Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 399–404

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Metabolic profile of the bioactive compounds of burdock (*Arctium lappa*) seeds, roots and leaves

Rosalia Ferracane, Giulia Graziani*, Monica Gallo, Vincenzo Fogliano, Alberto Ritieni

Department of Food Science, Via Università 100, University of Naples "Federico II", Parco Gussone Ed.84, 80055 Portici, Naples, Italy

ARTICLE INFO

Article history:
Received 5 December 2008
Received in revised form 2 March 2009
Accepted 4 March 2009
Available online 25 March 2009

Keywords:
Arctium lappa
Lignan
Caffeoylquinic acid
Tandem mass spectrometry
Antioxidant activity

ABSTRACT

In this work the bioactive metabolic profile, the antioxidant activity and total phenolic content of burdock (*Arctium lappa*) seeds, leaves and roots were obtained. TEAC values and total phenolic content for hydro-alcoholic extracts of burdock ranged from 67.39 to 1.63 μmol Trolox equivalent/100 g dry weight (DW), and from 2.87 to 45 g of gallic acid equivalent/100 g DW, respectively. Phytochemical compounds were analyzed by liquid chromatography coupled to electrospray tandem mass spectrometry (LC/MS/MS) in negative mode. The main compounds of burdock extracts were caffeoylquinic acid derivatives, lignans (mainly arctiin) and various flavonoids.

The occurrence of some phenolic acids (caffeic acid, chlorogenic acid and cynarin) in burdock seeds; arctiin, luteolin and quercetin rhamnoside in burdock roots; phenolic acids, quercetin, quercitrin and luteolin in burdock leaves was reported for the first time.

1. Introduction

Burdock, *Arctium lappa* (Asteraceae) is among the most popular plants in traditional Chinese Pharmacopoeia and it is associated to several biological effects [1]. This vegetable, which is consumed daily in beverages in China for centuries, was recently introduced in Japan and Taiwan [2].

In the literature, many health benefits associated to occurrence of different classes of bioactive secondary metabolites have been reported. These classes include, among others, flavonoids and lignans, and burdock is an important natural source of compounds from both families. The burdock beneficial effects observed are related to hypertension, gout, arteriosclerosis, hepatitis and other inflammatory disorders [3,4]. Many of the biological properties attributed to burdock, including antimutagenicity, anticarcinogenicity, and antiaging, may originate from the antioxidant ability of its component [5–7].

Pharmacological studies and clinical trials indicated that burdock roots have hepatoprotective, anti-inflammatory and free radical scavenging activities [8,9] attributed to the presence of caffeoylquinic acid derivatives [10]. On the other hand, the chemopreventive effects of burdock seeds are associated to lignans such as arctiin and arctigenin [11,12]. Recently, antiproliferative and apoptotic effects of lignans from *A. lappa* on leukemic cells [13] and antitumoral effects of arctigenin on pancreatic cancer cell lines were also described [14].

In the literature few studies on HPLC analysis of burdock lignans are reported [15,16]. Liu et al. [17] used single quadrupole mass spectrometry to characterize burdock lignans and reported the isolation and identification of arctiin in burdock leaves. The aim of this study was to study the antioxidant properties of polyphenolic compounds in different tissues of the burdock plant and to evaluate their LC/MS/MS profile by electrospray ionization (ESI).

2. Experimental

2.1. Materials

All reagents and solvents HPLC grade were purchased from Merck (Darmstadt, Germany). Burdock roots, leaves and seeds were obtained from organic certified agriculture. These materials were provided respectively from BIOPLANTA s.a.s. (Irsina, Matera, Italy), Rocca dei Fiori (Lizzano in Belvedere, Bologna, Italy) and agriculture company "Campovioletto" (Cernusco sul Naviglio, Milano, Italy). Standards used to identify burdock antioxidant compounds were obtained from different supplies: caffeic acid, chlorogenic acid, quercetin, luteolin, quercetin-rhamnoglucoside (rutin), luteolin glucoside, quercetin rhamnoside (quercitrin) and quercetin glucoside from Sigma (Milano, Italy). The dicaffeoylchinic acid (cynarin) was provided by ChromaDex (Laguna Hills, CA, USA) while lignan standards (arctiin, arctigenin, lappaol A, lappaol C, arctignan E and matairesinol) were purified with an HPLC-UV/VIS

