STUDIA UBB CHEMIA, LXII, 2,Tom I, 2017 (p. 125-143)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2017.2.10

Dedicated to Professor Costel Sârbu on the Occasion of His 65th Anniversary

COMPARATIVE CHEMOMAPPING OF PHYTOCONSTITUENTS FROM DIFFERENT EXTRACTS OF GLOBE ARTICHOKE - *CYNARA SCOLYMUS* L.

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ABSTRACT. Artichoke (*Cynara scolymus* L.) is a well-known herb for its efficiency in the prevention/treatment of liver injuries, among other human chronic diseases. The aim of present study was to analyse the phytoconstituents content of aqueous and hydro-alcoholic extracts obtained from the leaves of artichoke. The chemomapping was carried out using UHPLC-ESI-MS. Several new and some known phytoconstituents were identified in the two type of extracts that have slightly different composition profiles. The newly found phytoconstituents in artichoke, plead for multiple health promoting effects that have presumably more stochastic than determinative features. Therefore, further experiments are needed using such extracts, and based on a system biology approach to clarify the complexity of beneficial effects of artichoke.

Keywords: *globe artichoke, Cynara scolymus, phytoconstituents, bioactive compound, LC-ESI-MS*

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INTRODUCTION

Globe artichoke (*Cynara scolymus* L.) has been cultivated since the ancient times in the Mediterranean and North African regions. In the middle ages, its cultivation spread across Western Europe from Italy to Spain, France, The Netherlands, England, and later on, in the 1800s reaches the Southern parts of USA. Moreover, Northern African countries like Egypt, Algeria and Tunisia together with South American countries like Argentina and Peru did become important artichoke producers in recent times.

Globe artichoke is considered a healthy food due to its nutritive and phytoconstituent content. It contains proteins, minerals, a low amount of lipids, dietary fibre and a high proportion of phenolics [1-2]. Among phenolics there were identified compounds like cynarin (1,3-di-O-caffeoylquinic acid), luteolin, cynaroside (luteolin-7-O-glucoside), scolymoside (luteolin-7-rutinoside); phenolic acids such as caffeic, coumaric, hydroxycinnamic, ferulic, caffeoylquinic acid derivatives; mono- and dicaffeoylquinic acids, including chlorogenic; acid alcohols; flavonoid glycosides [2-3]. The content of phytoconstituents was shown to vary among different cultivars and conditions related to cultivation, harvest, post-harvest and cooking [4-5].

Globe artichoke features a relatively high antioxidant capacity [6-7], its hepatoprotective, bile-enhancing and lipid-lowering effects have been demonstrated [8], while its implications in preventing cardiovascular disease by its lipidic and glycemic-reducing action has also been confirmed [9-10]. Moreover, its putative anticancer effect has been studied, and some experimental data suggests that artichoke extracts could be applied as a nonconventional, adjuvant therapy for cancer chemoprevention and/or treatment [11-13].

In the present paper we are describing the comparative UHPLC-ESI-MS chemomapping of aqueous and hydro-alcoholic artichoke extracts that were found to inhibit significantly the proliferation of several human cancer cell lines [14]. Our study was meant to identify all possible phytoconstituents with the used experimental setup, and as a consequence 49 and 51 molecules were described in the aqueous and hydro-alcoholic artichoke extracts, respectively. Some of the newly identified compounds were confirmed by standards, while other compounds have already been reported by others [15-26].

RESULTS AND DISCUSSION

In this paper, we are describing the qualitative analysis performed for artichoke (*Cynara scolymus* L.) extracts by applying reversed phase UHPLC-ESI-MS using a gradient mobile phase consisting of acetonitrile and water. The

aqueous and the hydro-alcoholic extracts of artichoke leaves were investigated in positive and negative ionisation modes as described in Materials and Methods.

There have been 49 phytoconstituents identified in the aqueous artichoke extract as shown on the corresponding chromatograms (Figure 1-2.) and in Table 1.

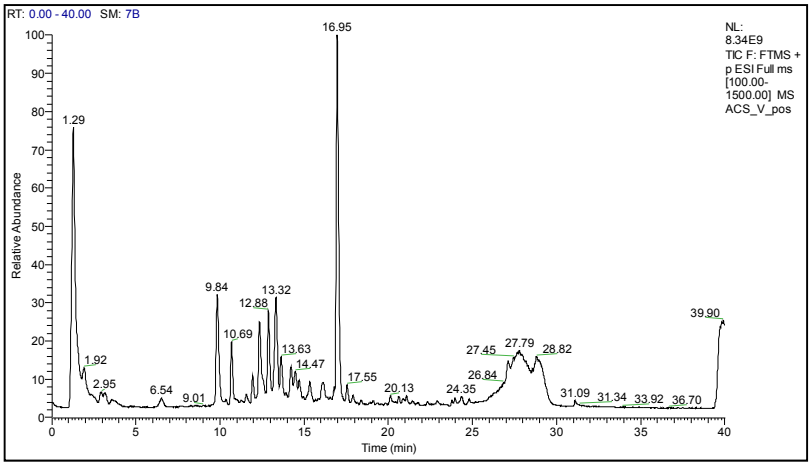

Figure 1. Total ion chromatogram of aqueous extract of artichoke in positive ionisation mode.

