Bioactive lignans and other phenolics from the roots, leaves and seeds of *Arctium lappa* L. grown in Egypt

Elsayed A. Aboutabl[a], Mona E. El Mahdy[b], Nadia M. Sokkar[a], Amany A. Sleem[c] and Manal M. Shams[b]

[a]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, [b]Department of Pharmacognosy, National Organization for Drug Control and Research, Cairo and [c]Department of Pharmacology, National Research Center, Giza, Egypt

Correspondence to Elsayed A. Aboutabl, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr-el-Aini Str., 11562 Cairo, Egypt
Tel: +20 100 242 8817; fax: +20 223 628 426; e-mail: eaboutabl@yahoo.com

Received 2 January 2012
Accepted 15 March 2012

Egyptian Pharmaceutical Journal 2012, 11:59–65

Aim

Investigation of the phenolic constituents of roots, leaves and seeds of *A. lappa* L. cultivated in Egypt.

Methods

Qualitative and quantitative analysis of lignans and other phenolics by LC-MS/MS and HPLC/PDA-LC-MS/MS, as well as evaluation of potential bioactivities.

Results and conclusions

LC-MS/MS of the ethyl acetate fractions from *Arctium lappa* seeds, leaves and roots showed the presence of 13 phenolics, including lignans, distributed in the different organs. The following phenolics were identified: caffeic acid **1**, genistein **2**, biochanin A **3**, luteolin **4**, chlorogenic acid **5**, materisenol **6**, arctigenin **7**, quercetin **8**, cynarin **9**, arctiin **10**, lappaol A **11**, rutin **12** and lappaol F **13**. Quantification of these compounds by HPLC-PDA-MS/MS showed that arctiin was the major compound in seeds. The antioxidant, antihepatotoxic, anti-inflammatory and cytotoxic activities of ethanolic extracts of the different organs were evaluated.

Keywords:

antihepatotoxicity, anti-inflammatory, antioxidant, *Arctium lappa* L., coumarin, cytotoxicity, lignans, phenolics

Egypt Pharm J 11:59–65

1687-4315

Introduction

Arctium lappa L. or burdock (Asteraceae) is native to Europe and north Asia. It is a safe and edible food product in Asia [1,2]. In traditional medicine, decoctions of powdered seeds, leaves and roots of burdock have been used in the treatment of cold, catarrh, gout and rheumatism, as a diuretic, diaphoretic and laxative, and for skin problems [3]. Burdock is one of the herbs widely used by cancer patients in some Canadian populations to improve the quality of life and reduce cancer progression [4]. Evaluation of the activities of extracts of different organs of burdock and the compounds isolated thereof were carried out including antipyretic, antimicrobial, diuretic, diaphoretic, antihyperglycaemic [5], in-vitro antioxidant [3,6–9], anti-inflammatory, antihepatotoxicity [10–13], antiulcer [14], antimutagenicity [15,16] and antitumour activities [17–19]. Phytochemical investigations indicated the presence of fixed oil (15–30%), phenolic acids, flavonoids and lignans [2,7], resin, mucilage, essential oil [20], polyacetylenes [20] and caffeoylquinic acid derivatives [8].

The present study aimed to investigate the constituents of roots, leaves and seeds of *A. lappa* L. cultivated in Egypt to explore any variations because of environmental changes. Lignans and other phenolics were investigated qualitatively and quantitatively by LC-MS/MS and HPLC/PDA-LC-MS/MS. The potential bioactivities of extracts from seeds, leaves and roots of the plant were evaluated, namely, in-vivo antioxidant, hepatoprotective, anti-inflammatory and cytotoxic activities.

Subjects and methods

Plant material

Seeds of *A. lappa* L. were kindly provided to Prof. Dr E. Aboutabl by the Botanical Garden, Bohn, Germany. The plant was raised in the Experimental Station, Faculty of Pharmacy, Cairo University. Seeds, leaves and roots were annually obtained from the cultivated plant collected during the fruiting stage in July, air-dried and kept in closed containers.

Reference standards

Standard samples of arctigenin and arctiin were kindly supplied to Prof. Dr E.A. Aboutabl by the late Prof. Dr Martin Luckner, Institute of Pharmaceutical Biology, Martin-Luther University, Halle/S, Germany. Chlorogenic acid, caffeic acid, coumarin, cynarin, quercetin, rutin and luteolin were obtained from the National Organization of Drug Control and Research Reference Standards Department, and genistein, biochanin A, carrageenan and alloxan were obtained from Sigma (USA). Indomethacin was obtained from Epico, (Egypt), α-tocopheryl acetate from Pharco Pharmaceutical Co. (Egypt) and carbon tetrachloride BDH (England).

Biochemical kits and reagents

Glutathione kits for the assessment of antioxidant activity and transaminase kits for the assessment of serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were obtained from Biodiagnostic Co. (Cairo, Egypt).

