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.

Keywords: Laurus nobilis L.; Cultivated/Wild; Chemical characterization; Antioxidant properties; Phenolic profile

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 flavor 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 (Ferreira, Proença, Serralheiro, & Araújo, 2006; Conforti, Statti, Uzunov, & Menichini, 2006; Ozcan, Esen, Sangun, Coleri, & Caliskan, 2010; Polovka & Suhaj, 2010; Ouchikh et al, 2011; Speroni et al, 2011; Ramos et al, 2012). Infusions of dry bay leaves are used in folk medicine for their stomachic and carminative remedies and also 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, Kefalas, & Boskou, 1998; Gómez-Coronado & Barbas, 2003; Gómez-Coronado, Ibañez, Ruperéz, & 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 (Škerget et al, 2005; Dall'Acqua et al., 2009; Lu, Yuan, Zeng, & Chen, 2011). 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 ^{o}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 ºC (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 ºC. 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 ºC) 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 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/ min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. 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 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 mm × 4.6 mm i.d.) normal-phase column from YMC Waters operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the injection volume was 20 µL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

2.4. Antioxidants contribution of wild and cultivated samples

2.4.1 Methanolic extract and infusion preparations. The methanolic extract was obtained from the wild and cultivated plant material. Each sample (1 g) was extracted twice by stirring with 30 mL of methanol (25 ºC at 150 rpm) for 1 h and subsequently filtered through Whatman No. 4 paper (Guimarães et al, 2013). The combined methanolic extracts were evaporated at 40 ºC (rotary evaporator Büchi R-210) to dryness.

For infusion preparation the plant material (1 g) was added to 200 mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered under reduced pressure (Guimarães et al, 2013). The obtained infusion was frozen and lyophilized.

2.4.2 Antioxidant activity evaluation. Methanolic extracts and lyophilized infusions were redissolved in methanol and water, respectively (final concentration 2.5 mg/mL) for antioxidant activity evaluation. The final solutions were further diluted to different concentrations to be submitted to the following assays. DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, USA), and calculated as a percentage of DPPH discolouration using the formula: $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance after 2h of assay/initial absorbance) × 100. Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decreasing in thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: $[(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the sample solution, respectively (Guimarães et al, 2013). The final results were expressed in EC_{50} values (mg/mL), sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay). Trolox was used as positive control.

2.4.3 Phenolic profile. Phenolic compounds were determined by HPLC (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, USA) as previously described by the authors (Rodrigues et al, 2012). Double online detection was carried out in the diode array detector (DAD) using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (API 3200 Qtrap, Applied Biosystems, Darmstadt, Germany) connected to the HPLC system via the DAD cell outlet. The phenolic compounds were characterized according to their UV and mass spectra and retention times, and comparison with authentic standards when available. For quantitative analysis, calibration curves were prepared from different standard compounds: catechin ($y=158.42x+11.38$; $R^2=0.999$); epicatechin ($y=129.11x+11.663$, $R^2=0.9999$); rutin ($y=281.98x-0.3458$; $R^2=1$); kaempferol-3-*O*-glucoside ($y=288.55x-4.05$; $R^2=1$); kaempferol-3-*O*-rutinoside ($y=239.16x-10.587$; $R^2=1$); apigenin-6-*C*-glucoside ($y=223.22x+60.915$, $R^2=1$); luteolin-6-*C*-glucoside ($y=508.54x-152.82$; $R^2=0.997$); luteolin-7-*O*-glucoside ($y=80.829x-21.291$; $R^2=0.999$); quercetin-3-*O*-glucoside ($y=253.52x-11.615$; $R^2=0.999$) and isorahmetin-3-*O*-rutinoside ($y=327.42x+313.78$; $R^2=0.999$) The results were expressed in mg per g of methanolic extract and lyophilized infusion.

2.5. Statistical analysis

For wild and cultivated plant material, three samples were used and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey’s HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 18.0 program.