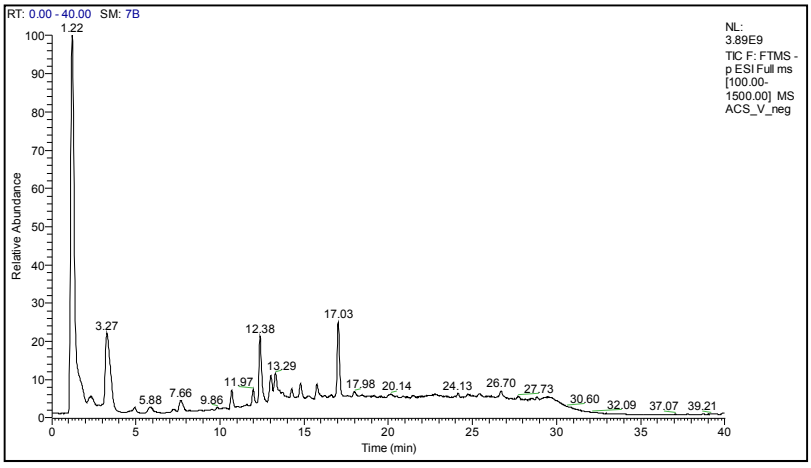

Figure 2. Total ion chromatogram of aqueous extract of artichoke in negative ionisation mode.

Table 1. Phytoconstituents identified in the aqueous artichoke extract.
Rt –retention time; [M+H]+ - molecular ion masses; [M+H]- - the found fragment ion mass; Ref- references; (*) [M]+; (**) confirmed by standards. The difference between measured and calculated molecular ion masses were always below 5 ppm.

Peak	R_t	$[M+H]^+$	$[M-H]^-$	Formula	Fragments found	Assignment	Ref.
1	1.22	104.10754*		$C_5H_{14}NO$	60.0814, 59.0736	Choline	
2	1.27	175.11951		$C_6H_{14}N_4O_2$	158.0922, 130.0975	Arginine**	
3	1.27		179.05557	$C_6H_{12}O_6$	113.0229, 101.0229	Glucose or galactose	
4	1.29	138.05550*		$C_7H_8NO_2$	110.0602, 96.0447	Trigonelline	
5	1.32	133.06132		$C_4H_8N_2O_3$	116.0344, 88.0397	Asparagine**	
6	1.43	324.05968		$C_9H_{13}N_3O_5$	112.0507, 95.0240	Cytidine**	
7	1.48	146.09296		$C_5H_{11}N_3O_2$	128.0817, 111.0555	4-Guanidinobutyric acid	
8	1.51	136.06233		$C_5H_5N_5$	119.0352, 94.0402	Adenine	
9	1.52		362.05018	$C_{10}H_{14}N_5O_8P$	211.0005, 150.0408	Guanosine 5'-monophosphate	
10	1.53	168.06607		$C_8H_9NO_3$	150.0548, 140.0705	Pyridoxal**	
11	1.57	124.03986		$C_6H_5NO_2$	96.0448, 80.0499	Nicotinic acid**	
12	1.59	144.10245*		$C_7H_{14}NO_2$	102.0554, 98.0968	Stachydrine	
13	1.71	170.08172		$C_8H_{11}NO_3$	152.0704, 134.0600	Pyridoxine**	
14	1.74		243.06171	$C_9H_{12}N_2O_6$	200.0557, 153.0291	Uridine	
15	1.76	113.03511		$C_4H_4N_2O_2$	96.0084, 95.0245	Uracil**	
16	1.78	182.08172		$C_9H_{11}NO_3$	165.0544, 147.0439	2-Hydroxyphenyl-alanine	
17	1.92	123.05584		$C_6H_6N_2O$	106.0291, 96.0447	Nicotinamide**	
18	2.34		346.05526	$C_{10}H_{14}N_5O_7P$	211.0006, 192.9902	Adenosine 5'-monophosphate	
19	2.62		282.08385	$C_{10}H_{13}N_5O_5$	150.0408, 133.0143	Guanosine	
20	2.94	268.10458		$C_{10}H_{13}N_5O_4$	136.0617, 119.0350	Adenosine**	

