Acta Biologica Hungarica 66(1), pp. 103–118 (2015)
DOI: 10.1556/ABiol.66.2015.1.9

DIVERSITY OF ACTIVE CONSTITUENTS IN *CICHORIUM ENDIVIA* AND *CYNARA CORNIGERA* EXTRACTS

Ahmad K. Hegazy,[1,2]* Shahira M. Ezzat,[3] Iman B. Qasem,[4]
Mohamed S. Ali-Shtayeh,[5]
Mohammed O. Basalah,[1] Hayssam M. Ali[1] and Ashraf A. Hatamleh[1]

[1]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[2]Department of Botany, Faculty of Science, Cairo University, Giza 12613, Egypt
[3]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
[4]Biodiversity and Environmental Research Center (BERC)/ Til, POB 696 Nablus, West Bank, Palestine
[5]Department of Biology, Faculty of Science, An-Najah University, West Bank, Palestine

(Received: February 25, 2014; accepted: June 16, 2014)

The present study attempts to explore the phytochemical constituents of different extracts from *Cynara cornigera* and *Cichorium endivia* plant materials. The two species studied are native in Egypt. Five different solvents, *viz.*, aqueous, methylene chloride, petroleum ether, ethyl acetate, and *n*-butanol were used. Phytochemical analysis revealed the presence of phenols, flavonoids, sterols (stigmasterol and beta-sitosterol), terpenes (α-amyrin, ursolic and oleanolic acid), and hydrocarbons (*n*-alkane), the latter found in low amount. The ethyl acetate and water extracts of *C. cornigera* root showed lower mass fractions of phenolic compounds ranged from 20 to 81 g/100 g, and higher amounts in ethyl acetate extract of the inflorescences and butanol extract of the root where values ranged from 195 to 399 g/100 g. The β-sitosterol and stigmasterol were present in all plant extracts. Oleanolic and ursolic acids were detected in roots, leaves and inflorescences of *C. cornigera* and in *C. endivia* shoot. The ethyl acetate extracts from *C. cornigera* leaf and inflorescence attained higher chemical diversity than the other extracts. Alternatively, sterols and triterpenes were the major constituents. The high chemical diversity of active constituents justifies the future potential use of the two species at commercial level.

Keywords: Sterols – triterpenes – flavonoids – phenolic acids – Egypt

INTRODUCTION

The increasing use of plant extracts in food, cosmetic and pharmaceutical industries suggests a systematic study of medicinal plants around the world [49]. Biotic and abiotic stresses exert a considerable influence on the secondary metabolite pool especially the qualitative and quantitative phenolic composition [1]. Polyphenols when associated with various carbohydrates and organic acids exhibit a wide range of pharmaceutical properties including antiallergic, antiatherogenic, antiinflammatory, antimicrobial, antioxidant, antithrombotic, cardioprotective, antioxidants, and vasodilatory effects [2, 5, 36].

*Corresponding author; e-mail address: akhegazy@yahoo.com

The wild cichory or endive, *Cichorium endivia* L. (Asteraceae) is a Mediterranean perennial herb indigenous to Europe, Western Asia and North Africa [17]. The genus *Cichorium* comprises seven species native to the Mediterranean basin [42]. Strong antioxidant activities were found in the polyphenolic fraction of two *Cichorium* varieties; *Cichorium endivia var. crispum* and *var. latifolium* [41], where both varieties contain chlorogenic acid derivatives [14, 26]. Alternatively, different active constituents of other *Cichorium* spp. were reported [27, 36a], and few other studies reported different active constituents and medicinal uses of *Cichorium endivia* [29, 45, 54].

The wild artichoke, *Cynara cornigera* Lindl. (Asteraceae) is a perennial herbaceous species, originating from the Mediterranean region. The species is widely cultivated for economic purposes all over the world [15]. Cynaroside, apigenin-7-O-glucoside, and scolymoside and two sesquiterpens, *viz.*, grosheimin and solstitalin A were identified from the leaf extracts [6, 15, 52, 57]. Extracts from *C. scolymus* are used in folk medicine against liver complaints and to improve liver regeneration after partial hepatectomy [9, 19]. Flower head of *C. cornigera* is eaten as a vegetable and prepared for different value-added products such as salad, concentrate, and canned beverages [56]. The flavonoid kaempferol 3-O-(6-O-malonyl) was reported from the root extracts of the two species [14].

Considering the reported chemical constituents in other species of Asteraceae, this study decided to screen for additional secondary metabolites that might be present in the wild cichory and artichoke native to Egypt. The aim is to explore the active constituents in the two species, particularly the phenolic compounds, terpenes and steroid contents.

MATERIALS AND METHODS

Plant extracts

Plant materials were collected from wadi Habis, about 30 km west of Matrouh city, Mediterranean coast, Egypt. Individual plants were excavated and separated into different organ components, *viz.*, roots, stems, leaves and inflorescences (flower heads) for wild artichoke and whole plant phytomasss for wild cichory. The plant materials were cut into pieces and air-dried in shade at room temperature. The dry material (792 gm) of wild cichory; and leaves (675 gm), roots (864 gm) and flower heads (630 gm) of wild artichoke were ground into powder. Extraction was performed in 90% ethyl alcohol at room temperature till exhaustion, and was then concentrated under reduced pressure to dry at 40 °C using a rotary evaporator. Materials were subjected to successive extraction with petroleum ether, methylene chloride, ethyl acetate, and *n*-butanol using separating funnel. The solvents were used in the order to increase polarity. The residue obtained from each solvent was dried and weighed. The extracts were kept at 4 °C till used for phytochemical screening.

