Journal of Food Composition and Analysis 24 (2011) 103–110

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Article

The effects of extraction method on the measured tocopherol level and antioxidant activity of *L. nobilis* vegetative organs

Olfa Ouchikh [a,*], Thouraya Chahed [a], Riadh Ksouri [b], Mouna Ben Taarit [a], Hanen Faleh [b], Chedly Abdelly [b], Mohamed Elyes Kchouk [a], Brahim Marzouk [a]

[a] *Laboratory of Bioactive Substances, Biotechnology Center in Borj Cedria Technopole, BP, 901, Hammam-Lif 2050, Tunisia*
[b] *Laboratory of Plant Adaptation to Abiotic Stress, Borj Cedria Biotechnology Centre (CBBC), Borj Cedria Ecopark, B.P. 901, 2050 Hammam-Lif, Tunisia*

ARTICLE INFO

Article history:
Received 22 November 2008
Received in revised form 17 April 2010
Accepted 22 April 2010
Available online 8 January 2011

Keywords:
Laurus nobilis vegetative organs
HPLC
Tocopherol
Probe sonication
Micro-scale saponification
Antioxidant activity
β-Carotene–linoleic acid system

ABSTRACT

Tocopherol contents of Tunisian *Laurus nobilis* vegetative organs were screened for antioxidant activity. Tocopherol isomers extracted by probe sonication and micro-scale saponification were analysed by reversed-phase high performance liquid chromatography (RP-HPLC) with UV detection to determine the optimum extraction method. Total phenolic, flavonoid and proanthocyanidin contents were determined spectrophotometrically. Significant variations in the amounts of α-, γ- and δ-tocopherols were observed with the two different extraction methods as well as with different plant parts. Leaf extract contained the highest amount of α-tocopherol (139 mg/100 g fresh weight), but root extract contained the highest content of flavonoids (11.12 mg catechin equivalents (CE)/g dry weight or DW), total phenolics (55.45 ± 2.9 mg GAE/g DW) and condensed tannins (9.76 ± 0.1 mg CE/g DW). Acetonic extract of laurel leaf exhibited the highest antioxidant activity response to lipid peroxidation in the β-carotene–linoleic acid system, which may have been due to the high content of α-tocopherol. These findings suggest that laurel leaf may be a source of natural α-tocopherol and that it may be increasingly important for human consumption, as well as for the agro-food, cosmetic and pharmaceutical industries.

1. Introduction

Tocopherols (vitamin E), synthesised only by photosynthetic organisms, are lipid-soluble antioxidants that share a common structure, with a chromanol head containing two rings, one phenolic and one heterocyclic, and a phytyl tail. Chemically, α-tocopherol is the most active form of vitamin E due to the substitution pattern of methyl groups on the chromanol ring that make the hydrogen of the C-6 hydroxy group especially active, which facilitates the transfer of the hydrogen to a peroxyl radical. Several studies suggest that use of vitamin E may contribute to help lower the risks of specific chronic and degenerative diseases such as Alzheimer's disease, age-related macular degeneration, some types of cancer, cataracts and ischemic heart disease (Hathcock et al., 2005). *Laurus nobilis* (also called bay-tree, bay laurel or bay leaves) is an evergreen tree known in Greek and Roman mythology as an emblem of victory and success. It is the only European representative of the Lauraceae family (Leung and Foster, 1999). This plant is cultivated in many temperate and warm parts of the world, particularly in the Mediterranean area including Greece, Spain, Portugal, Morocco and Turkey. In Tunisia, bay leaf grows at the edges of rivers, on mountains and on wet cliffs. It also grows in the humid and sub-humid bioclimatic areas, especially in Ain Draham, Tabarka, and Cap-Bon (Pottier-Alapetite, 1979). This species is endangered, and it is listed for environmental protection (Boussaid et al., 1998).

