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Lipophilic Extracts of *Cynara cardunculus* L. var. *altilis* (DC): A Source of Valuable Bioactive Terpenic Compounds

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Supporting Information

ABSTRACT: Lipophilic extracts of *Cynara cardunculus* L. var. *altilis* (DC) from the south of Portugal (Baixo Alentejo) were studied by gas chromatography−mass spectrometry. One sesquiterpene lactone, four pentacyclic triterpenes, and four sterols were reported for the first time as cultivated cardoon components, namely, deacylcynaropicrin, β- and α-amyrin, lupenyl and ψ-taraxasteryl acetates, stigmasterol, 24-methylenecholesterol, campesterol, and Δ^5-avenasterol. In addition, other new compounds were identified: ten fatty acids, eight long-chain aliphatic alcohols, and six aromatic compounds. Four triterpenyl fatty acid esters were also detected. Sesquiterpene lactones and pentacyclic triterpenes were the major lipophilic families, representing respectively 2−46% and 10−89% of the detected compounds. Cynaropicrin was the most abundant sesquiterpene lactone, while taraxasteryl acetate was the main pentacyclic triterpene. Fatty acids and sterols, mainly hexadecanoic acid and β-sitosterol, were present at lower amounts (1−20% and 1−11% of the detected compounds). Long-chain aliphatic alcohols and aromatic compounds were detected at reduced abundances (1−6% of the detected compounds).

KEYWORDS: *cultivated cardoon, Cynara cardunculus L. var. altilis (DC), GC−MS analysis, lipophilic extracts, pentacyclic triterpenes, sesquiterpene lactones*

INTRODUCTION

Cynara cardunculus L. (Asteraceae) is a Mediterranean plant species that comprises three varieties, namely, var. *sylvestris* (Lamk) Fiori (wild cardoon), var. *scolymus* (L.) Fiori (globe artichoke), and var. *altilis* (DC) (cultivated cardoon).[1] The wild cardoon grows spontaneously in clay soils in the Mediterranean basin and Macaronesia (Madeira and Canary Islands).[2] Several lines of evidence have shown that wild cardoon is the ancestor of both cultivated forms, characterized by different morphological traits, due to agricultural selection.[3] Globe artichoke is cultivated all over the world for its edible immature large capitula, with high economic importance in Italy, Spain, France, and Turkey.[2] Cultivated cardoon has been explored for its fleshy stems and leaf petioles. These are generally collected in late autumn−early winter and submitted to a blanching process before cooking. This "delicacy" is much appreciated in regional dishes in Spain, Italy, France,[4] and in the south of Portugal. Another traditional application, in the Iberian Peninsula, involves the use of cardoon's capitula as a source of aspartic proteinases (cardosins A and B) for milk clotting during ewe cheese manufacturing.[5] Furthermore, both artichoke and cardoon leaf extracts have been used since ancient times in folk medicine for hepatobiliar system regulation[6,7] due to their recognized hepatoprotective,[8] hypocholesterolemic,[9] choleretic and anticholestatic[10] actions.

In addition to the described traditional uses, several industrial applications have also been considered for cardoon biomass, namely, for pulp and paper production,[11−13] power generation,[11,14] and domestic heating.[15] Moreover, the oil from cardoon seed is suitable for biodiesel production.[11,16,17] Thus, cardoon has been regarded as a multipurpose perennial crop for sustainable economic development in southern European countries, well adapted to the Mediterranean climatic and soil conditions, with high biomass productivities (in the range of 15.2−24.2 t/ha).[11,18]

Besides the lignocellulosic fraction, cardoon plantation can be further valorized through the exploitation of high-value extractable compounds, addressing the key goal of the biorefinery concept, that is, the integrated use of all the fractions of any biomass resource.[19] The valorization of any plant biomass requires the detailed knowledge of its chemical composition, the establishment of relationships with traditional applications, and the search for new applications. Regarding dietary and nutraceutical applications, several studies have been focused on the relationship between phenolic composition and biological activity of globe artichoke and cardoon, specifically antioxidant[20] and antitumor[21,22] activities. So far, few studies have been devoted to the study of the lipophilic composition of globe artichoke, for instance, the isolation of sesquiterpene lactones from the leaves[23] and pentacyclic triterpenes from the capitula.[24] However, less attention has been devoted to the lipophilic fraction of *C. cardunculus* L. var. *altilis* (DC) beyond the fatty acid composition of the seeds' oil.[25] Finally, the

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isolation of pentacyclic triterpenes and sterols from cardoon has already been described,[26] but with no differentiation between wild and cultivated cardoon varieties. Therefore, considering the growing interest in the exploitation of cultivated cardoon as a biomass source in the south of Portugal and the lack of detailed information on the extractive composition, as well as our interest in the search for new bioactive plant components, we report here the detailed chemical characterization of the lipophilic fractions of different morphological parts of *C. cardunculus* L. var. *altilis* (DC) by gas chromatography−mass spectrometry (GC−MS), which led to the identification of 36 compounds for the first time in the extracts of this variety. This knowledge can open new perspectives for the valorization of cultivated cardoon, promoting regional and national economic development.

MATERIALS AND METHODS

Chemicals. Dichloromethane (p.a., ≥99% purity), methanol (p.a., ≥99.8% purity), and light petroleum (p.a., ≥99% purity) were supplied by Fischer Scientific (Pittsburgh, PA). Ethyl acetate (p.a., ≥99.8% purity) was purchased from Carlo Erba Reagents (Val de Reuil, France). Hydrochloric acid (p.a., ≥37%) was purchased from Fluka Chemie (Madrid, Spain). Potassium hydroxide (p.a., ≥88% purity), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (99% purity), trimethylchlorosilane (99% purity), hexadecane (99% purity), hexadecanoic acid (≥99% purity), nonadecan-1-ol (99% purity), vanillin (99% purity), cholesterol (99% purity), lupeol (≥94% purity), chloroform-*d* ($CDCl_3$) (99.8% D), tetramethylsilane (≥99.9% purity), and acetyl chloride (p.a., ≥99% purity) were obtained from Sigma Chemicals Co. (Madrid, Spain). Silica gel grade 60 for column chromatography (63−200 μm particle size) and for thin-layer chromatography (5−40 μm particle size) was purchased from Merck (Darmstadt, Germany). Pyridine (p.a., ≥99.5% purity) was purchased from Panreac (Castellar del Vallès, Spain). Cynaropicrin (≥97.5% purity) and β-amyrin (≥98.5% purity) were obtained from Extrasynthese (Genay Cedex, France). Taraxasteryl acetate (≥99.2% purity) was purchased from AvaChem Scientific (San Antonio, TX).

