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Phenolic compounds, antioxidant and antibacterial activities of three Ericaceae from Algeria

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ABSTRACT

Herbs of the Ericaceae family are commonly found in Algeria and used in traditional medicine as antiseptic, diuretic, astringent, depurative, and to treat scalds and wounds. The methanolic extracts of three species, *Arbutus unedo* L. (*A. unedo*, leaves), *Erica arborea* L. (*E. arborea*, flowered aerial parts), and *Erica multiflora* L. (*E. multiflora*, flowered aerial parts), were compared regarding their content in phenolic compounds, their antioxidant, and antibacterial activities. *A. unedo* harbors the highest content in total phenolics and flavonoids, followed by *E. arborea E. multiflora*. The contents in total phenolics and flavonoids showed a correlation with the measured antioxidant (hydrogen-donating) activities; this was particularly the case for flavonoids content. The *A. unedo* extract showed antibacterial activity against all the tested strains (*Staphylococcus aureus* ATCC 6538, *S. aureus* C100459, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 9027); however, the *E. arborea* and *E. multiflora* extracts showed antibacterial activity only against Gram positive bacteria. Some polyphenols were identified in the three herbs by thin-layer chromatography and high-performance liquid chromatography coupled with diode array and mass spectrometry detection; from these, caffeic acid, *p*-coumaric acid, naringin, quercetin and kaempferol are reported for the first time in *E. multiflora*.

1. Introduction

In many countries, including Algeria, folk medicines widely resort to medicinal plants as primary tools to treat many diseases; the study of such traditional practices has uncovered many drugs of importance and is still a cornerstone of modern drug discovery (Newman and Cragg, 2012). In the last decades, herbal medicines have received much attention in Western and Eastern countries as sources of biologically active substances, notably for the discovery of antioxidant, antimutagenic, anticarcinogenic and cytotoxic agents (Parejo et al., 2003).

The Ericaceae, a large cosmopolitan family represented by 4100 species regrouped in 124 genera, notably *Arbutus*, *Calluna* and *Erica*, present the highest diversity under the Mediterranean climates (Lhuillier, 2007). Medicinal properties have long been recognized for some Ericaceae which led to their inclusion in the list of species that may enter into the composition of herbal medicines (Bruneton, 2001). The therapeutic functions of Ericaceae species are generally attributed to their abundant (poly)phenolic compounds (Márquez-García et al., 2009).

From this family, *Erica arborea*, *Erica multiflora* and *Arbutus unedo* are commonly found in Algeria and used for various medicinal properties; the *Erica* species are notably used to treat wounds and scalds. Also named “tree heath”, *E. arborea* is a shrub that usually measures up to 4 m high and even more in the old bush (Meyer et al., 2004). This species is distributed in the Mediterranean region, in the west of Portugal, in the Canary Islands (La Mantia et al., 2007) and in Northern Africa; it is found in Morocco, Tunisia and Algeria, where it is common in altitude scrubland, in Aures mountains and Ksour Range (Ait Youssef, 2006). *E. arborea* is considered as

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an astringent plant (Bezanger-Beauquesne et al., 1990); its aerial parts have many traditional uses as antiulcer, antimicrobial, cytotoxic, anti-edema (Akkol et al., 2007; Márquez-García et al., 2009), antidiarrheal and healing agent (Ait Youssef, 2006). According to Ay et al. (2007), its leaves and flowers are used in many countries as diuretic, urinary antiseptic, and against constipation. Flavonoids and phenolics are the main compounds isolated from this species that also contains terpenoids, coumarins and essential oils (Ait Youssef, 2006; Garnier et al., 1961).

E. multiflora, known as “many-flowered heather”, a sub-shrub with evergreen needle-like foliage (Vilà and Terradas, 1998), is present in Northern Africa and France. In Algeria, the species is common all along the coast; it grows mainly in scrublands and is very rare in Kabylia (Ait Youssef, 2006). The flowering tops of *E. multiflora* are used to treat hyperlipidemia (Harnafi et al., 2007), atherosclerosis, prostate cancer (Ait Youssef, 2006), and as antiseptic, diuretic (Harnafi et al., 2007), anti-inflammatory (Sadki et al., 2010), astringent, sedative, and wound- healing agent (Rios et al., 1987). Tannins, proanthocyanidols and flavonoids represent major compounds of the flowers (Bruneton, 1987). The *E. multiflora* leaves ethyl acetate extract and its active compound lupenone (lup-20(29)-en-3-one) stimulate melanogenesis by increasing the tyrosinase enzyme expression at both the transcriptional and translational levels, making it a possible treatment for hypopigmentation diseases (Villareal et al., 2013).