Phenolic compounds

Qualitative and quantitative determinations of phenolic compounds were carried out according to Dimitrios et al. [12]. The retention time (RT) of the compounds isolated from *C. endivia* and *C. cornigera* was compared with that of corresponding standards under the same conditions and further confirmed by co-injection with isolated standards. The compounds were then injected to a High Performance Liquid Chromatography (HPLC) system at the same time as the solvent extracts and the chromatogram was run with a gradient of CH_3OH in acetic water (10 to 90% CH_3OH in water in the presence of 5% CH_3COOH). Throughout the experiment each peak detected in the range from 254 to 280 nm in 30 min at 20 °C corresponded, as a rule, to a single molecule. Most compounds exhibited absorption peaks in the low-wavelength region 245 nm.

All the HPLC samples (one gram each) were prepared by extraction with 50 ml methanol (Lab scan) and sub-coiled condensed for one hour. After evaporation, the residue was dissolved in methanol to give a final concentration of 0.0025–0.025% (w/v). Filtration over acrodisc-CR removed the insoluble particles. The samples were analysed at room temperature (20 °C) in a Brucker LC 42 (Brucker Franzen Analytic Gmbh) whith UV diode array detection, LC 21 pump, LC 51 automatic sample analyzer, LC 211 oven, using a spherisorb C16 ODS column, 5 µm , 250 mm × 4.6 mm (Bischoff, Leonberg). The flow rate was 1 ml/min, and the injection volume 5 µl. The operating system was the Chromstar Data system of the Brucker LC 42 connected to an Epson computer. Both the height and the area of a well-resolved peak in a chromatogram were proportional to the concentration of the compounds detected. For quantification, either peak height or peak area was used.

Lipids and triterpenes

Plant extracts were used for the investigation of lipid and triterpene contents using Unicam Pro-GC with [(Column: 3%OV-17(Methyl phenyl Silicone) on chromosorb-WHP); (Mesh: 100–120) and (Dimensions: 1.5 × 4 mm)]. The percentage composition of the extracts was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data base. The condtions of GC used are shown in Table 1.

Table 1
Separation conditions of unsaponifiable matters during GC analysis

Temperature Programing						Gases Flow Rate		
Initial temperature	Rate	Final temperature	Final time	Injector	Detector	N_2	H_2	Air
70 °C	10 °C/min	270 °C	50 min	250 °C	300 °C	30 ml/min	33 ml/min	330 ml/min

The quantification of Oleanolic acid (OA) and Ursolic acid (UA) followed the method described by Wang et al. [53]. Standard solutions of OA and UA were prepared in final concentration of 25 µg/ml of each compound. The petroleum ether extracts were dissolved in 5 ml methanol then an aliquot of 50 µl extract was diluted in 5 ml methanol. Sampels of 20 µl were injected in the HPLC apparatus. The peaks in the chromatogram were identified in comparison to their retention time with the corresponding standards under the same conditions.

RESULTS

Successive extracts yield

The yield of ethanolic extracts and the sucssive solvent extraction of *C. endivia* and *C. cornigera* are presented in Table 2. The highest yield of *C. endivia* was obtained from the ethanolic and water extracts with values 18.95 and 16.47 mg/100 g, respectively. The highest yield from *C. cornigera* was obtained from the ethanolic extract of roots (111.22 mg/100 g), followed by leaves (110.24 mg/100 g) and the least amounts in the flower heads (86.38 mg/100 g). Considering the order of extract dependent yield in *C. cornigera*, the order of root extract yield was ethanol > water > ethyl acetate > *n*-butanol > methylene chloride > petroleum ether, while for leaf and flower the yields were ethanol > water > n-butanol > ethyl acetate > methylene chloride > petroleum ether.

Phenolic compounds and flavonoids

Twenty phenolic compounds were detected in the two study species, eight flavonoids, *viz.*, Catechin, apigenin, luteolin, luteolin-3-methoxy-7-rutinoside, rutin, quercetin, chrysin and 5-7-dihydroxy-4-methisoflavone and 12 phenolic acids (Tables 3 and 4).

Table 2
The yield (mg/100 g) of *Cichorium endivia* and *Cynara cornigera* extracts obtained by the different solvents

Plants	Total extract of dry plant material						
	Dry weight (g)	Ethanol	Petroleum ether	Methylene chloride	Ethyl acetate	*n*-Butanol	Water
C. endivia	812	18.95	0.12	0.56	0.44	1.34	16.47
C. cornigera roots	675	111.22	0.06	0.19	1.14	1.13	108.68
C. cornigera leaves	864	110.24	0.05	0.59	1.13	1.96	106.48
C. cornigera flower heads	630	86.38	0.05	0.40	1.48	1.85	84.44

Values rounded to the nearest two decimals.

Table 3
Retention time (min) and percentage (%) of phenolic compounds in *Cynara cornigera* extrcts

