JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 10323–10330 10323
DOI:10.1021/jf9022096

Anatomical, Chemical, and Biochemical Characterization of Cladodes from Prickly Pear [*Opuntia ficus-indica* (L.) Mill.]

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Opuntia ficus-indica cladodes represent the green stem of the plant and are generally used as animal feed or disposed of in landfills. The present work investigated the anatomical and chemical composition of *Opuntia* cladodes, which form the basis of their pharmacological effects. Glucose and galacturonic acid were the main sugars of *Opuntia* cladodes, whereas high-performance liquid chromatography (HPLC) analysis showed the presence of mainly kaempherol and isorhamnetin glycosides (glucoside and rhamnoside). The presence of high amounts of calcium oxalate crystals was demonstrated by light microscopy on fresh and lyophilized cladodes. No antimicrobial activity was observed even after enzymatic treatment. *O. ficus-indica* cladodes may retain material tightly associated with cell-wall components, and this property will have the potential to greatly reduce the bioavailability of bioactive compounds.

KEYWORDS: *Opuntia ficus-indica*; cladodes; byproducts; flavonoids; phenolics; cell-wall structure

INTRODUCTION

More than 100 million tons of solid and liquid wastes are generated each year within member states of the European Union (EU). A consistent part of them, ca. 16 million tons per year, is spread on the land in agriculture, forestry, and land reclamation operations, with a cost of €320 million for environmental damage, while another significant portion is disposed of in landfill sites, with a cost of €25−30/ton, reaching €90 if incineration is used. Therefore, currently more and more effort is directed toward use of agro-industrial byproducts, before they become waste, to recover potential economic value and to address environmental issues.

The prickly pear cactus, *Opuntia ficus-indica* (L.) Mill., is a tropical or subtropical plant grown in arid climates, such as the Mediterranean and Central America areas; 94% of cultivated crops of the Italian production is grown in Sicily, where it represents an important food source (*1*). *O. ficus-indica* has gradually attained economic importance in both Sicilian agriculture and the wider international community through the United Nations (UN) Food and Agriculture Organization (FAO), which also contributes to increase the cultivation. However, the disposal of *O. ficus-indica* byproducts (including noncommercial fruits and cladodes) constitutes an environmental pollution problem, because of their fermentability. In Sicily, *O. ficus-indica* cladodes are generally used as animal feed or disposed of in landfills. Alternatively, the cladodes could be used as a fresh edible food ingredient, such as nopatolitos in Mexico, while other products from cladodes include jam, chutney, or pickles and candied nopales (*2*). The cladodes, while scarcely used in modern nutrition and medicine, contain bioactive compounds, well-known for their health-related properties (*3*). Extracted compounds have shown a number of pharmacological actions, including emollient and wound-healing effects, hypocholesterolemic effects, inhibition of stomach ulceration, neuroprotective effects through antioxidant actions, and anti-inflammatory effects (*4*−*6*). Effects on blood glucose control, serum lipid levels, and antiglycated activities have been related to the hypoglycaemic and antidiabetic properties of *Opuntia* cladodes (*7*, *8*).

The main constituent of *O. ficus-indica* cladodes is water (80−95%), followed by small amounts of carbohydrates (3−7%), fiber (1−2%), and protein (0.5−1%); other compounds are only partly known and have not been quantitatively determined (*3*). The sugar moiety includes mucilaginous components containing polymers, such as chains of (1−4)-linked β-D-galacturonic acid and α(1−2)-linked L-rhamnose residues (*9*, *10*). Mucilage is present in the characteristic slimy fluid secreted by cladodes and fruits and constitutes about 14% of the cladode dry weight. The physiological role of the plant mucilage is to regulate

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Published on Web 10/15/2009

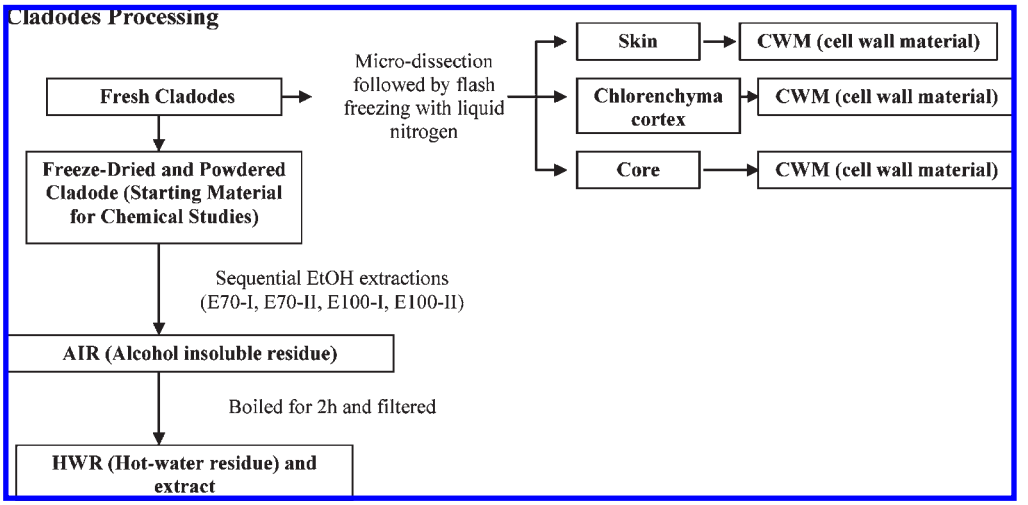

Figure 1. Cladode processing for microscopic and chemical analyses: flow diagram of the fractionation procedure.

the cellular water content during prolonged drought and to regulate the calcium fluxes of the plant.

O. ficus-indica cladodes also represent a source of phytochemicals, such as phenolics and flavonoids (*11*), minerals, and other nutrients. The potential benefits of the high calcium content are negligible because it is in the form of poorly bioavailable calcium oxalate crystals (*12*).