A. unedo, known as “strawberry tree”, is an evergreen shrub widely distributed in the Mediterranean basin (Ait Youssef, 2006; Fortalezas et al., 2010) and South-Western Asia (Ait Youssef, 2006). The fruits are used in the production of alcoholic beverages, jams, jellies and marmalades (Ayaz et al., 2000; Serçe et al., 2010; Takrouni and Boussaid, 2010); they are also used in folk medicine for the treatment of gastrointestinal, dermatological, urological, cardiovascular and gastritis disorders, and for their antimicrobial activity (Ruiz-Rodríguez et al., 2011). This organ is also a good source of antioxidants (Pallauf et al., 2008), including phenolic compounds (*e.g.*, flavonoids, anthocyanins, gallic acid derivatives and tannins), vitamin C, vitamin E, and carotenoids (Fortalezas et al., 2010). *A. unedo* leaves are used for astringent, antiseptic, urinary antiseptic, diuretic, antidiarrheal, depurative, anti-inflammatory and antioxidant properties, and in the therapy of hypertension, diabetes and gonorrhea (El Haouari et al., 2007; Kivcak and Mert, 2001; Oliveira et al., 2009). As mentioned by Ait Youssef (2006), *A. unedo* leaves are used for direct application on recurrent skin diseases, like eczema or fungal infections. The antiaggregant activity of leaves extracts has been proposed for the treatment and/or prevention of cardiovascular diseases (Andrade et al., 2009; Mariotto et al., 2008). The antihypertensive effect has been attributed to their richness in phenolic compounds, including tannins (Afkir et al., 2008; Pallauf et al., 2008). The roots are disinfectant of the urinary tract (Garnier et al., 1961), anti-inflammatory, laxative, carminative, digestive, odontalgic, and cardiotonic (Barros et al., 2010). Potentially bioactive compounds, including lipids, tannins, vitamin E (Pabuçcuoglu et al., 2003), triterpenoids, flavonoids (Fiorentino et al., 2007), aromatic acids, iridoids, monoterpenoids, phenylpropanoids, and sterols, have been described in the leaves of *A. unedo* (Ruiz-Rodríguez et al., 2011).

Based on their reported traditional uses and phenolic compositions, particularly flavonoids, proanthocyanidins and phenolic acids, Ericaceae may be a potential source for clinically relevant antioxidant and/or antibacterial agents. Given the widespread distribution and utilization of *E. arborea*, *E. multiflora* and *A. unedo* in Algeria, the present work aims at validating these folk uses by investigating polar extracts of local cultivars for total phenolics, flavonoids content, antioxidant, and antibacterial activities. Major compounds will be profiled through thin-layer chromatography (TLC) and high-performance liquid chromatography coupled with diode array and mass detection (HPLC–DAD–ESI-MS).

2. Material and methods

2.1. Chemicals and culture medium

Folin Ciocalteu’s, 2,2-diphenyl-1-pycryl hydrazyl (DPPH), apigenin, arbutin, epicatchin, salicylic acid, ethyl gallate, chlorogenic acid and sodium monocarbonate were obtained from Sigma–Aldrich (St. Louis, USA), catechin, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), tetrabutylammonium hexafluorophosphate (Bu_4NPF_6), dimethylformamide (DMF) and hesperidin were from Fluka (Sigma–Aldrich, St. Louis, USA), dimethylsulfoxyde (DMSO) and resorcinol from Merck (Darmstadt, Germany), quercetin from Riedel-de Haen (Seelze, Germany), aluminium chloride and methanol from Biochem Chemopharma (Quebec, Canada), kaempferol, galangin and 4-OH benzoic acid from Carl Roth (Karlsruhe, Belgium), rutin from Alfa Aesar (Ward Hill, USA), delphinidin from Extrasynthèse (Genay, France), caffeic acid from Janssen Chemica (Geel, Belgium), gallic acid, *m*-coumaric acid and *p*-coumaric acid from Koch Light Laboratories (Gauteng South Africa, South Africa), and potassium persulfate from Rhone-Povalenc (Paris, France). The Mueller Hinton broth was from Oxoid (Hampshire, UK). Cefotaxim (30 μg) and streptomycin were used as control for the microdilution assay (Taastrup, Denmark). *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027 were obtained from the American Type Culture Collection (ATCC, Manassas, USA). The *S. aureus* C100459 was a methicillin-resistant (MRSA) clinical isolate from the Centre Hospitalier Universitaire of Charleroi (Belgium).

2.2. Plant material

The leaves of *A. unedo* (November 2008; voucher specimen BR 000 000 5333 905), the flowered aerial parts of *E. arborea* (April 2009; voucher specimen BR 000 000 5334 223), and *E. multiflora* (November 2008; voucher specimen BR 000 000 5334 551) were collected in the Region of Ait Guendouze in the Boukhlifa township (Bejaia, Algeria). They have been dried at room temperature (in the shade), then ground and sieved in order to obtain a fine powder (<125 μm). The three species were identified by a local botanist, and confirmed by Prof. J. Lejoly (Université Libre de Bruxelles, Belgium). Voucher specimens were deposited in the herbarium of the National Botanic Garden of Belgium (Meise, Belgium).

