Phytochemical Investigation for the Main Active Constituents in *Arctium lappa* L. Cultivated in Iraq

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Abstract

Burdock **(** *Arctium lappa*), is among the most popular plants in traditional medicine and it is associated with several biological effects. Literature survey revealed the presence of phenylpropanoid compounds .The most widespread are hydroxycinnamic acids (mainly caffeic acid and chlorogenic acid) and lignans (mainly arctiin and arctigenin). This work will confirm the presence of these compounds in *Arctium lappa*, cultivated in Iraq, in both root and leaf samples. The dried plant samples were extracted by soxhlet with 80% methanol then separated the main constituents by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Identification of the isolated compounds was carried out by UV, IR, and compared with reference standards using TLC, HPLC and HPTLC.

Keywords: ***Actium lappa*, hydroxycinnamic acid, Lignan, Phytochemical.**

دراسة كيميائية للمواد الفعّالة في نبات الارقطيون المستزرع في العراق

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الخلاصة

يعد نبات الارقطيون والمعروف باسم البلسكاء , من النباتات الاكثر شعبية في الطب التقليدي ويرتبط بالعديد من الفعاليات البيولوجية . أظهرت الدراسات السابقة وجود مركبات الفنل بروبانويد . والاكثر انتشاراهي حوامض هيدروكسي سينامك (خصوصا حامض الكافيك وحامض الكلوروجنيك) واللكنان (بصورة رئيسية الأركتين و الأركتيجنين).وهذا العمل سوف يثبت وجود هذه المركبات في نبات الارقطيون المستزرع في العراق في كل من الجذور و الاوراق. وقد تم ذلك باخذ عينات من النبات الجاف واستخلاصها بواسطة الميثانول80٪ وفصل مركباتها بكروماتوكرافيا الطبقة الرقيقة مع كروماتوكرافيا عالية الأداء السائلة . ثم تم تشخيص المركبات المفصولة باستخدام طيف الاشعة فوق البنفسجية والاشعة تحت الحمراء بالاضافة الى مقارنتها مع المواد القياسية باستخدام كروماتوكرافيا الطبقة الرقيقة وكروماتوكرافيا عالية الاداء السائلة.

الكلمات المفتاحية: الارقطيون ، حامض هيدروكسي سينامك ، ليغنان ، دراسة كيميائية .

Introduction

Actium lappa (common name: Burdock) is a flowering plant that belongs to the family Asteraceae (Compositae). With the advancement of different state-of-the-art analytical techniques, more active ingredients of *Actium lappa* have been identified over the last decade[(1)]. The literature furnishes numerous data on their anti-inflmmatory, hepatoprotective, antitumor, antimicrobial, antifungal, anti-aging and hypoglycemic effects[(2)]. The main active ingredients isolated from this herb are: Caffeic acid (3, 4 – Dihydroxy - cinnamic acid); Chlrogenic acid (1*S*,3*R*,4*R*,5*R*)-3-{[(2*Z*)-3-(3,4 dihydroxyphenyl) prop -2 – enoyl] oxy }- 1, 4 ,5-trihydroxycyclohexanecarboxylic acid; Arctiin (3*R*,4*R*)-4-[(3,4-dimethoxyphenyl)methyl]-3-{[3-methoxy-4-[(2*S*, 3*R*, 4*S*, 5*S*, 6*R*) -3, 4, 5-trih ydroxy -6- (hydroxymethyl) oxan -2-yl] oxyphenyl] methyl}oxolan-2-one; Arctigenin (3*R*,4*R*)-4-[(3, 4-dimethoxyphenyl) methyl]-3-[(4- hydroxy -3- methoxy phenyl) methyl]-2-tetrahydrofuranone[(3)]. Dong WH. et al. (2006) [(4)] Study the condition for extraction of arctiin from fruits of *Arctium lappa* using supercritical fluid extraction, they found that optimal extraction conditions were: pressure 40 MPa, temperature 70 degrees C, using methanol as modifier carrier, while Liu S. et al.(2005)[(5)] isolated and identified arctiin and arctigenin in leaves of burdock (Arctium lappa L.) by polyamide column chromatography in combination with HPLC-ESI/MS.

