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Identification and determination of some fatty acid compounds and free phenolics from the seeds of *Cuminum cyminum L.* plant growing in Iraq

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Abstract

The current research aims at the separation and identification of many fatty acid compounds from the seeds of *cuminum cyminum L.* that are grown in Iraq using a continuous soxhelt extractor and sequence solvent systems depending on the polarity, Pet-ether extract (CU_I), chloroform extract (CU_2), ethyl acetate extract (CU_3) and ethanolic extract (CU_4). Hot aqueous extracts (CU_5) and saponification processes were also used to liberate the free pool of fatty acid compounds; (Palmatic, stearic, Linoleic, α - Linolenic and Oleic acids) .The increasing of concentration of fatty acids was cleared from CU_5→ CU_1 as a result of decreasing of the polarity. In addition to that, the extracts (CU_3, Cu_4 and Cu_5) were carried out by the acid hydrolysis to obtain the free phenolic compounds, which were identified by means of using HPLC technique .The phenolic compounds, which were presented in the seeds of *Cuminum cyminum L.* were: (Apigenin, Gallic acid, Syringic acid, Vanillic acid, Rutin, Quercetin, Kaempferol and P-coumaric acid .Apigenin, Gallic acid and Syringic acid were appeared in the extracts (CU_3, CU_4 and CU_5). Moreover, the Vanillic acid compound was presented in CU3 and CU4, Rutin and Quercetin were also presented in CU_3 and CU_5. Also the Kaempferol, it was presented only in CU_4. Finally, P-Coumaric acid was identified only in CU_5.

Keywords: Cuminum Cyminum, fatty acids, phenolics, saponification.

Introduction

Green cumin (*cuminum cyminum L.*) is native plant grown in the eastern Mediterranean and also in south of Asia and the dried seeds of this plant are used for culinary purposes in other many countries [1]. The meta analysis of data from six randomized controlled trials demonstrate that the consumption of cumin reduces the total plasma and LDL cholesterol levels and increases the density of the plasma lipoprotein (HDL) cholesterol levels in the persons who suffer from overweight, type2 diabetes and non-alcoholic steatohepatitis (0.025-3 gd-1 for 2-6 months) [2].

Cuminum cyminum L. is also called Jeera in India. It belongs to Apiaceae family and it is an annual shrub which can reach up to 10-50 cm in height. It is a native plant that grows in the Mediterranean region and it also exists in Turkistan and

Egypt. Nowadays this plant is widely cultivated in Pakistan, India, Saudi Arabia, United Arab Emirates, Iraq and Syria [3]. Several studies were conducted on a nti-diabetic, hypolipidemic [4] anti-oxidants [5], anti-bacterial [6], anti-fungal [7], anti-epileptic [8], anti-fertility [9], memory enhancing and anti-stress [10].

The study of deriving the chemicals from the plants is called phytochemistry, which is based on the chemical structure and chemical characteristics and there are six major categories of phytochemicals and these categories comprise various nitrogen-containing compounds, carbohydrates, alkaloids, phenolics, lipids and terpenoids are also compounds that exist in plants naturally and they are biologically active, which in turn provide more health benefits to the human compared to macronutrients and micronutrients [11].

Phytochemical analysis exhibited that *C. cyminum* contains anthraquinone, protein, alkaloids, glycoside, steroid, coumarin, saponin, flavonoid, resin and tannin [12]. The volatile oil components like cuminaldehyde, cinnamic acid, safranal beta pinene, carophyllene, alpha pinene, P-cymene thymoquinone, terpineol and flavonoids such as (quercetin, lutein and carotene), vanillic acid and resorcinol which are present in various portions in the plant [13,14].

Cuminum cyminum is recognized for its stringent stimulant, carminative, diuretic and antispasmodic properties [15,16]. Its oil shows a maximum antioxidant activity due to the presence of Linalool, carvacrol, flavonoids, monoterpene alcohols and other polyphenolic compounds [17].The seeds of cumin (*cuminum cyminum L.*) in two geographic regions; Tunisia (TCS) and India (ICS) were studied to identify the compositions of fatty acids and essential oils. Moreover, the contents of the oil were 17.77 and 15.40% for (TCS) and (ICS) respectively, while petroselinic acid (18: 1n-12) was the major fatty acid in both types and the higher proportion was found in TCS (55.90%) of total fatty acids (TFA) compared to ICS (41.42%) TFA and the most predominant fatty acids, were palmatic, petroselenic and linoleic acids that account for more than 91% FTA in both of the types. Moreover, the unsaturated fatty acids content was high (70.95%) TFA in ICS and 62.17% TFA in ICS [18].

