

## Separation of Dipetroselin from the Coriander Extracts

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### ABSTRACT

*This research is about the natural product of coriander, or the Coriandrum species. The bioactive molecules and the health effects of this plant are constantly discussed. This paper is parallel with those publications, investigating the composition of the herb. The results of current study indicates that the Coriandrum seed is a source of fatty acids. From the publications, petroselinic acid or (6Z)-octadecenoic acid, is the major fatty acid in the coriander extracts. Previous articles reported on coriander's chemical constituents, biological activities of the coriander's seeds and leaves, as well as the coriander products in the retail. Indeed, coriander has a lot of uses either as a traditional medicines or spices. Different forms of coriander samples were subjected to a simple maceration by using chloroform. From the chromatographic purification of the seed crude extract, its component could include anisaldehyde, as one of the aromatic aldehyde. It is concluded that the lipid-containing fraction of the coriander seed could also consist of dipetroselin, a diglyceride.*

**Keywords:** chromatography, Coriandrum, lipid, spectroscopy



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## INTRODUCTION

This is study that highlights the coriander or *Coriandrium* species. It is known as *ketumbar* (Malay) or *cilantro* (Spanish) [1]. This plant is also identified as an Ayurvedic medicinal tree [2]. It belongs to Umbelliferae/Apiaceae family in the order of Apiales, which contains about 300 genera and more than 3000 species. A number of reviews were made pertaining on the phytopharmacological properties of *C. sativum*, since the last half of this decade [1-4]. However, it is noted that some concomitant information is published [1, 2, 5]. Another publication was released on *C. sativum* as one of the functional food and nutraceutical [6]. This edible plant is non-toxic to human. The botanical aspects of *Coriandrum* species were explained by[7]. The photo of the locally available seed sample (Indian product) is shown in Figure 1. It is almost ovate globular, dry schizocarp, with two mericarps. Multiple longitudinal ridges are seen on the seed surface. It possesses a sweet, slightly pungent and citrus flavour and becomes more fragrant with age. The objectives of this study is to extract the coriander, in addition to the fractionation of this herbal samples.

*Coriandrum* species was used for relief of gastrointestinal maladies. Other historical uses include as an aphrodisiac, antibiotic, a remedy for respiratory ailments and pain, and a treatment for loss of appetite and memory [8]. Coriander was shown to possess antioxidant properties (Duarte *et al.*, 2016 [9]; Wei *et al.*, 2019[10]). Thangavel *et al.*, (2015)[11], Özkinali *et al.* (2017)[12] and Aelenei *et al.*, (2019)[13] concluded that the coriander extracts possessed the antimicrobial activity. In comparison with strong antioxidants such as ascorbic acid, rutin, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are conventionally used in cosmetics and food industry, the coriander fruit extract (Atanasova *et al.*, 2010)[14] however, demonstrated lower antioxidant activity. It was also reported to possess hepatoprotective activity and gives antidiabetic effect [3]. Emamghoreishi *et al.*, (2006)[15] published on the sedative and hypnotic activity of the coriander seeds. The coriander might also plays a protective role against the deleterious effects in lipid metabolism in experimental colon cancer [16].



**Figure 1: Dry *Coriandrum* Sample from India, showing the Halves and Whole Seeds (left). The Fresh Green Leaves are also used in Cooking (right)**  
(Source by author)

The phytochemical evaluation of *Coriandrum* flowers was also performed. The alkaloid could be detected in the methanol extract [17]. The isocoumarin from this species was also recorded [3]. The isolation of other biomolecules, for example, the essential oils could be achieved by using subcritical water and ethanolic microwave-assisted extractions [18, 19]. Atanasova *et al.*, (2010) [14] studied the chemical composition of the coriander by extracting the fruit with tetrafluoroethane and analysed the extract by using gas chromatography/mass spectrometry (GC/MS). The yields of both essential and fixed oil from different parts of *C. sativum*, originating from different countries, were compiled by [4]. Northwestern Tunisia gave the highest yield of the coriander's essential oil. Meanwhile, German's coriander would provide the highest amount of fixed oil. In addition, the fatty acid composition of the ripe fruits of *C. sativum* was described. The major fatty acids were identified as petroselinic acid or (6Z)-octadecenoic acid and linoleic acid [20].

## METHODOLOGY

### Herbal materials, chemicals and apparatus

The materials used in this study included three different forms of coriander samples. Firstly, they consisted of coriander seeds which were crushed by using mortar and pestle, in order to get the whole seeds cut, at least into halves. Secondly, they were dried coriander leaves and thirdly, a powdered form of the coriander. They were purchased from the local market.

