

Detection of Some Fatty Acids and Phenolic Compounds in the Seeds of Plant in Coriander (*Coriandrum Sativum* L.) Seeds Cultivated in Iraq

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ABSTRACT

Coriander sativum (Linn) is an important medicinal plant belonging to the family of Apiaceae. It is a well-recognized plant in the traditional medicine and used by people in treatment of gastrointestinal problems and rheumatism. In the present investigation, after phytochemical screening of the seeds of coriander results revealed the presence of four fatty acid compounds (Lauric, Palmatic, Stearic and Linoleic acids) in the Pet-ether extract, while two fatty acids (Palmatic and Stearic acids) were found in the Chloroform extract. Phenolic compounds and their fractions Quercetin and Benzoic extract were also identified in the Ethyl acetate.

Also, Quercetin was identified in the extracts of (F2) and (F3), Moreover, IMS extract was contained of high concentration of Gallic acid while Quercetin were identified the fractions (F4, F5 and F6) of IMS extract. Other phenolic compounds were presented as low concentrations in other extracts.

Keywords: fatty acid compounds, phenolic compounds.

التحري عن بعض الاحماض الدهنية والمركبات الفينولية في بذور نبات الكزبرة المزروعة في العراق

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المخلص

يعد نبات الكزبرة نباتاً طبيياً مهماً يعود إلى العائلة الخيمية ومعروف استعماله في الوسط التجاري والشعبي وفي علاج مشكلات الجهاز الهضمي والروماتيزم. دراستنا الحالية حققت بعد الكشف عن احتواء بذور نبات الكزبرة على أربع أحماض دهنية وهي اللوريك والستياريك واللينولك والبالمتيك ، اذ وجد حامض (اللوريك، الستياريك، واللينولك) في مستخلص الأثير البترولي بينما وجد حامض (البالمتك والستياريك) في مستخلص الكلوروفورم.

كذلك شخّصت المركبات الفينولية مستخلص خلات الاثيل وأجزائه، وأعلى تركيز كان للكورسيتين وحامض البنزويك في مستخلص خلات الاثيل ، كذلك الكورسيتين تم تشخيصه في المستخلصات (F2) وال (F3). وان مستخلص ال IMS احتوى على تركيز عالي من بارا هيدروكسي حامض البنزويك بين المركبات الفينولية المشخصة للنبات، فيما احتوى مستخلص ال IMS واجزائه (F4 ، F5 ، وال F6) على مستخلص الهيدروكينون بينما المركبات الفينولية الأخرى وجدت بتراكيز قليلة في المستخلصات الأخرى. في حين وجد حامض الكالكيك في كلا المستخلصين واجزائهما وبتراكيز مختلفة وزمن احتجاز متقارب جدا.

الكلمات الدالة: الاحماض الدهنية، المركبات الفينولية، نبات الكزبرة.

1. Introduction

Coriander (*Coriandrum sativum* L.) is an annual Apiaceae herb [1]. Recent studies have also demonstrated a hypoglycaemic action and effects on carbohydrate metabolism [2]. It has also been reported the antimicrobial effect of coriander leaves and seeds against several microorganism [3-4].

The yield and chemical composition of coriander seed essential oil varies both qualitatively and quantitatively in relation to the method of extraction type of the cultivar and the area of harvest [5]. Coriander seed essential oil is mainly composed of linalool, together with some other oxygenated monoterpenes and monoterpene hydrocarbons. The phenolic compounds, apigenin, catechin, coumaric acid, aliphatic alkenals and alkanal were reported in *Coriandrum sativum* of aerial parts [6, 7]. While linalool, geranyl acetate, and petroselinic acid were found in the fruits [8]. The fruits contain vegetable oil with a high concentration of monounsaturated fatty acids especially of petroselinic acid [9].

Moreover, multiple extracts of different polarity from leaves and seeds of coriander (*Coriandrum sativum*) and coriander oil were investigated for their antioxidant activity, total phenolic content was also quantified as well [2]. Also, coriander seed oil methyl esters as biodiesel fuel was investigated and contained an usual fatty acids hitherto unreported as the principle component in biodiesel fuels petroselinic acid and the remaining fatty acid profile consisted of common 18 carbon constituents such as Linoleic, Oleic and also Stearic acid [9].

2. Material and Methods:

2.1 Sample preparation:

Coriandrum sativum L., seeds packed bags was acquired from a local supermarket in Mosul-Iraq and also classified by Dr. Amer Mohson (Assist Prof. in Taxonomy of the Plants in Iraq-Mosul University, Biology Dept.).

2.2 Preparation of plant extracts:

A batch of 25 gm of coriander grinded seeds was soxhleted for 6-8 hrs with 500 ml of four solvents [petroleum-ether 60-80°C chloroform, Ethyl acetate and also IMS (95% abs-Etoh & 5% Meoh). All there extracts were concentrated to 20 ml, using a

vacuum rotary evaporator at 50°C. The crude of each extract was kept in Deep freeze till us it for further studies.