3. Results and Discussion

3.1. Nutritional contribution of wild and commercial samples

Data on the chemical composition of cultivated and wild samples of *L. nobilis* namely, macronutrients, sugars and organic acids are presented in **Table 1**. Carbohydrates (including fiber) were the major macronutrients found in both samples, followed by proteins, fat and ash. Both samples revealed similar contents of carbohydrates, fat, ash and energetic values whereas the wild sample showed higher protein contents.

Fructose, glucose and sucrose were the free sugars detected in the studied samples. The wild sample gave the highest contents in total free sugars and also in sucrose and glucose. Fructose was the major free sugar found in the cultivated sample. The wild sample also revealed the highest content of organic acids. Oxalic and malic acids were found in both samples, but ascorbic acid was only found in wild bay leaves (**Table 1**). The several processes applied to cultivated samples throughout the supply chain (pre-harvest conditions, postharvest handling, storage conditions, processing, and preparation) could contribute to degradation of ascorbic acid. Nonetheless, the maturity at harvest and the genetic variations between both samples could also influence ascorbic acid content (Howard, Wong, Peery, & Klein, 1999).

Up to twenty-five fatty acids were found in cultivated and wild samples of *L. nobilis* (**Table 2**). Palmitic acid (C16:0; SFA) was the major fatty acid present in the cultivated sample, followed by linolenic acid (C18:3n-3; PUFA), while in the cultivated sample the opposite was observed. Thus, the highest levels of saturated fatty acids (SFA) were found in the cultivated sample, while wild bay leaves gave the highest content of polyunsaturated fatty acids (PUFA). Ozcan et al. (2010) reported linoleic and lauric acids as the main fatty acids in *L. nobilis* seeds, followed by palmitic acid. For a “good nutritional quality” with high health benefits, ratio on PUFA/SFA should be higher than 0.45 and n-6/n-3 fatty acids should be lower than 4.0 (Guil et al, 1996). Both samples presented the required values, however wild sample of bay leaves presented a higher value of PUFA/SFA ratio and a lower value of n-6/n-3 fatty acids ratio. All the isoforms of tocopherols were found in both samples of bay leaves (**Table 2**). Once more, the wild sample showed the highest total tocopherols content, mainly γ-tocopherol followed by α-tocopherol. Previous studies conducted using different extraction methodologies including a saponification step (Demo et al., 1998; Ouchikh et al., 2011) and supercritical fluids (Gómez-Coronado, 2004), or even different extraction solvents (Gómez-Coronado & Barbas, 2003), reported much lower tocopherols content and not detecting all the isoforms reported herein.

3.2. Antioxidants contribution of wild and commercial samples

The antioxidant activity of methanolic extract and infusion of cultivated and wild *L. nobilis* was studied and the results are presented in **Table 3**. Both preparations were chosen because infusions and extracts of the leaves are widely used in medicinal practices, as stated in the introduction section. In general, infusions of both samples revealed higher antioxidant activity (lower EC_{50} values) than methanolic extracts. Cultivated *L. nobilis* showed higher DPPH scavenging activity, reducing power and TBARS inhibition than the wild sample. The samples studied herein showed higher DPPH scavenging activity than the aqueous-methanol and aqueous extracts of *L. nobilis* from Finland (EC_{50}=0.55 mg/mL; Koşar, Dorman, & Hiltunen, 2005) and Montenegro (EC_{50}=0.16 mg/mL; Kaurinovic et al., 2010). Santoyo et al (2006) showed that, in supercritical extraction fluids, the antioxidant activity of *L. nobilis* increases, with lower EC_{50} values for DPPH (EC_{50}=0.10 mg/mL) and β-carotene (EC_{50}=0.04 mg/mL) assays. As stated by Papageorgiou, Mallouchos, & Komaitis (2008), the use of different drying methods influences the antioxidant activity of bay leaves. Finally, Conforti et al. (2006) described the wild sample (but ethanolic extracts) as having higher antioxidant activity than cultivated bay leaves.

The HPLC phenolic profile of a wild sample of *L. nobilis* recorded at 280 and 370 nm is shown in **Figures 1** and **2**, respectively, and peak characteristics and tentative identities are presented in **Table 4**. Thirty-two compounds were detected, from which fourteen were flavan-3-ols (i.e., catechins and proanthocyanidins), fourteen flavonols and four flavones.