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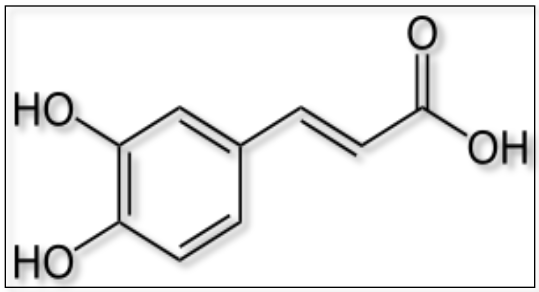
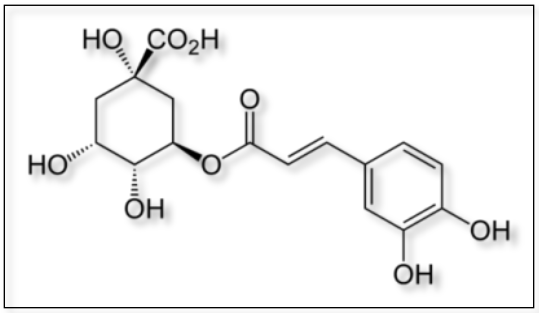
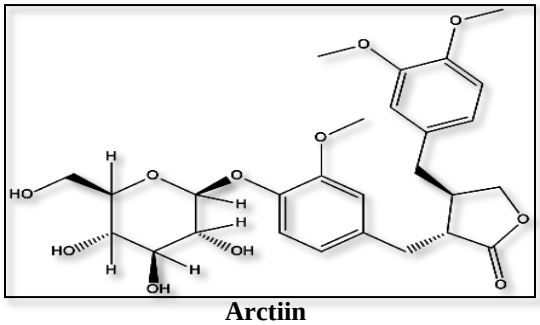
Ferracane R. et al. (2010) [6] were analyzed phytochemical compounds by liquid chromatography coupled to electro spray tandem mass spectrometry (LC/MS/MS) in negative mode.



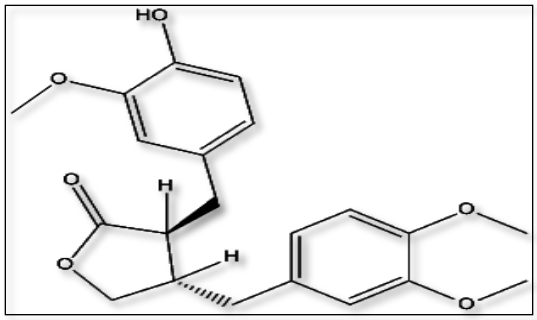
Caffeic acid



Chlorogenic acid



Arctiin



Arctigenin

Experimental

Plant Materials

The plant sample was collected from the department of medicinal plants, College of Agriculture, University of Baghdad. Identified by Dr.Saged Auda Mohammad , head of medicinal plant department. The plant leaves and roots were air dried in the shade at room temperature, coarsely powdered by mechanical grinder and weighed.

Chemicals

All reagents and solvents used were analytical grade. Standards used to identify the main active constituents (Arctiin, Arctigenin, Caffeic acid and Chlorogenic acid) were obtained from China (Cheng du Biopurify phytochemicals Ltd.).UV was done in methanol.

Instruments

Electrical sensitive balance: Sartorius/ Germany, Ultraviolet light : Desaga Heidelberg/ Germany, Rotatory evaporator: Buchi Rotatory evaporator, Chiller: Ultratemp 2000 , Water bath: Memmert/Germany, HPLC: Water/Irland, FTIR: Shimadzo FT-IR-84005 IR Spectrometer, HPTLC:Eike Reich/CAMAG – Laborator.