Taxonomical Classification [19,20]

Biological name	***Cuminum cyminum***
Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Apiales
Family	Apiaseae
Genus	*Cuminum*
Species	*Cuminum cyminum*

Also the objective of this research is to separate and identify fatty acid compounds and phenolic compounds from the seeds of *Cuminum cuminum L.*

Materials and Methods

Collection of seeds

C. cyminum seeds were collected from Mosul Dam area in May 2021 and they were classified in the Directorate of Medicinal Plants Development Project in Mosul Dar area, which is affiliated to the Ministry of Agriculture and Agricultural Reform. After that, the seeds were cleaned from all the dust and then put in paper bags and stored in a place away from moisture until using them.

Preparation of some plant extracts using continuous Soxhlet device:

The seeds *cuminum cyminum L*. were crushed by electric mill, where 25 gram of it. The well-ground powder was placed in the Soxhlet batch system and 400 ml of petroleum ether was added to the flax seeds extracted oil. The extraction process continued at the rate 7 hours per day until theent used in the device became colorless. In the end, the extract was concentrated using a rotary vacuum evaporator (RVE) [21].
Four solvents were used in the soxhlet device by the sequence solvent system concept; pet-ether (40-60) Cº (CU_1), chloroform (CU_2) Ethyl acetate (CU_3) and Ethanol (CU_4).Hot aqueous extract (CU_5) was employed using Grand method [22].

Saponification process [23]

The researcher took 5 ml of the two types by taking 5 ml of the each crude extracts using the solvents: Pet-ether (40-60) Cº, chloroform, ethyl acetate, ethanol and hot aqueous as crude extracts. Then 10 ml of IM KOH was added.
A reflex for 90 minutes at 100 Cº was used and then 100 ml of d.w was added and 50 ml of ether solvent was put in the separating funnel and then the aqueous layer was taken and concentrated sulfuric acid H_2SO_4 was added until the value of pH became 2. Finally, 50 ml of ether was added and put again in the separating funnel. Then the organic layer, which contains the free fatty acid compounds was obtained.

Identification of the fatty acids by using GLC analysis

The separated fatty acids were identified in the laboratories of the Ministry of Science and Technology / Dept. of Environment and Water by GLC model (Shimonezo, Japanese, 2010), using ionized flame detector and using the poetic column type (SE-30), with a length of (30 m), with different diameters (0.25mm, 0.5 mm). Also, the temperature values in the injection area and the detector were (280 Cº and 330 Cº), while the column temperature starts from (120-280 Cº) in the rate of 8 Cº/min. using the passive nitrogen gas as a carrier at the pressure rate of 100 Kp.

Phenols separation and purification using acid hydrolysis

We took 5 ml of the crude extracts of ethyl acetate, ethanol and hot aqueous separately, then 25 ml of IN (HCl) was added. After that the reflux was performed at a temperature of 100 Cº for one hour and the solution is cooled down and placed in a separating funnel and 50 ml of ethyl acetate is added twice with continuous shaking. Both the upper layer of the organic ethyl acetate and the bottom layer were obtained. The upper layer was taken and 3 g of $MgSo_4$ was added to it. Samples were kept in

well-sealed and opaque bottles and then kept in the refrigerator until they were identified by HPLC device [24].

Since the phenolic compounds were identified by depending on the area of separated compound and as the percentage ratio of the compound or they were converted to concentrations according to the previously approved equation [25].

Identification of the phenolic compounds using HPLC-UV device

The identification of the phenolic compounds was conducted in the laboratories of the Ministry of Science and Technology / Dept. of Environment and Water Resources after performing the acid hydrolysis process, according to the method conducted before [26]. By using high performance liquid chromatography device (HPLC) type Sykamn of German origin with a flow rate of 1.3 ($ml.min^{-1}$). The mobile phase is (A), which includes (methanol: D-W: formic acid, (70:25:5) with the column (18-ODS) has dimensions (25 cm*4.6 mm) and the responses were detected at the UV-280 nm wavelength.

Results and Discussions

The identification of fatty acid compounds of *cuminum cyminum L*. seeds by using GLC analysis.