Flavan-3-ols, peaks 1-13 and 16, were identified according to their UV spectra and pseudomolecular ions. Peaks 3, 5 and 8 were identified as (+)-gallocatechin, (+)-catechin and (-)-epicatechin, respectively, by comparison of their UV spectra and retention time with authentic standards. Peaks 1 and 2 presented a pseudomolecular ion $[M-H]^-$ at *m/z* 451, releasing an MS^2 fragment at *m/z* 289 ($[M-H-162]^-$, loss of an hexosyl moiety), corresponding to a catechin monomer. These compounds were tentatively identified as (epi)catechin hexosides, identity that was coherent with their earlier elution (higher polarity) compared with the parent aglycones (Santos-Buelga, Garcia-Viguera, & Tomas-Barberan, 2003). Other signals at *m/z* 577, 865 and 1153 (peaks 4, 6, 7, 11-13 and 16), can be respectively associated to B-type procyanidin dimers, trimers and tetramers (*i.e.*, (epi)catechin units with C4-C8 or C4-C6 interflavonoid linkages). Furthermore, peaks 9 and 10 showed a pseudomolecular ion $[M-H]^-$ at *m/z* 863 that could correspond to a procyanidin trimer containing one B-type and one A-type (i.e., C4-C8 or C4-C6 and C2-*O*-C7 or C2-*O*-C5) interflavonoid linkages (Dall'acqua et al., 2009; Rodrigues et al., 2012).

Fourteen flavonols derivatives were also detected, five of them derived from quercetin (λ_{max} around 350 nm and an MS^2 fragment at *m/z* 301), other five from isorhamnetin (λ_{max} around 354 nm and an MS^2 fragment at *m/z* 315) and four from kaempferol (λ_{max} around 347 nm and an MS^2 fragment at *m/z* 285) (**Table 4**). Quercetin 3-*O*-rutinoside (peak 18), quercetin 3-*O*-glucoside (peak 20), kaempferol 3-*O*-rutinoside (peak 22), kaempferol 3-*O*-glucoside (peak 24) and isorhamnetin 3-*O*-rutinoside (peak 25) were positively identified according to their retention, mass and UV-vis characteristics by comparison with a commercial standard.

Peaks 21, 23 and 26 ($[M-H]^-$ at *m/z* 463, 433 and 447, respectively) were assigned to quercetin (*m/z* at 301) derivatives; peaks 29 and 31 ($[M-H]^-$ at *m/z* 417 and 431, respectively) were assigned to kaempferol (*m/z* at 285) derivatives and peaks 27, 28, 30 and 32 ($[M-H]^-$ at *m/z* 477, 447 and 461, respectively) were assigned to isorhamnetin (*m/z* at 315) derivatives, presenting distinct losses of hexosyl (-162 mu), pentosyl (-132 mu) and rhamnosyl (-146 mu) moieties (Dias et al., 2013). Their elution order was coherent with the type of substituent sugars, according to their expected polarity, although the position and nature of the sugar moieties could not be identified, because their retention times did not correspond to any of the standards available.

The remaining phenolic compounds corresponded to *C*-glycosylated flavones, three apigenin derivatives (peaks 15, 17 and 19) and one luteolin derivative (peak 14), according to their UV spectra (λ_{max} around 337 for apigenin and 350 nm for luteolin) and MS^2 fragmentation pattern (**Table 4**). Peaks 15 and 19 showed the same pseudomolecular ion $[M-H]^-$ at *m/z* 431 giving place to two MS^2 fragment ions, a major one at *m/z* 341 $[M-90]^-$, and another one at *m/z* 311 $[M-120]^-$. This fragmentation pattern was characteristic of *C*-glycosylated flavones at C-6/C-8, and the relative abundance of fragments pointed out to sugar substitution at C-8 (peak 15) at C-6 (peak 19) according to the fragmentation patterns described by Ferreres, Silva, Andrade, Seabra, & Ferreira (2003) and Ferreres, Llorach, & Gil-Izquierdo (2004). These peaks were respectively identified as apigenin 8-*C*-glucoside and apigenin 6-*C*-glucoside; the identity of this latter was further confirmed by comparison with an authentic standard. Peak 17 showed a pseudomolecular ion $[M-H]^-$ at *m/z* 577, releasing typical MS^2