* Corresponding author. Tel.: +39 0812539351; fax: +39 0817762580.
E-mail addresses: liaferracane@unina.it (R. Ferracane), giulia.graziani@unina.it (G. Graziani), mongallo@unina.it (M. Gallo), fogliano@unina.it (V. Fogliano), ritialb@unina.it (A. Ritieni).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.03.018

working at 280 nm using the same chromatographic conditions described in the LC/MS/MS section from burdock seeds extracts. Fractions of the different compounds collected from HPLC were quickly freeze-dried, then dissolved in methanol at a concentration of 10 μg/mL and used for LC/MS/MS investigations. The (S)-(−)-6 hydroxy-2,5,7,8-tetramrthylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzothiazoline-6′-sulfonic acid) diammonium salt (ABTS) and potassium persulfate were obtained from Sigma (Milano, Italy).

2.2. Samples extraction

3 g of materials such as dried roots, leaves and seeds were extracted with 30 mL of methanol/water (70:30, v/v) by sonication at room temperature for 30 min. The extraction procedure was repeated twice for each sample. The mixtures were centrifugated at 4000 rpm, filtered through a Whatman filter papers (Whatman International Ltd., Maidstone, England) and then used for LC analysis, for antioxidant activity assay and for total phenolic content determinations.

2.3. Precision of the extraction procedure

The precision of the extraction procedure was validated using burdock samples. The samples (3 g of roots, 3 g of leaves and 3 g of seeds), were extracted twice as described above. An aliquot of each extract was then used for the analysis.

2.4. Accuracy

The accuracy of the extraction method was evaluated with the recovery test. The performance of the extraction was evaluated spiking samples with known quantities of standard compounds. In particular, chlorogenic acid was used for roots and leaves extraction and arctin was used for seeds extraction. The spiked samples were then extracted and analyzed with the LC/MS/MS protocol described below. The percentage recovery was determined by subtracting the values obtained for the control matrix from those samples with the added standards, divided by the amount added and then multiplied by 100.

2.5. Antioxidant activity assay

Antioxidant capacity assay was performed using an UV–VIS recording spectrophotometer (Shimadzu, Japan) by the improved $ABTS^{\bullet+}$ method as described by Re et al. [18]. $ABTS^{\bullet+}$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23 °C) in dark for 16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. The filtered sample was diluted with 70% methanol so as to give 20–80% inhibition of the blank absorbance with 0.1 mL of sample. 1 mL of $ABTS^{\bullet+}$ solution (with absorbance of 0.700 ± 0.050) was added to 0.1 mL of the tested samples and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 2.5 min and the absorbance was immediately recorded at 734 nm. Trolox standard solution (final concentration 0–15 μM) in methanol was prepared and assayed at the same conditions. The absorbance of the resulting oxidized solution was compared to that of the calibrated Trolox standard. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mmol Trolox equivalents per 100 g dry weight of plant).

2.6. Total phenolic contents determination

Total phenol concentration in plant extracts were determined spectrophotometrically by the Folin–Ciocalteu assay [19] using gallic acid as a standard. An aliquot of 125 μL of each plant extract was mixed with 125 μL of Folin–Ciocalteu phenol reagent and allowed to react for 6 min. After 6 min 1.25 mL of saturated Na_2CO_3 solution (7.5%) was added and allowed to stand for 90 min before the absorbance of the reaction mixture was measured in triplicate at 760 nm. The total phenolic contents of the plant extracts was expressed as mg gallic acid equivalents per 100 g of plant material.