The present study has been carried out to understand more fully the structural and chemical basis for the pharmacological effects of *O. ficus-indica* and to help in evaluating other potential uses of this byproduct. Light microscopy has been used to evaluate the internal structure of the cladodes to provide a structural basis to the chemical evaluation of the whole cladodes and the component epidermal, photosynthetic, and internal regions.

MATERIALS AND METHODS

Source of *O. ficus-indica* Cladodes. Fresh cladodes, consisting of a mix of the three major commercial cultivars, Surfarina, Muscaredda, and Sanguigna, were collected in Reggio Calabria (southern Italy), stored at −18 °C, and lyophilized for 5 days. Samples were further processed by powdering with a commercial food blender prior to the various extractions and analysis.

Chemicals. All solvents were of high-performance liquid chromatography (HPLC) grade, and all water was ultra-pure-grade. All carbohydrate and flavonoid standards (3-*O* glucoside and 3-*O* rutinoside of kaempferol, quercetin, and isorhamnetin) were obtained from either Sigma-Aldrich (Poole, U.K.) or Extrasynthese (Genay, France). Pectinase 690 L (endogalacturonase, 190.7 units/mL; cellulase, 188.9 units/mL from *Aspergillus* sp. and *Trichoderma* sp.) was obtained by Biocatalysts Ltd. (Cefn Coed, Wales, U.K.).

Light Microscopy. The structure of the skin, the underlying green chlorenchyma tissue, and the central core of a fresh cladode was examined by bright field microscopy. Transverse sections of the cladodes were cut by hand and examined unstained using an Olympus BX60 (Olympus, Japan) microscope with Acquis software (Syncroscopy, Cambridge, U.K.). An AIR sample of whole cladodes together with AIR samples of the skin, green chlorenchyma, and central core were examined, and the component structures were identified. Starch was identified after staining with iodine in potassium iodide.

Cell-Wall Fractionation. An alcohol-insoluble residue (AIR) and four liquid fractions (termed E70-I, E70-II, E100-I, and E100-II) were produced by sequential ethanolic fractionation of 100 g of dry weight of the powdered lyophilized cladodes using the method previously described by Mandalari et al. (*13*) (**Figure 1**). Briefly, the cladodes were homogenized using a Waring commercial blender in 1 L of 70% (v/v) ethanol (EtOH) for 5 min, boiled for 5 min, and filtered through nylon mesh (100 μm). The residues were sequentially re-extracted and re-homogenized with a further

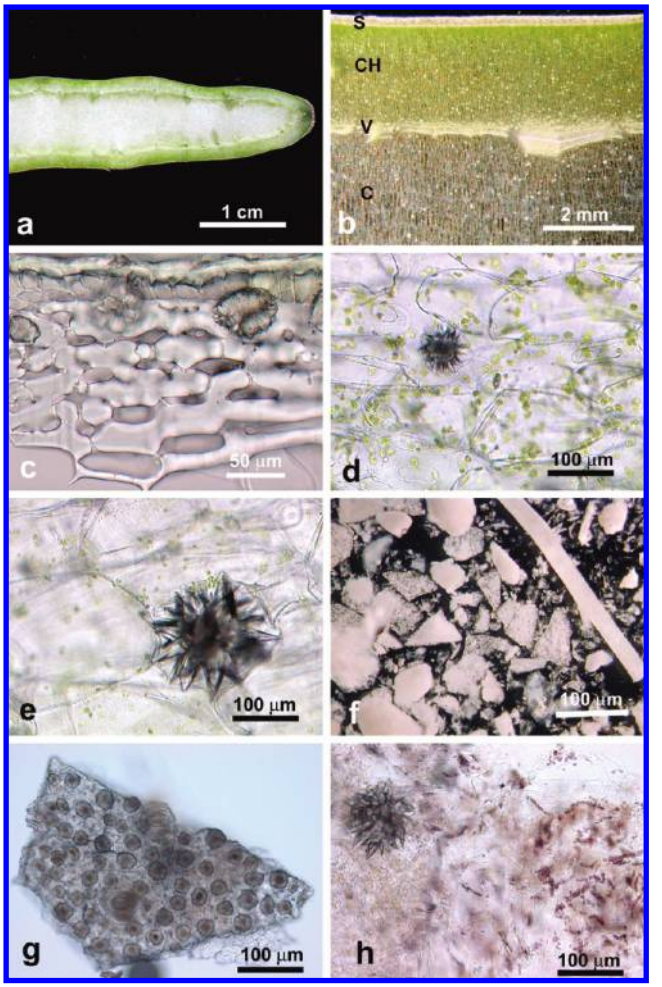

Figure 2. Morphology of *O. ficus-indica* cladodes. (a) Transverse section (TS) of part of the whole cladode. (b) TS of the outer layers of the cladode showing skin (S), chlorenchyma (CH), vascular (V), and core (C) tissue. (c) TS of the skin showing a thick-walled hypodermis. (d) TS of the chlorenchyma with plastids and oxalate crystal. (e) TS of the core tissue with oxalate crystal. (f) Whole alcohol-insoluble residue (AIR). (g) Skin fragment from the whole AIR showing numerous oxalate crystals. (h) Core fragment from the whole AIR showing iodine-stained starch.

1 L of 70% EtOH (v/v), followed by two extractions with 100% EtOH (v/v). After freeze-drying, all fractions were analyzed for sugars, uronic acid, and