Extraction of plant

100 g of dried milled leaves and roots were extracted separately in a soxhlet apparatus with 80% methanol (3:1 solvent/plant ratio), for 6 hours. The extracts obtained were evaporated to dryness at 45^{o}C under vacuum using rotary evaporator [7] .

General phytochemical screening by chemical tests

The different parts of the plant have been screened for the occurrence of saponines, flavonoids, tannins and alkaloids by the following chemical reactions

A- Ferric chloride test for tannins.
B- Froth test for saponin.
C- Conc H_2SO_4 with ammonia for flavonoids.
D- reagent for alkaloids [8] .

Isolation and identification Mayer's of active constituents

Separation of the main active constituents from both leaves and roots of *Arctium lappa* L. were carried out using Preparative TLC**:** PLC plates, 20x20cm and 1mm thickness of silica gel GF_{254} developed in solvent system ($CHCl_3$-MeOH 80:20) together with standard references

The chromatogram was visualized by UV lamp (at 254 nm and 366 nm) [9].

Identification of active constituents was done by:

1-Matching with standards by TLC using the following mobile phases:

S1: Chloroform: aceton: formic acid (75,16.5, 8.5), S2: Ethanol:acetic acid (85:15), S3: Chloroform:Methanol (80:20) [10]

2-High Performance Liquid Chromatography (HPLC): HPLC analysis was done using C_{18} – column (150 × 4.6 mm) with specific conditions for each compound:

- HPLC conditions for Arctiin, Arctigenin and Caffeic acid: The mobile phase is methanol: water (60:40) , flow rate: 0.5 ml/min at 280 nm [11] .
- HPLC conditions for Chlorogenic acid: The mobile phase is acetonitrile: acetic acid: water (15: 0.5:85), flow rate 1ml/min at 320 nm [12] .

3- Fourier transform infrared spectroscopy (FTIR) in KBr disk.

4-High performance thin layer chromatography (HPTLC): the mobile phase is ethanol: acetic acid (85:15), the list of standards: Arctiin std., Arctigenin std., Caffeic acid std., Chlorogenic acid std., then Leaf sample and Root sample.

Results and Discussion

The preliminary phytochemical investigation revealed the presence of saponins, flavonoids, tannins in both parts of the plant, while the alkaloids are absent as shown in table(1).

Table 1: The results of the general screening by chemical tests.

The plant sample	Plant part	Saponin	Alkaloid	Flavonoid	Tannin
Actium lappa L.	Root	+	-	+	+
	Leaf	+	-	+	+

Identification for each isolated compound depend on :

1- Accurate measurement of R_f values in TLC as shown in table 2 and figure 1

Table 2: R_f values of some plant constituents and their standards in different developing solvent systems in TLC.

Compounds	TLC R_f (Standard)			TLC R_f (Isolated compound)		
	S1	S2	S3	S1	S2	S3
Arctiin	0.71	0.68	0.67	0.72	0.67	0.66
Arctigenin	0.92	0.82	0.86	0.94	0.80	0.85
Caffeic acid	045	0.48	0.36	0.46	0.49	0.35
Chlorogenic acid	0.04	0.03	0.02	0.03	0.04	0.03

