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Analytical Methods

Nutritional and antioxidant contributions of *Laurus nobilis* L. leaves: Would be more suitable a wild or a cultivated sample?

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ABSTRACT

Medicinal and aromatic plants are used since ancient times in folk medicine and traditional food, but also in novel pharmaceutical preparations. The controversy lies in the use of cultivated and/or wild plants presenting both advantages and disadvantages in biological, ecological but also economic terms. Herein, cultivated and wild samples of *Laurus nobilis* L. were chemically characterized regarding nutritional value, free sugars, organic acids, fatty acids and tocopherols. Furthermore, the antioxidant activity (scavenging activity, reducing power and lipid peroxidation inhibition) and individual phenolic profile of *L. nobilis* extracts and infusions were evaluated. Data showed that the wild sample gave higher nutritional contribution related to a higher content of proteins, free sugars, organic acids, PUFA and tocopherols. It also gave better PUFA/SFA and $n-6/n-3$ ratios. Regarding antioxidant activity and phenolic compounds, it was the cultivated sample (mostly the infusion) that showed the highest values. The present study supports the arguments defending the use of wild and cultivated medicinal and aromatic plants as both present very interesting features, whether nutritional or antioxidant, that can be an assessed by their consumption. *In vitro* culture could be applied to *L. nobilis* as a production methodology that allows combination of the benefits of wild and cultivated samples.

1. Introduction

Currently, there is a major controversy concerning the use of wild or cultivated plants, presenting both advantages and disadvantages in biological and ecological, but also economic terms (Schippmann, Leaman, & Cunningham, 2002). Due to the growing demand of global market, FAO (Food and Agricultural Organization) recommended the cultivation of medicinal and aromatic plants, not only from the point of view of sustainability but also because it allows better control of biotic and abiotic production conditions, representing a reliable resource of raw material that has gained great economic importance (Schippmann et al., 2002). Being used since ancient times for their organoleptic characteristics, therapeutic and medicinal properties, it is crucial to preserve the genetic-pool resources that these plants represent (Guarrera & Savo, 2013). On the other hand, the use of wild medicinal and aromatic plants by many local populations provides herbal medicines for health care needs encouraging their protection and maintenance, not requiring the use of pesticides neither investments in infrastructures to produce them (Schippmann et al., 2002).

Laurus nobilis L., commonly known as bay leaves, belongs to Laureacea family, being a native plant from the warm Mediterranean region, including countries like Italy, France, Spain and Portugal. It is widely used as a spicy fragrance and flavour in traditional meat dishes, stews and rice (Camejo-Rodrigues, Ascenção, Bonet, & Valles, 2003; Gómez-Coronado & Barbas, 2003; Ouchikh et al., 2011). Its leaves and extracts are used to suppress high blood sugar, fungal and bacterial infections, to treat eructation, flatulence and gastrointestinal problems. It also exhibits anti-inflammatory, anticonvulsive, antiepileptic and antioxidant properties (Conforti, Statti, Uzunov, & Menichinia, 2006; Ferreira, Proença, Serralheiro, & Araújo, 2006; Ouchikh et al., 2011; Ozcan, Esen, Sangun, Coleri, & Caliskan, 2010; Polovka & Suhaj, 2010; Ramos et al., 2012; Speroni et al., 2011). Infusions of dry bay leaves are used in folk medicine for their stomachic and carminative remedies and also

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to treat gastric diseases (Afifi, Khalil, Tamimi, & Disi, 1997; Dall'Acqua et al., 2009).

Tocopherols content of *L. nobilis* was reported on aerial parts (Demo, Petrakis, Kefalasa, & Boskou, 1998; Gómez-Coronado & Barbas, 2003; Gómez-Coronado, Ibañez, Rupérez, & Barbas, 2004) and vegetative organs (Ouchikh et al., 2011); fatty acids composition was studied on seeds (Ozcan et al., 2010).

Antioxidant activity of wild *L. nobilis* leaves was previously reported on ethanol and aqueous extracts (Elmastaş et al., 2006; Emam, Mohamed, Diab, & Megally, 2010; Kaurinovic, Popovic, & Vlaisavljevic, 2010; Ramos et al., 2012), methanol/water extracts (Conforti et al., 2006) and infusions (Dall'Acqua et al., 2009). Flavonoids such as quercetin, luteolin, apigenin, kaempferol and myrcetin derivatives as well as flavan-3-ols have been reported as the most abundant phenolic compounds found in bay leaves (Dall'Acqua et al., 2009; Lu, Yuan, Zeng, & Chen, 2011; Škerget et al., 2005). The hydroxyl groups attached to the ring structure of flavonoids conferred them antioxidant properties, acting as reducing agents, hydrogen donators, metal chelators and radical scavengers, preventing oxidative stress, the main cause of cell death (Carocho & Ferreira, 2013).

In the present work, *L. nobilis* wild and cultivated samples were chemically characterized regarding nutritional value, free sugars, organic acids, fatty acids and tocopherols. Furthermore, as far as we know, this is the first study comparing antioxidant activity and phenolic compounds of extracts and infusions of *L. nobilis* cultivated and wild samples.

2. Materials and methods

2.1. Samples

The cultivated *Laurus nobilis* L. sample (air-dried leaves) was purchased from a local company, Ervital from Castro Daire, Portugal, which produces Mediterranean herbs using organic principles and methods. According to the label information, the leaves were collected in 2012. The wild sample (fresh leaves) was collected in 2012 autumn in Bragança, Portugal, and further air-dried. Both samples were lyophilized (FreeZone 4.5, Labconco, Kansas, USA) in order to preserve as most as possible their chemical composition until analysis. Afterwards, each sample was reduced to a fine dried powder (20 mesh) and mixed to obtain homogenate sample.

2.2. Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also were other individual fatty acid isomers, L-ascorbic acid, tocopherol, sugar and organic acid standards. Phenolic compound standards were from Extrasynthese (Genay, France). Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Nutritional contribution of wild and cultivated samples

2.3.1. Proximate composition and energetic value

The samples were analysed for proteins, fat, carbohydrates and ash using the AOAC procedures (AOAC, 1995). The crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein) + 3.75 × (g carbohydrate) + 9 × (g fat).

2.3.2. Sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), after an extraction procedure previously described by the authors (Guimarães et al., 2013) using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and an RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 °C (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards analysed in the same conditions. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight.

2.3.3. Organic acids

Organic acids were determined following a procedure previously described by the authors (Pereira, Barros, Carvalho, & Ferreira, 2013). The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation). Separation was achieved on a SphereClone (Phenomenex) reverse phase C_{18} column (5 μm, 250 mm × 4.6 mm i.d.) thermostatted at 35 °C. The elution was performed with sulphuric acid 3.6 mM using a flow rate of 0.8 mL/min. Detection was carried out in a PDA, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

2.3.4. Fatty acids

Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Guimarães et al., 2013). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 °C) and a Macherey–Nagel column (30 m × 0.32 mm × 0.25 μm). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 30 °C/min ramp to 125 °C, 5 °C/min ramp to 160 °C, 20 °C/ min ramp to 180 °C, 3 °C/min ramp to 200 °C, 20 °C/min ramp to 220 °C and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with those of standards. The results were recorded and processed using the CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.3.5. Tocopherols

Tocopherols were determined following a procedure previously described by the authors (Guimarães et al., 2013). Analysis was performed by HPLC (equipment described above), and a fluorescence detector (FP-2020; Jasco) programmed for excitation at