The identification of the Fatty acids in the pet-ether (CU_1), chloroform (CU_2), ethyl acetate (CU_3), ethanol (CU_4) and hot aqueous (CU_5) extracts after saponification process and five fatty acid compounds were identified (palmatic, stearic, linoleic, α-linoleic acids and Oleic acid), table (1) shows that the concentration of the palmatic acid is (2.15%) and the highest concentration in the pet.ether extract (CU_1) due to the non-polarity of this solvent and this shows the reason behind its high concentration. The lowest concentration n of this extract was (0.89%) in the ethanolic extract (CU_4). Moreover, the stearic acid compound was presented in pet.ether with (0.68%) as the highest concentration while presented with (0.25%) as the lowest concentration in (CU_4) extract. Also, the linoleic acid was found at (6.55%) with the highest concentration and (1.59%) at the lowest concentration also the α- linoleic acid was found at (19.58%) with the highest concentration and (9.26%) at the lowest concentration. Finally, Oleic acid was found at (20.99%) with the highest concentration and the lowest concentration was found at (10.56%) in the ethanolic extract (CU_4).

This result is in conformity with the study of [27], which indicated that the sequence of solvents system in the extraction showed the same result.

A chromatographic chart was obtained that showed that the retention time for each compound was determined by using a standard sample of fatty acid compound figures (1,2,3,4,5,6,7,8,9,10).

Identification of number of phenolic natural compounds using HPLC technique in *C. cyminum L*. in terms of quality and quantity.

The chart of analysis obtained shows that the retention time of each sample was obtained and compared with standard. The time for Apignin was (6.60 min), Gallic ac-

id (5.150 min), Syringic acid (3.28 min), , Vanillic acid (2.980 min), Rutin (8.00 min), Quercetin (11.02 min), Kaempferol (7.75 min) and P-couramic acid (4.44 min). table (2) and the figures (11,12,13,14,15,16,17,18,19,20,21). This indicates the presence of the phenolic compounds: in the seeds of *cuminum cyminum L*.

The Apigenin; the crystalline solid is a hexagonal ring in which there are three of hydroxyl groups and its molecular formula is ($C_{15}H_{10}O_5$), a secondary metabolic natural compound in this plant. Also, it is found in the seeds and it is a cure for several diseases such as cardiovascular diseases and some conces as it acts as an antioxidant [28]. It is also considered as a vital to treat Alzheimer's disease and it reduces the effects of bacteria, viruses and fungi [29].

Apigenin was showed in three extracts (CU_3, CU_4 and CU_5) after conducting the process of acid hydrolysis. The concentrations of Apigenin were (0.0064, 0.0054 and 0.0056). As for the Gallic acid the values were (min5.15), (3,4, 5-trihydroxy benzoic acid) is a cyclic aromatic compound containing for hydroxyl groups and its molecular formula is ($C_7H_6O_5$) has been found in various plants and was also present in three extracts (CU_3, CU_4 and CU_5) with the concentrations (0.0260, 0.0411 and 0.0358 mg/g). Syringic acid was also detected in the extracts with concentrations (0.0912, 0.0979 and 0.0402 mg/g), Vanillic acid was presented in the extracts (CU_3 and CU_4) with concentrations (0.095 and 0.126 mg/g).

Rutin was present at 8.00 min. and with concentrations of (0.048 mg/g in CU_3 and was not detected in CU_4, while it was present at 0.056 mg/g in CU_5.

Quercetin is a compound of flavonoids and it contains five groups of hydroxyl and its molecular formula is ($C_{15}H_{10}O_7$) with a retention time of (11.02 min.). it was present only in two extracts (CU_3 and CU_5) with concentrations (0.0090 and 0.0072 mg/g).

Kaempferol was detected in the ethanolic extract (CU_4) only with a concentration of (0.0182 mg/g). Finally, P-coumoric acid (4.44 min.) was detected in the hot aqueous extract (CU_5) at the concentration (0.0296 mg/g) and it was not detected in CU_3 and CU_4.

Table (1): The percentage ratio of concentration of fatty acid compounds presented in various extracts of *C. cyminum* seeds

Name %	CU_1	CU_2	CU_3	CU_4	CU_5
Palmatic	2.15	2.05	1.84	0.89	0.98
Stearic	0.68	`0.56	0.36	0.25	0.30
Linoleic	6.55	4.58	4.00	1.59	1.65
α-Linolenic	19.58	14.58	12.58	9.26	10.11
Oleic	20.99	16.22	13.66	10.56	10.98

