Industrial Crops and Products 64 (2015) 135–151

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Analysis of phenolic compounds in leaves from endemic trees from Madeira Island. A contribution to the chemotaxonomy of Laurisilva forest species

Eulogio J. Llorent-Martínez[a,b], Sandra Gouveia[a], Paula C. Castilho[a,*]

[a] Centro de Química da Madeira (CQM), Centro de Ciências Exactas e da Engenharia da Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal
[b] Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas S/N, E-23071 Jaén, Spain

ARTICLE INFO

Article history:
Received 24 July 2014
Received in revised form 29 October 2014
Accepted 30 October 2014
Available online 15 November 2014

Keywords:
Macaronesia
Tandem mass spectrometry
Flavonoid
Procyanidin
Endemic species

ABSTRACT

Phenolic compounds present high antioxidant activity and, therefore, health promoting effects, serving as a type of preventive medicine. Hence, research on the chemical composition of plants with potential antioxidant value is of high interest.

Forest cleaning, thinning, and pruning are beneficial activities that help maintaining healthy forests. In addition, they can provide vegetal material as source of valuable bioactive compounds that can have health promoting effects. In this work, the phenolic composition of several trees native to Madeira Archipelago (Portugal) was studied. Specifically, the leaves from *Olea europaea* ssp. *cerasiformis*, *Ilex perado* ssp. *perado*, *Clethra arborea*, and *Heberdenia excelsa* have been analyzed. The screening of the main phenolic compounds from their methanolic extracts has been carried out using high-performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC-ESI-MS^n). This is the first report on the phenolic composition of these Madeira native species, and more than 100 compounds have been detected and identified or tentatively characterized.

1. Introduction

Phenolic compounds, secondary metabolites synthesized by the plants, present high antioxidant activity and, therefore, important health benefits, such as protection against cancer, and cardiovascular and neurodegenerative diseases (Gouveia and Castilho, 2009; Ignat et al., 2011). Hence, many studies are being performed to characterize phenolic compounds from natural sources. For this purpose, liquid chromatography coupled to tandem mass spectrometry has proved to be a very powerful tool.

The Madeira 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 its chemistry remains unexplored, even though several species have been used for centuries in the preparation of folk remedies (Rivera, 1995). In this work, the most important non-lauraceae trees of the Laurisilva forest (*Olea europaea* ssp. *cerasiformis*, *Ilex perado* ssp. *perado*, *Clethra arborea*, and *Heberdenia excelsa*) have been selected, and their phenolic composition studied.

The olive tree (*Olea europaea* L., Oleaceae) is a fruit crop of high economic importance. Six subspecies of *O. europaea* have been described. Of them, ssp. *cerasiformis* Webb and Berth. ex Kunkel and Sunding (previously named *O. europaea* L. ssp. *maderensis* Lowe) is native to the Madeira Archipelago (Brito et al., 2008). It is locally known as "Oliveira brava" or "zambujeiro" and grows widely at altitudes between 0 and 200 m. Unlike the *O. europaea* cultivar developed for fruit and oil production, the fruits of the endemic wild species are inedible, but infusions of its leaves are used as an antihypertensive. Although previous studies have been carried out regarding the phenolic composition of *O. europaea* (Fu et al., 2010; Quirantes-Piné et al., 2013; Savarese et al., 2007), this is the first study for the Madeira native species.

The genus *Ilex* L. (Aquifoliaceae) includes over 400 species of trees and shrubs. *I. perado* Aiton, a complex of four subspecies distributed in different Macaronesian archipelagos, is represented in Madeira by *I. perado* ssp. *perado* Aiton (Sosa et al., 2013). In the *Ilex* genus, the phenolic composition of *I. paraguensis* has been previously reported (Bastos et al., 2007; Dartora et al., 2011; Peres et al.,

* Corresponding author. Tel.: +351 291705102.
E-mail address: castilho@uma.pt (P.C. Castilho).

http://dx.doi.org/10.1016/j.indcrop.2014.10.068
0926-6690/

2013), but no data are available regarding ssp. *perado*. It is known as Madeira Holy or "azevinho".

Clethra arborea Aiton, also known as the lily-of-the-valley-tree or "folhado", is a flowering plant in the genus *Clethra*. It is native to Madeira, extinct in the Canary Islands (Spain), and an invasive species in the Azores (Portugal). To our best knowledge, the phenolic composition of the *Clethra* genus has not been studied to date.

Heberdenia excelsa Aiton (*Ardisia excelsa* A.) is an uncommon species of Laurisilva, native to Madeira and to the Canary Islands, and known locally as "aderno". It is currently included in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2013). The phenolic composition of plants in the *Heberdenia* genus has been scarcely studied to date (de Mejía et al., 2006), the report presented here being the first one for the ssp. *excelsa*.

Being protected as part of UNESCO natural patrimony since 2000, every year the Laurisilva forest must be cleaned and thinned to prevent fire spread and to improve the growth of healthy trees. Presently, the felled specimens and cut branches are discarded or treated as biomass residue without further valorization. The present work is part of a prospective project that aims at finding uses for discarded material for forest valorization.

2. Experimental

2.1. Chemicals and reagents

HPLC grade acetonitrile (CH_3CN) (99%; LabScan; Dublin, Ireland), ultrapure water (Milli-Q Waters purification system; Millipore; Milford, MA, USA), and formic acid (analytical reagent grade; Sigma–Aldrich; St. Louis, MO, USA), were used in the LC-MS analysis. The methanol used for the extraction procedures, of analytical reagent grade, was obtained from Fisher (Lisbon, Portugal). Eluents LC-MS analysis were also filtered through 0.45 μm Nylon membranes (Millipore; Merck; Darmstadt, Germany). Charcoal activated powder was purchased from Sigma–Aldrich.

Quercetin (>99%) was obtained from ExtraSynthese (Lyon, France). Kaempferol (>99%) and 5-O-caffeoylquinic acid (99%) were purchased from Acros Organics (Geel, Belgium). 3,4-O-dicaffeoylquinic acid, 3,5-O-dicaffeoylquinic acid and 4,5-O-dicaffeoylquinic acid were purchased from Chengdo biopurity phytochemicals (Ltd China, Sichuan, China). Rutin (>94%) was obtained from Sigma–Aldrich.

2.2. Sample preparation and extraction of phenolic compounds

Samples of *Clethra arborea*, *Heberdenia excelsa*, *Ilex perado*, and *Olea europaea* were collected in the wild in Madeira Island, in June 2012, with the help of Professor Miguel Menezes de Sequeira from the Biology Department of Madeira University. Vouchers were deposited in the Madeira Botanical Garden Herbarium collection. Specimen collection was performed at full maturity of leaves, in a protected forest area, of restricted human access and free of contamination of introduced species. The leaves were de-stemmed, lyophilized to dryness (Savant vapour trap RVT400; Thermo Scientific Inc.; Waltham, MA, USA), ground to powder, and stored at −20 °C until analysis.

The phenolic compounds were extracted by ultrasound-assisted extraction. Using a sonicator Bandelin Sonorex (Germany), 1 g of plant material was extracted with 25 mL of methanol (room temperature) at 35 Hz and 200 W for 60 min. Then, chlorophylls 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 extracts were stored at −20 °C until use. For HPLC analysis, the extracts were dissolved in the initial HPLC mobile phase, to obtain solutions of 5 mg mL^{-1} concentrations.

2.3. Chromatographic conditions

The HPLC analysis was carried out 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 achieved on 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).

For HPLC-ESI-MS^n analysis, a Bruker Esquire model 6000 ion trap mass spectrometer (Bremen, Germany) with an ESI source was used. MS^n analysis was performed in negative and positive mode and scan range was set at *m/z* 100–1000 with speed of 13,000 Da/s. The conditions of ESI 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 with the auto MS^n mode, selecting an isolation width of 4.0 *m/z*, and a fragmentation amplitude of 1.0 V (MS^n up to MS^4). Samples were filtered through 0.45 μm PTFE membrane filters, and 10 μL were injected.

3. Results and discussion

For the analysis of the phenolic composition by HPLC-ESI-MS^n, both the positive and negative ionization modes were used. Practically all the information was obtained using the negative mode, and the positive mode was mainly used for confirmation purposes and for the screening of anthocyanidins. The base peak chromatograms of the methanolic extracts of each plant are shown in Figs. 1 and 2.

In general, in the negative ionization mode (ESI^-) MS^1 spectrum, the most intense peak corresponded to the deprotonated molecular ion $[M-H]^-$, allowing MS^n analysis. Losses of sugar moieties like hexosyl, deoxyhexosyl, pentosyl, rutinosyl, and glucuronyl (−162, −146, −132, −308, and −176 Da, respectively) were observed in conjugated phenolic compounds.

Compounds were numbered by their order of elution, maintaining the same numeration in all the samples. The structures of the most relevant compounds identified are shown in Figs. 3 and 4.

3.1. Olea europaea ssp. cerasiformis

The results obtained in the analysis of leaves extracts from *Olea europaea* are shown in Table 1 (ESI^-). Most of the compounds, as previously reported in scientific literature for other *Olea* subspecies, were secoiridoids and flavonoids (Fu et al., 2010; Quirantes-Piné et al., 2013).

3.1.1. Secoiridoids

Olea europaea L. was rich in oleosides, which are oleaceae-specific secoiridoids usually esterified to a phenolic moiety (Quirantes-Piné et al., 2013). Oleuropein (compound **61**) was the most abundant compound, which is in agreement with scientific bibliography (Altiok et al., 2008; Benavente-García et al., 2000; Briante et al., 2002; De Nino et al., 1997; Fu et al., 2010; Mylonaki et al., 2008; Pereira et al., 2007; Quirantes-Piné et al., 2013). The identification of oleuropein was based on its $[M-H]^-$ at *m/z* 539, and its characteristic fragmentation pattern (Bianco et al., 2001; Fu et al., 2010), with fragment ions at *m/z* 377, 307, and 275.

Table 1
Characterization of the methanolic extracts from leaves of *Olea europaea* ssp. *cerasiformis*.

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
1	2.8	683	MS^2[683]: 342 (10.3), 341 (100) MS^3[683 → 341]: 179 (100), 143 (10.1), 119 (15), 113 (26.8), 101 (12.9) MS^4[683 → 341 → 179]: 161 (65.3), 143 (50.7), 119 (48.2), 113 (61.3), 89 (100)	Hexose polymer	Brudzynski and Miotto (2011)
4	3.0	191	MS^2[191]: 173 (74.7), 127 (100), 111 (59.1), 93 (37.9), 85 (36.9) MS^3[191 → 127]: 109 (81.5), 85 (100)	Quinic acid	Gouveia and Castilho (2011)
5	3.1	731	MS^2[731]: 390 (11.3), 389 (100) MS^3[731 → 389]: 345 (100), 183 (33.9), 165 (86.6), 131 (27.9), 121 (38.8) MS^4[731 → 389 → 345]:151 (85.1), 121 (82.4), 119 (36), 113 (37.5), 101 (100)	Oleoside/secologanoside derivative	–
8	3.9	317	MS^2[317]: 225 (100), 165 (19.5), 125 (23.8) MS^3[317 → 225]: 207 (45.1), 165 (100), 125 (80.4), 95 (84.4), 81 (43.3)	Unknown	–
9	4.1	601	MS^2[601]: 404 (17.5), 403 (100), 223 (11.9), 179 (7.5) MS^3[601 → 403]: 371 (34.3), 333 (14.2), 223 (100), 179 (29.4), 121 (21.2) MS^4[601 → 403 → 223]: 161 (2.1), 121 (100), 101 (17.5)	Elenolic acid glucoside derivative	Eyles et al. (2007)
12	4.3	389	MS^2[389]: 345 (100), 227 (3.1), 209 (47.5), 183 (15.2), 165 (86.1), 121 (32.1) MS^3[389 → 345]: 183 (59.7), 179 (32.5), 165 (100),163 (50.6), 161 (16.3) MS^4[389 → 345 → 165]: 121 (100)	Oleoside/secologanoside	Cardoso et al. (2005); Fu et al. (2010)
13	4.3	593	MS^2[593]: 503 (29.2), 474 (24), 473 (100), 383 (32.4), 353 (88.3) MS^3[593 → 473]: 383 (20.1), 354 (16.4), 353 (100) MS^3[593 → 473 → 353]: 326 (10.1), 325 (100), 298 (21.6), 297 (80.8)	Vicenin-2 (apigenin-6,8-di-C-glucoside)	Barreca et al. (2011); Truchado et al. (2011)
14	4.4	447	MS^2[447]: 315 (100), 163 (60.4), 153 (51.1), 152 (78), 151 (31.5) MS^3[447 → 315]: 163 (12.2), 153 (100), 135 (12.8), 109 (24.6), 108 (73.3)	3,5-dihydroxybenzoicacid-glucopylxyloside	Han et al. (2008)
19	5	437	MS^2[437]: 402 (13.8), 401 (100) MS^3[437 → 401]: 270 (17), 269 (100), 233 (16.3), 161 (96.2) MS^4[437 → 401 → 269]: 161 (100), 159 (15.6), 126 (19)	Unknown	–
20	5.2	377	MS^2[377]: 153 (29.9), 197 (100) MS^3[377 → 197]: 153 (100)	Oleuropein aglycone derivative	Fu et al. (2010); Jemai et al. (2009)
24	5.8	807	MS^2[807]: 404 (13.2), 403 (100), 371 (14.5) MS^3[807 → 403]: 371 (100), 333 (7.4), 223 (58.1), 179 (28.4), 121 (18.6) MS^4[807 → 403 → 371]: 166 (17.3), 165 (19.5), 121 (100)	Elenolic acid glucoside	Fu et al. (2010)
26	6	739	MS^2[739]: 594 (20.7), 593 (100), 286 (14.8), 285 (68.6) MS^3[739 → 593]: 447 (20.5), 285 (100) MS^4[739 → 593 → 285]: 267 (86.8), 257 (55.8), 197 (30.5), 151 (100)	Kaempferol–rhamnoside–hexoside–rhamnoside	Bilia et al. (2004)
29	6.9	525	MS^2[525]: 481 (100), 389 (60.2), 345 (35.2), 195 (70), 165 (57.9) MS^3[525 → 481]: 345 (54.5), 319 (17.6), 301 (68.6), 255 (100), 195 (44.5), 139 (59.5)	Demethyloleuropein	Savarese et al. (2007)
30	6.9	593	MS^2[593]: 286 (13.4), 285 (100) MS^3[593 → 285]: 243 (76.4), 241 (56.7), 211 (100), 199 (78.8), 175 (73.8), 151 (19.8), 149 (91.4) MS^4[593 → 285 → 149]: 105 (100)	Luteolin 7-rutinoside	Cardoso et al. (2005); Quirantes-Piné et al. (2013)
33	7.2	609	MS^2[609]: 302 (11.5), 301 (100), 300 (25.3) MS^3[609 → 301]: 271 (22), 255 (30.9), 179 (94.2), 151 (100), 107 (10.5) MS^4[609 → 301 → 151]: 169 (54.1), 107 (100)	Rutin	[a]
37	7.5	623	MS^2[623]: 462 (17.6), 461 (100) MS^3[623 → 461]: 315 (34.2), 161 (10.3), 135 (100)	Verbascoside	Cardoso et al. (2005); Fu et al. (2010)
40	8.1	447	MS^2[447]: 286 (19.1), 285 (100) MS^3[447 → 285]: 243 (52.5), 241 (100), 217 (60.6), 175 (74.9), 151 (91.6) MS^4[447 → 285 → 241]: 213 (47.7), 201 (51.7), 200 (57), 199 (75.7), 197 (100)	Luteolin glucoside	Cardoso et al. (2005); Fu et al. (2010); Quirantes-Piné et al. (2013)
44	8.6	701	MS^2[701]: 540 (29.6), 539 (100), 307 (10.5) MS^3[701 → 539]: 377 (13.3), 371 (11.8), 307 (100), 275 (87.5) MS^4[701 → 539 → 307]: 275 (100), 139 (23.4)	Oleuropein diglucoside isomer	Fu et al. (2010); Quirantes-Piné et al. (2013)
47	9.5	701	MS^2[701]: 540 (21.8), 539 (100), 377 (13.4), 307 (18.7), 275 (16.2) MS^3[701 → 539]: 469 (16.7), 377 (16.8), 327 (12), 307 (100), 275 (59.2) MS^4[701 → 539 → 307]: 276 (16.4), 275 (100), 139 (27.7)	Oleuropein diglucoside isomer	Fu et al. (2010); Quirantes-Piné et al. (2013)

