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INFLUENCE OF THE EXTRACTION PARAMETERS OF ANTOCIANES FROM THE FRUITS OF *ARONIA MELANOCARPA* (MICHX.) ELLIOTT IN THE EXTRACTION ASSISTED WITH ULTRASOUND. PRELIMINARY RESULTS

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Abstract
In recent years, more and more studies have highlighted the potential of fruits of Aronia melanocarpa (Michx.) Elliott for the pharmaceutical and cosmetic industry, due to the high content of polyphenols and anthocyanins. Currently, the use of agricultural production of Aronia melanocarpa is mainly done through the use of fruit juice or powders in the food industry. In the present study we set out to achieve a superior route of fruit valorization by obtaining extracts for the pharmaceutical and cosmetics industry. For ultrasound-assisted extraction, Aronia melanocarpa fruits were pre-processed (drying, grinding, sieving) and then subjected to ultrasonic-assisted solvent extraction.
The study carried out in this phase of the experiment followed the influence of the concentration of the solvent (water/ethanol ratio) on the anthocyanin content and the chromatographic profile of the extract. The chromatographic profile was obtained using the HPTLC method. The analysis material was represented by 3 extracts obtained under various experimental conditions, from the species Aronia melanocarpa *(Michx.) Elliot fruits.*
The best HPTLC chromatographic imprint was shown for Aronia fruit extracts prepared in 3:1 water-ethanol mixture, at amplitude of 40%. In the other experimental variants the anthocyanin content was low.

Keywords: pharmaceutical and cosmetic industry, polyphenols and anthocyanins, ultrasonic assisted extraction.

1. INTRODUCTION

Aronia melanocarpa (Michx.) Elliott, black chokeberry black (*Rosaceae*), is a shrub native to North America, naturalized in Europe and Asia. It is widely grown as an ornamental plant (through flowers, leaves), but especially for its fruit with uses in the fruit growing/food industry (juices, jams, wines) (Valcheva-Kuzmanova et Belcheva, 2006; Kokotkiewicz et al., 2010; Banjari et al., 2017). From a phytochemical point of view, *Aronia melanocarpa* fruits (*Aroniae melanocarpae* fructus) mainly contain active antioxidant components: anthocyanosides (cyanidol-3-O-galactoside, cyanidol-3-O-glucoside, cyanidol-3-O-arabinoside, cyanidol-3-O-xyloside), proanthocyanidols, flavonols (quercetol), flavonozides (quercetol-3-O-glucoside, quercetol-3-O-rutinozide), resveratrol, catechin tannin [(-) - epicatechol], triterpenoids, polyphenolcarboxylic acids

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(chlorogenic acid, neochlorogenic acid), vitamins (ascorbic acid) (Valcheva-Kuzmanova et Belcheva, 2006; Yu et al., 2007; Kokotkiewicz et al., 2010; Banjari et al., 2017; Denev et al., 2018; Gralec et al., 2019; Kobus et al., 2019). The medicinal product *Aroniae melanocarpae* fructus, has remarkable pharmacological actions: antioxidant, antimutagenic, radioprotective, antitumor, immunomodulatory, cardioprotective, antidiabetic, hepatoprotective, uricosuric, gastroprotective, anti-inflammatory, antimicrobial and antiviral (Valcheva-Kuzmanova et Belcheva, 2006; Krajka-Kuźniak et al., 2009; Kokotkiewicz et al., 2010; Banjari et al., 2017; Cebova et al., 2017; Denev et al., 2018; Kobus et al., 2019; Wang et al., 2019). In recent years, there has been an increase in the demand for natural extracts obtained by "green", non-conventional raw material processing techniques. These techniques have also been developed to solve the problems that arise when using conventional extraction methods. The "green" technique can be defined as an "extraction method that is based on the discovery and design of extraction processes that will reduce energy consumption, allowing the use of alternative solvents and renewable natural products and ensuring a safe and high quality extract / product". Compared to the classic maceration, the reasons why ultrasound assisted extraction (UAE) is considered a "green" or "ecological" technique are represented by the reduced consumption of fossil energy and the reduction of solvent use resulting in higher extraction yields, in a relatively short time (Carrera et al., 2012; Chemat et al., 2015; Tiwari, 2015). Ultrasonic assisted extraction has high efficiency, although it requires low energy, small amounts of solvent and short periods of time for the extraction process. The main advantage of this method is the production of the cavitation phenomenon involving local pressures and temperatures of hundreds of atmospheres and thousands of degrees Celsius. The aim of this study was to identify the best extraction parameters in order to obtain an optimal amount of anthocyanins.