2.3. Extract preparation

The samples (5 g) were defatted with 15 mL of *n*-hexane, dried, and extracted by stirring at 130 rpm with 100 mL of methanol, at room temperature for 24 h, then filtered on cellulose (Soares et al., 2009). The filtrates were dried, reconstituted with methanol and stored at 4 °C until use.

2.4. Determination of total phenolics

The content of total phenolics was determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965). To 0.1 mL of the extract (1 g/L) or of gallic acid solution (29 mg/L), 2 mL of water and 0.5 mL of the Folin-Ciocalteu reagent were added. After 1 min, 1.5 mL of a saturated solution of sodium carbonate was added and the total volume adjusted to 10.0 mL with water. The solutions were mixed, allowed to stand (in the dark) for 2 h at room temperature. Then, the absorbance was measured at 760 nm *versus* a blank prepared without extract. The total phenolic content was expressed as milligram gallic acid equivalents per gram of dry weight (GAE/g of DW) (Bramorski et al., 2011).

2.5. Determination of flavonoids

The content in total flavonoids was determined by the $AlCl_3$ method of Lamaison and Carnet (1990). 1.0 mL of the extract (1 g/L, diluted by ½ for *E. arborea* and by 1/3 for *A. unedo*) or of quercetin (1.25–20 mg/L) was mixed with 1 mL of methanolic $AlCl_3$ (0.1 M), incubated in the dark for 10 min and measured at 455 nm *versus* a blank prepared without extract. The total flavonoids content was expressed as milligram quercetin equivalents per gram of dry weight (QE/g of DW).

2.6. Determination of the antioxidant activity

The antioxidant activity was measured by three *in vitro* methods, using free radicals DPPH$^\bullet$, ABTS$^{\bullet+}$ and electro-generated $O_2^{\bullet-}$ (anion superoxide radical).

2.6.1. Measurement of DPPH$^\bullet$ quenching

The hydrogen donating and/or radical-scavenging capacity of the samples was evaluated by their ability to scavenge the free radical DPPH$^\bullet$ according to Blanc et al. (2011). For each extract, five dilutions in methanol were prepared (1.93–9.67 mg/L). 2 mL of each dilution were added to 0.15 mL of a 10^{-3} M DPPH methanolic solution and maintained in the dark at room temperature for 1 h. The absorbance was measured at 517 nm *versus* a blank prepared without extract (Blois, 1958).

2.6.2. Measurement of ABTS$^{\bullet+}$ quenching

The ABTS$^+$ stock solution was prepared by dissolving 7 mM ABTS and 2.45 mM potassium persulfate and incubating for 12–16 h at room temperature in the dark. The working solution was obtained by diluting with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 10 μL of the extract (2–10 g/L) were added to 0.99 mL of diluted ABTS$^+$, incubated in the dark at room temperature for 30 min and the absorbance was measured at 734 nm *versus* a blank prepared without extract (Re et al., 1999).

2.6.3. Measurement of O_2^- quenching

2.6.3.1. Electrochemical material. Cyclic voltammetry experiments were performed on a dual potentio-galvanostat PGSTAT30 (Autolab instrument, Eco Chemie B.V., Utrecht, The Netherlands) driven by a GPES software (General Purpose Electrochemical System version 4.9, Eco Chemie B.V.). All measurements were carried out on a three- electrode thermostated cell. A glassy carbon disk working electrode (diameter 2 mm), a platinum wire counter electrode and a reference electrode, Ag/AgCl in EtOH saturated by LiCl, were used for all electrochemical experiments. The reference electrode was separated from the solution by a salt bridge containing 0.5 MBu_4NPF_6 in DMF. The glassy carbon disk working electrode was polished using silicon carbide 4000 paper with a LaboPol-5 (Struers, Ballerup, Denmark), washed with distilled water and then dried. For all measurements, the temperature was maintained at 21 ± 0.5 °C with a Julabo heating circulator MP-5 (Julabo, Seelbach, Germany).

2.6.3.2. Quenching of $O_2^{\bullet-}$. The methodology developed by Le Bourvellec et al. (2008) is based on the reaction kinetics of the antioxidant substrate with the superoxide anion radical ($O_2^{\bullet-}$). A cyclic voltammetry forward scan generates $O_2^{\bullet-}$ by reduction of molecular oxygen in an aprotic medium, *N*, *N*-dimethylformamide (DMF); the consumption of the radical O_2^- is directly measured at the backward scan by the anodic current decay from its oxidation in the presence of Ericaceae extracts.