Table 1 (*Continued*)

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
51	10.3	551	MS^2[551]: 508 (23.3), 507 (100), 389 (19.5), 341 (14.4), 281 (27.1) MS^3[551 → 507]: 393 (16.1), 345 (17.7), 323 (11.5), 179 (18.2), 161 (100) MS^4[551 → 507 → 161]: 133 (100)	6′-β-hexopyranosyloleoside	Cardoso et al. (2005); Damak et al. (2008)
55	11.3	447	MS^2[447]: 302 (14.6), 301 (100), 300 (33.2) MS^3[447 → 301]: 271 (16.9), 255 (15.7), 179 (94.4), 151 (100) MS^4[447 → 301 → 151]: 108 (22.5), 107 (100), 83 (26.1)	Quercetin-O-rhamnoside	–
57	12.9	569	MS^2[569]: 538 (19.1), 537 (100), 403 (44.4), 223 (17.5) MS^3[569 → 537]: 403 (12.5), 357 (27.5), 305 (12.2), 223 (82.4), 151 (100) MS^4[569 → 537 → 151]: 123 (100); MS^4[569 → 537 → 223]: 121 (100)	Unknown	–
59	13.3	447	MS^2[447]: 286 (16.8), 285 (100) MS^3[447 → 285]: 243 (18.7), 241 (56.5), 217 (17.2), 199 (73.9), 175 (100)	Luteolin glucoside	Cardoso et al. (2005); Fu et al. (2010); Quirantes-Piné et al. (2013)
61	13.7	539	MS^2[539]: 377 (56.1), 308 (12), 307 (100), 275 (93.7) MS^3[539 → 307]: 276 (14.8), 275 (100), 139 (27.5), 111 (13.8) MS^4[539 → 307 → 275]: 149 (59), 139 (100), 121 (16.3), 113 (89), 111 (58.4)	Oleuropein	Bianco et al. (2001); Fu et al. (2010)
64	15	539	MS^2[539]: 377 (36.8), 345 (22.6), 327 (17.9), 307 (100), 275 (98) MS^3[539 → 307]: 275 (100), 139 (25.9) MS^4[539 → 307 → 275]: 149 (70.4), 139 (70), 113 (100), 111 (31.7)	Oleuropein isomer	Fu et al. (2010); Quirantes-Piné et al. (2013)
68	15.9	539	MS^2[539]: 403 (16.8), 377 (16.8), 307 (88.2), 276 (21.6), 275 (100) MS^3[539 → 275]: 139 (100), 123 (11.9), 113 (23.1), 111 (12.4), 95 (57.4) MS^4[539 → 307 → 275]: 139 (100), 149 (46.4), 113 (94.7), 111 (58.3), 85 (16.1) MS^3[539 → 307]: 275 (100), 139 (15.3), 111 (10.4) MS^4[539 → 275 → 139]: 111 (17.5), 96 (11.3), 95 (100)	Oleuroside	Fu et al. (2010); Quirantes-Piné et al. (2013)
73	18.6	601	MS^2[601]: 403 (9.2), 223 (7.4), 197 (100) MS^3[601 → 197]: 153 (100)	Unknown	–
74	19.9	523	MS^2[523]: 362 (13.9), 361 (89.4), 291 (100), 260 (11.7), 259 (48.4) MS^3[523 → 291]: 171 (12.5), 143 (12.8), 139 (65.2), 127 (14.4), 111 (100) MS^3[523 → 361]: 291 (100), 259 (59.8) MS^4[523 → 361 → 291]: 259 (16.2), 171 (25.9), 143 (11.5), 139 (46.1), 111 (100)	Ligstroside	Briante et al. (2002); Laguerre et al. (2009)
79	23.8	557	MS^2[557]: 514 (16.3), 513 (100), 389 (1.8), 345 (35.4), 199 (23.2), 185 (39) MS^3[557 → 513]: 345 (100), 227 (50.8), 209 (52.4), 185 (86.6), 183 (61.5) MS^4[557 → 513 → 345]: 183 (100), 165 (53.1), 121 (42.2), 119 (40.8), 101 (46)	Oleoside/secologanoside derivative	–
80	25.2	301	MS^2[301]: 299 (13.8), 273 (13.5), 179 (93.3), 151 (100) MS^3[301 → 151]: 169 (45.4), 107 (100)	Quercetin	[a]
81	26.8	877	MS^2[877]: 715 (20), 701 (20.9), 613 (14.5), 540 (23.6), 539 (100) MS^3[877 → 539]: 377 (10.2), 371 (16.4), 308 (21.7), 307 (100), 275 (84.1) MS^4[877 → 539 → 307]: 276 (16.2), 275 (100), 139 (19.3)	Oleuropein derivative	–
82	27.3	925	MS^2[925]: 540 (16.7), 539 (100), 377 (12.9), 307 (11.7) MS^3[925 → 539]: 377 (50.7), 307 (100), 276 (11), 275 (77.1) MS^4[925 → 539 → 307]: 275 (100), 139 (22.1), 111 (10.9)	Oleuropein derivative	–
83	27.7	539	MS^2[539]: 377 (58), 308 (12.7), 307 (93.2), 276 (11.8), 275 (100) MS^3[539 → 275]: 139 (100), 113 (15.9), 111 (13.5), 95 (48.7) MS^4[539 → 275 → 139]: 111 (13.6), 96 (10.4), 95 (100) MS^3[539 → 307]: 276 (15.4), 275 (100), 139 (21.6) MS^4[539 → 307 → 275]: 149 (69.4), 139 (39.2), 135 (20), 113 (100), 111 (16.1)	Oleuropein isomer	–
86	29	539	MS^2[539]: 437 (11.9), 377 (58), 307 (100), 276 (13.7), 275 (87.5) MS^3[539 → 275]: 139 (100), 113 (21.4), 111 (46.5), 95 (51.7) MS^4[539 → 275 → 139]: 111 (81.4), 96 (100) MS^3[539 → 307]: 275 (100), 139 (13.6) MS^4[539 → 307 → 275]: 149 (45.6), 139 (100), 113 (63), 111 (98.9)	Oleuropein isomer	–

Table 1 (*Continued*)

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
90	31.8	615	MS^2[615]: 286 (14.4), 285 (100) MS^3[615 → 285]: 243 (53.9), 241 (61.3), 200 (58.4), 199 (73.6), 175 (100)	Luteolin derivative	–
102	39.1	587	MS^2[587]: 300 (12), 299 (100) MS^3[587 → 299]: 271 (100) MS^4[587 → 299 → 271]: 243 (74), 227 (84.9), 201 (32.1), 199 (100), 157 (26.6)	Unknown	–

[a] Compared with standard compound.

Compounds **64**, **68**, **83**, and **86** presented the same $[M-H]^-$ ion that oleuropein did, and similar fragmentation patterns. Compound **68** was identified as oleuroside, oleuropein secoxyloganin analog (Obied et al., 2007). The presence of oleuroside and another oleuropein isomer (compound **64**) has been previously described (Fu et al., 2010; Quirantes-Piné et al., 2013). However, to our best knowledge, compounds **83** and **86**, also oleuropein isomers considering MS/MS data, are here reported for the first time.

Compound **20** displayed an $[M-H]^-$ ion at *m/z* 377, with MS^2 fragment ions at *m/z* 197 and 153, and was identified as an oleuropein aglycone derivative (Fu et al., 2010; Jemai et al., 2009).

Compounds **44** and **47** displayed deprotonated molecules at *m/z* 701. Their MS^2 fragmentation profile showed base peak ion at *m/z* 539, formed by the loss of 162 Da (hexoside residue). The MS^3[701 → 539] fragmentation produced main fragment ions at *m/z* 377, 307 and 275, typical from oleuropein. These compounds were characterized as oleuropein diglucoside isomers (Fu et al., 2010; Quirantes-Piné et al., 2013). Compounds **81** and **82** were tentatively characterized as oleuropein derivatives considering the fragment ion at *m/z* 539, which displayed the fragmentation pattern of oleuropein.

Compound **12** exhibited $[M-H]^-$ at *m/z* 389, with MS^2 fragment ions at *m/z* 345 (base peak), 227, 209, and 183. The fragment ion at *m/z* 345 was formed by the neutral loss of 44 Da (CO_2 molecule from a carboxylic group). The fragment ions at *m/z* 227 and 209 corresponded to the loss of hexoside and hexoside + H_2O, respectively. This fragmentation pattern was consistent with oleoside or secologanoside (Cardoso et al., 2005; Fu et al., 2010). Compounds **5** and **79** presented fragment ions at *m/z* 389 and 345, with fragmentation patterns similar to oleoside/secologanoside, so they were tentatively characterized as derivatives.

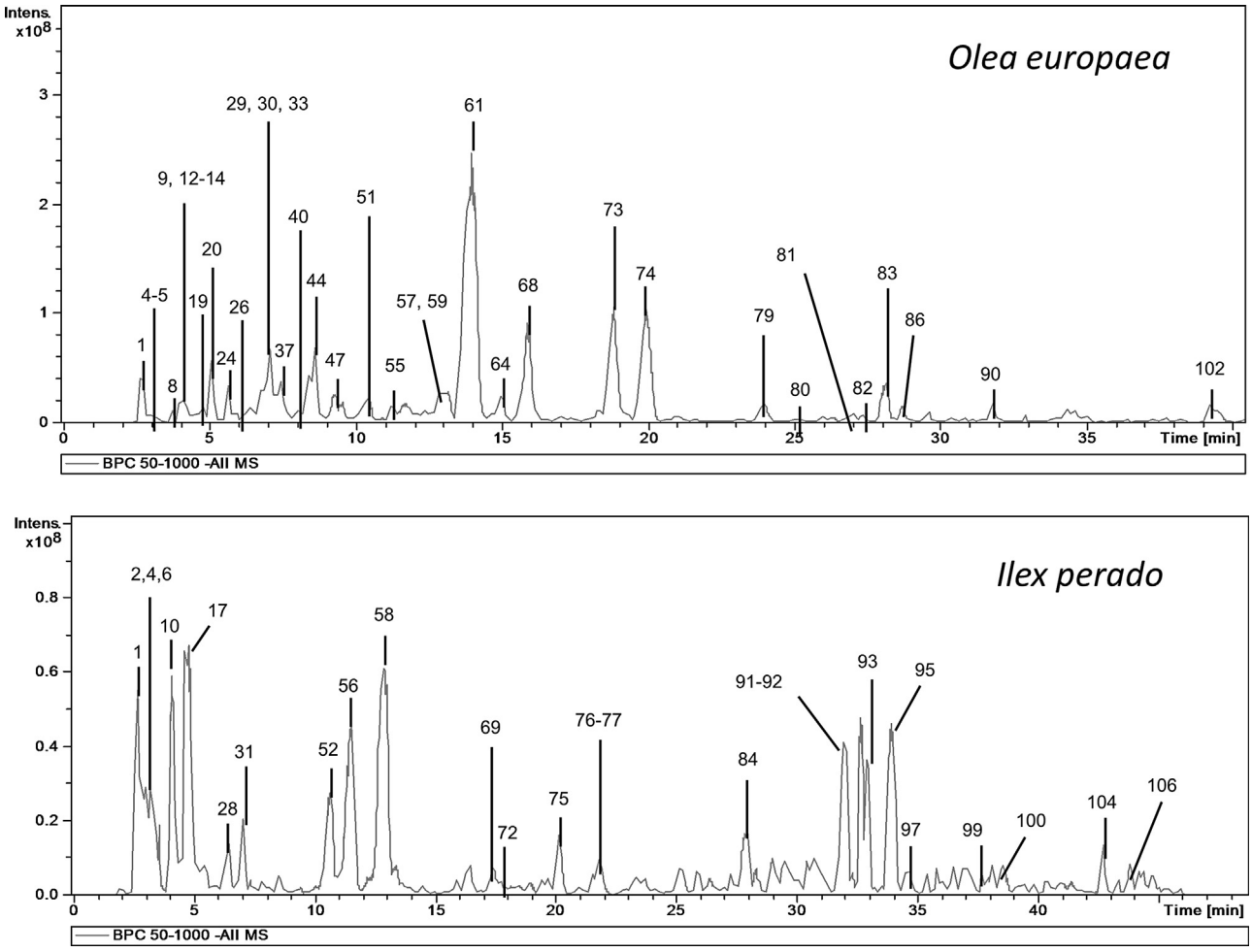


Fig. 1. HPLC-ESI/MS^n base peak chromatograms (BPC) of the methanolic extracts from *Olea europaea* and *Ilex perado*.