Peak	R_t	$[M+H]^+$	$[M-H]^-$	Formula	Fragments found	Assignment	Ref.
21	3.18	166.08681		$C_9H_{11}NO_2$	149.0602, 131.0492	Phenylalanine**	
22	3.27		353.08726	$C_{16}H_{18}O_9$	191.0552, 179.0342	Caffeoylquinic acid I	16
23	4,78	122.09698		$C_8H_{11}NO_2$	105.0702, 103.0546	Phenethylamine	
24	4.83	220.11850		$C_9H_{17}NO_5$	202.1073, 184.0967	Pantothenic acid**	
25	5.90		337.09234	$C_{16}H_{18}O_8$	191.0552, 163.0388	Coumaroylquinic acid I	17
26	6.52	205.09771		$C_{11}H_{12}N_2O_2$	188.0705, 170.0599	Tryptophan**	
27	7.66		353.08726	$C_{16}H_{18}O_9$	191.0552, 179.0338	Caffeoylquinic acid II	16
28	8.31	190.05042		$C_{10}H_7NO_3$	162.0547, 144.0442	Kynurenic acid	
29	8.84	341.08726		$C_{15}H_{16}O_9$	179.0338, 151.0388	Esculin	
30	8.86		335.07669	$C_{16}H_{16}O_8$	179.0339, 161.0231	Caffeoylshikimic acid I	
31	9.57	295.12940		$C_{14}H_{18}N_2O_5$	278.1119, 232.0961	γ-Glutamylphenyl-alanine	
32	9.97	298.09739		$C_{11}H_{15}N_5O_3S$	163.0422, 145.0313	5'-S-Methyl-5'-thioadenosine	
33	10.19		337.09234	$C_{16}H_{18}O_8$	191.0552, 163.0389	Coumaroylquinic acid II	17
34	11.53	191.07082		$C_{11}H_{10}O_3$	176.0466, 148.0518	7-Methoxy-4-methylcoumarin	
35	12.78		593.15065	$C_{21}H_{18}O_{11}$	473.1093, 383.0772	Vicenin-2	
36	13.02		335.07669	$C_{16}H_{16}O_8$	179.0339, 161.0232	Caffeoylshikimic acid III	
37	13.02		515.11896	$C_{25}H_{24}O_{12}$	335.0776, 191.0552	1,3-Di-O-caffeoylquinic acid (Cynarin)	
38	13.27	283.15455		$C_{15}H_{22}O_5$	265.1429, 247.1324	Cynaratriol	
39	13.54		461.07201	$C_{21}H_{18}O_{12}$	285.0404, 217.0501	Luteolin-7-O-glucuronide	18
40	13.54	146.06059		C_9H_7NO	118.0652,117.0573	Indole-4-carbaldehyde	
41	13.81	179.07082		$C_{10}H_{10}O_3$	161.0594, 147.0438	4-Hydroxy-3-methoxy-cinnamaldehyde	
42	14.60		445.07709	$C_{21}H_{18}O_{11}$	269.0454, 225.0546	Apigenin-7-O-glucuronide	19

Peak	R_t	$[M+H]^+$	$[M-H]^-$	Formula	Fragments found	Assignment	Ref.
43	14.74		593.15065	$C_{27}H_{30}O_{15}$	285.0404, 133.0275	Luteolin-7-O-rutinoside (Scolymoside)	20
44	14.79		447.09274	$C_{21}H_{20}O_{11}$	327.0509, 285.0403	Luteolin-7-O-glucoside (Cynaroside)	18, 19, 21
45	15.20		193.05009	$C_{10}H_{10}O_4$	178.0262, 149.0596	Ferulic acid	
46	15.67	433.11347		$C_{21}H_{20}O_{10}$	271.0600, 153.0180	Cosmosiin (Apigenin-7-O-glucoside)**	22, 23
47	17.98		285.03991	$C_{15}H_{10}O_6$	217.0499, 199.0393	Luteolin	
48	20.34		809.43235	$C_{42}H_{66}O_{15}$	647.3814, 603.3902	Cynarasaponin E	26
49	21.86		793.43744	$C_{42}H_{66}O_{14}$	631.3859, 587.3961	Cynarasaponin C	26

There have been 51 phytoconstituents identified in the hydro-alcoholic artichoke extract as shown on Fig.3-4 and in Table 2.