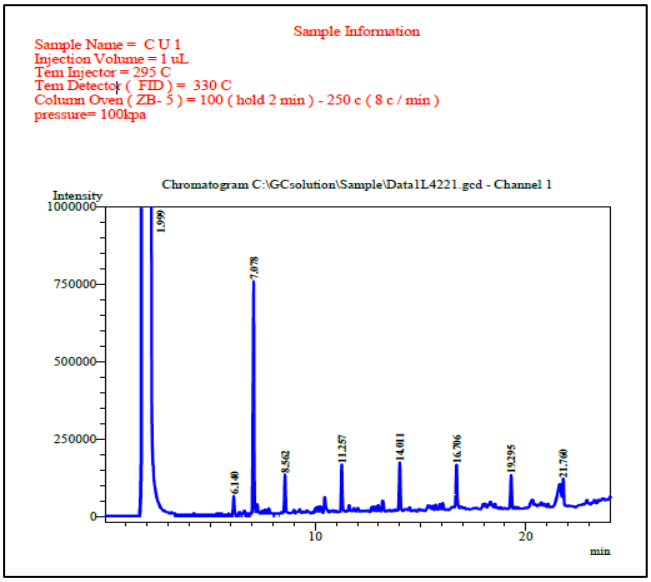

Figure (1): The fatty acid compounds from the saponified pet.ether extract (CU_1)

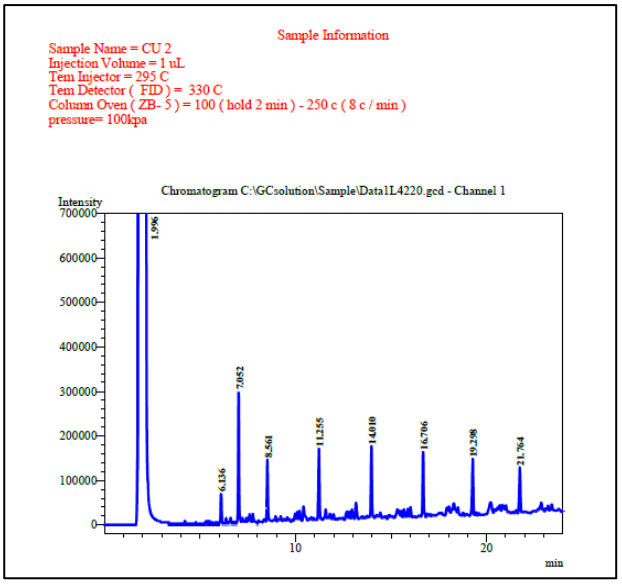

Figure (2): The fatty acid compounds from the saponified chloroform extract (CU_2)

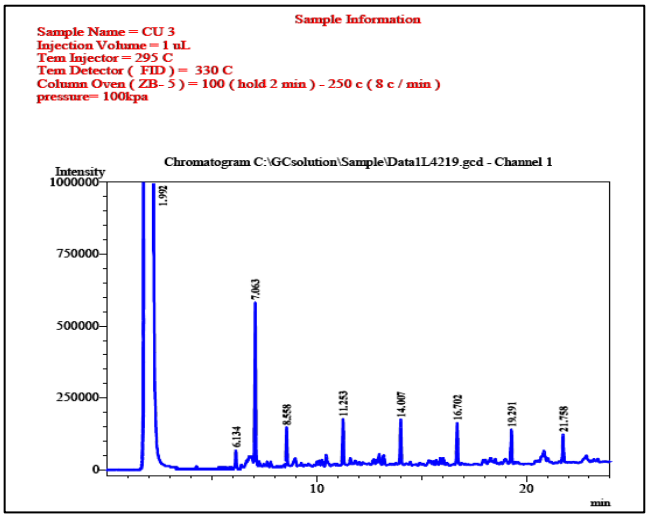

Figure (3): The fatty acid compounds from the saponified acetate extract (CU_3)

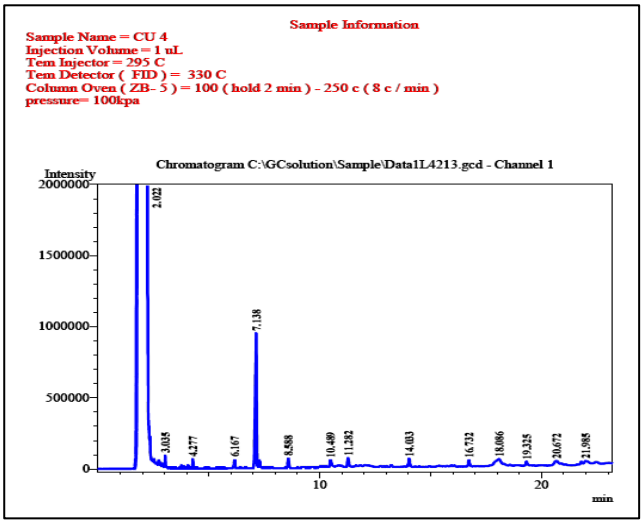

Figure (4): The fatty acid compounds from the saponified ethanol extract (CU_4)