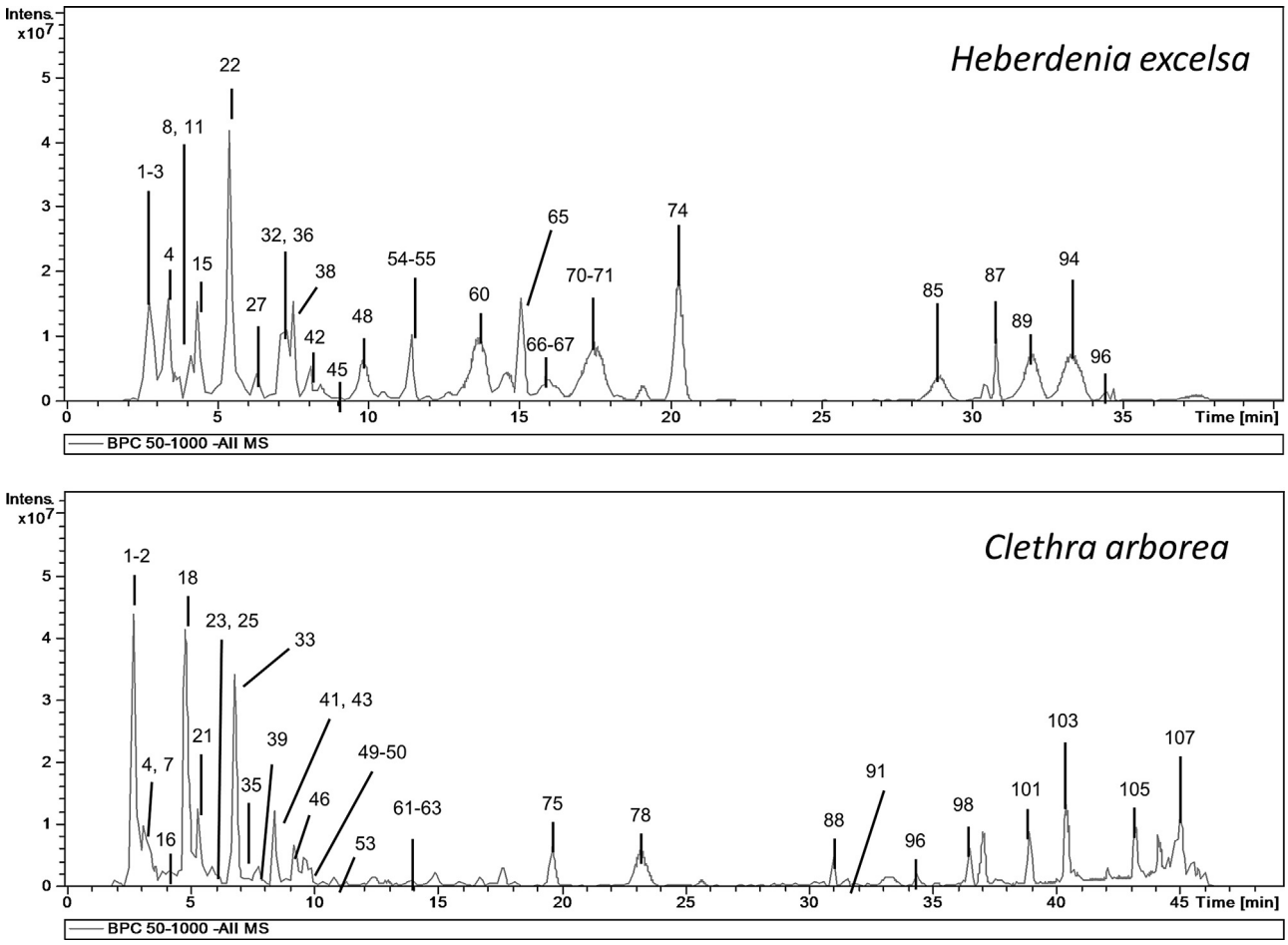


Fig. 2. HPLC-ESI/MS^n base peak chromatograms (BPC) of the methanolic extracts from *Heberdenia excelsa* and *Clethra arborea*.

Compound **24** showed [M−H]$^-$ at *m/z* 807, and an intense peak at *m/z* 403 in MS^1. Its MS^2 base peak ion was present at *m/z* 403, indicating that the compound was a dimer. The MS^3 spectrum exhibited fragment ions at *m/z* 371 (base peak), 223, and 179. This fragmentation pattern was previously reported for elenolic acid glucoside (Fu et al., 2010). Compound **9**, with [M−H]$^-$ at *m/z* 601, exhibited a base peak ion at *m/z* 403, that presented the same fragmentation pattern than elenolic acid glucoside, so it was characterized as a derivative (Eyles et al., 2007). Another oleoside derivative was compound **51**, which was identified as 6′-β-hexopyranosyloleoside (Cardoso et al., 2005; Damak et al., 2008).

Compound **29** was identified as demethyloleuropein, with [M−H]$^-$ at *m/z* 525 and MS^2 fragment ions at *m/z* 389 and 319 (Savarese et al., 2007). Compound **74** was a deoxy analog of oleuropein, ligstroside, also previously reported in olive leaves (Briante et al., 2002; Laguerre et al., 2009).

3.1.2. Flavonoids

Rutin (compound **33**) and quercetin (compound **80**) were detected as aglycones and characterized using analytical standards. In addition, several derivatives of apigenin, kaempferol, and luteolin (compounds **13**, **26**, **30**, **40**, **59**, **91**) were observed.

Compound **13** presented an [M−H]$^-$ ion at *m/z* 593, with MS^2 fragment ions at *m/z* 473 (base peak), 503, 383 and 353. It was identified as vicenin-2, an apigenin-6,8-di-C-glucoside (Barreca et al., 2011; Truchado et al., 2011). To our knowledge, this compound has not been previously reported in *Olea europaea*.

Compound **26** presented deprotonated molecular ion at *m/z* 739, and suffered neutral losses of 146 Da (rhamnoside) and 162 Da (hexoside) in its MS^n fragmentation to produce the fragment ions [M−H-rhamnose]$^-$ at *m/z* 593, [M−H-rhamnose-rhamnose]$^-$ at *m/z* 447, and [M−H-rhamnose-hexose-rhamnose]$^-$ at *m/z* 285. The fragment ion at *m/z* 285 corresponded to the aglycone kaempferol (main fragment ion at *m/z* 151, and absence of fragment ions at *m/z* 243 and 241, which are typical from luteolin). This compound was identified as kaempferol-rhamnoside-hexoside-rhamnoside (Del Rio et al., 2004).

Compounds **30**, **40**, and **59**, exhibited [M−H]$^-$ ions at *m/z* 593, 447, and 447, respectively, originating in all cases a base peak ion at *m/z* 285 (luteolin) in the MS^2 spectrum. Hence, these compounds were tentatively identified as luteolin-7-rutinoside, and luteolin glucoside isomers, based on bibliographic data previously reported in *Olea europaea* (Cardoso et al., 2005; Fu et al., 2010; Quirantes-Piné et al., 2013). Compound **90** was tentatively identified as a luteolin derivative.

Compound **55** was characterized as quercetin rhamnoside. It presented an [M−H]$^-$ ion at *m/z* 447, and an MS^2 fragment ion at *m/z* 301 by the loss of a rhamnoside moiety (146 Da). The fragment ion at *m/z* 301 corresponded to quercetin (characteristic fragment ion at *m/z* 151).

3.1.3. Phenolic acids

Compound **14** was characterized as a benzoic acid glucoside, 3-5,dihydroxy-benzoicacid-glucopylxyloside, based on its [M−H]$^-$ ion at *m/z* 447, and MS^2 and MS^3 base peaks at *m/z* 315 and 153, respectively (Han et al., 2008).

Compound **37** displayed an [M−H]$^-$ ion at *m/z* 623, and fragment ions at *m/z* 461, 315, 161 and 135. The fragment ions at *m/z*

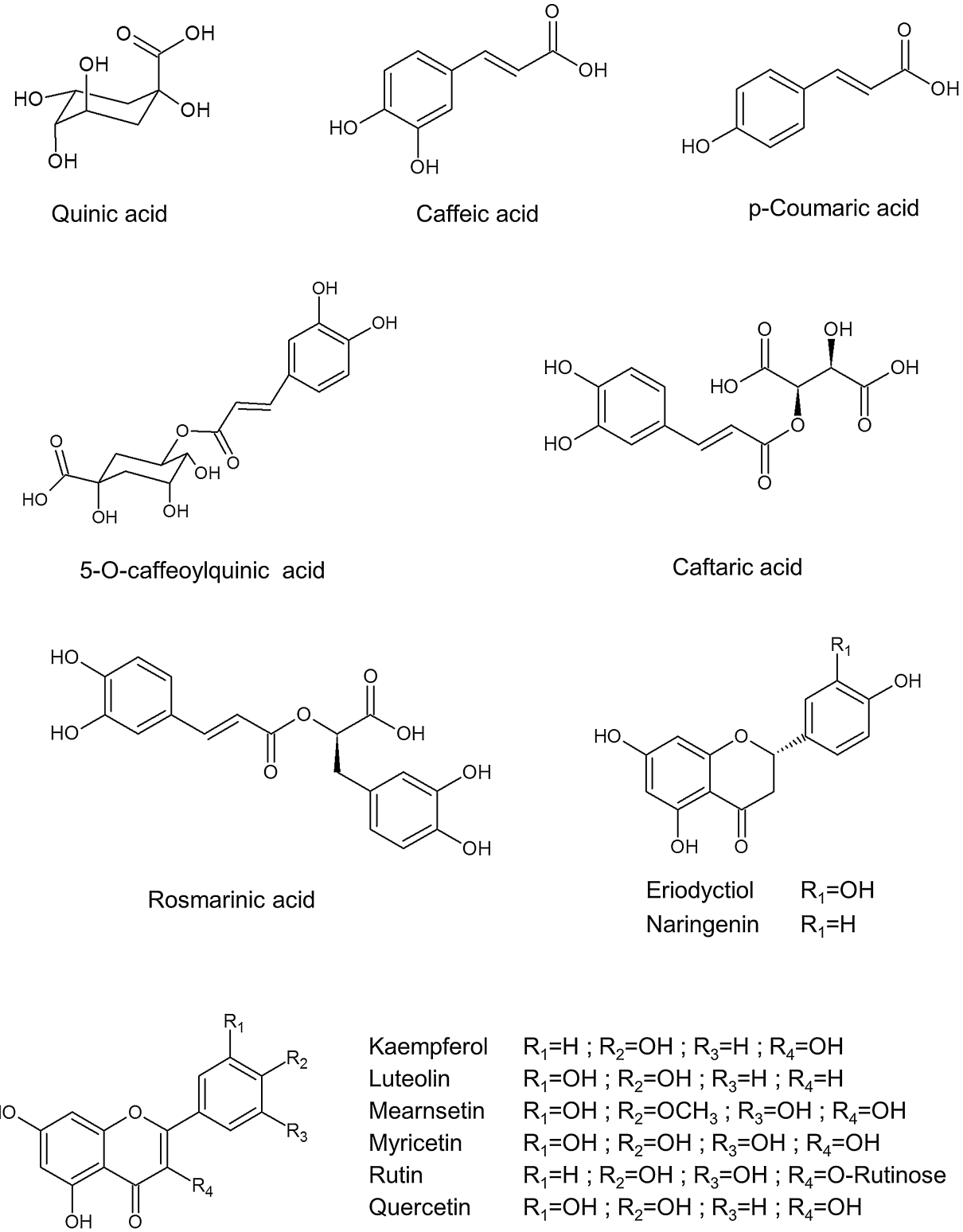


Fig. 3. Chemical structures of phenolic acids, flavonoid aglycones, and substitution groups found in the analyzed leave extracts.

461 and 315 were caused by the sequential neutral losses of hexoside (162 Da) and rhamnoside (146 Da). This fragmentation pattern is typical from verbascoside (Cardoso et al., 2005; Fu et al., 2010).

3.1.4. Other compounds

Compound **4** exhibited an $[M-H]^-$ ion at *m/z* 191. Its MS^2 fragmentation yielded a $[M-H-CO-2H_2O]^-$ ion at *m/z* 127 and a $[M-H-H_2O]^-$ ion at *m/z* 173. It was identified as quinic acid (Gouveia and Castilho, 2011). There were several compounds that could not be identified and were just stated as unknowns in Table 1.

