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Detection and Isolation of some Flavonoids and Aromatic Acid from Head(capsule) of *Cynara scolymus* Cultivated in Iraq

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Abstract

The target of this study was to study the natural phytochemical components of the head (capsule) of *Cynara scolymus* cultivated in Iraq. The head (capsule) of plant was extracted by maceration in70% ethanol for 72 hours, and fractioned by hexane, chloroform and ethyl acetate. Preliminary qualitative phytochemical screening was performed on the ethyl acetate fraction for capsule was revealed the presence of flavonoid and aromatic acids. These were examined by (high -performance liquid chromatography) (HPLC diodarray), (high—performance thin-layer chromatography)(HPTLC).

Flavonoids were isolated by preparative layer chromatography and aromatic acid was isolated by preparative high-performance liquid chromatography HPLC from the ethyl acetate fraction of capsule.

Then identified by High Performance Thin Layer Chromatography HPTLC, High performance liquid chromatography HPLC diode array , ultraviolet diode array UV-diode array and Liquid Chromatography /Mass Spectroscopy LC/MS. The chloroform fraction from the capsule was evaluated by Gas Chromatography//Mass Spectrometer(GC/MS). The different chromatographic and spectroscopic techniques revealed the presence of luteolin, apigenin and cinnamic acid in capsule of *Cynara scolymus*, also 9-octadecanoic acid (oleic acid), Oxalic acid, allyl tetradecyl ester, limonene, in chloroform of *Cynara scolymus*

The results of the current study proved the presence of luleolin, apigenin, and cinnamic acid in the ethyl acetate fraction of *Cynara scolymus* capsule.

Keywords: *Cynara scolymus*, Flavonoids, Aromatic acid , Gas Chromatography //Mass Spectrometry (GC/MS) ,High-performance Thin -layer chromatography(HPTLC), High-performance -layer chromatography(HPLC) and Liquid Chromatography//Mass Spectroscopy(LC/MS).

كشف وعزل الفلافونويد والحامض العطري من نبات الخرشوف المستزرع في العراق

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الخلاصة

الهدف من الدراسة هو دراسة المكونات الكيميائية للجزء العلوي (الكبسولة)لنبات الخرشوف المستزرع في العراق .تم استخلاص الجزء العلوي لنبات الخرشوف بواسطة عملية النقع البارد بنسبة ٧٠٪ايثانول لمدة ٧٢ساعه وبعدها تم تجزئة المستخلص الإيثانولي باالهكسان ,الكلوروفورم و خلات الأثيل. تم إجراء فحص كيميائي نباتي أولي على مستخلص خلات الإيثيل ، وتم كشف وجود الفلافونويد والحمض العطري وكذلك كروموتوكرافيا الطبقه الرقيقه (HPTLC) وكروموتوكرافيا السائل عالي الاداء (HPLC) تم عزل الفلافونويدات بواسطة كروموتوغرافيا الطبقه الرقيقه التحضيري (PLC) والحامض العطري بواسطه كروموتوغرافيا السائل عالي الاداء (HPLC)من جزء خلات الاثيل في الكبسولة و تم تحديد المواد المفصوله بواسطة كروموتوغرافيا الطبقه الرقيقه HPTLC و كروموتوغرافيا السائل عالي الاداء (ثنائي الصمام) HPLC و مطياف الكتلة (LC/MS) تم تحليل جزء الكلوروفورم من الكبسولة باستخدام كروماتوجرافيا الغاز / مطياف الكتلة (GC / MS).

الكلمات المفتاحية : الخرشوف الكروي، فلافنويد,حامض عطري، كروموتوغرافيا الغاز، مطياف الكتلة ، كروموتوكرافيا الطبقة الرقيقة ، كروموتوكرافيا السا ئل عالي الاداء، كروموتوغرافيا السائل ، مطياف الكتلة .

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Introduction

Medicinal plants commonly used as raw materials for extraction of active ingredients used in the production of different drugs [1] The therapeutic effectiveness of a medicinal plant is due to the presence of some bioactive constituents [2] Herbal medicine around the globe, is based on traditional medicine, pharmacological screening and exploration of the chemical constituents of the plants may provide us the basis for developing a lead molecule through herbal drug discovery. In modern medicine, the very important life-saving drugs have been provided by herbs [3] This awareness in Iraq was explained and enhanced by the Arab physicians [4-6] In many republics in the world, traditional medicine remains accordingly important to the formal health system [7] The Arab countries including Iraq are among these countries [8].

Artichoke (*Cynara scolymus*L. belong to Asteraceae familly) , the Asteraceae (Compositae, alternate name) with its approximately 1,620 genera and more than 23,600 species is the major family of flowering plants[9]. The family is distributed worldwide except for Antarctica but is especially diverse in the tropical and subtropical regions of North America, eastern Brazil, the Andes, southern Africa, the Mediterranean region, central Asia, and southwestern China. The globe artichoke in appearance is like a large, blue thistle [10]. Globe artichoke (*Cynarascolymus*L.), a perennial species of this family is grown for its big fleshy immature inflorescences flower heads[11].The globe artichoke *(Cynara scolymus)* is a unique vegetable, having fibrous, fleshy rhizomes with buds that develop into several tomentose and branched stems. The most vigorous varieties may reach 1.20-1.30 m in height.

The bearing stem is erected, ribbed, and rounded in cross-section, ending with a floral head (capitulum), capitulum it is composed of several tubular and bluish-violet fertile florets opening from the outside inwards[12].This plant an medicinal plant and golden harvest and, the therapeutic possible of which was known to the ancient Egyptians, Greeks and Roman [13], It contains (cynarin and chlorogenic acid) caffeoylquinic acid derivatives) and flavonoids (Apigenin and luteolin) , as well as the anthocyanidins such as 2-(4-Hydroxy-3-methoxyphenyl) chromenylium-3,5,7-Tirol (pending),2-(3,4,5-trihydroxyphenyl) chromenylium -3,5,7-Tirol (delphinidin) and 2-(3,4-dihydroxyphenyl) chromenylium-3,5,7-Tirol (cyanidin), these have been isolated in only in the capsule of artichoke [14,15] The above flavones luteolin and apigeninhas been identified in capsule and leaves of the plant in the form of rutinosides and glucosides and, whereas anthocyanin pigments are present only in capsule, in form of sophorosides and glucosides[14].

Pharmacological activities of *Cynara scolymus*, antioxidant ntimicrobial Activities[16],antiatherogenic and hypoglycemic effect[17] antispasmodic activity[18] ,cardiovascular protection [19,20]choleretic effects (stimulation of bile secretion[21,22], antifungal activity[23], anti-metabolic syndrome[24,25] and anti-cancer effect[26].The dominant study was studying the natural phytochemical components of the head(capsule) of *Cynara scolymus* cultivated in Iraq.

