Phytochemical Study of *Cynara scolymus* L. (Artichoke) (Asteraceae) Cultivated in Iraq, Detection and Identification of Phenolic Acid Compounds Cynarin and Chlorogenic Acid

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Abstracts

The leaves of globe artichoke, *Cynara scolymus* Family Asteraceae/ compositea have long – used in traditional medicine and now included in British and European Pharmacopeia, the British Harbal Pharmacopeia and complete German Commission E monographs.The plant originally comes from Mediterranean region and North Africa and cultivated around the world. The flowers are used worldwide for nutrition purposes and the leaves for medical purposes including hepatic affections. The plant wildly distributed in Iraq in the watery lines and boundary of the field.The plant contains many phytochemicals such as the bitter phenolic acids whose choleretic and hypocholestremic as these compounds are antioxidant. Other materials to have other pharmacologic effect specially flavonoids. Thus this work deals with phenolic acids cholrogenic acid and cynarin.This work includes cold extraction evaporation by freeze drying and separation inusing column chromatography, TLC and finally in High pressure liqued chromatography (HPLC), the two important compound; chlorogenic acid and cynarin were separated and identified.

Key words: Artichoke, *Cynara scolymus*, Poly phenolic acids, Chlorogenic acid, Cynarin.

دراسة كيمياوية للنبات المسمى *Cynara scolymus* المستزرع في العراق. أيجاد وتشخيص المركبات الحلقية الفنولية السينارين وحامض الكلوروجنك

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الخلاصة

أوراق الأرضي شوكي النبات المسمى *Cynara scolymus* من العائلة/Asteraceae/ compositae مستعملة من وقت طويل كعلاج تقليدي ومذكورة في الدستور البريطاني والأوربي كذلك في المجموعة الألمانية. النبات ينمو في حوض البحر الأبيض المتوسط وشمال أفريقيا ومنتشر في جميع أنحاء العالم تستعمل نورته كغذاء وأوراقه للأغراض الطبية والتي تشمل أمراض الكبد. النبات ينمو وينتشر في اطراف الحقول الزراعية وخاصة قرب مصادر المياه والأماكن الرطبة في العراق.النبات يحتوي على كيمياويات نباتية منها مجموعة الحوامض الفينولية المرة والتي لها خاصية علاج تخفيض مستوى الكوليسترول في الدم وحماية الكبد كذلك المركبات الفلافنودية والتي لها خواص طبية لذا فأن هذه الورقة تبحث في المركبات الحامضية الفينولية وخاصة Chlorogenic acid وكذلك Cynarin.أشتمل العمل على الأستخلاص الكحولي المائي البارد وتم أزالة السوائل بواسطة الـfreeze dryer ثم فصلت المواد بواسطة كرماتوغرافيا العمود السائل وفي كروماتوغرافيا الطبقة الرقيقة وتم التأكد من هذين المركبين بواسطة أجهزة الضغط العالي للكروماتوغرافيا السائلة (HPLC) وتم تشخيصها في الجهاز.

الكلمات المفتاحية : أرتيشوك(أرضي شوكي)، سينارا سكوليمص،الحوامض الحلقية الفينولية : سينارين وحامض الكلوروجنك .

Introduction

The importance of the plant *Cynara scolymus* which is called artichoke or globe artichoke steamed from its used as edible material for nutrition and from its content of phenolic acid constituent in particular cynarin and chlorogenic acid[1, 7, 10].The leaves of globe artichoke, *Cynara scolymus* L.Family Asteraceae / Compositae, have been long-used in traditional medicine and now included in British and European Pharmacopeia (BP / EP) , the British Harbal pharmacopeia (BHP) and the Complete German Commission E Monographs [1].The plant *Cynara scolymus* L. originally comes from Mediterranean region and north Africa and also cultivated around the world [2,3] .The flowers are used worldwide with nutrition purposes and the leaves with medical purposes , broadly used in Phytotherapy preparations with special indication in hepatic affections [3].The plant is widely distributed in Iraq and normally located and found in the outer lines of the fields, water lines and humid watery soil. The plant flourishes in winter and harvested in February and March.

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Received : 5/6/2011
Accepted : 24/10/2011

In North of Iraq and Kurdistan the people are used to eat the carpel of blooms [2]; because of its nutritional value.The leaves of *C. scolymus* are characterized by the composition and high content of bitter phenolic acid compounds whose choleretic, hypocholestremic and heptatoprotective activity attributed [4]. At least to the antioxidant potential of artichoke extracts and of their phenolic compounds. Constituents which are around 2% such as: caffeic acid, chlorgenic acid and cynarin, flavenoids (0.1 – 1 %) and essential oil [4]. Pharmacological studies demonstrated that the extracts of *C. scolymus* and active principle cynarin (1,3 di-caffeoyl quinic acid ($C_{25}H_{24}O_{11}$) posses choleretic and hypocholesterolemic activity [5] .The extract of the plant also protect hepatocytes treated with carbontetrachloride (CCl_4) from hepatic cellular necrosis. This activity related to the power antioxidant effect of phenolic acids [6].