2.3 Alkaline hydrolysis (Saponification) of pet-ether (60-80)°C and chloroform extracts [11]:

Pet-ether and Chloroform extracts as resulted from preparation of plant extracts, 10 ml of each extracts were added to 100 ml of 7.5 M of solution of KOH in Methanol: water (3:2) and refluxed in a round bottom flask for 90 min at 100°C. The mixture was allowed to cool at room temperature and 50 ml of distilled water added. The crude was extruded with diethyl ether (2×25 ml) to remove unhydrolyzed lipid. The aqueous layer was acidified with 10% H₂SO₄, up to PH=2. The fatty acids were extracted with diethyl ether (2×25 ml). Drying of two combined extracts with anhydrous sodium sulfate, filtered and concentrated in vacuo to give 1.92 gm of saponified pet-ether and 2.70 gm of saponified chloroform extracts respectively.

2.4 Esterification of fatty acid compounds:

To prepare of methyl esters, 0.5 ml of acetyl chloride was added to 125 ml of methanol with stirring. A sample of 2 ml of dry fatty acids was added to above mixture then boiled under reflux in water bath for 30 min, cooled then and for GLC . analysis [12].

2.5 GLC Analysis:

The esters were analyzed by using GLC, a type of (Shimadzo-14A) equipped with a denl flame ionization detectors held at 250°C A (3m × 1.8 inch) international diameter column packed with 3% SE-30 on Teflon (100-120 ml) was held at 100°C initially then raised at 5°C/min to 250°C. Samples of (1ml) was used for injection. The identification of fatty acids were determined by reference to a standard of a known composition [14].

2.6 Extraction of phenolic compounds from ethyl acetate and IMS extracts and fractions:

After defatted of coriander seeds, using pet-ether and chloroform, seed were-extracted with IMS to get the IMS extracts and their fractions which were obtained by using column chromatography (CC) of each extracts, ethyl acetate & IMS, using different solvents to get three fractions from Ethyl acetate (F₁, F₂, F₃) and also three fractions from IMS (F₄, F₅, F₆) using silica gel (60-120 mesh) as packing material. Also acid hydrolyses was carried out on ethyl acetate, IMS and also on there obtained fractions using 1N HCl and reflux for 30 min. After cooling the mixture, liberated free phenolic compounds were dissolving in ethyl acetate (2×10 ml), which also concentrated to 1 ml, using rotary vacuum evaporator [13].

HPLC – analysis:

Free phenolic compounds were investigated by HPLC instrument, type (Shimadzo, LC 2010 AHT), after purification with filters (0.1 μm) with 320 nm as wave length, with (Acetonitrile:H₂O, 85-15%) as mobile phase using a column C18 (4×240 mm) at 30°C and the runs carried out at Education college in Mosul university, identification of these phenolic compounds was carried out by using standard compounds as 0.1 gm was dissolved in 10 ml ethanol [14]. Samples were injected on to the HPLC bed manually with injection volume as 20 ml.

To identify the peaks, the spectral patterns and retention time of the samples were compared with standard. Phenolic compounds were quantified by comparing the peak areas and retention times obtained for the extracted sample with the peak areas and retention times of appropriate standards of phenolic compounds.

The content of phenolic compounds in the samples was calculated, using the following equation [15],

$$\text{Phenolic compound} \left(\frac{\text{mg}}{\text{g}} \right) = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{standard conc.} \left(\frac{\text{mg}}{\text{ml}} \right) \times \frac{\text{Ext. volume (ml)}}{\text{Dry weighting (gm)}}$$

3. Results and Discussion:

1- Chromatographic identification of fatty acid compounds presented in pet-ether & chloroform extracts using GLC – analysis. Petroselinic acid C₁₈:1n-12 was the fatty acid marker in edible plant glycolipid (GL) substances obtained from coriander seed oil, followed by linoleic acid C₁₈:2n-6 [16].

From this study, fatty acid compounds were identified from saponified of Pet-ether extract of the coriander seeds as following, Linoleic acid (0.00026), Stearic acid (0.002), Palmitic acid (0.0014) and also Lauric acid (0.0004). The above result was differed from the saponified chloroform extract which gave Plamitic acid (0.0082) and also Steraric acid (0.003). **Table (1)** and **Fig. (1,2,3,4,5,6,7,8,9)**.

Table (1): concentration (%) and Values of retention times of many fatty acids for pet-ether and chloroform extracts from coriander seeds comparing with standard compounds, using GLC – instrument.

Fatty acid compounds (Rt min)	Heptanoic acid 4.65		Octanoic acid 6.72		Lauric acid 12.45		Palmitic acid 13.96		Stearic acid 14.45		Oleic acid 15.39		Linoleic acid 15.64	
	R _t	Conc. (%)	R _t	Conc. (%)	R _t	Conc. (%)	R _t	Conc. (%)	R _t	Conc. (%)	R _t	Conc. (%)	R _t	Conc. (%)
Pet-ether					11.88	0.0004	13.78	0.0043	14.17	0.002			15.76	0.0026
Chloroform							13.88	0.0082	15.75	0.003				

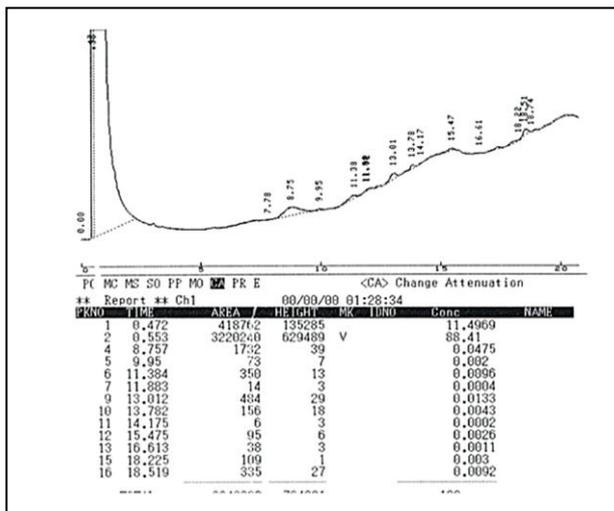


Fig. (1): Petroleum ether.