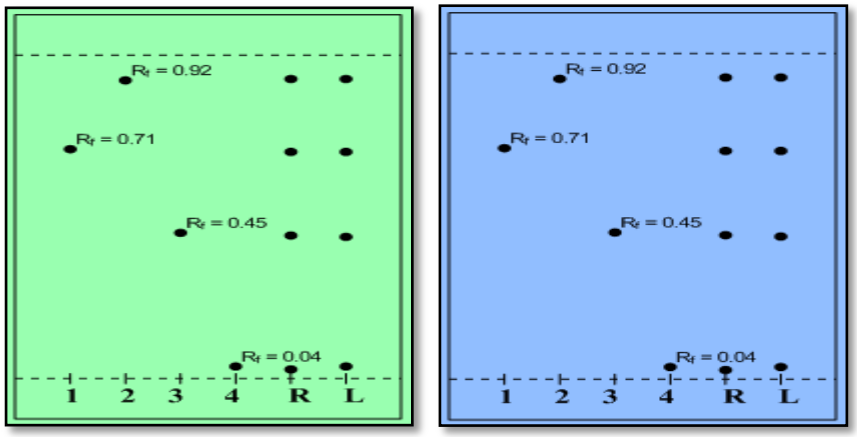
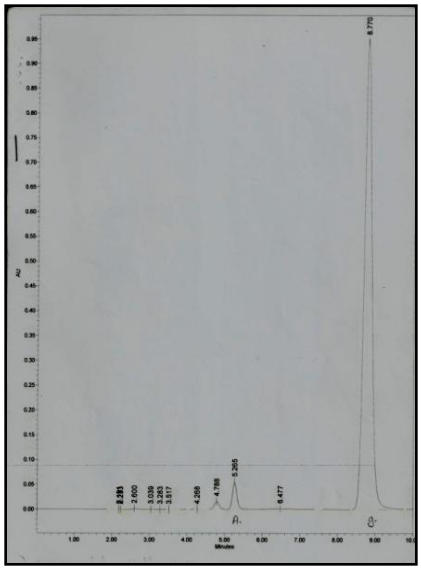


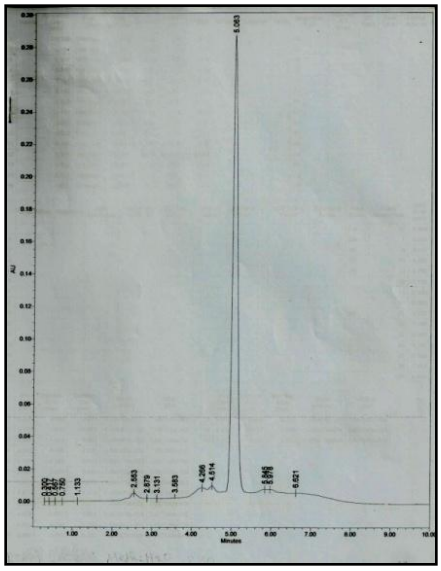
Figure 1 :Thin layer chromatograms of both root and leaf sample with four references standard on silica gel GF_{254}, developing in S1 solvent system and detection by UV light at 254 nm & 366 nm.[1:Std. Arctiin , 2: Std.Arctigenin , 3:Std. Caffeic acid , 4: Std.Chlorogenic, R: root extract, L: leaf extract.].

2-High performance thin layer chromatography HPLC

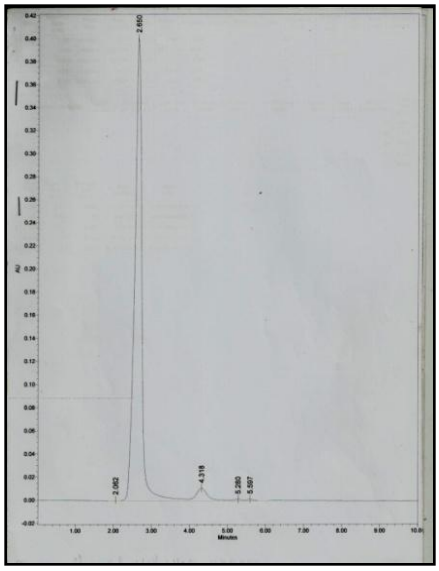
HPLC analysis can be carried out for qualitative analysis by comparison of retention times of analyzed samples and authentic standards at identical chromatographic conditions .the results are shown in Figures (2, 3) and table 3.



