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The economic potential of beach-cast seagrass – *Cymodocea nodosa*: a promising renewable source of chicoric acid

Abstract: Detrital and fresh specimens of the seagrass *Cymodocea nodosa* (Cymodoceaceae) from the Atlantic Ocean and Mediterranean Sea were screened for their phenolic content. For the first time, the major polyphenol was identified as chicoric acid (CA) by means of nuclear magnetic resonance, ultraviolet spectra, liquid chromatography/mass spectrometry, and comparison with standards. The CA content of the crude extracts was determined by quantitative high-performance liquid chromatography. The values found in the leaves ranged from 8.13 to 27.44 mg g⁻¹ dw. The concentration was smaller in the rhizomes, with 0.31–3.70 mg g⁻¹ dw. The phenolic profile was found to be similar whatever the geographical location in the Atlantic Ocean or the Mediterranean Sea. Considering the demonstrated therapeutic applications of CA, its high value on the nutraceutical market, and its rare occurrence in the plant kingdom, the high content found in *C. nodosa* detrital leaves makes this abundant biomass of interest for dietary and pharmaceutical applications.

Keywords: chicoric acid; *Cymodocea nodosa*; detritus; nutraceutical value; quantitative HPLC.

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Introduction

Plant secondary metabolites are economically important in the field of food additives, nutraceuticals, and drugs. This is especially the case for phenolic acids, which possess a broad spectrum of pharmacological properties. As a result, phenolic acids have been recommended as major bioactive compounds for preventing chronic diseases and promoting health (see, e.g., Peterson and Simmonds 2003, Jiang et al. 2005).

In the nutraceutical and drug segments, interest in herbal alternatives to synthetic products is increasing globally, driven by consumer demographics and health concerns (Siró et al. 2008). Phenolic acids are generally obtained from terrestrial plants, e.g., fruits, vegetables, and various medicinal plants, in which they occur in different concentrations and play several important functions (antibacterial, anticariogenic; Ferrazzano et al. 2011). However, the identification of novel sources of phenolic acids has become of scientific and economic interest, and marine ecosystems can offer a great potential in this field (e.g., Guinea et al. 2012).

Compared with other phenolic acids, chicoric acid (CA; Figure 1) is a rare and valuable natural product of special interest owing to its large spectrum of biological properties, and its success on the nutraceutical market. It has been shown to have immunostimulatory properties, promoting phagocyte activity *in vitro* and *in vivo* (Bauer et al. 1989), and to inhibit hyaluronidase, a key enzyme involved in bacterial infection (Bauer 1998). In addition, CA has antiviral activity (Pellati et al. 2004) and has been reported to inhibit HIV integrase and replication (King et al. 1999, Lin et al. 1999, Charvat et al. 2006, Liu et al. 2006). The activity of CA against herpes simplex virus has been demonstrated (Binns et al. 2002). The antioxidant activity of CA was found to be comparable with that of rosmarinic acid (Dalby-Brown et al. 2005).

CA is commercially available through extraction from the terrestrial angiosperm *Echinacea* (Asteraceae), but remains relatively expensive. The roots are used in dietary supplements, which are currently one of the best-selling herbs in North America, and have gained great attention owing to their increasing economic value (Liu et al. 2006). CA was found to be the main phenolic compound in *Echinacea purpurea*; however, it does not occur in appreciable amounts in the other species such as *E. pallida* and *E. angustifolia* (Pellati et al. 2004). The identification of any novel sources of CA is of economic interest.

The oceans are a potential source for a wide variety of non-drug nutritional natural products. Several species