3.2. Ilex perado

To our best knowledge, the determination of phenolic compounds in *I. perado* has not been previously reported. However, there are some available data in scientific bibliography regarding

Table 2
Characterization of the methanolic extracts from leaves of *Ilex perado* ssp. *perado*.

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
1	2.7	683	MS^2 [683]: 341 (100) MS^3 [683 → 341]: 179 (100), 161 (12.5), 143 (20.8), 131 (14.5), 119 (15.5), 113 (27.8) MS^4 [683 → 341 → 179]: 161 (100), 113 (99.6), 101 (31.2), 89 (69.9), 87 (31.7)	Hexose polymer	Brudzynski and Miotto (2011)
2	2.8	533	MS^2 [533]: 192 (4.7), 191 (100) MS^3 [533 → 191]: 173 (55.2), 171 (40.7), 127 (100), 109 (21.5), 85 (42) MS^4 [533 → 191 → 127]: 109 (100), 85 (68.5)	Quinic acid derivative	–
4	3.0	191	MS^2 [191]: 173 (100), 127 (99), 111 (63.1), 93 (47), 87 (31.9) MS^3 [191 → 173]: 127 (90), 111 (100), 109 (41.9), 94 (31), 93 (80.5) MS^3 [191 → 127]: 109 (43), 85 (100), 81 (26.2)	Quinic acid	Gouveia and Castilho (2011)
6	3.1	353	MS^2 [353]: 191 (100), 179 (33.9), 173 (11) MS^3 [353 → 191]: 173 (93.8), 127 (100), 111(112.3), 93 (41.8), 85 (40.2) MS^4 [353 → 191 → 127]: 110 (62.7), 109 (100), 85 (65)	Caffeoylquinic acid	–
10	4.2	353	MS^2 [353]: 191 (100), 179 (35.4), 135 (14.7) MS^3 [353 → 191]: 173 (39.5), 127 (100), 111 (45.4), 93 (83.1), 85 (62.5) MS^4 [353 → 191 → 127]: 110 (32.6), 109 (97.6), 85 (100)	3-O-Caffeoylquinic acid	Clifford et al. (2003)
17	4.7	707	MS^2 [707]: 354 (10.2), 353 (100) MS^3 [707 → 353]: 191 (100) MS^4 [707 → 353 → 191]: 173 (29.8), 127 (100), 111 (26.4), 85 (30.6)	5-O-Caffeoylquinic acid	Clifford et al. (2003) [a]
28	6.4	337	MS^2 [337]: 191 (24.3), 173 (100), 163 (8.3) MS^3 [337 → 173]: 155 (12.5), 111 (57.1), 93 (100), 81 (12.1), 71 (12.4) MS^3 [337 → 191]: 173 (46.1), 127 (100), 111 (17.9), 93 (24), 85 (28.5)	4-p-Coumaroylquinic acid	Clifford et al. (2006a)
31	7.1	551	MS^2 [551]: 506 (30.5), 505 (100) MS^3 [551 → 505]: 374 (20), 373 (100), 191 (12.1), 179 (7.2), 143 (9.7), 161 (21.6) MS^4 [551 → 505 → 373]: 161 (100)	Unknown	–
52	10.6	515	MS^2[515]: 353 (100), 335 (15.8), 191 (13), 179 (24.4), 173 (36) MS^3[515 → 353]: 192 (6.2), 191 (48.8), 179 (91.4), 173 (100), 135 (23.4) MS^4[515 → 353 → 173]: 137 (24.5), 129 (16.1), 111 (63), 94 (16.6), 93 (100)	3,4-dicaffeoylquinic acid	Clifford et al. (2005) [a]
56	11.6	515	MS^2[515]: 354 (11.3), 353 (100), 191 (6.7) MS^3[515 → 353]: 192 (4.3), 191 (100), 179 (36.9), 135 (8.3) MS^4[515 → 353 → 191]: 173 (94.3), 127 (38.2), 109 (79.9), 93 (100), 85 (37.8)	3,5-dicaffeoylquinic acid	Clifford et al. (2005) [a]
58	12.9	515	MS^2[515]: 354 (15), 353 (100), 203 (13.7), 179 (7.8), 173 (29.6) MS^3[515 → 353]: 191 (21), 180 (4.3), 179 (57.5), 173 (100), 135 (15) MS^4[515 → 353 → 173]: 155 (35.4), 111(100), 93 (76.7), 92 (18.8), 69 (15.9)	4,5-dicaffeoylquinic acid	Clifford et al. (2005) [a]
69	17.4	515	MS^2[515]: 354 (15), 353 (100), 191 (4.9), 179 (8.3), 173 (9.1) MS^3[515 → 353]: 192 (7.1), 191 (63.2), 179 (71.6), 173 (100), 135 (11.3) MS^4[515 → 353 → 173]: 155 (58.2), 111 (42), 109 (22.8), 93 (100), 83 (31.5)	Dicaffeoylquinic acid.	–
72	17.7	499	MS^2[499]: 338 (16.9), 337 (100), 173 (24.9), 163 (4.1) MS^3[499 → 337]: 173 (100), 163 (15.4), 119 (3.4) MS^4[499 → 337 → 173]: 137 (20.9), 111 (49.6), 109 (21), 93 (100), 71 (14.9)	3-O-caffeoyl-4-O-p-coumaroylquinic acid	Clifford et al. (2006a)
75	20.1	493	MS^2[493]: 448 (23.8), 447 (100) MS^3[493 → 447]: 315 (100), 161 (51.6), 159 (13.2), 149 (17.9), 131 (19.7) MS^4[493 → 447 → 315]: 161 (100), 159 (7.6), 143 (6), 113 (4.5), 101 (6.6)	Unknown	–
76	21.5	583	MS^2[583]: 538 (10.7), 537 (43.9), 536 (4.4), 430 (18.6), 429 (100) MS^3[583 → 429]: 369 (6.4), 327 (55.6), 195 (13.9), 153 (100), 152 (20.1) MS^4[583 → 429 → 153]: 125 (12.3), 110 (16.1), 109 (100), 108 (56.7), 107 (10.6)	Unknown	–
77	21.8	839	MS^2[839]: 677 (8.2), 665 (3.6), 664 (22.9), 663 (100), 501 (3.4) MS^3[839 → 663]: 543 (8.5), 502 (19.1), 501 (100), 484 (7.2), 483 (23.4), 439 (4.8) MS^4[839 → 663 → 501]: 484 (14.4), 483 (100), 441 (5.1), 439 (14.2), 437 (6.1), 421 (3.5)	Triterpenoid saponin	–
84	27.8	795	MS^2[795]: 440 (27.7), 439 (100), 421 (6.6) MS^3[795 → 439]: 422 (18.3), 421 (100), 395 (8.2) MS^4[795 → 439 → 421]: 376 (22.1), 375 (100)	Unknown	–

Table 2 (*Continued*)

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
91	31.8	327	MS^2[327]: 291 (51.3), 229 (100), 211 (74.8), 209 (25.1), 171 (72.8) MS^3[327 → 171]: 154 (100), 127 (63.5) MS^3[327 → 229]: 211 (100), 209 (85.2), 165 (51.3), 155 (33.9), 125 (25) MS^4[327 → 229 → 211]: 194 (51.4), 175 (100), 163 (94.4)	Oxo-dihydroxy-octadecenoic acid	Levandi et al. (2009); Van Hoyweghen et al. (2014)
92	31.9	663	MS^2[663]: 543 (24.6), 502 (24.6), 501 (100), 457 (46.8), 439 (22.2) MS^3[663 → 501]: 484 (17.4), 483 (100), 457 (25.5), 455 (21.9), 439 (13.7) MS^4[663 → 501 → 483]: 440 (24.8), 439 (100), 438 (7.2), 437 (31.3), 391 (6.4)	Triterpenoid saponin	Chen et al. (2011)
93	32.9	811	MS^2[811]: 765 (4.7), 603 (100), 604 (33.6) MS^3[811 → 603]: 586 (34.3), 585 (100) MS^4[811 → 603 → 585]: 571 (36.8), 453 (100)	Unknown	–
95	33.9	811	MS^2[811]: 765 (3.9), 603 (100), 604 (36.5) MS^3[811 → 603]: 586 (25.8), 585 (100), 543 (4.1) MS^4[811 → 603 → 585]: 571 (100), 453 (15.1)	Unknown	–
97	34.5	895	MS^2[895]: 734 (48.6), 733 (100) MS^3[895 → 733]: 571 (100), 572 (22.3), 553 (6.5) MS^4[895 → 733 → 571]: 539 (100), 439 (17)	Saponin-1	Khan et al. (1993)
99	37.6	793	MS^2[793]: 673 (10.2), 633 (9.4), 632 (28.7), 631 (100), 613 (15.7) MS^3[793 → 631]: 555 (7),456 (18.7), 455 (100) MS^4[793 → 631 → 455]: 419 (60.4), 408 (78.5), 407 (100), 394 (96.8), 191 (36.5)	Saponin-2	Gouveia and Castilho (2011)
100	38	793	MS^2[793]: 633 (7), 632 (38.7), 631 (100), 613 (9.3), 569 (4.6) MS^3[793 → 631]: 555 (8.2) 509 (4.3), 457 (6.7), 456 (49.1), 455 (100) MS^4[793 → 631 → 455]: 407 (48.5), 191 (100)	Saponin-3	Gouveia and Castilho (2011)
104	42.6	501	MS^2[501]: 484 (24.1), 483 (100), 456 (12.2), 455 (58.3), 439 (17.9) MS^3[501 → 483]: 455 (10.3), 440 (33.5), 439 (100) MS^4[501 → 483 → 439]: 409 (18.5), 367 (23.1), 356 (64.2), 355 (100), 339 (22)	Unknown	–
106	43.9	501	MS^2[501]: 484 (18.7), 483 (100), 456 (11.1), 455 (42.4), 439 (17.9) MS^3[501 → 483]: 455 (34.3), 440 (35.6), 439 (100), 425 (5.5), 423 (8.8) MS^4[501 → 483 → 439]: 439 (42.3), 409 (100), 367 (66.4), 355 (66.4), 309 (46.8)	Unknown	–

[a] Compared with standard compound.

the analysis of species of the *Ilex* genus, specifically *I. paraguariensis* (Bravo et al., 2007; Carini et al., 1998; Dartora et al., 2011). In these works, mono- and di-caffeoylquinic acids, along with other hydroxycinnamates, were the main detected compounds. In the present study, caffeoylquinic acids and other esters of quinic acid were also detected as the major components of *Ilex perado* (Table 2).

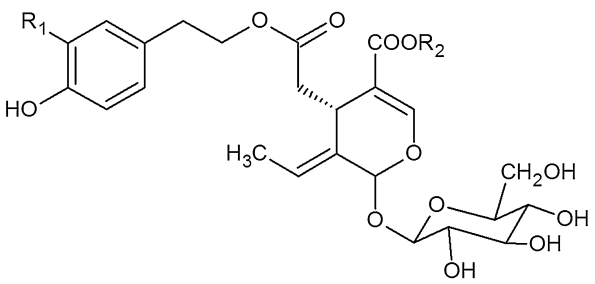
3.2.1. Phenolic acids

Compounds **6**, **10**, and **17**, were identified as mono-caffeoylquinic acids. All of them displayed $[M-H]^-$ at *m/z* 353 and a dimer at *m/z* 707. The MS^n fragmentation of the ion at *m/z* 353 resulted in the typical fragmentation pattern of caffeoylquinic acids. The MS^2 spectrum of compound **10** showed a fragment ion at *m/z* 191 as base peak and a relatively intense fragment ion at *m/z* 179. Based on the hierarchical key proposed by Clifford et al. (Clifford et al., 2003), compound **10** was characterized as 3-*O*-caffeoylquinic acid. Compound **17** also showed base peak at *m/z* 191, but an undetectable fragment ion at *m/z* 179. Thus, it was identified as 5-O-caffeoylquinic acid. This identification was confirmed by the retention time when injecting the corresponding analytical standard. Compound **6** showed the same fragmentation pattern than **10**, 3-caffeoylquinic acid, and could be an isomer, since UV exposure during sample preparation can sometimes induce some geometric isomerization in hydroxycinnamic acids (Clifford et al., 2006b). The presence of these caffeoylquinic acids was confirmed by the positive ionization mode, obtaining $[M+H]^+$ at *m/z* 355 with fragment ions at *m/z* 163 (base peak).

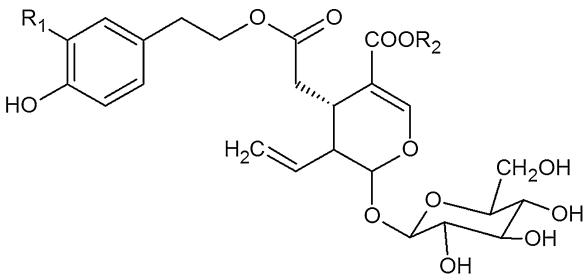
Compounds **52**, **56**, **58**, and **69** exhibited $[M-H]^-$ at *m/z* 515, and base peak in MS^2 at *m/z* 353, [M−H-162]. They were all identified as di-caffeoylquinic acids. For their characterization, the hierarchical key for the identification of di-caffeoylquinic acids was used (Clifford et al., 2005), taking into account the different MS^3 and MS^4 spectra of the *m/z* 353 ions. Compounds **52**, **58**, and **69** presented MS^3 base peak at *m/z* 173, which is indicative of a 4-OH substituted quinic acid. Compound **52** showed weak (*ca* 15%) MS^2 ion at *m/z* 335 (15.8%) and strong (>50% base peak) MS^3 ion *m/z* 179 (91.4%), being identified as 3-4-dicaffeoylquinic acid. Compound **58** presented undetectable MS^2 ion at *m/z* 335 and strong (>50% base peak) ion at *m/z* 179 (57.5%) and was characterized as 4-5-dicaffeoylquinic acid. Compound **56** was identified as 3,5-dicaffeoylquinic acid, considering the MS^3 base peak at *m/z* 191. These identifications were confirmed by the injection of analytical standards. Compound **69** showed a fragmentation pattern similar to 4-5-dicaffeoylquinic acid, but could not be fully characterized.