Bay leaf is widely used as a dried herb and gives a very fragrant and aromatic essential oil; it is used as a valuable spice and flavouring agent in the culinary and food industries and as an additive in cosmetics (Conforti et al., 2006). The bay leaf known *Rand* in Tunisia is used in several types of cooking as well as in folk medicine for its antirheumatic properties, for the treatment of anal fistulas and various dermatoses and for its efficacy as a stomachic and carminative remedy. Further, the decoct root was recognised for its expectorant properties. The drup was also used by the Jewish population of Jerba in the treatment of jaundice and rheumatic pain (Boukef, 1986).

L. nobilis has attracted renewed interest because of the pharmacological properties and potential health benefits related to several compounds present in the plant. Recent studies have shown that essential from laurel leaves possesses antimicrobial (De Corato et al., 2010; Derwich et al., 2009; Marzouki et al., 2009a,b), antiviral (Loizzo et al., 2008; Simic et al., 2004) and antioxidant activities (Politeo et al., 2007). The phenolic constituents and

* Corresponding author. Tel.: +216 714 30855; fax: +216 71412 836.
E-mail address: ouchikholfa@yahoo.fr (O. Ouchikh).

0889-1575/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2010.04.006

bioactivities of organic and aqueous extracts from leaves have been studied extensively (Conforti et al., 2006; Dall'Acqua et al., 2009; De Marino et al., 2004; Liu et al., 2009; Papageorgiou et al., 2008; Škerget et al., 2005). However, vitamin E has been isolated only from leaves (Demo et al., 1998; Gómez-Coronado and Barbas, 2003; Puoci et al., 2007) and fruits (Frega et al., 1982); other parts of the plant have not been investigated previously.

Due to the nutritional importance of tocopherol, research studies have been performed to develop a simple and sensitive method for routine determination of tocopherol. The extraction of tocopherols from the sample is the most critical and time-consuming step in the quantification of tocopherols using both reversed-phase and normal phase HPLC. Depending on the nature of the sample, various extraction methods have been employed, including saponification, soxhlet extraction and direct solvent extraction, to release vitamin E (Eitenmiller and Lee, 2004; Lee and Lee, 2006; Lim et al., 2007; Ryynänen et al., 2004; Sánchez-Machado et al., 2002).

To the best of our knowledge, this is the first study to describe tocopherol extraction from *L. nobilis* stems, roots and leaves, and to evaluate the effects of different extraction methods on the efficiency of the extraction of tocopherols. The present work includes a comparison of tochopherols extracted either by microscale saponification or by probe sonication as well as a discussion of the concentration of these compounds, known for their high antioxidant capacity, in laurel parts. An investigation of the antioxidant activity of laurel stems and roots has not been reported to date. To address this data gap, antioxidant activity has been assayed, and the content of total phenolics has been determined, in order to characterise and establish the value of *L. nobilis* for other applications in the pharmaceutical, cosmetic and agroalimentary industries. Increased use of *L. nobilis* in new applications could result in preservation and intensified cultivation *of L. nobilis* in Tunisia.

2. Experimental procedures

2.1. Chemicals and standards

All solvents (HPLC grade quality), γ- and δ-tocopherols, linoleic acid and β-carotene were purchased from Sigma–Aldrich (Germany). Folin–Ciocalteu reagent, Na_2CO_3, vanillin, catechin, gallic acid, sodium nitrite, $AlCl_3$, NaOH, potassium hydroxide, ammonium molybdate, ascorbic acid, sodium phosphate, Tween 40, BHT and α-tocopherol were purchased from Fluka (Biochemika; Buchs, Switzerland). All other chemicals used were of analytical grade.

2.2. Apparatus

The HPLC system used was an Agilent Technologies 1100 Series liquid chromatograph (RP-HPLC) coupled with a UV–vis multi-wavelength detector, equipped with a G1328A manual injector (DE54003304), a G1311A Agilent quaternary pump (DE03012126), a G1322A Agilent vacuum solvent delivery degasser (JP 05026203) and a G1314A Agilent UV–Vis photodiode array detector. Chromatographic analysis was performed using an analytical scale (250 mm × 4.6 mm) C_{18} hypersil ODS column with a particle size of 5 μm (Agilent Technologies, Palo Alto, CA, USA).

