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Aronia leaves at the end of harvest season — Promising source of phenolic compounds, macro- and microelements

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ABSTRACT

Aronia (*Aronia melanocarpa*) leaves at the end of harvest season (in the leaves senescence vegetative stage), when it represents an agricultural waste material, have been extracted under different conditions in order to assess potential of this plant material in valuable bioactive compounds recovering. Response surface methodology was used in screening of the most important parameters effecting extraction (ethanol concentration, extraction time and solvomodule), as well as for mathematical modeling and process optimization. The optimal extraction conditions (ethanol concentration of 49.18%, extraction time of 75.26 min and solvomodule of 0.19 g/mL) that simultaneously maximize extractive matter yield and total phenolic content, minimizing the EC50 value, were proposed by desirability function approach. Finally, in further assessment of aronia agricultural waste as a potential source of bioactive compounds, the extract obtained under proposed optimal conditions was analyzed by UHPLC-MS, while the content of macro- and microelements was determined by ICP-OES technique.

1. Introduction

There is a strong tendency in application of plant extracts as functional ingredients in food, beverages, pharmaceutical and cosmetic preparations. Modifications of traditionally used recipes by addition of natural plant products can convert products of food industry into the high quality food products with additional functionality. These products, besides nutritional needs satisfying, exert health beneficial effects having a great importance for modern man in various diseases prevention. Nowadays, different natural bioactive compounds and herbal extracts more often replace synthetic antioxidants since phenolic compounds inside them are extremely effective antioxidants and widely used as high-quality bioactive ingredients in the functional food products (Moure et al., 2001). Therefore, bioactivity investigations and chemical characterization of untested plant material, with the aim of biologically valuable polyphenolic compounds discovering, are of great scientific and practical interest.

The content of polyphenolic bioactive compounds depends on type of plant material, genetic predisposition of plant species, climate conditions, soil composition, and vegetative stage of the plant (Wei et al., 2013). In some plants, *Foeniculum vulgare* (Salami et al., 2017), *Anethum graveolens* L. (Lisiewska et al., 2006), *Rhizophora mangle* (Habermann et al., 2016) and *Carthamus tinctorius* L. (Abdallah et al., 2013), the content of total phenolics and flavonoids in the leaves, as well as the antioxidative activity, increase from early vegetative stage to full mature fruits stage. High content of bioactive compounds in the leaves after reaping (fruits harvesting), when the leaves represent a waste material causing environmental and ecological problems, classifies this raw material in a group of highly attractive and inexpensive raw materials for obtaining of natural polyphenolic compounds with potential application in food, pharmaceutical and cosmetic industries. Also, along to the leaves vegetative cycle the concentration of minerals (macroelements and microelements) being changed. In general, the mineral concentration largely depends on the plant species, but also on the soil characteristics, climate and growth conditions (Niskanene, 2002; Milošević and Milošević, 2018). Therefore, the stage after fruit reaping (leaves senescence vegetative stage) might be an optimal harvesting time to obtain a high valuable plant material, which still contains a high content of bioactive compounds, without endangering the proper fruits ripening.

Besides the above-mentioned, efficacy of polyphenolic compound extraction from different plant material also depends on extraction conditions, such as extraction time, temperature, solvomodule, concentration and solvent polarity which could significantly affect the extraction process and the yield of extracted compounds. So, the optimization procedure and an optimal level of process parameters finding is a very important step in extraction efficacy maximizing and scale up procedure. One-factor-at-a-time optimization method is laborious, time

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consuming and is not able to assess the combined impact of the considered factors. On the other hand, mathematical and statistical methods, such as response surface methodology (RSM), are increasingly used for the design, modeling, analysis and optimization of solvent extraction processes (Granato and de Araújo Calado, 2014).

The leaves of aronia (*Aronia melanocarpa* (Michx.) Elliott, chokeberry) have been chosen as a raw material in this work. Aronia, belonging to the rose family (*Rosaceae*), is a shrub originating from North America; it's 90–180 cm high, with purple-black colored pomes, about 6 mm in diameter in clusters up to 14 fruits on red pedicels. The leaves are lustrous and glabrous, 3–7 cm long (Kokotkiewitcz et al., 2010). It has been traditionally used in Native American Medicine by Forest Patawotami Native Americans. During the past century aronia begins to cultivate in the Soviet Union and Eastern European countries where became popular as a food ingredient and herbal medicine (Kokotkiewitcz et al., 2010; Brand et al. 2017). The harvest period is from August to October, determined by the pomes ripening based on stable berry color, uniformity of pigmentation across all berries in a cluster, etc. (Brand et al. 2017).

Aronia is one of the richest natural sources of polyphenols (hydroxycinnamic acids, flavonoids and anthocyanins) (Kulling and Rawel, 2008). A considerable number of researchers have investigated the aronia fruits used as antiarterosclerotics, antidiabetic, anti-inflammatory, antiviral, antimutagenic agent and immunomodulator; it also shows anti-proliferative and anti-carcinogenic effects (Kulling and Rawel, 2008; Thi and Hwang, 2014). However, the leaves of aronia, that are considered as a waste material (in the leaves senescence vegetative stage), representing a source of bioactive compounds with possible useful biological effects, are much less studied. Leaves of several aronia species have been used in traditional medicine as anti-inflammatory, antiviral, antimicrobial and anti-proliferative agents; the biological activity of aronia leaves probably comes from polyphenols, flavonoids and chlorophylls (Lee et al. 2014; Thi and Hwang, 2014). Some authors investigating aronia emphasize that high content of polyphenolic compounds (chlorogenic acid, neochlorogenic acid, and caffeic acid) could be found in the leaves, but intelligibly in a lower concentration than in the fruit (Thi and Hwang, 2014).