2. MATERIALS AND METHODS

The material to be analyzed was represented by the fruits of the species *Aronia melanocarpa* (Michx.) Elliott (NERO variety) cultivated at the Institute for Research and Development for Pomiculture (ICDP) Pitești-Mărăcineni, Arges County. For ultrasound-assisted extraction, the fruits of *Aronia melanocarpa* (Michx.) Elliot were preliminary processed by drying, grinding and sieving. The extract was accomplished with an ultrasonic extraction equipment model HIELSCHER UIP 1000hDT in order to obtain the following experimental variants:
Aronia_1: water:alcohol ratio – 3:1; plant:solvent ratio – 1:10; Time: 5 min; Amplitude: 40%;
Aronia_2: water:alcohol ratio – 3:1; plant:solvent ratio – 1:10; Time: 5 min; Amplitude: 60%;
Aronia_3: water:alcohol ratio – 3:1; plant:solvent ratio – 1:10; Time: 5 min; Amplitude: 80%.
High-performance Thin Layer Chromatography (HPTLC) can separate and identify a number of anthocyanin derivatives for fingerprint analysis under the following experimental conditions (Filip et al., 2012; Bräunlich et al., 2013; Lee, 2019):

- stationary phase HPTLC silica gel G 60 F254, preformed glass plates 20 × 10 cm (Merck, Darmstadt, Germania);
- stationary phase pre-wash: chloroform-methanol mixture (1:1, v/v);
- activation of the stationary phase: drying in the oven (110°C, 30 minutes);
- mobile phase: mixture of ethyl acetate-methyl ethyl ketone-water-formic acid (7:3:2:2, v/v/v/v), 20 ml in the chromatographic tank, with supersaturation for 20 minutes at 25°C;
- samples to be analyzed, for HPTLC fingerprint: extracts of *Aroniae melanocarpae* fructus, diluted 1:4 (dilution factor 5) with ethanol;

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- migration distance: 62 mm (the sample application line was set at 8 mm and the solvent front set at 70 mm);
- the volumes applied to the starting line: 1 μl of each sample to be analyzed (diluted previously with 1: 4 with ethanol);
- the samples to be analyzed were applied by spray, in the form of strips with a length of 5 mm, using the semi-automatic system CAMAG Linomat 5 (CAMAG, Muttenz, Switzerland): gas-air spray, syringe volume - 100 μl, solvent in syringe - ethanol, application speed - 30 nl / s, predose volume - 0.2 μl;
- drying of the chromatographic plate, after development: 5 minutes, at 25°C (using a cold air blower);
- examination (detection): in visible (VIZ) (for photographing the chromatographic plate and at λ 520 nm, for obtaining the densitograms), without derivatization (respectively chemical treatment), using the CAMAG TLC Scanner 3 photo-densitometer: the chromatographic plate scan speed - 20 mm / s, resolution - 100 μm, lamp - deuterium and tungsten, measurement mode - absorption. The solvents used for HPTLC analysis (LiChrosolv®) were sourced from Merck – Millipore (Darmstadt, Germany).

3. RESULTS AND DISCUSSIONS

The analysis material was represented by 3 extracts obtained under various experimental conditions, from the species *Aronia melanocarpa* (Michx.) Elliot fruits cultivated at the Research - Development Institute for Pomiculture (ICDP) Piteşti–Mărăcineni, judeţul Argeş.

By high performance thin layer chromatography (HPTLC – *High-Performance Thin Layer Chromatography*) a series of anthocyanin-derivatives can be separated and identified, for fingerprint analysis, in the following experimental conditions (Bräunlich *et al.*, 2013; Filip *et al.*, 2012; Lee, 2019).

Data on HPTLC fingerprint analysis for anthocyanin-derived content of *Aroniae melanocarpae* fructus extracts, obtained under various experimental conditions (No.1, No.2, No. 3), are presented, as follows, in Figures 1-3 and in Table 1.

Table 1 - HPTLC fingerprint analysis results for anthocyanin-derived content of Aroniae melanocarpae fructus extracts (according to densitograms)

The extract analyzed	Peak No.	R_f	[AU – *Area Units*] × 10^{-3}	Area [%]	Observations
*Aronia*_1	1.	0.24	0.96	6.87	
	2.	0.55	9.23	66.43	Cyanidol derivative
	3.	0.67	3.71	26.7	
*Aronia*_2	1.	0.56	2.91	69.76	Cyanidol derivative
	2.	0.68	1.26	30.24	
*Aronia*_3	1.	0.56	3.06	69.03	Cyanidol derivative
	2.	0.68	1.37	30.97	

By HPTLC coupled with photodensitometry, in 3 extracts obtained under various experimental conditions, from the fruits of the species *Aronia melanocarpa*, anthocyanin-like components (mainly a cyanidol derivative) were separated and identified from the phytochemical footprint). The best HPTLC chromatographic imprint was shown for Aronia fruit extracts prepared in 3:1 water-ethanol mixture, at amplitude of 40%. The experimental results are in agreement with the

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information from the specialized works regarding the anthocyanin-derivatives content of the fruits of the species *Aronia melanocarpa* (Michx.) Elliot (Bräunlich et al., 2013; Denev et al., 2018; Gralec et al., 2019; Kobus et al., 2019). Chromatographic analysis is preliminary in nature, following only the specific fingerprint for anthocyanin derivatives, without the use of specific reference substances (standards).