The liquid chromatographic system was controlled, and the data were collected and processed by HP Chemstation for LC 3D software (Agilent Technologies Palo Alto, CA, USA).

2.3. Plant material

L. nobilis bay leaves, roots and stems (or branches) were collected from five trees in November 2006 from the region of Menzel Bouzalfa, in the Northeast of Tunisia (36°41′02 70″N and 10°34′56 86″E) at about 44 m above sea level. The plant material was botanically characterised by Prof. Abderrazak Smaoui (Biotechnology Center in Borj-Cedria Technopark, Tunisia) according to the morphological description presented in Tunisia flora (Pottier-Alapetite, 1979). A control specimen has been kept in our laboratory for future reference.

2.4. Determination of tocopherol content in L. nobilis

2.4.1. Drying procedure

After collection, approximately 5 g of laurel leaves, branches and roots were weighed in triplicate before and after drying in a microwave oven at 800 W to constant weight. The drying time for each organ was optimised to 75 s for young branches, 95 s for leaves and 135 s for roots, which corresponded to water levels of 53, 50.5, and 32%, respectively. The efficacy of this microwave method for drying has been demonstrated previously Gómez-Coronado et al. (2004). Malheiro et al. (2009) reported an increase in the content of α-tocopherol early during the process of microwave heating olive oil, which may be explained by the destruction of other compounds that were linked to α-tocopherol.

2.4.2. Extraction procedure

2.4.2.1. Probe sonication. Tocopherols from *L. nobilis* leaves, branches and roots were extracted by probe sonication, according to the method described by Rupérez et al. (2001). Briefly, 0.25 g of ground *L. nobilis* material was added to 4 mL of acetone and sonicated for 1 min at 50–60 Hz at ambient temperature. The solvent was evaporated under nitrogen. Leaf, branch and root residues, 70.55 ± 1.45, 45.50 ± 1.5 and 71.80 ± 1.2 mg, respectively, were re-dissolved in 1 mL of methanol, and 20 μL of the sample were injected into the HPLC column.

2.4.2.2. Microscale saponification. Tocopherols from *L. nobilis* leaves, branches and roots were also extracted by the method of Sánchez-Machado et al. (2002) with minor modifications. Briefly, 1 mL of L-ascorbic acid solution (6 mM in methanol) and 5 mL of KOH solution (0.5 M in methanol) were added to screw-top assay tubes containing 0.40 g of *L. nobilis* samples. The tubes were heated at 80 °C for 15 min, and then 1 mL of distilled water and 5 mL of hexane were added after cooling. The upper phase was dried under nitrogen, residue was redissolved in 1 mL of methanol and 20 μL of each laurel sample was injected into the HPLC column. Before injection extracts were kept at −10 °C in the dark until analysis.

2.4.3. Analytical HPLC

Tocopherol separation was performed on a 250 mm × 4.6 mm, 5 μm Hypersil ODS C18 reversed-phase column with an isocratic elution; the solvent flow rate was maintained at 1 mL min^{-1} with column temperature of 25 °C and a backpressure of approximately 400 bar.

The mobile phase consisted of methanol:acetonitrile at a ratio of 95:5 (v/v). The UV detection wavelength was set at 292 nm. Tocopherols were identified by comparison of the retention times with those of authentic standards. Peaks were observed at 5.25 ± 0.15 min for δ-tocopherol, 5.94 ± 0.15 min for γ-tocopherol and 6.57 ± 0.13 for α-tocopherol. RP-HPLC chromatograms of a standard solution and typical tocopherols from *L. nobilis* organs are shown in Fig. 1. Tocopherols were quantified by an external standard method such that quantification was based on peak areas (Ryynänen et al., 2004; Schwartz et al., 2008). Calibration curves with eight points were obtained daily by standard injections. The method was validated by determining the following parameters: detection limit, range of linearity, precision and accuracy. The detection limit was calculated as the concentration corresponding to a signal three times the standard deviation of the baseline noise (American Chemical