Compound	Ethyl acetate (Flower head)		Ethyl acetate (Leaf)		Ethyl acetate (Root)		*n*-Butanol (Root)		Water (Root)	
	Retention time (min)	%	Retention time (min)	%	Retention time (min)	%	Retention time (min)	%	Retention time (min)	%
Gallic acid	14.38	0.09	ND	ND	ND	ND	14.68	0.14	14.68	8.52
Pyrogallic			ND	ND	ND	ND	17.87	0.11		
Chlorogenic acid	22.02	1.75	ND	ND	ND	ND	ND	ND	ND	ND
Resorcinol	ND	ND	ND	ND	ND	ND	22.80	1.11	22.43	5.55
Catechin	23.59	0.42			23.87	2.10	ND	ND	23.83	3.60
p-Hydroxy benzoic acid	26.39	12.41	26.27	0.69	ND	ND	ND	ND	26.41	1.23
Luteolin-3-methoxy-7-rutinoside	ND	ND	27.01	2.46	26.89	1.30	27.06	0.17	ND	ND
Caffeic acid	29.40	4.40	29.34	2.25	29.49	8.86	29.6	12.65	29.51	1.81
Vanillin	29.88	0.42	30.00	26.37	30.04	23.03	29.83	16.74	30.15	0.80
Ferulic acid	30.96	2.87	30.98	4.82	ND	ND	30.86	16.33	ND	ND
Rutin	ND	ND	31.53	0.23	31.47	4.81	ND	ND	ND	ND
Phenol	32.16	0.95	32.16	7.70	32.21	2.12	32.22	0.41	32.25	0.36
p-Coumaric acid	32.39	4.07	32.72	1.33	ND	ND	ND	ND	ND	ND
Luteoline	ND	ND	34.30	0.15	34.88	0.30	34.42	0.05	ND	ND
Quercetin	36.32	0.57	36.54	2.71	36.44	1.67	ND	ND	36.63	0.16
Salicylic acid	ND	ND	37.53	0.43	ND	ND	37.35	1.11	ND	ND
Apigenin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Euganol	ND	ND	ND	ND	ND	ND	41.44	0.03	ND	ND
5,7-Dihydroy 4-methoxy isoflavone	43.68	0.35	43.60	0.38	43.49	0.63	ND	ND	ND	ND
Chrysin	ND	ND	ND	ND	ND	ND	ND	ND	44.24	1.80

Values rounded to the nearest two decimals. ND = Not detected.

Table 4
Retention time (min) and percentage (%) of phenolic compounds of *Cichorium endivia* extracts

Compound	Ethanol		Ethyl acetate		Water	
	Retention time (min)	%	Retention time (min)	%	Retention time (min)	%
Gallic acid	14.657	4.04	ND	ND	14.62	6.26
Pyrogallic	17.70	2.33	ND	ND	17.72	0.41
Chlorogenic acid	22.03	1.90	ND	ND	21.95	0.51
Resorcinol	ND		ND	ND	22.61	2.24
Catechin	23.69	2.10	ND	ND	23.94	16.63
p-Hydroxy benzoic acid	26.71	18.66	26.83	7.62	26.65	0.89
Luteolin-3-methoxy-7-rutinoside	ND	ND	ND	ND	26.94	0.58
Caffeic acid	29.40	1.50	ND	ND	27.31	0.76
Vanillin	ND	ND	30.06	1.65	29.69	5.35
Ferulic acid	30.98	0.65	30.96	0.60	ND	ND
Rutin	31.23	1.31	ND	ND	31.21	0.55
Phenol	ND	ND	32.02	2.29	ND	ND
p-Coumaric acid	32.46	1.15	32.50	0.90	32.41	0.01
Luteoline	34.85	0.09	ND	ND	34.73	0.06
Quercetin	36.28	3.10	ND	ND	ND	ND
Salicylic acid	36.98	0.56	ND	ND	37.36	0.08
Apigenin	ND	ND	ND	ND	39.75	0.06
Euganol	ND	ND	41.37	0.49	ND	ND
5,7-dihydroy 4-methoxy isoflavone	ND	ND	43.71	0.50	43.37	0.04
Chrysin	44.050	0.7659	ND	ND	ND	ND

Values rounded to the nearest two decimals. ND = Not detected**.**

All plant organs contained phenolic compounds in different concentrations, ranging between 0.01 and 135.63 mg/100 g (Table 5). Ethyl acetate extracts displayed more phenolic content (0.476–135.63 mg/100 g), while that of the water and *n*-butanol extracts, only approached 38.03 mg/100 g, and 84.89 mg/100 g, respectively.

Only in the butanol root extract of *C. cornigera* the presence of euganol and pyrogallic acid was observed. Chlorogenic acid, ferulic acid, phenol and *p*-coumaric acid were detected in the organic solvent extracts, while were absent in the aqueous extract. Significant amounts of chlorogenic acid, *p*-hydroxyl benzoic acid, *p*-coumaric acid, caffeic acid, and ferulic acid were detected in ethyl acetate extract of the flower heads, with values 135.63, 121.17, 56.66, 28.22 and 22.76 mg/100 g, respectively. Caffeic acid, vanillin, phenol, and quercetin were present in all *C. cornigera*

Table 5
The quantitative determination of phenolic compounds (mg/100 g) in different extracts of *Cynara cornigera* and *Cichorium indivia*

Phenolic compound	*Cynara cornigera*					*Cichorium indivia*		
	Ethyl acetate			Butanol	Water	Ethyl acetate	Water	Ethanol
	Flower head	Leaf	Root	Root	Root			
Gallic acid	1.65	ND	ND	1.64	5.84	ND	6.05	20.35
Pyrogallic	ND	ND	ND	9.66	ND	ND	2.94	86.26
Chlorogenic acid	135.63	ND	ND	ND	ND	ND	2.26	44.03
Resorcinol	ND	ND	ND	2.43	7.49	ND	4.27	ND
Catechin	16.99	ND	15.65	ND	5.84	ND	38.03	25.14
p-Hydroxy benzoic acid	121.17	3.24	ND	ND	0.49	41.01	0.50	54.45
Luteolin-3-methoxy-7-rutinoside	ND	6.92	1.42	0.68	ND	ND	0.19	ND
Caffeic acid	28.22	6.93	10.56	53.14	0.47	ND	0.27	2.87
Vanillin	2.67	79.79	27.06	69.31	0.20	5.76	1.93	ND
Ferulic acid	22.76	18.32	ND	84.89	ND	2.64	ND	1.55
Rutin	ND	2.22	17.58	ND	ND	ND	0.62	7.71
Phenol	12.43	48.01	5.14	3.50	0.19	16.41	ND	ND
p-Coumaric acid	56.66	8.87	ND	ND	ND	6.92	0.01	4.81
Luteoline	ND	0.63	0.47	0.32	ND	ND	0.03	0.23
Quercetin	5.09	11.57	2.77	ND	0.06	ND	ND	8.27
Salicylic acid	ND	8.27	ND	ND	ND	ND	ND	ND
Apigenin	ND	ND	ND	ND	ND	ND	0.01	ND
Euganol	ND	ND	ND	0.45	ND	5.96	ND	ND
5,7-Dihydroy 4-methoxy isoflavone	1.53	0.79	0.50	ND	ND	1.19	0.01	ND
Chrysin	ND	ND	ND	ND	0.72	ND	ND	2.25
Sum of compounds	404.84	195.61	81.20	255.34	21.33	79.91	57.38	264.76

