Industrial Crops & Products 107 (2017) 1–12

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Phenolic profiles of Lauraceae plant species endemic to Laurisilva forest: A chemotaxonomic survey

Eulogio J. Llorent-Martínez[a,*], Vítor Spínola[b], Paula C. Castilho[b]

[a] University of Castilla-La Mancha, Regional Institute for Applied Chemistry Research (IRICA), Ciudad Real 13071, Spain
[b] CQM —Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9020-105 Funchal, Portugal

ARTICLE INFO

Keywords:
Macaronesia
Lauraceae
Flavonoids
Procyanidins
Epicatechin

ABSTRACT

In this work, the phenolic composition of several trees endemic to Madeira archipelago (Portugal) was studied. Specifically, the leaves of the most relevant species of the Lauraceae family (*Laurus novocanariensis*, *Apollonias barbujana*, *Ocotea foetens*, and *Persea indica*) have been analyzed. The screening of the main phenolic compounds in their methanol extracts has been performed by high-performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC-ESI–MS^n), identifying or tentatively characterizing almost 100 compounds, including a high number of proanthocyanidins (A- and B-type), which have been reported to present remarkable health benefits. Thirty-four compounds have been quantified, observing total individual phenolic contents (TIPCs) between 18.43 and 88.99 mg g^{-1} dry extract, with the lowest TIPC in *O. foetens* and the highest in *A. barbujana*.

1. Introduction

Madeira (Madeira archipelago, Portugal) laurel forest, Laurisilva, is a subtropical forest with a very rich bryophyte and vascular flora. It is well characterized from the botanical point of view, but little attention has been paid to the composition of most plant species, even though several species have been used for centuries in folk medicine (Rivera and Obón, 1995). Among the different compounds that are present in plants, phenolic compounds are of special interest to scientists, as they exhibit important biological activities, such as anti-oxidant, anti-microbial, anti-cancer and anti-mutagenic. Therefore, the characterization of these compounds, mainly uninvestigated in wild plants, is an important research field nowadays. The chemical and biological characterization of these plants may lead to promising sources of biologically active compounds.

In a previous study (Llorent-Martínez et al., 2015a), our research group established the phytochemical composition of the most important non-lauraceae trees of the Laurisilva forest (*Olea europaea* ssp. cerasiformis, *Ilex perado* ssp. perado, *Clethra arborea*, and *Heberdenia excelsa*). In this sense, the aim of the present work was to investigate the phenolic profile of selected plants from the Laurisilva belonging to the Lauraceae family: *Laurus novocanariensis*, *Apollonias barbujana*, *Ocotea foetens*, and *Persea indica*. Other species from the Lauraceae family have been reported to present health benefits, due to their phytochemical profile. For instance, cinnamon (*Cinnamomum zeylanicum* and *C. cassia* barks) has been the most studied species, which extracts are recognized by their high levels of procyanidins (Rao and Gan, 2014). The study of other non-edible *Lauraceae* species may lead to new sources of proanthocyanidins.

Laurus is a genus of evergreen trees belonging to the Lauraceae family, and three autochthonous species [*Laurus nobilis* L., *Laurus azorica* (Seub.) Franco and *Laurus novocanariensis* Rivas-Mart., Lousã, Fern. Prieto, E. Díaz, J.C. Costa & C. Aguiar] are described in Portugal (Vinha et al., 2015). *L. novocanariensis* is the most abundant endemic laurel from the Madeira archipelago and can grow from 3 to 20 m tall, presenting aromatic, shiny dark-green foliage. It presents male and female flowers on separate plants (Press and Short, 1994); the latter produce ovoid berries (1–1.5 cm) black when ripe. These berries derive a fatty oil that has been used in traditional medicine to treat skin ailments (Viciolle et al., 2012). Additionally, leaves (non-edible) are used in traditional cuisine to flavor dishes (Vinha et al., 2015), and to prepare infusions to relieve common cold and as sudorific (Rivera and Obón, 1995). A previous investigation on *L. novocanariensis* leaves documented monomeric and oligomeric flavan-3-ols as major phenolics (Vinha et al., 2015).

