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 الاحماس الدهنية والمركبات الفينولية في بذور نبات الكزيرة المزروعة في العراق

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الملخص

بعد نبات الكزيرة نباتاً طبياً مهماً يعود إلى العائلة الخمية ومعروف استعماله في الوسط التجاري والشعبي وفي علاج مشكلات الجهاز الهضمي والروماتيزم. دراستنا الحالية حققت بعد الكشف عن احتواء بذور نبات الكزيرة على أربع أحماض دهنية وهي الوليوريك والستيروك واللبزورك والبريماتيك، إذ وجد حامض (الوليورك، الستيروك، واللبزورك) في مستخلص الأثير البترولي بينما وجد حامض (البريماتيك والستيروك) في مستخلص الكلوروفورم.

كذلك شُكِّلت المركبات الفينولية مستخلصات خلات الأليل وأجزائه، وعلى تركيز كان للكورسيتين وحامض البنزويك IMS في مستخلصات خلات الأليل، كذلك الكورسيتين تم تشخيصه في المستخلصات (F2) وال (F3). وإن مستخلص ال IMS احتوى على تركيز عالي من بارا هيدروكسي حامض البنزويك بين المركبات الفينولية المشخصة للنبات، فيما احتوى مستخلص ال IMS واجزائه (F6، وال F5، F4) على مستخلص الهيدروكسي ببنما المركبات الفينولية الأخرى وجدت بتركيز قليلة في المستخلصات الأخرى. في حين وجد حامض الكاليك في كلا المستخلصين واجزائهما وتركيز مختلفه وزمن احتجاج مقارب جدا.

الكلمات الدالة: الاحماس الدهنية، المركبات الفينولية، نبات الكزيرة.
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 monoterpane 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 unhydrolylized 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 (F_1, F_2, F_3) and also three fractions from IMS (F_4, F_5, F_6) using silica gel (60-120 mesh) as packing malaria. 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:H_2O, 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],

\[
\text{Phenolic compound} \left( \frac{\text{mg}}{\text{g}} \right) = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \frac{\text{standard conc. (mg/ml)}}{\text{Dry weighting(gm)}} \times \frac{\text{Ext. volume (ml)}}{\text{mg}}
\]
3. Results and Discussion:

1- Chromatographic identification of fatty acid compounds presented in pet-ether & chloroform extracts using GLC – analysis. Petroselinic acid C\textsubscript{18}:1n-12 was the fatty acid marker in edible plant glycolipid (GL) substances obtained from coriander seed oil, followed by linoleic acid C\textsubscript{18}: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 | Octanoic acid | Lauric acid | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid |
|-----------------------------|----------------|--------------|-------------|--------------|-------------|-----------|--------------|
| Pet-ether                   | 11.88          | 0.0004       | 13.78       | 0.0043       | 14.17       | 0.002     | 15.76        |
| 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): Palmatic acid

Fig. (7): Stearic acid

Fig. (8): Oliec 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 \((F_3)\) was identified of four similar compounds, Quercetin \((2.701 \text{ mg/g})\), Benzoic acid \((2.145 \text{ mg/g})\), Phenol \((0.141 \text{ mg/g})\) and Gallic acid \((0.018 \text{ mg/g})\). From the above results we showed that Quercetin was the main compound of high concentration in the two fractions \((F_2 \& F_3)\). 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 phenic | Phenol | Resorcinol | Hydroquinone | Quercetin | Salicylic acid | p-Hydroxybenzoic acid | Benzaic 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 | \(R_t\) | 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 | 1.150 | 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 |

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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).

**Table (2)**

| Compound                | F4    | F5    | F6    |
|-------------------------|-------|-------|-------|
| Benzoic acid            | 6.271 | 3.120 | 2.980 |
| Phenol                  | 2.720 | 0.074 | 0.074 |
| Hydroquinone            | 1.926 | 1.930 | 2.281 |
| Gallic acid             | 0.021 | 1.826 | 0.0038|
| P-Hydroxybenzoic acid   | 6.105 | 6.150 | 6.145 |

**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-Hydroxybenzoic acid
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