1687-4315 © 2012 Division of Pharmaceutical and Drug Industries Research, National Research Centre DOI: 10.7123/01.EPJ.0000415466.17860.6a

Animals

Albino mice weighing 25–30 g and adult albino rats of the Sprague–Dawley strain (130–150 g) were kept under hygienic conditions and on standard laboratory diet and water, supplied *ad libitum*.

Methods

Thin-layer chromatography (TLC) was performed on precoated silica gel F254 plates (Fluka, Germany). The solvent systems used were: (S1) chloroform–methanol (9 : 1); (S2) ethyl acetate–water–formic acid (85 : 15 : 10) for lignans, phenolic acids and coumarin; and (S3) hexane–ethyl acetate–formic acid (20 : 19 : 1) for flavonoids. Visualization of flavonoids and phenolic acids was carried out under UV (254 and 366 nm), that of lignans was performed by spraying with 50% H_2SO_4 and that of coumarins by spraying with 10% alcoholic KOH solution.

Column chromatography was performed using silica gel H 60 (Sigma); for VLC, Sephadex LH-20 (Pharmacia, Sweden) and silica gel 60 (Fluka, Germany) were used.

NMR spectra were run on JOEL GLM, and JOEL TMS route instrument, 500 MHz (Japan).

EI-MS, mass spectrophotometer (FINNIGAN MAT SSQ.70000, USA) and (Shimadzu QP 1000EX, Japan) ionization mode 70 eV equipment were used.

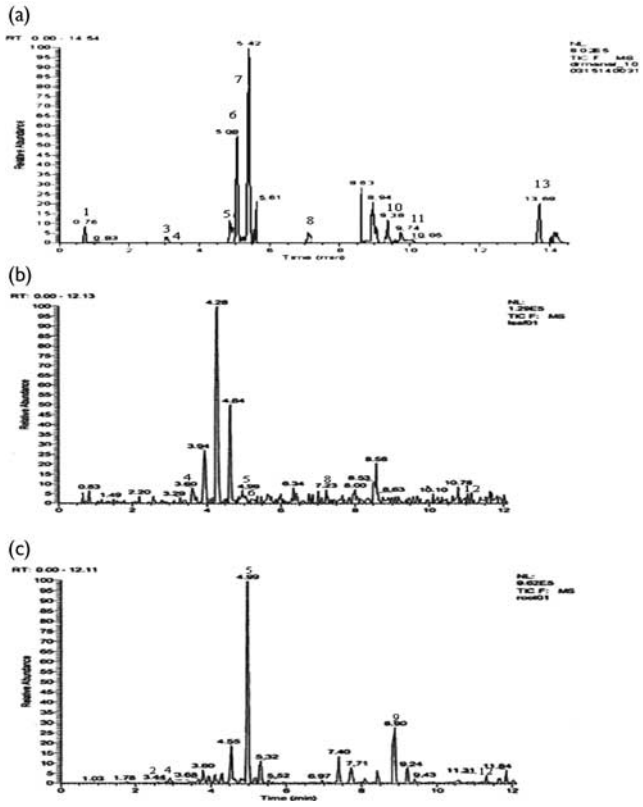
LC-MS/MS was performed on a Thermo ion trap mass spectrophotometer LCQ Advantage. The analysis was performed using the following settings: drying gas (air) was heated to 400°C; capillary voltage was set at 4 kV; air was the nebulizer gas; the curtain gas was N_2; the collision gas was He; ionization was performed in negative mode $[M–H]^-$ and collision energy was 35%. The full-scan mass infusion was performed using a syringe pump (Hamilton syringe, 500 µl) directly connected to electrospray ionization at a flow rate of 10 µl/min. The total ion mapping technique was used in LC-MS/MS (Fig. 1, Table 1).

HPLC analysis was performed using a PDA detector and an Intersil ODS-2 C18 column, 2.1 × 50 mm, particle size 3 µm (Alltech, Deerfield, Illinois, USA), using the solvent system A: water 0.2% formic acid and B: acetonitrile/methanol (60:40 v/v). The gradient program was as follows: 70% A (2 min), 50% A (4 min), 30% A (3 min) and 70% A (9–12 min); a flow rate of 0.2 ml/min; and an injection volume of 20 µl. X calibur version 1.4 (Thermo Fisher Scientific Inc., USA) was the software linked to the instrument for the calculation of the corresponding concentrations.

HPLC-PDA-LC-MS/MS quantification of major phenolics

Defatted air-dried powdered seeds, leaves and roots (1 g, each) were separately extracted with 70% methanol by sonication at room temperature. Stock solutions were prepared by dissolving each of the concentrated extracts in 5 ml methanol. The sample solutions were injected into the mass detector against isolated compounds considered as external standards of caffeic acid, cynarin, chlorogenic acid, arctigenin and arctiin at different

Figure 1



Total Ion chromatograms of LC-MS/MS of (a) seed extract, (b) leaf extract and (c) root extract.

dilutions (0.031–2 mg/ml, each) and arctigenin (0.018–2 mg/ml). The stock solutions were stored at –20°C. The stock and standard solutions were filtered through 0.45 µm filters, before injection, and diluted as necessary with methanol. Each concentration of the standards was analysed in triplicate. Quantification of the compounds was performed by measuring the peak area against six concentrations of the standards, plotting the standard curves and determining the compounds as the mean values of three replicate injections. Quantitative determination of caffeic acid, cynarin, arctigenin, chlorogenic acid and arctiin was carried out (Figs 1 and 2; Table 2).