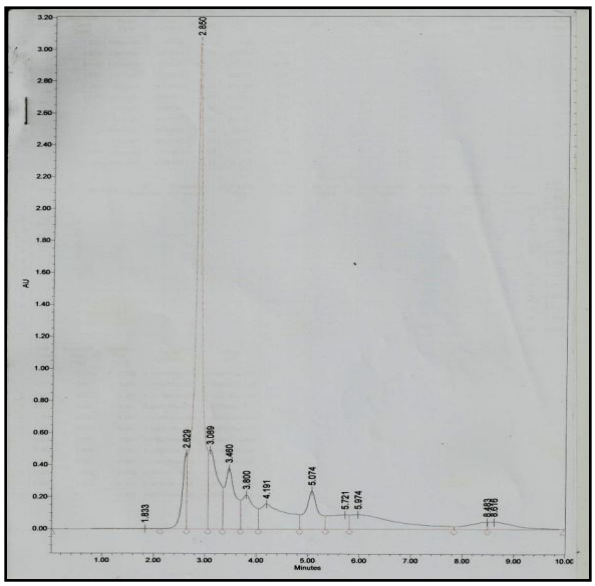
HPLC of std. Arctigenin



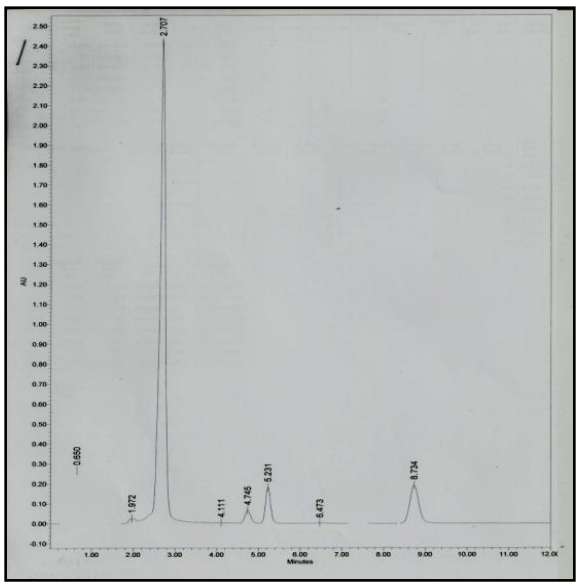
HPLC of std. Arctin



HPLC of std. Caffeic acid

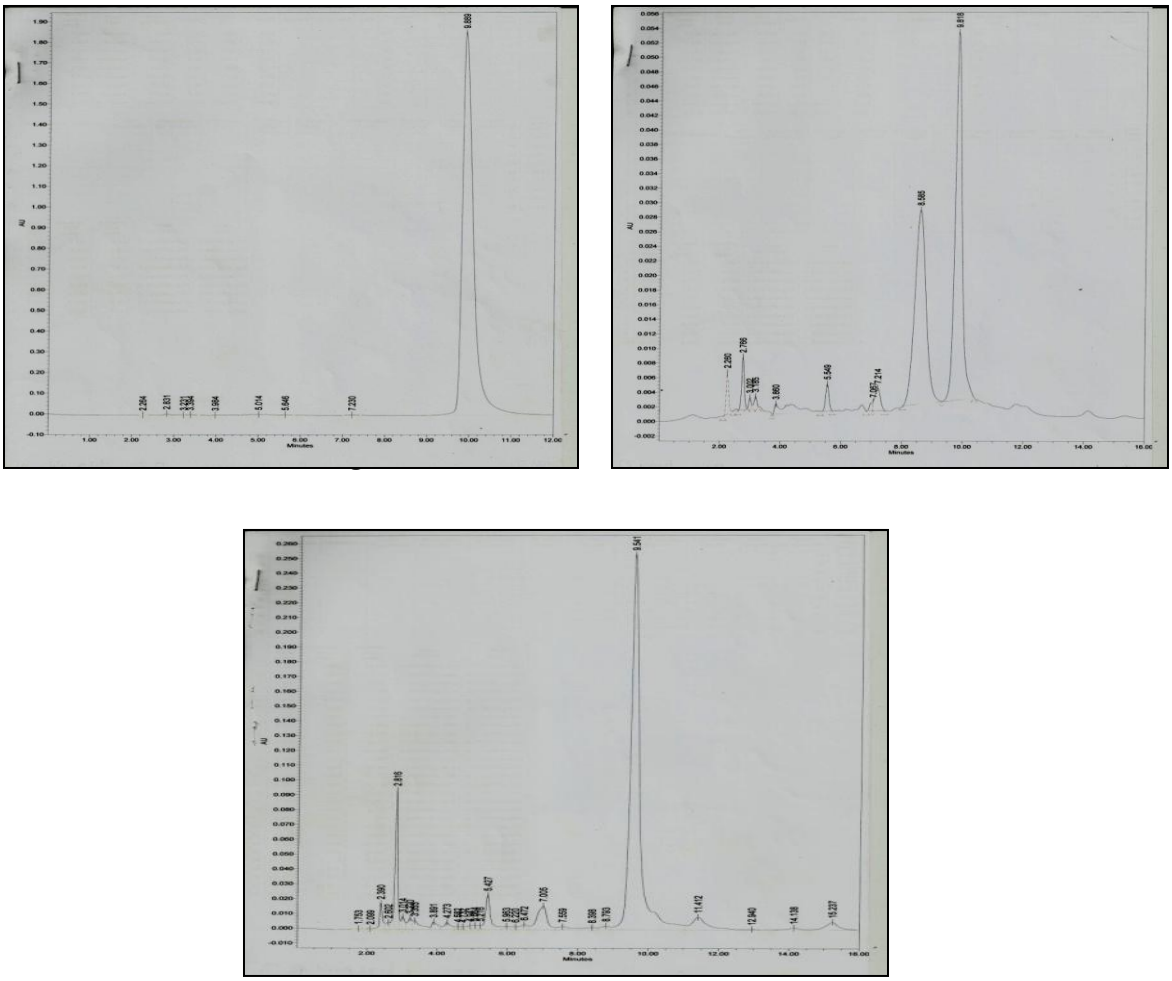


HPLC of methanolic Leaf extract



HPLC of methanolic Root extract

Figure 2: HPLC analysis of std. Arctiin, std. Arctigenin, std.Caffeic acid and methanolic extract of leaf &root sample.



Methanolic Leaf extract

Figure 3: HPLC analysis of std. chlorogenic acid and methanolic extract of root & leaf sample.

Table 3: The retention time of each isolated compound with that of reference standards

Compound	R_t of standard	R_t of compounds in root extract	R_t of compounds in leaf extract
Arctiin	5.063	5.231	5.074
Arctigenin	8.770	8.734	8.616
Caffeic acid	2.650	2.707	2.850
Chlorogenic acid	9.889	9.818	9.541

3-FT-IR spectroscopy