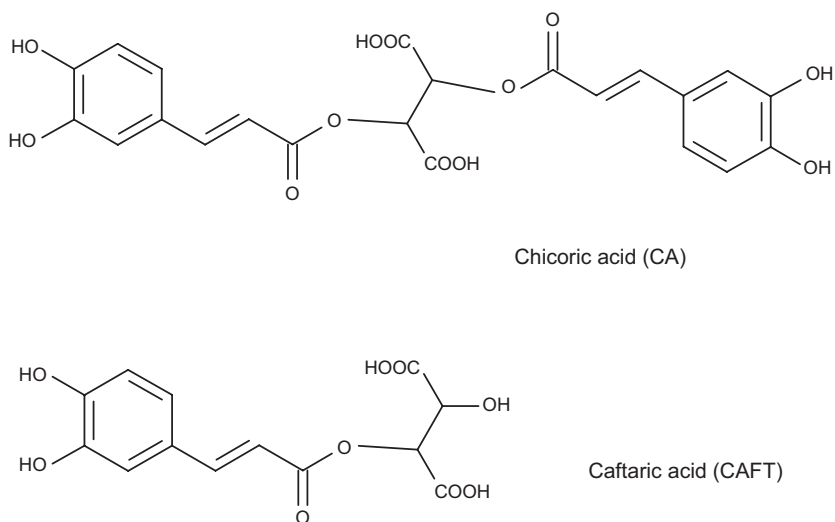


Figure 1 *Cymodocea nodosa*: structural formulae of CA and CAFT.

of seaweeds are used as human food or as raw material for the production of compounds of nutritional interest (Cardozo et al. 2007). Compared with algae, seagrasses remain very little exploited despite the tremendous opportunities they offer to find new commercially valuable phytochemicals (Achamlale et al. 2009, Nuissier et al. 2010).

Seagrass beds generate considerable standing biomass. Storms and autumn leaf drop result in large accumulations of detritus along shorelines, which could be exploited as raw material. Local managers are under great public pressure to remove this material whenever it accumulates on beaches and shorelines used for recreational purposes. In most cases, the collected biomass is disposed of in waste disposal sites; however, according to the European Community guidelines on environmental protection, land filling is no longer possible without prior treatment. It has therefore become necessary to recycle the seagrass detritus.

We have recently reported about the isolation of CA from the dead biomass of the tropical seagrass *Syringodium filiforme*, which belongs to the family Cymodoceaceae (Nuissier et al. 2010). Considering the high value properties of CA, it appears of interest to investigate other members of this family growing in temperate seas. We report here our results related to *Cymodocea nodosa*, which is found throughout the Mediterranean Sea and extends into the Atlantic Ocean, along the coast from Portugal to Senegal, and around the Canary, Madeira, and Cape Verde Islands (Afonso-Carrillo and Gil-Rodríguez 1980, Pavon-Salas et al. 2000, Green and Short 2003).

The presence in *C. nodosa* of soluble sugars, polyamines, steroids, and diarylheptanoids is well documented (Drew 1983, Kontiza et al. 2008 and references therein); however, little consideration has been given to the phenolic content of *C. nodosa*, and detrital plant material was never studied despite the huge amount available. Only low amounts of quercetin and isorhamnetin derivatives were previously isolated from *C. nodosa* (Cariello et al. 1979). *Cymodocea nodosa* is one of the most important macrophytes in the Mediterranean Sea, with a relatively fast growth rate and a high turnover (Perez and Romero 1994). Large amounts of *C. nodosa* detritus often accumulate on beaches (e.g., Mateo 2010) and, for this reason, determining its chemical composition is of practical and ecological interest. Seagrasses have been shown to be more resistant to decomposition than are algae or freshwater angiosperms (Godshalk and Wetzel 1978, Harrison 1989), and phenolic compounds remain (Achamlale et al. 2009). This feature could justify significant exploitation of such a low-cost, underutilized, but very abundant renewable resource. Harvesting it in a reasonable manner, concentrating on areas where it is a nuisance to other economic activities, could be of benefit to all.

Considering the economic potential of phenolic acids within the pharmaceutical, cosmetic, and food industries, it appears of interest to reconsider the content of *C. nodosa*. This work reports the first quantitative characterization of polyphenols in detritus collected in Gran Canaria (Atlantic Ocean) and different locations of the Mediterranean coastline, with a view to exploiting the flotsam, otherwise deposited in landfill, as a new source of phytochemicals.

Materials and methods

Sample collection

Dead biomass of *Cymodocea nodosa* (Ucria) Ascherson (Cymodoceaceae, common name: lesser Neptune grass) was collected from piles in the intertidal zone at four field sites: San Agustín, Gran Canaria, Canary Islands (three seasonal collections); Cadiz Bay, Spain; Zeytineli, Turkey; and Sahline Sebkha beach, Tunisia. In addition, a living sample was collected in the western Mediterranean at Alfacs Bay, Spain. Collection sites and dates are listed in Table 1.

After collection, the samples were thoroughly rinsed in seawater, and then quickly washed in freshwater to remove sand and salt. The collected material was hand-picked to remove associated debris. Then, plant material was air-dried at room temperature to constant weight. The moisture content of the dried material was <1%. When both were present, leaves and rhizomes were processed separately. They were manually ground using a mortar and pestle immediately before extraction.