fragments ions. The loss of 120 mu (ion at *m/z* 457 ([M-H-120]$^-$) is characteristic of *C*-hexosyl flavones (Ferreres et al., 2003), while the loss of 164 mu, releasing the fragment at *m/z* 413 ([M-H-146-18]$^-$) can be associated to an *O*-glycosylation on the hydroxyl group at position 2 of the *C*-glycosylating sugar (Ferreres, Gil-Izquierdo, Andrade, Valentão & Tomás-Barberán, 2007). The remaining ions at *m/z* 341 ([aglycone + 71)]$^-$, *m/z* 311 ([aglycone + 41)]$^-$ and *m/z* 293 ([aglycone + 41-18]$^-$) are usual in mono-*C*-glycosyl derivatives *O*-glycosylated on 2'' position (Ferreres et al., 2007). According to this fragmentation pattern the compound was tentatively identified as 2''-*O*-rhamnosyl-*C*-hexosyl-apigenin.

Peak 14 was assigned to a luteolin derivative. It showed a pseudomolecular ion [M-H]$^-$ at *m/z* 447 giving place to three MS^2 fragment ions, a major one at *m/z* 357 [M-H-90]$^-$, and other two at *m/z* 327 [M-H-120]$^-$ and at *m/z* 297 [M-H-30]$^-$. This fragmentation pattern and the relative abundance of fragments was characteristic of *C*-glycosylated flavones at C-6 (Ferreres et al., 2003, 2004). The peak was identified as luteolin-6-*C*-glucoside, which was further confirmed by comparison to a standard.

The cultivated sample presented higher concentration of phenolic compounds, especially flavonol and flavone derivatives, when compared to the wild sample; on the other hand, the flavan-3-ols concentration was very similar in both types of samples. Flavan-3-ols were the major phenolic compounds present in both wild and commercial samples (**Table 5**), being (-)-epicatechin and a procyanidin trimer with an A-type linkage the most abundant ones. Škerget et al. (2005) reported the identification of flavonols such as quercetin and kaempferol derivatives and flavan-3-ols in the methanolic extract of *L. nobilis* from Slovenia, but in much lower concentrations than in our samples. Dall'acqua et al. (2009) identified ten major peaks in the infusion of *L.*

nobilis from Italy corresponding to kaempferol and quercetin glycosides derivatives and flavan-3-ols (mainly catechin and proanthocyanidins), although these latter in very low amounts. Lu et al. (2011) reported the presence of flavonoids and low concentrations of phenolic acids in ethanolic extracts of *L. nobilis* from China, but with a single identification of rutin; all the phenolic acids were indicated as unknown. No relevant amounts of phenolic acid derivatives were detected in the samples here analysed.

Overall, the wild sample showed the highest 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 interesting nutraceutical features: the wild sample gave higher nutritional contribution, but it was the cultivated sample that showed higher bioactivity. *In vitro* culture could be applied to *L. nobilis* as a production methodology that allows combination of the benefits of wild and cultivated samples.

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Table 1. Macronutrients, free sugars and organic acids of cultivated and wild *Laurus nobilis*.

	Cultivated	Wild
Fat (g/100 g dw)	5.47 ± 0.00^{a}	5.41 ± 0.00^{a}
Proteins (g/100 g dw)	10.44 ± 0.02^{b}	13.24 ± 0.03^{a}
Ash (g/100 g dw)	4.83 ± 0.05^{a}	5.09 ± 0.41^{a}
Carbohydrates (g/100 g dw)	79.27 ± 0.03^{a}	76.26 ± 0.31^{a}
Energy (kcal/100 g dw)	408.06 ± 0.14^{a}	406.69 ± 1.16^{a}
Fructose	1.68 ± 0.02^{a}	1.40 ± 0.12^{b}
Glucose	1.17 ± 0.17^{b}	1.78 ± 0.32^{a}
Sucrose	1.34 ± 0.10^{b}	2.60 ± 0.61^{a}
Total sugars (g/100 g dw)	4.19 ± 0.09^{b}	5.79 ± 0.41^{a}
Oxalic acid	0.43 ± 0.01^{b}	0.55 ± 0.00^{a}
Malic acid	0.25 ± 0.03^{a}	0.35 ± 0.00^{a}
Ascorbic acid	nd	0.03 ± 0.00
Total organic acids (g/100g dw)	0.68 ± 0.02^{a}	0.90 ± 0.01^{a}

nd- not detected; dw- dry weight. In each row different letters mean significant differences ($p<0.05$).