A solution of 10 mL of an extra dry DMF ($[H_2O] \leq 0.01\%$), stored over molecular sieve (3 Å) containing the supporting electrolyte 0.1 M Bu_4NPF_6 was saturated by dry air during 10 min. In these conditions, the solubility of oxygen was assumed to be $C_{O_2} \approx 9.4 \times 10^{-4}$ mol/L, this value corresponding to a partial pressure of 0.2 bar at 293 k (Dapremont-Avignon et al., 1991). The cyclic voltammogramm (CV) of the oxygen reduction was then recorded at a scan rate 0.1 V/s, with the initial potential at 0 V and the reverse one at −1.3 *versus* Ag/AgCl. Stock solutions of the standard antioxidant or herbal extracts were prepared at about 4 g/L for *A. unedo* and *E. arborea* extracts and 6 g/L for *E. multiflora* extract. For each extract, aliquots of stock solution were successively added to 10 mL of oxygen solution in order to get an extract concentration in the range of 30–450 mg/L. After each aliquot addition, the CV plot of the oxygen solution was recorded at a scan rate 0.1 V/s. The measurement of the antioxidant activity is estimated by the antioxidant index values AI or AI_{50}, defined as the phenolic compound or extract concentration needed to consume, respectively, 30% or 50% of the electrogenerated radical [corresponding to $(Ip_a{}^0 - Ip_a{}^S)/Ip_a{}^0 = 0.3$ or 0.5 where $Ip_a{}^0$ is the intensity of the anodic current peak of O_2^- and $Ip_a{}^S$ the intensity of the anodic current peak of O_2^- for the concentration *S* of the sample]. With this characterization, the lower the AI_{30} or AI_{50} value, the more the substrate has a strong reactivity toward the superoxide.

2.7. Antibacterial assay

Each plant extract was dissolved in DMSO (80 g/L), then diluted to 5.0 mL with Mueller Hinton broth; on 96-well micro-plates, this solution was ½ serially diluted with the same broth, added with 0.1 mL/well of bacterial inoculum (10^6 bacteria/mL) and incubated at 37 °C for 24 h in ambient atmosphere. The MIC (the minimum inhibitory concentration) was defined as the lowest antimicrobial concentration that completely inhibited growth as detected by the naked eye (Brantner and Grein, 1994; Chérigo et al., 2009). The MBC (the minimal bactericidal concentration) was defined as the lowest concentration that yielded negative sub-cultures (Mandal et al., 2010; Okusa et al., 2007).

2.8. Synergy between plant extracts and antibiotics

The eventual synergy between plant extracts and antibiotics was evaluated on *S. aureus* C100459 and *P. aeruginosa* ATCC 9027 by a broth microdilution method according to Okusa et al. (2007). Compounds were placed into 96-wells culture plates to obtain mixtures covering a broad range of suboptimal concentrations of both compounds. The values of fractional inhibitory concentrations (FIC) and FIC index were determined to evaluate if the interaction extract/antibiotic is synergistic, antagonistic or indifferent. The FIC index was calculated according to the equation: FIC index = FICA + FICB = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone). The FIC index was evaluated as follows: synergy (FIC index ≤ 0.5), additive (0.5 < FIC index ≤ 1), indifference (1 < FIC index ≤ 2) and antagonism (FIC index > 2) (Jarrar et al., 2010; Mackay et al., 2000; Okusa et al., 2007).

2.9. TLC analysis

For both analyzes, TLC and HPLC, the extracts were analyzed before and after acid hydrolysis (with HCl 1.2 M, 100 °C, 1 h).

For TLC, the analysis was performed on Silicagel 60 F254 plates (Merck, Germany). The solvent system used was ethyl acetate, formic acid and water (90:6:6, v/v/v); plates were sprayed with a solution of aminoethanol diphenylborate (1% MeOH) then a solution of macrogol 400 (5% MeOH) and visualized under UV 366 nm (Wagner and Bladt, 1996).

2.10. HPLC–DAD–ESI-MS profiling of polyphenolic compounds

Major phenolic compounds were identified by comparing retention times, UV spectra and mass spectra of samples peaks with those of standards. LC–DAD analysis was carried out using a quaternary pump (TSP P4000), a diode array detector (TSP UV 6000), an online vacuum degasser, a column oven and an autosampler (TSP). The LC–DAD system was connected to a Finnigan LCQ DUO mass spectrometer *via* an electrospray ionization (ESI) interface using a post-column passive splitter. Chromatographic separations were achieved at a constant flow rate of 1.2 mL/min on an Altima C18 column (250 × 4.6 mm i.d.; 5 μm; Alltech, Deerfield, USA) maintained at 40 °C. A linear gradient elution was performed with mobile phase A (19% acetonitrile, 80% water, 1% formic acid) and mobile phase B (59% acetonitrile, 40% methanol, 1% formic acid) as follows: 0 min, 0% B; 5 min, 0% B; 15 min, 15% B; 20 min,15% B; 40 min, 60% B; 45 min, 100% B. By solvent splitting, 1 mL/min flow rate was delivered into the DAD detector and 0.2 mL/min into the mass spectrometer ionization source. DAD detection wavelengths were set at 280, 320 and 350 nm with peak scanning between 190 and 800 nm. Full scan mass spectra were registered in negative mode between 50 and 800/*z*. Chromatographic and mass spectrometry data were acquired and processed using the instrument built-in LCQ software.