Sample Preparation. *C. cardunculus* L. var. *altilis* (DC) was collected in June 2010 at the Experimental Center of Agriculture School of the Instituto Politécnico de Beja, southern Portugal. The plants were separated into stalks, capitula, and leaves and preserved at −20 °C until analysis. Before extraction, the samples were freeze-dried. Then the stalks were separated into outer and inner parts and capitula into receptacle, bracts, and florets.

Extraction. All plant fractions were ground to a granulometry of 40−60 mesh prior to extraction. Each sample (6 g of dry weight) was Soxhlet extracted with dichloromethane (150 mL) for 7 h. The solvent was evaporated to dryness at low pressure. The dried extracts were weighed, and the results are expressed as percentages of dry biomass material. Dichloromethane was chosen because it is a fairly specific solvent for lipophilic extractives.[27]

Alkaline Hydrolysis. About 20 mg of each extract was dissolved in 10 mL of 1 M KOH in 10% aqueous methanol. The mixture was heated at 100 °C, under a nitrogen atmosphere, for 1 h. The reaction mixture was cooled, acidified with 1 M HCl to pH ≈ 2, and then extracted three times with dichloromethane, and the solvent was evaporated to dryness.[27] The alkaline hydrolysis reaction was performed to detect indirectly esterified compounds, e.g., triglycerides, steryl esters, etc.

GC−MS Analysis. Before GC−MS analysis, nearly 20 mg of each dried sample was converted into trimethylsilyl derivatives.[27] Each sample was dissolved in 250 μL of pyridine containing 1 mg of hexadecane (internal standard), and compounds with hydroxyl and carboxyl groups were converted into trimethylsilyl (TMS) ethers and esters, respectively, by adding 250 μL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide and 50 μL of trimethylchlorosilane. The mixture was maintained at 70 °C for 30 min.

GC−MS analyses were performed using a Trace gas chromatograph (2000 series) equipped with a Thermo Scientific DSQ II mass spectrometer (Waltham, MA). Separation of compounds was carried out in a DB-1 J&W capillary column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness) using helium as the carrier gas (35 cm s^{-1}). The chromatographic conditions were as follows: initial temperature, 80 °C for 5 min; temperature rate, 4 °C min^{-1} up to 260 °C, 2 °C min^{-1} up to 285 °C, which was maintained for 8 min; injector temperature, 250 °C; transfer-line temperature, 290 °C; split ratio, 1:33. The mass spectrometer was operated in the electron impact (EI) mode with an energy of 70 eV, and data were collected at a rate of 1 scan s^{-1} over a range of m/z 33−700. The ion source was kept at 250 °C.[27]

To detect the presence of esterified structures, dichloromethane extracts were also analyzed in a DB-1 J&W short capillary column (15 m × 0.32 mm inner diameter, 0.25 μm film thickness); the chromatographic conditions were as follows: initial temperature, 100 °C for 3 min; temperature gradient, 5 °C min^{-1}; final temperature, 340 °C for 12 min; injector temperature, 290 °C; transfer-line temperature, 290 °C; split ratio, 1:33.[27]

Chromatographic peaks were identified by comparing their mass spectra with the equipment mass spectral library (Wiley-NIST Mass Spectral Library, 1999) and with literature data (as mentioned in the Results and Discussion and Supporting Information) and, when needed, by injection of standard samples. In some cases, identification was also confirmed on the basis of characteristic retention times (RTs) under the described experimental conditions.[27−29] For quantitative analysis, GC−MS was calibrated with pure reference compounds, representative of the major lipophilic extractive components (namely, hexadecanoic acid, nonadecan-1-ol, vanillin, cynaropicrin, lupeol, and cholesterol) relative to hexadecane (the internal standard). The respective response factors were calculated as the average of four GC−MS runs. The compound contents are expressed as milligrams per kilogram of dry weight of plant biomass.

Two aliquots of each extract were analyzed before alkaline hydrolysis, and another two aliquots were analyzed after alkaline hydrolysis. Each aliquot was injected in duplicate. The presented results are the average of the concordant values obtained for each sample (less than 5% variation between injections of the same aliquot and between aliquots of the same sample).

NMR Experiments. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 spectrometer (Wissembourg, France) (300.13 and 75.47 MHz for ^1H and ^{13}C, respectively) using chloroform-*d* as the solvent and tetramethylsilane as the internal reference. Unequivocal ^1H and ^{13}C assignments were made with the aid of unidimensional (1D) distortionless enhancement polarization transfer (DEPT) and bidimensional (2D) correlation spectroscopy (COSY; $^1H/^1H$), heteronuclear single quantum coherence (HSQC; $^1H/^{13}C$), and heteronuclear multiple bond correlation (HMBC; $^1H/^{13}C$) experiments. The low-pass *J*-filter portion of the HMBC experiment was optimized for an average of one-bond heteronuclear coupling of 145 Hz; the delay for evolution of long-range couplings was optimized for 7 and 2 Hz.

Lupenyl Acetate Standard Preparation. Lupenyl acetate standard was prepared by acetylation of lupeol with acetyl chloride in pyridine at room temperature for 12 h. The reaction product was analyzed by GC−MS and the identification confirmed by comparing its EI-MS (Table S1, Supporting Information) with literature data.[30]

Isolation and Characterization of Cynaropicrin and Grosheimin from Leaf Extracts. About 50 g of cultivated cardoon leaves was Soxhlet extracted with dichloromethane (750 mL) for 7 h to afford 7.5 g of crude extract. About 1 g of dried extract was dissolved in dichloromethane, subjected to column chromatography on silica gel, and continuously eluted with a gradient of ethyl acetate in light petroleum, collecting 50 mL of each fraction: fractions F1 and F2 were collected with 10% ethyl acetate, fractions F3 and F4 with 20%, fractions F5 and F6 with 40%, fractions F7 and F8 with 50%, and fractions F9−F11 with 100%. An aliquot of each fraction was submitted to derivatization and analyzed by GC−MS. Fractions F10

and F11, accounting for 363.7 mg, presented the higher relative abundances of cynaropicrin and grosheimin, being mixed together.

