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ORIGINAL ARTICLE

Sea buckthorn (*Hippophae rhamnoides* L.) vegetative parts as an unconventional source of lipophilic antioxidants

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β-Carotene

Abstract The profile of lipophilic antioxidants in different vegetative parts (leaves, shoots, buds and berries) was studied in sea buckthorn (*Hippophae rhamnoides* L.) male and female plants collected in the end of spring. Five lipophilic compounds, i.e. three tocopherol homologues (α, β and γ), plastochromanol-8 and β-carotene, were identified in each vegetative part of male and female sea buckthorn plants at the following concentrations: 7.25–35.41, 0.21–2.43, 0.41–1.51, 0.19–1.79 and 4.43–24.57 mg/100 g dry weight basis. Additionally, significant amounts of α-tocotrienol (1.99 mg/100 g dry weight basis) were detected in buds. The α-tocopherol and β-carotene were predominant lipophilic antioxidants in each vegetative part, accounting for 78.3–97.0% of identified compounds. The greatest amounts of lipophilic antioxidants were found in leaves, especially of female plants. Nevertheless, apart from leaves, also shoots of plants of both sexes seem to be a good source of α-tocopherol and β-carotene.

1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is widely grown all over the world for its valuable berries. Nevertheless, presently not only berries, but also leaves are focused on by scientists due to the presence of nutritionally beneficial hydrophilic and lipophilic compounds (Górnaś et al., 2014d; Guan et al., 2005; Kumar et al., 2011; Sajfrtov and Sovova, 2012; Šnē et al., 2013a,b). Moreover, Šnē et al. (2013a,b) reported that not only leaves, but also other vegetative parts, e.g. shoots, may be used as a valuable source of phenolic compounds. In the last decade an increased interest has been observed in the use of phenolic compounds from non-traditional sources

Abbreviations: DW, dry weight basis; NP-HPLC/FLD/DAD, normal phase-high-performance liquid chromatograph/fluorescence detection/diode-array detection; PC-8, plastochromanol-8; SD, standard deviation; T, tocopherol; T3, tocotrienol

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(Šnē et al., 2013a,b), but also in lipophilic antioxidants such as tocochromanols (Ciftci et al., 2011; Górnaś et al., 2014a,b), β-carotene and plastochromanol-8 (Górnaś et al., 2014c, 2013). Those bio-compounds, especially tocopherols and tocotrienols, are reported to possess a wide spectrum of biological activities, such as antioxidant and inflammatory properties (Jiang et al., 2001; Nogala-Kałucka et al., 2013). Therefore, since the amount of information concerning lipophilic compounds in sea buckthorn vegetative parts (leaves, shoots and buds) is still limited or lacking, the aim of this study was to evaluate their profile.

2. Materials and methods

2.1. Reagents

Tocopherol (T) and tocotrienol (T3) homologues (α, β, γ and δ) standards (>95% of purity) were purchased from Merck (Darmstadt, Germany) and LGC Standards (Teddington, Middlesex, UK), respectively. Methanol, ethanol, *n*-hexane, ethyl acetate (HPLC grade), β-carotene (≥97.0%), pyrogallol, sodium chloride and potassium hydroxide were obtained from Sigma–Aldrich (Steinheim, Germany). Since, the standard of plastochromanol-8 (PC-8) is not commercially available, was isolated from flaxseed oil using a semi-preparative HPLC to obtain the pure PC-8 according to Siger et al. (2014).

2.2. Plant material

Vegetative parts of sea buckthorn (*H. rhamnoides* L.) were collected in Baltplant Ltd., Latvia, GPS location: N: 56°36′39″; E: 23°17′50″, at two different harvest times, in the second part of April (buds) and the middle of June (leaves, shoots and berries) 2012. Sea buckthorn vegetative parts were picked from ten randomly selected 7-year old male and female trees, grown at a 2 × 4 m spacing without fertilization and irrigation. Two female cultivars 'Botanicheskaya Lubitelskaya' and 'Prozrachnaya' (selected by T. T. Trofimov, the Moscow Botanical Garden) and male plants (open pollinated seedlings of unknown origin) were used in the experiment. About 20 ± 1 g of each vegetative part of sea buckthorn was harvested from each tree (both female and male) by sampling from the lower and upper parts around the tree circumference (between 08:30 and 09:30 a.m. local time) and transported immediately (15 min) to the laboratory. All sea buckthorn vegetative parts, separately for material collected from male and from female trees were carefully mixed, frozen in liquid nitrogen and immediately powdered using a Knifetec 1095 laboratory mill (Foss, Höganäs, Sweden).