The aim of this work is to find an optimal extraction conditions for aronia agricultural waste material (leaves at the end of harvest season, i.e. in the senescence vegetative stage) in order to recover valuable bioactive compounds with antioxidant properties. Since aronia is a perennial plant, it is possible to collect the senescence leaves every year without any consequences. The criterion for optimization was maximal extractive matter yield with maximal content of polyphenolic compounds and highly expressed antioxidant activity.

2. Material and methods

2.1. Plant material

Aronia (*Aronia melanocarpa*) was cultivated in the garden located in the village Togočevce near Lebane, Southeast Serbia (42°56′22″ N; 21°51′11″ E; 346 m a.s.l.). Plant material was identified by Prof. Zora Dajic Stevanovic (University of Belgrade, Faculty of Agriculture) and herbarium voucher was deposited at Department of Applied Botany, University of Belgrade, Faculty of Agriculture. The aronia leaves were collected in November 2015, two months after fruits harvesting, in the leaves senescence vegetative stage. The fresh plant material was milled in a laboratory disintegrator (laboratory electric mill "BRAUN AROMATIC KSM2"), immediately before extraction.

2.2. Reagents

Absolute ethanol (Zorka Šabac, Serbia), Folin-Ciocalteu's reagent, gallic acid, rutin, chlorogenic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, aluminum (III) chloride hexahydrate, potassium acetate, (Sigma Chemical Company, St. Louis, USA), formic acid (Carlo Erba, France), methanol and water (Fisher Chemical, LC–MS and HPLC grade, respectively).

2.3. Extraction

Grinded plant material and aqueous ethanol (30%, 50% and 75%, v/v) in solvomodule 0.10, 0.15 and 0.20 m/v (plant material/solvent ratio; g/mL) were placed in Erlenmeyer flasks. The extraction was performed for 30, 60 and 90 min at 25 °C. The obtained extracts were filtered under a weak vacuum at 50 °C and dried in the vacuum dryer at 40 °C till constant mass; extractive matter (EM) yield was calculated based on dry residue content and expressed as a gram of EM per 100 g of fresh plant material (g/100 g PM).

2.4. Total phenolic content

Spectrophotometric method with Folin–Ciocalteu reagent was used for rapid total phenolic content (TPC) quantification (Singleton et al., 1999). This method is based on color changes measuring (at 765 nm) as a consequence of reagent reduction in the presence of phenolic compounds. Distilled water (4.5 mL) and Folin-Ciocalteu reagent (0.5 mL) was added in 0.5 mL of extract (0.2 mg/mL). After 5 min, 5 mL of 7% Na_2CO_3 was added in the sample solutions. The reaction mixture was vigorously shaken and left for 90 min at room temperature. The absorbance of the reaction mixture was measured on VARIAN Cary-100 spectrophotometer and obtained results were expressed as milligram of gallic acid equivalents (GAE) per gram of plant material.

2.5. DPPH-test

Antioxidant activity of extracts obtained from aronia leaves was determined by DPPH test (Stanojević et al., 2009). Ethanolic solution of DPPH radicals (1 mL, 3×10^{-4} mol/L) was added in 2.5 mL of extract of various concentrations (3–200 µg/mL). Absorbance at 517 nm was measured after 20 min of incubation at room temperature in the dark. The absorbance was also measured for pure ethanolic solution of DPPH radical diluted in adequate proportion (1 mL of DPPH radical, 3×10^{-4} mol/L, in 2.5 mL of ethanol - "control"), and for the extract without DPPH radical (2.5 mL of extract diluted with 1 mL of ethanol - "blank"). Free radical scavenging capacity is calculated according to equation:

$$\textit{The extent of DPPH radicals neutralization}\ (\%) = 100 - \left[(A_U - A_B) \times \frac{100}{A_C} \right]$$

A_u – absorbance of „sample“; A_B – absorbance of „blank“; A_C – absorbance of „control“.

The concentration of extract required for a half of the initial DPPH radicals neutralization is EC_{50} value. These values were mathematically calculated according to the experimental data by nonlinear regression analysis.

2.6. Ultrahigh performance liquid chromatography-diode array-mass spectrometry method (UHPLC-MS method)

The chromatographic separation was performed on Hypersil gold C18 column (50 × 2.1 mm, 1.9 µm) at 25 °C using a Dionex Ultimate 3000 UHPLC+ system equipped with a diode array (DAD) detector and LCQ Fleet Ion Trap Mass Spectrometer, Thermo Fisher Scientific, USA. Mobile phase composed of two solvents, 0.1% (v/v) formic acid in water (A) and methanol (B) yielding a gradient program at 0.250 mL/min flow rate: 0–2 min (10-30% B), 5-8 min (40-50% B), 9-11 min (80-90% B), followed by isocratic period 11-12 min (90% B), returned on 10% B in 12.0-12.1 min and finished by 10% B in 12.1-15 min.