Isolation of Cynaropicrin. About 312.4 mg of the F10 and F11 combined fractions was dissolved in dichloromethane and further fractionated by column chromatography on silica gel eluting with 50% ethyl acetate in light petroleum to collect five fractions, F1a–F5a (50 mL each). The column was then eluted with 60% ethyl acetate in light petroleum to collect fractions F6a and F7a (50 mL each) and F8a–F11a (25 mL each). Fraction F11a yielded 10 mg of pure cynaropicrin that was identified by comparing its EI-MS (Table S2, Supporting Information) and NMR data (Table S3 and Figure S1, Supporting Information) with literature data.[31]

Isolation of Grosheimin. About 51.3 mg of the F10 and F11 combined fractions was dissolved in dichloromethane and applied to thin-layer chromatography (TLC) plates, which were eluted with dichloromethane/methanol (95:5). A main spot was detected at R_f = 0.31 and isolated from the TLC plates, yielding 7 mg of pure grosheimin which was identified by comparing its EI-MS data (Table S2, Supporting Information) with the equipment mass spectral library.

RESULTS AND DISCUSSION

Extraction Yield. Dichloromethane extraction yields (%, w/w) of *C. cardunculus* L. var. *altilis* (DC) morphological parts (Table 1) ranged between 1.0% and 2.0% for stalks and 3.3–4.5% for capitula parts, while for the leaves an extraction yield of 17.3% was obtained. The extraction yields of the outer and inner parts of the stalks are in good agreement with previously reported results (0.75–0.98%),[32] while the yield reported here for the leaves is considerably higher than the previously published value (2.7–4.4%).[32]

Table 1. Dichloromethane Extraction Yields (%, w/w) for Different Morphological Parts of *C. cardunculus* L. var. *altilis* (DC)

morphological part of *C. cardunculus* L. var. *altilis* (DC)		extraction yield (%, w/w)
stalks	outer part	1.0
	inner part	2.0
capitula	receptacle and bracts	3.3
	florets	4.5
leaves		17.3

Chemical Composition of the Extracts. The different morphological parts of *C. cardunculus* L. var. *altilis* (DC) presented similar lipophilic fraction compositions in regard to qualitative analysis, either before or after alkaline hydrolysis (Figure 1). However, the abundances of certain compounds are quite different between the different parts of the plant. Table 2 shows the different families and individual compounds identified in the dichloromethane extracts of cultivated cardoon, as well as their quantification before and after alkaline hydrolysis. Sesquiterpene lactones and pentacyclic triterpenes are the main families of lipophilic components present in cultivated cardoon. Fatty acids and sterols, long-chain aliphatic alcohols, and some aromatic compounds were also detected in smaller amounts. The abundances of the different families of lipophilic components in the five parts of cultivated cardoon are shown in Figure 2.

Sesquiterpene Lactones. Guaianolide-type sesquiterpene lactones (Table 2) were found to be the major family of lipophilic components of cultivated cardoon, being mainly concentrated in the leaves (95 g/kg) and representing 52% of the total amount of detected compounds. The stalk parts showed considerably lower amounts of sesquiterpene lactones, while the capitula only presented residual amounts.

Cynaropicrin (Figure 3) was identified as the main sesquiterpene lactone in all morphological parts, except in capitula florets, ranging from about 84% to nearly 100% of the total sesquiterpene lactone content in the inner part of the stalks and capitula receptacle and bracts, respectively. More-

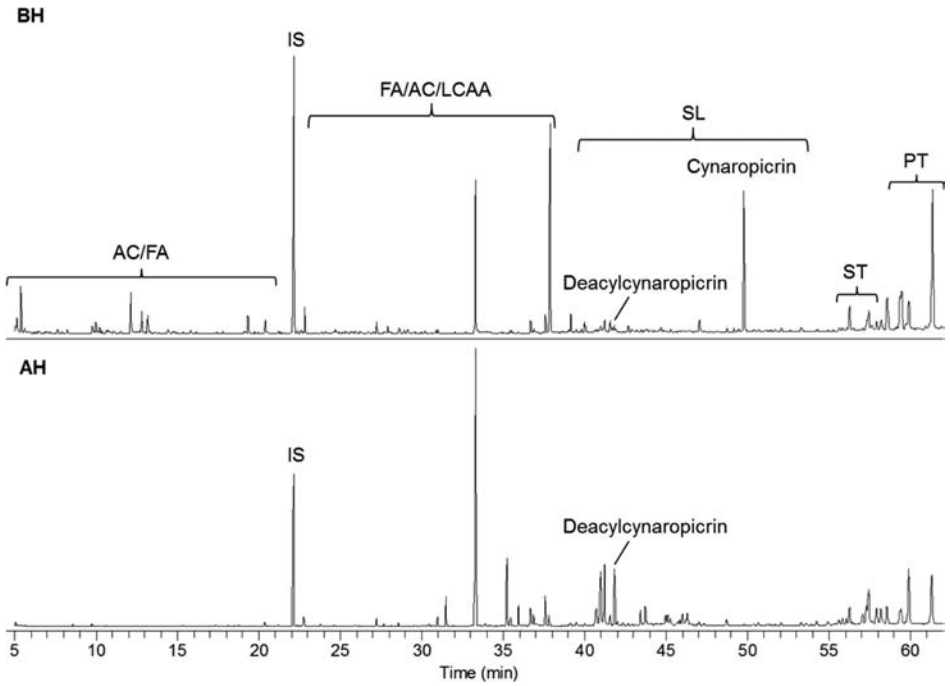

Figure 1. GC–MS chromatogram (DB-1 30 m column) of the TMS-derivatized dichloromethane extract of the outer part of the stalks from *C. cardunculus* L. var. *altilis* (DC) before (BH) and after (AH) alkaline hydrolysis. Abbreviations: AC, aromatic compounds; FA, fatty acids; IS, internal standard (hexadecane); LCAA, long-chain aliphatic alcohols; SL, sesquiterpene lactones; ST, sterols; PT, pentacyclic triterpenes.