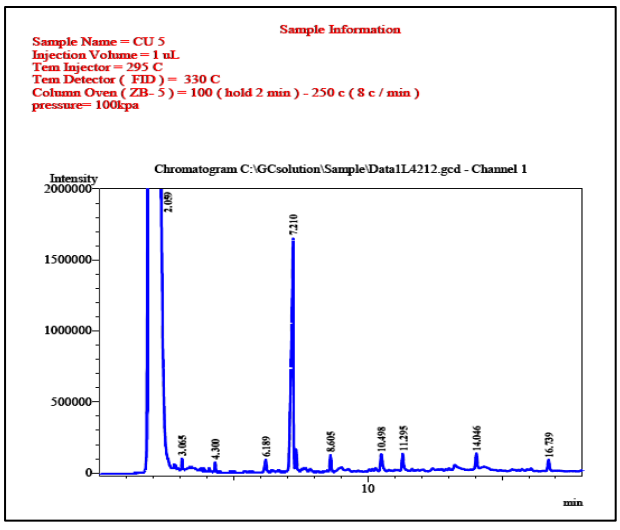

Figure (5): The fatty acid compounds from the saponified Hot aqueous extract (CU_5)

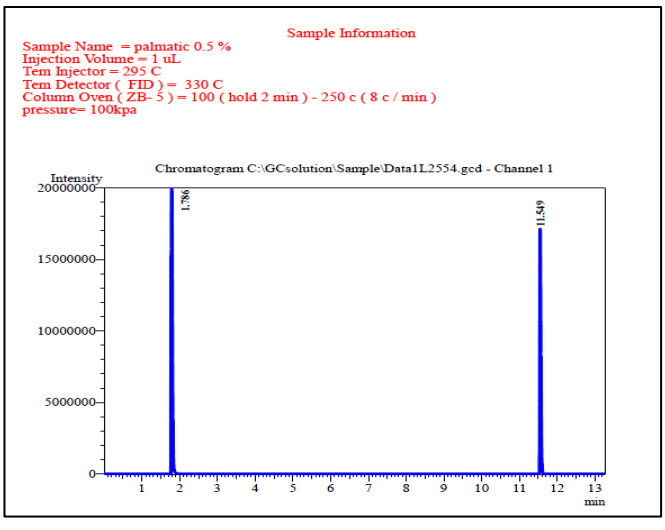

Figure (6): The standard curve of palmitic acid

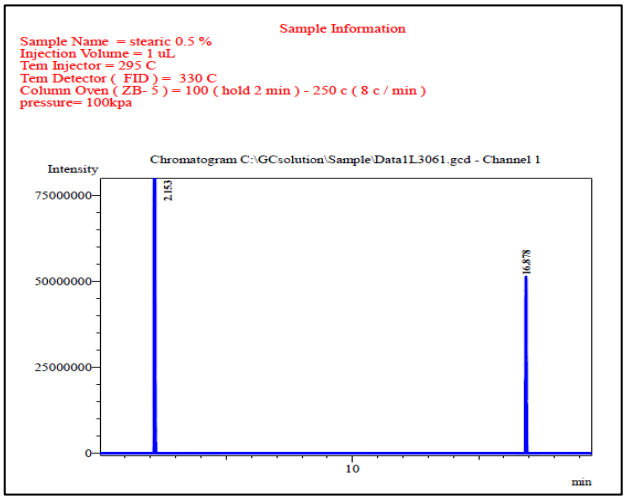

Figure (7): The standard curve of stearic acid

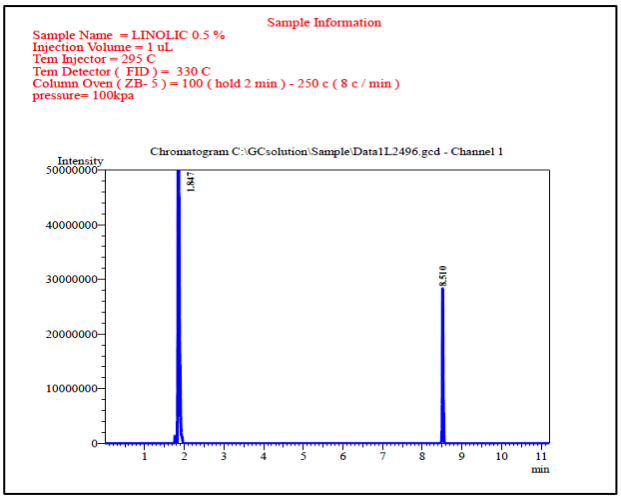

Figure (8): The standard curve of Linolic acid

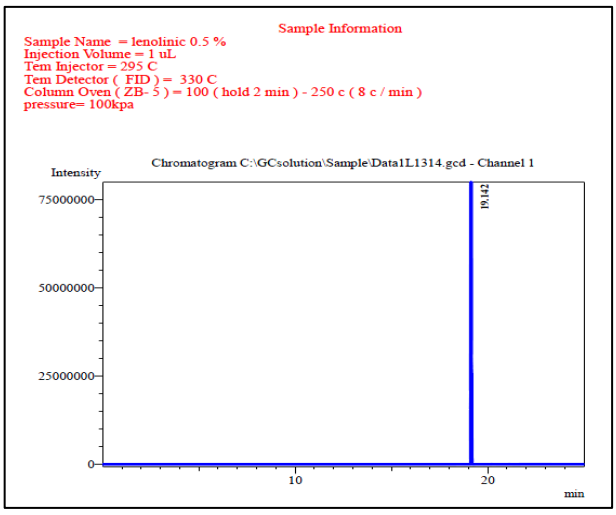

Figure (9): The standard curve of α-Linolinic acid

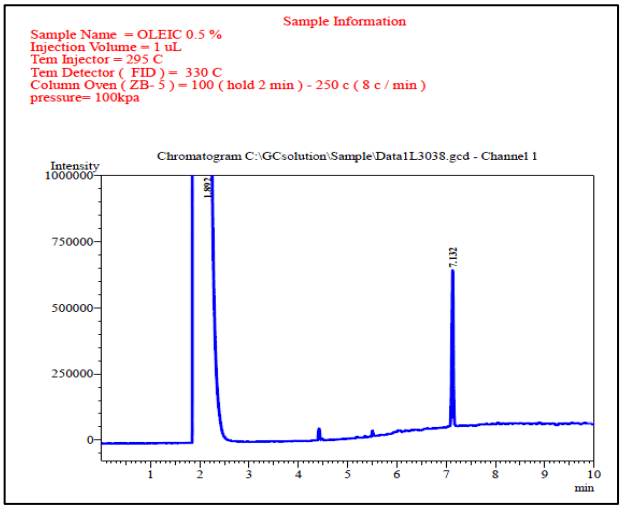

Figure (10): The standard curve of oleic acid

Table (2): Indicates the standard retention times and the concentration of some phenolic compounds by using HPLC technique of *C. cyminum*.