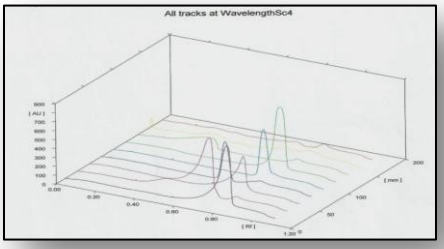
IR spectroscopy is most frequently used in phytochemical studies as a'finge printing' device, for comparing a natural with a synthetic reference standard .Such comparisons are very important in the complete identification of many types of plant constituents as shown in table (4).

Table 4: IR absorption bands of isolated compounds (in cm^{-1}).

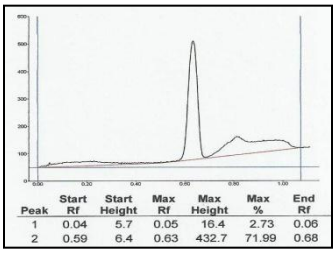
Compound	Aproximate positions of characteristic bands.
Arctiin	3470(OH), 1780(C=O),1594,1520(Ar-H), 1080, 1030, 1020(β-glycopyranoside), 2930,2850, 1465, 1420,1262, 670.
Arctigenin	3470(OH), 1770(C=O), 1610, 1520, 3030(Ar-H), 1465, 1390 , 1270.
Caffeic acid	974,576 3433, 3234, 2910, 1645, 1620, 1448, 1278 , 1217 , 1120,
Chlorogenic acid	3421, 2929, 1697, 1635, 1456, 1398 ,1278,1182, 812.