Table 2. Composition (mg of compound/kg of dry weight) of Dichloromethane Extracts from Different Morphological Parts of *C. cardunculus* L. var. *altilis* (DC) Obtained before (BH) and after (AH) Alkaline Hydrolysis[a]

		stalks				capitula					
		outer part		inner part		receptacle and bracts		florets		leaves	
RT (min)	compound	BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
	aromatic compounds	**114**	**112**	**223**	**248**	**373**	**460**	**487**	**477**	**516**	**2084**
10.29	benzoic acid	16	13	32	31	53	63	63	66	254	282
19.07	vanillin	17	15	32	31	53	63	ND	ND	ND	279
23.75	syringaldehyde	16	16	32	31	54	64	83	76	ND	277
24.02	2,6-dimethoxyhydroquinone	16	ND	32	30	52	ND	63	ND	ND	ND
26.14	vanillic acid	16	13	32	31	53	63	63	66	ND	278
27.75	3-vanillylpropanol	ND	13	ND	ND	ND	ND	ND	66	262	360
29.35	vanillylpropanoic acid	ND	ND	ND	ND	ND	ND	64	67	ND	ND
29.44	syringic acid	16	13	32	31	52	64	65	68	ND	ND
29.96	*cis*-ferulic acid	ND	13	ND	31	ND	63	ND	ND	ND	281
33.81	*trans*-ferulic acid	16	16	32	34	56	79	ND	69	ND	328
54.88	scopolin	ND	ND	ND	ND	ND	ND	87	ND	ND	ND
	fatty acids	**712**	**1499**	**1200**	**2391**	**1354**	**2748**	**2164**	**3542**	**3721**	**16220**
	saturated	340	913	559	1244	960	1852	1186	2153	2753	8489
28.54	tetradecanoic acid	16	18	27	31	101	63	54	64	210	306
30.94	pentadecanoic acid	15	28	30	44	44	61	55	65	ND	311
33.22	hexadecanoic acid	174	604	266	765	490	1038	545	1009	638	4011
35.41	heptadecanoic acid	15	23	26	32	44	88	52	79	203	290
37.54	octadecanoic acid	31	67	47	92	90	179	98	162	222	765
39.53	nonadecanoic acid	13	11	24	24	ND	49	48	53	ND	237
41.50	eicosanoic acid	21	39	29	41	50	72	72	101	224	544
43.40	heneicosanoic acid	13	18	25	25	41	51	52	56	ND	238
45.21	docosanoic acid	14	23	27	34	48	69	91	191	221	408
46.99	tricosanoic acid	14	16	29	28	ND	57	54	67	209	255
48.69	tetracosanoic acid	15	21	28	34	51	72	65	104	251	347
50.50	pentacosanoic acid	ND	13	ND	25	ND	53	ND	57	ND	ND
52.19	hexacosanoic acid	ND	17	ND	38	ND	ND	ND	71	345	430
56.01	octacosanoic acid	ND	17	ND	31	ND	ND	ND	76	230	347
	unsaturated	360	573	616	1122	394	896	917	1389	968	7731
32.50	*cis*-9-hexadecenoic acid	13	11	25	25	41	52	48	52	ND	220
32.61	*trans*-9-hexadecenoic acid	13	12	26	25	ND	51	49	54	ND	233
36.64	9,12-octadecadienoic acid	181	349	374	749	163	420	511	887	233	1358
36.70	9,12,15-octadecatrienoic acid	47	64	108	169	81	190	175	214	260	4878
36.84	*cis*-9-octadecenoic acid	88	121	53	112	63	121	71	106	232	683
37.00	*trans*-9-octadecenoic acid	17	17	30	41	45	62	62	76	243	359
	hydroxy fatty acids	13	13	25	26	ND	ND	61	ND	ND	ND
15.27	2-hydroxyheptanoic acid	13	13	25	26	ND	ND	ND	ND	ND	ND
24.29	2-hydroxyundecanoic acid	ND	ND	ND	ND	ND	ND	61	ND	ND	ND
	long-chain aliphatic alcohols	**181**	**182**	**146**	**305**	**332**	**977**	**385**	**866**	**1764**	**3170**
31.46	hexadecan-1-ol	26	23	49	51	ND	190	96	154	391	470
35.19	*cis*-9-octadecen-1-ol	28	24	ND	ND	ND	238	ND	ND	ND	ND
35.89	octadecan-1-ol	25	22	49	49	81	147	96	143	ND	456
40.03	eicosan-1-ol	ND	21	ND	48	ND	98	ND	104	ND	ND
43.87	docosan-1-ol	26	23	ND	48	83	99	96	107	ND	494
47.44	tetracosan-1-ol	25	21	48	51	85	103	97	111	442	550
50.79	hexacosan-1-ol	26	24	ND	ND	83	102	ND	124	509	588
54.45	octocosan-1-ol	25	24	ND	58	ND	ND	ND	123	422	612
	sesquiterpene lactones	**301**	**244**	**184**	**408**	**16**	**46**	**ND**	**9**	**94571**	**103028**
39.91	grosheimin	13	ND	20	ND	ND	ND	ND	ND	6248	819
41.75	deacylcynaropicrin	9	244	10	397	ND	46	ND	9	841	102208
49.73	cynaropicrin	230	ND	154	TR	16	ND	ND	ND	87482	ND
	sterols	**320**	**591**	**470**	**694**	**1102**	**1295**	**1441**	**1727**	**1328**	**3346**
53.62	cholesterol	10	13	ND	ND	ND	ND	ND	63	ND	276
55.45	24-methylenecholesterol	ND	7	ND	17	ND	ND	54	65	ND	193
55.61	campesterol	26	46	41	56	81	117	150	190	151	248
56.24	stigmasterol	129	221	252	324	523	542	459	461	338	588

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Table 2. continued

		stalks				capitula					
		outer part		inner part		receptacle and bracts		florets		leaves	
RT (min)	compound	BH	AH	BH	AH	BH	AH	BH	AH	BH	AH
57.42	*β*-sitosterol	131	275	140	257	392	637	498	708	639	1716
57.62	*β*-sitostanol	25	30	38	40	106	ND	ND	ND	ND	ND
57.78	Δ^5-avenasterol	ND	ND	ND	ND	ND	ND	280	239	200	325
	pentacyclic triterpenes	**933**	**937**	**7934**	**7941**	**22107**	**22114**	**27556**	**27558**	**13851**	**13857**
57.30	*β*-amyrin	33	100	81	1090	2348	3520	1197	6041	290	1828
57.91	*α*-amyrin	41	81	63	1144	1886	2796	757	3030	80	300
58.16	lupeol	62	136	48	774	1397	2265	372	1892	524	2853
58.55	*β*-amyrin acetate	63	TR	1003	TR	1164	TR	4844	TR	1538	TR
59.38	*α*-amyrin acetate	40	TR	1081	TR	910	TR	2271	TR	214	TR
59.43	lupenyl acetate	74	TR	727	TR	868	TR	1521	TR	2330	TR
59.70	*ψ*-taraxasterol	46	105	143	1331	2530	3962	1351	6014	705	2925
59.88	taraxasterol	178	515	146	3601	5306	9571	1638	10581	1144	5950
61.12	*ψ* -taraxasteryl acetate	59	TR	1188	TR	1432	TR	4663	TR	2221	TR
61.31	taraxasteryl acetate	336	TR	3455	TR	4265	TR	8942	TR	4806	TR
	others	**4**	**ND**	**11**	**2**	**8**	**ND**	**12**	**ND**	**1221**	**528**
33.02	inositol	Q	ND	Q	ND	Q	ND	Q	ND	34	ND
45.00	2,3-dihydroxypropyl hexadecanoate	4	ND	11	2	8	ND	12	ND	23	ND
48.55	*trans*-squalene	ND	ND	ND	ND	ND	ND	ND	ND	158	130
54.11	*α*-tocopherol	ND	ND	ND	ND	ND	ND	ND	ND	1007	399
	not identified	**419**	**714**	**609**	**515**	**825**	**643**	**1648**	**1152**	**65572**	**17773**
	total detected compds	**2985**	**4280**	**10776**	**12504**	**26116**	**28283**	**33692**	**35331**	**182544**	**160007**