Recently pharmacological investigations and clinical reports published showed the efficacy and safety of artichoke extracts in treatment of hepatobiliary dysfunctions and abdominal pain[7].

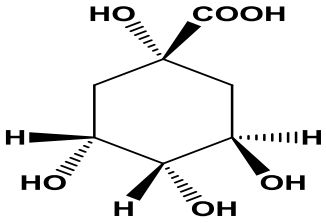
Cynara scolymus leaves

Cynara folium is the whole drug; consist of the dried or fresh basal leaves with coarsely toothed margin.The cut drug is composed of grayish green, tomintose and fleet like aggregate with pithy fragment of petioles and nervature as well as very long fine fibers. The lower leaf surface is densely pubescent, grayish and matted with pinnate venation. The upper leaf surface is glabrous and green [2]. The plant thistle – like *Cynara scolymus* is perennial herb about 1.5 meter height; with pinnatified leaves 8 – 15 cm with wide flower head has obtuse – ovate fleshly involucres bracts [3]; the pharmaceutical grad material consists exclusively of basal leaves, which up to 50 cm long and 25 cm wide; the deeply pinnotified leaf laminas is rarely entire margined but from flat, lance late segment with crossly serrate or finely toothed margin.The Iraqi plant has thrown margin. No regular cultivation was adopted in Iraq.

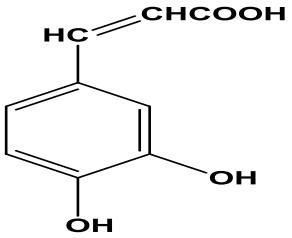
The chemical constituents of leaves of *Cynara scolymus*

The chemicals which from important constituent of *C. scolymus* include three classes. [8,9,10]:

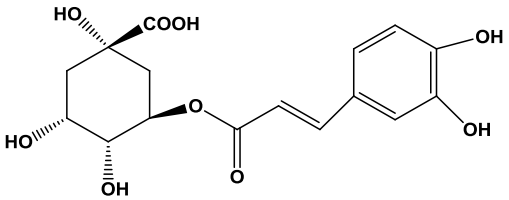
1. Phenolic acids which include a combinations of caffeic acid and quinic acid. *C. scolymus* have the two important anti oxidant cynarin and chlorogenic acid, by the combination of 1, 3 – 0 – quinic acid with two molecules of caffeic acid to from 1, 3 - di – 0 caffeoyl quinic acid (cynarin) and 5 – 0 – caffeoyl quinic acid (chrogenic acide).



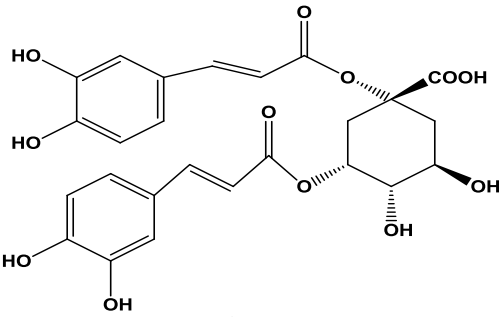
Quinic acid



Caffeic acid

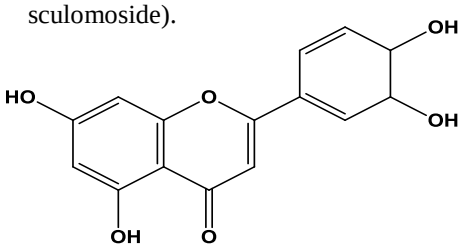


Chlorogenic acid



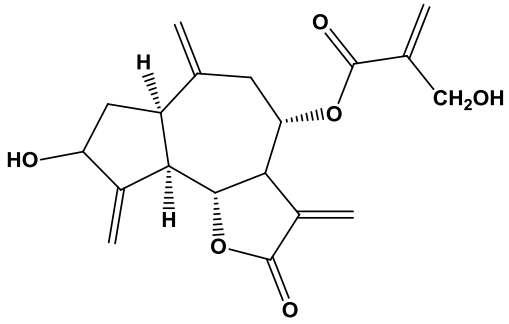
Cynarin

2. The flavenoids particularly glycoside of luteolin including luteoline-7- glycoside (cynaroside) and luteoline-7- rutinoside (sculomoside).