Table 1. Monosaccharide Composition of Cladodes and Extracts of *Opuntia*[a]

		sequential fractions of 100 g of cladodes					
	whole dry cladodes	E70-I	E70-II	E100-I	E100-II	AIR	HWR
yield (%)	100	6.20	7.60	2.43	1.98	63	(47.25)
rhamnose	7.13 ± 1.28	7.71 ± 1.14	4.47 ± 1.27	4.66 ± 0.96	4.20 ± 0.71	6.37 ± 1.44	3.12 ± 0.16
fucose	0.74 ± 0.11	4.42 ± 2.42	0.72 ± 0.56	0.62 ± 0.19	0.69 ± 0.07	0.77 ± 0.31	1.50 ± 0.06
arabinose	39.64 ± 1.96	3.65 ± 0.16	5.34 ± 0.19	20.04 ± 1.48	24.51 ± 2.43	45.80 ± 1.80	47.72 ± 2.79
xylose	18.64 ± 0.84	3.27 ± 0.21	3.00 ± 0.16	9.89 ± 1.21	12.47 ± 1.65	18.38 ± 4.14	17.47 ± 0.98
mannose	13.64 ± 0.81	30.74 ± 4.69	15.76 ± 0.14	5.21 ± 1.91	4.85 ± 0.75	6.12 ± 0.94	6.17 ± 0.43
galactose	33.69 ± 2.89	8.73 ± 0.93	8.89 ± 1.67	22.09 ± 6.87	27.55 ± 4.00	33.97 ± 7.96	42.66 ± 1.72
glucose	153.15 ± 0.25	179.21 ± 22.56	117.31 ± 9.11	195.15 ± 10.23	208.72 ± 19.34	122.42 ± 9.73	127.87 ± 10.35
GalA	96.26 ± 6.54	14.12 ± 1.90	22.02 ± 3.88	32.11 ± 20.93	45.51 ± 11.92	141.28 ± 11.12	209.12 ± 10.05
total (μg/mg)	362.89	251.85	177.51	289.77	328.52	375.11	455.63

[a] Composition is expressed as total μg/mg of extracted and dried material, and values represent mean ± standard deviation (SD) ($n = 3$). The yield was calculated as the dry weight recovered from the extraction volume as a percentage of the initial volume. GalA, galacturonic acid; AIR, alcohol-insoluble residue obtained from whole dry cladodes after sequential extraction in ethanol; HWR, hot-water residue obtained from AIR. The percentage yield from AIR is shown in parentheses.

flavonoids. A hot-water residue (HWR) was prepared from AIR by heating 10 g with water (150 mL) at 100 °C for 2 h and then immediately filtered through a GF-C membrane. The HWR was washed out with acetone and dried under a fumehood overnight.

Furthermore, fresh cladodes were dissected into three separate fractions consisting of skin, green chlorenchyma, and central core (**Figure 1**). All fractions were frozen in liquid nitrogen, and cell-wall material (CWM) was prepared following a modified method previously reported (*14*). Each weighed fraction (25 g) was blended in 1.5% (w/v) sodium dodecyl sulfate (SDS) using a Ystral homogenizer (Ystral GmbH, Dottingen, Germany) for 5 min. A few drops of octanol were added to reduce foaming. The homogenate was filtered through 70 μm nylon mesh, and the residue was ball-milled at 0 °C in 0.5% (w/v) SDS at 60 rpm for 5 h. The iodine test was performed to ensure that no starch or intracellular material was present. The CW residue was suspended in 200 mL of deionized water and then filtered through 70 μm nylon mesh. After several washes with EtOH and acetone, the CWM was allowed to dry overnight, and all samples were analyzed for sugars and uronic acid.

Extraction and Liquid Chromatography−Mass Spectrometry (LC−MS) Analysis of Phenolics and Flavonoids in Whole Cladodes and Ethanolic Extracts. Extraction of phenolics and flavonoids was performed as previously described (*13*). Triplicate samples (3 × 40 mg) of the freeze-dried, powdered cladodes were extracted in 2 mL screw-top microtubes with 1 mL of 70% (v/v) methanol, centrifuged, and filtered (0.2 μm) into HPLC vials prior to analyses using the previously described method (*13*). Subsamples (1 mL) of the ethanolic extracts were filtered (0.2 μm) and transferred to HPLC vials and analyzed for the methanol extracts (*13*). The UV−vis spectra and MS data for each phenolic and flavonoid are available as Supporting Information.

Cell-Wall Phenolics Content. Alkali-extractable phenolic acids in the AIR and HWR were analyzed by HPLC following the method of Waldron et al. (*15*).

Neutral Sugar Composition. Neutral sugars were released by Saeman hydrolysis and analyzed as their alditol acetates by gas chromatography (GC) using the method previously described (*16*). A second set of samples was hydrolyzed with 1 M sulphuric acid only, to exclude cellulose.

Uronic Acid Content. Total uronic acid content was determined by the method by Blumenkranz and Asboe-Hansen (*17*) and expressed as galacturonic acid equivalents.

Total Protein Assay. The total protein content of AIR was determined by a micro-Kjeldahl procedure according to the standard Association of Official Analytical Chemists (AOAC) method (*18*), with protein values expressed as $N \times 6.25$.

Lipid Content. The total lipid content of AIR and HWR was determined gravimetrically by Soxhlet extraction with *n*-hexane and expressed as a percentage of dry weight.

Klason Lignin Analysis. Klason lignin analysis was performed using the method adapted from Theander and Westerlund (*19*). AIR (50 mg) was dispersed in 0.75 mL of 72% (w/w) H_2SO_4 and incubated at room temperature for 3 h while shaking. The sample was further incubated for 2.5 h after dilution with water (9 mL) in a temperature-controlled oven set at 100 °C. The residue was recovered by filtration through preweighed sintered glass funnels (10 mm in diameter, Fisher Scientific) under vacuum. The insoluble material was washed 3 times with warm water (< 40 °C) until the residue was free of acid. The glass filter was dried at 50 °C in a temperature-controlled oven overnight or until a constant weight was obtained. Klason lignin was calculated gravimetrically as follows:

$$\text{Klason lignin (\%)} = ((W_1 - W_2) \times 100)/S$$

where W_1 is the weight of the glass filter + dried residue, W_2 is the weight of the glass filter, and S is the weight of the initial sample.

Starch Analysis. Starch was analyzed as total starch, assuming an initial starch content of 1−10% sample AIR, using a standard analytical kit as per the protocol of the manufacturer (Megazyme kit K-TSTA, Megazyme, Bray, Ireland). In brief, AIR samples were dispersed in 95% DMSO at 100 °C and then diluted to 40% DMSO prior to digestion with a thermostable α-amylase at pH 7.0 in 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer for 6 min. Digestion was completed using an amyloglucosidase at 50 °C (prepared at pH 4.5 in 200 mM sodium acetate buffer). The glucose released was measured at 510 nm using the GOPOD reagent (Megazyme kit K-GLUC, Bray, Ireland) (*20*).

