The Journal of Supercritical Fluids 128 (2017) 173–181

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Chemical and biological insights on aronia stems extracts obtained by different extraction techniques: From wastes to functional products

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ARTICLE INFO

Keywords:
Subcritical water extraction
Plant waste
Aronia stems
Natural bioactive agents

ABSTRACT

The present study aimed to evaluate chemical and biological potential of aronia stems for providing new raw material for food and pharmaceutical industries. Aqueous extracts of aronia stems were prepared by three different techniques (microwave-assisted, ultrasound-assisted and subcritical water extraction). For biological activities, antioxidant, antimicrobial and cytotoxic activities were evaluated. For chemical characterisation, phenolic compounds and essential elements were analysed as well as total phenolic contents. Subcritical water extracts exhibited the strongest antioxidant activity with the highest content of phenolics. Minimum inhibitory concentrations for analysed extracts were in the range from 9.76 to 156.25 μg/mL in antimicrobial assays. The extracts exerted prominent cytotoxicity against different cell lines. Rutin was detected as the major compound in the studied extracts and these extracts were also rich in essential elements. Our study suggested that subcritical water extract of aronia stem could be considered as a new raw material in developing novel functional/industrial products.

1. Introduction

Aronia (*Aronia melanocarpa*), commonly known as black chokeberry, represents one of the richest sources of dietary phytochemicals [1]. This plant has long traditional use, and different health benefts have been reported for it. Inter alia, it has been reported that fresh aronia berries demonstrate significantly stronger antioxidant properties compared to other berries and fruits [2]. Scientific evidence showed that both aronia and its extracts may be utilized in the prevention of diabetes and diabetes-associated complications [3], cardiovascular diseases [4] and colon cancer [3]. Other studies demonstrated its antiinflammatory, antimutagenic, hepatoprotective, cardioprotective, anticancer and lipid-lowering effects [2,5–7].

Besides its traditional use, aronia berries are widely used for industrial production of fruit syrups, juices, jams, marmalades, jellies, alcoholic and nonalcoholic drinks [2]. Moreover, this plant and its extracts are also often used as food preservatives since their polyphenolic constituents inhibit lipid peroxidation [8,9]. In recent years it has been highlighted as natural food colorant [2]. Current technology of aronia processing does not anticipate the use of stems after their separation from berries. To our knowledge there are no reported scientific evidence on the bioactivity and possible valorization of these aronia wastes.

Plant wastes are growing rapidly providing new raw materials both in scientific and industrial areas. Plant waste can be a significant source of valuable compounds such as bioactive molecules useful in the preparation of dietary supplements or nutraceuticals, food ingredients, pharmaceuticals and cosmetic products.

Isolation of bioactive compounds from plant waste is usually performed by using organic solvents. This approach has numerous drawbacks which are primarily related to the toxicity of organic solvents and negative impact on the environment. In last decades, the efforts have been directed to the development of environmentally-benign technologies and thus many studies focuses on the development and application of green technologies. Unconventional extraction techniques contain different approaches such as ultrasonic or micro-

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http://dx.doi.org/10.1016/j.supflu.2017.05.023
Received 10 February 2017; Received in revised form 15 May 2017; Accepted 16 May 2017
Available online 24 May 2017
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wave extraction.

Subcritical water extraction (SCW) represents an innovative green technology of exceptional potential. In this technique, water remains in its liquid state at temperature above boiling point under high pressure. The intermolecular hydrogen bonds begin to weaken with increase of temperature, thus reducing water polarity. Consequently, the polarity of water at these conditions becomes equivalent to that of common organic solvents. More precisely, the dielectric constant of water at ambient conditions is approximately 80, whereas the dielectric constant of methanol is 33, and that of ethanol 24. With the increase in temperature and pressure the dielectric constant of water decreases. At 250 °C and 50 bar the dielectric constant of water is 27 Because of this wide range of possible polarity changes, subcritical water has the ability to efficiently solubilize numerous compounds. In contrast, the solubility of compounds which are well soluble in ambient water doesn't change so dramatically with water heating and pressurisation [10]. Another important advantage of subcritical water has not any negative environmental impact. Within these perspectives, SCW has gained great interest in both scientific and industrial areas.

Within this article, new insights on aronia stems, which is a waste in aronia processing technologies, were investigated. To the best of our knowledge, there are no literature data about this waste applications and its biological potential. Thus, this study is the first report on the biological and chemical profiles of aronia stems. The recovery of bioactive molecules was compared with three different extraction techniques (ultrasound-assisted, microwave-assisted and subcritical water extraction) were compared. The extracts were also characterized with respect to phenol, flavonoid and mineral contents as well as to their antioxidant, antimicrobial and anticancer properties.