Apollonias barbujana (Cav.) Bornm. is an evergreen tree of 3–30 m tall. The simple leaves are alternate, elliptic, entire and petiolate, 6–8 cm length and 3–4 cm width. This tree produces panicles of white six-stellate flowers from June to September. Its berries are ovoid, approximately 15 mm long and brownish-grey color when ripe (Press

* Corresponding author.
E-mail addresses: Eulogio.Llorent@uclm.es, ellorent@ujaen.es (E.J. Llorent-Martínez).

http://dx.doi.org/10.1016/j.indcrop.2017.05.023
Received 20 January 2017; Received in revised form 28 March 2017; Accepted 15 May 2017

and Short, 1994). This species has been used in folk medicine as diuretic, analgesic, antiulcerogenic, cytostatic, cardiotonic, expectorant, stomachic, sedative or carminative effects, and against rheumatic pain (Rivera and Obón, 1995). The total phenolics content and antioxidant activity of *A. barbujana* have been determined before (Tavares et al., 2010), but not the individual composition of phenolics.

Ocotea foetens (Aiton) Baill is an evergreen tree up to 30 m height. It usually grows with multiple trunks branched from its base. Leaves are 9–12 cm long and 3–5 cm wide, oblong lanceolate. Flowering season is from June to August. It produces hard and fleshy berries, dark-green, approximately 3-cm long. Its leaves have been traditionally used to prepare infusions, used as antihypertensive. It has also been used to treat malignant diseases with poultices made of tender leaves and branchlets (Rivera and Obón, 1995). Its total phenolics content and antioxidant activity have been previously reported (Tavares et al., 2010), but its phytochemical profile remains unknown.

The genus *Persea*, belonging to the family Lauraceae, comprises about 190 species including its main species *P. americana* Miller (avocado) (Alvárez et al., 2016). *Persea indica* (L) Spreng is an evergreen tree up to 15–20 m tall, with a broad, rounded crown. It presents leaves without glands, 10–20 × 3–8 cm, elliptic. It grows berries of about 2 cm, ellipsoid, bluish-black when ripe (Press and Short, 1994). The phytochemical composition of *P. indica* has been scarcely studied to date; only the presence of diterpenes has been reported (Alvárez et al., 2016).

The Laurisilva is part of UNESCO natural patrimony since 2000, and the forest is cleaned and thinned every year to prevent fire spread, and to improve the growth of healthy trees. Considering that the felled specimens and cut branches are discarded, the present work is part of a project aiming to validate traditional claims in order to promote applications of the discarded material from the forest.

2. Experimental

2.1. Chemicals and reagents

All reagents and standards were of analytical reagent (AR) grade unless stated otherwise. Caffeic acid (≥98%), diosmin (≥90%), kaempferol (≥97%), protocatechuic acid (98%) and rutin (≥95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). (+)-catechin hydrated (> 99%), apigenin (≥99%) and hesperidin (≥98.5%) were obtained from Extrasynthese (Genay, France); and quercetin dihydrate (> 99%) from Riedel-de Haen. Stock solutions of 200 mg/L were prepared in ethanol (HPLC grade; Sigma). LC–MS grade acetonitrile (CH_3CN, 99%) (LabScan; Dublin, Ireland) and ultrapure water (Milli-Q Waters purification system; Millipore; Milford, MA, USA) were used for the HPLC–MS analyses.

2.2. Sample preparation and extraction of phenolic compounds

Plant material was collected in different locations of Madeira Island (Portugal) as described in Table 1. Branches were cut from healthy plants in the mentioned locations.