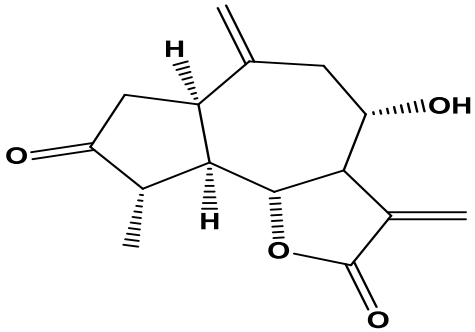


Cynaroside – luteolin-7- glylosyl
Scolomoside – luteolin-7- rutinosyl

3. Sesequiterpens; cynaropicrin and grosheimin lactons[13,14]. .



Cynaropicrin



Grosheimin

Other chemicals like sesequiterpin B- selinene and caryphyllene also present [2].

The plant was registered by Counsel of Europe as a natural source of food flavoring (category N_2). In USA the leaves used in beverage only with a maximum concentration (16 part per million).The German commission E recommended an average daily dose of 6 G. or equivalent dose of the extract for the treatment of dyspeptic problems.Because the plant available in Iraq as a weed and some has been cultivated and because the importance of such plant which extend worldwide; there for it was important to study such plant for its phenolic acid content and flavenoids as an important approach to use the plant medicinally in the health care and as a medicine, starting to study the phenolic acid content cynarin and chloragaic acid first.

Materials and Methods

250 Grams of dry basal leaves of *Cynara scolymus* were collected from Botanical Garden of Pharmacy College, University of Baghdad and authenticated; were dried under shade and powdered; the dry powder was macerated with 1 Liter of 80% methanol/ water for three days with occasional shaking. The methanolic extract was filtered and kept in the refrigerator the marc was macerated with another two 500 ml volumes of 80% methanol/ water each for two days, the filtrates were collected and mixed together with the first filtrate and evaporated under vacuum at 40°C in rotary evaporator (Stuart Rotatory Evaporator; UK) to remove the methanol; the left collected water extract which was about 400 ml were separated into two parts, the first one (about 200 ml) shacked with three successive portion of 100 ml n-butanol and the other shacked with three successive portion of 100 ml ethyl acetate the two portions were subjected to lyophilizati using Virtis lyophilized USA 4.28 G. obtained from n- butanol portion and 2.74 G. obtained from ethylacetate portion dried and kept in refrigerator[9]. The same procedure was applied to 250 Gm.of basal leaves of *Cynara scolymus* which was obtained from controlled cultivated field in Greaat district by Ministry of Agriculture.The quantities of the lyophilized n-butanol and ethylacetate extracts were 7.78G. and 5.88 respectively. [10]. Samples from two plant extracts were chromatographed and matched with standard cynarin obtained from china (Changdu Biopurify; China) using three mobile phases as fallow:

S_1= Water: methanol: acetic acid (78.5: 20: 2.5 ml)

S_2= n- hexan: aceton: chloroform: methanol (25: 25: 25: 25 ml)

S_3= ethyl acetate: Formic acid: water (80: 10: 10 ml)

The separated spots were detected on the chromatogram by using ultraviolet light at (254 – 366 nm); then sprayed by sulpheric acid and heating the plat to 110°C for five minutes.The R_f values were calculated and the results were shown in table 1 and 2.To separate the phenolic acid in *Cynara scolymus* column chromatography was used and gradient elution technique was applied, using two organic solvents ethyl acetate: methanol 100: 0; 80: 20; 60: 40; 40: 60; 20: 80; 0: 100 twelve fractions of 50ml were collected, using TLC to group the fraction; fraction 1; 2; 3; 4 and 5 show one compound, its R_f value was 0.75 so they are collected together and called F_1.Fractions 6, 7, 8, and 9 showed nearly three spots having R_f values 0.28, 0.29 and 0.3 respectively which were grouped together as F_2 . Fraction 10, 11 and 12 showed only two spots. High pressure liquid chromatography (HPLC) was used under the fallowing conditions [11]:-

The instrument name: KNAUR (Advance Scientific Instrument Germany).

The column used: Hyperclone ODS5 us; C18 V- 250 X4.6 mm.

Program used: Chromoget.

The loop capacity: 20 ml injection value.

Detector: Varian detector of variable Ultraviolet light.

The HPLC Chromatographic conditions applied.

The flow rate of the mobile phase 1.3 ml/ minute.

The mobile phase was: water: methanol: acetic acid (78.5: 20: 2.5 V/V).

The detection: UV at 316nm

Injection volume: 20 µℓ of sample, filtered by Millipore filter.

The results obtained shown in Table 3; 4 and 5:

Table -3- shows the retention time of *Cynara scolymus* standard.