2.11. Statistical analysis

All determinations were carried out in triplicate. Data were compared by a variance analysis (ANOVA) carried out with the software Statistica 5.5 and *P* values less than 0.05 were considered significant.

3. Results and discussion

3.1. Total phenolics content

Table 1 presents the total phenolics and flavonoids along with the antioxidant capacity of the three Ericaceae extracts. The *A. unedo* extract presents the highest concentration of total phenolic compounds, amounting to 179.6 ± 6.7 mg GAE/g; this is however, lower than previously reported in Portuguese samples (193 mg GAE/g, 329 mg GAE/g in ethanolic and acetonic extracts, respectively) (Andrade et al., 2009; Oliveira et al., 2009). Data from *Erica* species are difficult to compare as published studies express the total phenolics either in pyrogallol equivalents (PE) for *E. arborea* from Turkey (145 mg PE/g) (Ay et al., 2007), or in catechin equivalents (CE) for the same plant from Spain (249 mg CE/g) (Ammar et al., 2005); and for *E. multiflora* flowers from Morocco (105 mg CE/g) (Harnafi et al., 2007). In another study on the ethanol extract of the leaves of *E. arborea* (harvested in Morocco), the content of total polyphenols is about 78.49 ± 0.05 mg GAE/g DM (Amezouar et al., 2013).

3.2. Total flavonoids content

From Table 1, *A. unedo* leaves also contain the highest amount of flavonoids; samples from Croatia have previously been found to range from 5 to 20 mg QE/g (Maleš et al., 2006). From published data, the flavonoids content of *E. arborea* seems quite variable. In Turkish samples, Ay et al. (2007) reported 35 mg QE/g (methanolic extract); in Moroccan samples, Harnafi et al. (2007) reported 0.13 mg QE/g (methanolic extract) and Amezouar et al. (2013), 54 mg QE/g (ethanolic extract). The reasons for such differences are not clear. But as stated by Xia et al. (2014), the geographical and climatic conditions can lead to significant differences in both the concentrations of bioactive compounds in plants and their bioactivity for human health; they reported significant differences in total flavonoids in fern samples obtained from different regions.

3.3. Antioxidant activity

For DPPH and ABTS tests, the results were expressed in terms of IC_{50} value, the concentration of extract required to quench 50% of test radical (Andrade et al., 2009; Bougatef et al., 2009). As expected, an excellent correlation was observed between the DPPH and ABTS results ($r = 0.96$); this is due to the similarity of the two methods that measure the ability of antioxidants to donate an H atom (Huang et al., 2005). There were significant differences between the herbs for both assays.

The $O_2^{\bullet-}$ quenching activity is estimated through an electrochemical method that determines the antioxidant index values AI_{30} or AI_{50} (see Section 2.6.3). This recently developed electrochemical method has been applied successfully to seaweed extracts (Audibert et al., 2010; Blanc et al., 2011). Here, the method to determine the peak currents is different than in these previous works as the cyclic voltammograms were exploited by using convolution time semi-derivative transformation for the peak current measurements (Oldham and Spanier, 1973). The resulted convolution curves are much better resolved compared to the asymmetric voltammetric curves. As the baselines are simpler to define, the oxidation current before and after polyphenolic extract additions can be more easily measured (Fig. 1).

The values of AI_{30} and AI_{50} for each Ericaceae extract are consigned in Table 1. Although the number of species is low, a weak logarithmic relationship was observed between the measured antioxidant activities and the contents in total phenolics

Table 1
Content of total phenolic compounds, flavonoids, and antioxidant activity of Ericaceae species.

Sample	Total phenolic compounds (mg GAE/g DW)[a,b]	Total flavonoids (mg QE/g DW)[b,c]	Antioxidant activity		
			Quenching of DPPH• IC_{50} (mg/L)	Quenching of ABTS•+ IC_{50} (mg/L)	Quenching of $O_2^{\bullet-}$ AI_{50} AI_{30} (mg/L)
E. arborea flowering aerial parts	70.8 ± 2.5	9.5 ± 0.1	5.7 ± 0.08	6.8 ± 0.1	213 ± 7 115 ± 3
E. multiflora flowering aerial parts	68.2 ± 3.2	6.5 ± 0.3	10.2 ± 0.3	9.0 ± 0.1	261 ± 4 149 ± 6
A. unedo leaves	179.6 ± 6.7	21.4 ± 0.01	3.8 ± 0.2	4.2 ± 0.4	185 ± 5 96 ± 4

[a] GAE – gallic acid equivalents.
[b] DW – dry weight.
[c] QE – quercetin equivalents.