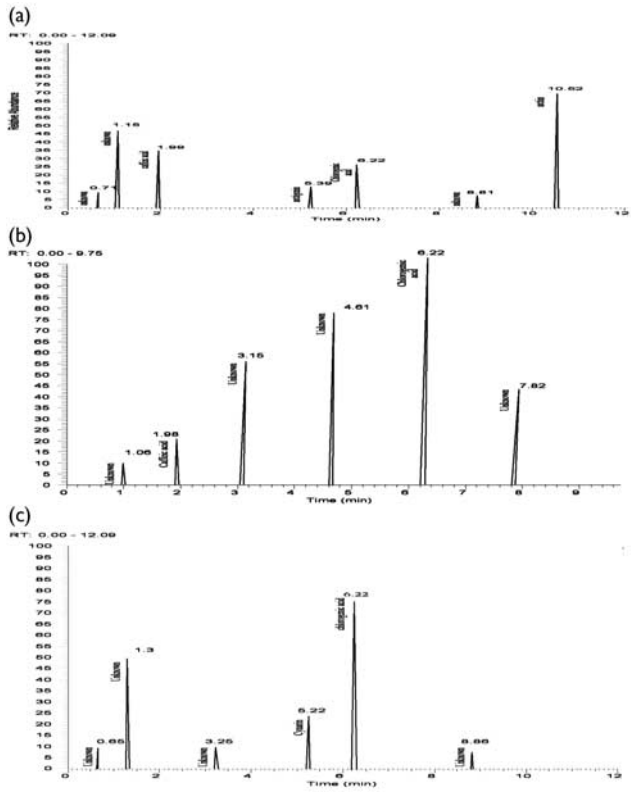
Colorimetric determination of total phenolics

Samples of air-dried and defatted powdered seeds, leaves and roots (5 g, each) were extracted with 70% methanol by sonication at room temperature. A stock solution (1 mg/ml) was prepared from the concentrated residue by dissolution in distilled water. Phenolics were determined using the Folin–Ciocalteau colorimetric method [21] and were expressed as mg of gallic acid equivalent/g of the air-dried plant material. The standard solution was prepared by dissolving 50 mg of gallic acid in 100 ml water. A calibration curve was constructed over the range of 15–100 µg/ml by diluting the stock solution in water. The prepared solutions from seeds, leaves and roots (0.1 ml, each) were separately mixed with 0.5 ml Folin–Ciocalteau reagent and diluted to 25 ml using an aqueous

Table 1 LC-MS/MS of phenolics in the extracts of *Arctium lappa* L. grown in Egypt

Number	Compound	R_t (min)	UV_{max}	$[M-H]^-$ *m/z*	Product ions	Seeds	Leaves	Roots
1	Caffeic acid	0.71	310, 285	179	135	+	+	
2	Genistein	2.93	259	269	–	+		+
3	Biochanin A	3.26	260	283	238.02, 267	+		
4	Luteolin	3.34	350	285	269		+	+
5	Chlorogenic acid	4.96	325 (sh), 295	353	190	+	+	+
6	Matareisinol	5.03	280	357	313,342	+		
7	Arctigenin	5.39	280	371	356	+		
8	Quercetrin	7.21	349, 256	447	301	+	+	
9	Cynarin	8.87	326	515	179,203,255,298.8, 352.87			+
10	Arctiin	9.33	280	533	371	+		
11	Lappaol A	9.35	283	535	373	+		
12	Rutin	11.14	361, 258	609	300.99		+	+
13	Lappaol F	13.64	282	713	677	+		

Figure 2



HPLC-PDA chromatogram of (a) seed extract, (b) leaf extract, (c) root extract.

Table 2 Quantitative HPLC determination of phenolics (mg/g powder)[a] in the seeds, leaves and roots of *Arctium lappa L.* grown in Egypt

	Seeds	Leaves	Roots
Caffeic	2.75 ± 0.09	0.973 ± 0.003	NQ
Cynarin	NQ	NQ	0.52 ± 0.02
Arctigenin	0.0003	NQ	NQ
Chlorogenic	4.26 ± 0.014	6.533 ± 0.01845	0.615 ± 0.026
Arctiin	47.54 ± 0.16	NQ	NQ

HPLC, high-performance liquid chromatography; NQ, not quantified.
[a]Results are means of three determinations ± SD

sodium carbonate solution (290 g/l). The absorbance was measured after 30 min at 730 nm against a blank prepared at the same time using 0.5 ml water as a compensating liquid. From the linear regression analysis ($y = 0.0169x + 0.0358$), $r^2 = 0.9871$, the phenol content of each extract was expressed as mg gallic acid/g. Results (Table 2) were the means of three determinations.