The chemicals used included analytical grade of organic solvents, including chloroform, toluene and ethyl acetate were utilised. Anisaldehyde was used as the spraying reagent. Meanwhile, the apparatus consisted of glass beakers, Erlenmeyer flasks, test tubes, stirring plate, magnetic stirrer bars, filter funnel, glass rod, dropper, evaporating dish and TLC developing tanks. Silica gel F<sub>254</sub> plates (thickness = 0.25 mm; Merck, Darmstadt, Germany) was used for the Thin Layer Chromatography (TLC) technique.

### Extraction of the herbal sample

Five grams of the coriander crushed seeds, dried leaves, and powder were put in different beakers. Later, 20 ml of chloroform was added. Magnetic stirrer bars were put in each beaker before placing all the beakers on a stirring plate for one hour. This maceration procedure provided the extracts, which were later dried, by evaporating the solvent under reduced pressure.

### Thin Layer Chromatography (TLC)

The identification of the compound can be determined by TLC technique. There were two types of mobile phase, (i) toluene-ethyl acetate and (ii) chloroform-ethyl acetate. In order to get a very good separation of spots, some modifications of the proportion of the mobile phases were made. The mobile phase was adjusted to become more or less polar, in order to have separated and well resolved spots. It was found that the best solvent system for the chloroform extracts was toluene-ethyl acetate (9:1).



Ten microlitres of the extracts were drawn using capillary tube, and were plotted on a dry TLC plate, about 1 cm from the bottom. Next, the TLC plates were left in a tank until the solvent reached the solvent front. Then, they were removed from the developing solvent and the solvent were allowed to evaporate, by using a dryer. The positions of the spot were determined by placing the plate under a short and long wave of UV light. Later, the plates were sprayed with anisaldehyde and heated at 150°C for two minutes. The chloroform extract of the crushed seeds was subjected to preparative TLC, for the purpose of purification. The TLC were developed in a big tank consisted of toluene (90%) and ethyl acetate (10%). Upon spraying with anisaldehyde and dried, five bands were observed. The most intense band was labelled as Band 1 (Retardation factor,  $R_f = 0.5$ ). Then, all bands were scrapped carefully and placed in five different small, clean beakers. Then, they were washed with chloroform thoroughly before they were filtered several times into five test tubes.

### **Nuclear Magnetic Resonance (NMR) spectroscopy**

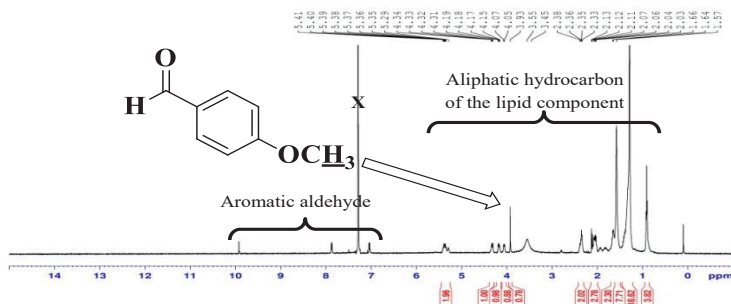
All purified compounds were analysed by using Bruker 500 MHz Ultrashield Nuclear Magnetic Resonance (NMR) spectrometer. Deuterated chloroform ( $\text{CDCl}_3$ ) was used as the solvent. The spectra were reviewed to elucidate the structure of the compounds.

## **RESULT AND DISCUSSION**

The percentage yield of the extracts were recorded as follows; 20.7%, 21.7% and 20.0%, respectively for the crushed coriander seeds, dried leaves and the powdered sample. It was noted that the crushed seeds and leaves extracts displayed approximately similar TLC spots with comparable retention factors. The TLC plate showed a few spots when observed under both short and long waves of UV light. In addition, some spots were observed when the plate was sprayed with anisaldehyde.