Table -4- shows the separated components of n – Butanol layer fractions

Table -5- shows Comparison of retention times of standard phenolic acids and plant phenolic acid.

Using the same conditions the fraction of the n- butanol extract and the retention times of F_2-6; F_2-7; F_2-8; F_2-9 was recorded in Table 4 and 5.

Results and Discussion

Experimental studies in vitro and in vivo support some of the reputed use of Artichoke. Traditionally the choleretic and cholesterol lowering activity of globe artichoke have been attributed to cynarin and chlorogenic acid present in the leaves and carpel of the plant[2,7,10].Clinical trials investigating the use of globe artichoke powder and cynarin in treatment of hyperlipidaemia generally reports positive results; the benefits of such heptoprotective and heptoregenarating activity have been documented to cynarin in vitro and in animals. The flavonoid content of the plant have antioxidant activity and it was up- regulate endothelial type Nitric- oxy synthese gene expressions in human endothelial cells. N. oxide (NO) produced by endothelial nitric oxide synthese (eNOS) represent and antithrombotic and anti- atheroseclorotic principle in vasculature which lead to provide protection against cardiovascular diseases [20].The results obtained from the extract of the two organic solvent n- butanol and ethyl acetate support of the separation the two most important compounds the phenolic acid and the flavonoid the difference in polarity of the two compounds lead to phenolic acid prefer the n- butanol and the flavonoids prefer the ethyl acetate portion. Another attempt to separate the more polar compound by shaking the two organic solvent extract with water but the results obtained (Table 1 and 2) showed similar components together and worked as n-butanol extract and ethyl acetate extract. It is obvious that the plant with controlled cultivation which include using fertilizers and herbicides will give more extractable materials and this need more investigation to evaluate the two important compounds phenolic acid and flavonoids with other components[13].TLC and HPLC result confirm the presence of the two phenolic acid compounds cynarin and chlorogenic acid[11,12, 15, 16, 17, 18, 19]; Table 1, 2, 3, 4 and 5 and Figers 1, 2, 3 for the standard cynaria and chlorogenic acid and their combination, Figure (4) for fraction (1) of butanol extract Figure (5) for fraction (6) of butanol which shows clearly the two phenolic acid compound plus other compound including other chemicals. Figs 6, 7, 8, show some phenolic acids and other compounds which needs more investigation in future.Tables 3, 4, 5 shows the retention time of the standard two phenolic acids and the retention time of butanol extract Table (4) and the standard retention time[21].In Table (4) many unidentified compounds are present which need further work to understand the complete photochemical found in the Iraqi plant. The lack of some instruments and standard will hinder the investigation of other appeared component of the chromatogram.

Table 1: Results of TLC (Thin Layer Chromatography) for Artichoke leaves from the (Medicinal Garden of the College of Pharmacy/ University of Baghdad)

Sample	S_1	S_2	S_3
Ethyl acetate layer	R_f 1= 0.85 R_f 2= 0.69 R_f 3= 0.36	R_f 1= 0.79 R_f 2= 0.62	R_f 1= 0.81
Aqueous layer of Ethyl acetate layer	R_f =0.87	R_f = 0.79	R_f = 0.53
Aqueous layer of Butanol layer	R_f = 0.91	R_f = Nil	R_f 1= 0.82 R_f 2= 0.65 R_f 3= 0.56
Standard cynarin	R_f = 0.83	R_f = 78	R_f 1= 0.85

Note: The results above calculated according to UV. & chemical identification using H_2So_4 in alcohol heating for 5 minutes in oven at 110°C.

Table 2: Results of TLC for Artichoke leaves from (Greaat controlled farm by Agriculture College of Baghdad University)

Sample	S_1	S_2	S_3
Ethyl acetate layer	R_f 1= 0.86 R_f 2= 0.71	R_f 1= 0.8 R_f 2= 0.78	R_f 1= 0.86 R_f 2= 0.68 R_f 3= 0.55
Aqueous layer of Ethyl acetate	R_f 1= 0.84	R_f 1= 0.81 R_f 2= 0.79	R_f 1=0.87 R_f 2= 0.66 R_f 3= 0.52 R_f 4= 0.25
Butanol layer	R_f 1= 0.86 R_f 2= 0.70	R_f 1= 0.82 R_f 2= 0.77	R_f 1=0.86 R_f 2= 0.75 R_f 3= 0.58 R_f 4= 0.49 R_f 5= 0.26
Aqueous layer of butanol	R_f 1= 0.88 R_f 2= 0.72 R_f 3= 0.20	R_f 1= 0.82 R_f 2= 0.76	R_f 1=0.86 R_f 2= 0.75 R_f 3= 0.68 R_f 4= 0.60 R_f 5= 0.50 R_f 6= 0.25
Standard cynarin	R_f = 0.83	R_f = 0.78	R_f = 0.85

Table 3: Standard of *Cynaria scolymus* phenolic compounds after separation in HPLC.