Leaves were lyophilized to dryness (Alpha 1–2 LD Plus freeze dryer, CHRIST), ground to powder, and stored at −20 °C. Phenolic extraction followed a previous procedure (Spínola et al., 2014): 1 g of dry material was extracted with 25 mL of methanol in an ultrasonic bath (Bandelin Sonorex, Germany) at 35 kHz and 200 W for 60 min (room temperature). Chlorophylls (which can interfere in the analyses) were removed by adsorption on activated charcoal and extracts were filtered and concentrated to dryness in a rotary evaporator (Buchi Rotavapor R-114; USA) at 40 °C. The resulting extracts were stored at 4 °C until further analysis.

2.3. Chromatographic conditions

The HPLC-DAD analysis was performed on a Dionex ultimate 3000 series instrument (Thermo Scientific Inc.) coupled to a binary pump, an autosampler and a column compartment (kept at 20 °C). Separation was carried out in a Phenomenex Gemini C_{18} column (5 µm, 250 × 3.0 mm i.d.) using a mobile phase composed by CH_3CN (A) and water/formic acid (0.1%, v/v) at a flow rate of 0.4 mL min^{-1}. The following gradient program was used: 20% A (0 min), 25% A (10 min), 25% A (20 min), 50% A (40 min), 100% A (42–47 min) and 20% A (49–55 min). Sample solutions (5 mg mL^{-1}) were prepared by dissolving the dried extract in the initial HPLC mobile phase; after filtration through 0.45 µm PTFE membrane filters, 5 µL was injected.

For HPLC-ESI–MS^n analysis, a Bruker Esquire model 6000 ion trap mass spectrometer (Bremen, Germany) with an ESI source was used in hyphenation with the former described Dionex HPLC system. MS^n analysis was performed in negative and positive modes and scan range was set at *m/z* 100–1000 with a speed of 13,000 Da/s. The ESI conditions were as follows: drying and nebulizer gas (N_2) flow rate and pressure, 10 mL min^{-1} and 50 psi; capillary temperature, 325 °C; capillary voltage, 4.5 keV; collision gas (He) pressure and energy, 1×10^{-5} mbar and 40 eV. The acquisition of MS^n data was made in auto MS^n mode, with an isolation width of 4.0 *m/z*, and a fragmentation amplitude of 1.0 V (MS^n up to MS^4). Esquire control software was used for the data acquisition and Data Analysis for processing.

2.4. Quantification of polyphenols

For this experiment, one polyphenol was selected as the standard for each group, and it was used to calculate individual concentrations by HPLC-DAD (Spínola et al., 2014). Caffeic and protocatechuic acids were used, respectively, for hydroxycinnamic and hydroxybenzoic acids determinations. Quercetin, (+)-catechin, hesperidin and apigenin were the standards used for flavonols, flavanols, flavanones and flavones, respectively. Stock standard solutions (1000 mg L^{-1}) were prepared in methanol, and calibration curves were built by diluting the stock solutions with the initial mobile phase. Six concentrations (5–100 mg L^{-1}) were used for the calibration, plotting peak area versus concentration ($R^2 \geq 0.967$ in all cases). Total individual phenolic contents (TIPC) were defined as the sum of the quantified phenolic compounds.

2.5. Statistical analysis

All samples were assayed in triplicate and results are given as means ± standard deviations. Data analysis was carried out by means

Table 1
List of analyzed species and nomenclature used.

Species	Common name		Collection Area	Collection data	Voucher number
L. novocanariensis	Loureiro	L1	Ribeiro Frio	March 2013	MADJ 9677
		L2	Chão dos Louros	March 2013	MADJ 11285
		L3	Ponta do Pargo	July 2013	MADJ 14155
A. barbujana	Barbusano	AJ	Ribeiro Frio	March 2013	MADJ 4765
O. foetens	Til	OF	Ribeiro Frio	March 2013	MADJ 13159
P. indica	Vinhático	PI	Ribeiro Frio	March 2013	MADJ 13157