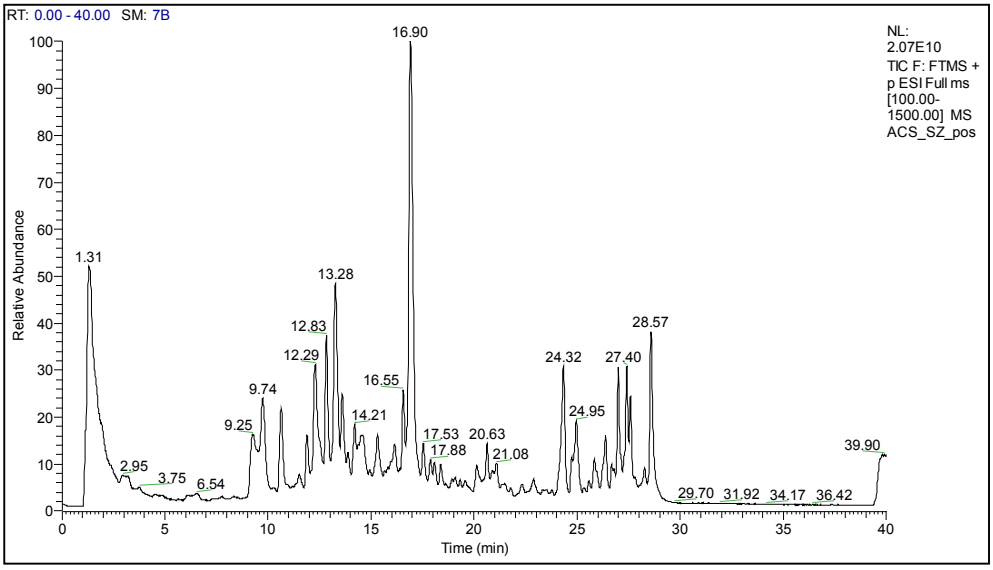

Figure 3. Total ion chromatogram of hydro-alcoholic extract of artichoke in positive ionisation mode.

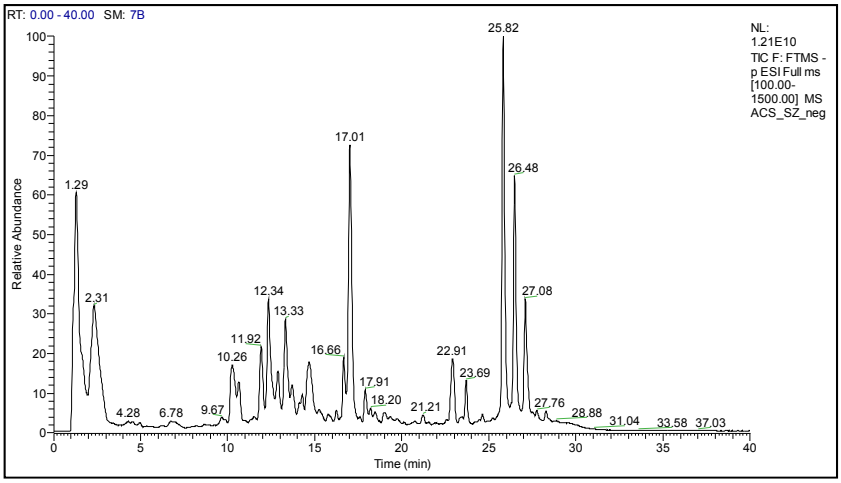

Figure 4. Total ion chromatogram of hydro-alcoholic extract of artichoke in negative ionisation mode.

Table 2. Phytoconstituents identified in the hydro-alcoholic artichoke extract. Rt –retention time; [M+H]+ - molecular ion masses; [M+H]- - the found fragment ion mass; Ref- references; (*) [M]+; (**) confirmed by standards. The difference between measured and calculated molecular ion masses were always below 5 ppm.

Peak	R_t	$[M+H]^+$	$[M-H]^-$	Formula	Fragments found	Assignment	Ref.
1	1.26	138.05550*		$C_7H_8NO_2$	110.0603, 96.0449	Trigonelline	
2	1.28	104.10754*		$C_5H_{14}NO$	60.0814, 59.0736	Choline	
3	1.30	175.11951		$C_6H_{14}N_4O_2$	158.0923, 130.0976	Arginine**	
4	1.35	133.06132		$C_4H_8N_2O_3$	116.0343, 88.0397	Asparagine**	
5	1.38		179.05557	$C_6H_{12}O_6$	113.0229, 101.0230	Glucose or galactose	
6	1.50	324.05968		$C_9H_{13}N_3O_5$	112.0507, 95.0243	Cytidine**	
7	1.52	146.09296		$C_5H_{11}N_3O_2$	128.0815, 111.0554	4-Guanidinobutyric acid	
8	1.53	136.06233		$C_5H_5N_5$	119.0353, 94.0403	Adenine	
9	1.62	124.03986		$C_6H_5NO_2$	96.0448, 80.0500	Nicotinic acid**	