Sample preparation for the screening of phenolics by LC-MS/MS

Air-dried and defatted powdered seeds, leaves and roots (5 g, each) were extracted with 70% methanol by sonication at room temperature. The concentrated aqueous residue was fractionated between chloroform and ethyl acetate. The ethyl acetate fraction of each of the three organs was filtered over anhydrous sodium sulphate, concentrated to dryness and dissolved in 100 ml methanol (HPLC grade) for LC/MS analysis, as it contained lignans, flavonoids and phenolic acids as determined by TLC.

Isolation of phenolics

Air-dried and defatted powdered seeds (600 g) and roots (300 g) were repeatedly suspended in 70% methanol until exhaustion. The concentrated extracts (250 and 135 g; yield = 41.7 and 45%, respectively) were separately suspended in water and successively fractionated with chloroform and ethyl acetate. The concentrated fractions yielded 27.6 and 4 g for seeds and 0.7 and 2 g for roots, respectively. The chloroform fraction of seed (10 g) was subjected to VLC (30 × 5 cm, silica gel G). Gradient elution was carried out using *n*-hexane; *n*-hexane/diethyl ether till 100% diethyl ether; diethyl ether/dichloromethane till 100% dichloromethane. Fractions (100 ml, each) were collected; similar fractions were pooled as monitored by TLC. The diethyl ether subfraction (140 mg) of seeds was subjected to preparative layer chromatography, followed by filtration through Sephadex and crystallization from methanol to yield compound **7** (20 mg). The concentrated dichloromethane subfraction (640 mg) was extracted with ethyl acetate to yield a yellow residue (500 mg); the latter was placed in a polyamide column eluted with 30% aqueous methanol and crystallization from a mixture of chloroform/ethyl acetate to yield compound **10** (100 mg). The ethyl acetate fractions of seeds (4 g) and roots (2 g) were placed in polyamide and Sephadex LH20 columns to yield compound **6** (80 mg) and compound **1** (6 mg) from seeds and compound **14** (10 mg) and compound **9** (20 mg) from roots.

Evaluation of bioactivities

Air-dried powdered seeds, leaves and roots (100 g, each) were separately macerated in 70% ethanol until exhaustion. The ethanol extracts of seed (EES), leaf (EEL) and root (EER) were separately concentrated for bioactivity evaluation.

Toxicity

The median lethal dose (LD_{50}) of each of the plant extracts was determined [22]. Antioxidant activity: glutathione in blood was determined [23]. Thirty rats were intraperitoneally administered Alloxan (150 mg/kg body weight) to induce hyperglycaemia [24]. Hyperglycaemia was assessed after 72 h by measuring the blood glucose level [25]. The results are presented in Table 3.

Hepatoprotective activity

Liver damage in rats was induced [26] by an intraperitoneal injection (5 ml/kg body weight) of 25% CCl_4 in liquid paraffin. Serum ALT, AST [27] and ALP [28] were determined. Animals were randomly divided into five groups: the first group received a daily dose of 1 ml saline for 1 month before and after liver damage was induced (control); in groups 2–5, liver-damaged rats were pretreated daily with ethyl acetate extract of seeds, leaves and roots (EES, EEL and EER, respectively) (100 mg/kg body weight), as well as silymarin (25 mg/kg body weight), respectively, for 2 weeks. Administration of the extract was continued after liver damage for another 2 weeks, followed by overnight fasting; whole blood was obtained from the retro-orbital venous plexus through the eyes canthus of anaesthetized rats. Blood samples were collected at zero time, 72 h and 2 weeks after the CCl_4 injection and at 1-month intervals. Serum was isolated by centrifugation (Table 4).

Acute anti-inflammatory activity [29]

Thirty male albino rats were divided into five groups (six animals each). Group 1 received 1 ml of saline and served as the control group; groups 2–5 received ethyl acetate extract of seeds, leaves and roots (EES, EEL and EER, respectively) (100 mg/kg body weight) and indomethacin (20 mg/kg body weight), respectively. One hour later, all the animals received a subplanter injection of a 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Four hours after drug

Table 3 Antioxidant activity of ethyl acetate extract of the seeds (EES), leaves (EEL) and roots (EER) of *Arctium lappa L.* grown in Egypt in comparison with vitamin E in diabetic male albino rats ($n=6$)

	Blood glutathione (mg%)	Difference from control (%)
Control	36.1 ± 1.1	36.1
Diabetic	21.7 ± 0.4[a]	39.8
Diabetic + vitamin E	35.3 ± 0.8[a]	2.2
Diabetic + EES	34.2 ± 0.7[a]	5.26
Diabetic + EEL	27.9 ± 0.5[a]	22.71
Diabetic + EER	26.3 ± 0.6[a]	27.14

[a]Statistically significant difference from the control group at $P<0.01$; data presented as mean ± SE; the extracts are in 100 mg/kg and vitamin E in 7.5 mg/kg.