[a]Results are the average of the concordant values obtained (less than 5% variation between injections) for the two aliquots of each sample injected in duplicate. Abbreviations: ND, not detected; Q, quantified by coelution with *trans*-9-hexadecenoic acid; TR, traces.

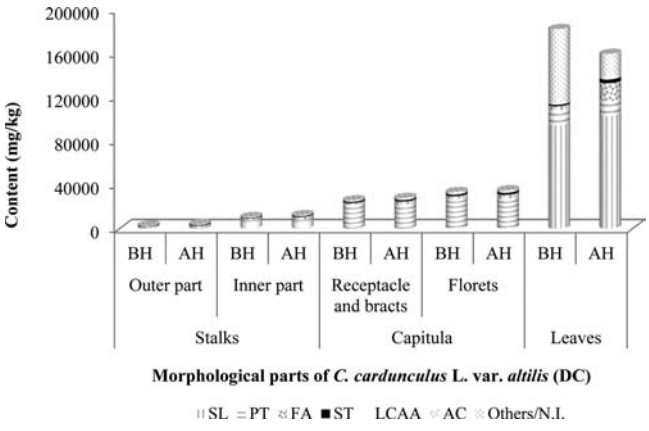

Figure 2. Major families of lipophilic compounds identified in dichloromethane extracts of *C. cardunculus* L. var. *altilis* (DC) before (BH) and after (AH) alkaline hydrolysis. Abbreviations: SL, sesquiterpene lactones; PT, pentacyclic triterpenes; FA, fatty acids; ST, sterols; LCAA, long-chain aliphatic alcohols; AC, aromatic compounds; N.I., not identified.

over, the leaves showed the highest content of this compound, accounting for 87 g/kg. Cynaropicrin was isolated from the leaf extract by preparative column chromatography and identified by NMR, which was a key step in its identification, since the EI-MS of the corresponding TMS derivative was not previously reported in the literature. NMR data of the isolated compound (Table S3 and Figure S1, Supporting Information) were closely comparable to published data for cynaropicrin.[31] Additionally, the EI-MS of the isolated cynaropicrin (Table S2, Supporting Information) showed a low-abundance molecular ion at *m*/*z* 346 and an intense fragmentation peak at *m*/*z* 244 which may have resulted from the loss of the ester unit $C_4H_5O_3$ and a hydrogen atom.[6] EI-MS of the cynaropicrin TMS derivative (Table S2, Supporting Information) showed a molecular ion at *m*/*z* 490, an intense peak at *m*/*z* 316 from the loss of the silylated ester unit derivative and a hydrogen atom, and a base peak at *m*/*z* 73 due to $[(CH_3)_3Si]^+$, typical of silylated compounds. Cynaropicrin was previously isolated from *C. cardunculus* L. varieties, namely, globe artichoke[33,34] and cardoon,[34] although with no differentiation between cultivated or wild cardoon.

Grosheimin and deacylcynaropicrin (Figure 3) represented respectively 4−11% and 1−5% of the total sesquiterpene lactone content (Table 2). Grosheimin was found in considerable quantities in the leaves (6.2 g/kg) and in minor amounts in the inner and outer parts of the stalks. This compound was previously detected in globe artichoke and cardoon.[35] Deacylcynaropicrin was also concentrated in the leaves of cultivated cardoon, but at lower abundance (0.84 g/kg). This compound is reported here for the first time as a *C. cardunculus* L. var. *altilis* (DC) component, although it was previously detected in *C. cardunculus* L. var. *scolymus*.[36] These compounds were identified by comparing the EI-MS data (Table S2, Supporting Information) with the equipment mass spectral library and literature data.[31]

After alkaline hydrolysis, higher amounts of sesquiterpene lactones were observed in the extracts of all morphological parts, except the outer part of the stalks, which may be related to the deacylcynaropicrin content increment. The content of this compound in hydrolyzed extracts was much higher than the total content of cynaropicrin and deacylcynaropicrin in the respective dichloromethane extracts, mainly in the inner part of the stalks. In this way, deacylcynaropicrin or cynaropicrin may be esterified to high molecular weight compounds. On the