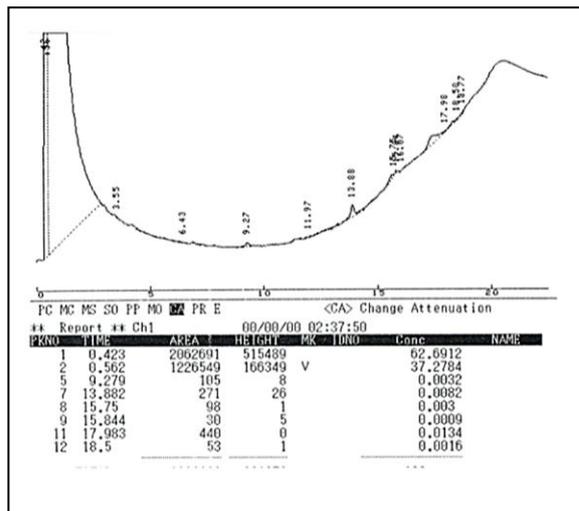


Fig. (2): Chloroform.

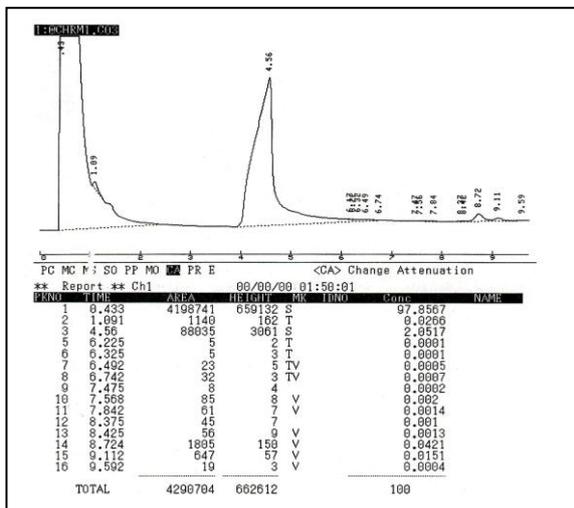


Fig. (3): Heptanoic acid

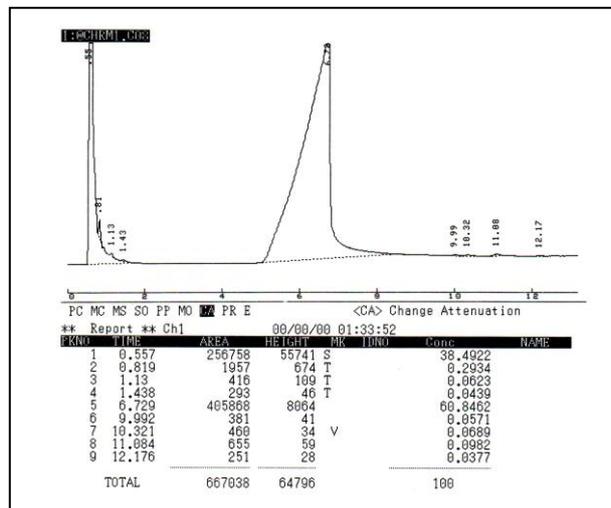


Fig. (4): Octanoic acid

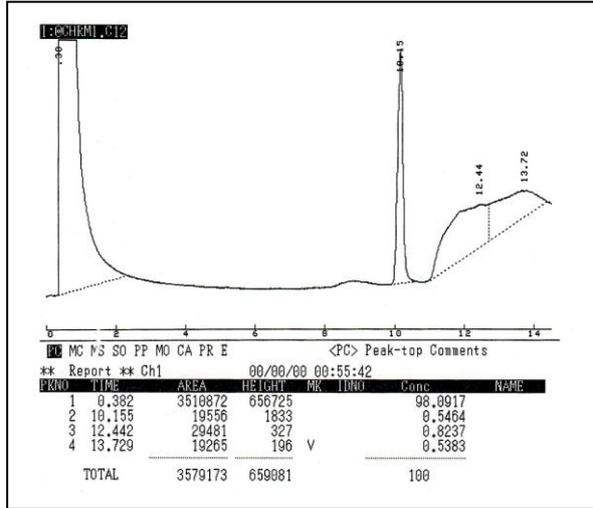


Fig. (5): Lauric acid