2.7. LC/MS/MS system

Chromatographic separation was performed using an HPLC apparatus equipped with two Micropumps Series 200 (PerkinElmer, Shellton, CT, USA), a UV/VIS series 200 (PerkinElmer, Shellton, CT, USA) detector setted at 280 nm and a Prodigy ODS3 100 Å column (250 mm × 4.6 mm, particle size 5 μm) (Phenomenex, CA, USA). The eluents were: A water 0.2% formic acid; B acetonitrile/methanol (60:40, v/v). The gradient program was as follows: 20–30% B (6 min), 30–40% B (10 min), 40–50% B (8 min), 50–90% B (8 min), 90–90% B (3 min), 90–20% B (3 min) at a constant flow of 0.8 mL/min. The LC flow was split and 0.2 mL/min was sent to the mass spectrometry. Injection volume was 20 μL. Three injections were performed for each sample. MS and MS/MS analyses of burdock extracts were performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonSpray source working in the negative ion mode. The analyses were performed using the following settings: drying gas (air) was heated to 400 °C, capillary voltage (IS) was setted to 4000 V, nebulizer gas (air) 12 (arbitrary units), curtain gas (N_2) 14 (arbitrary units), collision gas (N_2) 4 (arbitrary units). The declustering potential (DP), focus potential (FP) and the collision energy (CE) were optimized for each compound infusing directly into the mass spectrometer standard solutions (10 μg/mL) at a constant flow rate of 5 μL/min using a model 11 syringe pump (Harvard Apparatus, Holliston, MA, USA).

2.8. Identification of metabolites and peak purity

Lignans were identified based on their UV absorption at 280 nm and by Information Dependent Acquisition (IDA) [20]. This acquisition method generates a survey scan, single MS spectra with molecular mass information, product ion spectra (MS^2) and extracted ion fragmentgrams (XICs).

IDA was carried out in the range m/z 50–1100 and the identified compounds were then analyzed in MRM (multiple reaction monitoring). Others phytochemicals such as chlorogenic acid, cynarin, caffeic acid, luteolin and quercetin were identified by IDA acquisition based on their molecular weight, fragmentation pattern and comparison of their retention time and UV absorption with those of commercial standards. Peak purity test was performed using mass spectrometer coupled to the HPLC system, comparing the UV spectra of each peak with those of authentic reference samples.

3. Results and discussion

3.1. Total phenolic content and antioxidant activity of seeds, root and leaves of burdock

The antioxidant activity and total polyphenolic content on different tissues of burdock are shown in Fig. 1.

The free radical scavenging activity of the different parts of the plant was assessed by $ABTS^{\bullet+}$ free radical scavenging assay. Burdock tissues had significant different free radical scavenging activity indicating that its active components are concentrated in the seeds. The amount of total phenolics in the different tissues ranged from 2.87 to 45.76 g of gallic acid/100 g of dry material. The highest total phenolic levels were detected in seeds extracts (45.76 g/100 g) and

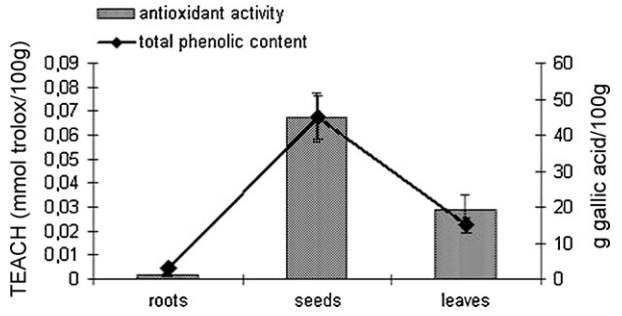

Fig. 1. Antioxidant activity and total polyphenolic content of burdock roots, seeds and leaves.

the lowest in roots extracts (2.87 g/100 g). A total polyphenolic content of 15.32 g/100 g was observed in the leaves, whereas leaves antioxidant activity was of 0.0289 mmol Trolox/100 g of dry weight (DW).

The amount of total phenolic compounds in the burdock seed extracts was very high when compared to that found in other medicinal plants. In the literature it was reported that phenolic contents on methanol extracts of 112 traditional Chinese medicinal plants (roots, stems or barks, leaves, flowers, fruits/seeds and whole plants) ranged from 0.22 to 50.3 g of gallic acid equivalent/100 g DW [21].

Antioxidant activities of hydro-alcoholic burdock extracts are shown in Fig. 1. The antioxidant activities ranged from 0.029 to 0.0016 mmol of Trolox/100 g DW. As expected, TAA and total phenolic contents of burdock extracts are strongly correlated ($R^2 = 0.98$). In the literature, only the antioxidant activity of roots was evaluated; Chen et al. [22] reported as the free radical scavenging activity of root of burdock was affected from different heating and peeling treatments, using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The authors claimed that burdock possessed significant free radical scavenging activity and while peeling have deleterious effects, heating decrease only slightly the antioxidant activity of burdock roots. Duh [23], reported that water extract and hot water extract of burdock roots exhibited an 80% scavenging effect on α-α-diphenyl-β-picrylhydrazyl radical and marked reducing power showing that burdock polyphenols act as primary antioxidants.