No.	Standard phenolic compounds	Standard retention times	Ethyl acetate extract CU3		Ethanolic extract CU4		Ho aqueous extract CU5	
			Cons. mg/g	Rt. Min.	Cons. mg/g	Rt. Min.	Cons. mg/g	Rt. Min.
1	**Apigenin**	6.60	0.006488	6.77	0.00548	6.55	0.00568	6.60
2	**Gllic acid**	5.150	0.026072	5.26	0.041112	5.32	0.0358	5.70
3	**Syringic acid**	3.28	0.091216	3.15	0.097944	3.10	0.040208	3.20
4	**Vanillic acid**	2.980	0.095184	2.88	0.126392	2.82	------	------
5	**Rutin**	8.00	0.048888	8.19	------	------	0.056544	8.10
6	**Quercetin**	11.02	0.00908	11.05	------	------	0.007256	11.00
7	**Kaempferol**	7.75	------	------	0.018224	7.72	------	------
8	**P-coumaric acid**	4.44	------	------	------	------	0.029616	4.79

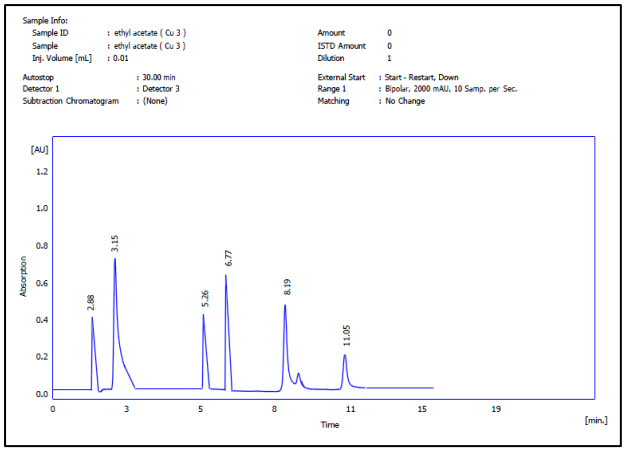

Figure (11): The phenolic compounds from the ethyl acetate extract (CU_3)