Mixed Linkage β-Glucan Analysis. Mixed linkage β-glucan was analyzed using a standard analytical kit (Megazyme kit BBG), with the assay procedure modified to extract a 10 mg sample weight in 10 mL. In brief, samples of AIR were dispersed in phosphate buffer (20 mM, pH 6.5 at 100 °C) prior to digestion with lichenase [specific endo-(1−3)(1−4)-β-D-glucan 4-glucanohydrolase] (40 units/mL, 10 units/assay tube, at 40 °C) for 1 h. An aliquot of each digest was then dispersed in 50 mM sodium acetate buffer (pH 4.0), digested with β-glucosidase (2 units/mL, 0.2 unit/assay tube, at 40 °C) for 15 min. The glucose released was quantified using the GOPOD reagent at 510 nm as described above for starch analysis.

Antimicrobial Activity of *O. ficus-indica* Phytochemical Fractions. The following bacteria were used for the antimicrobial testing and were obtained from the IFR in-house culture collection (Norwich, U.K.): *Escherichia coli* MG1655 (K12), *Salmonella enterica* var. Typhimurium LT2, *Pseudomonas putida* ATCC 795, *Listeria innocua* ATCC 33090, *Lactococcus lactis* MG1614, and *Staphylococcus aureus* FI10139 (food isolate supplied by the Food Processing Group, Unilever R&D, Bedford, U.K.). The strains were grown following the method previously described (*21*). The minimum inhibitory concentrations (MICs) of the cladode fractions E70-I, E70-II, E100-I, and E100-II were determined using a Bioscreen C system (Labsystems, Helsinki, Finland). The test organisms were grown for 24 h in appropriate media, and the optical density (OD, 600 nm) was measured at 10 min intervals. All assays were performed in duplicate, and growth in the presence of ethanol (maximum 1%, v/v) acted as controls. The MIC of each of the ethanol fractions or flavonoid fraction was considered as the lowest concentration that completely inhibited bacterial growth (OD cutoff point ≤0.1 = no growth) after 16 h.

To increase the antimicrobial activity (and to investigate the release of the potentially more active flavonoid aglycones), the E70-I fraction (0.5 g) was incubated with 10 units of Pectinase 690 L (endogalacturonase,

190.7 units/mL; cellulase, 188.9 units/mL) in 50 mM Na-acetate buffer at pH 5.0 for 2 h in a shaking incubator (37 °C, 100 rpm) in a final volume of 50 mL. The fraction obtained was tested against all strains mentioned above.

RESULTS AND DISCUSSION

Representative results of the microscopic examinations of cladodes are shown in **Figure 2**. Cladodes of *O. ficus-indica* are typically fleshy with a thick hard skin surrounding an outer layer of chlorenchyma and a central core (panels **a** and **b** of **Figure 2**). The vascular tissue lies in a single layer at the junction of the green chlorenchyma tissue and the colorless central core tissue. In the transverse section, the skin consists of a waxy cuticle covering a hypodermis of three or four layers of cells with heavily thickened walls (**Figure 2c**). The stomata are sunken (not shown). The chlorenchyma consists of thin-walled parenchyma containing green plastids (**Figure 2d**) and abundant starch if the cladode is freshly harvested. In the central core tissue, the cells are also thin-walled but they are colorless (**Figure 2e**). In accordance with a previous report (*22*), the cladodes of *O. ficus-indica* contain significant amounts of calcium oxalate crystals and these are present in all tissues. At low magnification, they are visible as small white spots throughout the cladode (**Figure 2b**). At higher magnification, they are clearly visible as rounded crystals in the epidermis (panels **c** and **g** of **Figure 2**) and, in the other tissues, as striking crystal rosettes (panels **e** and **g** of **Figure 2**). Calcium has an important role in water retention of succulent tissues to regulate the osmotic pressure in the cells. It has been shown that oxalate crystal size increased as a function of maturation (*23*). The existence of calcium oxalate could limit the calcium bioavailability, which could also have maturation dependence. The calcium was estimated to be 18−57 mg/100 g of dry weight (*12*, *22*). Starch is also usually present in freshly harvested material, and there are always mucilage ideoblasts (not shown).

Table 2. Noncellulosic Monosaccharide Composition of the Cladodes Sections of *Opuntia*[a]

	skin	chlorenchyma cortex	core
yield (%)	5.47	1.03	0.4
rhamnose	7.23 ± 0.21	11.26 ± 0.99	9.92 ± 1.25
fucose	2.67 ± 0.21	4.15 ± 0.21	3.21 ± 0.48
arabinose	101.86 ± 5.59	43.46 ± 2.73	34.77 ± 6.32
xylose	23.42 ± 1.01	34.10 ± 1.24	41.11 ± 6.34
mannose	12.59 ± 0.72	11.08 ± 1.33	8.28 ± 0.79
galactose	26.49 ± 0.73	98.24 ± 1.11	107.10 ± 3.45
glucose	53.81 ± 1.68	66.17 ± 2.11	59.36 ± 8.08
GalA	187.62 ± 10.05	223.46 ± 8.25	218.65 ± 9.09
total (μg/mg)	415.69	491.93	535.12

[a] Composition is expressed as total μg/mg material after 1 M H_2SO_4 hydrolysis, and values represent mean ± SD (n = 3). The yield was calculated as the dry weight recovered from the extraction volume as a percentage of the initial volume. GalA = galacturonic acid.