3.2. Accuracy

The accuracy of the method was evaluated with the recovery test. The extraction of samples with a mixture methanol/water (70:30, v/v) allowed to obtain a percentage of recovery of 98.20 ± 1.76, 96.54 ± 1.46 for chlorogenic acid respectively in roots and leaves. The analysis carried out on seeds showed a recovery value of 99.5 ± 1.87 for arctin. These results of the recovery test showed that the extractive method was highly accurate.

3.3. LC/MS/MS characterization of phenolic compounds of burdock seeds

In the literature, it has been reported that burdock seeds are particularly rich in lignans, a group of compounds formed

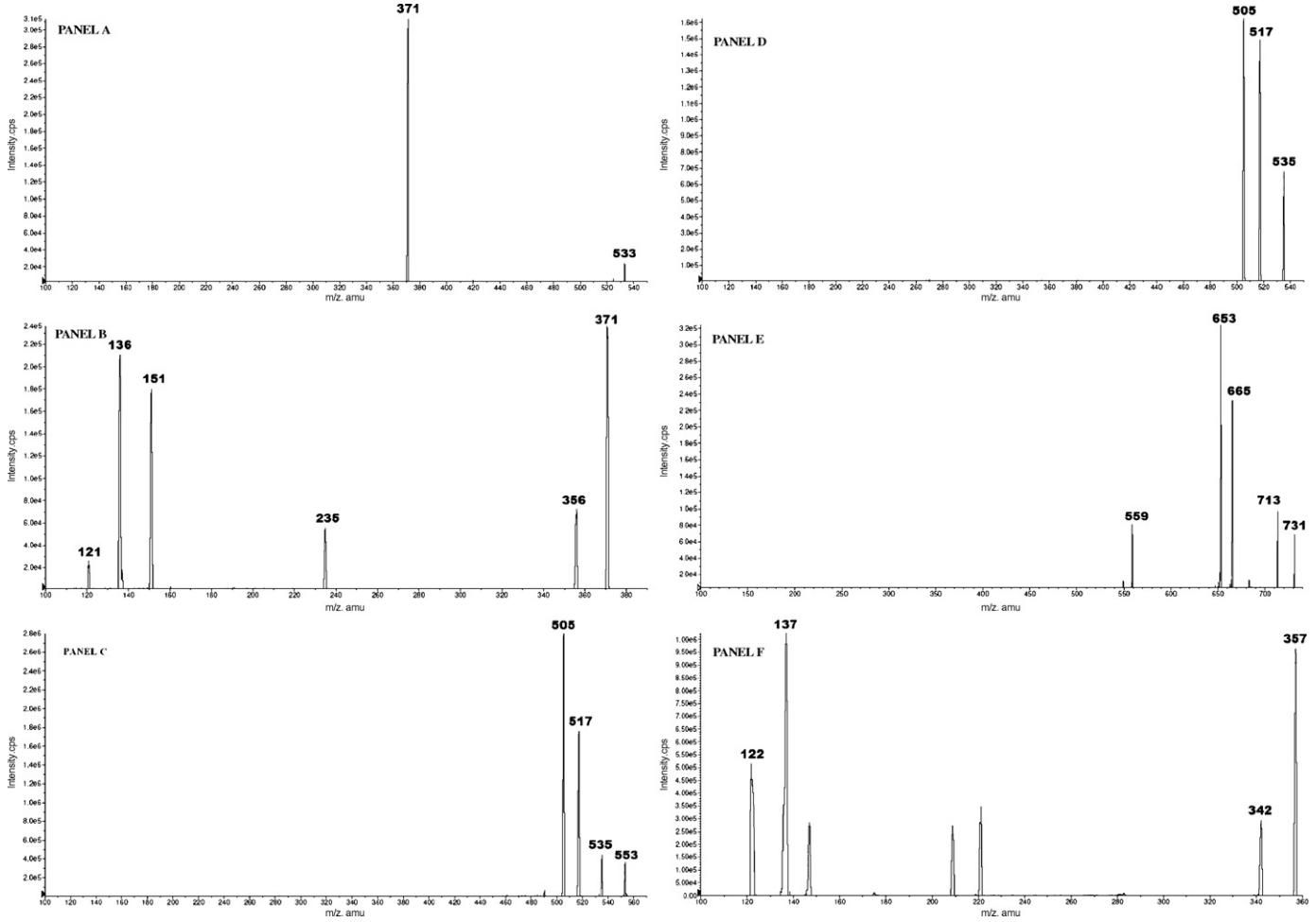

Fig. 2. MS/MS spectra of arctiin (A), arctigenin (B), lappaol C (C), lappaol A (D), arctignan E (E) and matairesinol (F).

Table 1
LC/MS/MS characteristics of phenols present in the extracts of *Arctium lappa* seeds.

Compound	Peak ID Nr.	Precursor ion [M–H]$^-$ (*m*/*z*)	Product ions (*m*/*z*)	DP (V)	CE (V)
Chlorogenic acid (caffeoylquinic acid)	1	353	191	40	30