ND = Not detected.

organs while gallic acid, luteoline, luteolin-3-methoxy-7-rutinoside, *p*-hydroxyl benzoic acid, 5-7-dihydroxy-4-methisoflavone and ferulic acid detected in three out of five extracts of *C. cornigera* (Table 5).

The flavonoid composition including catechin rutin and quercetin was the most abundant in *C. endivia*, while chrycin, 5,7-dihydroy 4-methoxy isoflavone, luteolin, luteolin-3-methoxy-7-rutinoside and apigenin detected as traces. On the other hand, the total flavonoids content found in *C. endivia* was 43.61 mg/100 g and 38.90 mg/100 g in ethanol and water extracts, respectively, followed by ethyl acetate in *C. cornigera* root (38.43 mg/100 g).

The order of yield in the most abundant constituents of different *C. cornigera* extracts was catechin > luteolin-3-methoxy-7-rutinoside > rutin > quercetin > 5.7-dihydroy 4-methoxy isoflavone, followed by, luteolin-3-methoxy-7-rutinoside, luteoline and chrycin. Flavonoid content ranged from traces to 38.039 mg/100 g (Table 5). It was abundant in ethyl acetate root extract except for quercetin in leaf extract and 5,7-dihydroy 4-methoxy isoflavone in flower head extract. Total flavonoids of plant organs are in the order of root (54.59 mg/100 g) > flower head (23.62 mg/100 g) > leaf (22.15 mg/100 g). Luteolin was extremely low in all organs of *C. cornigera* and also showed an almost zero level of apigenin. Phenolic compounds were abundant in ethanolic extract of *C. endivia* with about 19 out of 20 compounds. In general, *C. cornigera* extracts had higher phenol concentration than *C. endivia*. The most abundant compounds were pyrogallic acid in the ethanolic extract (86.26 mg/100 g) and catechin in the aqueous extract of *C. endivia* (38.03 mg/100 g).

Lipids and triterpenes

The plant extracts contained 22 *n*-alkane hydrocarbons (Tables 6 and 7). The identified compounds of methylene chloride formed 75.2–84.7%, where eicosane, octadecane, tridecane and heneicosane were found to be the major constituents in *C. cornigera* (Table 6). The petroleum ether fractions of the leaf and root were present in quite similar amounts, like the methylene chloride fractions. The amounts of octadecane (C_{17}, C_{18}) and tridecane were significantly higher in the roots (13.97, 12.18 and 11.85%, respectively) than flower heads (4.41, 5.77% and 4.54%, respectively), followed by leaves. The amount of eicosane was higher in leaves (18.99%) than in the flower heads (10.27%). Petroleum ether extract of the flower heads contained eight compounds higher than the leaves & roots which were characterized by high content of eicosane, C_{18} and docosane (18.02, 16.6 and 11.7%), while the roots contained high amounts of tridecane and tetradecane (16.2–11.2%). The identified compounds in petroleum ether extract constituted 83.4–95.1% of the total content. Octadecane, eicosane, docosane, tridecane and tetracosane were the major constituents in *C. endivia* (Table 7). The petroleum ether extract contains more hydrocarbons (93.43%) than the crude ethanol extract (62.60%).

Two sterol compounds were identified as stigmasterol and β-sitosterol. The lowest contents were measured in the methylene chloride leaf extract. The major sterol

Table 6
Percentage composition of hydrocarbon and sterol components in *Cynara cornigera* obtained by GC

Hydrocarbons	Relative %					
	Root		Leaf		Flower head	
	Petroleum ether	Methylene chloride	Petroleum ether	Methylene chloride	Petroleum ether	Methylene chloride
n-Nonane (C_9)	ND	ND	ND	4.99	ND	3.26
n-Decane (C_{10})	ND	2.64	1.32	4.95	ND	1.28
n-Henedecane (C_{11})	1.15	2.22	1.26	2.25	ND	4.67
n-Dodecane (C_{12})	2.40	2.71	2.07	3.11	ND	6.70
n-Tridecane (C_{13})	16.19	11.85	4.55	4.23	ND	4.54
n-Tetradecane (C_{14})	11.17	1.92	7.53	2.06	2.87	2.56
n-Pentadecane (C_{15})	4.25	1.02	9.34	7.67	7.71	6.72
n-Hexadecane (C_{16})	1.66	1.32	3.47	3.75	10.43	4.45
n-Heptadecane (C_{17})	0.73	13.97	1.51	2.88	ND	4.41
n-Octadecane (C_{18})	12.95	12.18	14.49	4.52	16.63	5.76
n-Nonadecane (C_{19})	0.65	2.11	0.53	4.23	3.72	3.16
n-Eicosane (C_{20})	12.92	3.72	12.48	18.99	18.02	10.26
n-Heneicosane (C_{21})	ND	9.50	0.30	4.98	1.21	4.07
n-Docosane (C_{22})	8.06	2.54	10.10	4.33	11.71	3.17
n-Tricosane (C_{23})	1.98	ND	0.34	3.37	ND	4.75
n-Tetracosane (C_{24})	6.96	6.07	7.09	2.91	9.55	2.66
n-Pentacosane (C_{25})	0.48	1.95	0.52	2.14	ND	2.44
n-Hexacosane (C_{26})	4.30	3.67	4.30	1.32	5.91	1.77
n-Heptacosane (C_{27})	0.21	1.24	0.55	1.51	ND	1.75
n-Octacosane (C_{28})	3.03	2.37	3.48	1.20	3.70	1.29
n-Triacontane (C_{30})	0.32	ND	2.55	ND	ND	1.90
n-Hentriacontane (C_{31})	ND	ND	1.54	ND	1.23	ND
Total	89.50	83.09	89.42	85.50	92.75	81.66
Sterols and triterpene						
α-Amyrin	0.36	0.90	0.49	0.71	ND	1.32
β-Setosterol	1.52	0.68	0.45	0.96	1.84	1.35
Stigmasterol	2.19	1.20	3.05	0.19	2.49	1.30