Name of the standard	Retention time (Minutes)	Retention time (Minutes)	Notes
1,3- Dicaffeoyl- quinic acid (cynarin)	4.783	—	—
Caffeoyl quinic acid (chlorogenic acid)	—	5.467	—
Mixture of 1, 3 Dicaffeoyl quinic acid cynarin and caffoyol-quinic acid chlorogenic acid standard	4.683 Second reading 4.467	6.583 Second reading 6.067	at the same date

Table 4 : Butanol layer fractions and their content from phenolic acids, after separation in HPLC

Fraction	Peak number	Retention time (minutes)	Notes
F_2 (6)	1	1.650	Unknown-need more Investigation
	2	2.483	Unknown-
	3	3.983	Unknown-
	4	4.383	Cynarin-
	5	5.050	Unknown
	6	5.817	Chlorogenic acid
F_2 (7)	1	1.683	Unknown-
	2	1.817	Unknown-
	3	2.517	Unknown-
	4	4.483	Cynarin
	5	5.150	Unknown-
	6	5.967	Chlorogenic acid
	7	7.900	
F_2 (8)	1	1.650	Unknown
	2	2.550	Unknown
	3	2.867	Unknown
	4	4.717	Cynarin
	5	6.550	Chlorogenic acid
	6	7.583	Unknown
F_3 (9)	1	2.75	unknown

F1: include fractions of the column 1, 2, 3, 4, 5. shows one peak at retention time 3:2 minutes which need further investigation in HPLC. Fig – 4 -

Fractions of the column 10, 11, 12 show negative results in HPLC.

Table 5: Comparison of retention times of standard phenolic acid with those of the plant n- butanol extracts after separation in HPLC.

Name	Retention time	Name	Retention time
Standard	——	n- butanol extractFractions	——
1,3- Dicaffeoyl- quinic acid (Cynarin)	4.783	F_2 (6)	4.383
5-0-Caffeoyl- quinic acid (Chlorogenic acid)	5.467	——	5.817
Cynarin	——	F_2 (7)	4.483
Chlorogenic acid	——	——	5.967
Cynarin	——	F_2 (8)	4.717
Cholrogenic acid	——	——	6.550

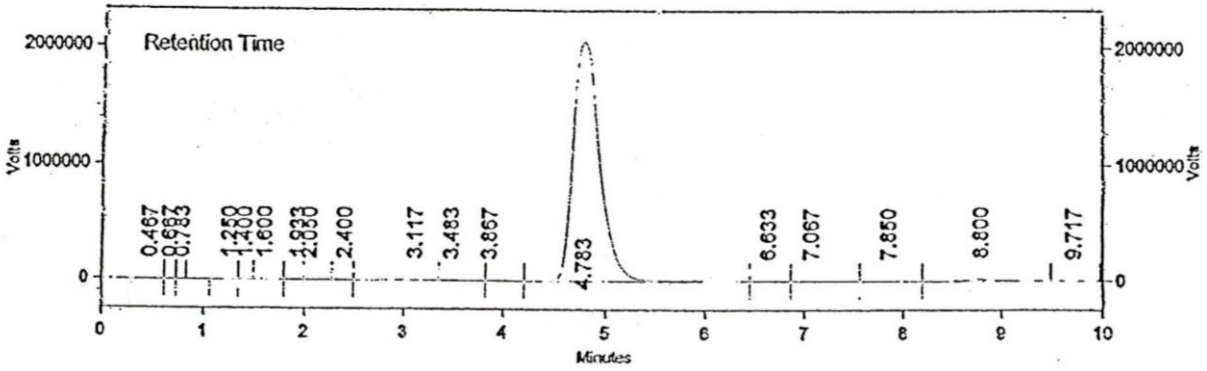


Figure 1 : HPLC analysis of Cynarin standard.