Table 2. Fatty acids and tocopherols of cultivated and wild *Laurus nobilis*.

Fatty acid	Cultivated	Wild
C6:0	0.64 ± 0.01^a	0.45 ± 0.10^b
C8:0	0.37 ± 0.03^a	0.08 ± 0.01^b
C10:0	0.35 ± 0.05^a	0.29 ± 0.08^b
C12:0	1.73 ± 0.08^a	0.54 ± 0.14^b
C13:0	2.46 ± 0.15^a	1.36 ± 0.37^b
C14:0	5.27 ± 0.05^a	1.31 ± 0.22^b
C14:1	0.60 ± 0.03^a	0.41 ± 0.07^b
C15:0	0.95 ± 0.02^a	0.36 ± 0.11^b
C15:1CIS-10	0.17 ± 0.01^a	0.15 ± 0.04^a
C16:0	25.97 ± 0.25^a	13.47 ± 0.57^b
C16:1	0.58 ± 0.07^a	0.50 ± 0.10^a
C17:0	1.32 ± 0.00^a	0.62 ± 0.00^b
C17:1CIS-10	0.13 ± 0.01^b	0.29 ± 0.02^a
C18:0	8.77 ± 0.12^a	3.39 ± 0.01^b
C18:1n9	9.00 ± 0.01^a	3.78 ± 0.36^b
C18:2n6	9.64 ± 0.10^b	12.40 ± 0.51^a
C18:3n6	0.42 ± 0.11^a	0.20 ± 0.13^b
C18:3n3	13.40 ± 0.07^b	51.59 ± 1.12^a
C20:0	1.57 ± 0.02^a	1.11 ± 0.00^b
C20:1CIS-11	0.38 ± 0.04^a	0.15 ± 0.04^b
C20:3n3+C21:0	0.54 ± 0.07^a	0.32 ± 0.01^b
C22:0	2.58 ± 0.05^a	1.06 ± 0.00^b
C23:0	1.18 ± 0.02^a	0.44 ± 0.01^b
C24:0	11.96 ± 0.03^a	5.71 ± 0.31^b
SFA	65.11 ± 0.10^a	30.23 ± 1.92^b
MUFA	10.70 ± 0.10^a	5.12 ± 0.20^b
PUFA	24.01 ± 0.01^b	64.50 ± 1.76^a
PUFA/SFA	0.37 ± 0.02^b	2.14 ± 0.14^a
n6/n3	0.72 ± 0.00^a	0.24 ± 0.01^b
α - tocopherol	304.74 ± 16.89^b	370.05 ± 0.56^a
β - tocopherol	45.14 ± 0.77^a	13.53 ± 0.15^b
γ - tocopherol	302.33 ± 6.47^b	395.76 ± 2.64^a
δ - tocopherol	3.49 ± 0.02^a	0.78 ± 0.12^b
Total tocopherols (mg/100 g dw)	655.70 ± 22.62^b	780.12 ± 2.36^a

nd- not detected; dw- dry weight. Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Tridecanoic acid (C13:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); *cis*-10-Pentadecenoic acid (C15:1); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0) cis-10-Heptadecenoic acid (C17:1); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6c); γ-Linolenic acid (C18:3n6); Linolenic acid (C18:3n3); Arachidic acid (C20:0); *cis*-11-Eicosenoic acid (C20:1); *cis*-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3+C21:0); Behenic acid (C22:0); Tricosanoic acid (C23:0); Lignoceric acid (C24:0). SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. In each row different letters mean significant differences between species (p<0.05).