Values rounded to the nearest two decimals. ND = Not detected.

components in *C. cornigera* were 4.33% in petroleum ether flower head extract, followed by root extract (4.08%) then leaf extract (3.99%). The main component of methylene chloride extract is α-amyrin found in the flower head (1.36%), followed by β-sitosterol and stigmasterol (1.35% and 1.30%, respectively). α-Amyrin was not found in petroleum ether flower head extract, while β-sitosterol was detected in flower head and root extracts with values1.84% and 1.52%, respectively. Stigmasterol

Table 7
Percentage composition of hydrocarbon and sterol components in *Cichorium endivia* obtained by GC

Hydrocarbons	Relative %	
	Ethanol	Petroleum ether
n-Octane (C_8)	ND	2.59
n-Nonane (C_9)	ND	7.44
n-Decane (C_{10})	ND	9.24
n-Henedecane (C_{11})	10.72	11.12
n-Dodecane (C_{12})	5.20	8.71
n-Tridecane (C_{13})	1.70	7.38
n-Tetradecane (C_{14})	2.29	5.85
n-Pentadecane (C_{15})	4.01	ND
n-Hexadecane (C_{16})	2.15	7.38
n-Heptadecane (C_{17})	2.88	0.37
n-Octadecane (C_{18})	3.80	7.51
n-Nonadecane (C_{19})	2.11	ND
n-Eicosane (C_{20})	4.19	5.77
n-Heneicosane (C_{21})	2.75	1.67
n-Docosane (C_{22})	1.41	4.62
n-Tricosane (C_{23})	1.93	ND
n-Tetracosane (C_{24})	5.68	4.20
n-Pentacosane (C_{25})	1.45	3.40
n-Hexacosane (C_{26})	6.03	1.30
n-Heptacosane (C_{27})	2.51	2.48
n-Octacosane (C_{28})	3.65	1.69
n-Triacontane (C_{30})	ND	0.63
n-Hentriacontane (C_{31})	ND	ND
Total	93.43	62.60
Sterols and triterpene		
α-Amyrin	11.33	1.17
β-Setosterol	6.76	0.97
Stigmasterol	3.27	1.61

Values rounded to the nearest two decimals. ND = Not detected.

was high in petroleum ether shoot extract of *C. endivia* (3.05%), followed by that of the flower head extract (2.49%) and root extract (2.19%) of *C. cornigera*.

Two types of triterpenes were detected in both *C. cornigera* and *C. endivia* extracts. Petroleum ether extract of *C. cornigera* flower heads showed the highest amount (54.7%) of oleanolic acid (Table 8), while *C. endivia* had the highest amount of ursolic acid (88.5%) (Table 9).

Table 8
Retention time (min) and percentage (%) of triterpene compounds in *Cynara cornigera* extracts of petroleum ether and ethanol

Compound	*Cynara cornigera*					
	Root		Leaf		Flower head	
	Retention time (min)	%	Retention time (min)	%	Retention time (min)	%
Oleanolic acid	2.21	1.70	2.60	2.20	0.98	54.70
Ursolic acid	0.98	95.5	0.96	96.3	0.26	32.00

Table 9
Retention time (min) and percentage (%) of triterpene compounds in *Cichorium endivia* extracts of petroleum ether and ethanol

Compound	*Cichorium endivia*			
	Petroleum ether		Ethanol	
	Retention time (min)	%	Retention time (min)	%
Oleanolic acid	2.58	3.40	2.26	4.40
Ursolic acid	0.93	96.60	0.93	88.50

DISCUSSION

The results of phenolic compounds and flavonoids showed that apigenin was not detected in the extracts of *C. cornigera*, while traces of chrysin was found in water extract of the root. These two compounds are reported here for the first time in the two species studied. Apigenin and luteolin were identified in *C. cardunculus* [31]. The chlorogenic acid was identified in the aqueous and alcoholic extracts of *Aster ageratoides* flower buds [11].

The results indicate that *C. cornigera* organs, especially the flower heads could represent an important source of polyphenols with therapeutic activity [34]. According to Gupta et al. [22], flavonoids are generally found in substantial concentrations in plant leaves or flowers. Brás et al. [8] reported the presence of quercetin and rutin in the leaves of *Eupatorium littorale* (Asteraceae). Twelve biologically active flavonoids, mainly flavonol and flavone derivatives, including two glycosides were detected in the glandular trichomes of *Chromolaena* species [25], while a total of 135 different compounds were reported from the Cichorieae (Lactuceae) tribe of the Asteraceae [44]. In *C. cornigera* and *C. endivia*, the high and variable active compound concentrations are related to the arid climate conditions, such as, hot temperature, high solar exposure, drought and salinity which stimulate the biosynthesis of secondary metabolites as polyphenols [13].