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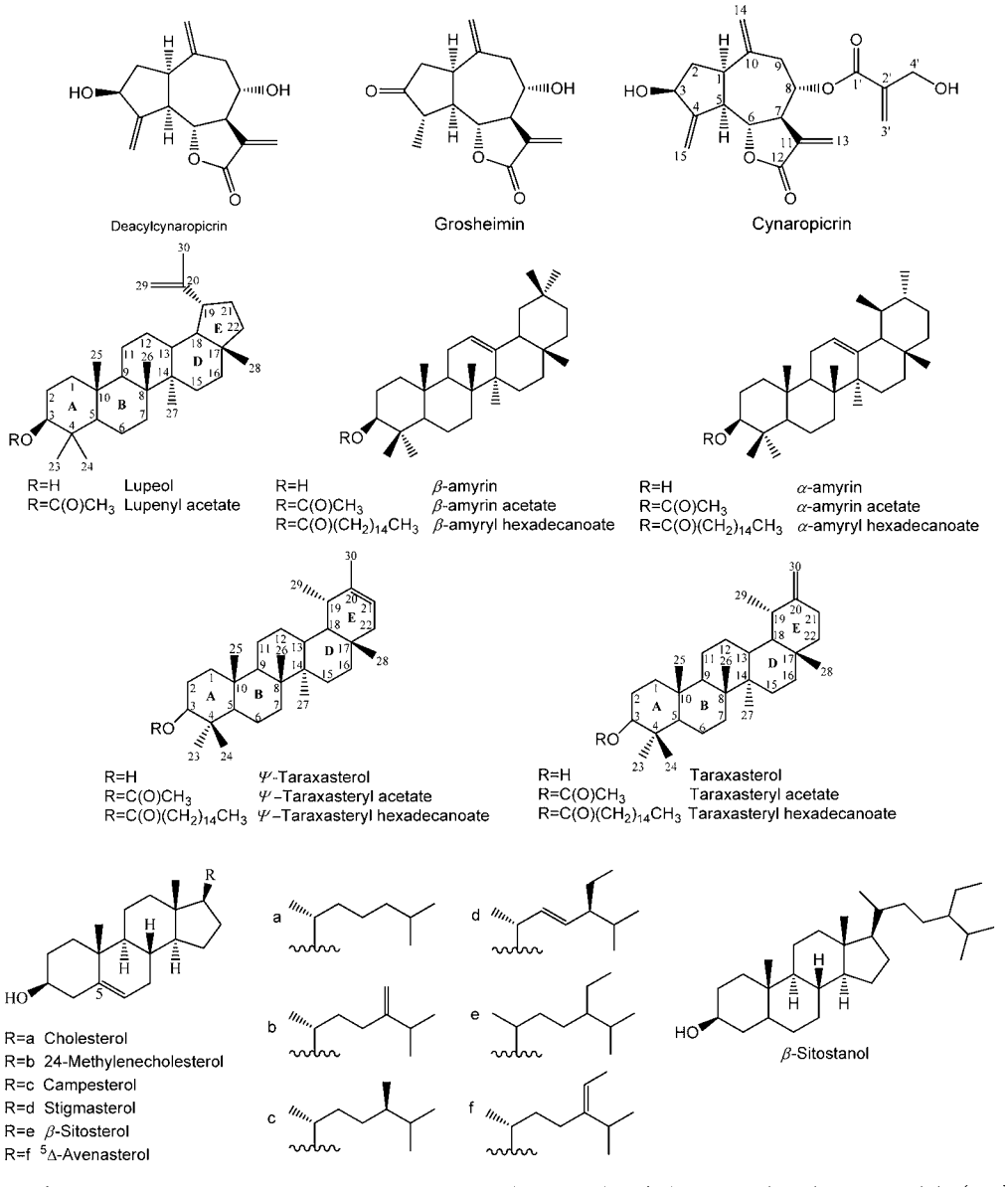

Figure 3. Structures of sesquiterpene lactones, pentacyclic triterpenes, and sterols identified in *C. cardunculus* L. var. *altilis* (DC).

contrary, the grosheimin abundance decreased after alkaline hydrolysis, probably being retained in the aqueous phase.

Pentacyclic Triterpenes. Pentacyclic triterpenes (Table 2) represented the major family of lipophilic components of all morphological parts of *C. cardunculus* L. var. *altilis* (DC), with the exception of the leaves, ranging from 0.93 g/kg in the outer part of the stalks to 28 g/kg in the capitula florets. This family ranged from 8% to 84% of the total amount of detected compounds in the leaves and capitula receptacle and bracts, respectively. Taraxastane-, lupane-, oleanane- and ursane-type triterpenes (Figure 3) were found in the different parts of cultivated cardoon.

These compounds were identified by comparing the EI-MS data of the corresponding TMS derivatives (Table S1, Supporting Information) with reference compounds run under the same experimental conditions or with literature data as discussed below.

Taraxastane-type compounds revealed the highest abundance among pentacyclic triterpenes in *C. cardunculus* L. var. *altilis* (DC), accounting for 60−66% of the total pentacyclic triterpene content. Taraxasteryl acetate (Figure 3) was detected as the main taraxastane triterpenic component in all morphological parts of cultivated cardoon, except in the receptacle and bracts of the capitula, ranging from 0.34 g/kg in the outer part of the stalks to 8.9 g/kg in the capitula florets (Table 2). On the other hand, taraxasterol (Figure 3) was the major taraxastane-type triterpene in the receptacle and bracts of the capitula (5.3 g/kg). Lupane-type triterpenic compounds such as lupeol and lupenyl acetate (Figure 3) were found in considerable amounts (7−21% of the total pentacyclic triterpene content), mainly in extracts of the capitula.

TMS derivatives of taraxasterol, ψ-taraxasterol, and lupeol showed similar EI-MS profiles (Table S1, Supporting Information), namely, a molecular ion at *m/z* 498 and two characteristic product ions due to C9−C11 and C8−C14 bond cleavages, containing A and B rings (*m/z* 279), as well as D and E rings and part of the C ring (*m/z* 218), respectively. Furthermore, the product ion at *m/z* 189 (base peak)

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contained D and E rings, due to C12−C13 and C8−C14 bond cleavages. Other characteristic product ions were observed at m/z 408, 203, and 175.[37,38] The unambiguous identification of these compounds was complemented by comparing their elution order with literature data,[39] while lupeol identification was further confirmed with the injection of a standard sample. Similarly, taraxasteryl, ψ-taraxasteryl, and lupenyl acetates were identified by comparing the EI-MS data with literature data.[30] EI-MS of these compounds (Table S1, Supporting Information) showed a molecular ion at m/z 468 and two fragments at m/z 249 and 218, resulting respectively from the bond cleavages at C9−C11 and C8−C14, similar to those for TMS derivatives of the corresponding alcohol derivatives. A product ion at m/z 204 resulted from two bond cleavages at C11−C12 and C8−C14, containing D and E rings. Other characteristic product ions from EI-MS of these compounds were also found at m/z 408 and 189.[30] The identification of lupenyl and taraxasteryl acetates was further confirmed by injection of standard samples; furthermore, in the case of ψ-taraxasteryl acetate, its identification was based on the 370 similar EI-MS fragmentation and elution order compared to 371 those of taraxasteryl acetate.[39]

Lupenyl acetate is identified here for the first time as a component of *C. cardunculus* L. species. The remaining triterpenic compounds were previously reported as components of the capitula[24] and leaves[33] of *C. cardunculus* L. var. *scolymus*. Furthermore, taraxasterol and its acetate derivative were previously isolated from cardoon's roots,[26] while both taraxastane alcohols and lupeol were detected in cardoon's capitula,[40] although no specification of either wild or cultivated varieties was reported. However, ψ-taraxasteryl acetate was detected here for the first time as a *C. cardunculus* L. var. *altilis* (DC) component.