Table 4 Effect of ethyl acetate extract of the seeds (EES), leaves (EEL) and roots (EER) of *Arctium lappa* L. and silymarin on serum enzyme level (AST, ALT and ALP) in liver-damaged rats ($n=6$)

	AST (U/l)				ALT (U/l)				ALP (KAU)			
	Zero	15 days	72 h	15 days	Zero	15 days	72 h	15 days	Zero	15 days	72 h	15 days
Control	29.4 ± 0.9	28.6 ± 0.4	136. ± 5.1	151.7 ± 5.9[ab]	31.6 ± 1.1	30.9 ± 0.7	143.0 ± 6.1	149.0 ± 5.7[b]	6.8 ± 0.1	7.1 ± 0.1	57.9 ± 1.8	63.4 ± 2.3[b]
EES	28.7 ± 1.1	28.1 ± 0.6	63.9 ± 2.4	38.9 ± 1.3[ab]	27.6 ± 0.4	27.1 ± 0.3	68.9 ± 2.6	36.8 ± 1.7[ab]	7.1 ± 0.1	6.8 ± 0.1	24.2 ± 0.8	19.8 ± 0.6[b]
EEL	30.9 ± 0.8	29.8 ± 0.7	67.3 ± 2.5	43.6 ± 1.7[ab]	31.4 ± 1.1	30.7 ± 0.9	74.9 ± 3.2	48.2 ± 2.1[ab]	7.4 ± 0.1	7.2 ± 0.1	28.7 ± 0.6	23.9 ± 0.7[ab]
EER	31.6 ± 1.2	30.8 ± 1.1	82.4 ± 2.7	56.8 ± 2.1[ab]	33.2 ± 1.2	33.5 ± 1.1	91.6 ± 3.8	73.2 ± 3.4[ab]	7.2 ± 0.1	7.4 ± 0.1	34.9 ± 1.4	29.6 ± 0.9[b]
Silymarin	32.3 ± 1.1	30.6 ± 0.9	49.2 ± 1.3	29.7 ± 0.6[a]	27.8 ± 0.5	26.8 ± 0.4	56.2 ± 1.8	29.2 ± 0.8[a]	7.3 ± 0.1	6.9 ± 0.1	18.3 ± 0.6	6.9 ± 0.1[a]

[a]Statistically significant from 72 h at $P<0.01$.
[b]Statistically significant from zero time at $P<0.01$ silymarin.

administration, the rats were sacrificed; both hind paws were separately excised and weighed (Table 5).

$$\%\,\text{Oedema} = \left(\frac{\text{weight of right paw} - \text{weight of left paw}}{\text{weight of left paw}}\right) \times 100.$$

Cytotoxicity

The potential cytotoxicity of the ethyl acetate extract of seeds, leaves and roots (EES, EEL and EER, respectively) of *A. lappa* L. was tested [30] on liver, breast and colon cancer cell lines. IC_{50} for each extract was calculated (Table 6). The relations between the surviving fractions and extracts concentrations were tabulated (Table 7) and plotted to construct the survival curves of each tumour cell line (Fig. 3).

Table 5 Acute anti-inflammatory activity of ethyl acetate extract of the seeds (EES), leaves (EEL) and roots (EER) of *Arctium lappa* L. grown in Egypt and indomethacin in male albino rats ($n=6$)

	%Oedema	
	Mean + SE	% of change[b]
Control	60.3 ± 2.1	
EER	41.6 ± 1.3[a]	54.2
EEL	27.6 ± 0.7[a]	31.01
EES	38.4 ± 0.9[a]	36.3
Indomethacin	22.4 ± 0.4[a]	62.8

[a]$P<0.01$ versus the control group.
[b]Percentage change with reference to the control group; extracts: 100 mg/kg; indomethacin: 20 mg/kg.

Table 6 IC_{50} of ethyl acetate extract of the seeds (EES), leaves (EEL) and roots (EER) of *Arctium lappa* L.[a] on cell lines

	IC_{50}		
Extracts	HCT116	HEPG2	MCF7
EES	1.59	0.817	4.69
EEL	2.23	0.85	5.53
EER	1.48	0.617	5.98

IC_{50}, dose of the sample that reduces survival to 50%; HCT116, colon carcinoma cell line; HEPG2, liver carcinoma cell line; MCF7, breast carcinoma cell line.
[a]Concentration μg/well.

Table 7 Cytotoxic activity of ethyl acetate extract of the seeds (EES), leaves (EEL) and roots (EER) of *Arctium lappa* L.