Figure 1. Fresh head(capsule) of *Cynara scolymus* .

Material and Methods

Plant material

Plant material of *Cynara scolymus* capsule (head) was obtained from University of Baghdad/ the College of Pharmacy during June/2018.The plant was identified and authenticated by D.r Khansaa. Al-Joboury in Iraqi Natural History Center Museum in Baghdad University. All parts that were obtained were washed thoroughly, dried in the shade, followed grinding by an electrical grinder to a fine powder.

Extraction of plant

100 grams of the powdered plant material was extracted by maceration in 70% ethanol for 72hours with frequent shaking, at room temperature, the extract was filtered off, this procedure was repeated three times. The filtrates were mixed together and evaporated under vacuum by a rotary evaporator. There mains (100ml) were partitioned successively with hexane, chloroform and ethyl acetate (3X100). The hexane, chloroform and ethyl acetate fraction were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness using a rotary evaporator.

Phytochemical examination for fractions of plant

Phytochemical analysis for screening and identification of bioactive chemical constituents in the medicinal plants as described [27].

Test for flavonoids: (a) 0.5 gm of fractions were suspended in ethanol mixed with few drops of 1% aluminum chloride in methanol in a test tube, and the color was observed. Formation of yellow color indicates the presence of flavonoids.

(b)0.5 gm of fractions were suspended in ethanol mixed with few drops of 1% potassium hydroxide in a test tube, and the color was observed. A dark yellow color indicated the presence of flavonoids.

Test for Phenols: 0.5 gm of each fractions were suspended in ethanol in a test tube, then few drops of 5% ferric chloride was added and a deep green to black color was observed for formation.

Examination of ethyl acetate fraction (capsule) by high performance thin layer chromatography (HPTLC)[28]

The presence of phenolic compounds in the analyzed fractions was confirmed by using a modern technique of HPTLC, using Eike Reich/CAMAG-Laborator/ Switzerland.

►**Samples**

Ethyl acetate fraction of the capsule

►**Standards**

1.Apigenin
2.Luteolin
3.Cinnamic acid

►**Preparation of standards and samples for HPTLC:** The standards (1mg) and samples (few milligrams) were prepared by dissolving them individually in 1 ml of absolute methanol.

►**Developing solvent system:**

The mobile phase used was composed of: chloroform: methanol: formic acid (16:3.5:0.5)

►**Detection:** Detection was done under UV light at 254 nm .

-Examination of ethyl acetate fraction by high performance liquid chromatography (HPLC)

HPLC conditions for analyzed fraction: show in Table 1.

Table 1 . Conditions of analytic HPLC[29].

Stationary phase	C18 (250X10) 5 µm particles size.
Mobil phase	solvent (0.05% TFA in HPLC grad water) and solvent (acetonitrile).
Standard	Cinnamic acid.
Sample	Ethyl acetate fraction.
Detection	Monitoring on 225 nm
Injected volume	Sample loop (200 µl) and injector.
Flow rate	3ml/min.

Isolation of flavonoids and aromatic acid from ethyl acetate fraction from the capsule of plant

-Isolation of flavonoids were done by preparative layer chromatography (PLC), from the ethyl acetate fraction of *C*ynara *scolymus* capsule the conditions of isolation show in table 2

Table 2. Conditions of preparative layer chromatography (PLC)

Stationary phase	Silica gel GF_{254}
Mobil phase	Chloroform: Methanol: Formic acid(16:3.5:0.5)
Standards	Apigenin and luteolin
Sample	Ethyl acetate fraction
Detection	U V 254nm

Isolation of aromatic acid by preparative (HPLC) from an ethyl acetate fraction of *Cynara scolymus* capsule: conditions of isolation of HPLC show in table 3

Table 3. Conditions of isolation of HPLC[29].

Stationary phase	C18 (250X10) 5 µm particles size.
Mobil phase	solvent (0.05% TFA in HPLC grad water) and solvent (acetonitrile).
Standard	Cinnamic acid.
Sample	Ethyl acetate fraction.
Detection	Monitoring on 225 nm
Injected volume	Sample loop (200 µl) and injector.
Flow rate	3ml/min.

Identification of separated compounds Identification of flavonoids (A4)(1)and (A6)(3) isolated by(PLC)and aromatic acid(A1) isolated by preparative (HPLC):

HPTLC: The sample was prepared by dissolving 0.5 mg of the isolated compound in 1 ml of absolute

methanol and examined under same previously mentioned conditions.

HPLC(diodarray): The sample was prepared by dissolving 0.5 mg of the isolated compound in 0.5 ml of absolute methanol and examined under same previously mentioned conditions.

UV-diodarray: 0.5 mg was dissolved individually in 1 ml absolute methanol, and the UV absorbance was scanned from 200-400 nm.

LC/MS

Mobil phase solvent acetonitrile and water Colum 0.19 mm external diameter (75 mm i.d)and 200mmlength wave packed with thermo scientific hypersil gold c18 with 5mmpartical size. Sample were run under the following condition /z rang was 250 to 1000.200k resolution, top 5 configuration with one m/s scan and five ms/ms scans, and dynamic exclusion set to 1 with a limit of 90 second.150 femtomole of angiotensin standard mix from micron biosciences was loaded on column per injection .A 2.5 hour LC/MS separation was used for all blank and standard sample.[30]

GC/ MS analysis of* the chloroform fraction *for capsule

GC/ MS analysis of the chloroform fraction for capsule was done using Agilent GC-MS model with the below Conditions: Agilent 190915-433UI, Hp-5ms Ultra Inert, In Front SSZ Inlet He, Out MSD, Initial 60c, Pressure 7.037 psi, Flow 0.9ml/min, Average Velocity 34772 cm/Sec, Holdup time 1.4379 min

Results and Discussion

The results of preliminary phytochemical analysis in different fractions of the plant shown in table 4.

Table 4. Phytochemical analysis for fractions of *C.scolymus*

Fractions	Phenols	Flavonoids
Hexane	+	+
Ethyl acetate	+	+

The present study done for the *Cynara scolymus* cultivated in Iraq showed the presence of medicinally active constituents phytochemical active compounds were qualitatively analyzed and the results are presented in Table 4 the positive results based on the presence or absence of color change. In this screening process, flavonoids and phenols give positive (+) results.