4-HPTLC analysis

HPTLC was carried out for further identification of main active constituents present in methanolic extract of both leaves and root of *Actium lappa*, by measuring the R_f values and UV spectrum :The results obtained are shown in figure 4.

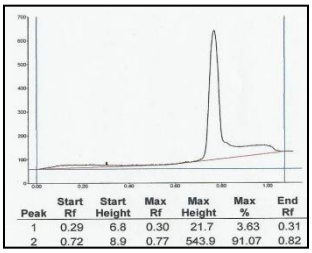


All tracks at wavelengtth 254 nm



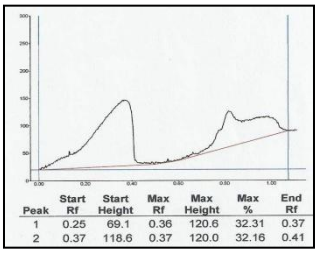
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf
1	0.04	5.7	0.05	16.4	2.73	0.06
2	0.59	6.4	0.63	432.7	71.99	0.68

Arctiin std.



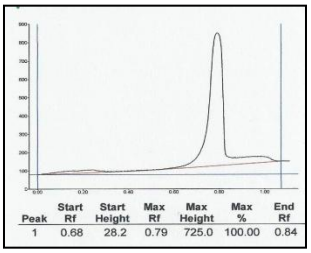
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf
1	0.29	6.8	0.30	21.7	3.63	0.31
2	0.72	8.9	0.77	543.9	91.07	0.82

Arctigenin std.



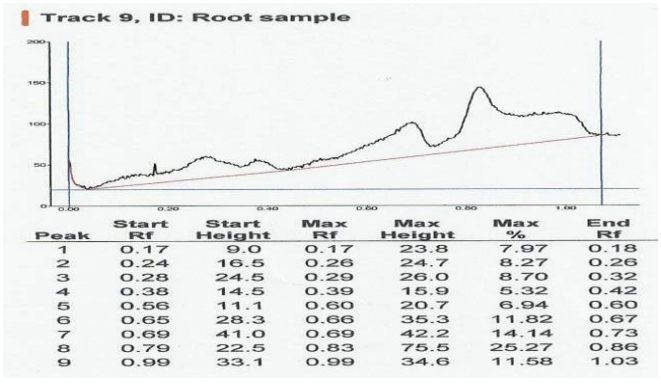
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf
1	0.25	69.1	0.36	120.6	32.31	0.37
2	0.37	118.6	0.37	120.0	32.16	0.41

Chlorogenic acid

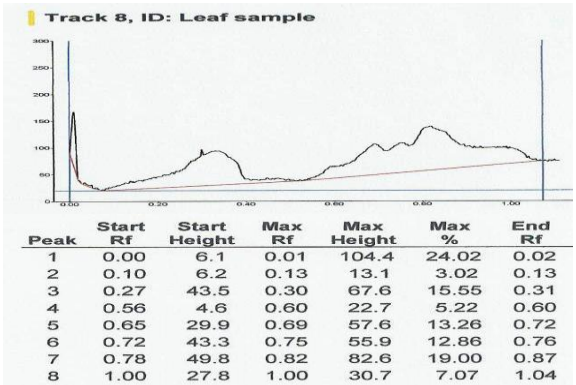


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf
1	0.68	28.2	0.79	725.0	100.00	0.84

Caffeic acid std.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf
1	0.17	9.0	0.17	23.8	7.97	0.18
2	0.24	16.5	0.26	24.7	8.27	0.26
3	0.28	24.5	0.29	26.0	8.70	0.32
4	0.38	14.5	0.39	15.9	5.32	0.42
5	0.56	11.1	0.60	20.7	6.94	0.60
6	0.65	28.3	0.66	35.3	11.82	0.67
7	0.69	41.0	0.69	42.2	14.14	0.73
8	0.79	22.5	0.83	75.5	25.27	0.86
9	0.99	33.1	0.99	34.6	11.58	1.03