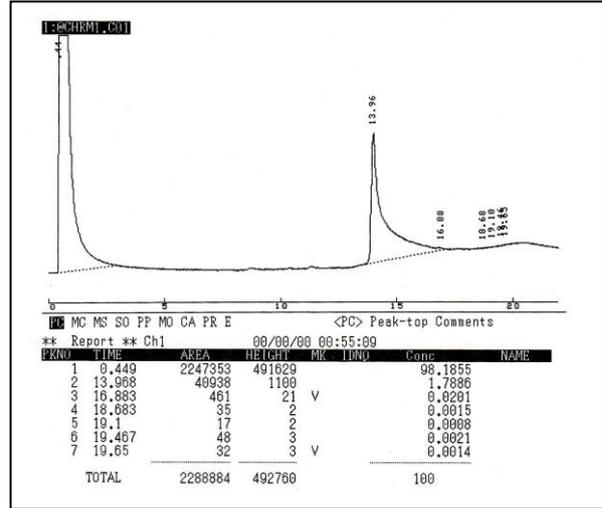


Fig. (6): Palmitic acid

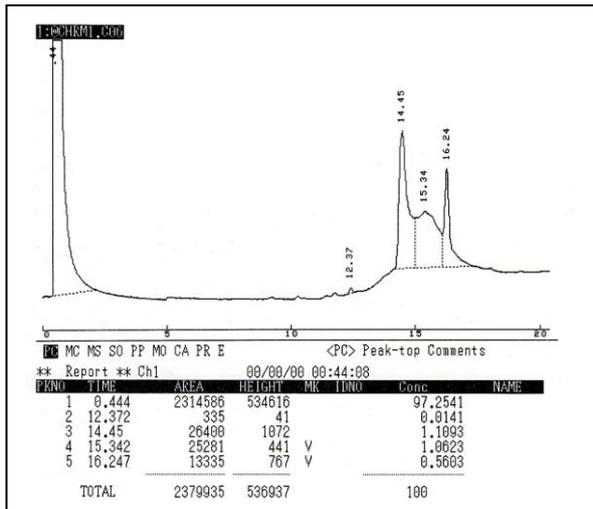


Fig. (7): Stearic acid

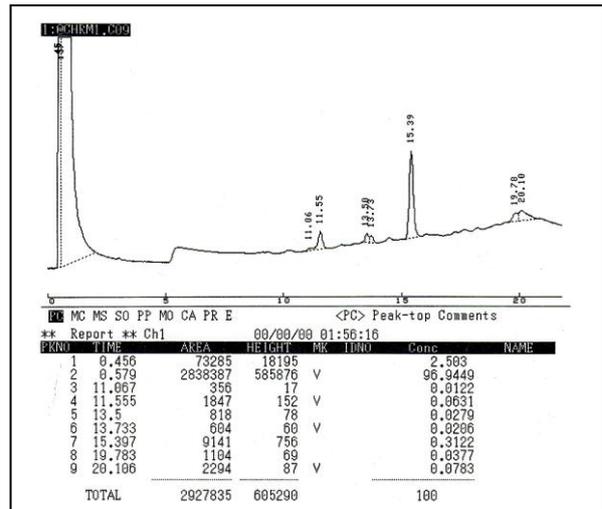


Fig. (8): Oleic acid

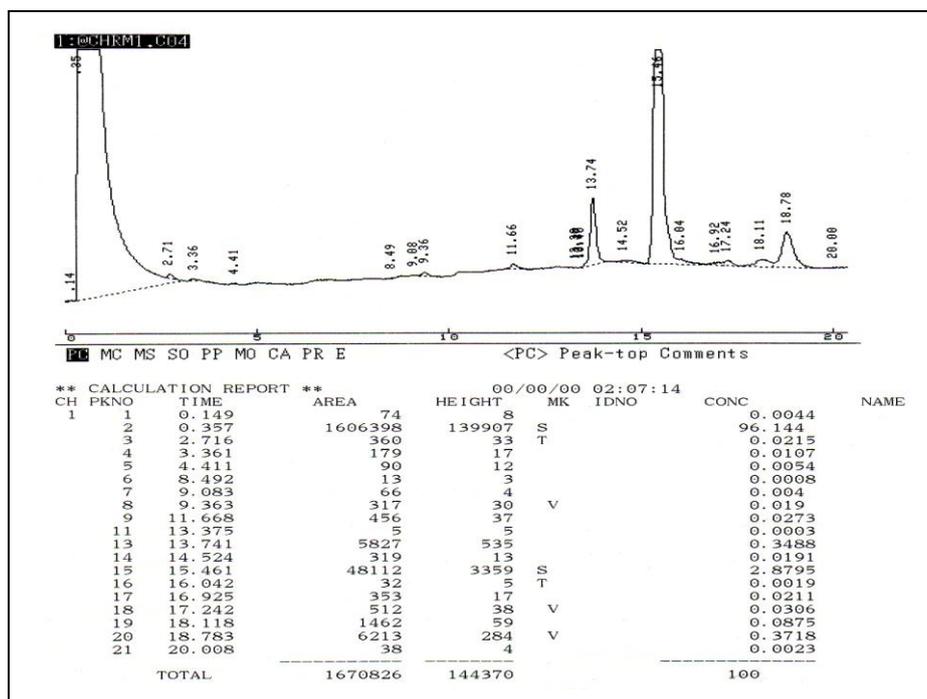


Fig. (9): Lenolic acid.