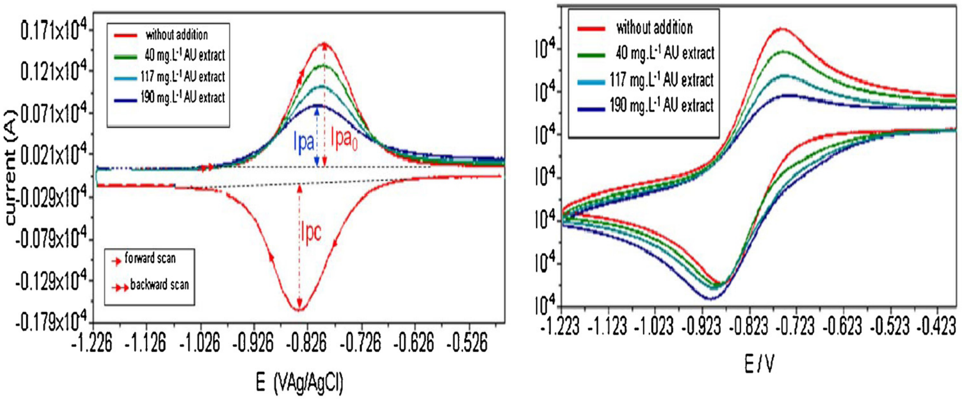

Fig. 1. Cyclic voltammograms of O_2 in absence and presence of increasing concentrations of *A. unedo* phenolic extract at a steady glassy carbon disk electrode in DMF/0.1 M Bu_4NPF_6. Scan rate 0.1 Vs^{-1}. (A) Time semi-derivative convoluted curves; (B) CV curves.

Table 2
The MICs (mg/L) of the different plant extract and control antibiotics.

Plant extract and antibiotics	*S. aureus* ATCC 6538	*S. aureus* C 100459 (MRSA)	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 25922
E. arborea flowering aerial parts	500	250	2000	>2000
E. multiflora flowering aerial parts	250	250	2000	>2000
A. unedo leaves	125	125	1000	1000
Cefotaxim	1	4	16	nd[a]
Penicillin	0.125	4	>64	64

[a] nd: not determined.

($R^2 = 0.57$, 0.82, 0.64, respectively; $n = 3$) and flavonoids ($R^2 = 0.82$, 0.98, 0.88, respectively; $n = 3$); the correlation was somewhat better for flavonoids, indicating that the quality of polyphenols/flavonoids in a herb is certainly more important than their content. This is in line with correlation data "DPPH scavenging *versus* total flavonoids" previously obtained on Chinese fern samples (Xia et al., 2014). Vasco et al. (2008) indicate that the correlation effectively depends on the extraction solvent, the hydrophilicity of the compounds, the sample, and the type of phenolic compound. By evidence, not all antioxidant characteristics are assessed by the tests performed here; notably, the ability to quench *in vivo* oxidative damage and lipid peroxidation largely depends on the lipophilicity of the compounds (phenols, tocopherols, carotenoids, flavonoid aglycones) and the chelation of metals (ascorbic acid, tannins, flavonoid aglycones and glycosides) (Bramorski et al., 2011). As for polyphenols and flavonoids content, the scarce data from the literature appear quite difficult to compare. For Moroccan *E. arborea*, Amezouar et al. (2013), with a much higher total flavonoids content, measured a lower DPPH radical scavenging potential ($IC_{50} = 10$ mg/L for a total flavonoids content of 54 mg QE/g), compared to the present study (5.7 mg/L for a total flavonoids content of 9.5 mg QE/g); for Portuguese *A. unedo*, Mendes et al. (2011) report a much lower DPPH radical scavenging potential ($IC_{50} = 87$ mg/L), compared to the present study (3.8 mg/L). Such apparently discording data should be more closely investigated and, indeed the qualitative and quantitative differences in polyphenol profiles should be correlated with their antioxidant power. The structure of polyphenols is certainly the most important parameter, with structural features strongly conditioning the redox power (Öztürk et al., 2007; Williams et al., 2004); the presence of 2, 3 unsaturation in conjugation with a 4-oxo- function in the C-ring and the presence of functional groups capable of binding transition metal ions indicate the possibilities of oxidation to quinoid forms and consequent high reduction power. Given the complexity of polyphenols and flavonoids profiles in the three species, only the coupling of antioxidant and metabolomics studies of samples harvested in different locations will be able to sort out the qualitative and quantitative features most important for biological activity (Hernández et al., 2009). Although studies are being carried out, for example on *Acacia* species (Abdel-Farid et al., 2014) or on tomato (Bovy et al., 2007), a practical method for correlating profiles with antioxidant capacity is not yet available.

3.4. *Antibacterial activity*

Tables 2 and 3 detail the antibacterial effects of the three tested herbs. According to Okusa et al. (2007), extracts displaying a MIC below 500 mg/L are considered worthy of further investigation; from 500 to 1000 mg/L, the antimicrobial activity is judged weak and, over 1000 mg/L, the extract is considered inactive. All these extracts have an interesting activity against Gram positive bacteria but not against Gram negative bacteria which are well known for their higher resistance, related to lipopolysaccharides in their outer membrane (Murray et al., 2009). Table 3 shows that the effects observed on Gram-positive bacteria are rather bactericidal (MBCs within a two-fold dilution of the MICs) than bacteriostatic

Table 3
The MBCs (mg/L) of the different plant extracts.