Table 3. Phenolics and Flavonoids in *O. ficus-indica* Cladode Material[a]

phenolic/flavonoid[b]	amount in 100 g[c] (mean ± σ_{n-1}) (70% MeOH)	total recovery in E70-I (mg)	total recovery in E70-II (mg)	total recovery in E100-I (mg)	total recovery in E100-II (mg)	percent remaining in the residue after EtOH sequence (%)
P1 (piscidic acid)	131 ± 2.29	57.4 (43.8)	34.1 (26.0)	1.5 (1.1)	0.46 (0.35)	28.75
P2 (unknown)	10 ± 0.23	8.6 (86)	1.2 (12)	0	0	2.00
P3 (eucomic acid)	17 ± 1.7	12.3 (72.4)	2.6 (15.3)	0.12 (0.7)	0.02 (0.12)	11.48
F1: Q-3-*O*-Glc-Rha/(Xyl or Ara)	26 ± 0.3	5.6 (21.5)	0.5 (1.92)	0.04 (0.15)	0.01 (0.04)	76.39
F2: iso-3-*O*-Glc-Rha/Glc	10 ± 0.24	1.5 (15)	0.1 (1)	0.01 (0.1)	0	83.9
F3: K-3-*O*-Glc-Rha/Rha I	20 ± 0.33	4.4 (22)	0.5 (2.5)	0.16 (0.8)	0.05 (0.03)	74.67
F4: K-3-*O*-Glc-Rha/Rha II						
F5: iso-3-*O*-Glc-Rha/Rha	79 ± 0.66	12.4 (15.7)	1.4 (1.8)	0.03 (0.04)	0.09 (0.11)	82.35
F6: iso-Glc-Rha (Xyl or Ara) I	159 ± 1.03	24.9 (15.7)	3.4 (2.1)	0.38 (0.24)	0.12 (0.08)	81.88
F7: iso-3-*O*-Glc-Rha/Rha						
F8: iso-Glc-Rha (Xyl or Ara) II	26 ± 0.14	3.6 (13.8)	0.4 (1.5)	0.05 (0.19)	0.01 (0.04)	84.47
F9: Q-3-*O*-Glc-Rha (rutin)	15 ± 0.24	2.9 (19.3)	0.3 (2.0)	0.04 (0.27)	0.01 (0.07)	78.36
F10: K-Glc/(Xyl or Ara)	5 ± 0.18	1.1 (22.0)	0.1 (2.0)	0.05 (1.0)	0.01 (0.2)	74.8
F11: iso-3-*O*-Glc-(Xyl or Ara)	38 ± 0.99	7.5 (19.7)	0.9 (2.4)	0.12 (0.32)	0.03 (0.08)	77.5
F12: Q-3-*O*-Glc (isoquercitrin)	7 ± 0.84	1.8 (25.7)	0.1 (1.4)	0.02 (0.29)	0	72.61
F13: K-3-*O*-Glc-Rha	6 ± 0.43	1.2 (20.0)	0.1 (1.7)	0.05 (0.8)	0.01 (0.17)	77.33
F14: iso-Glc-Rha	9 ± 0.54	1.4 (15.6)	0.2 (2.2)	0.08 (0.89)	0.02 (0.22)	81.09
F15: iso-3-*O*-Glc-Rha	76 ± 0.39	14.2 (18.7)	1.8 (2.4)	0.25 (0.33)	0.07 (0.09)	78.48
F16: iso-3-*O*-Glc	6 ± 0.2	1.5 (25.0)	0.1 (1.7)	0.02 (0.33)	0	72.97

[a] One set of samples was extracted with 70% MeOH (3 × 40 mg in 1 mL of 70% MeOH = 100% content), and a second set was sequentially extracted with 1000 mL of EtOH: 70% (v/v) EtOH I (970 mL recovered), 70% EtOH II (1000 mL recovered), 100% EtOH I (980 mL recovered), and 100% EtOH II (970 mL recovered). Values represent mean ± SD (n = 3). P1−P3, phenolics; F1−F16, flavonoids. [b] Abbreviations: Q, quercetin; iso, isorhamnetin; K, kaempferol. Flavonoids in bold text were additionally confirmed with pure standards. [c] Compounds expressed as total milligrams per fraction and as a percentage of the total in 100 g of starting material (in parentheses for each fraction). Phenolics (P1−P3) were expressed as 4-hydroxybenzoic acid equivalents. All flavonoids were determined using calibration curves with the closest appropriate standard, e.g., kaempferol-Glc/Rha/Rha quantified with K-3-*O*-Glc-Rha. Note the co-elution of flavonoids F3/F4 and F6/F7; therefore, quantification data are for each pair of flavonoids.

The whole AIR preparation consisted of a white powder in which vascular bundles (source of lignin), clumps of parenchyma from the flesh, and sheets of skin material are recognizable (**Figure 2f**). At higher magnification, the skin material comprises the cuticle, underlying hypodermis, stomatal complexes, and an almost continuous layer of calcium oxalate crystals (**Figure 2g**). Flesh parenchyma derived from the chlorenchyma or central core contains starch (stained blue/brown with iodine/potassium iodide) and associated calcium oxalate crystals (**Figure 2h**).

The yield (%) and the sugar composition of the initial *O. ficus-indica* cladodes, AIR, HWR, and ethanol extracts are reported in **Table 1**. A total of 36% and 37% of the dry weight of whole cladodes and AIR, respectively, were carbohydrate. It was previously reported that the level of carbohydrates in dry cladodes was between 64 and 71 g/100 g (*23*). Variations may reflect agronomic and environmental factors as well as the age of the cladodes used in the studies; younger cladodes have higher carbohydrate content (*23*). As expected, in all samples tested, there was a prevalence of glucose and galacturonic acid, while arabinose, galactose, mannose, xylose, and rhamnose were present in smaller quantities. Only traces of fucose were detected. Pectins have a number of applications in the pharmaceutical, cosmetic, and food industries, and new sources of these polysaccharides are constantly sought (*10*). Pectins extracted from *Citrus* peel have also shown functional food properties and could be used as potential sources of prebiotic functional foods (*24*). The percentage of different monomeric sugars (of total sugars content) of whole cladodes including galacturonic acid, glucose, arabinose, and galactose were 24, 41, 13, and 9%, respectively. A high level of glucose in the cladodes (41%) and AIR (32%) was detected. Mannose is the main sugar removed by ethanol treatment; in fact, the percentage of the mannose in AIR is low (1.62%).