Caffeic acid	2	179	135	40	21
Cynarin (dicaffeoylquinic acid)	3	515	353 191 179	70	20 54 41
Lappaol C	4	553	517 505 411	70	28 24 24
Arctiin	5	533	371	70	19
Arctignan E	6	731	665 653 559	60	35 39 42
Matareisinol	7	357	122 137 342	50	44 29 16
Lappaol A	8	535	517 505	60	25 25
Lappaol F	9	713	665 653 559	60	35 39 42
Arctigenin	10	371	151 136 121 356	60	26 35 60 23

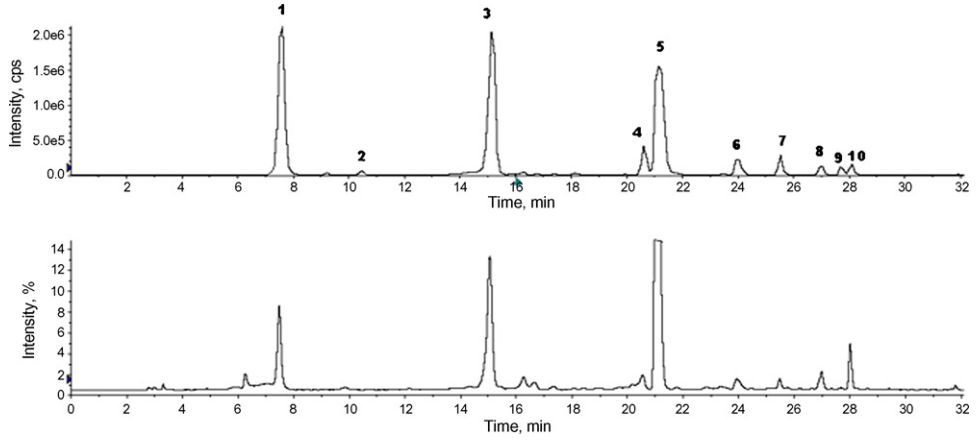

Fig. 3. Chromatogram TIC (top) and UV (bottom) at 280 nm of burdock seeds extract. For peak identification number see Table 1.

Table 2
LC/MS/MS characteristics of phenols present in the extracts of *Arctium lappa* roots.

Compound	Peak ID Nr.	Precursor ion [M–H]$^-$ (*m*/*z*)	Product ions (*m*/*z*)	DP (V)	CE (V)
Chlorogenic acid (caffeoylquinic acid)	1	353	191	40	30
Caffeic acid	2	179	135	40	21
Cynarin (dicaffeoylquinic acid)	3	515	353 191 179	70	20 54 41
Quercitrin (quercetin rhamnoside)	4	447	301	60	32
Arctiin	5	533	371	70	19
Quercetin	6	301	151 179	60	28 24
Luteolin	7	285	151 133	60	32 44