Plant extract	*S. aureus* ATCC 6538	*S. aureus* C 100459 (MRSA)	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 25922
E. arborea flowering aerial parts	500 (bactericidal)	1000 (bactericidal)	2000	nd
E. multiflora flowering aerial parts	1000 (bactericidal)	500 (bactericidal)	2000	>2000
A. unedo leaves	250 (bactericidal)	500 (bactericidal)	2000	>2000

Table 4
Interaction between plant extracts and antibiotics.

Antibiotic-plant extract	*S. aureus* C 100459 (MRSA)		*P. aeruginosa* ATCC 9027	
	FIC index	Interaction	FIC index	Interaction
Cefo/*E. arborea*[a]	1	Additive	1.125	Indifference
Cefo/*E. multiflora*	1	Additive	1.5	Indiferrence
Cefo/*A. unedo*	1.5	Indifference	1.25	Indifference
Strep/*E. arborea*	1	Additive	nd	nd
Strep/*E. multiflora*	1	Additive	nd	nd
Strep/*A. unedo*	1.5	Indifference	nd	nd

[a] Cefo: cefotaxim; Strep: streptomycin.

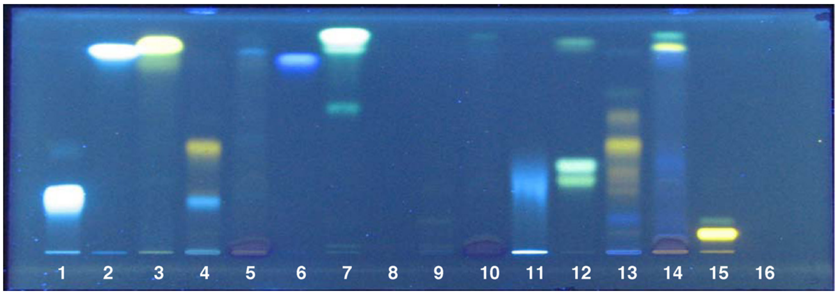

Fig. 2. TLC profiles of the three Algerian Ericaceae (1: chlorogenic acid, 2: caffeic acid, 3: quercetin, 4: *E. arborea*, 5: hydrolysed *E. arborea*, 6: gallic acid, 7: kaempferol, 8: arbutin, 9: *E. multiflora*, 10: hydrolysed *E. multiflora*, 11: ellagic acid, 12: 7-glucosid apigenin, 13: *A. unedo*, 14: hydrolysed *A. unedo*, 15: rutin, 16: epicatechin). The analysis was performed on Silicagel 60 F254 plates. The solvent system used was ethyl acetate, formic acid and water (90:6:6, v/v/v); plates were sprayed with a solution of aminoethanol diphenylborate (1% MeOH) then a solution of macrogol 400 (5% MeOH) and visualized under UV 366 nm. The extracts were analyzed before and after acid hydrolysis (with HCl 1.2 M, 100 °C, 1 h).

(MBCs values at least within an eight-fold dilution of the MICs). *E. multiflora* has already been reported for weak antibacterial effect against *S. aureus* with a MIC of 1000 mg/L (Rios et al., 1987) and this activity was attributed to phenolic compounds (flavonoids and phenolic acids) and sesquiterpene lactones; the latter phytochemicals class is infrequent in Ericaceae that are rather known for their richness in polyphenols. Polyphenols and tannins possess a strong binding ability to different molecular structures like proteins or glycoproteins (Wagner and Ulrich-Merzenich, 2009). They may bind to bacterial adhesins and, by doing so; disturb the exposition of receptors on the cell surface.

Havsteen (2002) has noted that many of the bacterial strains commonly encountered by humans are killed by flavonoids. The bactericidal effect of the flavonoids may well be the result of a metabolic perturbation. Ion channels, which are components of both bacterial and animal cells, are especially sensitive points of inhibition and likely targets of flavonoids.

Arbutoside, a hydroquinone glycoside, has been reported in *E. arborea* (Ay et al., 2007) and *A. unedo* (Fiorentino et al., 2007); its hydrolysis by bacteria and spontaneous oxidation to benzoquinone may account for observed antimicrobial effects. Follow-up purification studies are however, required to determine which compounds exactly may be responsible for the observed antibacterial activities.

3.5. Interaction between plant extracts and antibiotics

S. aureus C100459 and *P. aeruginosa* ATCC 9027 were used to test the effect of combination of plant extracts with two antibiotics (cefotaxim and streptomycin). Table 4 indicates that *E. arborea* and *E. multiflora* extracts were additive with cefotaxim and streptomycin (FIC index = 1) against *S. aureus* only. The *A. unedo* extract showed indifference effects against all the tested microorganisms/antibiotics combinations. Hatano et al. (2005) report the antibacterial effects of various plant phenolics, including flavonoids and tannins, some flavonoids and xanthones being effective against MRSA. These phenolics act either directly or by restoring the antibacterial effects of antibiotics.