Table 3. Antioxidant activity of methanolic extracts and infusions of cultivated and wild *Laurus nobilis*.

	Cultivated		Wild	
	Methanolic extract	Infusion	Methanolic extract	Infusion
DPPH scavenging activity (EC_{50}, mg/mL)	0.15 ± 0.00^{b}	0.09 ± 0.00^{d}	0.20 ± 0.00^{a}	0.13 ± 0.01^{c}
Reducing power (EC_{50}, mg/mL)	0.12 ± 0.00^{b}	0.09 ± 0.00^{c}	0.14 ± 0.00^{a}	0.12 ± 0.00^{b}
β-carotene bleaching inhibition (EC_{50}, mg/mL)	0.18 ± 0.02^{a}	0.16 ± 0.02^{a}	0.10 ±0.01^{b}	0.20 ± 0.03^{a}
TBARS inhibition (EC_{50}, mg/mL)	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.03 ± 0.00^{a}	0.02 ± 0.01^{b}

EC_{50} values correspond to the sample concentration achieving 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. In each row different letters mean significant differences ($p<0.05$).

Table 4. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification of flavonoids in *Laurus nobilis*.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	5.12	278	451	289(100)	(Epi)catechin-hexoside
2	5.88	278	451	289(100)	(Epi)catechin-hexoside
3	6.49	276	305	219(13),179(24),125(10)	(+)-Gallocatechin
4	7.49	278	1151	865(11), 713(16), 577(7),575(35),561(5), 289(44)	Procyanidin tetramer
5	8.05	279	289	245(79), 203(58), 137(24)	(+)-Catechin
6	8.57	280	577	451(28), 425(60), 407(83), 289(61), 287(13)	Procyanidin dimer
7	9.08	279	577	451(49), 425(82), 407(100), 289(69), 287(15)	Procyanidin dimer
8	10.97	278	289	245 (83), 205(46), 151(24), 137(26)	(-)-Epicatechin
9	11.71	276	863	711(53), 573(27), 451(30), 411(43), 289(22), 285(9)	Procyanidin trimer (B- and A-type linkages)
10	12.36	278	863	711(46), 573(27), 451(34), 411(46), 289(20), 285(8)	Procyanidin trimer (B- and A-type linkages)
11	13.66	280	1153	865(9), 713(4), 577(29),575(14),561(6), 289(23)	Procyanidin tetramer
12	14.06	280	1153	865(13), 713(9), 577(33),575(30),561(5), 289(61)	Procyanidin tetramer
13	14.74	280	865	739(8),713(17), 695(9), 577(16), 575(25), 425(8),407(16), 289(7), 287(15)	Procyanidin trimer
14	16.50	350	447	357(72), 327(74), 297(14)	Luteolin 6-*C*-glucoside
15	18.12	337	431	341(16),311(100)	Apigenin 8-*C*-glucoside
16	18.91	280	577	451(49), 425(85), 407(97), 289(89), 287(22)	Procyanidin dimer
17	19.18	338	577	457(8),413(49),341(7),311(6),293(34)	2''-*O*-Rhamnosyl-*C*-hexosyl-apigenin
18	19.59	355	609	301(100)	Quercetin 3-*O*-rutinoside
19	20.21	336	431	341(76),311(100)	Apigenin 6-*C*-glucoside
20	20.51	356	463	301(100)	Quercetin 3-*O*-glucoside
21	20.92	355	463	301(100)	Quercetin *O*-hexoside
22	23.14	347	593	285(100)	Kaempferol 3-*O*-rutinoside

23	23.36	344	433	301(100)		Quercetin *O*-pentoside
24	23.56	350	447	285(100)		Kaempferol 3-*O*-glucoside
25	24.15	354	623	315(100)		Isorhamnetin *O*-rutinoside
26	24.71	348	447	301(100)		Quercetin *O*-rhamnoside
27	25.01	356	477	315(100)		Isorhamnetin *O*-hexoside
28	25.60	354	477	315(100)		Isorhamnetin *O*-hexoside
29	26.72	347	417	285(100)		Kaempferol *O*-pentoside
30	28.49	355	447	315(100)		Isorhamnetin *O*-pentoside
31	29.23	343	431	285(100)		Kaempferol *O*-hexoside
32	29.85	350	461	315(100)		Isorhamnetin *O*-rhamnoside

Table 4. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification of flavonoids in *Laurus nobilis*.