Peak	R_t	$[M+H]^+$	$[M-H]^-$	Formula	Fragments found	Assignment	Ref.
10	1.75	170.08172		$C_8H_{11}NO_3$	152.0704, 134.0601	Pyridoxine**	
11	1.83	113.03511		$C_4H_4N_2O_2$	96.0084, 95.0245	Uracil**	
12	1.84	182.08172		$C_9H_{11}NO_3$	165.0542, 147.0439	2-Hydroxyphenyl-alanine	
13	1.94	123.05584		$C_6H_6N_2O$	106.0290, 96.0448	Nicotinamide**	
14	2.68		282.08385	$C_{10}H_{13}N_5O_5$	150.0408, 133.0142	Guanosine	
15	2.99	268.10458		$C_{10}H_{13}N_5O_4$	136.0617, 119.0347	Adenosine**	
16	3.23	166.08681		$C_9H_{11}NO_2$	149.0601, 131.0493	Phenylalanine**	
17	4.85	122.09698		$C_8H_{11}N$	105.0702, 103.0546	Phenethylamine	
18	4.87	220.11850		$C_9H_{17}NO_5$	202.1070, 184.0967	Pantothenic acid**	
19	6.54	205.09771		$C_{11}H_{12}N_2O_2$	188.0705, 170.0598	Tryptophan**	
20	8.26	190.05042		$C_{10}H_7NO_3$	162.0547, 144.0442	Kynurenic acid	
21	8.79	341.08726		$C_{15}H_{16}O_9$	179.0337, 151.0389	Esculin	
22	9.55	295.12940		$C_{14}H_{18}N_2O_5$	278.1121, 232.0964	γ-Glutamylphenyl-alanine	
23	9.95	298.09739		$C_{11}H_{15}N_5O_3S$	163.0422, 145.0318	5'-S-Methyl-5'-thioadenosine	
24	11.49	174.11302		$C_8H_{15}NO_3$	156.1010, 132.1019	N-Acetylisoleucine	
25	11.51	191.07082		$C_{11}H_{10}O_3$	176.0462, 148.0517	7-Methoxy-4-methylcoumarin	
26	12.03	174.11302		$C_8H_{15}NO_3$	156.1012, 132.1019	N-Acetylleucine	
27	12.52		593.15065	$C_{21}H_{18}O_{11}$	473.1084, 383.0770	Vicenin-2	
28	12.57		461.07201	$C_{21}H_{18}O_{12}$	285.0403, 217.0499	Luteolin-7-O-glucuronide	18
29	12.86	193.05009		$C_{10}H_8O_4$	178.0258, 165.0544	Scopoletin	
30	13.05		515.11896	$C_{25}H_{24}O_{12}$	335.0770, 191.0552	1,3-Di-O-caffeoylquinic acid (Cynarin)	
31	13.27	283.15455		$C_{15}H_{22}O_5$	265.1429, 247.1324	Cynaratriol	

Peak	R_t	$[M+H]^+$	$[M-H]^-$	Formula	Fragments found	Assignment	Ref.
32	13.32	163.07591		$C_{10}H_{10}O_2$	131.0492, 103.0545	Methyl cinnamate	
33	13.38		581.18703	$C_{27}H_{34}O_{14}$	297.1768, 167.0337	Naringin dihydrochalcone	
34	13.52	146.06059		C_9H_7NO	118.0652,117.0572	Indole-4-carbaldehyde	
35	13.65		445.07709	$C_{21}H_{18}O_{11}$	269.0454, 225.0550	Apigenin-7-O-glucuronide	19
36	13.80	179.07082		$C_{10}H_{10}O_3$	161.0595, 147.0439	4-Hydroxy-3-methoxy-cinnamaldehyde	
37	14.71		593.15065	$C_{27}H_{30}O_{15}$	285.0404, 133.0279	Luteolin-7-O-rutinoside (Scolymoside)	20
38	14.73		447.09274	$C_{21}H_{20}O_{11}$	327.0507, 285.0400	Luteolin-7-O-glucoside (Cynaroside)	18, 19, 21
39	15.19		193.05009	$C_{10}H_{10}O_4$	178.0259, 149.0595	Ferulic acid	
40	15.22		269.04500	$C_{15}H_{10}O_5$	225.0550, 201.0550	Apigenin**	21, 24
41	15.54	579.17139		$C_{27}H_{30}O_{14}$	271.0595, 153.0176	Isorhoifolin (Apigenin 7-O-rutinoside)	25
42	15.66	433.11347		$C_{21}H_{20}O_{10}$	271.0594, 153.0179	Cosmosiin (Apigenin-7-O-glucoside)**	22, 23
43	17.89		285.03991	$C_{15}H_{10}O_6$	217.0498, 199.0391	Luteolin	
44	18.94		539.04618	$C_{25}H_{16}O_{14}$	269.0453, 201.0548	Unknown Apigenin derivative	
45	19.03	301.07121		$C_{16}H_{12}O_6$	286.0466, 258.0515	Diosmetin	
46	21.76	329.10251		$C_{18}H_{16}O_6$	314.0781, 313.0697	Salvigenin	
47	25.38	291.23241		$C_{19}H_{30}O_2$	259.2035, 241.1949	Stearidonic acid methyl ester	
48	25.85	305.24806		$C_{20}H_{32}O_2$	259.2058, 241.1939	Stearidonic acid ethyl ester	
49	27.20	457.36818		$C_{30}H_{48}O_3$	439.3553, 411.3619	Ursolic acid	
50	19.43		809.43235	$C_{42}H_{66}O_{15}$	647.3806, 603.3903	Cynarasaponin E	26
51	21.20		793.43744	$C_{42}H_{66}O_{14}$	631.3851, 587.3955	Cynarasaponin C	26

The identification of the phytoconstituents was achieved by comparing individually the retention time, accurate mass, isotopic distribution and fragmentation pattern of every single newly detected molecule with artichoke compounds already reported in literature, and by screening MS databases like Metlin, mzCloud and Massbank. The identified molecules belong to twelve classes of phytoconstituents, and besides similarities, there are some striking differences among the aqueous and hydro-alcoholic artichoke extracts regarding their content as summarized in Table 3.