2- Chromatographic identification of phenolic compounds presented in ethyl acetate, IMS extracts and their fractions, using HPLC – analysis. It was known that coriander seeds had very effective antioxidant profile showing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical Scavenging activity due to its high total phenolic content [17], and from HPLC – analysis of ethyl acetate extract, we identified of the different phenolic compounds, Quercetin (4.572 mg/g), Benzoic acid (2.563 mg/g), Phenol (0.030 mg/g) and also Gallic acid (0.015 mg/g).

The fractions of Ethyl acetate (F₁, F₂, F₃) were also contained the phenolic compounds, the (F₁) fraction was identified four phenolic, P- Hydroxybenzoic acid (2.847 mg/g), Benzoic acid (2.760 mg/g), Hydroquinone (1.848 mg/g) and Gallic acid (0.019 mg/g), while the fraction (F₂) was identified three phenolic compounds, Quercetin (3.150 mg/g), Gallic acid (0.839 mg/g) and Resorcinol (0.034 mg/g).

Also, the fraction (F₃) was identified of four similar compounds, Quercetin (2.701 mg/g), Benzoic acid (2.145 mg/g), Phenol (0.141 mg/g) and Gallic acid (0.018 mg/g). From the above results we showed that Quercetin was the main compound of high concentration in the two fractions (F₂ & F₃). **Table (2)** and **Figs. (10-17)**.

Table (2): Values retention times (min.) and concentration (mg/gm) of many phenolic compounds from ethyl acetate (IMS) extracts and their fractions from coriander seeds with standard compounds, using HPLC analysis.

Standard phenolic	Phenol		Resorcinol		Hydroquinone		Quercetin		Salicylic acid		p-Hydroxybenzoic acid		Benzoic acid		Gallic acid	
Retention times R _t (min.)	2.372		2.726		2.833		3.028		3.062		3.200		3.645		4.240	
Extracts and fractions	R _t	Conc. mg/g	R _t	Conc. mg/g	R _t	Conc. mg/g	R _t	Conc. mg/g								
Ethyl acetate	2.117	0.030					3.014	4.572					3.570	2.563	4.471	0.015
F1					2.943	1.848					3.281	2.847	3.485	2.760	4.366	0.019
F2			2.681	0.034			3.023	3.150							4.163	0.839
F3	2.584	0.141					2.928	2.701					3.499	2.145	4.362	0.018
IMPS					2.933	2.972							3.460	0.048	4.363	3.053
F4	2.423	2.720			2.921	1.926					3.459	6.271			4.3455	0.021
F5	2.093	0.055			2.933	1.930							3.455	3.120	4.145	1.826
F6	2.144	0.074			2.919	2.281							3.458	2.980	4.355	0.038

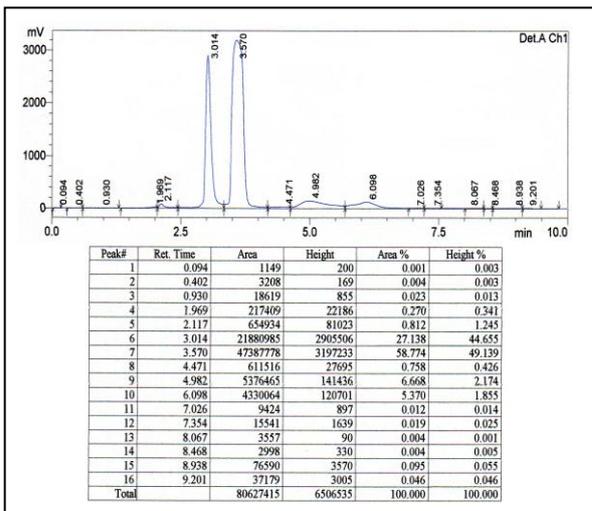


Fig. (10):

(Extract Ethyl acetate)

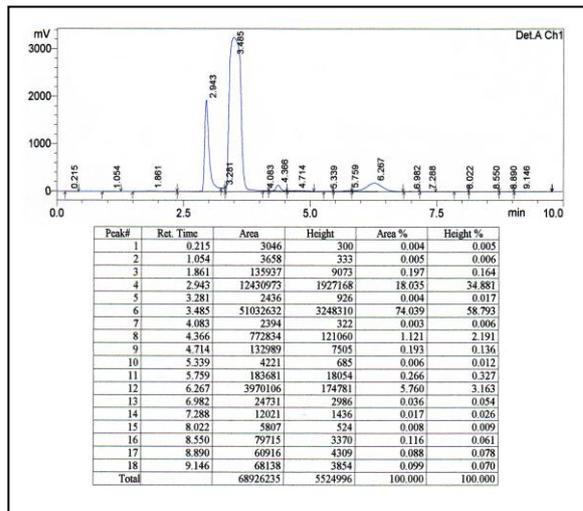


Fig. (11): F1

(fraction (1) of Ethyl acetate)