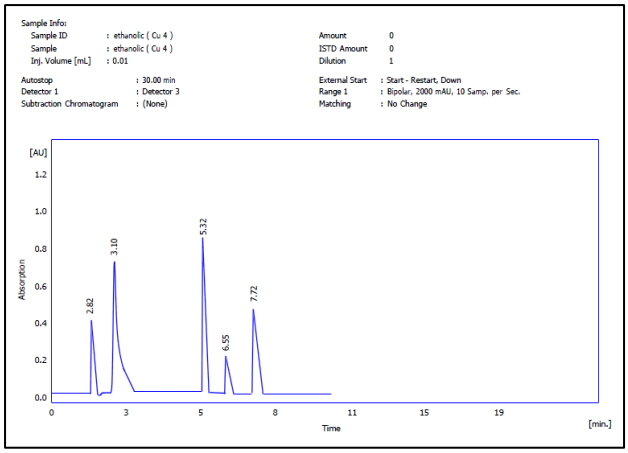

Figure (12): The phenolic compounds from the acid hydrolysis ethanolic extract(CU_4)

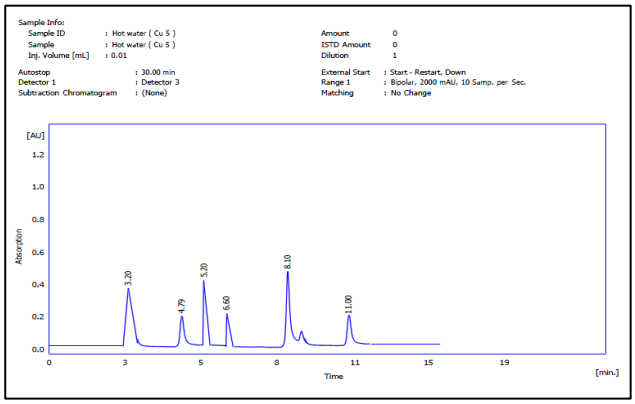

Figure (13): The phenolic compounds from the acid hydrolysis Hot water(CU_4)