Test		Surviving fraction		
Extract	Concentration (μg/well)	HCT116	HEPG2	MCF7
EES	0.000	1.000	1.000	1.000
	1.000	0.606	0.365	0.857
	2.500	0.346	0.25	0.963
	5.000	0.169	0.185	0.433
	10.000	0.109	0.088	0.336
EEL	0.000	1.000	1.000	1.000
	1.000	0.783	0.365	0.932
	2.500	0.444	0.136	0.806
	5.000	0.253	0.105	0.515
	10.000	0.124	0.084	0.355
EER	0.000	1.000	1.000	1.000
	1.000	0.692	0.201	0.893
	2.500	0.439	0.112	0.972
	5.000	0.221	0.074	0.541
	10.000	0.116	0.239	0.334

HCT116, colon carcinoma cell line; HEPG2, liver carcinoma cell line; MCF7, breast carcinoma cell line.

Results

Seeds were found to be the richest in total polyphenols (20.35%), followed by the leaves and roots (9.28 and 5.33%, respectively). Screening of phenolics in ethyl acetate extracts of seeds, leaves and roots of *A. lappa* was run on LC-MS/MS. Total ion chromatograms (Fig. 1a–c) using the negative ion mode showed the following phenolics (Table 1): caffeic acid **1**, genistein **2**, biochanin A **3**, luteolin **4**, chlorogenic acid **5**, materisenol **6**, arctigenin **7**, quercetin **8**, cynarin **9**, arctiin **10**, lappaol A **11**, rutin **12** and lappaol F **13**. As no commercial standards for materisenol **6**, lappaol A **11** and lappaol F **13** were available, they were identified by their fragmentation pattern and comparison with literature data [7]. The other compounds were identified by a comparison of their TLC, UV and MS data with the reference samples and literature data. Six compounds were isolated from the seeds and roots of *A. lappa* and identified as arctigenin **7**, arctiin **10**, chlorogenic acid **5**, caffeic acid **1**, coumarin **14** and cynarin **9** by comparing their UV, MS and ^{1}HNMR data with the reference samples and literature [7,31,32].

Although *A. lappa* L. is known to be rich in lignans and phenolic acids, no studies in the literature has reported the presence of flavonoids in the seeds. This study dealing with the plant recently introduced in Egypt has shown that seeds contain biochanin A, genistein and quercetrin. However, arctiin was found to be absent in the root of the plant grown in Egypt, which is significantly different from that reported in a previous work [7]. Quantitative analysis of the isolated compounds caffeic, cynarin, arctigenin, chlorogenic acid and arctiin by HPLC-PDA-LC-MS/MS showed that arctiin (Tables 1 and 2; Figs 1 and 2) was the major compound and concentrated mainly in seeds, followed by chlorogenic acid in different concentrations in the three organs.