General extraction methods

The solvents used for extractions were all high-performance liquid chromatography (HPLC) grade. Standards were purchased from Extrasynthèse (Genay, France), TFA from Aldrich Chemical Company (Saint-Louis, Missouri, USA), and analytical-grade water from Sodipro Company (Echirolles, France). Nuclear magnetic resonance (NMR) spectra were recorded on an AVANCE 300 MHz, Bruker, Jasco (Easton, USA) in DMSO, D₂O, or CD₃OD (Euriso-Top, Gif-Sur-Yvette, France). Ultraviolet (UV) spectra were recorded on a V-630 UV-VIS spectrophotometer (Jasco) in HPLC-grade water. Authentic samples of CA and caftaric acid (CAFT) were given by Eburon Organics (B-2310 Rijkvorsel, Belgium). The standards, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside,

and isorhamnetin-3-*O*-glucoside, were from Extrasynthèse. Other compounds for which no standards were available, such as coumarate and tartaric acid, were assigned by HPLC-diode array detection (HPLC-DAD), liquid chromatography-mass spectrometry (LC-MS) [electrospray ionization (ESI)], and comparison with the literature (Maier et al. 2006). Chlorogenic acid (Extrasynthèse) was used for the HPLC quantification of the phenolic content.

Preparation of extracts

To determine the best extraction parameters for assessing the maximum yield of CA from *Cymodocea nodosa*, different sequential extractive procedures were tested with leaves from sample D-1 (Table 2): extraction by MeOH then 50% aqueous MeOH, followed by water at room temperature (A) or reflux (B), twice soaking at room temperature in 50% aqueous methanol (C) or in water (D). The extraction yields and CA content of the extracts are reported in Table 2. The optimal conditions for extraction of CA were obtained with procedure C, which was used for all the other samples (Table 2, Figure 2).

Selected typical procedure C

Dried ground leaves or rhizomes (10 g) were extracted at room temperature with aqueous methanol (50:50; 120 ml, 24 h). The process was repeated, and then the extracts were pooled, evaporated to dryness, and analyzed by NMR and HPLC. Yields are given as percentage of the seagrass dry weight (Table 3).

Isolation of CA from a crude extract

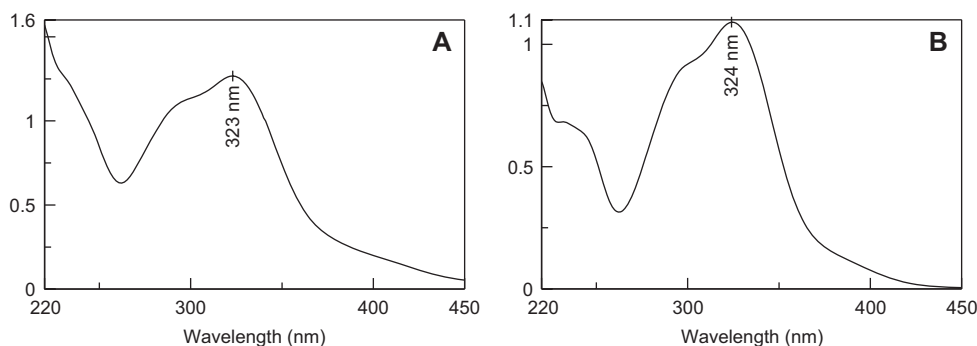
Crude aqueous methanolic extract obtained from batch D-1 was suspended in water and defatted in a separating

Table 1 *Cymodocea nodosa*: sites and dates of collection.

Collection place	Date	Batch code	Material collected
Gran Canaria (27°46 N, 15°32 W)	14/07/2007	D-1	Detrital (leaves and rhizomes)
Gran Canaria (27°46 N, 15°32 W)	30/11/2007	D-2	Detrital (leaves and rhizomes)
Gran Canaria (27°46 N, 15°32 W)	10/04/2008	D-3	Detrital (rhizomes)
Cadiz Bay (36°23 N, 6°10 W)	10/05/2008	D-4	Detrital (leaves and rhizomes)
Sahline Sebkha beach (35°46 N, 10°43 E)	18/07/08	D-5	Detrital (leaves and rhizomes)
Zeytineli (36°12 N, 26°24 E)	28/07/08	D-6	Detrital (leaves and rhizomes)
Alfacs Bay (40°43N, 0°52 E)	21/05/2008	F-7	Fresh (leaves and rhizomes)

Table 2 *Cymodocea nodosa*: influence of extraction protocols on yields (% dw) and CA content (mg g⁻¹ dw) for leaves from batch D-1 (Gran Canaria, July collection).