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf
1	0.00	6.1	0.01	104.4	24.02	0.02
2	0.10	6.2	0.13	13.1	3.02	0.13
3	0.27	43.5	0.30	67.6	15.55	0.31
4	0.56	4.6	0.60	22.7	5.22	0.60
5	0.65	29.9	0.69	57.6	13.26	0.72
6	0.72	43.3	0.75	55.9	12.86	0.76
7	0.78	49.8	0.82	82.6	19.00	0.87
8	1.00	27.8	1.00	30.7	7.07	1.04

Figure 4: HPTLC analysis of methanolic extract with reference standards.

End R_f values recorded for standards in HPTLC which have small peak area, are excluded because these are due to impurities. All chromatographic data coincide with that reported and shown by the standards and so as the obtained UV & FTIR results. This confirms the presence of arctiin, arctigenin, caffeic acid & chlorogenic acid in both leaves & root of *Arctium lappa.*

Conclusion

The Iraqi plant , *Actium lappa*, is a good source for many phenolic compounds with antioxidant activity. 80% methanol is the best solvent suitable for the extraction of the main active constituents from both root and leaf of the plant .The same constituents were obtained from both parts, however; the results showed that there was a slight different in the amount of each plant constituents. This study confirms the presence of Chlorogenic acid, Caffeic acid, Arctiin and Arctigenin in *Actium lappa* plant cultivated in Iraq.

References

1. James A.Duke; Mary Jo Bogenschutz-Godwin "Hand Book of Medicinal Herbs", second Edition, 2002; by CRC.
2. SheldonHendlerPH.D.M.D.; David Rorvik, "PDR for Nutritional Supplements" Second Edition, 2008(11): 156-159.
3. Joanne Barnes; Linda A. Anderson and J.D. Phillipson, "Herbal Medicines", 2007; Sep.
4. Dong WH; Liu B., "Study on condition for extraction of arctiin from fruits of Arctium lappa using supercritical fluid extraction", Zhongguo Zhong Yao Za Zhi. 2006 (31): 1240-1276.
5. Liu S, Chen K; Schliemann W; Strack D," Isolation and identification of arctiin and arctigenin in leaves of burdock (Arctium lappa L.) by polyamide column chromatography in combination with HPLC-ESI/MS", Phytochem Anal. 2005(16): 86-90.
6. 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.
7. Fabio Bahls Machado; Rafael Eidi Yamamoto; Karine Zanoli.; "Evaluation of the Antiproliferative Activity of the Leaves from Arctium lappa by a Bioassay-Guided Fractionation",Molecules, 2012(17): 1852-1859.
8. FabriciaSPredes; AnaLTG Ruiz; JoaoE Carvalho; Mary A Foglio; "Antioxidant and invite anti proliferative activity of Arctium lappa root extracts", Complementary and Alternative Medicine , 2011(10): 1186-1472.
9. Long-Ze Lin; James M Harnly; " Identification of hydroxycinnamoylquinic acids of arnica flowers and burdock roots using a standardized LC-DAD-ESI/MS profiling method", Journal of Agricultural and Food Chemistry 2008(56): 10105-10114.
10. Liu S et al. " Isolation and identification of arctiin and arctigenin in leaves of burdock (Arctium lappa L.) by polyamide column chromotography in combination with HPLC-ESI/MS", Phytochemical Anal 2005(16): 86-89.
11. Xiao Wang; Fuwei Li; Qinglei Sun; Jingpeng Yuan; Ting Jiang; Chengchao Zheng ; " Application of preparative high-speed counter-current chromatography for separation and purification of arctiin from Fructus Arctii", Journal of Chromatography A 2005 (10): 247-251.
12. Rosalia ferracane; Giulia Graziani; Monica Gallo; " Metabolic profile of the bioactive compounds of burdock (Arctium lappa)seeds,root and leaves", Journal of pharmaceutical and Biomedical Analysis 2010(51): 399-404.