Table 3. Phytoconstituents identified in the aqueous and hydro-alcoholic artichoke extracts.

Phytoconstituents		Aqueous artichoke	Hydro-alcoholic artichoke
Alkaloids	Kynurenic acid	+	+
	Trigonelline	+	+
	Stachydrine[a]	+	
Aminoacids	2-Hydroxyphenylalanine	+	+
	4-Guanidinobutyric acid	+	+
	Arginine	+	+
	Asparagine	+	+
	L-Phenylalanine	+	+
	γ-Glutamilphenylalanin	+	+
	Tryptophan	+	+
	N-Acetylisoleucine[b]		+
	N-Acetylleucin[b]		+
Coumarins	7-Methoxy-4-methylcoumarin	+	+
	4-hidroxy-3-methoxy-cinnamaldehyde	+	+
	Scopoletin[b]		+
Flavonoids	Unknown Apigenin derivative[b]		+
	Apigenin[b]		+
	Cosmosiin (Apigenin-7-O-glucoside)	+	+
	Diosmetin[b]		+
	Luteolin	+	+
	Luteolin-7-O-glucoside (cynaroside)	+	+
	Luteolin-7-O-glucuronide	+	+
	Apigenin-7-O-glucuronide	+	+
	Luteolin-7-O-rutinoside (scolymoside)	+	+
	Isorhoifolin (Apigenin-7-O-rutinoside)[b]		+
	Salvigenin[b]		+
	Naringin dihydrochalcone[b]		+
	Vicenin-2 (6,8-Di-C-glucosylapigenin)	+	+

Phytoconstituents		Aqueous artichoke	Hydro-alcoholic artichoke
Polyphenols	1,3-Di-O-caffeoylquinic acid (Cynarin)	+	+
	5-O-Caffeoylshikimic acid I[a]	+	
	5-O-Caffeoylshikimic acid II[a]	+	
	Esculin	+	+
	Ferulic acid	+	+
	Caffeoylquinic acid I[a]	+	
	Caffeoylquinic acid II[a]	+	
	Coumaroylquinic acid I[a]	+	
	Coumaroylquinic acid II[a]	+	
Other metabolites	Indole-4-carbaldehyde	+	+
	Choline	+	+
	Methyl cinnamate[b]		+
	Phenethylamine	+	+
Purines and pyrimidines	5'-S-Methyl-5'-thioadenosine	+	+
	Adenine	+	+
	Adenosine	+	+
	Adenosine 5'-monophosphate (AMP)[a]	+	
	Cytidine	+	+
	Guanosine	+	+
	Guanosine 5'-monophosphate (GMP)[a]	+	
	Uracil	+	+
	Uridine[a]	+	
Saponins	Cynarasaponin E	+	+
	Cynarasaponin C	+	+
Terpenoid	Cynaratriol	+	+
	Ursolic acid[b]		+
Sugars	Glucose or Galactose	+	+
Steroids	Stearidonic acid methyl ester[b]		+
	Stearidonic acid ethyl ester[b]		+
Vitamines	Nicotinamide	+	+
	Nicotinic acid (B3)	+	+
	Pantothenic acid (B5)	+	+
	Pyridoxal[a]	+	
	Pyridoxine (B6)	+	+

[a] Compounds to be found only in aqueous extract
[b] Compounds found only in hydro-alcoholic extracts

According to our current knowledge, we were the first to identify the kynurenic acid, trigonelline and stachydrine as the major alkaloids present in both artichoke extracts. The neuroprotective role of kynurenic acid has been already

demonstrated, and is achieved via the kynurenine pathway by metabolizing the tryptophan amino acid that is also present in both of ours artichoke extracts [27]. The presence of trigonelline in plant extracts like coffee and fenugreek was demonstrated, and some experimental data did indicate its Nrf2 inhibitory effect together with the blocking of Nrf2-dependent expression of proteasomal genes, and reduced proteasome activity in some pancreatic carcinoma cell lines [28]. Stachydrine is a prolinebetaine type of alkaloid that was suggested to play an important role in prevention of cardiovascular diseases by inhibiting the deleterious effect of high-glucose on endothelial cells through the modulation of SIRT1 pathway [29].

With the exception of phenylalanine and asparagine, all the other amino acids listed in Table 3. are reported for the first time in the case of artichoke extracts [30].