	Step 1		Step 2			Step 3			Global yield	Global CA content	
	Extraction conditions	Yield	CA content	Extraction conditions	Yield	CA content	Extraction conditions	Yield			CA content
A	MeOH, rt	10.4	11.79	MeOH/water 50:50, rt	10.9	12.76	Water, rt	2.6	0.58	23.9	25.13
B	MeOH, reflux	11	2.99	MeOH/water 50:50, reflux	8.5	8.44	Water, rt	2	1.04	21.5	12.47
C	MeOH/water 50:50, rt	14	15.73	MeOH/water 50:50, rt	9.5	11.71	–	–	–	23.5	27.44
D	Water, rt	16	11.90	Water rt	5.3	2.94	–	–	–	21.3	14.84

**Figure 2** *Cymodocea nodosa*: UV absorption spectra (water) of (A) crude extract obtained from detrital leaves (sample D-1) and (B) sample of authentic CA.

funnel with chloroform then ethyl acetate. The resulting aqueous solution was extracted three times with n-butanol. The combined butanolic extracts were evaporated to dryness leading to a creamy powder, which was flash-chromatographed on a silica gel column using ethyl acetate-MeOH (50:50 v/v) as eluant. CA was isolated as a cream powder. Its identity was evident from its ¹H and ¹³C NMR spectra recorded in MeOD, which were identical with data obtained in the same conditions for the standard (Figure 3). The $[\alpha]_D^{25}$ value $[-343^\circ (c 1, CH_3OH)_{D25}]$ indicates

that CA obtained from *Cymodocea nodosa* leaves is the laevorotatory antipode with the (2*R*, 3*R*) configuration.

HPLC analysis

Separation and quantification of phenolics in the crude extracts were performed using HPLC, consisting of an LC system (Thermo Electron) equipped with a SCM 1000 solvent degasser, a thermostatically controlled column

Table 3 *Cymodocea nodosa*: extraction yields and amounts of CA and CAFT from different samples using extraction protocol C (Table 2).

Collection site	Batch ¹	Extraction yields (% dw)		Phenolic content (mg g ⁻¹ dw)			
		L ¹	R ¹	CA		CAFT	
				L ¹	R ¹	L ¹	R ¹
Gran Canaria	D-1	23.5	–	27.441±0.425	–	0.909±0.018	–
	D-2	16.6	45.6	8.132±0.191	0.311±0.061	0.287±0.011	0.029±0.006
	D-3	–	43.5	–	0.342±0.059	–	0.042±0.002
Cadiz Bay	D-4	29.6	32.2	17.936±0.213	0.896±0.019	0.317±0.009	0.896±0.016
Izmir Bay	D-5	31	48	12.110±0.221	1.979±0.043	0.202±0.007	Traces
Monastir	D-6	35.6	46.1	17.661±0.242	3.698±0.083	0.501±0.013	0.896±0.011
Alfacs Bay	F-7	30	51	18.524±0.254	3.190±0.065	2.922±0.062	0.615±0.017

Values are means±SD of three replicates. ¹D, detrital material collected on the beach; F, fresh material collected in the seagrass bed; L, leaves; R, rhizomes.