Analysis of fraction and standards by high-performance thin layer chromatography (HPTLC)

HPTLC chromatography for standards and ethyl acetate fraction. HPTLC is a valuable tool for reliable identification because it can provide chromatographic finger prints that can be visualized and stored as electronic images [31]. HPTLC chromatography show max R_f value for standards (apigenin ,luteolin and cinnamic acid) and ethyl acetate fractions shown in Figure2.

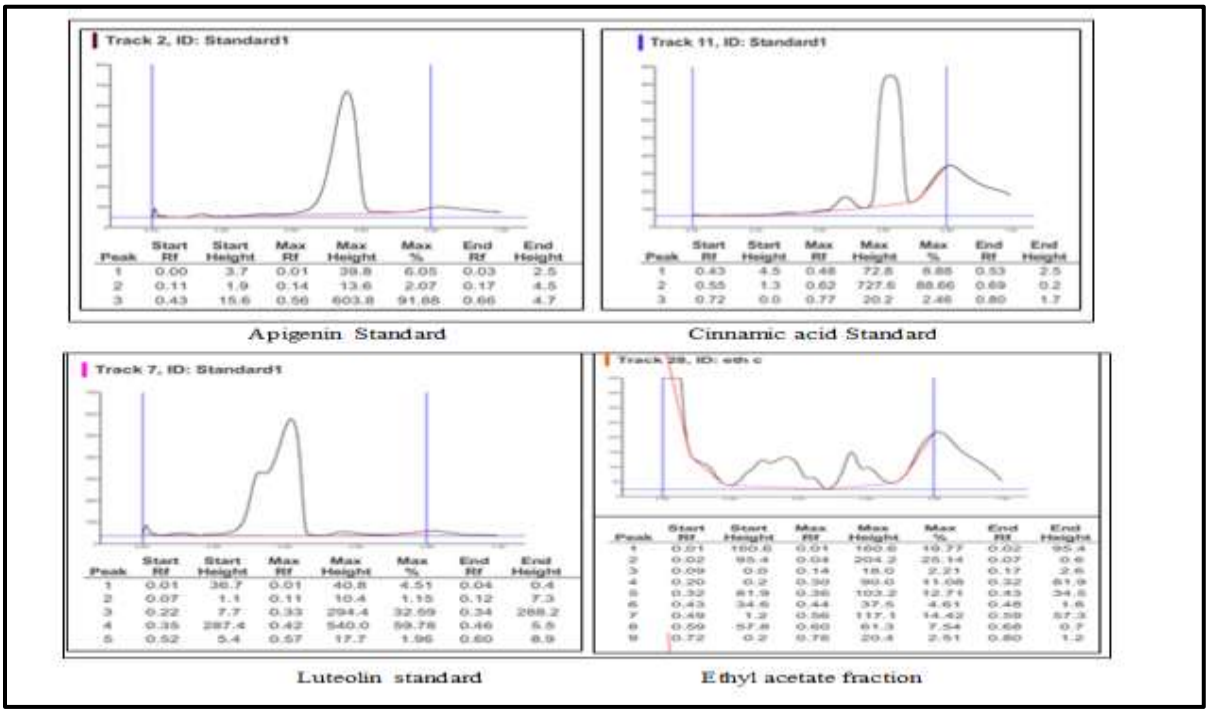


Figure 2. HPTLC chromatography show max R_f value for standards and fraction.

Analysis of ethyl acetate fraction by high-performance liquid chromatography (HPLC)

The HPLC results of the weight of main compounds in ethyl acetate fraction (capsule) shown in Table 5: Weight of main compounds in ethyl acetate fraction by calibration curve

Table 5. Weight of main compounds in ethyl acetate fraction

Name of compounds	Ethyl acetate Fraction of (capsule) w.t (µg / ml)
Cinnamic acid	15.939
Luteolin	69.6949
Apigenin	103.71325

Isolation compounds by preparative layer chromatography (PLC) from the ethyl acetate fraction

Preparative layer chromatography(PLC) was done utilizing ethyl acetate fraction of maceration method was developed in mobile phaseS12: chloroform: methanol: formic acid(16:3.5:0.5).2 bands separated symbolized as (A4)(1) and (A6)(3) shown in Figure3.

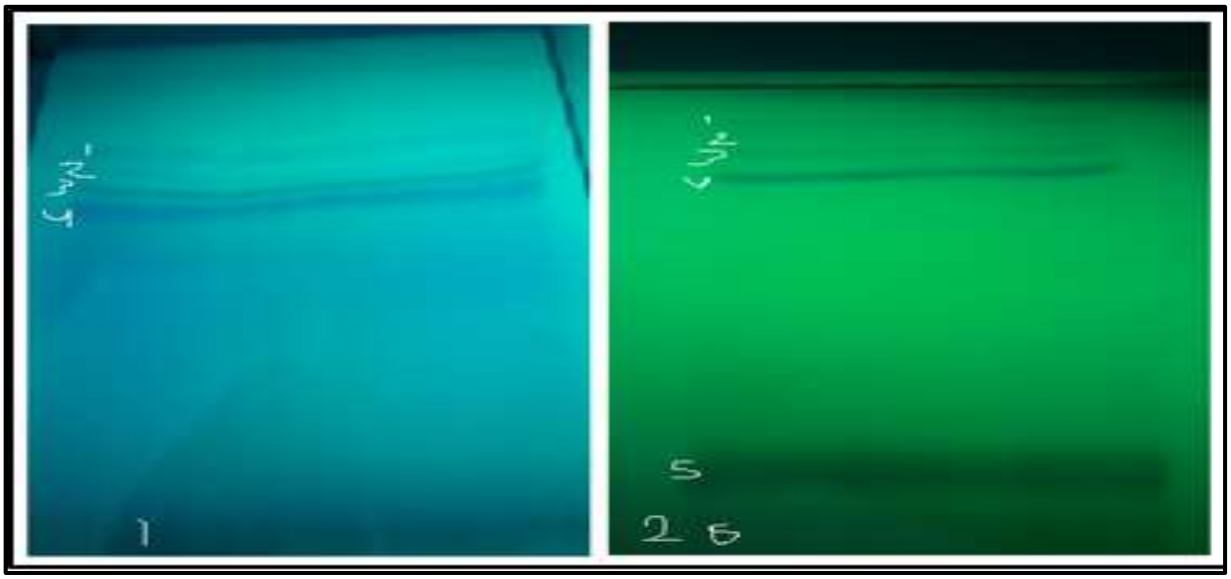


Figure 3. Preparative layer chromatography of ethyl acetate fraction developed in mobile phase (S12) chloroform: methanol: formic acid(16:3.5:0.5) observed at 254 nm.

HPTLC for isolated compounds A4(1) and A6(3) isolated by (PLC)and A1 isolated by (HPLC) and standards.