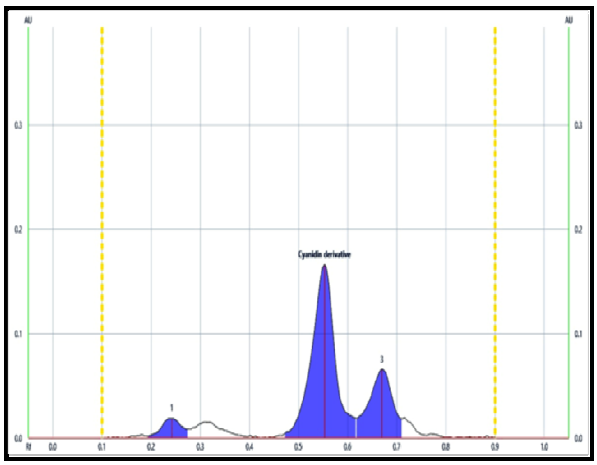

Figure 1 - Densitogram of the anthocyanin derivatives separated from the extract of Aroniae melanocarpae fructus No.1 (Aronia_1) (VIZ λ 520 nm, without derivatization)

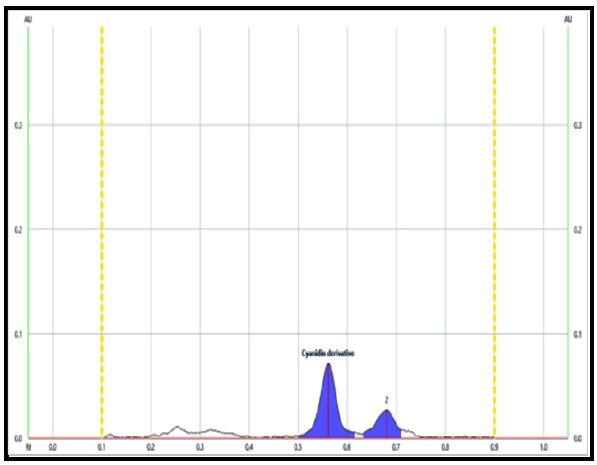

Figure 2 - Densitogram of the anthocyanin derivatives separated from the extract of Aroniae melanocarpae fructus No.2 (Aronia_2) (VIZ λ 520 nm, without derivatization)

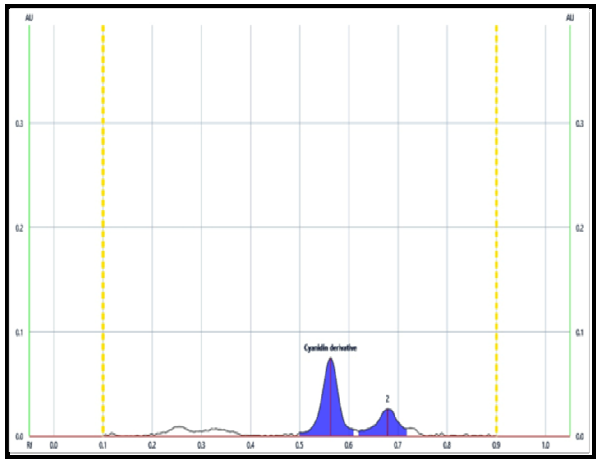

Figure 3 - Densitogram of the anthocyanin derivatives separated from the extract of Aroniae melanocarpae fructus No.3 (Aronia_3) (VIZ λ 520 nm, without derivatization)

4. CONCLUSIONS

The best HPTLC chromatographic imprint is recorded for Aronia fruit extract prepared in 3:1 water-ethanol mixture at an amplitude of 40%.

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The experimental results are in agreement with the information from the specialized papers regarding the anthocyanin-derivative content of the fruits of *Aronia melanocarpa* (Michx.) Elliot.
A considerable advantage of the ultrasonic assisted extraction method is that it is a versatile technique, which can be used both in research experiments in experimental laboratories, on a small scale and on an industrial scale. Also, this method demonstrates that it is an efficient extraction technique of plant compounds that can be used at industrial level.

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