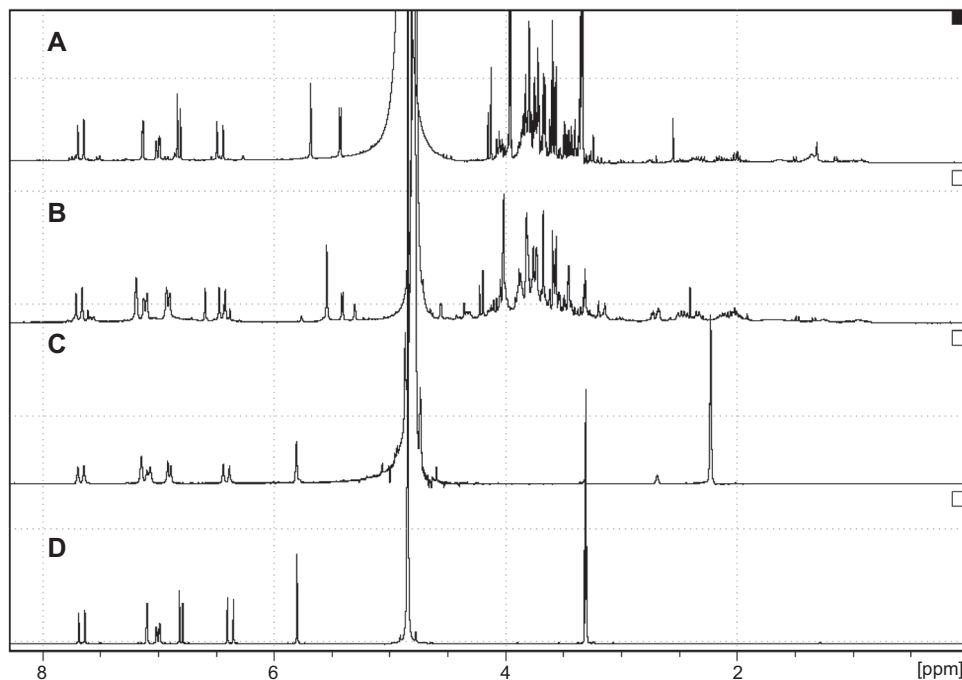


Figure 3 ^1H NMR spectra.

(A) Crude aqueous-methanolic extract obtained from D-1 (MeOD- D_2O); (B) crude aqueous extract from D-1 (D_2O , stars indicate the CAFT signals); (C) CA purified from the aqueous-methanolic extract obtained from D-1 (MeOD- D_2O); (D) sample of authentic CA (MeOD- D_2O).

compartment, an AS 3000 autosampler with a 100- μl loop, a PDA UV6000LP detector, and a Chromquest Chromatography Workstation. Separations were carried out at 40°C on a Hypersil GOLD C8 column (Thermo Finnigan, Villebon-sur-Yvette, France), 175-Å pore size, 5- μm particle size, 250 \times 4.6 mm i.d. column. The analytes were eluted at a flow rate of 1 ml min^{-1} using the binary gradient 0.1% (v/v) TFA in water (A) and methanol (B). The following linear gradient was used: 0 min, 1% B; 60 min, 99% B. Run time was 60 min, stop time 60 min, and post time 10 min. UV spectra were collected over the range of 220–400 nm, and the chromatograms were recorded at 270, 328, and 350 nm with a resolution of 1 nm and no smoothing. In addition, the data were processed to create a chromatogram in which each chromatographic peak represents absorbance of the eluting substance at its λ_{max} (max-plot chromatogram). The injection volume was 20 μl . The data were integrated using the Chromquest automated software system. Stock solutions of the dried extracts were prepared in H_2O at a concentration of 0.5 mg ml^{-1} . All solutions were filtered before analysis through a 0.2- μm syringe filter and injected three times into the HPLC. The products present in the sample were identified by matching the retention time and spectra of standards.

External standard calibration with chlorogenic acid dissolved in ethanol/water (70:30) with the aid of

sonication was established on six data points, covering the concentration range 0.0619–0.00619 mg ml^{-1} . Linear regression on the HPLC analyses gave R^2 values of 0.9994.

Chromatographic peaks were checked for peak purity, and identification was achieved by comparing retention times and UV spectra with those of standards. Quantitative determinations were carried out by peak area measurements at 328 nm, using the calibration curve of chlorogenic acid at the same wavelength and the correction factors, which take into account the differences in the responses of the HPLC detector to CA, CAFT, and chlorogenic acid. These factors have been determined by the Institute for Nutraceutical Advancement (INA) in the frame of the INA Methods Validation Program (INA Method 106.000 2000). The data presented in Table 3 are the average from three experiments, calculated using the following equation and correction factors:

$$\% \text{ W/W individual phenol compound} = (\text{C} \times \text{F} \times 100) / \text{C}_s$$

where C is the concentration of the tested phenolic compound (mg ml^{-1}) in the analyzed extract, calculated as chlorogenic acid from peak areas and linear regression; F is the correction factor of phenolic response against chlorogenic acid ($F=0.888$ for CAFT and 0.695 for CA); and C_s is the concentration of the sample (mg ml^{-1}), diluted in deionized water for analysis.