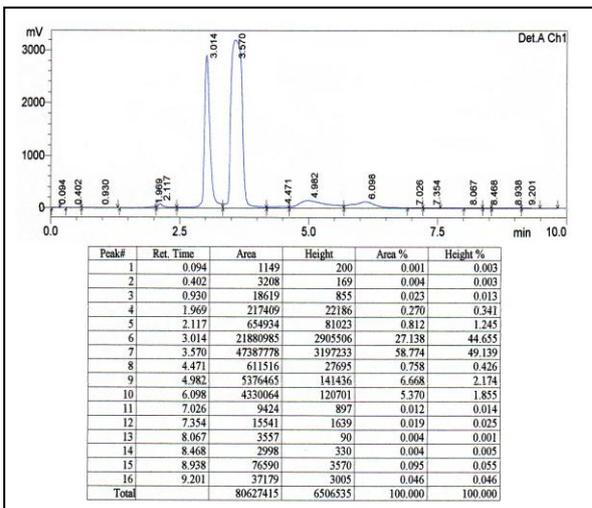


Fig. (12): F2

(fraction (2) of Ethyl acetate)

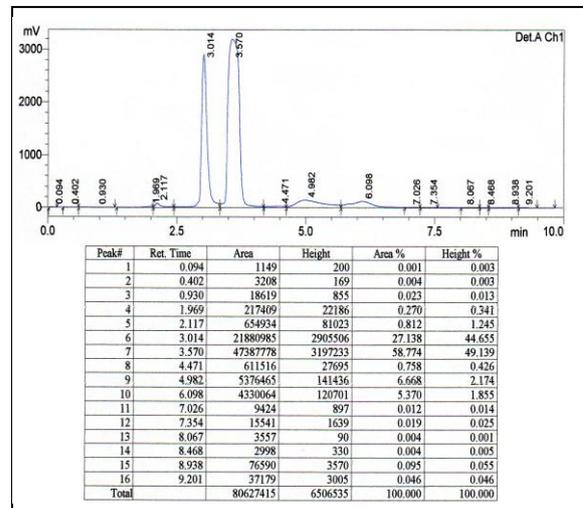


Fig. (13): F3

(fraction (3) of Ethyl acetate)

The IMS extract was also identified of three phenolic compounds; Gallic acid (3.053 mg/g), Hydroquinone (2.972 mg/g) and Benzoic acid (0.048 mg/g). The fractions of IMS extract (F4, F5, F6) were also contained phenolic compounds; the fraction (F4) was identified of four compounds; P-Hydroxybenzoic acid (6.271 mg/g), Phenol (2.720 mg/g), Hydroquinone (1.926 mg/g) and Gallic acid (0.021 mg/g), while the fraction (F5) was identified of four compounds; Benzoic acid (3.120 mg/g), Hydroquinone (1.930 mg/g), Gallic acid (1.826 mg/g) and Phenol (0.055 mg/g).

Also, the fraction (F6) was identified of four phenolic compounds; Benzoic acid (2.980 mg/g), Hydroquinone (2.281 mg/g), Phenol (0.074 mg/g) and Gallic acid (0.0038 mg/g). From the above result, we noticed the absence of Quercetin from the IMS extracts and their fractions while Hydroquinone was the main compound that presented and also identified of these mentioned extracts. Table (2) and Figs. (18-25).