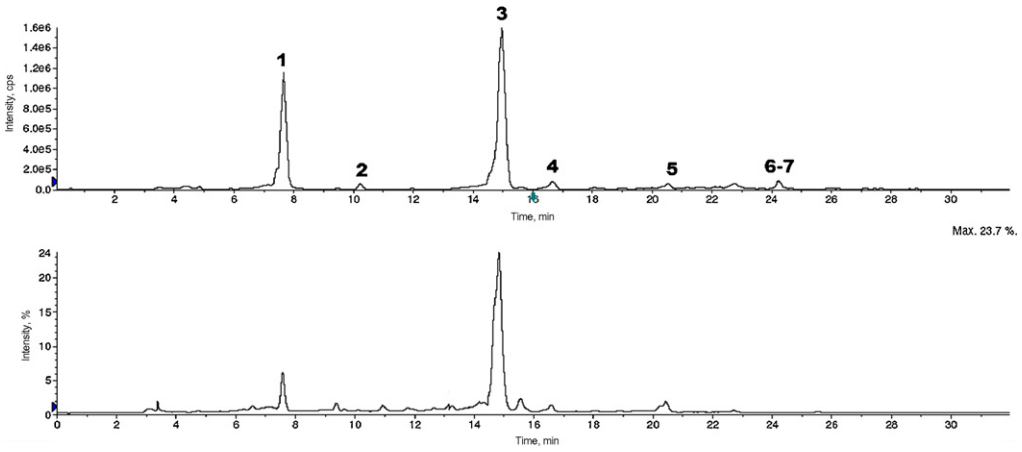

Fig. 4. Chromatogram TIC (top) and UV (bottom) at 280 nm of burdock roots extract. For peak identification number see Table 2.

by enzyme-mediated condensation of pairs of phenylpropane units. The main lignans reported in burdock seeds are arctiin, arctigenin, matairesinol, arctignan E and lappaols (A, C and F) [24,25].

No commercial standards of these lignans standards are available, therefore they were purified from seeds' extracts by preparative HPLC. Lignans were identified based on their UV absorption at 280 nm and by IDA analysis. Peaks at retention time of 20.6, 21.1, 23.9, 25.5, 27, 27.9 and 28.1 min corresponding respectively to lappaol C, arctiin, arctignan E, matairesinol, lappaol A, lappaol F and arctigenin, were collected, freeze-dried and used for identification and quantification purposes.

Others phytochemicals such as chlorogenic acid, cynarin, caffeic acid, luteolin and quercetin were identified by IDA acquisition based on their molecular weight, fragmentation pattern and comparison of their retention time and UV absorption with those of commercial standards.

The *optimum* LC/MS/MS conditions were set up using standard solutions 10 μg/mL in infusing experiments, in particular declustering potential (DP), focus potential (FP) and collision energy (CE) were optimized for each compound.

Phenolic compounds of burdock seed extracts were identified by IDA analyses and by comparison of their fragmentation patterns with literature data [26–29]. For arctigenin and matairesinol previous MS/MS studies are reported [28,29], whereas for arctigenin, lappaol C, lappaol A and arctignan F no MS/MS data were available. In Fig. 2 MS/MS spectra of six lignans purified and investigated are reported while in Table 1 the precursor ion, the MS/MS product ions, CE and DP for each compound are given. In Fig. 3 the chromatograms TIC and UV of burdock seeds extract were reported. Results showed that phenolic acids such as cynarin, chlorogenic acid, and caffeic acid were present in the extracts at relevant amount. This is the first study demonstrating caffeoylquinic acid derivatives occurrence in the burdock seed extracts.

3.4. LC/MS/MS characterization of phenolic compounds of burdock roots

Literature data shown that burdock roots contain caffeic acid derivates (chlorogenic acid, cynarin) [10] and quercetin [30]. Phenolic compounds were identified by IDA acquisition and by comparison of retention time of standards. Successively root extracts were analyzed in MRM.

In Table 2 the LC/MS/MS data for each identified compound were resumed. In Fig. 4 the chromatograms TIC and UV of an extract of burdock roots were reported. Results confirmed literature findings reporting that cynarin, chlorogenic acid, caffeic acid and quercetin are the main metabolites present in the extracts (Fig. 4). This study, based on a spectrometric approach, showed for the first time the occurrence of quercetin rhamnoside, arctiin and luteolin in burdock roots.

3.5. LC/MS/MS characterization of phenolic compounds of burdock leaves

The burdock leaves are poorly characterized and few data are reported in literature by Liu et al. [17–31] on arctiin and clorogenic acid occurrence. Experiments performed in IDA mode and

Table 3
LC/MS/MS characteristics of phenols present in the extracts of *Arctium lappa* leaves.