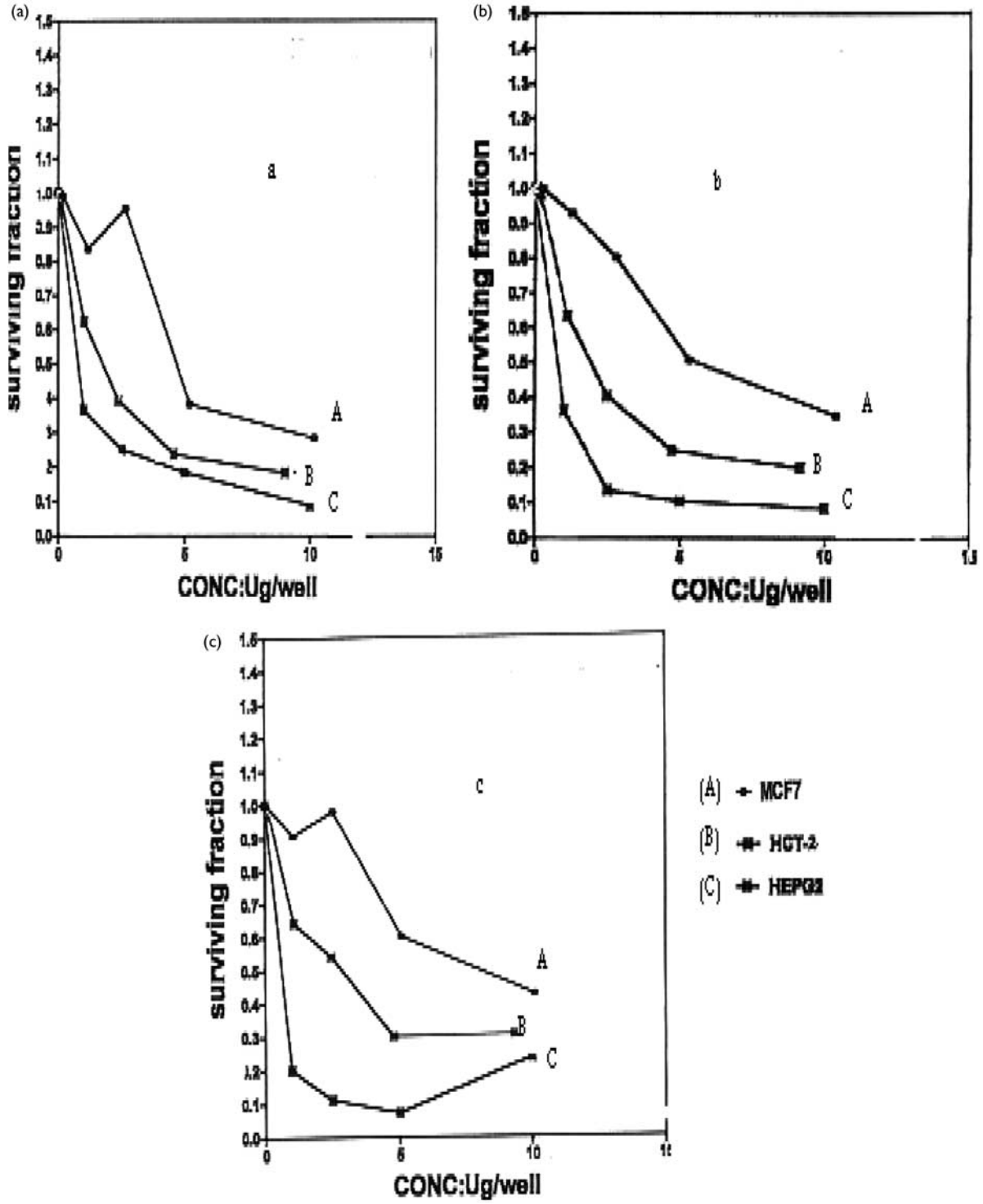
Toxicity

The results indicated the safety of seeds (LD_{50} up to 9.3 mg extract/kg body weight), the leaves and roots (up to 10 mg extract/kg body weight).

Antioxidant activity

The results (Table 3) showed that the blood glutathione level was considerably reduced in the untreated diabetic rats. The blood glutathione level was restored by the treatment of animals with vitamin E (with percentage change = 2.2). EES was the most potent extract, exerting almost the same effect as that of vitamin E, with percentage change = 5.26, followed by EEL and EER. Previous in-vitro antioxidant studies have shown a higher potency of roots [8] and leaves [6] of *A. lappa*; this variation can possibly be attributed to differences in the concentrations of flavonoids and lignans in seeds.

Figure 3



Cytotoxic activity of ethyl acetate extract of seeds (a), leaves (b) and roots (c) of *Arctium lappa* L. on liver (A), colon (B) and breast (C) carcinoma.

Antihepatotoxic activity

Liver damage by 25% CCl_4 (5 ml/kg) showed an increase in the levels of AST, ALT and ALP in blood. A significant decrease in serum enzymatic levels was observed (Table 4) after the administration of different extracts of *A. lappa* (100 mg/kg, daily) for 1 month, in the order seeds > leaves > roots. Previous investigations have reported the antihepatotoxicity of roots [10] and total herb [11].

Anti-inflammatory activity

All the extracts (Table 5) showed significant in-vivo anti-inflammatory effects as compared with the control group. The potency increased in the order: seeds > leaves > roots, showing percentage change of 54.2, 36.3 and 31.01%, respectively. In-vitro anti-inflammatory activity has been attributed to arctigenin [12]. Previous studies [10,13] have studied the anti-inflammatory activity of root extracts.

Cytotoxic activity

Ethanolic extracts of different organs of *A. lappa* (Fig. 3 and Tables 6 and 7) showed highly potent cytotoxic activities in liver carcinoma. Ethanolic extract of seeds showed more potency in colon carcinoma, while that of leaves being the most potent in breast carcinoma. The antiproliferative activity of the prepared extracts was mainly because of arctigenin and arctiin [4,17,33]. An anticarcinogenic effect was reported for seed [19] (using the cell line LNCaP) and root extracts [9] (using cell lines K562, MCF7 and 786-0).

Conclusion

A. lappa L. was successfully cultivated in Egypt. In this report, its phytochemical and bioactive profile is presented as a comparative study of its different organs in order to investigate its safe consumption. Evaluation of antioxidant, hepatoprotective, anti-inflammatory and cytotoxicity activities showed that the most significant activities were found for extracts of seeds, which can possibly be attributed to its high content of phenolics, including flavonoids, lignans and phenolic acids.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Bown D. *Encyclopedia of herbs & their uses*. 240-1: Dorling Kindersley, London; 1995.

2 Leung AY, Foster S. *Encyclopedia of common natural ingredients used in food, drugs and cosmetics*. 2nd ed. New York: Wiley-Blackwell; 1996.

3 Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004; 74:2157–2184.

4 Tamayo C, Richardson MA, Diamond S, Skoda I. The chemistry and biological activity of herbs used in flor-essence(TM) herbal tonic and essiac(TM). Phytother Res 2000; 14:1–14.

