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Antioxidant capacity, phenolic compounds and minerals content of blackcurrant (*Ribes nigrum* L.) leaves as influenced by harvesting date and extraction method

Violeta Nour[a,*], Ion Trandafir[b], Sina Cosmulescu[a]

[a] Department of Horticulture & Food Science, Agriculture & Horticulture Faculty, University of Craiova, A.I.Cuza Street, 13, 200585 Craiova, Romania
[b] Department of Chemistry, Sciences Faculty, University of Craiova, Calea Bucuresti Street, 107, 200529 Craiova, Romania

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ABSTRACT

The efficiencies of 80 °C water, 40% ethanol and 80% ethanol in the extraction of phenolics from black currant leaves, as well as the antioxidant capacity of the obtained extracts, were investigated. Aqueous ethanol (40%) was the most effective in the extraction of phenolics followed by 80 °C water while the antioxidant capacity of the investigated extracts correlated with their phenolic content. Seven phenolic acids (gallic, chlorogenic, caffeic, p-coumaric, ferulic, sinapic and salicylic) and three flavonoids (rutin, myricetin and quercetin) were identified and quantified using HPLC with PDA detector. Also, total phenolics, minerals and trace elements content and antioxidant capacity were determined in black currant leaves of six different cultivars, over five harvesting dates (from June to August). Significant differences were observed among cultivars and sampling dates but the patterns of variation during the harvesting period were similar for all cultivars. The highest total phenolic contents and antioxidant capacities among samplings were recorded in mid-June for all the investigated cultivars, followed by a considerable decrease until early August. The black currant leaves registered maximum contents of Ca, Mg, Fe, Mg, Al, Cr and B in mid-June while the highest content of K was reached on June 1st. The results suggested that black currant leaves should be further explored as a potential source of natural antioxidants in certain food applications and for the cosmetic and pharmaceutical industries.

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1. Introduction

Black currant (*Ribes nigrum* L.) is a deciduous shrub found in Europe, Asia, and America that belongs to *Grossulariaceae* family and *Ribes* species (Chrzanowski, 2008). It grows 1–2 m tall and produces purple-black, sweet, aromatic, edible and seed-containing berries up to 12 mm in diameter (Gopalan et al., 2012). The leaves are palmately lobed, up to 10 cm (4 in.) per side, glabrous above, with serrated margins, slightly pubescent with numerous sessile, aromatic glands beneath (Hummer and Barney, 2002).

Black currants represent an abundant source of phytochemicals with potent antioxidant, anti-inflammatory and anti-microbial properties (Nour et al., 2011; Tabart et al., 2006). Black currant leaves contain considerable amounts of minerals and trace elements which perform essential roles in the activation of enzymatic systems or have involvement in the metabolism of biomolecules. Many preclinical and clinical studies provide substantial evidence that black currant phytoconstituents possess tremendous potential in the prevention as well as therapy of a myriad of disease conditions (Bishayee et al., 2011; Gopalan et al., 2012). In traditional folk medicine not only fruits but also black currant leaves were recommended for their therapeutic potential being used for their diaphoretic and diuretic properties as well as for treatment of inflammatory disorders such as rheumatic disease (Raudsepp et al., 2010; Tabart et al., 2012). Therefore, dried leaves of black currants are widely used in herbal teas or tea blends (Raudsepp et al., 2010), and several scientific studies have been published with respect to their various therapeutic applications and the composition of the chemical compounds responsible for their health beneficial effects (Cyboran et al., 2011, 2012; Garbacki et al., 2005; He et al., 2010). Declume (1989) showed that the hydroalcoholic extract of black currant leaves and the lyophilisate revealed significant anti-inflammatory activity, without ulcerogenic potential, even at high doses during long treatments. Their anti-inflammatory effect was mostly assigned to the phenolic compounds that are originally synthesized by plants to protect themselves against ultraviolet radiation or aggression by pathogens (Raudsepp et al., 2010). Chenah et al. (1986) found that the biological activity of a mixture of flavonoids extracted from currant leaves was higher than the activity of its two main constituents, i.e. rutin and isoquercetin.

* Corresponding author. Tel.: +40 722791987; fax: +40 251414541.
E-mail addresses: vionor@yahoo.com, vionour@central.ucv.ro (V. Nour).

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Also, Cyboran et al. (2011) found that the extracts from black currant leaves exhibit a high antioxidant activity which depends not only on the overall phenolic composition, but also on the kind and amount of the individual phenolic compounds, mainly those of quercetin. These authors concluded that the high antioxidant activity of the extracts may also be due to other nonphenolic substances that occur in leaves such as alkaloids, carotenoids, tocoferols or ascorbic acid.

Phenolic compounds can be delivered to the organism in the form of plant extracts as medicines, dietary supplements and cosmetics. The extractive efficiency of phenolic compounds from plant material is greatly depended on the solvent (Jakopic et al., 2009). Various solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used in previous studies for the extraction of phenolics from plant materials, often with different proportions of water, in order to establish their extractive efficiency (Dai and Mumper, 2010; Fernández-Agulló et al., 2013; Vongsak et al., 2013). For the extraction of total phenolics from black currant leaves, aqueous acetone was found to be more effective than methanol and water (Tabart et al., 2007). Having in view the prospective use of the extracts as food additives, medicinal components or dietary supplements, a first goal of the present research was to investigate the hydroalcoholic extraction of phenolics and antioxidant capacity from black currant leaves.