HPTLC results shown in Figure4: (A4)(1)and apigenin(standard),(A6)(3) and luteolin(standard) and (A1) and cinnamic acid (standard).

HPLC for isolated compounds A4(1) and A6(3) isolated by (PLC) and A1 isolated by (HPLC) and standards.

The HPLC diode array results of analyzing separated compounds and standards are demonstrated in Table 6.The identification of compounds by HPLC is usually performed by comparing the obtained retention times with the ones of related standards.

Table 6. Retention time of the standards and the isolated compounds

Retention time of isolated compounds (min)	Isolated compounds	Retention time of Standards(min)	Standards
Apigenin	(17.16)	A4(1)	(17.15)
Luteolin	(16.18)	A6(3)	(16.18)
Cinnamic acid	(16.8)	A1	(16.7)

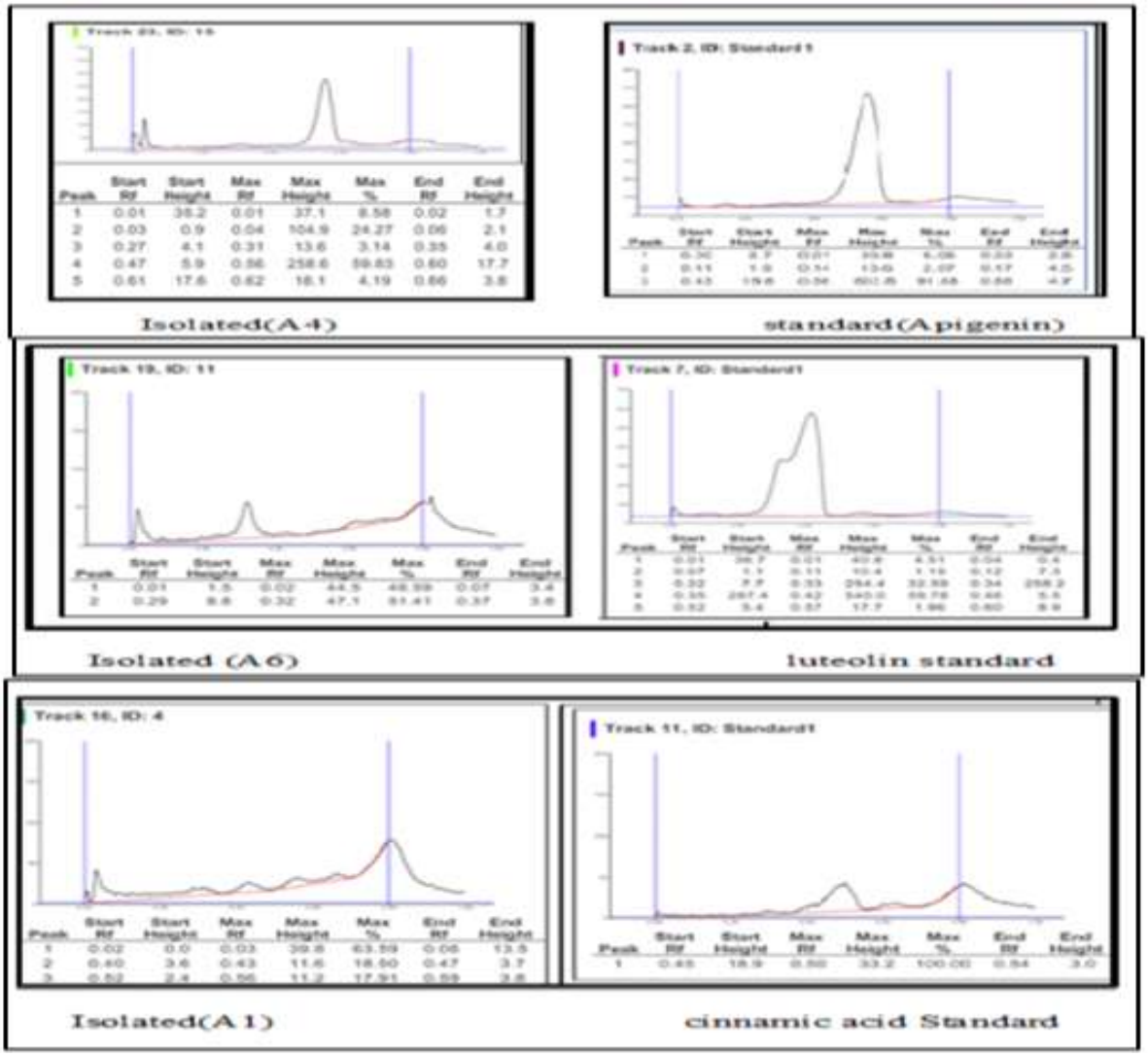


Figure4. HPTLC for isolated (A4)(1) standard(apigenin),(A6)(3) and standard(luteolin) and (A1) and and standard(cinnamic acid).

HPLC for isolated compound A4(1) and apigenin standard HPLC results show in Figures (5 -6)

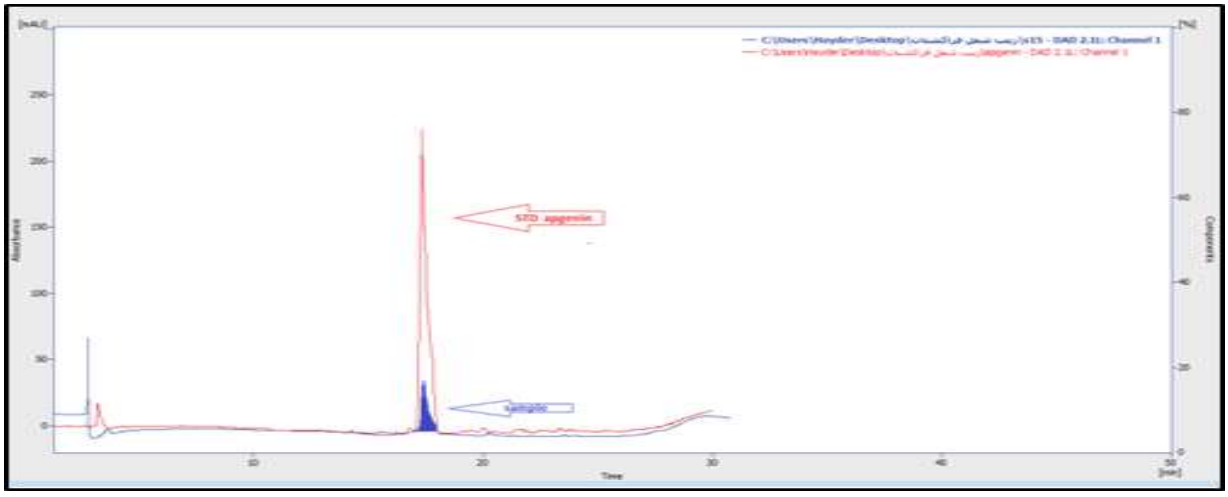


Figure 5.Analytic apigenin standard and isolated compound A4(1)