Data are expressed in milligrams per gram of dry matter of *Cymodocea nodosa* [mg g⁻¹ dw; mean±standard deviation (SD) of three determinations; Table 3].

LC-MS analysis

LC-MS was performed using an HP1100 (Hewlett-Packard, Palo-Alto, CA, USA) equipped with an Agilent MSD 1946B simple quad mass spectrometer and HP Chemstation software. Positive mode ESI spectra of the column eluate were recorded in the range of *m/z* 100–1000 a.m.u. Absorbance was measured at 280 and 320 nm. Compounds were separated using an MN Nucleodur C18 column (Macherey-Nagel, Düren, Germany): 125 mm×2 mm i.d., 3- μ m particle size. The analytes were eluted at a flow rate of 0.3 ml min⁻¹ using the binary gradient (v/v) formic acid in water (pH 2.55, A) and methanol (B). The following linear gradient was used: 15% B to 100% B (15 min). Separation of the analytes was carried out at 50°C. The injection volume was 2 μ l. For MS analysis, compounds were detected using the following conditions: nebulizing gas pressure, 60 psi; drying gas flow rate, 12 l min⁻¹; drying gas temperature, 350°C; capillary voltage, 4000 V; temperature source,

350°C. Data were acquired in full scan mode (*m/z* 100–1000) at a fragmentor voltage of 70 V.

Results

To ensure extraction of all the phenolics whatever their polarity, different sequential extractive procedures were tested with sample D-1-L (Table 2). Global extraction yields were found quite similar whatever the extraction method (21.3–23.9%, Table 2). However, examination of the yields and CA content for each step showed differences in the extraction efficiency (Table 2). The third extraction by water in protocols A and B appeared useless (step 3: low yields and low CA content). Comparison of protocols A and B shows the dramatic effect of heating on the CA content: 25.13 mg at room temperature versus 12.47 mg at reflux of the solvent. In addition, the third step appeared ineffective (low yield and low CA content). Water alone (protocol D) was found less efficient than aqueous methanol 50:50 (14.84 and 27.44 mg, respectively). From these results, protocol C was selected for the comparative evaluation of the different samples. Comparison of the UV spectrum of the