Compound **28** presented an $[M-H]^-$ ion at *m/z* 337, with MS^2 base peak at *m/z* 173. According to the hierarchical key for identification of chlorogenic acids (Clifford et al., 2003), it was identified as 4-p-coumaroylquinic acid.

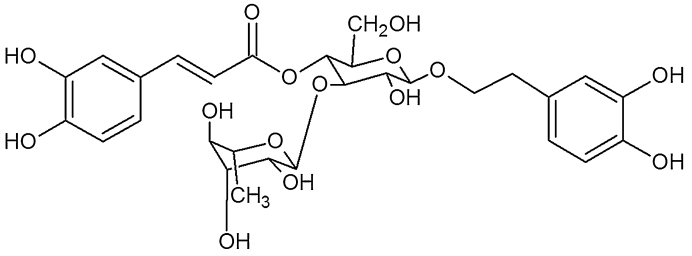
Compound **72** exhibited $[M-H]^-$ ion at *m/z* 499, and lost a caffeoyl moiety (162 Da) to form a base peak ion at *m/z* 337 in the MS^2 spectrum. Considering the sequential MS^n fragmentation



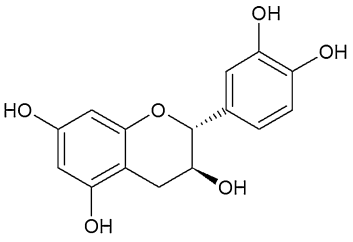
Oleuropein R_1=OH; R_2=CH_3
Demethyloleuropein R_1=OH; R_2=H
Ligstroside R_1=H; R_2=CH_3



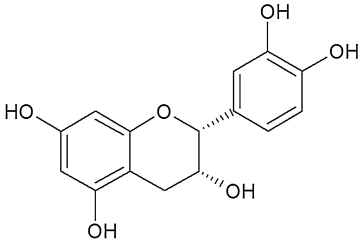
Oleuroside



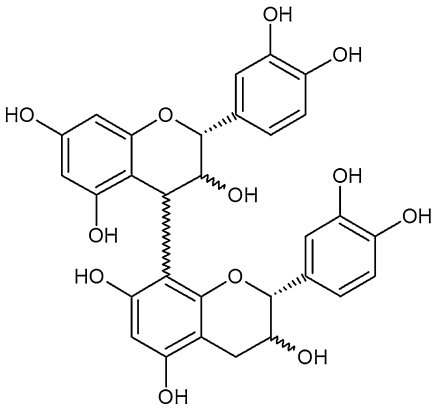
Verbascoside



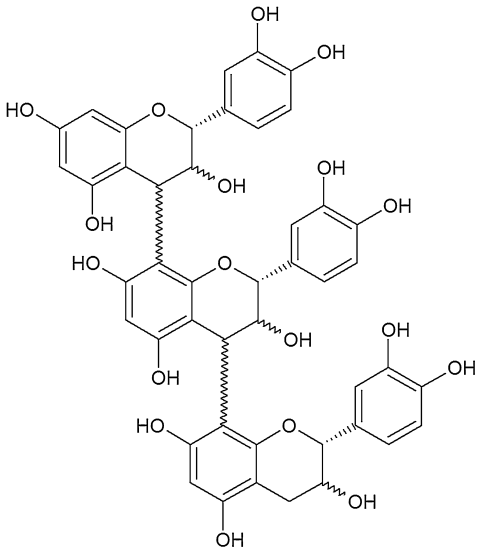
(+) Catechin



(-) Epicatechin



Procyanidin dimer



Procyanidin trimer

Fig. 4. Chemical structures of the main compounds found in the leaves of *Olea europaea* and *Clethra arborea*.

pattern, the compound was identified as 3-O-caffeoyl-4-O-p-coumaroylquinic acid (Clifford et al., 2006a).

3.2.2. Terpenoid saponins

Compound **77**, with $[M-H]^-$ ion at *m/z* 839, suffered the neutral loss of 176 Da (probably glucuronide) in MS^2 to yield a fragment ion at *m/z* 663 (base peak). In MS^3, fragment ions at *m/z* 501 (base peak), 483, and 439 were observed. MS^4 fragmentation exhibited fragment ions at *m/z* 483 (base peak), 439, and 421. Compound **92**, with $[M-H]^-$ ion at *m/z* 663, displayed a similar fragmentation. This fragmentation pattern has been previously described for triterpenoids in *Ilex* species (Chen et al., 2011).

Compound **97** produced a $[M-H]^-$ ion at *m/z* 895. Fragment ions appeared at *m/z* 733 $[M-H-162]^-$ in MS^2; 571 $[M-H-162-162]^-$ in MS^3; and 439 $[M-H-162-162-132]^-$ in MS^4. This fragmentation pattern has been previously described for a saponin (Khan et al., 1993).

Compounds **99** and **100** presented $[M-H]^-$ ions at *m/z* 793, with ion fragments at *m/z* 631 (MS^2), 455 (MS^3), and 407 and 191 (MS^4). This fragmentation pattern has been previously reported for saponins (Gouveia and Castilho, 2011).

3.2.3. Other compounds

Compound **2**, with $[M-H]^-$ ion at *m/z* 533, showed a direct loss of 341 Da at MS^2, yielding a fragment ion at *m/z* 191, which presented a fragmentation pattern similar to quinic acid and, therefore, was characterized as a derivative.

Compound **75** displayed an $[M-H+HCOOH]^-$ ion at *m/z* 493. After loss of 46 Da (formic) and 132 Da (pentoside), it yielded a fragment ion at *m/z* 315, which probably corresponded to the aglycone. However, the exact nature of the aglycone could not be determined.

Compound **91**, with a $[M-H]^-$ ion at *m/z* 327 and MS^2 base peak ion at *m/z* 229, was identified as oxo-dihydroxy octadecenoic acid (oxo-DHODE), an oxylipin (Levandi et al., 2009; Van Hoyweghen et al., 2014).

Compounds **93** and **95** showed similar mass spectra presenting $[M-H+HCOOH]^-$ at *m/z* 811. In MS^2, they suffered a neutral loss of 208 Da (46 + 162), which could correspond to the loss of formic acid and a hexoside residue, yielding a fragment ion at *m/z* 603. In MS^3, they suffered a loss of 18 Da (water) and yielded an ion at *m/z* 585, which suffered further loss producing ions at *m/z* 571 (−14 Da) and 453 (−132 Da). These two compounds may be isomers, but their nature could not be elucidated due to the absence of bibliographic data.

3.3. Heberdenia excelsa

To our best knowledge, there are not bibliographic data to compare the obtained results in the analysis of *Heberdenia* leaves. Most of the detected compounds corresponded to phenolic acids, flavonoids (mainly rhamnosides) and isoflavonoids, although other compounds such as phenylethanoid glycosides were identified (Table 3).

3.3.1. Phenolic acids

Compound **11** displayed $[M-H]^-$ at *m/z* 385, and in MS^2 suffered the loss of the glucose unit and the loss of the acid group from the aglycone, yielding the fragment ions at *m/z* 223 and 179. It was identified as sinapic acid-O-glucoside (Sánchez-Rabaneda et al., 2004). Compound **32** also exhibited fragment ions at *m/z* 223 and 179 in its MS^n fragmentation pattern, and was characterized as a sinapic acid derivative.

Compound **27**, with $[M-H]^-$ at *m/z* 435, showed in his fragmentation characteristic ions of protocatechuic acid, at *m/z* 153 and 109 (Gouveia and Castilho, 2012), and was tentatively identified as a derivative.

Compound **48** showed $[M-H]^-$ at *m/z* 247. It suffered a neutral loss of 80 Da (sulphate) in MS^2, yielding a fragment ion at *m/z* 167, which displayed a fragmentation pattern typical from vanillic acid. It was therefore characterized as vanillic acid sulphate, taking into account bibliographic data (Suárez et al., 2011).

3.3.2. Flavonoids

Glycosylated flavonoids from myricetin, kaempferol, mearnsetin, and quercetin were identified. Compounds **42**, **55**, and **65** displayed deprotonated molecular ions at *m/z* 463, 447, and 431, respectively. All of them suffered the neutral loss of a rhamnoside unit (146 Da) in MS^2, yielding fragment ions that corresponding to the aglycones myricetin, quercetin, and kaempferol. Compound **54**, with $[M-H]^-$ ion at *m/z* 477, also suffered the neutral loss of 146 Da in MS^2 producing a fragment ion at *m/z* 331, which corresponded to mearnsetin (typical fragment ion at *m/z* 316) (Han et al., 2008). Hence, the compound was characterized as mearnsetin-O-rhamnoside.

3.3.3. Isoflavonoids

Compound **15**, with $[M-H]^-$ at *m/z* 429, lost 162 Da (hexoside) in MS^2, yielding a fragment ion at *m/z* 267. Compound **22** showed $[M-H]^-$ ion at *m/z* 581 and suffered the neutral loss of 152 Da (galloyl moiety) and 162 Da (hexoside), producing also the aglycone at *m/z* 267. Compound **36** also produced the same aglycone after several fragmentations. According to Prasain et al. (2003), the aglycone is most likely formononetin, a methoxylated isoflavonoid. Compound **15** showed a similar pattern to formononetin-7-O-glucoside and was identified as such. Compounds **22** and **36** were characterized as formononetin derivatives.

3.3.4. Other compounds

Compounds **38** and **45** exhibited the same deprotonated molecular ion, at *m/z* 623, and same MS^n fragmentation, yielding ions at *m/z* 461, 315, and 135. This fragmentation pattern has been previously described for phenylethanoid glycosides (Guo et al., 2007). Considering their retention time, **38** and **45** were identified as acteoside and forsythoside A, respectively

Compound **60** displayed the deprotonated molecular ion at *m/z* 287, and suffered the loss of 80 Da (sulphate) in MS^2. This fragmentation has been previously reported for dihydroxyphenyl-valerolactone sulphates (Urpi-Sarda et al., 2009a,b).

Compounds **66** and **70** showed $[M-H]^-$ ion at *m/z* 457, and displayed the main fragment ions at *m/z* 260, 231, and 97. Considering their similar fragmentation patterns, they can be considered isomers. This fragmentation has been previously reported in leaves from *Helichrysum melaleucum* (Gouveia and Castilho, 2010), but their identification could not be carried out.

Compound **96** was identified as trihydroxy-octadecenoic acid, considering its $[M-H]^-$ ions at *m/z* 329 and its fragmentation pattern (Van Hoyweghen et al., 2014).

3.4. Clethra arborea

The main groups of compounds found during the analysis of the extracts from *Clethra* leaves were phenolic acids, procyanidins (dimers and trimers), and flavonoids, mainly glucuronides. The results (ESI^-) are shown in Table 4. ESI^+ was also used for confirmation purposes and for the identification of anthocyanidins.

3.4.1. Phenolic acids

Compound **25**, with $[M-H]^-$ at *m/z* 311, displayed both tartaric and caffeic acid as MS^2 fragment ions at *m/z* 149 and 179, respectively. Fragment ions at *m/z* 135 (decarboxylated caffeic acid) and *m/z* 87 (characteristic of tartaric acid) were observed in MS^3.

Table 3
Characterization of the methanolic extracts from leaves of *Heberdenia excelsa*.