In this paper we are describing also for the first time the presence of some coumarins in artichoke extracts. The newly identified 7-methoxy-4-methylcoumarin was shown by others to behave like the multidrug resistant modulator verapamil that was more cytotoxic against tumor cells than normal cells [31]. Cinnamaldehyde is found in both of our artichoke extracts, and it was shown by others to ameliorate the induced cardiac dysfunction in rats by inhibiting ROS production and autophagy through TLR4-NOX4 pathway and exhibits anti-inflammatory activity [32]. Similarly to others [33], we were able to identify the scopoletin in artichoke leaves hydro-alcoholic extracts, and it was suggested to have an important anti-inflammatory activity by inhibiting the phosphorylation of NF-κB and p38 MAPK in mice [34].

Flavonoids like apigenin, apigenin-7-O-glucoside, apigenin-7-O-glucuronide, luteolin-7-O-glucuronide, luteolin-7-O-glucoside and apigenin-7-rutinoside had been already reported [35-38]. However, flavonoids like diosmetin, salvigenin, naringin dihydrochalcone and vicenin-2 have been for the first time identified, and are mostly present in the hydro-alcoholic artichoke extract (see Table 3.). Diosmetin was shown by others to inhibit the metastasis of hepatocellular carcinoma cells [39,40], while salvigenin antitumor and immunomodulatory effects on tumor bearing mice had been demonstrated [41]. The naringin dihydrochalcone biological effects were not analysed to present days, however its major constituent the naringin was suggested to be the main component of Ganshuang granule that plays an anti-fibrotic role through deactivation of hepatic stellate cells in cirrhotic mouse model [42], and through the attenuation of EGFR/ERK signalling could suppress cancer cell growth [43]. In the case of vicenin-2 has been recently shown that can suppress high-glucose induced vascular inflammatory processes in human umbilical vein endothelial cells and mice, thereby suggesting its effectiveness as a therapeutic agent for vascular inflammatory diseases [44, 45].

The polyphenol content of artichoke was extensively analysed, and several papers were published comparing mature and baby plants in raw or cooked forms with the a relevant phytoconstituent like cynarin -1,3-Di-O-caffeoylquinic acid [46]. Our aqueous artichoke extract contained much more polyphenols than the hydro-alcoholic extract, and several bioactive constituents were identified for the first time in artichoke, including 5-O-caffeoylshikimic acid, esculin and coumaroylquinic acids (see Table 3.). At present, no data are available regarding the biological effects of 5-O-caffeoylshikimic acid. Esculin has been found to feature gastroprotective effect in mice presumably through the inhibition of NF-κB activation [47], and its protective role against the genotoxicity induced by mitomycin C on liver and kidney mice cells was also described [48]. Ferulic acid is considered the methylated derivate of caffeic acid, and it was suggested that together with other flavonoids and polyphenols to contribute to the antioxidant, anti-inflammatory and anti-septic potential of *Lolium multiflorum* extracts [49].

Among metabolites we could identify indole-4-carbaldehyde that has not been descried in previously by others, while the incidence of choline, methyl cinnamate and phenethylamine are shown for the first time in the case of artichoke extracts. Methionine- and choline-deficient diet leads to nonhydro-alcoholic fatty liver diseases in mouse, rat and swine model systems, therefore, it is expected that the administration of choline would contribute to the prevention of nonhydro-alcoholic steatohepatitis and fibrosis. Methyl cinnamate is a safe antibacterial and flavouring agent used in food industry, and was shown to inhibit the gastrointestinal contractility [50], PPARγ activity and adipocyte differentiation in part, by the CaMKK2-AMPK signalling pathway [51]. Phenethylamine is widely used in weight-loss type of dietary supplements [52].

We were able to confirm the finding of others with respect to the presence of saponins like cynarasaponin C, E, B and K in artichoke extracts [26, 53], while their biological effects remained totally elusive.

Among terpenoids the cynaratriol was already reported in artichoke extracts, while the ursolic acid is a newly identified phytoconstituent. The cynaratriol biological effects are not elucidated, while for ursolic acid has been demonstrated to exert anti-oxidative and anti-inflammatory effects on mouse brain injury model by activating the Nrf2-ARE pathway [54], moreover its anti-cancer and anti-metastatic effects were also proven [55,56].

We were also able to identify carbohydrates in artichoke extracts, though the applied method did not allow us to distinguish between glucose and galactose.

According to our current knowledge, steroids like stearidonic acid methyl ester and stearidonic acid ethyl ester were not reported in the case of previously studied artichoke extracts. However, the steroids detected by us

are derivates of the stearidonic acid (18:4n-3), a plant-derived dietary n-3 PUFA, whose impact on tissue n-3 PUFA content are lacking.

The identification of vitamin C and some vitamins belonging to the B group (thiamine, riboflavine, nicotinamide and nicotinic acid) in artichoke extracts was already reported [57]. It has been demonstrated that the nicotinic acid can inhibit lipolysis, acutely reducing plasma free fatty acid concentrations, and my act in much the same manner as cynarin [58]. We are describing for the first time the incidence of pantothenic acid, pyridoxal and pyridoxine in artichoke extracts, while the above mentioned B5 and B6 vitamins were suggested to act as cancer risk reduction agents [59], and having anti-inflammatory effects associated with atherosclerosis and autoimmunity [60].