2. Materials and methods

2.1. Chemicals and reagents

Cirsimarin, resazurin, amricin, nystatin, sabourand dextrose, Tween 20 and 80 and *cis*-diamminedichloroplatinum (*cis*-DDP) were purchased from Tedia Company (USA). Folin-Ciocalteu reagent, trichloroacetic acid, 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH), galic acid, linoleic acid, thiobarbituric acid (TBA), 2-deoxyribose, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sodium phosphate, ammonium molybdate, ammonium thiocyanate, Butylated hydroxytoluene (BHT), α-tocopherol, sulfuric acid, iron(II) chloride, nystatin, β-carotene and rutin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Iron(II) sulfate was obtained from Zorka (Šabac, Serbia). Amracin was purchased from Galenika a.d. (Belgrade, Serbia). All chemicals and reagents were of analytical reagent grade.

2.2. Plant material

Aronia (*Aronia melanocarpa*) was collected in Southeast region of Serbia in August 2014. Separated stems were stacked in a crate with perforated bottom, in order to ensure air flow. Drying was performed naturally in the draft and dark until moisture content of 10%. Dry stems were packed in glass jar and stored in the dark until use.

2.3. Sample preparation

2.3.1. Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) was performed in ultrasonic water bath (Branson, USA) during 30 min. In volumetric flask 4 g of stems were mixed with 100 mL of deionised water and sonificated. Obtained extracts were filtrated.

2.3.2. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) was performed in an open system by using a modified domestic microwave oven. Stems were mixed with the water in the ratio as in the case of UAE. The extraction procedure was performed at 580 W during 30 min.

2.3.3. Subcritical water extraction (SCW)

Subcritical water extraction (SCW) was performed in a homemade subcritical water extractor/reactor previously described by Cvetanović et al. [11]. Pressurization of the vessel was performed with 99.99% nitrogen in order to prevent oxidation at high temperatures. The operating pressure was 40 bar while the process temperature was 140 °C. Stirring was at the frequency range of 3 Hz. Extraction duration was 30 min. Obtained extracts were filtrated and stored in the refrigerator until analysis.

2.4. Determination of total extraction yield, total phenolic content (TPC)

In order to determine the total extraction yield, certain volume of liquid extracts was evaporated under vacuum. Evaporated extracts were dried at 105 °C until a constant mass. Based on the mass difference extraction yield was calculated as expressed in percentage (%).

The total phenolic content (TPC) in obtained aronia stem extracts was determined by Folin–Ciocalteu procedure [12,13] using chlorogenic acid as a standard. Absorbance was measured at 750 nm. Content of phenolic compounds was expressed as mg of chlorogenic acid equivalent (CAE) per g of dry extract (mg CAE/g). All experiments were performed in triplicate.

2.5. Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using aluminum chloride colorimetric assay [14]. Results were expressed as mg of rutin equivalent (RE) per g of dry extract (mg RE/g). All experiments were performed in triplicate.

2.6. Determination of antioxidative capacity

2.6.1. Determination of lipid peroxidation

Determination of inhibitory activity against lipid peroxidation was carried out according to the thiocyanate method [15]. Dried extracts were dissolved in water to the final concentration of 1 mg/mL of dried extract. This solution was further used for preparation of serial dilutions, and 0.5 mL of each diluted solution was added to linoleic acid emulsion (2.5 mL, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid and 0.2804 g of Tween 20 in 50 mL of 40 mM phosphate buffer. The mixture was incubated at 37 °C for 72 h. After that, 0.1 mL of the reaction solution was mixed with 4.7 mL of ethanol (75%), 0.1 mL of $FeCl_2$ (20 mM), and 0.1 mL of ammonium thiocyanate (30%). The mixture was stirred for 3 min and absorbance was measured at 500 nm. Ascorbic acid, gallic acid, α-tocopherol and BHT were used as reference compounds. All tests were performed in triplicate, and the results were expressed as IC_{50} values (the concentration of the test solution for inhibiting 50% of linoleic acid oxidation).

2.6.2. Measurement of ferrous ion chelating ability

The ferrous ion chelating ability was measured by the decrease in absorbance at 562 nm of the iron(II)-ferrozine complex [16]. Reaction mixture was prepared by mixing sample solutions (1 mL) with 0.125 mM $FeSO_4$ (1 mL). Immediately after, 1 mL of ferrozine (0.3125 mM) was added. The prepared mixture was allowed to equilibrate for 10 min before absorbance measurement. The chelating activity was calculated in the following way (Eq. (1)):

$$I\ (\%) = (A_0 - A_1)/A_0 \times 100 \quad (1)$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the

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