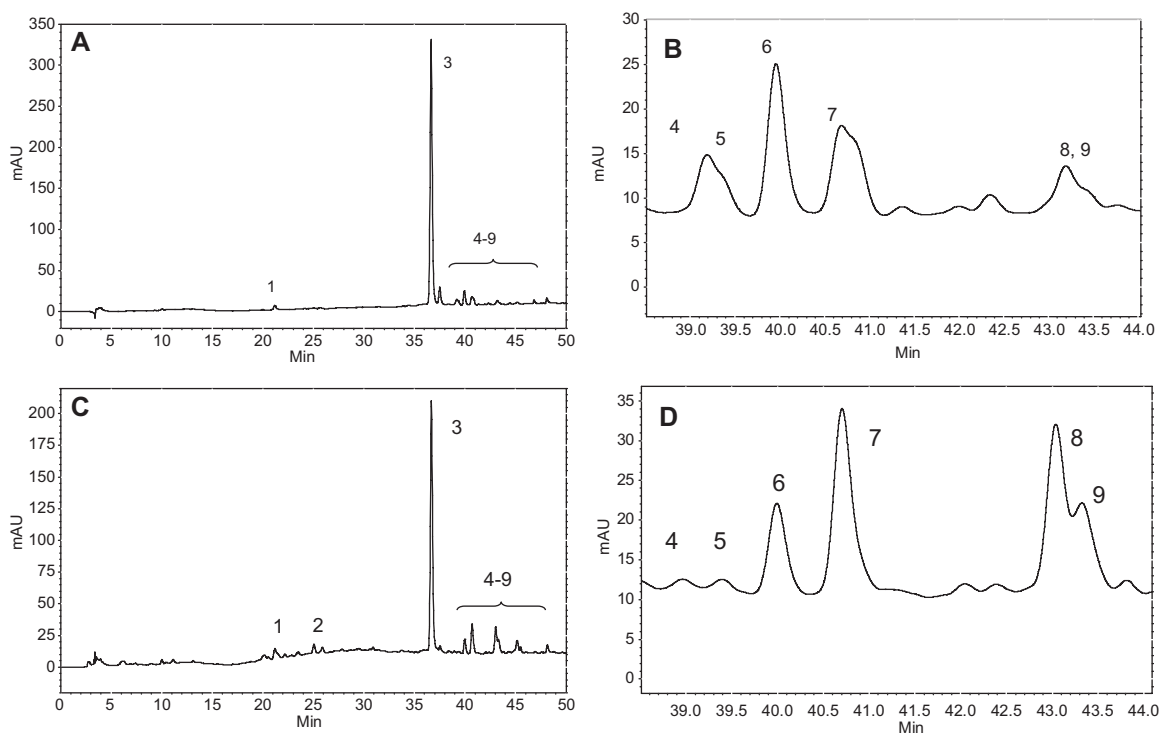


Figure 4 *Cymodocea nodosa*.

HPLC profiles of crude extracts obtained from sample D-5: (A, B) leaves and (C, D) rhizomes. Retention times (Rt, min), assignment: (1) 21.6, CAFT; (2) 25.6, coumaric acid; (3) 36.7, CA; (4) 39.2, rutin; (5) 39.3, quercetin-3-*O*-glucoside; (6) 40.0, di-coumaroyl-tartaric acid; (7) 40.6, unidentified caffeate; (8) 43.1, isorhamnetin-3-*O*-rutinoside; (9) 43.4, isorhamnetin-3-*O*-glucoside.

crude extract obtained from D-1-L using protocol C with those recorded for the authentic sample of CA (Figure 2) clearly shows the efficiency of the extraction process and the high CA content of this sample.

All the extracts were systematically analyzed by NMR and quantitative HPLC, which both gave a clear indication of their metabolic content (Figures 3 and 4). The NMR spectra of the aqueous-methanolic crude extracts (Figure 3A) showed a well-defined typical pattern of caffeate moiety and singlet at 5.45 ppm, in good accordance with CA as the major phenolic (comparison with authentic sample, Figure 3D). The presence of a minor product with a *trans*-caffeoyl unit and two broad singlet protons at 4.45 and 5.22 ppm, suggesting a tartaric acid moiety, was also observed in the ^1H NMR spectrum of the aqueous extracts obtained from sample D-1-L when using protocol D (Figure 3B). This pattern is in good agreement with data reported in the literature for *trans*-CAFT (Lu and Yeap Foo 1999, Maier et al. 2006), and confirmed by LC/MS.

HPLC analysis confirmed the large predominance of CA in all the extracts (Table 3, Figure 4). All the extracts prepared from living or detrital leaves showed a phenolic profile largely dominated by CA, which was eluted at 36.7 min. Small amounts of CAFT were found in all extracts at 21.6 min. This assignment is supported by a typical caffeate UV spectrum, in good agreement with CAFT and CA (online λ_{max} 328 and 330 nm, respectively). In addition, LC-DAD–ESI-MS analyses were performed using peak mass and UV spectra. Results confirmed the structure assignments, i.e., CAFT (EISMS m/z : 335 $[\text{M}+23]^+$; main product ion at $m/z=163$ for the first eluted caffeate) and CA (EISMS m/z : 497 $[\text{M}+23]^+$; main product ion at $m/z=163$ for the second).

Depending on the extracts, variable amounts of other minor peaks were also detected (Figure 4). On the basis of LC/MS and comparison with standards and the literature (Maier et al. 2006), they were assigned to coumaric acid, rutin, quercetin-3-*O*-glucoside, di-coumaroyl-tartaric acid, isorhamnetin-3-*O*-rutinoside, and isorhamnetin-3-*O*-glucoside. Altogether, these minor flavonoids and hydroxycinnamates of tartaric acid represented 9–11% of the total phenolics detected in the leaves, and up to 30% in the rhizomes.

The HPLC profile was the same for detritus and fresh specimens, regardless of collection site.

Quantification of CA and CAFT by HPLC

Whatever the collection site, CA was found to be the most important phenolic in all the extracts (Table 3). Compared with CA, the CAFT content was always low, as shown by

the CA/CAFT ratio, which varied from 6 to 56 in the leaves and 1 to 10 in the rhizomes. The CA content in the leaves was between 8.13 and 27.44 mg g^{-1} dw (Table 3). The highest value was found in sample D-1 collected in July (Gran Canaria) and the lowest in sample D-2 collected in November (Gran Canaria). Compared with the leaves, the concentrations of CA were smaller in the rhizomes (0.31–3.70 mg g^{-1} dw), but remain worthy of attention considering the high value properties of CA and the abundance of rhizomes in the detritus after storms. To our knowledge, this is the first time that CA was found in *Cymodocea nodosa*.