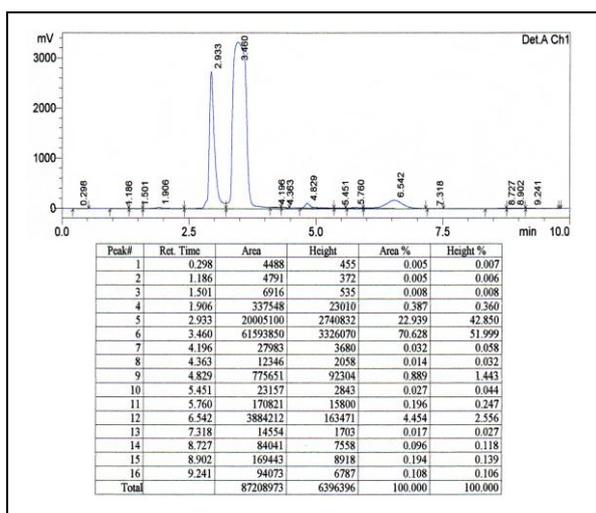


Fig. (14): extract IMS

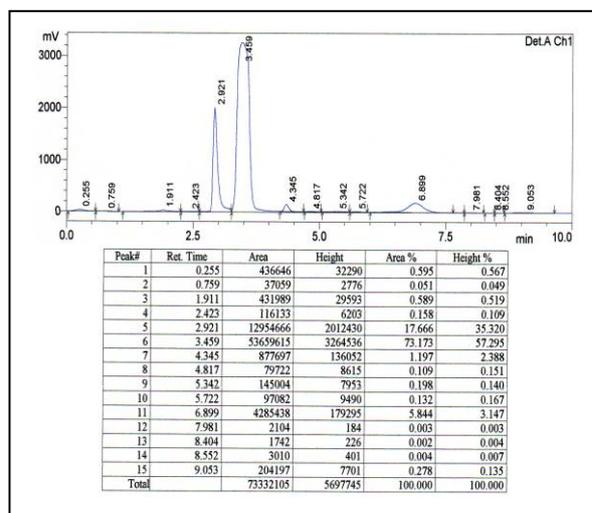


Fig. (15): F4

Fraction (F4) for extract IMS

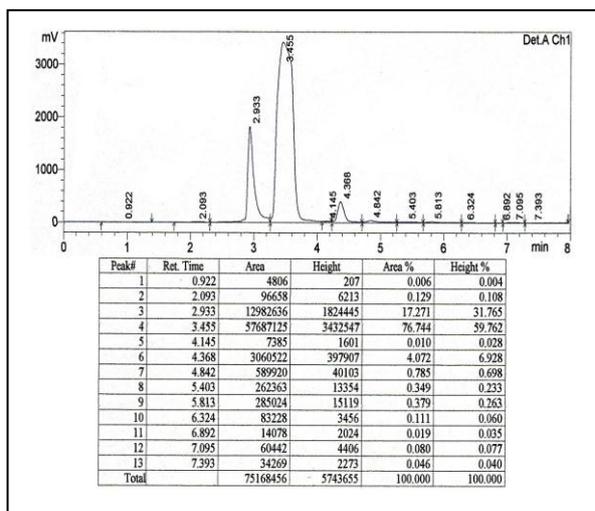


Fig. (16): F5

Fraction (7) for extract IMS

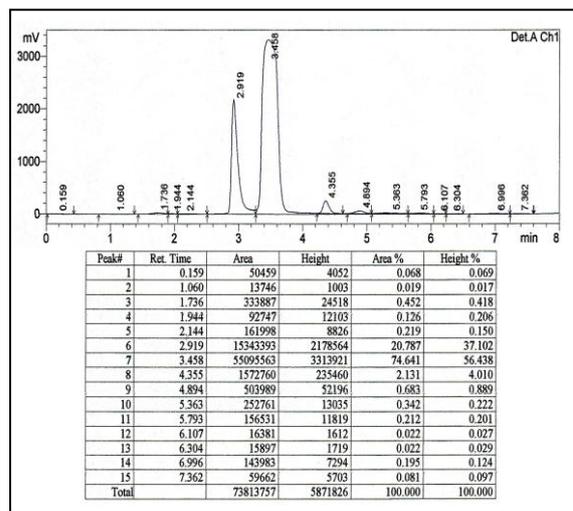


Fig. (17): F6

Fraction (6) for extract IMS

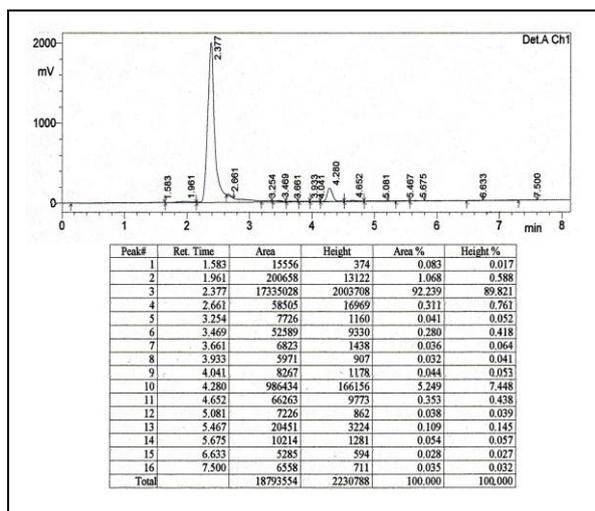


Fig. (18): Phenol

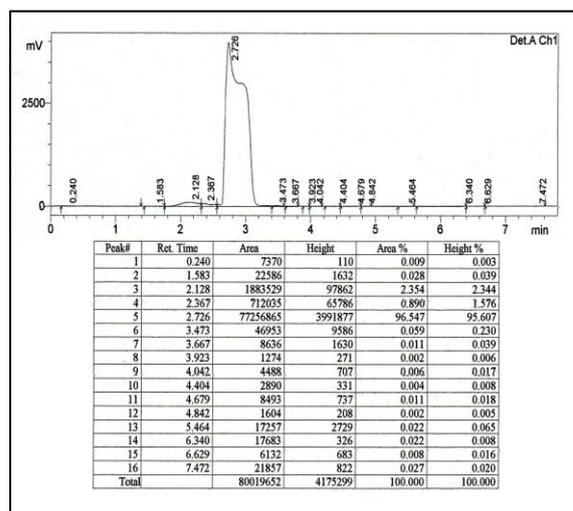


Fig. (19): Resorcinol

