. This technique was successively used in analysis of plant extract [10].

The adsorbent and the eluent composition for the determination of triterpenes in *Vaccinium myrtillus* were optimized experimentally. The obtained spots were dense, compact and well separated from the other compounds (Fig. 1, 2).

The identification of triterpenic acids was done based on hR_f values. The purity of the peaks in the sample was ascertained by comparison of absorption spectra with those obtained from the standards.

A calibration plot was established by analysis of standard solution at five different concentrations in the ranges 115-2875 ng/spot for oleanolic acid and 45-450 ng/spot for ursolic acid. The mean peak areas from ten different plates were taken for the construction of calibration curve. The data were analyzed by linear regression least square model and showed a good linear relationship over the tested range

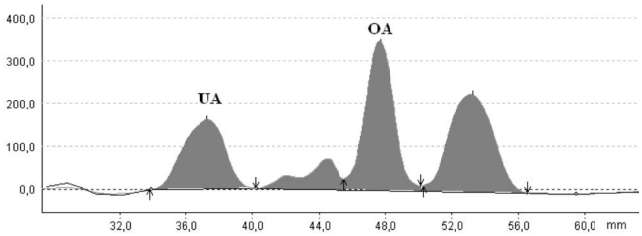

Fig. 1. Densitogram of *Vaccinium myrtillus* extract (leaves) obtained after derivatization with 10% H_2SO_4 in flurescence mode at λ = 400 nm

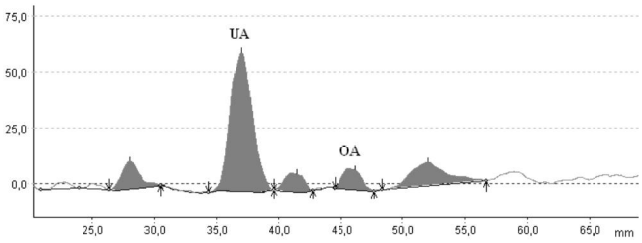

Fig. 2. Densitogram of *Vaccinium myrtillus* extract (fruit) obtained after derivatization with 10% H_2SO_4 in flurescence mode at λ = 400 nm

(*r* = 0.9973 for oleanolic acid and *r* = 0.9984 for ursolic acid). The linear regression equations for oleanolic and ursolic acids were y = 0.24x – 8.66 and *y* =1.25x – 9.03, respectively. An example of calibration curve is presented on Fig. 3. Limit of detection and limit of quantification were calculated by the use of the equations: LOD =3xN/b and LOQ=10x N/b, where N is the standard deviation of the peak areas taken as a measure of noise, and b is the slope of the calibration curve. The limits of detection were 31 ng/spot for oleanolic acid and 13 ng/spot for ursolic acid. The limits of quantification were 103 ng/spot and 43 ng/spot, respectively.

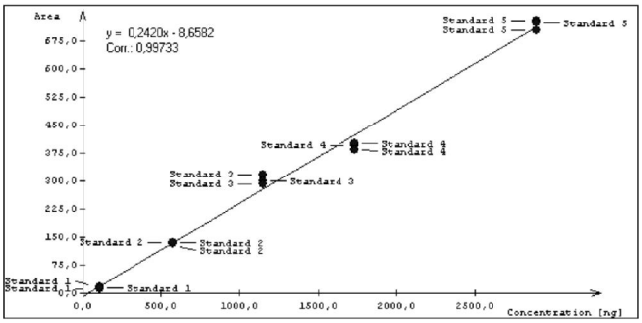

Fig. 3. The calibration curve for determination of oleanolic acid

The obtained results of quantitative analysis of the extracts are presented in Table 1.

Table 1. The results of quantification of triterpenic acids in *Vaccinium myrtillus* (mg/g of dry plant material)

Sample of extract	Oleanolic acid	Ursolic acid
Vaccinium myrtillus L. (leaves)	1.16 mg/g	0.283 mg/g
Vaccinium myrtillus L. (fruits)	0.18 mg/g	0.331 mg/g

CONCLUSION

HPTLC combined with densitometry is a rapid and cost-effective tool for analysis of plant extracts. This method was successfully applied to identification and quantification of oleanolic and ursolic acid in fruits and leaves of *Vaccinium myrtillus*. Both triterpenes can influence the biological activity of bilberries.

REFERENCES

1. Anonymous: Monograph. *Vaccinium myrtillus* (bilberry). *Altern. Med. Rev*., 6, 500, 2001.
2. Baj A et al.: Qualitative and quantitative evaluation of *Vaccinium myrtillus* anthocyanins by high-resolution gas chromatography and high-performance liquid chromatography. *J. Chromatography, 279, 365,* 1983.
3. Canter PH, Ernst E: Anthocyanosides of *Vacinnium myrtillus* (bilberry) for night visions a systematic review of placebo controlled trials. *Surv. Ophthalmol.,* 49, 38, 2004.
4. Garzón GA et al.: Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (Vaccinium meridionale Swartz) from Colombia. *Food Chem*., 122, 980, 2010.
5. Liu J: Pharmacology of oleanolic acid and ursolic acid. *J. Ethnopharmacol*., 49, 57, 1995.
6. Liu J: Oleanolic acid and ursolic acid: research perspectives. *J. Ethnopharmacol*., 100, 92, 2005.
7. Madhavi DL et al.: Isolation of bioactive conctituents from *Vaccinium myrtillus* (bilberry) fruits and cell cultures. P*lant Sci.*, 131, 95, 1998.
8. Noculak-Palczewska A: Porównanie wartości terapeutycznej preparatów z owoców borówki. Post. Fitoter., 1, 10, 2004.
9. Valentová K et al.: Cytoprotective effect of a bilberry extract against oxidative damage of rat hepatocytes. *Food Chem*., 101, 912, 2007.
10. Wójciak-Kosior M: Separation and determination of closely related triterpenic acids by high performance thin-layer chromatography after iodine derivatization. *J. Pharm. Biomed. Anal*., 45, 337, 2007.