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
1	2.7	683	MS^2 [683]: 341 (100) MS^3 [683 → 341]: 179 (100), 161 (19.8), 143 (90.9), 131 (29.9), 119 (28.0), 113 (26.4) MS^4 [683 → 341 → 179]: 161 (38.1), 101 (100), 89 (71.4)	Hexose polymer	Brudzynski and Miotto (2011)
2	2.8	533	MS^2[533]: 191 (100) MS^3[533 → 191]: 173 (49.6), 127 (50.1), 111 (32.4), 93 (100), 85 (91.6)	Quinic acid derivative	–
3	2.8	549	MS^2[549]: 503 (100) MS^3[549 → 503]: 323 (18.2), 221 (60.7), 179 (100), 143 (11.8) MS^4[549 → 503 → 179]: 161 (100), 143 (20.9), 119 (92), 101 (59.5)	Hexose polymer	Brudzynski and Miotto (2011)
4	3	191	MS^2[191]: 173 (86.7), 127 (79.5), 111 (100), 93 (38.2), 85 (57.5) MS^3[191 → 111]: 93 (100) MS^3[191 → 127]: 109 (100)	Quinic acid	Gouveia and Castilho (2011)
8	3.9	317	MS^2[317]: 225 (100), 165 (25.6), 153 (14.7), 125 (18) MS^3[317 → 225]: 207 (93.1), 165 (100), 143 (40.5), 125 (59.1), 81 (20)	Unknown	–
11	4.2	385	MS^2[385]: 223 (100), 179 (70.4) MS^3[385 → 223]: 179 (100) MS^4[385 → 223 → 179]: 150 (100), 138 (79.4), 124 (91.3), 123 (61.9), 109 (92.6)	Sinapic acid-O-glucoside	(Sánchez-Rabaneda et al. (2004)
15	4.4	429	MS^2[429]: 267 (100), 249 (25.7), 231 (12.3) MS^3[429 → 267]: 249 (100), 231 (35), 205 (11.8), 139 (10.4) MS^4[429 → 267 → 249]: 231 (100), 205 (19), 187 (24.7)	Formononetin-7-O-glucoside	Prasain et al. (2003)
22	5.5	581	MS^2[581]: 430 (14.9), 429 (100), 313 (29.1), 267 (80.6), 249 (11.6) MS^3[581 → 429]: 267 (100), 249 (16.3), 231 (14.3) MS^4[581 → 429 → 267]: 249 (100), 231 (34.7)	Formononetin derivative	–
27	6.2	435	MS^2[435]: 298 (15.1), 297 (100), 153 (44.9) MS^3[435 → 297]: 195 (12.3), 177 (12.7), 153 (100), 109 (12.9) MS^4[435 → 297 → 153]: 109 (100), 108 (15.1)	Protocatechuic acid derivative	–
32	7.1	521	MS^2[521]: 477 (16.4), 255 (16.4), 223 (100), 205 (29), 179 (61.7) MS^3[521 → 223]: 179 (100), 163 (9.3), 161 (9.2) MS^3[521 → 179]: 138 (77.2), 137 (27.9), 125 (33.1), 124 (100) MS^4[521 → 223 → 179]: 151 (86.9), 138 (99.7), 124 (29.8)	Sinapic acid derivative	–
36	7.3	565	MS^2[565]: 429 (22.7), 297 (15.6), 267 (100), 249 (35.2), 231 (22.6) MS^3[565 → 267]: 249 (100), 231 (40.8), 205 (16.5), 187 (14.2) MS^4[565 → 267 → 249]: 231 (100)	Formononetin derivative	–
38	7.6	623	MS^2[623]: 477 (1.4), 461 (100) MS^3[623 → 461]: 315 (93.9), 297 (17), 161 (16.7), 151 (15.1), 135 (100) MS^4[623 → 461 → 315]: 135 (100), 91 (76.6)	Acteoside	Guo et al. (2007)
42	8.2	463	MS^2[463]: 317 (77.3), 316 (100) MS^3[463 → 316]: 287 (15), 271 (100), 270 (35), 179 (67.6), 151 (14.9) MS^4[463 → 316 → 179]: 151 (66.2) MS^4[463 → 316 → 271]: 244 (27.4), 243 (52.5), 242 (100), 215 (24.5)	Myricetin-O-rhamnoside	–
45	9	623	MS^2[623]: 462 (22.1), 461 (100) MS^3[623 → 461]: 315 (44.8), 297 (12.2), 135 (100) MS^4[623 → 461 → 315]: 135 (100)	Forsythoside A	Guo et al. (2007)
48	9.6	247	MS^2[247]: 167 (100), 139 (22.4), 123 (51.2) MS^3[247 → 167]: 123 (100) MS^4[247 → 167 → 123]: 81 (100)	Vanillic acid sulphate	Suárez et al. (2011)
54	11.2	477	MS^2[477]: 331 (100), 316 (26.5), 315 (24.3) MS^3[477 → 331]: 316 (100) MS^4[477 → 331 → 316]: 287 (45.1), 271 (95.7), 179 (100), 164 (24.6)	Mearnsetin-O-rhamnoside	–
55	11.3	447	MS^2[447]: 302 (14.2), 301 (100), 300 (31.4) MS^3[447 → 301]: 271 (24.7), 255 (17.5), 179 (100), 151 (85.5) MS^4[447 → 301 → 179]: 169 (16.3), 151 (100)	Quercetin-O-rhamnoside	–
60	13.5	287	MS^2[287]: 208 (14.3), 207 (100) MS^3[287 → 207]: 163 (92.1), 123 (100), 121 (10.5)	Dihydroxyphenyl-valerolactone sulphate	Urpi-Sarda et al. (2009a,b)
65	15.1	431	MS^2[431]: 286 (16.9), 285 (100), 284 (24.6) MS^3[431 → 285]: 257 (50.6), 256 (35.1), 255 (100), 229 (22), 213 (14.3) MS^4[431 → 285 → 256]: 239 (36.5), 229 (92.7), 227 (100), 213 (40.2), 163 (70.4)	Kaempferol-O-rhamnoside	–
66	15.7	457	MS^2[457]: 329 (28.3), 261 (17.9), 260 (100) MS^3[457 → 260]: 245 (21.5), 231 (34.9), 179 (16.8), 110 (17.9), 97 (100)	Unknown	Gouveia and Castilho (2010)

Table 3 (*Continued*)

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-ESI-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
67	15.9	499	MS^2[499]: 485 (15.1), 484 (100), 453 (16.3), 419 (16) MS^3[484]: 453 (100), 369 (22.9), 167 (45.9)	Unknown	–
70	17.5	457	MS^2[457]: 275 (92.5), 261 (20.1), 260 (100), 231 (10.1) MS^3[457 → 260]: 245 (10.7), 231 (63.4), 179 (35), 110 (27.6), 97 (100)	Unknown	Gouveia and Castilho (2010)
71	17.5	439	MS^2[439]: 425 (25.4), 424 (100), 393 (11.6), 359 (52.3), 316 (12.3) MS^3[439 → 424]: 394 (44.2), 393 (100), 312 (44.2)	Unknown	–
74	20.1	523	MS^2[523]: 362 (24.9), 361 (100), 292 (14.2), 291 (92), 259 (52.4) MS^3[523 → 361]: 292 (18.6), 291 (100), 259 (77.5) MS^4[523 → 361 → 291]: 259 (22.9), 231 (19.8), 171 (11.1), 139 (58.5), 127 (14.6), 111 (100)	Ligstroside	Briante et al. (2002); Laguerre et al. (2009)
85	28.8	305	MS^2[305]: 290 (100), 275 (18.3), 111 (14.4), 97 (41.8) MS^3[305 → 290]: 275 (100), 209 (48.8), 195 (14.1), 97 (44.4) MS^4[305 → 290 → 275]: 195 (100), 97 (80.1)	Unknown	–
87	30.4	601	MS^2[601]: 556 (23), 555 (100), 393 (42.4) MS^3[601 → 555]: 394 (16.3), 393 (100) MS^4[601 → 555 → 393]: 209 (11.1), 183 (100), 139 (16.1)	Unknown	–
89	31.8	439	MS^2[439]: 421 (20.2), 410 (19), 409 (100) MS^3[439 → 409]: 394 (53.3), 330 (12.8), 329 (100), 314 (38.9) MS^4[439 → 409 → 329]: 315 (11.6), 314 (100)	Unknown	–
94	33.2	439	MS^2[439]: 409 (100), 299 (26.9), 259 (22.0), 223 (16.0) MS^3[439 → 409]:395 (83.2), 329 (100), 315 (94.3), 314 (64.6)	Unknown	–
96	34.3	329	MS^2[329]: 311 (35.3), 293 (27.1), 229 (93.5), 211 (85.4), 171 (100) MS^3[329 → 171]: 127 (100), 125 (86.8) MS^3[329 → 229]: 211 (100), 209 (81.5), 155 (10.2), 125 (20.1)	Trihydroxy-octadecenoic acid	Van Hoyweghen et al. (2014)

This fragmentation was consistent with caftaric acid (Schütz et al., 2005).

For the characterization of compound **53**, the positive-ion mode was used due to the more informative fragment ions. This compound presented the protonated molecular ion at *m/z* 373, and fragment ions at *m/z* 211, 193, 175 and 135. The fragment ions at *m/z* 211 and 193 corresponded to the losses of a dehydrated hexose and a hexose, respectively. The ions at *m/z* 193, 175 and 135 were indicative of a hydroxyferulic acid residue. Hence, this compound was tentatively characterized as a hydroxyferulic acid glycoside (Ma et al., 2007).

Compound **62** was characterized as rosmarinic acid, considering its $[M-H]^-$ ion at *m/z* 359, and MS^n fragment ions at *m/z* 197, 179, 161, and 133 (Liu et al., 2007).

3.4.2. Procyanidins

Several procyanidin dimers and trimers were detected in the analyzed extracts. Procyanidin dimers (**7**, **18**, **39**) showed characteristic $[M-H]^-$ ions at *m/z* 577, with MS^2 fragment ions at *m/z* 451, 425, 407, 289, and 287. Procyanidin trimers (**16**, **21**, **35**) displayed $[M-H]^-$ ions at *m/z* 865, and typical MS^2 fragment ions at *m/z* 739, 713, 695, 577, 407, and 289. These fragment patterns have been previously reported (Ruiz et al., 2005; Tomás-Barberán et al., 2001). In addition, $[M+H]^+$ ions at *m/z* 579 and 865 were observed in ESI^+ for procyanidin dimers and trimers, respectively, confirming the results obtained in ESI^-.

3.4.3. Flavonoids

Compound **23** was characterized as catechin, based on its $[M-H]^-$ ion at *m/z* 289 and the comparison of its fragmentation pattern with bibliographic data (Rockenbach et al., 2012). Compound **78**, with $[M-H]^-$ at *m/z* 543, suffered a neutral loss of 288 Da, yielding a fragment ion at *m/z* 255. This loss is probably due to an (epi)catechin unit (González-Paramás et al., 2006; Mateos-Martín et al., 2012). However, the exact nature of the compound could not be elucidated.

Rutin (compound **33**) was identified, using an analytical standard, due to its characteristic $[M-H]^-$ ion at *m/z* 609 and MS^2 base peak at *m/z* 301.

Compound **41** exhibited $[M-H]^-$ ion at *m/z* 607 and suffered the neutral loss of 322 Da, yielding a fragment ion at *m/z* 285, which corresponded to kaempferol (Ye et al., 2005). The exact nature of this compound could not be determined and it was identified as a kaempferol derivative. Compound **43** displayed an $[M-H]^-$ ion at *m/z* 593 and yielded an MS^2 fragment ion at *m/z* 285 by loss of 308 Da (hexose-rhamnose), and was identified as kaempferol 3-rutinoside (Del Rio et al., 2004). The fragmentation pattern of kaempferol was also confirmed by the analysis of a commercial standard.

Several glucuronides were identified in *Clethra* extracts. Compound **49** exhibited an $[M-H]^-$ ion at *m/z* 477 and suffered a neutral loss of 176 Da (glucuronide), yielding the aglycone at *m/z* 301. The fragmentation of the ion at *m/z* 301 yielded fragments at *m/z* 273, 257, 179, and 151, typical from quercetin, and therefore it was identified as a quercetin glucuronide (Downey and Rochfort, 2008). Compound **50** also suffered a neutral loss of 176 Da in MS^2, yielding a fragment ion at *m/z* 287 (eriodictyol), so it was identified as eriodictyol glucuronide (Fabre et al., 2001). Compound **63**, with the aglycone at *m/z* 271, was characterized as naringenin glucuronide (Sánchez-Rabaneda et al., 2004).

3.4.4. Other compounds

Oleuropein (compound **61**) was identified, as previously discussed, based on its $[M-H]^-$ at *m/z* 539, and fragment ions at *m/z* 377, 307, and 275.

Compounds **91** and **96** were characterized as oxo-dihydroxy-octadecenoic and trihydroxy-octadecenoic acids, respectively, taking into account the fragmentation data, previously described in scientific literature (Levandi et al., 2009; Van Hoyweghen et al., 2014).

Using ESI^+, it was possible to characterize compound **34** as delphinidin glucuronide, showing M^+ ion at *m/z* 479 and suffering a neutral loss of 176 Da in MS^2.

Table 4
Characterization of the methanolic extracts from leaves of *Clethra arborea*.

