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Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves

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ARTICLE INFO

Article history:
Received 23 November 2014
Received in revised form 20 February 2015
Accepted 24 February 2015
Available online

Keywords:
Leaves
Fruit
Polyphenolic compounds
Antioxidant activity

ABSTRACT

The aim of this study was to determine and compare a polyphenolic profile and antioxidant activity in leaves and fruits of 7 selected species: *Malus domestica* (2 cultivars), *Cydonia oblonga* (4 cv.), *Chaenomeles japonica* (3 cv.), *Ribes nigrum* (3 cv.), *Aronia melanocarpa* (1 cv.), *Vaccinium macrocarpon* (11 cv.) and *Vaccinium myrtillus*. Polyphenolic profile was determined by LC-MS and UPLC-PDA-FL and antioxidant activity were analysed by ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and FRAP (ferric reducing ability of plasma) test. The results showed that the leaves contained significantly higher polyphenol compounds than the fruits. The highest concentration of total polyphenols were characterized by quince leaves (major group: polymeric proanthocyanidins) followed by cranberry > apple > chokeberry > Japanese quince > bilberry > and blackcurrant leaves (major group: flavonols). Also the strongest antioxidant potential was represented for quince leaves: 116.49 and 65.25 mmol trolox equivalents (TE)/100 g of dry matter (dm) in ABTS and FRAP tests, which correlated with the content of polymerized proanthocyanidins and flavonols.

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1. Introduction

As some studies show, leaves of well-known crops and wild growing plants (such as blackcurrant, chokeberry and bilberry) are a valuable source of antioxidant substances, especially polyphenols (Skupień, Kostrzewa-Nowak, Oszmiański, & Tarasiuk, 2008; Tabart et al., 2007; Witzell, Gref, & Näsholm, 2003).

It has been proven that chokeberry leaves (*Aronia melanocarpa*) extracts inhibit lipid and protein peroxidation in the brain of rats treated with oxidative stress-inducing factors (Cuvorova, Davydov, Prozorovskii, & Shvets, 2005). Skupień et al. (2008) described antileukaemic activity of chokeberry leaves against human promyelocytes of several cell lines (HL60, HL60/VINC and HL60/DOC), while Maslov et al. (2002) in a rat study revealed their antidiabetic properties.

Potential pharmacological properties are also exhibited by quince leaf extract (QLE). Khademi, Danesh, Mohammad Nejad, Ghorbani, and Soleimani Rad (2013) investigated the ability of QLE in preventing atherosclerosis progression and determined the lipid-lowering effect (on tested rabbits). The effect of the examined extract (dose: 50 mg/kg) on lipid profiles was assessed by measuring total cholesterol, triacylglycerol, low- and high-density lipoprotein (LDL, HDL) and liver enzyme levels in plasma. In this respect, the properties of QLE were similar to those of atorvastatin (a medication used in hypercholesterolaemia). A reduction of lipid profile, increase

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http://dx.doi.org/10.1016/j.jff.2015.02.041
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of HDL cholesterol and decrease of liver enzyme levels were observed.

Aslan, Orhan, Orhan, and Ergun (2010) examined antidiabetic properties of hydro-ethanolic extracts from quince leaves. They reported a significant reduction of glucose level in diabetic rats' blood by oral administration of QLE (dose: 250 and 500 mg/kg; time: 0–3 h). The same effect was observed when an antidiabetic drug (tolbutamide) in a dose of 100 mg/kg was employed.

High biological potential has also been observed in sea buckthorn leaves (*Hippophae rhamnoides* L.). It is related to their ability to accelerate tissue regeneration and radioprotective, anti-inflammatory, immunomodulatory and adaptogenic activity (Chawla et al., 2007; Ganju et al., 2005; Geetha et al., 2005; Gupta, Kumar, Pal, Banerjee, & Sawhney, 2005; Saggu et al., 2007; Upadhyay, Kumar, Siddiqui, & Gupta, 2011).

The knowledge about the active compounds of leaves, their cytotoxicity or potential pharmacological properties, is still too limited. The starting point in the study of plants' health-promoting activity is to know the composition of bioactives, including polyphenols. Because of the wide action spectrum of these compounds, they may be complementary, natural raw material in fruit processing, enriching the health and sensory values of products, e.g. juices or purees (Teleszko, 2011). However, the available literature data indicate that this issue has rarely been addressed. For example, in the research of Kolniak-Ostek, Oszmiański, and Wojdyło (2013), apple leaves (*Malus domestica* Borkh.) in the amount of 0.5, 1.0 and 5.0% were added to cloudy apple drink. It was found that the use of leaves had a positive impact on the content of polyphenolic compounds in the final product (including phenolic acids, dihydrochalcones and flavonols) and significantly increased its antioxidant capacity.