During our study, we also came across other phytoconstituents like vitamin C, thiamine, rutin, luteolin and quercetin. The molecular peaks have been identified for the above mentioned phytoconstituents, and the corresponding specific isotopic patterns confirm their molecular structure, but their fragmentation profiles do not corroborate with the values previously reported in scientific literature.

CONCLUSIONS

In the current paper, we are describing the comparative chemomapping of aqueous and hydro-alcoholic extracts of artichoke leaves. Some previously reported phytoconstituents presence was confirmed, while many other newly identified compounds are reported for the first time to be specific to artichoke. The currently described phytoconstituent profile strongly supports the liver and gallbladder tonic effect of artichoke by interfering with lipid metabolism. Moreover, some kind of anti-cancerous effect could also be expected based on some phytoconstituents. Indeed we were able to demonstrate that the aqueous and hydro-alcoholic extracts of artichoke presented in this paper possess anti-cancerous effects [14]. Based on individual effects of the identified phytoconstituents, multiple mechanisms could be evoked to explain the artichoke health promoting effects like the inhibition of cholesterol synthesis and lipolysis, together with the activation of anti-inflammatory, anti-tumour growth cellular pathways. It seems therefore likely that due to the plethora of phytoconstituents found in artichoke, the health promoting effect of the analysed extracts, might have a more stochastic than determinative nature. Further experiments are needed based on a system biology type of approach to clarify the complexity of the beneficial effects including the correlations with chemical composition.

EXPERIMENTAL SECTION

x. Materials and methods

x.1. Chemicals and reagents

HPLC-MS grade acetonitrile, water and formic acid were purchased from Fisher Scientific (Geel, Belgium). HPLC grade ammonium acetate and ammonium formate were purchased from Sigma-Aldrich (Munich, Germany).

x.2. Plant material

The artichoke dried leaves were obtained from TTDR 2000 Ltd., Hungary.

x.3. Sample preparation

Aqueous (AE) extract: Artichoke dried leaves (5 g) were cooked (5 min) in boiling water (100 ml). After cooling at room temperature, the extract was centrifuged (10 min, 4000 rpm) and filtered through Whatman filter paper (Sigma Aldrich).

Hydro-alcoholic (HE) (ethanol : water 1:1) extract: 50 g artichoke dried leaves were extracted two times with 500 ml ethanol – water (50:50) by stirring for 4h at 40 °C. This artichoke solution were centrifuged at 4000 rpm for 10 min at room temperature and moved the ethanol from the sample in a rotation vacuum evaporator.

Both types of samples were filtered through a 45 µm filter and stored at 4 °C until analysis.

x.4. HPLC-MS analysis

The UHPLC system (Dionex Ultimate 3000RS equipped with a Thermo Accucore C18 column, 100/2.1 with a particle size of 2.6 µm) was coupled to a Thermo Q Exactive Orbitrap mass spectrometer equipped with an electrospray ionization source (ESI). Eluent A (500 ml of water containing 10 ml of acetonitrile, 0.5 ml of formic acid and 2.5 mM of ammonium formate) and eluent B (500 ml of acetonitrile containing 10 ml of water, 0.5 ml of formic acid and 2.5 mM of ammonium formate) were used in the HPLC separation in positive ionization mode, and eluent A (500 ml of water containing 10 ml of acetonitrile and 2.5 mM of ammonium acetate) and eluent B (500 ml of acetonitrile containing 10 ml of water and 2.5 mM of ammonium acetate) were used in the HPLC separation in negative ionization mode. Flow rate was 200 µl/min. The following gradient elution program was used both positive and negative ionization mode: 0-1 min,

95% A, 1-22 min, 20% A; 22-24 min, 20% A; 24-26 min, 95% A; 26-40 min, 95% A. 5 µl of samples were injected in every run. The Q Exactive hybrid quadrupole-orbitrap mass spectrometer was operated with the following parameters: capillary temperature 320 °C, spray voltage 4.0 kV in positive mode and 3.8 kV in negative mode, the resolution was set to 35000 in the case of MS and to 17500 in the case of MS/MS. The mass range scanned was 100-1000 m/z. Collision energy was 40NCE in the MS/MS scans. The used UHPLC-ESI-MS measurement accuracy is within 5ppm. The difference between measured and calculated molecular ion masses were always below 5 ppm.

ACKNOWLEDGMENTS

We would like to thank to the members of the Agricultural and Molecular Research Institute of University of Nyíregyháza for their helpful technical assistance. We are thankful to Dr. Neli Kinga Olah for her comments and suggestions regarding phytoconstituents of artichoke and manuscript preparations. The research expenses were covered by the “In vitro study of some plant extracts of natural origin with emphasis on their anti-tumor effects.” HURO/0801 Hungarian-Romanian Cross Border Cooperation 2007-2013 grant.

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