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-DAD-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
1	2.7	683	MS^2[683]: 341 (100) MS^3[683 → 341]: 179 (100), 161 (11.4), 143 (20.3), 119 (36.3), 113 (33.9) MS^4[683 → 341 → 179]: 161 (18.2), 119 (15.1), 115 (15.5), 107 (26.6), 89 (100)	Hexose polymer	Brudzynski and Miotto (2011)
2	2.8	533	MS^2[533]: 191 (100) MS^3[533 → 191]: 173 (46.7), 171 (26), 127 (100), 109 (39.9), 85 (42.7) MS^4[533 → 191 → 127]: 110 (100), 109 (17.3), 85 (71.8)	Quinic acid derivative	–
4	3.2	191	MS^2[191]: 173 (57), 127 (100), 109 (32.3), 93 (37.7), 85 (35) MS^3[191 → 127]: 109 (100), 99 (67.1)	Quinic acid	Gouveia and Castilho (2011)
7	3.2	577	MS^2[577]: 451 (19.5), 425 (100), 407 (92.4), 289 (23.6), 287 (7.5) MS^3[577 → 407]: 389 (31.3), 285 (100), 284 (25.2), 283 (46.1), 281 (53.1) MS^4[577 → 407 → 284]: 283 (23.1), 257 (100), 255 (14.6), 241 (23.1), 213 (35.5) MS^3[577 → 425]: 408 (15.2), 407 (100) MS^4[577 → 425 → 407]: 285 (77.9), 281 (100), 256 (28.3), 255 (25), 243 (39.2)	Procyanidin dimer	Ruiz et al. (2005); Tomás-Barberán et al. (2001)
16	4.5	865	MS^2[865]: 739 (26.9), 713 (27.9), 695 (100), 577 (64.9), 575 (45.8), 407 (58), 289 (10.4) MS^3[865 → 695]: 677 (40.2), 544 (32.5), 543 (100), 525 (70.3), 243 (74.8) MS^4[865 → 695 → 543]: 526 (37.7), 525 (100), 499 (15), 404 (13.9), 392 (18.3) MS^4[865 → 695 → 677]: 610 (11.5), 600 (100), 555 (12), 554 (48.6), 467 (7)	Procyanidin trimer	Ruiz et al. (2005); Tomás-Barberán et al. (2001)
18	4.7	577	MS^2[577]: 451 (20.1), 425 (100), 407 (95.5), 289 (26.2), 287 (14.2) MS^3[577 → 407]: 285 (100), 281 (65.3), 257 (46.3), 255 (37.4), 243 (35.6) MS^4[577 → 407 → 285]: 283 (100), 257 (51.6), 256 (54.1), (155 (10.8) MS^3[577 → 425]: 408 (18.8), 407 (100) MS^4[577 → 425 → 407]: 285 (50.8), 283 (29.9), 281 (100), 257 (29.3), 256 (33.8)	Procyanidin dimer	Ruiz et al. (2005); Tomás-Barberán et al. (2001)
21	5.4	865	MS^2[865]: 739 (28.6), 713 (27.1), 695 (100), 577 (85), 425 (30.9), 407 (53.6), 289 (7.8) MS^3[865 → 695]: 543 (100), 525 (64.8), 451 (30.6), 405 (29.8), 243 (54.8) MS^4[865 → 695 → 543]: 526 (31.5), 525 (100), 405 (8.6), 391 (26), 243 (9)	Procyanidin trimer	Ruiz et al. (2005); Tomás-Barberán et al. (2001)
23	5.6	289	MS^2[289]: 247 (14.5), 246 (10.9), 245 (100), 205 (36.5), 179 (16.2) MS^3[289 → 245]: 227 (11.8), 203 (100), 187 (16.8), 175 (7.7), 161 (13.6) MS^4[289 → 245 → 203]: 188 (35.3), 175 (16.3), 174 (100), 161 (53.3), 160 (15.9)	Catechin	Rockenbach et al. (2012)
25	5.8	311	MS^2[311]: 179 (44.5), 177 (34.1), 149 (100), 135 (5.9) MS^3[311 → 149]: 131 (71.6), 103 (66.5), 87 (100), 59 (18.4)	Caftaric acid	Schütz et al. (2005)
33	6.9	609	MS^2[609]: 307 (12.6), 301 (100) MS^3[609 → 301]: 273 (9.6), 229 (10.5), 179 (100), 151 (94.3), 107 (11.1) MS^4[609 → 301 → 179]: 169 (19.7), 151 (100)	Rutin	–
34	7.0	479 (+)	MS^2[479]: 304 (15.6), 303 (100) MS^3[479 → 303]: 285 (33.1), 257 (71.4), 229 (100)	Delphinidin glucuronide	Cooke et al. (2006)
35	7.2	865	MS^2[865]: 739 (33), 713 (24.1), 695 (100), 577 (39.9), 543 (56), 407 (40), 289 (16.3) MS^3[865 → 695]: 543 (100), 525 (54.3), 451 (25.8), 408 (27.3), 407 (86.9) MS^4[865 → 695 → 543]: 525 (100), 499 (14.9), 392 (30.9), 391 (18), 255 (14.5)	Procyanidin trimer	Ruiz et al. (2005); Tomás-Barberán et al. (2001)
39	7.7	577	MS^2[577]: 451 (19.8), 426 (19), 425 (100), 407 (74.7), 289 (12.4), 287 (13.1) MS^3[577 → 425]: 408 (22.3), 407 (100) MS^4[577 → 425 → 407]: 285 (100), 283 (28.5), 281 (63.8), 257 (35.4), 256 (28.4)	Procyanidin dimer	Ruiz et al. (2005); Tomás-Barberán et al. (2001)
41	8.1	607	MS^2[607]: 321 (8.1), 285 (100) MS^3[607 → 285]: 257 (28.3), 241 (26.3), 229 (25), 213 (44.1), 151 (100) MS^4[607 → 285 → 151]: 107 (100)	Kaempferol derivative	–
43	8.4	593	MS^2[593]: 307 (12), 286 (13), 285 (100) MS^3[593 → 285]: 267 (28.9), 257 (82.3), 229 (46.1), 223 (50.7), 151 (100) MS^4[593 → 285 → 151]: 169 (52.4), 107 (100)	Kaempferol 3-rutinoside	Del Rio et al. (2004)

Table 4 (*Continued*)

No	t_R (min)	$[M-H]^-$ (*m/z*)	HPLC-DAD-MS^n; *m/z* (% base peak)	Assigned identity	Ref.
46	9.4	563	MS^2[563]: 281 (100), 279 (9.7), 237 (19.6), 193 (12), 161 (9.3) MS^3[563 → 281]: 237 (48.1), 193 (57.6), 191 (17.8), 161 (44.6), 109 (100)	Unknown	–
49	9.7	477	MS^2[477]: 302 (16.4), 301 (100) MS^3[477 → 301]: 273 (12), 257 (7), 179 (100), 151 (85.7) MS^4[477 → 301 → 151]: 170 (13.4), 169 (46.7), 107 (100), 83 (6.2), 65 (11.1) MS^4[477 → 301 → 179]: 169 (72.2), 152 (35.1), 151 (100)	Quercetin-3-O-glucuronide	Downey and Rochfort (2008)
50	9.9	463	MS^2[463]: 288 (12.4), 287 (100), 151 (18) MS^3[463 → 287]: 151 (100), 135 (4.7) MS^4[463 → 287 → 151]: 107 (100)	Eriodictyol glucuronide	Fabre et al. (2001)
53	10.9	373 (+)	MS^2[373]: 211 (100), 193 (65.0), 175 (23.7), 135 (41.1), 119 (26.8) MS^3[373 → 211]: 193 (100), 175 (65.3), 135 (41.7), 119 (72.1), 109 (20.0) MS^4[373 → 211 → 193]: 175 (100), 135 (36.4), 121 (16.5), 119 (73.6)	Hydroferuloylglucose	Ma et al. (2007)
61	13.8	539	MS^2[539]: 377 (21.2), 327 (15.3), 307 (61.8), 275 (100) MS^3[539 → 275]: 139 (100), 95 (83.8)	Oleuropein	Bianco et al. (2001); Fu et al. (2010)
62	13.8	359	MS^2[359]: 197 (20.4), 179 (21.8), 161 (100) MS^2[359 → 161]: 134 (54.3), 133 (100)	Rosmarinic acid	Liu et al. (2007)
63	14	447	MS^2[447]: 272 (34.5), 271 (100), 175 (45.8) MS^3[447 → 271]: 177 (41.1), 166 (16.5), 151 (100), 125 (10.3), 93 (13.3) MS^4[447 → 271 → 151]: 107 (100)	Naringenin glucuronide	Sánchez-Rabaneda et al. (2004)
75	19.6	493	MS^2[493]: 448 (27.2), 447 (100) MS^3[493 → 447]: 315 (100), 191 (15), 161 (86.7), 149 (23.6), 131 (15.4) MS^4[493 → 447 → 315]: 161 (100), 143 (12.3), 113 (10.8), 101 (10.3),	Unknown	–
78	22.8	543	MS^2[543]: 255 (100) MS^3[543 → 255]: 211 (11.3), 193 (52.3), 175 (100), 97 (27.5), 81 (12.2) MS^4[543 → 255 → 175]: 157 (94), 113 (59.8), 103 (81.1), 83 (24.2), 71 (100)	Catechin derivative	–
88	30.9	625	MS^2[625]: 602 (100), 464 (12.5), 463 (60.8) MS^3[625 → 602]: 463 (100) MS^4[625 → 602 → 463]: 397 (100)	Unknown	–
91	31.5	327	MS^2[327]: 292 (15.5), 291 (33.6), 229 (83.4), 211 (33.1), 171 (100) MS^3[327 → 229]: 211 (100), 209 (82.4), 125 (23.1) MS^4[327 → 229 → 209]: 165 (100), 95 (15.9)	Oxo-dihydroxy-octadecenoic acid	Levandi et al. (2009); Van Hoyweghen et al. (2013)
96	34.3	329	MS^2[329]: 311 (24.8), 293 (22.4), 229 (100), 211 (73.6), 193 (7.1), 171 (22.8) MS^3[329 → 229]: 211 (85.6), 209 (36.7), 167 (27.2), 127 (49.5), 125 (100)	Trihydroxy-octadecenoic acid	Van Hoyweghen et al. (2014)
98	36.4	503	MS^2[503]: 486 (29), 485 (100), 453 (41.5) MS^3[503 → 485]: 453 (100), 439 (83.9), 403 (90) MS^4[503 → 485 → 453]: 423 (100), 409 (25.5)	Unknown	–
101	38.9	503	MS^2[503]: 486 (29.3), 485 (100) MS^3[503 → 485]: 441 (100), 439 (32.5), 421 (89.5), 403 (68.4) MS^4[503 → 485 → 441]: 421 (100), 419 (53.8) MS^4[503 → 485 → 421]: 403 (100), 393 (90.6)	Unknown	–
103	40.2	503	MS^2[503]: 486 (25), 485 (100) MS^3[503 → 485]: 453 (32.6) 439 (100), 421 (79.2) MS^4[503 → 485 → 439]: 423 (61.1), 421 (24.1), 407 (100)	Unknown	
105	43.1	487	MS^2[487]: 469 (100) MS^3[487 → 469]: 437 (66.8), 435 (100), 424 (17.1), 392 (34.8) MS^4[487 → 469 → 435]: 375 (100)	Unknown	–
107	44.6	487	MS^2[487]: 470 (30.3), 469 (100), 467 (12.3) MS^3[487 → 469]: 423 (100), 421 (21.4), 406 (34.4), 393 (15.2), 389 (26.4) MS^4[487 → 469 → 423]: 407 (34.4), 405 (69.4), 403 (42), 393 (24.9), 389 (100)	Unknown	–

3.5. Potential applications of the main phenolic compounds identified

The main goal of this research was to find potential applications to the discarded vegetal material during forest cleaning tasks, particularly to leaves with high phenolic contents, which may represent a source of valuable bioactive compounds.

In *O. europaea*, the most abundant compound was oleuropein, as well as different derivatives or isomers. Due to its beneficial biological activities, the possibility of preparing oleuropein-enriched

functional foods has been recently reported (Zoidou et al., 2014). Hence, discarded leaves from *O.europaea* may represent a source of oleuropein with applications in the food industry.

I. perado presented high levels of mono- and di-caffeoylquinic acids. Dried leaves of *I. paraguariensis* are used to prepare infusions rich in phenolic acids (Bastos et al., 2007), so further experiments could confirm the usefulness of *I. perado* for similar purposes.

Finally, different flavonoid glycosides, and procyanidin dimers and trimers were found in *H. excelsa* and *C. arborea*. However, the levels of these compounds were not as high as those found in *O. europaea* or *I. perado*, so further research would be necessary to confirm the potential uses of leaves from these plants.

4. Conclusion

In this work, a report on the phenolic composition of leaves of several relevant plants endemic to Madeira Archipelago is presented for the first time. Using HPLC-ESI-MS^n, different families of compounds were identified.

O. europaea ssp. *cerasiformis* extract was mainly constituted by oleuropein derivatives, like other varieties of *O. europaea*, and a simple flavonoid pattern of luteolin, apigenin and quercitin glucosides. A kaempferol triglycoside was the differentiating component, being previously reported in tea and citrus but not in *Olea* ssp. Caffeic acid derivatives and triterpenoid saponins were the main components of *Ilex perado*. *Clethra arborea* most relevant components in the methanolic extract were catechin derivatives, dimers and trimers, type B procyanidins, and flavonoid glucuronides. The identification of components of *Heberdenia excelsa* was not fully achieved and several components remain unknown, although several glycosylated flavonoids were identified. Phenylethanoids and isoflavoids were the differentiating components of this extract.

In total, over 100 compounds were detected in the analyzed extracts. Taking into account that there are no bibliographic data available in scientific literature for this species of plants, more research will be carried out in our research group, focusing in the identification of some of the unknowns, and the isolation and bioevaluation of relevant components.

Acknowledgments

This research was supported by Fundação para a Ciência e a Tecnologia (FCT) with funds from the Portuguese Government (Project PEst-OE/QUI/UI0674/2011). The mass spectrometer used in this work is part of the Portuguese National Mass Spectrometry Network (Contract RNEMREDE/1508/REM/2005) and was purchased in the framework of the National Programme for Scientific Re-equipment, with funds from POCI 2010 (FEDER) and FCT. EJLM also acknowledges the financial support from Campus de Excelencia Internacional Agroalimentario (ceiA3) and University of Jaén, from Spain.

References

Altiok, E., Bayçin, D., Bayraktar, O., Ülkü, S., 2008. Isolation of polyphenols from the extracts of olive leaves (*Olea europaea* L.) by adsorption on silk fibroin. Sep. Purif. Technol. 62, 342–348.

Barreca, D., Bellocco, E., Caristi, C., Leuzzi, U., Gattuso, G., 2011. Distribution of C- and O-glycosyl flavonoids, (3-hydroxy-3-methylglutaryl)glycosyl flavanones and furocoumarins in *Citrus aurantium* L. juice. Food Chem. 124, 576–582.

Bastos, D.H.M., Saldanha, L.A., Catharino, R.R., Sawaya, A.C.H.F., Cunha, I.B.S., Carvalho, P.O., Eberlin, M.N., 2007. Phenolic antioxidants identified by ESI-MS from yerba maté (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. Molecules 12, 423–432.

Benavente-García, O., Castillo, J., Lorente, J., Ortuño, A., Del Rio, J.A., 2000. Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. Food Chem. 68, 457–462.

Bianco, A., Buiarelli, F., Cartoni, G., Coccioli, F., Muzzalupo, I., Polidori, A., Uccella, N., 2001. Analysis by HPLC-MS/MS of biophenolic components in olives and oils. Anal. Lett. 34, 1033–1051.

Bravo, L., Goya, L., Lecumberri, E., 2007. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Res. Int. 40, 393–405.

Briante, R., Patumi, M., Terenziani, S., Bismuto, E., Febbraio, F., Nucci, R., 2002. *Olea europaea* L. leaf extract and derivatives: antioxidant properties. J. Agric. Food Chem. 50, 4934–4940.

Brito, G., Loureiro, J., Lopes, T., Rodriguez, E., Santos, C., 2008. Genetic characterisation of olive trees from Madeira Archipelago using flow cytometry and microsatellite markers. Gen. Res. Crop Evol. 55, 657–664.

Brudzynski, K., Miotto, D., 2011. Honey melanoidins: analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. Food Chem. 127, 1023–1030.