β-amyrin and its acetate derivative (Figure 3) were detected in considerable abundance (10−22% of the total pentacyclic triterpenic content) in all morphological parts of cultivated cardoon, followed by α-amyrin and its acetate derivative (Figure 3) (2−14% of the total pentacyclic triterpenic content). These compounds were identified by comparing the EI-MS fragmentation of their TMS derivatives (Table S1, Supporting Information),[30,41] retention time,[28] and elution order[39] with literature data. α- and β-amyrin and the corresponding acetates were previously reported in the capitula of *C. cardunculus* L. var. *scolymus*,[24] while α- and β-amyrin were detected in cardoon's capitula,[40] without specification of cultivated or wild cardoon varieties. α- and β-amyrin acetate derivatives were reported here for the first time as *C. cardunculus* L. var. *altilis* (DC) components.

The content of pentacyclic triterpene alcohols increased in all hydrolyzed extracts of cultivated cardoon. This variation can be related to alkaline hydrolysis from either the corresponding acetates or triterpenyl fatty acid esters.

Indeed, triterpenyl fatty acid esters were detected in GC−MS chromatograms obtained with short-length columns within the 44−46 min range (Figure S2, Supporting Information). The EI-MS of the peak at RT = 44.39 min showed a molecular ion at m/z 664 and two characteristic product ions at m/z 649 and 409, the last one due to the loss of the hexadecanoic acid (Table S1, Supporting Information).[42] Comparing the EI-MS data and elution order with literature data, this compound can be tentatively assigned as β-amyryl hexadecanoate.[42] Furthermore, other product ions were observed at m/z 218, 203 and 189, resulting from the EI fragmentations of β-amyrin[43] after hexadecanoic acid loss.[42] The chromatographic peak at RT = 44.78 min seems to result from the coelution of two triterpenyl hexadecanoates. Its EI-MS spectrum (Table S1, Supporting Information) showed a molecular ion at m/z 664 and characteristic product ions at m/z 649 $[M - CH_3]^+$ and 409 $[M - CH_3(CH_2)_{14}COO]^+$. However, two fragmentation peaks at m/z 189 and 218 with 100% relative abundance were observed; these are generally the base peaks of distinct triterpene classes, namely, lupane and ursane. Considering the pentacyclic triterpenols identified here, this peak is most probably composed of lupenyl hexadecanoate (for m/z 189) and α-amyryl hexadecanoate (for m/z 218),[44] since, although the base peaks of the taraxasterol and ψ-taraxasterol EI-MS spectra are the same as that of lupeol (m/z 189), it is not probable that taraxasteryl or ψ-taraxasteryl hexadecanoate (or a mixture) coelutes with α-amyryl hexadecanoate, as it is known that taraxasteryl hexadecanoate elutes after α-amyryl hexadecanoate.[45]

The EI-MS data (Table S1, Supporting Information) of the chromatographic peak at RT = 45.50 min showed a molecular ion at m/z 664, followed by characteristic product ions of a triterpenyl hexadecanoate at m/z 649 $[M - CH_3]^+$ and 408 $[M - CH_3(CH_2)_{14}COOH]^+$, as well as other fragments at m/z 393, 207, 189, and 175, resulting from taraxasterol fragmentation.[42] In addition to EI-MS data, this compound elutes after α-amyryl hexadecanoate. Therefore, it is most probable that this peak corresponds to taraxasteryl hexadecanoate. Although ψ-taraxasteryl fatty acid esters have not been reported before, given the similarity of the EI-MS spectra of both taraxasterol and ψ-taraxasterol, the possibility of this peak being ψ-taraxasteryl hexadecanoate or a mixture of both cannot be excluded.

The presence of triterpenyl fatty acid esters as components of *C. cardunculus* L. species was reported here for the first time.

Fatty Acids. Fatty acids (Table 2) were mainly concentrated in the leaves (3.9 g/kg). Moreover, this family represented the second major group of lipophilic compounds derived from the outer and inner parts of the stalks, corresponding respectively to 24% and 12% of the total amount of detected compounds. Both saturated and unsaturated fatty acids were identified in *C. cardunculus* L. var. *altilis* (DC) in appreciable amounts, accounting respectively for 44−70% and 25−49% of the total fatty acid content. Saturated fatty acids were predominantly observed in the leaves, while unsaturated fatty acids were detected in both the outer and inner parts of the stalks. Hexadecanoic, 9,12-octadecadienoic, and 9,12,15-octadecatrienoic acids were the predominant fatty acids and were previously detected in the seeds' oil from cultivated cardoon.[25] Finally, odd-numbered saturated fatty acids, namely, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, and tricosanoic acids, were also found in low amounts in the studied extracts. Apart from heptadecanoic acid, these compounds were identified here for the first time as *C. cardunculus* L. components. Finally, two 2-hydroxy fatty acids, namely, 2-hydroxyheptanoic and 2-hydroxyundecanoic acids, were detected among the minor components. To the best of our knowledge, both compounds were identified for the first time as *C. cardunculus* L. components.

After alkaline hydrolysis, increased fatty acid contents were observed in all morphological parts, particularly in leaf extracts, with a 4-fold increment. 9,12,15-Octadecatrienoic acid was the major fatty acid present in leaf extracts after hydrolysis, followed by hexadecanoic acid. Furthermore, the hydrolyzed

extracts presented higher contents of odd-numbered saturated fatty acids.

Sterols. Several Δ^5-sterols (Table 2) were detected in all morphological parts of cultivated cardoon at low abundance, varying between 1% and 11% of the total detected compounds. These compounds were mainly found in the capitula florets (1.4 g/kg), followed by the leaves (1.3 g/kg). Four classes of Δ^5-sterols (Figure 3) were identified here, namely, cholestane, ergostane, campestane, and stigmastane sterols, with the last group representing 86−93% of the total sterol content. β-Sitosterol was the major stigmastane-type sterol detected in the outer part of the stalks, capitula florets, and leaves, while stigmasterol was mainly concentrated in the remaining parts. Campesterol was the single campestane-type sterol found in *C. cardunculus* L. var. *altilis* (DC), accounting for 7−11% of the total sterol content. Ergostane-type sterols such as 24-methylenecholesterol were detected in very low amounts in the capitula florets. Cholestane-type sterols such as cholesterol, an intermediary in sterol biosynthesis, was also detected in very low amounts in the outer part of the stalks.