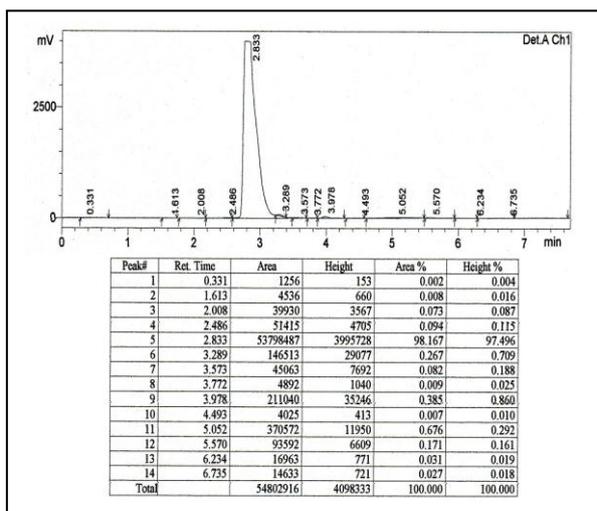


Fig. (20): Hydroquinone

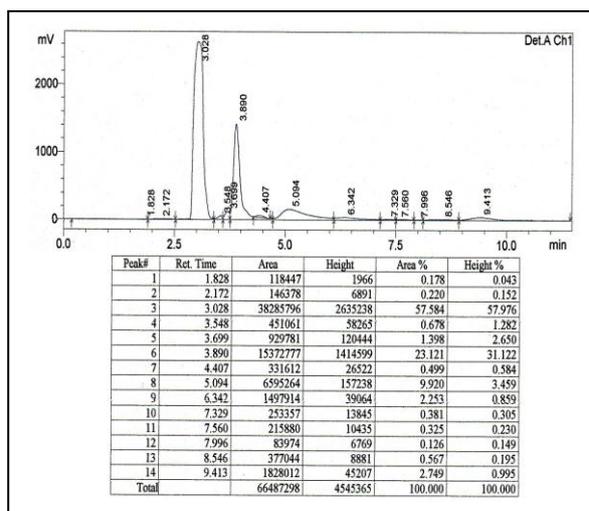


Fig. (21): Qurecetin

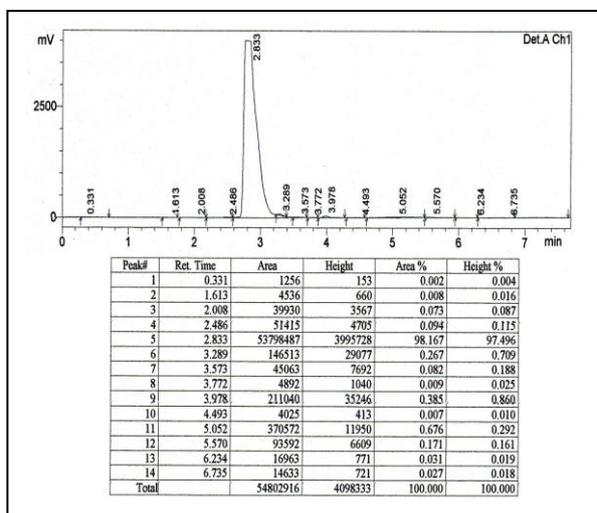


Fig. (22): Salicylic acid

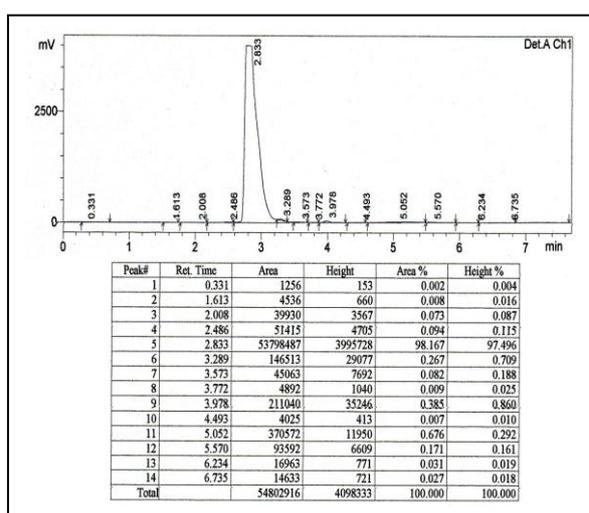


Fig. (23): P-Hydroxybanzoic acid

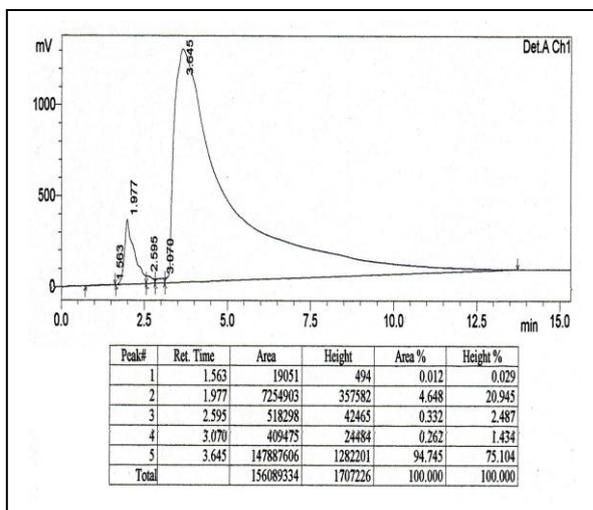


Fig. (24): Benzaic acid

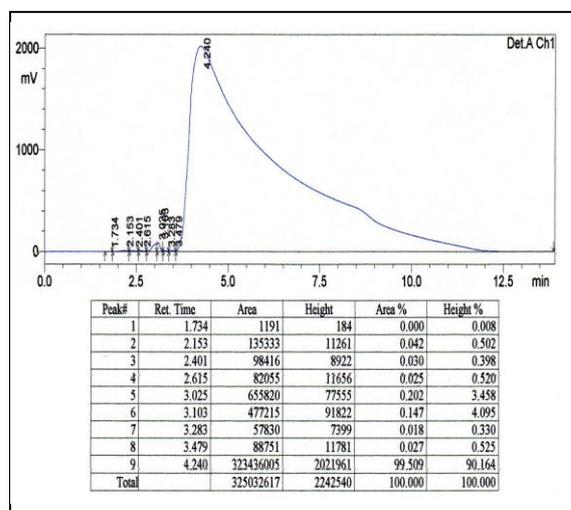


Fig. (25): Gallic acid

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