The neutral sugar composition of the EtOH fractions showed that the main components were glucose and mannose in the 70% extracts, while glucose, arabinose, and galactose were prevalent in 100% extracts. A small percentage of glucose and rhamnose in the ethanol fractions is derived from the low levels of flavonoid glycosides (glucoside and rhamnoside).

The chemical composition of the mucilage has been described as a "core" chain of (1−4)-linked β-D-galacturonic acid and α(1−2)-linked L-rhamnose with trisaccharide side chains of β(1−6)-linked D-galactose attached at O(4) of L-rhamnose residues (*9*). Branching occurs at different positions. The mucilage content found in the cactus cladodes is influenced by temperature and irrigation. For its capacity to absorb water, mucilage can be considered as a functional component with industrial potential, such as a source of hydrocolloids (*25*).

To provide information on the nature of the polysaccharides present in AIR and to solubilize the major part of noncellulosic polysaccharides, AIR was extracted with hot water. As a function of the original fresh weight, the carbohydrate yield of the HWR was only 9% less than that of the AIR, indicating that little CWM had been solubilized. The sugar composition of the HWR was therefore similar to that of the AIR, containing mainly galacturonic acid, followed by glucose, galactose, and arabinose (**Table 1**). It is known that the chemical composition of *O. ficus-indica* may change depending upon edaphic factors at the cultivation site, such as the season and the age of the plants, and this may influence the carbohydrate profiles in terms of the individual sugar contents and polysaccharide structures (*23*).

To provide further detail on the anatomical basis for the data in **Table 1**, the cell wall was purified to a much higher degree from the main tissue zones of *Opuntia* cladodes (skin, chlorenchyma cortex, and core) by extraction in SDS. These were evaluated for their carbohydrate compositions, and the results are shown in **Table 2**. The methodology used for purifying CWM will remove all water- and detergent-soluble components. The yield of CWM differed considerably between the tissue types. Skin CWM exhibited a very high yield of 5.47% (w/w), reflecting the small cells with heavily thickened cell walls (**Figure 2c**). In contrast, the relatively thin-walled cells of the chlorenchyma cortex and inner core gave a much lower CWM yield of 1.03 and 0.4% (w/w), respectively. The carbohydrate compositions of the CWM preparation showed that the three tissues were all rich in uronic acid but differed mainly in relation to the neutral noncellulosic sugars of pectin/mucilage-related components. The skin CWM preparation had much arabinose and considerably less galactose, while the CWM preparations of the other two tissues contained more galactose and less arabinose.

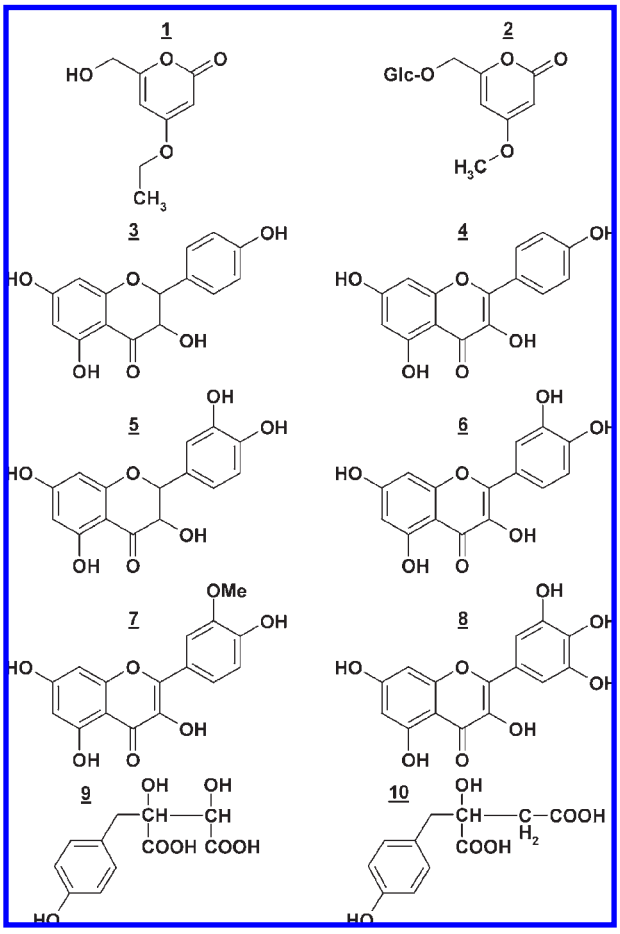

Figure 3. Structures of phytochemicals previously identified in *O. ficus-indica* cladodes. 1, 4-ethoxy-6-hydroxy-methyl-α-pyrone; 2, opuntioside; 3, aromadendrin; 4, kaempferol; 5, taxifolin; 6, quercetin; 7, isorhamnetin; 8, myricetin; 9, piscidic acid; and 10, eucomic acid.

Samples of AIR treated with I_2/KI were stained blue/brown, suggesting that significant amounts of either starch or xyloglucan were present. The proportion of xylose present in the profile of sugars from the AIR indicated that xyloglucans were unlikely to be a major polysaccharide component. The apparent high proportion of glucose released using 1 M acid hydrolysis, however, suggested that a noncellulosic glucan was present. Analysis for starch indicated that the AIR had a starch content of 89.5 ± 4.4 mg/g, consistent with the reported (~14%) starch content from *Opuntia* forage analysis. However, in related species, such as *Aloe vera*, starch has not been detected (*26*).