In view of the above, the aim of our study was to determine the polyphenolic profile in leaves of chosen plants and to compare leaves and fruits of examined species in terms of antioxidant contents.

2. Materials and methods

2.1. Reagents and chemicals

Quercetin and kaempferol 3-*O*-glucoside, cyanidin 3-rutinoside, *p*-coumaric acid, (+)-catechin, (–)-epicatechin were purchased from Extrasynthese (Lyon Nord, France). Chlorogenic acid and neochlorogenic acid were supplied by TRANS MIT GmbH (Giessen, Germany). Acetic acid, phloroglucinol, ascorbic acid, acetonitrile and methanol were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Plant material

Leaves and ripe fruits of apples (*Malus domestica* Borkh.; cv.: 'Szampion', 'Ozark Gold') cultivated in Poland were obtained from Research Station for Cultivar Testing in Zybiszów, chokeberry (*Aronia melanocarpa* (Michx.) Elliott; cv.: 'Galicjanka') from Orchards Company Trzebnica, cranberry (*Vaccinium macrocarpon* L.; cv.: 'Bain Favorite', 'Ben Lear', 'Bergman', 'Drewer', 'Early Richard', 'Hollister Red', 'Howes', 'McFarlin', 'Pilgrim', 'Stankiewicz', 'Stevens') from Berry Crops Experimental Field of Warsaw University of Life Sciences in Błonie, blackcurrant (*Ribes nigrum* L.; cv.: 'Titania', Tiben', Tisel') and Japanese quince (*Chaenomeles japonica* L.; cv.: 'Dębosz', 'Witaminnyj', 'Żółtogaraczyj') from Institute of Horticulture in Skierniewice. Quince leaves and fruits (*Cydonia oblonga* Mill.; cv.: 'Kaszczenko', 'Marija', 'Późna Rejmana', 'Ronda') were obtained from the Garden of Medicinal Plants, at the Wrocław Medical University. Bilberry leaves and fruits (*Vaccinium myrtillus* L.) were obtained from Forest District Oleśnica. The morphological parts of each plant species were collected at the same time, ie. when the fruits were ripe. Before harvesting, fruits and leaves were prepared (inspection, washing), frozen at –70 °C and then lyophilized in ALPHA 1–4 LSC freeze drier (Christ, Osterode, Germany). The parameters of the process are: vacuum: 0.960 mbar, temperature of the shelves: +26 °C, drying time: 20 h.

2.3. Plant extract preparation to polyphenols content analysis

Extracts were prepared by spreading about 0.2 g of freeze-dried leaves or 0.5 g of fruits by 10 mL of mixture containing HPLC-grade methanol (30 mL/100 mL), ascorbic acid (2.0 g/100 mL) and acetic acid in an amount of 1.0 mL/100 mL of reagent. The extracts were sonicated for 15 min (Sonic 6D, Polsonic, Poland), left for 24 h at 4 °C without light and then sonicated again for 15 min. The samples were centrifuged (MPW-380R; MPW Med. Instruments, Poland) for 10 min at 4 °C and 20,000 rpm. The resulting extract was analysed.

Determination of polyphenols by UPLC coupled to PDA and FL detector.

The analysis of polyphenolic compounds (including proanthocyanidins) was carried out on a UPLC system Aquity (Waters, USA) consisting of a binary solvent manager, sample manager, photodiode array detector (PDA) and fluorescence detector (FL, model λe). Empower 3 software was used for chromatographic data gathering and integration of chromatograms. A UPLC analyses were performed on a BEH Shield C18 analytical column (2.1 mm × 5 mm; 1.7 μm). The flow rate was 0.45 mL/min. A partial loop injection mode with a needle overfill was set up, enabling 5 μL injection volumes when 10 μL injection loop was used. Acetonitrile was used as a strong wash solvent and 10% acetonitrile as a weak wash solvent. All incubations were done in triplicate.

2.4. Analysis of polyphenols compounds

Analysis was previously described by Wojdyło, Oszmiański, and Bielicki (2013). The analytical column was kept at 30 °C by column oven, sample manager at 4 °C. The mobile phase used for the separation was composed of aqueous formic acid 4.5% (A) and acetonitrile (B) in gradient mode set as follows: initial conditions 1% B; from 0 to 5 min 75% B; from 5.0 to 6.5 min 100% B; from 6.5 to 7.5 min the composition was kept constantly at 100% B; from 7.5 to 8.5 min reconditioning the column to initial gradient (1% B). The runs were monitored at the following wavelengths: flavan-3-ols (FL) at 280 nm, phenolic acid (FA) at 320 nm, and flavonol glycosides (F) at 360 nm, and were measured over the wavelength range of 200–600 nm in steps of 2 nm.

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