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10	12.36	278	863	711(46), 573(27), 451(34), 411(46), 289(20), 285(8)	Procyanidin trimer (B- and A-type linkages)
11	13.66	280	1153	865(9), 713(4), 577(29),575(14),561(6), 289(23)	Procyanidin tetramer
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17	19.18	338	577	457(8),413(49),341(7),311(6),293(34)	2''-*O*-Rhamnosyl-*C*-hexosyl-apigenin
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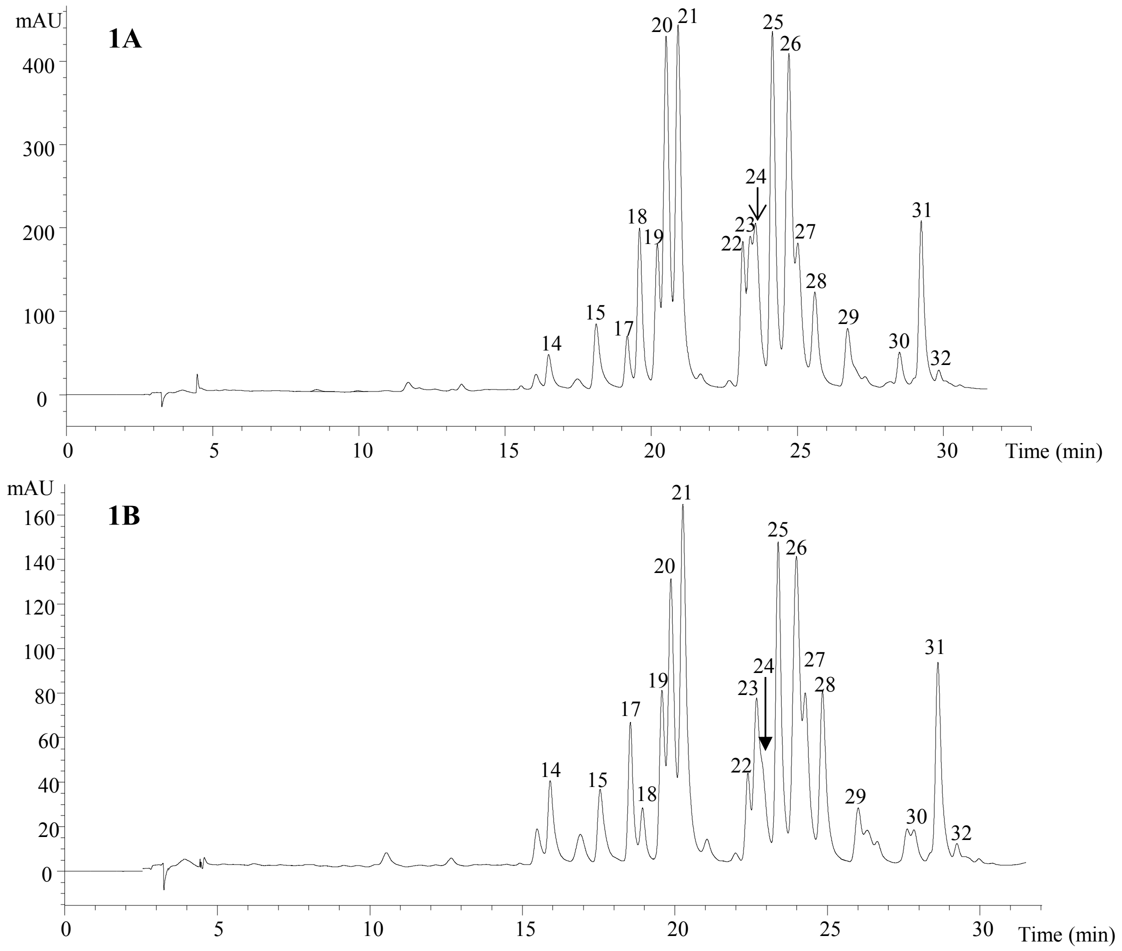

Figure 1. HPLC phenolic profile (flavone/ols) of cultivated (A) and wild (B) *Laurus nobilis*, obtained at 370 nm. Identification of peaks 14, 15 and 17-32 is presented in Table 4.

