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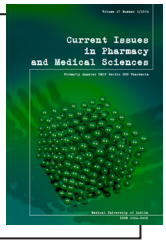

Identificatin and quantiative analysis of amyrins in *Humulus lupulus* L.

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ABSTRACT

In the paper, the identification and quantification of α- and β-amyrin in five varieties of *Humulus lupulus* is described. The plant samples were extracted with ethyl acetate and analyzed using of high performance liquid chromatography (HPLC) with PAD detection. The separation was achieved on RP 18 column, at 2 mL/min flow rate and at temperature of 35°C. Acetonitrile was used as a mobile phase. The established calibration curves and the other validation parameters: linearity (correlation coefficient r > 0.9988) and precision (RSD values ranged from 0.14 to 1.81%) were found to be satisfactory for the proposed method. The content of α- and β-amyrin strongly depended on varieties of *H. lupulus* and amounted from 72.7 to 232.5 μg/g and from 77.9 to 176.9 μg/g of dry plant material, respectively.

INTRODUCTION

Humulus lupulus L. (*Cannabaceae*) popularly known as a hop is a flowering plant, native to Europe, western Asia and North America. Its female inflorescences are commonly called cones. In Poland, it commonly occurs on the moist thickets, alder and alluvial forests, and in old parks, gardens and slums [6]. The plant is well known in the brewing industry because it provides the characteristic aroma and flavor and is used to preserve beer. Moreover, the cones extracts are used in medicine for ulcers and chronic wounds, due to strong antibacterial and antifungal action [3]. They are also applied during inflammation of face and scalp for people with allergy, especially for skin with seborrhea and acne. The hop alleviates hyperactivity and is recommended as a mild sedative in sleeping disorders [5,11]. In recent years, the estrogenic, antioxidant and potential anticancer properties was also reported [1,2,7,8]

The therapeutic activity of *H. lupulus* is related to a presence of biologically active substances such as: terpenes, chalcones, flavonol glycosides, aromatic resin (lupulin), volatile oil, bitter acids and catechins [10,12].

β- and α-amyrin (Fig. 1) belong to triterpenoids which widely exist in food, medicinal herbs and other plants. There are a lot of reports about their antitumor, anti-inflammatry, analgesic, antiplatelet and antibacterial effect [4,9,13].

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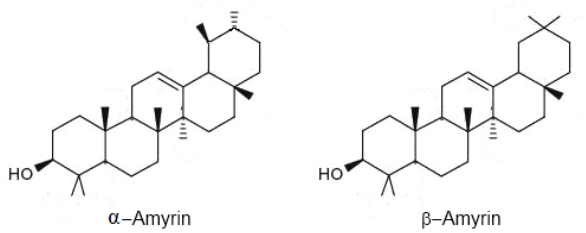

Figure 1. The chemical structure of α-amyrin and β-amyrin

The objective of our investigation was analysis of extract from different varieties of *H. lupulus* for presence of amyrins. These triterpenes can influence on anti-inflammatory and antioxidant activity of extract from *H. lupulus*.

MATERIALS AND METHODS

Chemicals

β- and α-amyrin standards were purchased from Sigma (St. Louis, MO, USA). The purities of standards were 98.5%. HPLC-grade methanol and acetonitrile were from Merck (Darmstadt, Germany). Water was deionized and purified by ULTRAPURE Milipore Direct-Q® 3UV-R (Merck).

Standard and sample preparation

Stock solutions of α- and β-amyrin were prepared by dissolving 0.48 mg α-amyrin and 0.57 mg β-amyrin in 10 mL of methanol (final concentrations were: 48 μg/mL and

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57 µg/mL, respectively). Standard working solutions were prepared by dilution of the stock solutions with methanol.

Five varieties of *H. lupulus* cones: *magnost*, *magnum, junga, sybilla* and *lubelski* were collected by IUNG from Puławy (October 2013). Plant material was pulverized and accurately weighted (5 g). Samples were extracted two times with 2 × 25 mL portion of ethyl acetate in ultrasonic bath at temperature of 35°C (2 × 30 min.). The obtained extracts were combined, evaporated to dryness, dissolved in small amount of methanol and then filtered to measuring flask. Finally, the volume was made up by methanol to 10 mL.

Chromatographic conditions

Quantitative HPLC analysis was conducted using VWR Hitachi Chromaster 600 chromatograph (Merck, Darmstadt, Germany) with pump (5160), a degasser, thermostat (5310), autosampler (5260), DAD detector (5430) and EZChrom Elite software.

The extracts were separated on LiChrospher 100 (Merck, Darmstadt, Germany) C18 reversed-phase column (25 cm × 4.0 mm i.d., 5 µm particle size) at flow rate of 2.0 mL/min with use of isocratic elution. Mobile phase consisted of acetonitrile. The temperature of autosampler and thermostat was 35°C. The quantification was conducted at 200 nm.