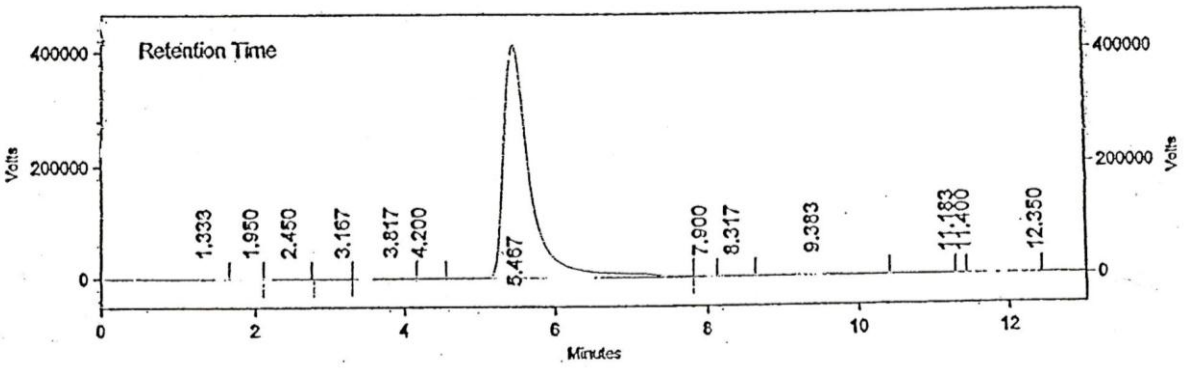


Figure2 : HPLC analysis of chlorogenic acid standard.

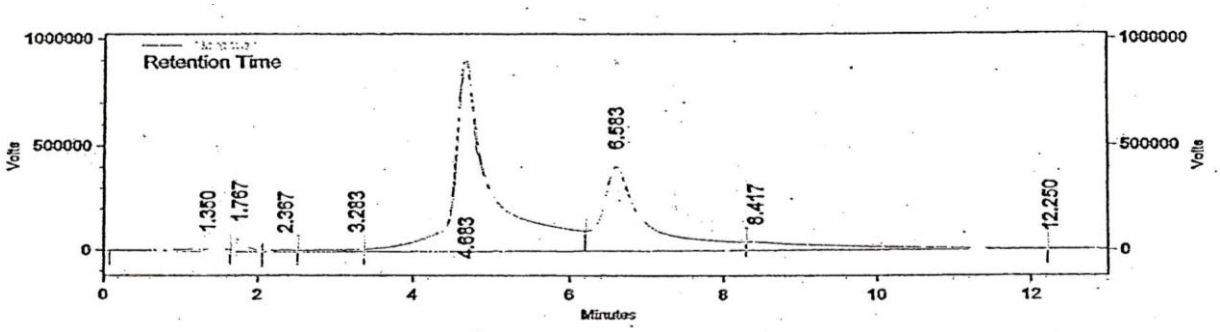


Figure 3: HPLC analysis ofmixture of cynarin and chlorogenic acid standard.

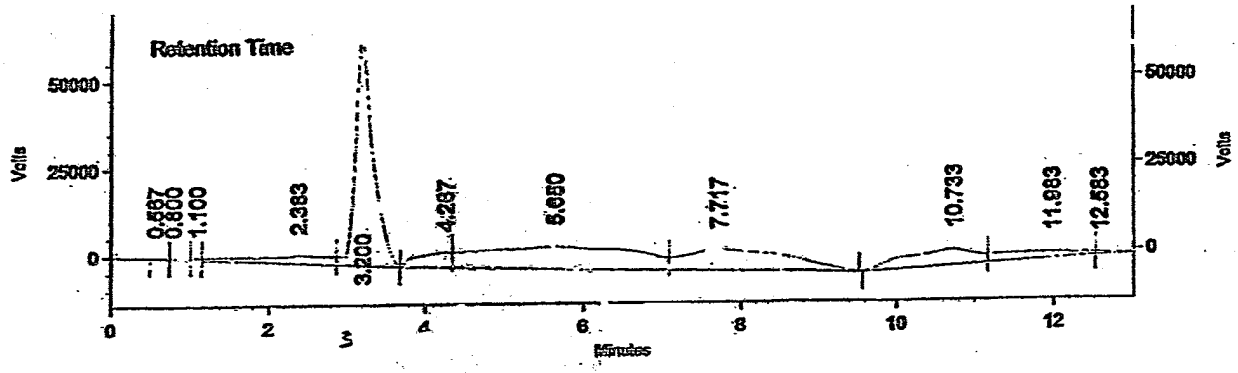


Figure 4: HPLC analysis of F1 of butanol extract of *Cynaria scolyus* of Pharmacy College Baghdad University. Fraction of the column № 1,2,3,4,5.