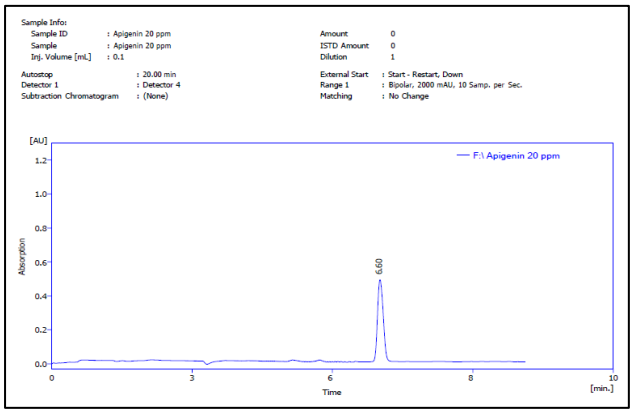

Figure (14): The standard curve of Apigenin

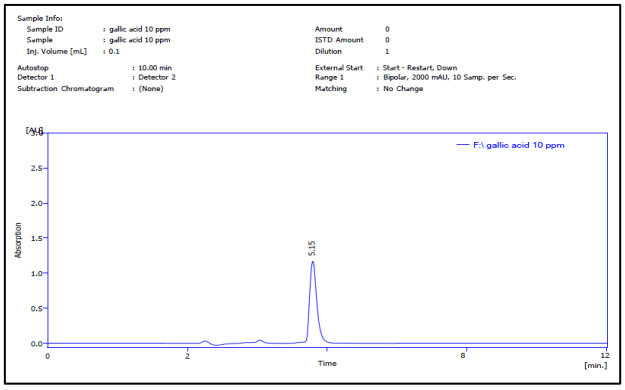

Figure (15): The standard curve of gallic acid

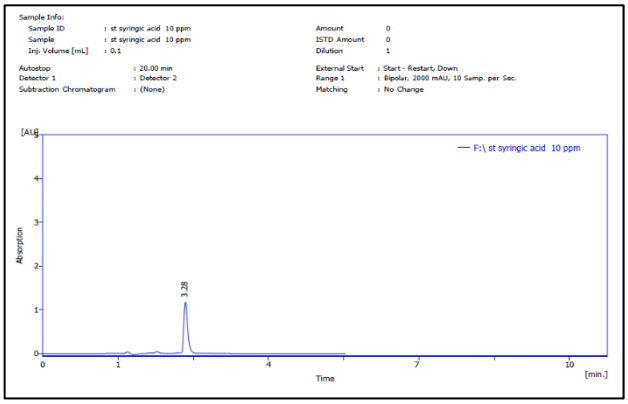

Figure (16): The standard curve of syringic

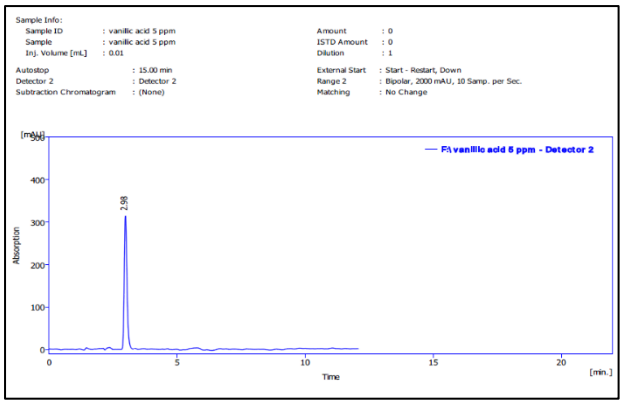

Figure (17): The standard curve of vanillic acid

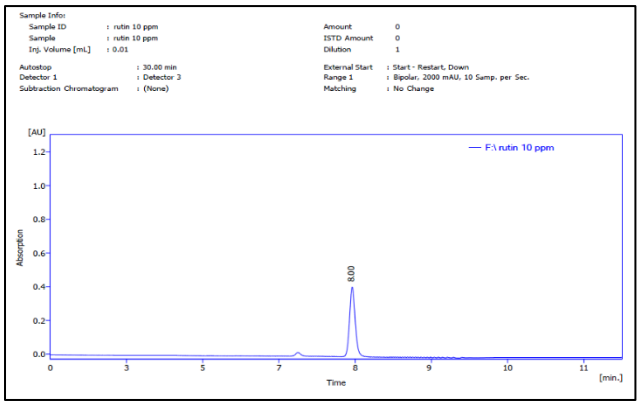

Figure (18): The standard curve of rutin acid

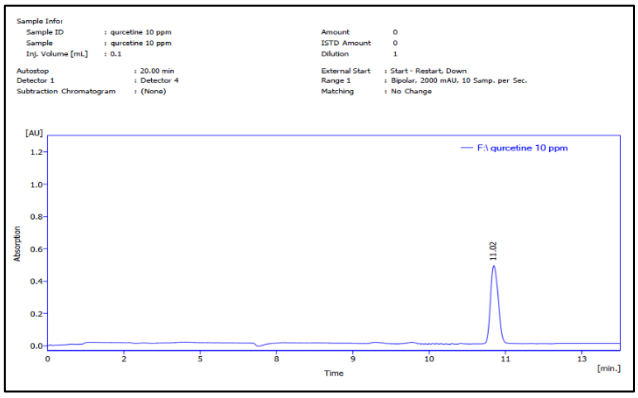

Figure (19): The standard curve of qurcetine acid

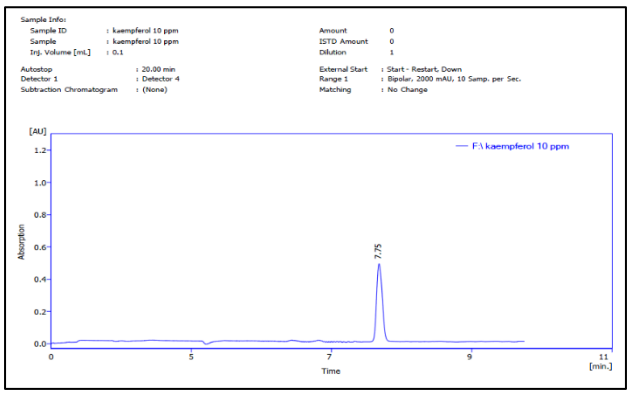

Figure (20): The standard curve of kaempferol acid

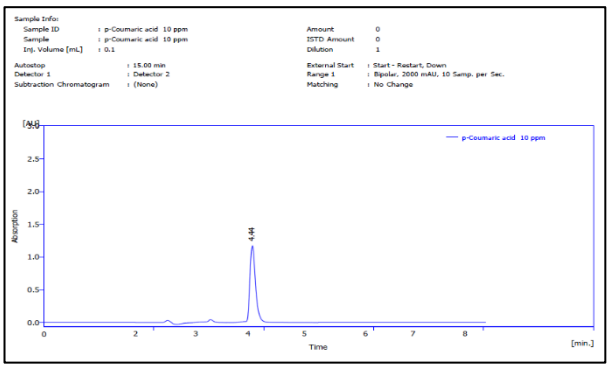

Figure (21): The standard curve of P-coumaric acid

From the results which have been obtained, it is confirmed that *C. cyminum* seeds were among the seeds of plants, which are rich with fatty acids and phenolic compounds because the seeds involve materials that belong to the secondary metabolism natural compounds.

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