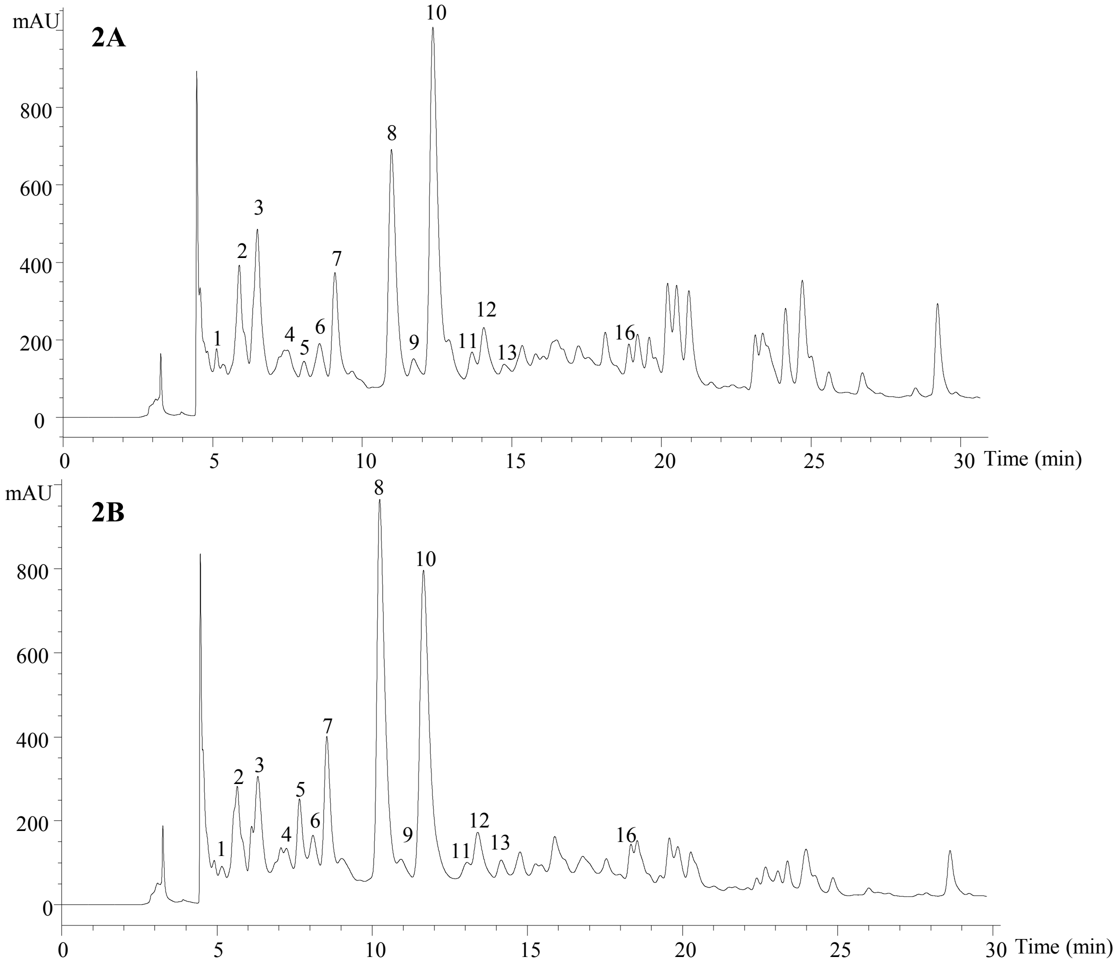

Figure 2. HPLC phenolic profile (flavan-3-ols) of cultivated (A) and wild (B) *Laurus nobilis*, obtained at 280 nm. Identification of peaks 1-13 and 16 is presented in Table 4.