Phenolic compounds are biosynthesized in the plants as secondary metabolites, and their concentration in each plant may be influenced by several factors including physiological variations, environmental conditions, geographic variation, genetic factors and evolution (Figueiredo et al., 2008). Seasonal variation in leaf phenolic composition is considered to be important for acclimation of plants to seasonal changes in their biotic and abiotic environment (Kotilainen et al., 2010). The influence of environmental conditions on quantity of active constituents in various plants has been widely reported. Environmental factors such as light, temperature, water availability, mineral nutrition, grafting, elevated atmospheric CO_2, elevated ozone levels and agricultural technologies have a direct impact on biochemical pathways, thus affecting the metabolism of these secondary products. On the other hand the genetic background may play a pivotal role in determining the antioxidant, micronutrient and phytochemical composition of plants. Moreover, phenolic profiles can be used for cultivar identification, which was applied for many species (Treutter, 2010).

Previously published data concerning the influence of harvesting date and cultivar on the antioxidant activity, total and individual phenolic content and mineral content of black currant leaves are scarce. Therefore, a second goal of the present study was to determine the antioxidant activity, the total and individual phenolics content and the mineral content in leaves of six black currant cultivars grown under the same agricultural, geographical and climatic conditions at different harvesting dates during the plant vegetation period.

2. Materials and methods

2.1. Plant material

Leaves from six black currant cultivars ('Blackdown', 'Bogatar', 'Tenah', 'Record', 'Tinker' and 'Deea') were collected on five sampling dates (June 1st, June 15th, July 1st, July 15th, August 1st) in the experimental field of Banu Maracine Didactical Station of University of Craiova, located in the region of Oltenia, Romania (44°20′N, 23°49′E). Fully developed healthy leaves from 6 bushes of each cultivar were selected from the mid part of shoots or stems for the samples used in this study (10 leaves per bush). The leaves were air dried in shade at room temperature and then ground in the electric grinder.

2.2. Sample preparation

Triplicate dried leaf samples (2.5 g) from each cultivar were subjected to each of the following extraction procedures: (a) extraction in 100 mL water at 80 °C for 15 min; (b) sonicated-assisted extraction in 100 mL 40% ethanol at 20 °C for 55 min; (c) sonicated-assisted extraction in 100 mL 80% ethanol at 20 °C for 55 min. The extracts were centrifuged at 3000 g/10 min and the supernatants were then filtered through 0.45 μm filter and stored at 4 °C until analysis.

2.3. Determination of total phenolics contents

The total phenolic content was determined according to the Folin–Ciocalteau phenol reagent method (Singleton and Rossi, 1965) using gallic acid as a standard. The filtered extracts were diluted with methanol (1:4) at ambient temperature and 100 μL of each methanolic extract were mixed with 5 mL of distilled water and 500 μL of Folin–Ciocalteau reagent. After that, 1.5 mL of sodium carbonate (20%, w/v) was added and the reaction mixture was diluted with distilled water to a final volume of 10 mL. The same procedure was also applied to the standard solutions of gallic acid. The absorbance at 765 nm of each mixture was measured on a Varian Cary 50 UV spectrophotometer (Varian Co., USA) after incubation for 30 min at 40 °C. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry leaves.

2.4. Evaluation of antioxidant capacity

The antioxidant capacity was measured in the extracts diluted in methanol (1:4) using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by Hatano et al. (1988), with some modifications (Cosmulescu and Trandafir, 2012). Briefly, each methanol diluted extract (50 μL) was mixed with 3 mL of a 0.004% (v/v) DPPH methanolic solution. The mixture was incubated for 30 min at room temperature in the dark and the absorbance was measured at 517 nm on Varian Cary 50 UV–Vis spectrophotometer (Varian Co., USA). The inhibition of the DPPH radical by the samples was calculated according to the following formula: DPPH scavenging activity (%) = [1 − Abs.sample/Abs.blank] × 100. The DPPH scavenging activity was subsequently calculated with respect to the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), which was used as a standard reference. Six calibration solutions of Trolox (0.5, 1, 1.5, 2, 2.5 and 3.5 mM) were also tested to establish a standard curve. A blank control of methanol/water mixture was run in each assay. All assays were conducted in triplicate. Results were expressed as micromoles Trolox equivalents per gram of dry leaves.

2.5. HPLC analysis of phenolic acids and flavonoids

The phenolic compounds for chromatographic analysis were extracted from 0.5 g of air-dried black currant leaves with 10 mL of 70% ethanol and 10 mL of 4 N HCl for 30 min at 80 °C under reflux conditions. The chromatographic separation of the individual phenolic compounds was carried out using a Surveyor Thermo Electron system (Thermo Electron Corporation, San Jose, CA, USA) including vacuum degasser, Surveyor Plus LCPMPP pump, Surveyor Plus ASP autosampler, PDA5P diode array detector and Chrom Quest 4.2 system manager as data processor. Separation was achieved by a reversed-phase Hypersil Gold C18 column (5 μm particle size, 250 mm × 4.6 mm) provided by Thermo Electron Corporation (USA). HPLC analysis was performed according to our previously reported method (Nour et al., 2012). The mobile phase consisted of 1.5% aqueous acetic acid solution (A) and methanol (B). The samples were eluted with the following gradient: 90% (A) from 0 to

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