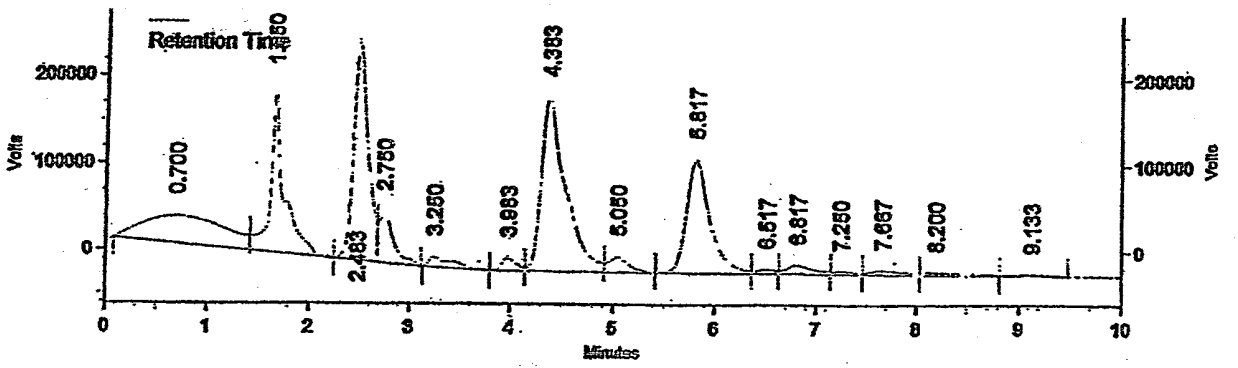


Figure 5: HPLC analysis of F2 of butanol extract of *Cynaria scolymus* of Pharmacy College Baghdad University. Fraction of the column № 6.

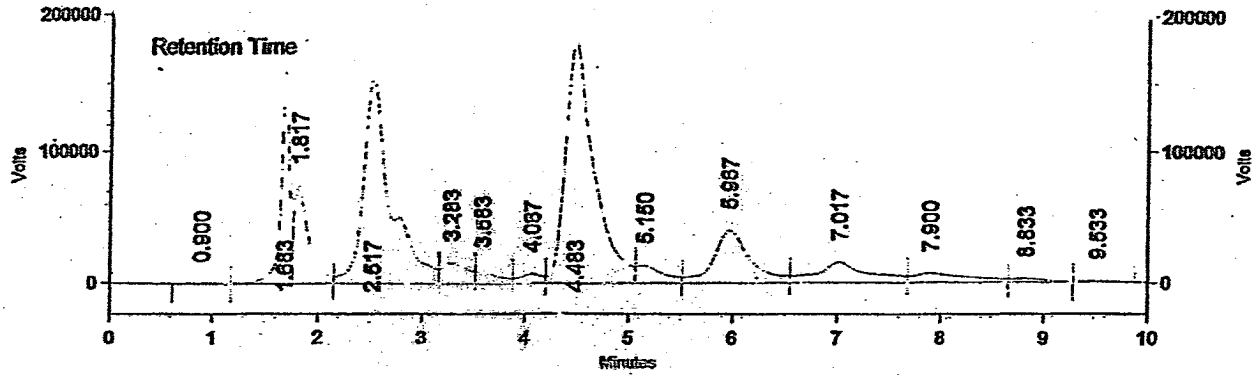


Figure 6: HPLC analysis of F1: of butanol extract of *Cynaria scolymus* of Pharmacy College Baghdad University. Fraction of the column № 7.

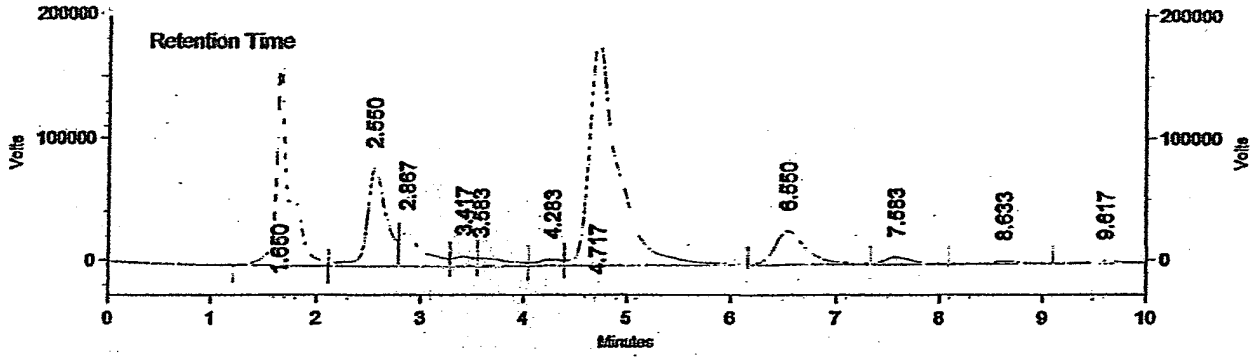


Figure 7: HPLC analysis of F2: of butanol extract of *Cynaria scolymus* of Pharmacy College Baghdad University. Fraction of the column № 8.