24-Methylenecholesterol, β-sitostanol, and Δ^5-avenasterol were identified by comparing the EI-MS data and elution order of the corresponding TMS derivatives with literature data.[46,47] The remaining sterols were identified by comparing the EI-MS data of the corresponding TMS derivatives with the equipment mass spectral library.

Only a few studies have previously reported the sterol composition of *C. cardunculus* L. plants. β-sitosterol was previously isolated from involucral bracts of the capitula and roots of cardoon,[26] but with no reference to wild or cultivated varieties. Moreover, stigmasterol was identified in the capitula and leaves of *C. cardunculus* L. ssp. *cardunculus*.[48] Therefore, this compound was here identified for the first time as a *C. cardunculus* L. var. *altilis* (DC) component. Furthermore, 24-methylenecholesterol, campesterol, and Δ^5-avenasterol are mentioned here for the first time as components of *C. cardunculus* L. species.

After alkaline hydrolysis, the sterol content increased between 1.2- and 2.5-fold in extracts of all morphological parts of *C. cardunculus* L. var. *altilis* (DC). This is in agreement with the identification of esterified sterol structures in the GC−MS chromatograms obtained with a short-length column (Figure S2, Supporting Information). It is also worth mentioning that the sterol content variation with hydrolysis is not related to the presence of steryl glycosides.[49] Hydrolyzed extracts of the leaves showed the highest sterol content, mainly composed of β-sitosterol, representing 51% of the total sterol content.

Long-Chain Aliphatic Alcohols. Long-chain aliphatic alcohols (Table 2) were detected in quite low amounts, accounting for 1−5% of the total detected compounds in all morphological parts of *C. cardunculus* L. var. *altilis* (DC). Hexacosan-1-ol was the major compound of this family before alkaline hydrolysis. After alkaline hydrolysis, increased contents of long-chain aliphatic alcohols were detected in all morphological parts of cultivated cardoon. Moreover, eicosan-1-ol was only detected after alkaline hydrolysis in all morphological parts, with the exception of the leaves. To the best of our knowledge, these compounds were found here for the first time in *C. cardunculus* L. var. *altilis* (DC) extracts.

Aromatic and Other Compounds. Aromatic compounds (Table 2) were also detected in low amounts (between 0.3% and 4% of the total detected compounds) in extracts of *C. cardunculus* L. var. *altilis* (DC). 3-Vanillylpropanol was the major aromatic compound found in the leaves, followed by benzoic acid. Scopolin was only detected in the capitula florets. Besides 3-vanillylpropanol and benzoic acid, vanillin, syringaldehyde, 2,6-dimethoxyhydroquinone, and vanillylpropanoic acid were reported here for the first time as *C. cardunculus* L. var. *altilis* (DC) components. Scopolin was previously identified in cardoon's involucral bracts,[26] while the remaining aromatic compounds identified here were detected in cardoon's leaves.[50]

Aromatic compounds were identified on the basis of the EI-MS data of the TMS derivatives, by comparison to the equipment mass spectral library and literature data.[27,29,51]

With the exception of the outer part of the stalks and capitula florets, hydrolyzed extracts of *C. cardunculus* L. var. *altilis* (DC) showed increased contents of aromatic compounds after alkaline hydrolysis, in particular leaf extracts (4-fold). 3-Vanillylpropanol was still the most abundant aromatic compound in the hydrolyzed extracts, followed by *trans*-ferulic acid. Moreover, *cis*-ferulic acid was only detected after alkaline hydrolysis in all extracts, except in the capitula florets, which can be related to the fact that hydroxycinnamic acids tend to be found in plants esterified with lignin- and polysaccharide-like components.[27,49,52]

Finally, 2,3-dihydroxypropyl hexadecanoate (monoglyceride) and *trans*-squalene were also detected in *C. cardunculus* L. var. *altilis* (DC) in reduced amounts; α-tocopherol was only detected in the leaves (1.0 g/kg).

The present study shows, to the best of our knowledge, one of the first detailed studies about the lipophilic composition of the different morphological parts of *C. cardunculus* L. var. *altilis* (DC) from the south of Portugal. The outer and inner parts of the stalks, the receptacle, bracts, and florets of the capitula and, particularly, the leaves present high lipophilic extractive contents. The leaves of cultivated cardoon were shown to be a rich source of sesquiterpene lactones and pentacyclic triterpenes. Cynaropicrin was the most abundant sesquiterpene lactone in cultivated cardoon, especially in the leaves. Moreover, this work showed for the first time that deacylcynaropicrin is present in *C. cardunculus* L. var. *altilis* (DC). Regarding pentacyclic triterpenes, taraxasteryl acetate was the main compound of this family, followed by taraxasterol, essentially observed in the capitula components. Lupenyl acetate was detected here for the first time in *C. cardunculus* L. species, while β- and α-amyrin and ψ-taraxasteryl acetates were found for the first time in *C. cardunculus* L. var. *altilis* (DC). Since sesquiterpene lactones and pentacyclic triterpenes, namely, cynaropicrin and taraxasteryl acetate, are known respectively for their tumor antiproliferative[31] and anti-inflammatory[24] properties, this study is an important contribution for *C. cardunculus* L. var. *altilis* (DC) valorization as a source of bioactive compounds. Finally, the extraction of high-value bioactive compounds can be perfectly integrated with the large-scale applications of cardoon (as a source of biomass for energy conversion and for paper pulp production), contributing to the integrated valorization of the species and therefore to the development of Mediterranean regions where this species is produced.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

EI-MS data of the pentacyclic triterpenes identified in the form of TMS derivatives and sesquiterpene lactones identified in *C. cardunculus* L. var. *altilis* (DC), ^{1}H and ^{13}C NMR data of

cynaropicrin, key HMBC correlations found for cynaropicrin, and GC−MS chromatogram of the TMS-derivatized dichloromethane extract of the outer part of the stalks of *C. cardunculus* L. var. *altilis* (DC). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS

AH, after hydrolysis; BH, before hydrolysis; EI, electron impact; GC−MS, gas chromatography−mass spectrometry; HMBC, heteronuclear multiple bond correlation; NMR, nuclear magnetic resonance; RT, retention time; TLC, thin-layer chromatography; TMS, trimethylsilyl

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