Compound	Peak ID Nr.	Precursor ion $[M-H]^-$ (m/z)	Product ions (m/z)	DP (V)	CE (V)
Chlorogenic acid (caffeoylquinic acid)	1	353	191	40	30
Caffeic acid	2	179	135	40	21
Rutin (quercetin rhamnosil glucoside)	3	609	301 271	60	49 74
Cynarin (dicaffeoylquinic acid)	4	515	353 191 179	70	20 54 41
Quercitrin (quercetin rhamnoside)	5	447	301	60	32
Arctiin	6	533	371	70	19
Quercetin	7	301	151 179	60	28 24
Luteolin	8	285	151 133	60	32 44

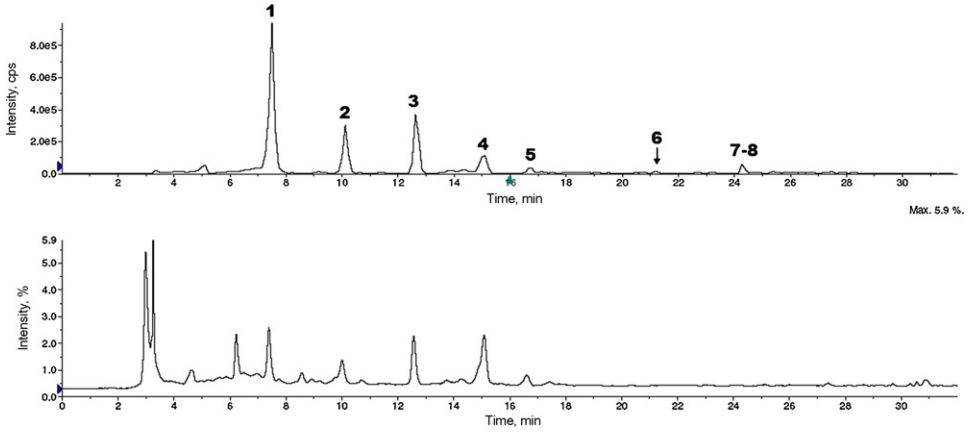

Fig. 5. Chromatogram TIC (top) and UV (bottom) at 280 nm of burdock leaves extract. For peak identification number see Table 3.

the comparison with standards retention time showed in the leaves extracts the presence of caffeic acid, chlorogenic acid, cynarin, rutin, quercitrin, quercetin and luteolin, besides arctiin and chlorogenic acid. Acquisition was performed in MRM. In Table 3 the MS/MS characteristics of phenolic compounds identified in extracts of *A. lappa* leaves were reported.

In Fig. 5. the chromatogram TIC and UV of an extract of burdock leaves was shown. As in roots, lignans are scarcely represented in leaves where cynarin and rutin are the most abundant compounds.

4. Conclusions

Several plants are used in the Chinese medicine as a source of natural drugs and among them burdock has recognized healthy effect on human. The main achievement of this research was to identify phenolic compounds of burdock seeds, roots and leaves using LC/MS/MS analysis, antioxidant activity and total polyphenolic content assays. The present study showed that burdock tissues are strong radical scavengers and can be considered as good sources of natural antioxidants for side dishes, medicinal and commercial uses.

The research identified some phenolic components for the first time from this plant, in particular phenolic acids (caffeic acid, chlorogenic acid and cynarin) in burdock seeds; arctiin, luteolin and quercetin rhamnoside in burdock roots; phenolic acids, quercetin, quercitrin and luteolin in burdock leaves.

The knowledge of antioxidant capacity and bioactive metabolites profile is a fundamental prerequisite to use this plant in medical formulation, as a functional ingredient in new healthy food and to increase its use in dietary habits.