The presence of biologically active secondary metabolites such as tannins (gallic acid and catechin), sterols and terpenes contributes to its medicinal value [47]. Tannins have been reported to have several pharmacological activities such as spas-

molytic activity in smooth muscle cells [50]. Gallic acid has antineoplastic and bacteriostatic activities and anticancer properties in prostate carcinoma cells [28], and anti-inflammatory and antibacterial activity [46]. The present study revealed that gallic acid is in range from 1.64 mg/100 g in *C. cornigera* parts to 20.35 mg/100 g in *C. endivia*. There were variations in total phenolic content of different aromatic and medicinal plants ranging between 6.80–32.10 mg gallic acid/g dry weight bases [4, 21]. *p*-Coumaric and ferulic acids are also present in combination with betanidin monoglucoside in fruits of *Basella rubra* [20]. Hydroxycinnamic acid derivatives were found in *C. endivia* var. *crispum* and var. *latifolium*; three derivatives (5-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, and 5-O-feruloylquinic acid) were found in both chicories, while 3,5-di-O-caffeoylquinic acid was typical of var. crispum and cis-caftaric acid of var. latifolium [40].

The presence of kaempferol 3-O-(6-O-malonyl) glucoside in endive is reported here for the first time. Methanol extract of *Lactuca sativa* L. varieties (Romaine, Iceberg and Baby) and *C. endivia* L. variety "escarole" have yielded a high phenolic content, where chicory extracts being the richest with 50 mg phenolics/g of freeze-dried extract [14, 35]. Tannins, sesquiterpene lactones, cinnamic acid, caffeic and chlorogenic acids were reported in chicory [27]. Monocaffeoyl tartaric acid, chlorogenic acid, and chicoric acid were detected in different varieties of *Cichorium intybus*, while cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, and cyanidin 3-O-glucoside were the main phenolic compounds in the red varieties [26]. Also, apigenin and apigenin-7-0-α-1-arabinoside were isolated from *Cichorium intybus* aerial parts [55].

The *p*-hydroxybenzoic acid (4-hydroxybenzoic acid) found in *C. cornigera* showed a broad range of pharmacological activities, for instance, antifungal, antimutagenic, antisickling, estrogenic and antimicrobial effects [10, 43]. Chlorogonic acid has been reported to have a number of biological properties, such as antibacterial, antioxidant, hepatocyte protective and antimutagenic activity [51]. Artichoke capitula was characterized by significant amounts of chlorogenic acid and various dicaffeoylquinic esters, especially 1,3-dicaffeoylquinic acid, known as cynarin [7].

Eight phenolic compounds were isolated from *n*-butanol leaf fraction of *Cynara scolymus* which is known for its antimicrobial activity [56, 57]. Also, Orlovskaya et al. [39] have previously reported qualitative and quantitative results on phenolic compounds on raw material and extracts of *C. scolymus* leaves. The phenolic contents in leaf and seed of *Cynara cardunculus* were similar and two times higher than those in flower heads [16]. The organic subfraction of artichoke is rich in polyphenolic compounds, with caffeoylquinic acids and flavonoids as the major chemical constituent [32]. In leaf extracts of commercially available artichoke *(C. scolymus)* a number of compounds were identified, including 8-deoxy-11-hydroxy-13-chlorogrosheimin, cryptochlorogenic acid, chlorogenic acid, neochlorogenic acid, cynarin, cynaratriol (tentatively), grosheimin, 8-deoxy-11,13-dihydroxygrosheimin, luteolin-7-O-rutinoside, luteolin-7-O-glucoside, and cynaropicrin [18]. Phenolic acids, up to 2%, caffeic acid, mono- and dicaffeoylquinic acid derivatives, e.g., cynarin, and chlorogenic acid

were reported to be present in artichoke [24]. The flavonoids, triterpenoids and saponines were reported to possess hepatoprotective activity in animals [3].

As for lipid and triterpene content, twenty-two compounds of the hydrocarbons were identified in petroleum ether of *C. cornigera* parts. The major components constitute up to 85% of the plant chemical composition, while the minor components reported in trace amounts [37]. A high content of derivatives was also characteristic in *C. endivia* extracts, including henedecane, decane, dodecane, octane and heptadecane. The presence of terpenoids in *C. cornigera* and *C. endivia* has also been reported to show several biological and pharmacological activities such as antioxidative [48], anti-inflammatory [38], hepato-protective effects [33]. The mechanism of action of terpenes is not fully understood but it is speculated to involve membrane disruption by the lipophilic compounds [23].

In conclusion, our findings indicate that *C. cornigera* and *C. endivia* are rich sources of eight important biologically active flavonoids, twelve phenolic acids, tow triterpenes and three steroids, which were detected for the first time in the two species studied. Considering that chlorogenic acid and rutin are highly valuable natural polyphenol compounds used as medical and industrial materials, the identification and quantification of these main polyphenols in the present study can be important for potential use at commercial level in the pharmaceutical industry.

ACKNOWLEDGEMENT

We thank the Deanship of Scientific Research, College of Science Research Center, King Saud University, Riyadh, Saudi Arabia for supporting this publication.