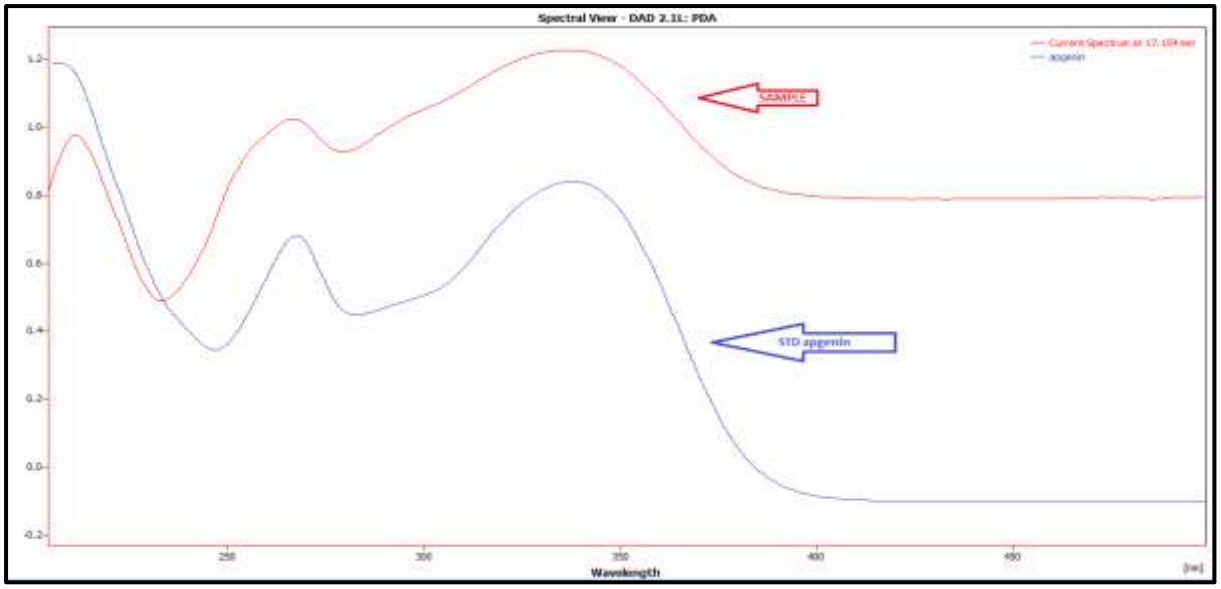


Figure 6. UV spectrum for apigenin standard and isolated compound A4(1)

HPLC for isolated compound (A6) (3) and. luteolin standard HPLC results show in Figures (7-8)**:**

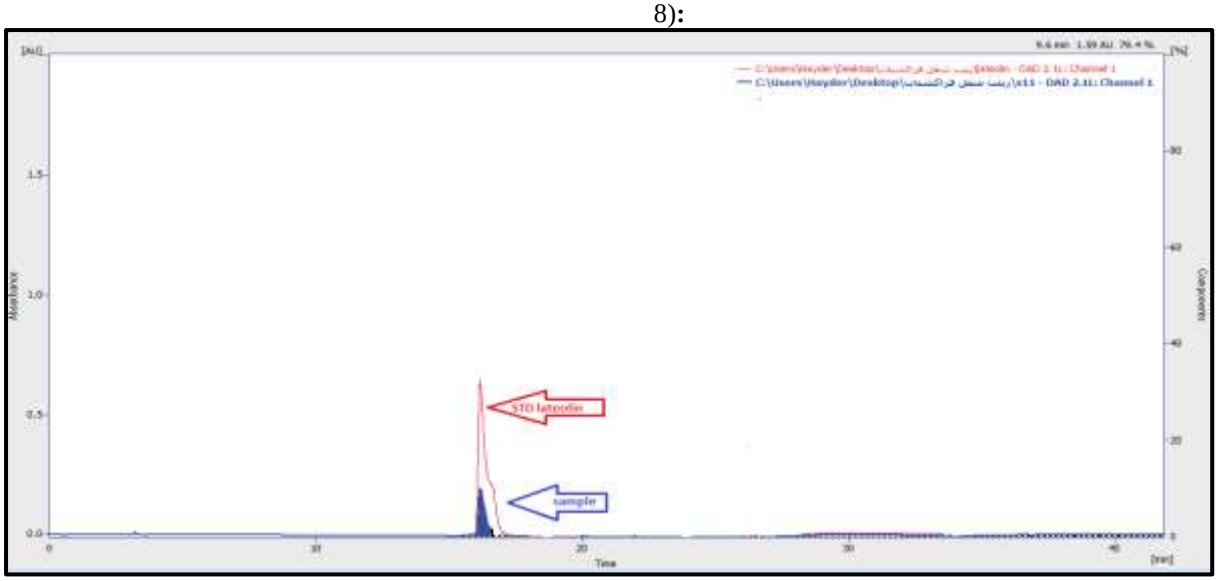


Figure 7.Analytic luteolin standard and isolated compound A6(3)

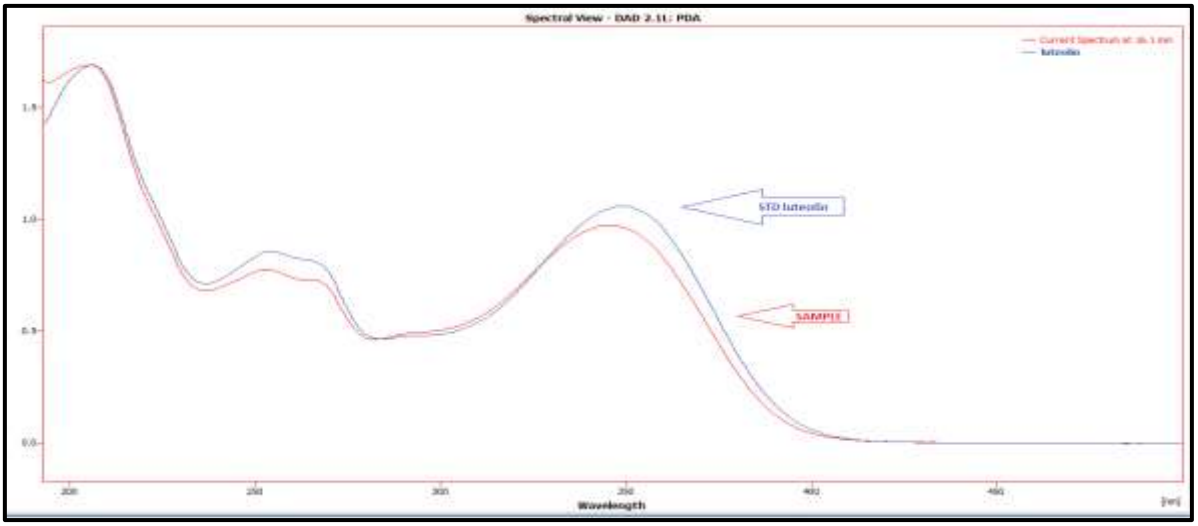


Figure 8. UV spectrum for luteolin standard and isolated compound A6(3).