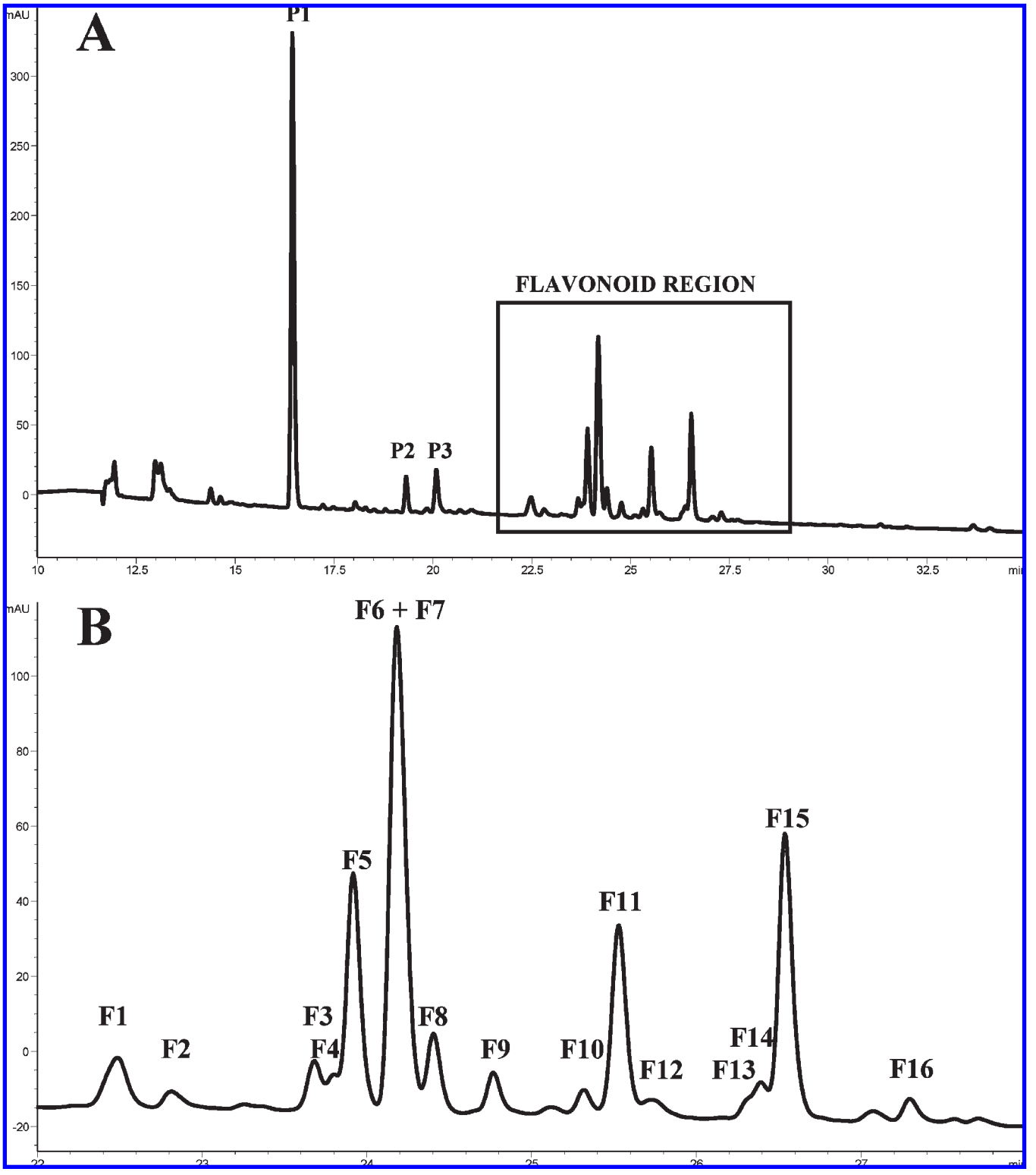

Figure 4. HPLC chromatograms of (A) 70% methanol extracts of *O. ficus-indica* cladodes at 270 nm and (B) detail of the flavonoid region of the chromatogram. For peak identifications, see **Table 3**.

Mixed linkage β-glucans tend to be associated with graminaceous cereals; therefore, it was not unexpected that only trace levels were detected in the *Opuntia* AIR (5.5 ±0.7 mg/g of AIR). The levels detected may reflect the presence of a minor glucan with $\beta(1-3)$ linkages and/or the release of a water-soluble glucose-containing polysaccharide with $\beta(1-4)$ linkages because of its (partial) digestion by β-glucosidase during the assay procedure.

The concentration of the soluble phenolic components in the whole cladodes was very low. Total phenolic acid contents in AIR and HWR were 6.9 and 2.7 μg/mg, respectively. Piscidic acid was the major component, representing 90 and 75% of the total amounts in AIR and HWR, respectively. Traces of *p*-hydroxy benzaldehyde and *trans-p*-coumaric acid were detected in AIR, while *trans*-ferulic acid and *cis*-coumaric acid were additionally found in HWR. While the latter phenolics are commonly found in

Table 4. Phytochemicals Present in Fraction E70-I before and after Treatment with Pectinase 690 L (0.5 g of Freeze-Dried E70-I Incubated with Pectinase in a Final Volume of 50 mL of Buffer)[a]