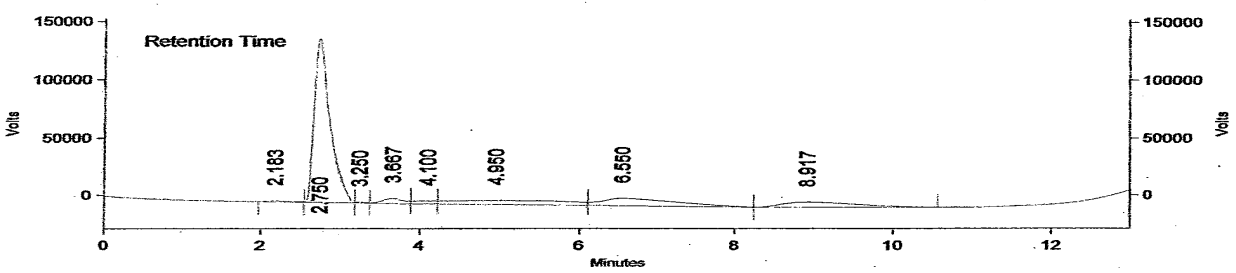


Figure 8: HPLC analysis of F3 of butanol extract of *Cynaria scolymus* of Pharmacy College Baghdad University. Fraction of the column № 9.

References

1. Trease and Evans; Phormacognosy; Artichoke leaf 16th Ed. 2009 P. 184.
2. Maria Rosario ALONSO; Maria del Carmn GARCIA Claudia Garcia BONELLI: Validated HPLC Method for Cynarin Dctermination in Biological Sample: Acta Farm. Bonaerense; 2006; 25(2):267 – 70.
3. P. Perziosi: II Farmaco 1962; 17: 701 – 45.Antioxident potential of artichoke axtract.
4. S. Gorzaczany, R. Filip, M. Alonso 2001; Choleretic and hypocholesterolemic activity of cynarin; J. Ethenopharmacol 75: 291 – 4.through Acta Farm Bonaerense 2006;25(2): 267-70 .
5. M. Faure, E. Lissi, R. Torres and L. videla activity related to the power antioxidant effect of phenolic acids; Phytochem Pharmacol,1990; 29: 3773 – 5.
6. S. M. Wittemer; M. Ploch, T. Winddeck, S. C. Muller, 2005, Phytomedicin 12 (1 – 2): 28 – 38.
7. Herbal Drugs and Phytopharmaceutical; 2004. Cynara folium (Artichoke leaf) Third ed. P.173 – 175.
8. Neung Tae Jun, Ki Chang Jang; J. Appl. Boil. Chem.; 2007; 50(4) 244 -284 Radical Scavenging Activity and Content of cynarin.
9. Kevin Robards; J. of chromat Graphy A. 2003; Strategies for Determination of Bioactive phenols in Plants, Fruit and Vegetable,2003; 657 – 691.
10. Herbal Medicine; Artichoke, third Ed. 2007; P.67 – 71. Pharmaceutical press.
11. E. Hvattum, Rapid commun. 2002; 16; 655.
12. Kevin Robards; Strateyies for determination of bioactive phenols in plants, fruit and vegetable; Journal of chromatography A, 1000 ,2003; 657 – 691.
13. N - Brand z. Constituents of cynara folium flavenoids; Phytother ,1999; 20: 292 – 302. Through herbal drugs and phytopharmacutical.
14. N- Brand z. Constituent of cynara folium bitter compound sesequitapine, Phytother ,1990;11: 169 – 175.
15. BBV (Bretange Biotechnologie Vegetable) FAV Health, Quebee City Dr. Serge Mabean, Applied Research Center for Plant Breeding, Biotechnology and Quality. Antioxidant activity of extracts of artichoke and by – products, 2005.
16. P. Mattila, K. Konko, M. Eurola, Concentration of phenols in the plant, J. Agri Food Chem, 2001; 49: 2343.
17. W. K, Li, HH. S Fong, K. W. Singletary; JF. Fitzooff. J. Lig. Chromatogr. Relat. Technol ,2002;25: 397. Gradient elution technique.
18. A. M. Torres, T. Man- Lastovicka, R. Rezaaiyan, J. Agric Food Chemi; 1987;35: 921.
19. X. Q Mu, Q. Shi, A. Duan, T. T. X. Dong; J. Agric Food Chem, 2002;50: 4816.
20. Huige Li, Ning Xia, Isolde Brausch, Ying Yao and Ulrich Forstermann Flavenoid from Artichoke (Cynara Scolymus); UP Regulate Endothelial Type Nitric – Oxide synthase Gen Experssion in Human Endothelial Cell. Jpet. aspetjournals. org. ;2007.
21. Jan Frissche. Christaon M. Bindorff; Eur. Food Res. Technol, Isolation, chroctevization and determination of minor artichoke (cynara scolymus L.) leap extract compounds. 2002;215: 149 – 157.