HPLC for isolated compound (A1) and cinnamic acid standard HPLC results show in figures (9-10):

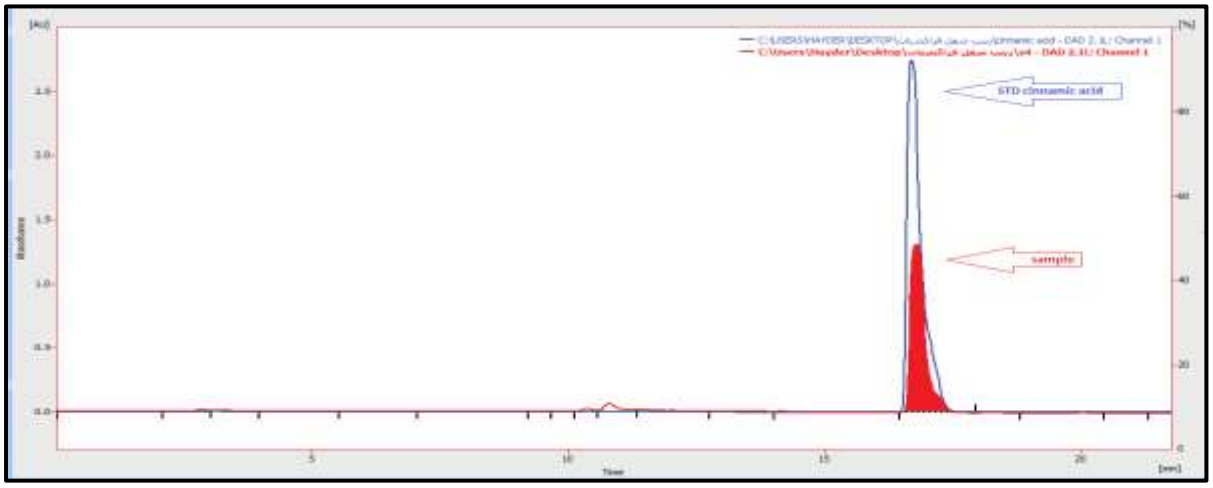


Figure 9.Analytic cinnamic acid standard and isolated compound(A1).

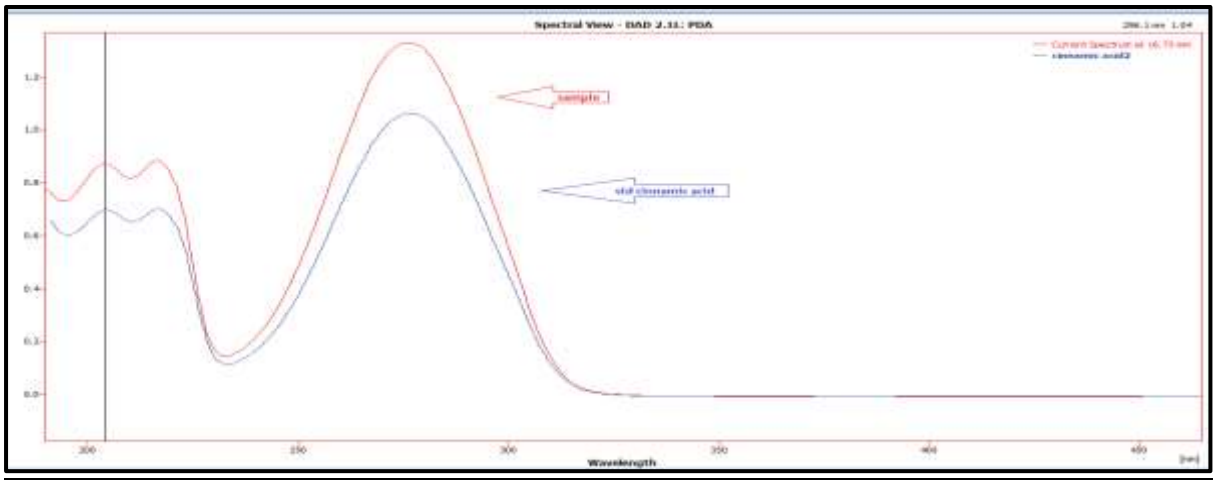


Figure 10. UV spectrum for cinnamic acid standard and isolated compound(A1).

LC/MS of isolated compounds A4(1) and A6(3) isolated by (PLC) and A1 isolated by (HPLC).

LC/MS of isolated compoundsA4(1): the result show in Figure11.