phenolic/flavonoid[b]	MW	control	post-pectinase (flavonoid aglycone percentage relative to the estimated) (%)	estimated aglycone production (mg)
P1 (piscidic acid)	256	2.50 ± 0.06	2.47 ± 0.09	
P2 (unknown)	326	0.38 ± 0.004	0	
P3 (eucomic acid)	240	0.43 ± 0.002	0.44 ± 0.022	
F1: Q-3-*O*-Glc-Rha/(Xyl or Ara)	742	0.72 ± 0.11	0	0.119 Q
F2: iso-3-*O*-Glc-Rha/Glc	786	0.20 ± 0.04	0	0.032 iso
F3: K-3-*O*-Glc-Rha/Rha I	740	0.73 ± 0.03	0	0.109 K
F4: K-3-*O*-Glc-Rha/Rha II	740			
F5: iso-3-*O*-Glc-Rha/Rha	770	2.41 ± 0.09	0	0.406 iso
F6: iso-Glc-Rha (Xyl or Ara) I	756	5.31 ± 0.20	0	(using MW 763) 0.911 iso
F7: iso-3-*O*-Glc-Rha/Rha	770			
F8: iso-Glc-Rha (Xyl or Ara) II	756	0.89 ± 0.04	0	0.155 iso
F9: Q-3-*O*-Glc-Rha (rutin)	610	0.38 ± 0.03	0	0.093 Q
F10: K-Glc/(Xyl or Ara)	580	0.23 ± 0.01	0	0.056 K
F11: iso-3-*O*-Glc-(Xyl or Ara)	610	1.34 ± 0.08	0	0.360 iso
F12: Q-3-*O*-Glc (isoquercitrin)	464	0.05 ± 0.01	0	0.021 Q
F13: K-3-*O*-Glc-Rha	594	0.13 ± 0.02	0	0.030 K
F14: iso-Glc-Rha	624	0.25 ± 0.04	0	0.064 iso
F15: iso-3-*O*-Glc-Rha	624	2.48 ± 0.07	0	0.636 iso
F16a: iso-3-*O*-Glc	478	0.13 ± 0.01		0.057 iso
F16b: (residual post-enzyme)	478		0.53 ± 0.08	0.232 iso
				totals
quercetin (aglycone)	302	0	0.05 ± 0.002 (21.4)	0.234
kaempferol (aglycone)	286	0	0.06 ± 0.003 (30.8)	0.195
isorhamnetin (aglycone)	316	0	0.20 ± 0.01 (adjusted to residual iso-3-*O*-Glc) (8.4)	2.621

[a] Each phytochemical is expressed as mg/g of E70-I; Mean ± σ_{n-1}. [b] Abbreviations: P1−P3, phenolics; F1−F16, flavonoids (flavonols); Q, quercetin; iso, isorhamnetin; K, kaempferol.

small quantities in plant cell walls, piscidic acid is not. It is possible that intracellular piscidic acid (see below) was co-precipitated with intracellular protein and partially resistant to alcohol extraction. Free phenolic and flavonoid moieties from fresh cladode material are presented in **Table 3**. Piscidic acid is the main simple phenolic acid found in the *O. ficus-indica* extracts. The other simple phenolics, eucomic acid and an unknown phenolic acid with a retention time of 19 min, are present in smaller quantities. The predominant flavonoids in the cladodes of *O. ficus-indica* were an isorhamnetin glucosyl-rhamnosyl-rhamnoside (F5), and a mixture of two flavonoids, an isorhamnetin glucosyl-rhamnosyl-(xyloside or arabinoside) (F6) and a second isorhamnetin glucosyl-rhamnosyl-rhamnoside isomer (F7). Isorhamnetin was the predominant core aglycone for the *Opuntia* flavonoids, with smaller concentrations of flavonoids with a kaempferol or quercetin core aglycone (**Table 3** and Figure **4**). All ethanol fractions had the same compounds but in decreasing concentration from E70-I to E100-II; E70-I contained the highest amount of phenolics and flavonoids. The results showed a high percentage (72−85% depending upon the flavonoid) of flavonoids remaining in the residue (AIR) after ethanol extraction (**Table 3**). This suggests that some component in the structure of the *Opuntia* cladode cell walls is affecting the solubility of these low-molecular-weight (MW) components.

Eight flavonoids have been recently identified from stems and fruits of *O. ficus-indica* var. *saboten* (*11*) (**Figure 3**). Flavonoids are secondary metabolites known as internal physiological regulators in plants. In addition, *in vivo* and *in vitro* experiments demonstrated beneficial health activities of flavonoids as protective agents against cancer, cardiovascular, inflammatory, and allergic disorders, capillary fragility, and human platelet aggregation (*27*).

In addition to the above phenolic and carbohydrate components, 6.42% of the AIR dry weight comprised protein but only 0.63% consisted of lipid. In previous reports, the concentration of the protein and lipid in the dry matter of cladodes was about 4−10 and 1−4%, respectively (*25*). It was interesting to note a high percentage of calcium oxalate and Klason lignin (16%) in the cladodes. In the Klason lignin method, 72% (w/w) H_2SO_4 was used and it was impossible to separate the values of Klason lignin from that of calcium oxalate because the sulphuric acid used could not break up the crystals. The amount of calcium oxalate is likely to be much higher than Klason lignin. Klason lignin content is expected to be around 4−5% on the basis of previous studies (*25*).

The antimicrobial activities of various *O. ficus-indica* phytochemical fractions were evaluated. The minimum inhibitory concentrations (MICs) of *Opuntia* ethanol fractions, E70-I (before and after treatment with Pectinase 690 L), E70-II, E100-I, and E100-II, against the Gram-positive and Gram-negative bacteria listed above and *S. cerevisiae* were studied. In recent studies, Pectinase 690 L was used to solubilize bergamot peel and to hydrolyze the flavonoid glycosides to their corresponding aglycones (*21*). Results of controls containing ethanol (maximum 1%, v/v) indicated the complete absence of inhibition of all strains tested (results not shown). All *Opuntia* ethanol fractions showed no inhibitory activity against the strains tested. In addition, the antimicrobial properties of E70-I did not increase after treatment with Pectinase 690 L (**Table 4**). This is probably due to either the very poor conversion of the glycosides to the corresponding aglycones or subsequent binding or decomposition of the released aglycones; i.e., post-690 L isorhamnetin aglycone is 0.20 mg/g (8.4% of the potential maximum), followed by quercetin aglycone (0.05 mg/g; 21.4% of the potential maximum), and kaempferol aglycone (0.06 mg/g; 30.8% of the potential maximum) (**Table 4**).

In summary, this paper provides further information on the chemical composition of the *O. ficus-indica* cladodes, which forms the basis for its pharmacological properties.

ACKNOWLEDGMENT

We thank Biocatalysts Limited for providing pectinolytic enzyme samples and Kathryn Cross for preparing **Figure 2**.

Supporting Information Available: UV−vis and MS spectra of all phenolics and flavonoids found in the cladodes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received February 27, 2009. Revised manuscript received August 26, 2009. Accepted September 25, 2009. This research was funded by the Biotechnology and Biological Research Sciences Council (BBSRC, U.K.) and the University of Messina (Italy).