RESULTS AND DISCUSSION

Chromatographic conditions to determine α- and β-amyrin were established experimentally. Both amyrins have a strong retention on octadecyl silica, thus the solvents with high elution strength such as methanol, acetonitrile or propanol should be used for their analysis. The best shape of chromatographic peaks as well as sensitivity of determination were obtained for pure acetonitrile. Under conditions described above, amyrins were well separated from the other components of the samples (Fig. 2). Peaks were identified by comparison of retention times and UV spectra with those of the corresponding standards. Times of retention for β-amyrin and α-amyrin were: 17.14 ± 0.12 min and 19.14 ± 0.18 min, respectively.

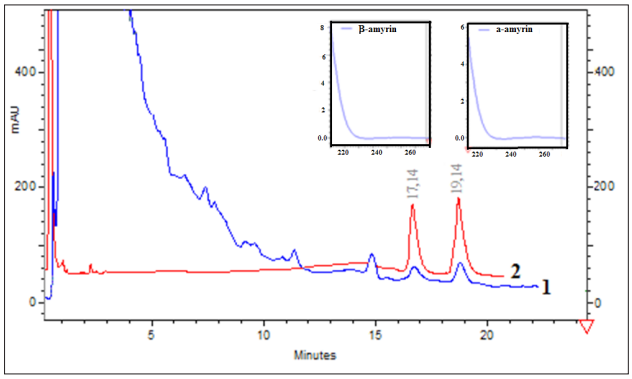

Figure 2. The chromatogram of extract from *H. lupulus* (1) and standard of α-amyrin and β-amyrin (2)

The calibration curves were constructed on the basis of peak areas (n=3). The obtained linear regression equations and the validation parameters such as: linearity, precision expressed as a relative standard deviation, limit of detection and quantification are summarized in Table 1. Samples of extracts were analyzed in triplicate and the amounts of triterpenes were calculated from the calibration plot. The data were analyzed by linear regression equation.

Table 1. The obtained validation parameters

Component	α-amyrin	β-amyrin
Regression equation	y = 17269701x – 140355	y = 20683915x – 106522
Correlation coefficient	r = 0.9988	r = 0.9993
RSD values	0.14-1.70%	0.30-1.81%
Limit of detection (LOD) (µg/mL)	0.49	1.12
Limit of quantification (LOQ) (µg/mL)	1.49	3.39

The both investigated compounds were detected in cones of all varieties of *H. lupulus*. The content of α- and β-amyrin found in cones of *H. lupulus* using the presented method were from 72.7 to 232.5 µg/g and from 77.9 to 176.9 µg/g of dry plant material, respectively. The obtained results are presented in Table 2. The high variation of amyrin content was observed depending on varieties. The highest total amount of investigated triterpenes was found in var. *junga* while the lowest concentration was determined in var. *magnost* (Fig.3).

Table 2. The results of quantification of investigated triterpenes in *H. lupulus* L.

Plant material	α-amyrin (µg/g of dry plant material ±SD)	β-amyrin (µg/g of dry plant material ±SD)
H. lupulus var. *magnost*	72.7 ± 1.2	80.2 ± 0.8
H. lupulus var. *magnum*	116.7 ± 0.5	140.8 ± 0.59
H. lupulus var. *junga*	232.5 ± 1.0	176.9 ± 1.36
H. lupulus var. *sybilla*	143.4 ± 2.9	77.9 ± 1.30
H. lupulus var. *lubelski*	151.2 ± 2.4	80.7 ± 0.80

The investigated compounds may have a positive influence, directly or indirectly by synergistic action, on its anti-inflammatory and antioxidant properties.

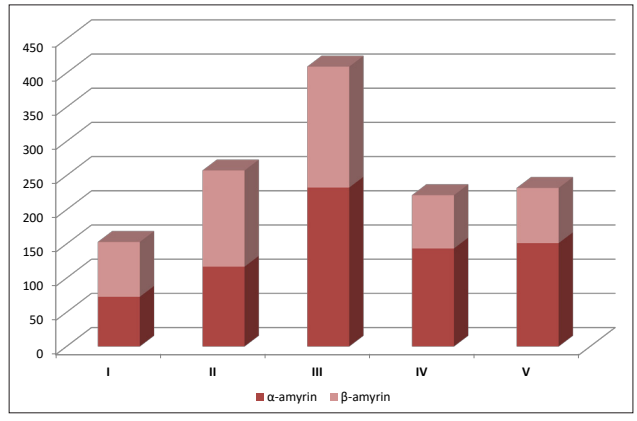

Figure 3. The comparison of α-amyrin and β-amyrin content in different varieties of *H. lupulus*: I – *magnost*, II – *magnum*, III – *junga*, IV – *sybilla* and V – *lubelskis*

CONCLUSION

High performance liquid chromatography with spectrophotometric detection (HPLC-DAD) was successfully

applied for analysis of two amyrins in different varieties of *H. lupulus*.

The obtained validation parameters such as high linearity (correlation coefficient $r \geq 0.997$) and high precision (RDS < 1.70% and < 1.81% for α- and β-amyrin, respectively) occurred satisfactory for quantitative determination. Both α- and β- amyrin were detected in investigated plant material. Their contents were from 72.7 to 232.5 μg/g and from 77.9 to 176.9 μg/g of dry herb, respectively. The significant variation of investigated compounds amount between varieties was found.

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