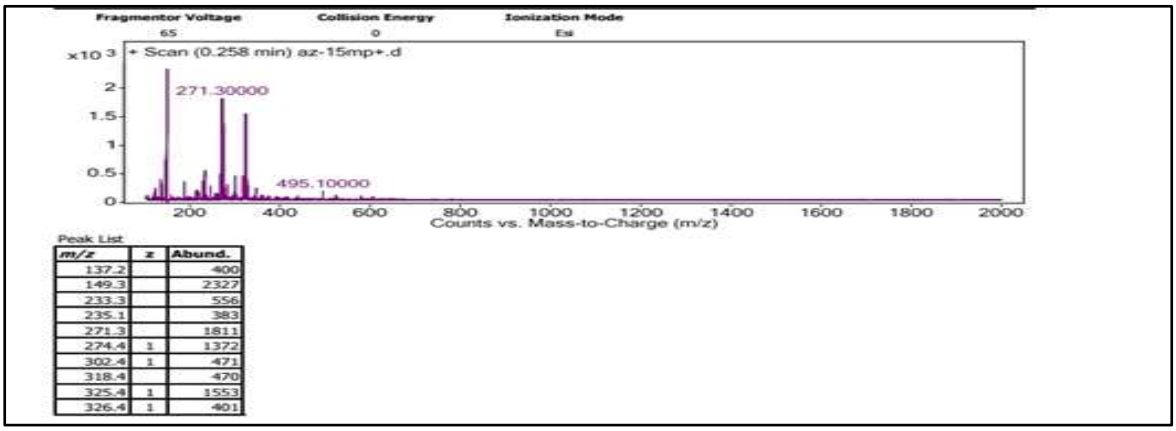
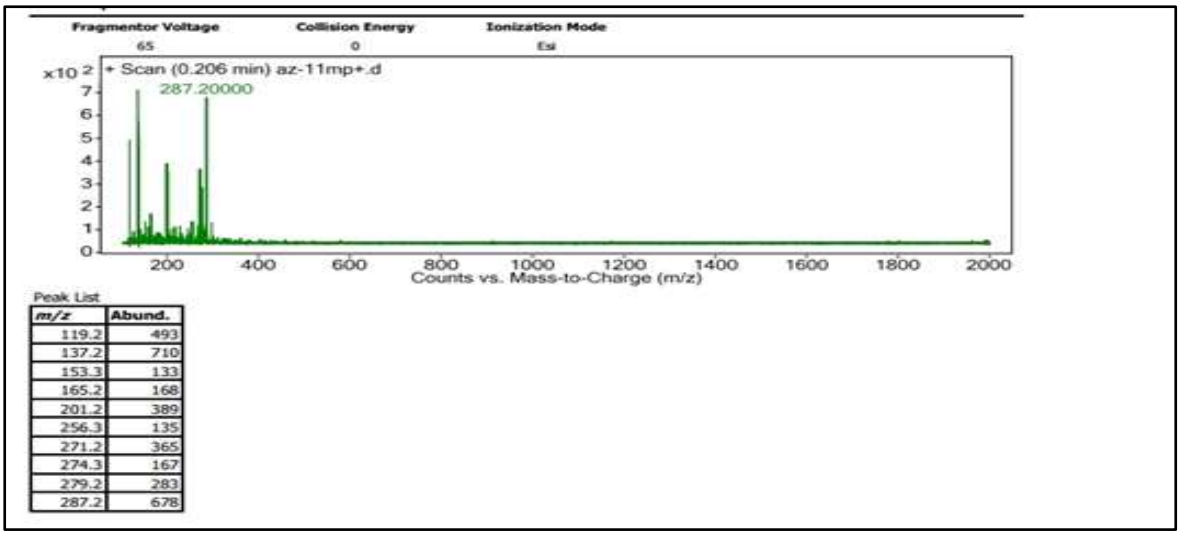


Figure 11.LC/MS for isolated compound A4(1)

LC/MS for isolated(A6)(3): the result show in Figure12:

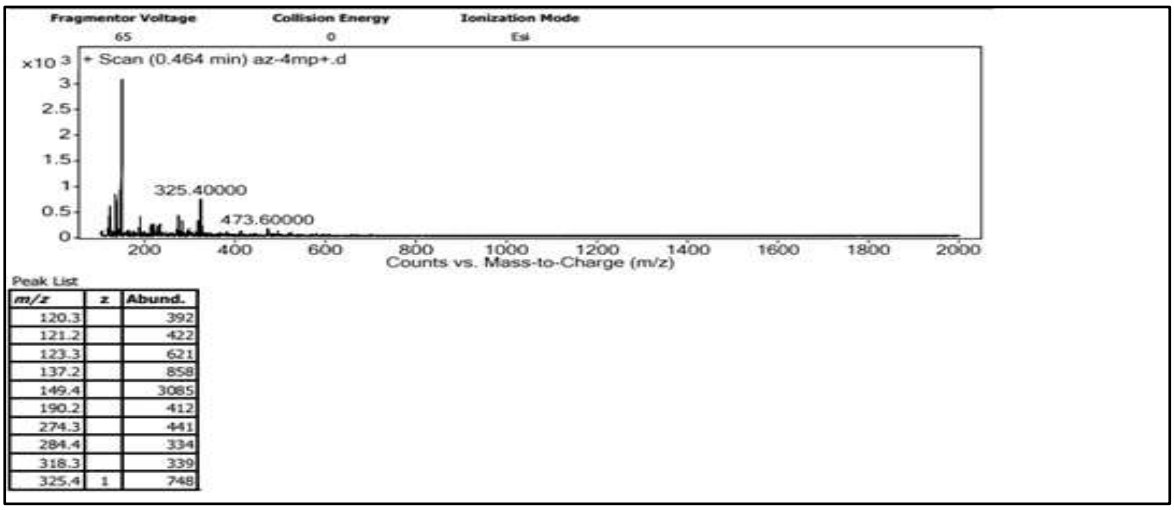


Peak List

m/z	Abund.
119.2	493
137.2	710
153.3	133
165.2	168
201.2	389
256.3	135
271.2	365
274.3	167
279.2	283
287.2	678

Figure 12:LC/MS for isolated compound A6(1)

LC/MS of isolated compound (A1): the result show in Figure13.



Peak List

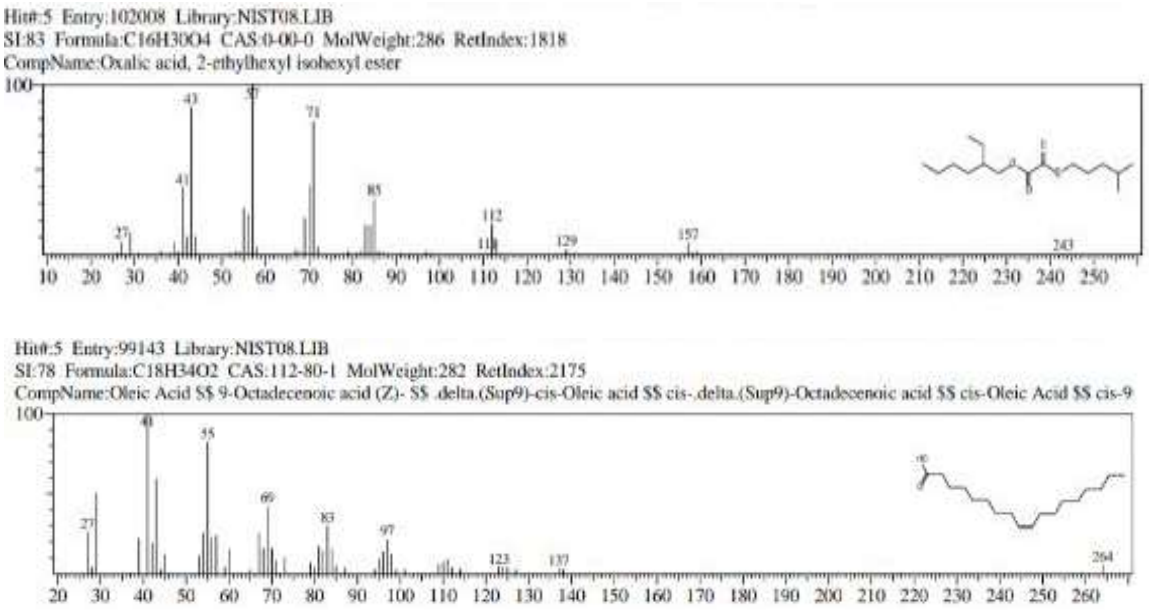
m/z	z	Abund.
120.3		392
121.2		422
123.3		621
137.2		858
149.4		3085
190.2		412
274.3		441
284.4		334
318.3		339
325.4	1	748