References

[1] G. Spignoli, V. Mercati, E. Boncompagni, S.S. Aboca (Eds.), Guida Bibliografica ai più noti fitoterapici, Italy, 1999, pp. 37–42.
[2] C.M. Han, Agric. World 145 (1995) 55–57.
[3] C.S. Lieber, Gastroenterology 106 (1994) 1085–1105.
[4] C. Tamayo, M.A. Richardson, S. Dimond, I. Skoda, Phytother. Res. 14 (2000) 1–14.
[5] J.W. Mclarty, in: H.S. Garewal (Ed.), Antioxidants and Disease Prevention, CRC Press, New York, 1997, pp. 45–66.
[6] E. Niki, in: H. Ohigashi, T. Osawa, J. Terao, S. Watanebe, T. Yoshikawa (Eds), Food Factors for Cancer Prevention, Springer, Tokyo, 1997, pp.55–57.
[7] C.S. Yang, J.M. Landau, M.T. Huang, H.L. Newmark, Annu. Rev. Nutr. 21 (2001) 381–406.
[8] C.C. Lin, J.M. Lu, J.J. Yang, S.C. Chuang, T. Ujiie, Am. J. Chin. Med. 24 (1996) 127–137.
[9] S.C. Lin, C.H. Lin, C.C. Lin, Y.H. Lin, C.F. Chen, I.C. Chen, L.Y. Wang, J. Biomed. Sci. 9 (2002) 401–409.
[10] Y. Maruta, R. Kawabata, R. Niki, J. Agric. Food Chem. 43 (1995) 2592–2595.
[11] S.Y. Ryu, J.W. Ahn, Y.H. Kang, B.H. Han, Arch. Pharm. Res. 18 (1995) 462–463.
[12] M. Hirose, T. Yamaguchi, C. Lin, N. Kimoto, M. Futakuchi, T. Kono, S. Nishibe, T. Shirai, Cancer Lett. 155 (2000) 79–88.
[13] S. Awale, J. Lu, S.K. Kalauni, Y. Kurashima, Y. Tezuka, S. Kadota, H. Esumi, Cancer Res. 66 (2006) 1751–1757.
[14] T. Matsumoto, K. Hosono-Nishiyama, H. Yamada, Planta Med. 72 (2006) 276–278.
[15] H.Y. Li, S.R. Lu, Yaoxue Xuebao 30 (1995) 41–45.
[16] B.S. Yu, X.P. Yan, J. Xiong, Q. Xin, Chem. Pharm. Bull. 51 (2003) 421–424.
[17] S. Liu, K. Chen, W. Schliemann, D. Strack, Phytochem. Anal. 16 (2005) 86–89.
[18] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C.A. Rice-Evans, Free Radic. Biol. Med. 26 (1999) 1231–1237.
[19] J.D. Box, Water Res. 17 (1983) 511–525.
[20] T.N. Decaestecker, S.R.V. Casteele, P.E. Wallemacq, C.H. Van Peteghem, D.L. Defore, J.F. Van Bocxlaer, Anal. Chem. 76 (2004) 6365–6373.
[21] Y. Cai, Q. Luo, M. Sun, H. Corke, Life Sci. 74 (2004) 2157–2184.
[22] F.A. Chen, A.B. Wu, C.Y. Chen, Food Chem. 86 (2004) 479–484.
[23] P.D. Duh, JAOCS 75 (1998) 455–461.
[24] H.Y. Wang, J.S. Yang, Acta Pharm. Sin. 28 (1993) 911–917.
[25] D.S. Ming, E.S. Guns, A. Eberding, G.H.N. Towers, Pharm. Biol. 42 (2004) 44–48.
[26] K. Schütz, D.R. Kammerer, R. Carle, A. Schieber, Rapid Commun. Mass. Spectrom. 19 (2005) 179–186.
[27] F. Sanchez-Rabaneda, O. Jauregui, I. Casals, C. Andres-Lacueva, M. Izquierdo-Pulido, R.M. Lamuela-Raventos, J. Mass Spectrom. 38 (2003) 35–42.
[28] A. Smeds, K. Hakala, J. Chromatogr. B 793 (2003) 297–308.
[29] P.C. Eklund, M.J. Backman, L.A. Kronberg, A.I. Smeds, R.E. Sjöholm, J. Mass Spectrom. 43 (2008) 97–107.
[30] G.K. Dy, L. Bekele, L.J. Hanson, A. Furth, S. Mandrekar, J.A. Sloan, J. Clin. Oncol. 22 (2004) 4810–4815.
[31] S. Liu, K. Chen, W. Schliemann, J. Schmidit, D. Strack, Chin. J. Pharm. Anal. 23 (2003) 140–143.