3.6. TLC and HPLC–DAD-ESI-MS analysis

The polyphenolic composition of the polar extracts from the three Ericaceae species was investigated using TLC (Fig. 2) and HPLC–DAD-ESI-MS (Table 5). The major polyphenols of *E. multiflora* (Fig. 3) are reported here for the first time.

Márquez-García et al. (2009) reported the following phenolic compounds in *E. arborea* exposed to different degrees of metal pollution in soils: ellagic acid, vanillic acid, cinnamic acid derivate, *m*-coumaric acid, caffeic acid and its derivate, 2 *p*-coumaric acid derivate, catechin and 8 of its derivates, epicatechin, rutin and 4 of its derivates, kaempferol and myricetin. In this species were

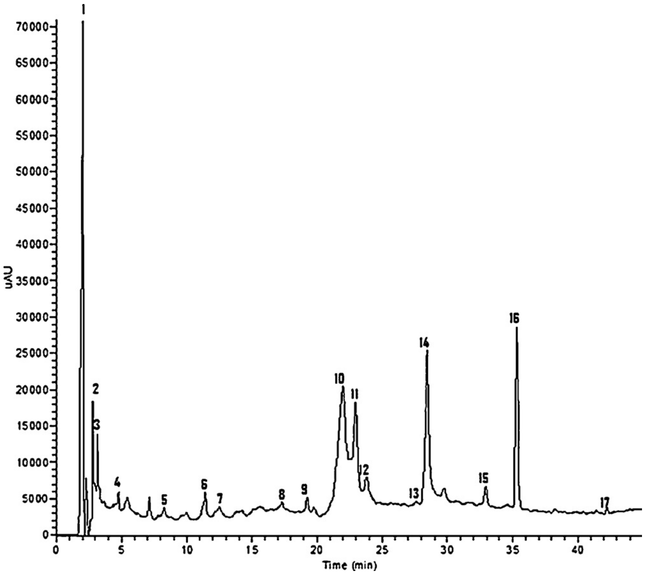

Fig. 3. Total ion chromatogram of the *E. multiflora* methanolic extract (6: naringin, 10: quercetin and 14: kaempferol).

Table 5
Major polyphenols identified in the three Algerian Ericaceae.

Compounds	Tr (min)	*m*/*z*	Species
Epicatechin	4.28	289.10	*A. unedo*
Caffeic acid	4.95	179.00	*E. arborea*, *E. multiflora*, *A. unedo*
p-Coumaric acid	8.33	163.18	*E. arborea*, *E. multiflora*, *A. unedo*
Naringin	11.47	507.50	*E. multiflora*
Quercetin	21.74	301,10	*E. arborea*, *E. multiflora*, *A. unedo*
t-Cinnamic acid	15.70	147.00	*E. arborea*, *A .unedo*
Kaempferol[a]	28.42	285.00	*E. multiflora*, *A. unedo*

[a] This peak was identified as kaempferol after acidic hydrolysis of the extract; the structure of the glycoside has not been determined.

also reported epicatechin, quercetin, arbutoside, tannins (Ay et al., 2007), proanthocyanidols, and coumarins (Ait Youssef, 2006).

Regarding *E. multiflora*, Akkol et al. (2007) and Harnafi et al. (2007), have simply noted that this species contains flavonoids, tannins, proanthocyanidins and coumarins. Ozcan and Haciseferogullan (2007) and Pallauf et al. (2008) have noted that *A. unedo*, contains gallic, ellagic and *p*-hydroxybenzoic acids. According to Maleš et al. (2006), it also contains vanillic, syringic and chlorogenic acids. This plant is also reported to contain some flavonoids, including arbutoflavonol A and B, afzelin, juglanin, avicularin, quercetin, isoquercetin, hyperoside and anthocyanosides (Fiorentino et al., 2007; Maleš et al., 2006), tannins, notably proanthocyanidins, ethyl gallate and catechin (Ayaz et al., 2000; Fiorentino et al., 2007; Maleš et al., 2006). Arbutoside, a phenolic glycoside, is also described in *A. unedo* (Fiorentino et al., 2007; Garnier et al., 1961; Valnet, 1992).

4. Conclusion

Although the polar extract of *A. unedo* leaves presents sensibly higher polyphenols and flavonoids levels and lower IC_{50} than the two *Erica* species investigated, all the three species present relatively potent antibacterial and antioxidant activities that could explain their use for the treatment of scalds and wounds in traditional Algerian medicine. The profiling of phenolic acids and flavonoids by TLC and HPLC–DAD-MS has allowed identifying some of the major polyphenols but should be pursued to obtain a better comprehension of polyphenolic compounds distribution in Ericaceae.

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