Cardoso, S.M., Guyot, S., Marnet, N., Lopes-da-Silva, J.A., Renard, C.M.G.C., Coimbra, M.A., 2005. Characterisation of phenolic extracts from olive pulp and olive pomace by electrospray mass spectrometry. J. Sci. Food Agric. 85, 21–32.

Carini, M., Maffei Facino, R., Aldini, G., Calloni, M., Colombo, L., 1998. Characterization of phenolic antioxidants from Mate (*Ilex paraguayensis*) by liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 12, 1813–1819.

Clifford, M.N., Johnston, K.L., Knight, S., Kuhnert, N., 2003. Hierarchical scheme for LC-MSn identification of chlorogenic acids. J. Agric. Food Chem. 51, 2900–2911.

Clifford, M.N., Knight, S., Kuhnert, N., 2005. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MSn. J. Agric. Food Chem. 53, 3821–3832.

Clifford, M.N., Marks, S., Knight, S., Kuhnert, N., 2006a. Characterization by LC-MSn of four new classes of p-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. J. Agric. Food Chem. 54, 4095–4101.

Clifford, M.N., Zheng, W., Kuhnert, N., 2006b. Profiling the chlorogenic acids of aster by HPLC-MSn. Phytochem. Anal. 17, 384–393.

Chen, X.-Q., Zan, K., Yang, J., Liu, X.-X., Mao, Q., Zhang, L., Lai, M.-X., Wang, Q., 2011. Quantitative analysis of triterpenoids in different parts of *Ilex hainanensis*, *Ilex stewardii* and *Ilex pubescens* using HPLC-ELSD and HPLC-MSn and antibacterial activity. Food Chem. 126, 1454–1459.

Cooke, D.N., Thomasset, S., Boocock, D.J., Schwarz, M., Winterhalter, P., Steward, W.P., Gescher, A.J., Marczylo, T.H., 2006. Development of analyses by high-performance liquid chromatography and liquid chromatography/tandem mass spectrometry of bilberry (*Vaccinium myrtilus*) anthocyanins in human plasma and urine. J. Agric. Food Chem. 54, 7009–7013.

Damak, N., Bouaziz, M., Ayadi, M., Sayadi, S., Damak, M., 2008. Effect of the maturation process on the phenolic fractions, fatty acids, and antioxidant activity of the Chétoui olive fruit cultivar. J. Agric. Food Chem. 56, 1560–1566.

Dartora, N., De Souza, L.M., Santana-Filho, A.P., Iacomini, M., Valduga, A.T., Gorin, P.A.J., Sassaki, G.L., 2011. UPLC-PDA-MS evaluation of bioactive compounds from leaves of *Ilex paraguariensis* with different growth conditions, treatments and ageing. Food Chem. 129, 1453–1461.

de Mejía, E.G., Chandra, S., Ramírez-Mares, M., Wang, W., 2006. Catalytic inhibition of human DNA topoisomerase by phenolic compounds in *Ardisia compressa* extracts and their effect on human colon cancer cells. Food Chem. Toxicol. 44, 1191–1203.

De Nino, A., Lombardo, N., Perri, E., Procopio, A., Raffaelli, A., Sindona, G., 1997. Direct identification of phenolic glucosides from olive leaf extracts by atmospheric pressure ionization tandem mass spectrometry. J. Mass Spectrom. 32, 533–541.

Del Rio, D., Stewart, A.J., Mullen, W., Burns, J., Lean, M.E.J., Brighenti, F., Crozier, A., 2004. HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. J. Agric. Food Chem. 52, 2807–2815.

Downey, M.O., Rochfort, S., 2008. Simultaneous separation by reversed-phase high-performance liquid chromatography and mass spectral identification of anthocyanins and flavonols in Shiraz grape skin. J. Chromatogr. A 1201, 43–47.

Eyles, A., Jones, W., Riedl, K., Cipollini, D., Schwartz, S., Chan, K., Herms, D., Bonello, P., 2007. Comparative phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). J. Chem. Ecol. 33, 1430–1448.

Fabre, N., Rustan, I., de Hoffmann, E., Quetin-Leclercq, J., 2001. Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. J. Am. Soc. Mass Spectrom. 12, 707–715.

Fu, S., Arráez-Roman, D., Segura-Carretero, A., Menéndez, J.A., Menéndez-Gutiérrez, M.P., Micol, V., Fernández-Gutiérrez, A., 2010. Qualitative screening of phenolic compounds in olive leaf extracts by hyphenated liquid chromatography and preliminary evaluation of cytotoxic activity against human breast cancer cells. Anal. Bioanal. Chem. 397, 643–654.

González-Paramás, A.M., Lopes Da Silva, F., Martín-López, P., Macz-Pop, G., González-Manzano, S., Alcalde-Eon, C., Pérez-Alonso, J.J., Escribano-Bailón, M.T., Rivas-Gonzalo, J.C., Santos-Buelga, C., 2006. Flavanol–-anthocyanin condensed pigments in plant extracts. Food Chem. 94, 428–436.

Gouveia, S., Castilho, P.C., 2011. Antioxidant potential of Artemisia argentea L'Hér alcoholic extract and its relation with the phenolic composition. Food Res. Int. 44, 1620–1631.

Gouveia, S., Castilho, P.C., 2012. *Helichrysum monizii* Lowe: phenolic composition and antioxidant potential. Phytochem. Anal. 23, 72–83.

Gouveia, S.C., Castilho, P.C., 2009. Analysis of phenolic compounds from different morphological parts of *Helichrysum devium* by liquid chromatography with on-line UV and electrospray ionization mass spectrometric detection. Rapid Commun. Mass Spectrom. 23, 3939–3953.

Gouveia, S.C., Castilho, P.C., 2010. Characterization of phenolic compounds in *Helichrysum melaleucum* by high-performance liquid chromatography with on-line ultraviolet and mass spectrometry detection. Rapid Commun. Mass Spectrom. 24, 1851–1868.

Guo, H., Liu, A.-H., Ye, M., Yang, M., Guo, D.-A., 2007. Characterization of phenolic compounds in the fruits of *Forsythia suspensa* by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun. Mass Spectrom. 21, 715–729.

Han, J., Ye, M., Qiao, X., Xu, M., Wang B.-r., Guo, D.-A., 2008. Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. J. Pharm. Biomed. Anal. 47, 516–525.

Ignat, I., Volf, I., Popa, V.I., 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem. 126, 1821–1835.

IUCN, 2013. IUCN Red List of Threatened Species. Version 2013.2, http://www.iucnredlist.org/ (accessed 29.04.14).

Jemai, H., Bouaziz, M., Sayadi, S., 2009. Phenolic composition, sugar contents and antioxidant activity of Tunisian sweet olive cuitivar with regard to fruit ripening. J. Agric. Food Chem. 57, 2961–2968.

Khan, M.N.I., Nizami, S.S., Khan, M.A., Ahmed, Z., 1993. New saponins from *Mangifera indica*. J. Nat. Prod. 56, 767–770.

Laguerre, M., López Giraldo, L.J., Piombo, G., Figueroa-Espinoza, M.C., Pina, M., Benaissa, M., Combe, A., Rossignol Castera, A., Lecomte, J., Villeneuve, P., 2009. Characterization of olive-leaf phenolics by esi-ms and evaluation of their antioxidant capacities by the cat assay. J. Am. Oil Chem. Soc. 86, 1215–1225.

Levandi, T., Püssa, T., Vaher, M., Toomik, P., Kaljurand, M., 2009. Oxidation products of free polyunsaturated fatty acids in wheat varieties. Eur. J. Lipid Sci. Technol. 111, 715–722.

Liu, A.-H., Guo, H., Ye, M., Lin, Y.-H., Sun, J.-H., Xu, M., Guo, D.-A., 2007. Detection, characterization and identification of phenolic acids in Danshen using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. J. Chromatogr. A 1161, 170–182.

Ma, C., Xiao, S.-y., Li, Z.-g., Wang, W., Du, L.-j., 2007. Characterization of active phenolic components in the ethanolic extract of *Ananas comosus* L. leaves using high-performance liquid chromatography with diode array detection and tandem mass spectrometry. J. Chromatogr. A 1165, 39–44.

Mateos-Martín, M.L., Fuguet, E., Quero, C., Pérez-Jiménez, J., Torres, J.L., 2012. New identification of proanthocyanidins in cinnamon (*Cinnamomum zeylanicum* L.) using MALDI-TOF/TOF mass spectrometry. Anal. Bioanal. Chem. 402, 1327–1336.

Mylonaki, S., Kiassos, E., Makris, D.P., Kefalas, P., 2008. Optimisation of the extraction of olive (*Olea europaea*) leaf phenolics using water/ethanol-based solvent systems and response surface methodology. Anal. Bioanal. Chem. 392, 977–985.

Obied, H.K., Bedgood Jr., D.R., Prenzler, P.D., Robards, K., 2007. Chemical screening of olive biophenol extracts by hyphenated liquid chromatography. Anal. Chim. Acta 603, 176–189.

Pereira, A.P., Ferreira, I.C.F.R., Marcelino, F., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., Pereira, J.A., 2007. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. Molecules 12, 1153–1162.

Peres, R.G., Tonin, F.G., Tavares, M.F.M., Rodriguez-Amaya, D.B., 2013. HPLC-DAD-ESI/MS identification and quantification of phenolic compounds in ilex paraguariensis beverages and on-line evaluation of individual antioxidant activity. Molecules 18, 3859–3871.

Prasain, J.K., Jones, K., Kirk, M., Wilson, L., Smith-Johnson, M., Weaver, C., Barnes, S., 2003. Profiling and quantification of isoflavonoids in kudzu dietary supplements by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. J. Agric. Food Chem. 51, 4213–4218.

Quirantes-Piné, R., Lozano-Sánchez, J., Herrero, M., Ibáñez, E., Segura-Carretero, A., Fernández-Gutiérrez, A., 2013. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterising phenolic compounds in olive-leaf extracts. Phytochem. Anal. 24, 213–223.

Rivera, D.O.C., 1995. The ethnopharmacology of Madeira and Porto Santo Islands, a review. J. Ethnopharmacol. 46, 21.

Rockenbach, I.I., Jungfer, E., Ritter, C., Santiago-Schübel, B., Thiele, B., Fett, R., Galensa, R., 2012. Characterization of flavan-3-ols in seeds of grape pomace by CE, HPLC-DAD-MS n and LC-ESI-FTICR-MS. Food Res. Int. 48, 848–855.

Ruiz, D., Egea, J., Gil, M.I., Tomás-Barberán, F.A., 2005. Characterization and quantitation of phenolic compounds in new apricot (*Prunus armeniaca* L.) varieties. J. Agric. Food Chem. 53, 9544–9552.

Sánchez-Rabaneda, F., Jáuregui, O., Lamuela-Raventós, R.M., Viladomat, F., Bastida, J., Codina, C., 2004. Qualitative analysis of phenolic compounds in apple pomace using liquid chromatography coupled to mass spectrometry in tandem mode. Rapid Commun. Mass Spectrom. 18, 553–563.

Savarese, M., De Marco, E., Sacchi, R., 2007. Characterization of phenolic extracts from olives (*Olea europaea* cv. Pisciottana) by electrospray ionization mass spectrometry. Food Chem. 105, 761–770.

Schütz, K., Kammerer, D.R., Carle, R., Schieber, A., 2005. Characterization of phenolic acids and flavonoids in dandelion (*Taraxacum officinale* WEB. ex WIGG.) root and herb by high-performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun. Mass Spectrom. 19, 179–186.

Sosa, P.A., González-González, E.A., González-Pérez, M.A., Pérez de Paz, P.L., 2013. Contrasting patterns of genetic differentiation in macaronesian lineages of ilex (aquifoliaceae). Bot. J. Linn. Soc. 173, 258–268.

Suárez, M., Valls, R.M., Romero, M.P., Maclà, A., Fernández, S., Giralt, M., Solà, R., Motilva, M.J., 2011. Bioavailability of phenols from a phenol-enriched olive oil. Br. J. Nutr. 106, 1691–1701.

Tomás-Barberán, F.A., Gil, M.I., Cremin, P., Waterhouse, A.L., Hess-Pierce, B., Kader, A.A., 2001. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J. Agric. Food Chem. 49, 4748–4760.

Truchado, P., Vit, P., Ferreres, F., Tomas-Barberan, F., 2011. Liquid chromatography–tandem mass spectrometry analysis allows the simultaneous characterization of C-glycosyl and O-glycosyl flavonoids in stingless bee honeys. J. Chromatogr. A 1218, 7601–7607.

Urpi-Sarda, M., Garrido, I., Monagas, M.a., Goímez-Cordovés, C., Medina-Remoín, A., Andres-Lacueva, C., Bartolomé, B.a., 2009a. Profile of plasma and urine metabolites after the intake of almond [*Prunus dulcis* (Mill.) D.A. Webb] polyphenols in humans. J. Agric. Food Chem. 57, 10134–10142.

Urpi-Sarda, M., Monagas, M., Khan, N., Llorach, R., Lamuela-Raventós, R.M., Jáuregui, O., Estruch, R., Izquierdo-Pulido, M., Andrés-Lacueva, C., 2009b. Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1216, 7258–7267.

Van Hoyweghen, L., De Bosscher, K., Haegeman, G., Deforce, D., Heyerick, A., 2014. In vitro inhibition of the transcription factor NF-κB and cyclooxygenase by bamboo extracts. Phytother. Res. 28, 224–230.

Ye, M., Yan, Y., Guo, D.A., 2005. Characterization of phenolic compounds in the Chinese herbal drug Tu–Si–Zi by liquid chromatography coupled to electrospray ionization mass spectrometry. Rapid Commun. Mass Spectrom. 19, 1469–1484.

Zoidou, E., Magiatis, P., Melliou, E., Constantinou, M., Haroutounian, S., Skaltsounis, A.L., 2014. Oleuropein as a bioactive constituent added in milk and yogurt. Food Chem. 158, 319–324.