5 Barnes J, Anderson L, Philipson J. *Herbal medicines*. 3rd ed. 102-3: Pharmaceutical Press; 2008.

6 Kardosová A, Machová E. Antioxidant activity of medicinal plant polysaccharides. Fitoterapia 2006; 77:367–373.

7 Ferracane R, Graziani G, Gallo M, Fogliano V, Ritieni A. Metabolic profile of the bioactive compounds of burdock (Arctium lappa) seeds, roots and leaves. J Pharm Biomed Anal 2010; 51:399–404.

8 Maruta Y, Kawabata J, Niki R. Antioxidative caffeoylquinic acid derivatives in the roots of burdock (*Arctium lappa* L.). J Agric Food Chem 1995; 43: 2592–2595.

9 Predes FS, Ruiz AL, Carvalho JE, Foglio MA, Dolder H. Antioxidative and in vitro antiproliferative activity of *Arctium lappa* root extracts. BMC Complement Altern Med 2011; 11(1):25.

10 Lin CC, Lin JM, Yang JJ, Chuang SC, Ujiie T. Anti-inflammatory and radical scavenge effects of *Arctium lappa*. Am J Chin Med 1996; 24:127–137.

11 Lin SC, Lin CH, Lin CC, Lin YH, Chen CF, Chen IC, *et al*. Hepatoprotective effects of *Arctium lappa* Linne on liver injuries induced by chronic ethanol consumption and potentiated by carbon tetrachloride. J Biomed Sci 2002; 9:401–409.

12 Tsai WJ, Chang CT, Wang GJ, Lee TH, Chang SF, Lu SC, *et al*. Arctigenin from *Arctium lappa* inhibits interleukin-2 and interferon gene expression in primary human T lymphocytes. Chin Med 2011; 6:12.

13 Sohn EH, Jang SA, Joo H, Park S, Kang SC, Lee CH. Anti-allergic and anti-inflammatory effects of butanol extract from *Arctium lappa* L. Clin Mol Allergy 2011; 9:4–14.

14 Os'kina OA, Pashinskiĭ VG, Kanakina TA, Povet'eva TN, Gribel' NV. The mechanisms of the anti-ulcer action of plant drug agents. Eksp Klin Farmakol 1999; 62:37–39.

15 Ching Kuei H, Ching Chuan K, Wenchang C. Changes in chemical composition and antimutagenicity effect of burdock extract under various cooking duration. Shipin Kexue 1996; 23:693–701.

16 Ling L, Lili T. Studies on antimutagenicity of burdock. Acta Acad Med Nanjing 1997; 17:343–345.

17 Hirose M, Yamaguchi T, Lin C, Kimoto N, Futakuchi M, Kono T, *et al*. Effects of arctiin on PhIP-induced mammary, colon and pancreatic carcinogenesis in female Sprague–Dawley rats and MeIQx-induced hepatocarcinogenesis in male F344 rats. Cancer Lett 2000; 155:79–88.

18 Kasai H, Fukada S, Yamaizumi Z, Sugie S, Mori H. Action of chlorogenic acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxyguanosine formation in vitro and in a rat carcinogenesis model. Food Chem Toxicol 2000; 38:467–471.

19 Ming DS, Guns ES, Eberding A, Towers GHN. Isolation and characterization of compounds with anti-prostate cancer activity from *Arctium lappa* L. using bioactivity-guided fractionation. Pharm Biol 2004; 42:44–48.

20 Penelope O. *The complete medicinal herbal: a practical guide to the healing properties of herbs, with more than 250 remedies for common ailments*. 58: Dorling Kindersley, N.Y.; 1993.

21 Singleton VL, Orthofer R, Lamuela Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol 1998; 299:152–178.

22 Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983; 54:275–287.

23 Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882–888.

24 Eliasson SG, Samet JM. Alloxan induced neuropathies: lipid changes in nerve and root fragments. Life Sci 1969; 8:493–498.

25 Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969; 6:24–28.

26 Klaassen CD, Plaa GL. Comparison of the biochemical alterations elicited in livers from rats treated with carbon tetrachloride, chloroform, 1,1,2-trichloroethaneand 1,1,1-trichloroethane. Biochem Pharmacol 1969; 18:2019–2027.

27 Reitman S, Frankel S. A colourimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56–62.

28 Belfield A, Goldberg D. Colourimetric determination of alkaline phosphatase activity. Enzyme 1971; 12:561–566.

29 Winter GA, Risley EA, Nuss GW. Carragenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 3:1544–1547.

30 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al*. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990; 82:1107–1112.

31 Mabry TJ, Markham KR, Thomas MB. *The systematic identification of flavonoids*. 1st ed. Verlag, New York, Heidelberg, Berlin: Springer; 1970.

32 Han BH, Kang YH, Yang HO, Park MK. A butyrolactone lignan dimer from Arctium lappa. Phytochemistry 1994; 37:1161–1163.

33 Ryu SY, Ahn JW, Kang YH, Han BH. Antiproliferative effect of arctigenin and arctiin. Arch Pharmacol Res 1995; 18:462–463.