REFERENCES

1. Abdelly, C. (2007) Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritime*. *Plant Physiol. Biochem. 45,* 244–249.
2. Amic, D., Davidovic-Amic, D., Beslo, D., Trinajstic, N. (2003) Structure-radical scavenging activity relationships of flavonoids. *Croat. Chem. Acta 76,* 55–61.
3. Anusha, M., Venkateswarlu, M., Prabhakaran, V., Taj, S. S., Kumari, B. P., Ranganayakulu, D. (2011) Hepatoprotective activity of aqueous extract of Portulaca oleracea in combination with lycopene in rats. *Indian J. Pharmacol. 43,* 563–567.
4. Bajpai, M., Pande, A., Tewari, S. K., Prakash, D. (2005) Phenolic contents and antioxidant activity of some food and medicinal plants. *Int. J. Food Sci. Nutr. 56,* 287–291.
5. Balasundram, N., Sundram, K., Sammar, S. (2006) Phenolic compounds in plants and agri-industrial by-products. Antioxidant activity, occurrence, and potential uses. *Food Chem. 1,* 191–203.
6. Barbetw, P., Fardella, G., Chiappini, I., Scarcia, V., Brent, J. A., Rumack, B. H. (1993) Role of free radicals in toxic hepatic injury II. *Clin. Toxicol. 31,* 173–196.
7. Ben Hod, G., Basnizki, Y., Zohary, D., Mayer, A. M. (1992) Cynarin and chlorogenic acid content in germinating seeds of globe artichoke (*Cyanara scolymus* L.). *J. Plant Breed. Genet. 46,* 63–68.
8. Brás, H., de Oliveira, H., Nakashimab, T., Filhoc, J., de Souza, D., Frehse, F. L. (2001) HPLC analysis of flavonoids in *Eupatorium littorale. J. Brazil Chem. Soc. 12,* 243–246.
9. Chinou, I., Harvala, C. (1997) Polyphenolic constituents from the leaves of two Cynara species growing in Greece. *Planta Med. 63,* 469–470.

10. Chong, K. P., Rossall, S., Atong, M. (2009) In vitro antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against *Ganoderma Boninense*. *J. Agric. Sci. 1,* 15–20.
11. Clifford, M. N., Zheng, W., Kuhnert, N. (2006) Profiling the chlorogenic acids of aster by HPLC-MS^n. *Phytochem. Anal. 17,* 384–393.
12. Dimitrios, K. P., Constantin, E., Harrala, C. (2000) Achemometric comparison of three taxa of *Scabiosa* L.s.1. *Plant Biosyst. 134,* 67–70.
13. Djeridane, M., Yousfi, B., Nadjemi, D., Boutassouna, P., Stocker, N. (2006) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food. Chem. 97,* 654–660.
14. DuPont, M. S., Mondin, Z., Williamson, G., Price, K. R. (2000) Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J. Agr. Food Chem. 48,* 3957–3964.
15. Elsayed, S. M., Nazif, N. M., Hassan, R. A., Hassanein, H. D., Elkholy, Y. M., Gomaa, N. S., Shahat, A. A. (2012) Chemical and biological constituents from the leaf extracts of the wild Artichoke *(Cynara cornigera). Int. J. Pharm. Sci. 4,* 396–400.
16. Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., Abdelly, C. (2008) Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *C. R. Biol. 331,* 372–379.
17. Fernald, M. L. (1950) *Gray's Manual of Botany. A Handbook of the Flowering Plants and Ferns of the Central and Northeastern United States and Adjacent Canada, Eighth (Centennial) Edition Illustrated.* American Book Company, New York.
18. Fritsche, J., Christaan, M., Dachtler, B. M., Zhang, H., Jan, G., Lammers, A. (2002) Isolation, characterization and determination of minor artichoke (*Cynara scolymus* L.) leaf extract compounds. *Eur. Food Res. Technol. 215,* 149–157.
19. Gebhardt, R. (1997) Antioxidative protective properties of extract from leaves of the Artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicol. Appl. Pharm. 144,* 279–286.
20. Glässgen, W. E., Metzger, J. W., Heuer, S., Strack, D. (1993) Betacyanins from fruits of Basella rubra. *Phytochemistry 33,* 1525–1527.
21. Gruz, J., Ayaz, F. A., Torun, H., Strand, M. (2011) Phenolic acid content and radical scavenging activity of extracts from medlar (*Mespilus germanica* L.) fruit at different stages of ripening. *Food Chem. 124,* 271–277.
22. Gupta, V. K., Garg, M., Gupta, M. (2010) Recent updates on free radicals scavenging flavonoids: An overview. *Asian J. Plant. Sci. 9,* 108–117.
23. Gutierrez-Lugo, M. T., Singh, M. P., Maiese, W. M., Timmermann, B. N. (2002) New antimicrobial cycloartane triterpenes from Acalypha communis. *J. Nat. Prod. 65,* 872–875.
24. Hammouda, F. M. (1993) Quantitative determination of the active constituents in Egyptian cultivated Cynara scolymus. *Int. J. Pharmacog. 31,* 299–304.
25. Helena, S., Taleb-Contini, S. K., Batista Da Costa, F., Rodrigues de Oliveira, D. C. (2007) Detection of flavonoids in glandular trichomes of Chromolaena species (Eupatorieae, Asteraceae) by reversed-phase high-performance liquid chromatography. *Braz. J. Pharmaceut. Sci. 43,* 220–228.
26. Innocenti, M., Gallori, S., Giaccherini, C., Ieri, F., Franco, F., Vincieri, M. N. (2005) Evaluation of the phenolic content in the aerial parts of different varieties of *Cichorium intybus* L. J. *Agric. Food Chem. 53,* 6497–6502.
27. Judentiene, A., Budiene, J. (2008) Volatile constituents from aerial parts and roots of *Cichorium intybus L.* (chicory) grown in Lithuania. *Chemija 19,* 25–28.
28. Khadem, S., Robin, J., Marles, K. (2010) Monocyclic phenolic acids; hydroxy- and polyhydroxybenzoic acids: occurrence and recent bioactivity studies. *Molecules 15,* 7985–8005.
29. Kisiel, W., Michalska, K., Szneler, E. (2004) Norisoprenoids from aerial parts of *Cichorium pumilum*. *Biochem. Syst. Ecol. 32,* 343–346.