Discussion

CA has been found to be the main phenolic compound in *Echinacea purpurea* roots (19.27 mg g^{-1} dw); however, it does not occur in appreciable amounts in other species, such as *E. pallida* and *E. angustifolia* (Pellati et al. 2004). Commercial production from *Echinacea* suffers limitations owing to variables such as environmental pollutants, fungi, bacteria, and other contaminants (Murch et al. 2006). As a result, discrepancies in the composition of commercial herbal medicines from *Echinacea* are observed (Pellati et al. 2004). In addition, care must be taken to avoid the degradation of CA during storage and extraction of *Echinacea* (Kim et al. 2000, Pellati et al. 2004). Indeed, CA is sensitive to the enzymes present in *Echinacea* in moist conditions.

Variable amounts of CA have also been found in other members of the Asteraceae: *Taraxacum officinale* (Ckhikvishvili and Kharebava 2001), *Leontodon autumnalis* (Grass et al. 2006), *Lactuca sativa* (Nicolle et al. 2004), and in *Ocimum basilicum* (Lamiaceae; Lee and Scagel 2009, 2010). Making direct comparisons between our results and the CA amounts reported in the literature for these plants is difficult because it is not always clear how the calculations have been done, some of the results are expressed on a fresh weight basis, and concentrations were often obtained without taking into account the difference in the responses of the HPLC detector toward CA and the standards used for quantification. Using the method developed by the INA (INA Method 2000) for *Echinacea* eliminates these problems, and the data obtained represent the real amounts of CA and CAFT in the dry plant. Our results show that detrital material of *Cymodocea nodosa*, especially the leaves, can compete with the traditional sources of CA.

Seagrasses colonized the sea from terrestrial angiosperm ancestors, which necessitated a number of key

adaptations. However, the phenolic secondary chemistry in the seagrasses appears not to have been fundamentally modified by this adaptation, as shown by the frequent occurrence of caffeic derivatives (Achamlale et al. 2009, Nuissier et al. 2010, Papenbrock 2012). Compared with terrestrial plants, using dead biomass of seagrass as a source of phytochemicals offers some particular advantages. First, the phenolic profile in seagrasses is generally less complex, and largely dominated by one or two components, and this facilitates extraction and analysis. Second, the only cost for this raw material is harvesting the beached detritus. Finally, in contrast to terrestrial plants, vegetative reproduction is more important in seagrasses than pollination. This limits the plant-to-plant variation and consequent genetic inconsistencies in the type and level of phytochemicals produced by terrestrial plants.

From these results, detrital leaves of *C. nodosa* appear to be one of the most concentrated sources of CA and probably also the cheapest. This hitherto unused but readily available and renewable marine resource constitutes a promising new raw material for the production of health products and provides a solution to an environmental problem.

In addition to their economic value, these results are of ecological and taxonomic interest. The characterization of the individual flavonoids in *Zostera noltii* permitted the demonstration of different chemotypes, which fit well with the existence of geographically distinct populations of *Z. noltii* (Coyer et al. 2004, Grignon-Dubois and Rezzonico 2012). In the case of *C. nodosa*, genetic differentiation across the Mediterranean-Atlantic transition region has been reported (Alberto et al. 2008). The constancy of the phenolic profile of *C. nodosa* throughout its biogeographic

range shows that this genetic differentiation has no influence on the phenolic secondary chemistry of this species. Also noticeable is the fact that *C. nodosa* and *Syringodium filiforme* share the same phenolic fingerprints, so that it is difficult to distinguish these two species on the basis of only their HPLC-DAD chromatograms or their NMR spectra (Nuissier et al. 2010). Although other members of Cymodoceaceae should be analyzed, these results already suggest that CA and CAFT could be used as taxonomic markers for the Cymodoceaceae. Among the four families of seagrasses, the Cymodoceaceae encompasses the highest variety with 15 species in five genera. Except for the two species we have studied, the full characterization of the phenolic content of the 13 others still needs to be established. Fingerprinting of other members of the genus *Cymodocea* and *Syringodium* is now in progress in our team, and will be pursued by the screening of members of the genera *Amphibolis*, *Halodule*, and *Thalassodendron*.

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