Dry weight basis (dw) in samples was measured gravimetrically according to Ruiz (2005).

2.3. Extraction of tocochromanols, PC-8 and β-carotene

Tocochromanols, PC-8 and β-carotene from the sea buckthorn vegetative parts were extracted according to the previously validated method (Górnaś et al., 2014a) with minor modifications. Since this saponification method was shown in a previous study to be the best to recover lipophilic antioxidants from sea buckthorn leaves (Górnaś et al., 2014d), it was also applied in the present study to determine the profile of lipophilic compounds in leaves, shoots, berries and buds of sea buckthorn plants. Briefly, 0.1 g of powdered sea buckthorn vegetative parts was placed in a 15 mL tube, next pyrogallol (0.05 g), ethanol (2.5 mL) and potassium hydroxide (0.25 mL, 600 g/L) were added, vortexed (10 s) and incubated in a water bath (25 min, 80 °C). During incubation in the water bath (after 10 min) the test tubes were vortexed (10 s). After the completion of incubation the samples were immediately cooled in an ice-water bath, subsequently supplemented with sodium chloride (2.5 mL, 10 g/L) and vortexed (5 s). Then *n*-hexane:ethyl acetate (2.5 mL, 9:1; v/v) was added, the samples were vortexed (15 s) and centrifuged (5 min, 1000*g*, at 4 °C). The organic layer was collected to a round bottom flask and the residues were retracted (×3) as described above. The combined organic layer fractions were evaporated using a Laborota 4000 vacuum rotary evaporator (Heidolph, Schwabach, Germany), dissolved in *n*-hexane (2 mL) and analysed immediately by NP-HPLC/FLD/DAD.

2.4. Chemical analysis

The lipophilic compounds were separated on a Water system (Milford, MA, USA) consisting of a pump (Waters 600), a fluorescence detector (Waters 474) and a diode-array detector (Waters 2998 PDA) according to Górnaś et al., 2014c. Analyses were run on a LiChrosorb Si 60, 4.6 × 250 mm, 5 μm column (Merck, Darmstadt, Germany) at the column thermostat set at 20 °C. The mobile phase was a mixture of *n*-hexane and 1,4-dioxane (97:3 v/v) at a flow rate of 1.5 mL/min. For tocopherol and PC-8 measurements the fluorescence detector was set at the following wavelengths, excitation λ = 295 nm and emission λ = 330 nm. β-Carotene was detected using a photo-diode array detector at a wavelength λ = 450 nm. Tocopherols and β-carotene were identified and quantified based on commercial standards whereas, PC-8 was identified using the PC-8 standard isolated from flaxseed oil and expressed as equivalents of the γ-T commercial standard. The results of chromatographic separation, with the exception of β-carotene, are presented in Fig. 1.

2.5. Statistical analysis

The results are presented as means ± standard deviation from three replicates of each experiment. A p-value <0.05 was used to denote significant differences between mean values determined by the analysis of variance (ANOVA). The homogeneity of variance was verified by Levene's test. Homogeneous groups were determined by the Bonferroni post hoc test with the assistance of Statistica 10.0 (StatSoft, Tulsa, OK, USA) software.

3. Results and discussion

Five lipophilic compounds, i.e. α-T, β-T, γ-T, PC-8 and β-carotene, were identified in each vegetative part of sea buckthorn (leaves, shoots, berries and buds) male and female plants. Additionally, significant amounts of α-T3 (1.99 mg/100 g dw) were detected in buds. In each vegetative part of both male and female sea buckthorn plants α-T was a predominant homologue of tocopherol (81.9–96.9%).