Figure 13.LC/MS for isolated compound A1

The compounds detected in chloroform fraction for capsule of *Cynara scolymus* show in Table7 and Figure 14:

Table 7. GC/MS analysis of the chloroform fraction of *Cynara s colymus*

Compounds name	Molecular weight g/Mol	Molecular formula
Oxalic acid, allyl tetra decyl ester	326	C19H34O4
Oleic Acid $$ 9-Octadecenoic acid	282	C18H34O2
Limonene	136.23	C10h16



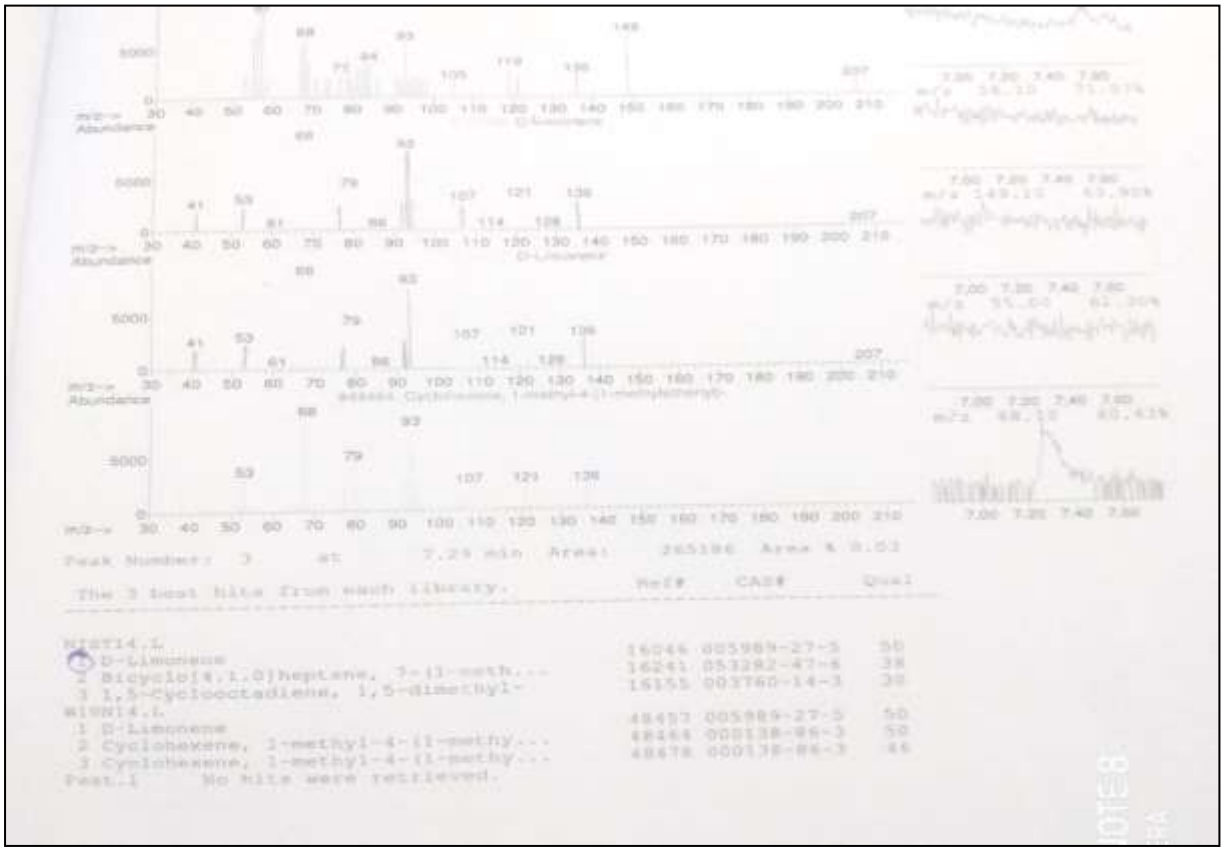


Figure 14.GC/MS chromatography of chloroform fraction of plant

Discussion

The preliminary phytochemical analysis confirmed the presence of flavonoids and aromatic acid. The HPLC results show the occurrence of flavonoids and aromatic acid in the capsule of *C*ynara *scolymus*, such as luteolin, apigenin, and aromatic acid cinnamic acid ,apigenin more concentration, then luteolin and finally cinnamic acid.

The consequences of the present study show the isolation of flavonoids (apigenin and luteolin) from ethyl acetate fraction by PLC and aromatic acid (cinnamic acid) by preparative HPLC .HPTLC results revealed the presence of apigenin, luteolin and cinnamic acid LC/MS systems facilitate the analysis of samples that traditionally have been difficult to analyze.

Liquid chromatography (LC) separates the sample components and then introduces them to the mass spectrometer (MS). The MS creates and detects charged ions. The LC/MS data may be used to provide information about the molecular weight. Electrospray is a soft ionization technique that produces a large number of molecular adduct ions. Adduct ions are typically protonated parent ions [M+H]+.[32] LC/MS results give m/z for apigenin isolated compound (271) molecular weight of apigenin standard (270), m/z for luteolin (287), molecular weight of luteolin standard (286) and m/z for cinnamic acid (149), molecular weight of cinnamic acid standard(148) .So from these all data, isolated compound could be identified

Conclusions

Based on the results, the following points may be concluded:

1. Phytochemical screening of *Cynara scolymus* cultivated in Iraq demonstrates the presence of flavonoids and aromatic acid which were separated from head (capsule)of plants according to differences in their chemical nature.

2 . In this study, Two chromatographic analyses were carried out to isolate in a pure form: one: preparative TLC for flavonoids (apigenin and luteolin) apigenin more concentration than luteolin isolate from head (capsule) and second : preparative(HPLC) for isolated aromatic acid(cinnamic acid).

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