31. Kukic, J., Popović, V., Petrović, S., Mucaji, P., Ćirić, A., Stojković, D., Sokovic, M. (2008) Antioxidant and antimicrobial activity of Cynara cardunculus extracts. *Food Chem. 107,* 861–868.
32. Li, H., Xia, N., Brausch, I., Yao, Y., Forstemann, U. (2004) Flavonoids from artichoke (*Cynara Scolymus L.*) up-regulate endothelial-type nitric-oxide synthase gene expression in human endothelial cells. *J. Pharmacol. Exp. Ther. 310,* 926–932.
33. Liu, Y., Hartley, D. P., Liu, J. (1998) Protection against carbon tetrachloride hepatotoxicity by oleanolic acid is not mediated through metallothionein. *Toxicol. Lett. 95,* 77–85.
34. Llorach, R., Espin, J. C., Tomas-Barberan., F. A., Ferreres, F. (2002) Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *J. Agric. Food Chem. 50,* 3458–3464.
35. Llorach, R., Tomás-Barberán, F. A., Ferreres, F. (2004) Lettuce and chichory byproducts as a source of antioxidant phenolic extracts. *J. Agric. Food Chem. 52,* 5109–5111.
36. Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L. (2004) Polyphenols, food sources and bioavailability. *Amer. J. Clin. Nutr. 79,* 727–747.
36a. Michalska, K., Kisiel, W. (2007) Further sesquiterpene lactones and phenolics from *Cichorium spinosum. Biochem. Syst. Ecol. 35,* 714–716.
37. Miguel, M. G. (2010) Antioxidant activity of medicinal and aromatic plants. *Flav. Frag. J. 25,* 291–312.
38. Mix, K. S., Mengshol, J. A., Benbow, U., Vincenti, M. P., Sporn, M. B., Brinckerhoff, C. E. (2001) Asynthetic triterpenoid selectively inhibits the induction of matrix metalloproteinases 1 and 13 by inflammatory cytokines. *Arthritis Rheum. 44,* 1096–1104.
39. Orlovskaya, T. V., Luneva, I. L., Chelombit¢ko, V. A. (2007) Chemical composition of *Cynara scolymus leaves. Chem. Nat. Compd. 43,* 239–240.
40. Papetti, A., Daglia, M., Aceti, C., Sordelli, B., Spini, V., Carazzone, C., Gazzani, G. (2008) Hydroxycinnamic acid derivatives occurring in *Cichorium endivia* vegetables. *J. Pharmaceut. Biomed. 48,* 472–476.
41. Papetti, A., Daglia, M., Gazzani, G. (2002) Anti- and pro-oxidant water soluble activity of *Cichorium* genus vegetables and effect of thermal treatment. *J. Agric. Food Chem. 50,* 4696–4704.
42. Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E., Romani, A. (2007) Simultaneous quantification of cafeoyl esters and flavonoids in wild and cultivated cardoon leaves. *Food Chem. 105,* 1695–1701.
43. Pugazhendhi, D., Pope, G. S., Darbre, P. D. (2005) Oestrogenic activity of p-hydroxybenzoic acid (common metabolite of paraben esters) and methylparaben in human breast cancer cell lines. *J. Appl. Tox. 25,* 301–309.
44. Sareedenchai, V., Zidorn, C. (2010) Flavonoids as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Biochem. Syst. Ecol. 38,* 935–957.
45. Seto, M., Miyase, T., Umehara, K., Ueno, A., Hirano, Y., Otani, N. (1988) Sesquiterpene lactones from *Cichorium endivia* L., and *C. intybus* L. and cytotoxic activity. *Chem. Pharm. Bull. 36,* 2423–2429.
46. Singh, S., Srivastava, R., Choudhary, S. (2010) Antifungal and HPLC analysis of the crude extracts of *Acorus calamus*, *Tinospora cordifolia* and *Celestrus paniculatus*. *J. Agric. Technol. 6,* 149–158.
47. Sofowara, A. (1993) *Medicinal Plants and Traditional Medicine in Africa.* Spectrum Books Ltd., Ibadan, Nigeria.
48. Somova, L. O., Nadar, A., Rammanan, P., Shode, F. O. (2003) Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomedicine 10,* 115–121.
49. Stanojević, L., Stanković, M., Nikolić, V., Nikolić, L., Ristić, D., Čanadanovic-Brunet, J., Tumbas, V. (2009) Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. *Sensors 9,* 5702–5714.

50. Tona, L., Kambu, K., Mesia, K., Cimanga, K., Apers, S., de Bruyne, T., Pieters, L., Totte, J., Vlietinck, A. J. (1999) Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. *Phytomedicine 6,* 59–66.
51. Wang, J., Dingqianglu, H., Jiang, B., Wang, J., Ling, X., Ouyang, H. C. P. (2010) Discrimination and classification of tobacco wastes by identification and quantification of polyphenols with LC–MS/MS. *J. Serbian Chem. Soc. 75,* 875–891.
52. Wang, M., Simon, J. E., Aviles, I. F., He, K., Zheng, Q. Y., Tadmor, Y. (2003) Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *J. Agric. Food Chem. 51,* 601–608.
53. Wang, H., Wang, Z., Guo, W. (2008) Comparative determination of ursolic acid and oleanolic acid of *Macrocarpium officinalis* (Sieb. et Zucc.) Nakai by RP-HPLC. *Industrial Crops and Products 28,* 328–332.
54. Warashina, T., Miyase, T. (2006) Sesquiterpenes from the roots of Cichorium endivia. *Fitoterapia 77,* 354–357.
55. Zaman, R., Akhtar, M. S., Khan, M. S. (2006) Anti-ulcerogenic screening of *Cichorium intybus* L. leaf in indomethacin treated rats. *Int. J. Pharm. 2,* 166–170.
56. Zhu, X., Zhang, H., Lo, R. (2004) Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *J. Agric. Food Chem. 52,* 7272–7278.
57. Zhu, X. F., Zhang, H. X. (2004) Flavonoids of *Cynara scolymus*. *Chem. Nat. Comp. 40,* 600–601.