Band 1 provided  $^1\text{H-NMR}$  data ( $\delta_{\text{H}}$  0.00 – 10.00 ppm), as shown in Figure 2. The spectral interpretation is displayed in Table 1. Based on  $^1\text{H-NMR}$  spectral analysis, the peak that appeared in the region of  $\delta_{\text{H}}$  5.37 ppm (*m*), (Figure 2, Table 1) could indicate the presence of two units of the olefinic bonds ( $\text{C}=\text{C}$ ). They could be originated from two units of petroselinic acid component [19]. The confirmations for the glyceride backbone were provided by the data at  $\delta_{\text{H}}$  5.29, 4.33 and 4.18 ppm. A single, glycerol proton of C-2 could resonate at  $\delta_{\text{H}}$  5.29 (*m*). Meanwhile, the glycerol  $-\text{CH}_2$  protons of C-1/3 would give a double of doublets at  $\delta_{\text{H}}$  4.33 ppm (*dd*,  $J = 12, 4$  Hz) and  $\delta_{\text{H}}$  4.18 ppm (*dd*,  $J = 9, 6$  Hz). It is noteworthy that the absence of  $^1\text{H-NMR}$  signal in  $\delta_{\text{H}}$  2.5 – 3.5 ppm, could indicate the absence of  $\text{CH}_2$  group ( $=\text{CH}-\text{CH}_2-\text{CH}=\text{}$ ) as seen in linoleic acid.



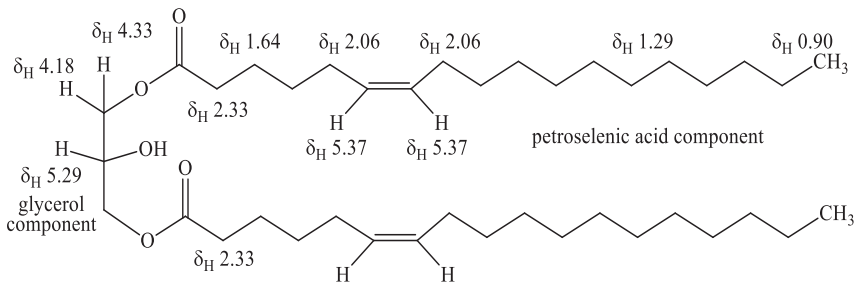
**Figure 2: The  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ) Spectrum of Band 1. The Signals Include *Para*-anisaldehyde's Aromatic Doublets ( $\delta_{\text{H}}$  7.00 – 8.00 ppm), Near the Solvent Peak**

The signal at  $\delta_{\text{H}}$  2.33 ppm (*t*,  $J = 8$  Hz, 4H) could be assignable to the fatty acids component, indicating only 2 chains of fatty acid. Therefore, Band 1 could consist of a diglyceride (Figure 3). Next, the signal at  $\delta_{\text{H}}$  2.06 ppm (*m*) could be dedicated to the protons, close to the olefinic bonds. A proof for the  $-\text{CH}_2\text{CH}_2\text{COO}-$  of fatty acids, is shown by the chemical shift at  $\delta_{\text{H}}$  1.64 ppm (*m*). The methylene moieties ( $-\text{CH}_2-$ ) of the fatty acids component were also displayed by the resonance at 1.57 ppm (*m*). Finally, two units of the terminal methyl ( $\text{CH}_3\text{CH}_2-$ ) of the fatty acids chain, could be detected at  $\delta_{\text{H}}$  0.90 ppm (*t*,  $J = 6.5$  Hz).

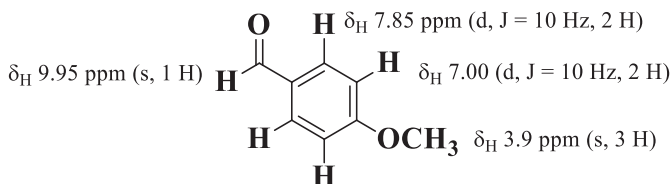
**Table 1: The  $^1\text{H-NMR}$  Signals and the Functional Groups of the Seed's Component**

$\delta_{\text{H}}$ , ppm	The multiplicity *	Integration	Functional groups
5.37	<i>m</i>	4 H = 2 units of C=C	-CH=CH-
5.29	<i>m</i>	1 H	-CH(OH)(CH <sub>2</sub> )O-
4.33	<i>dd</i> , J = 12, 4 Hz	2 H	-O(CH <sub>2</sub> ) <sub>2</sub> CH(OH)
4.18	<i>dd</i> , J = 9, 6 Hz	2 H	-O(CH <sub>2</sub> ) <sub>2</sub> CH(OH)
2.33	<i>t</i> , J = 8 Hz	4 H = 2 units of CH <sub>2</sub>	-CH <sub>2</sub> COO- (4H)
2.06	<i>m</i>	8 H	-CH <sub>2</sub> -CH=CH-
1.64	<i>m</i>	4 H	-CH <sub>2</sub> -
1.29 - 1.57	<i>m</i>	CH <sub>2</sub> units	-CH <sub>2</sub> -
0.90	<i>t</i> , J = 6.5 Hz	4 x 2 H = 2 x CH <sub>3</sub>	CH <sub>3</sub> CH <sub>2</sub> -

br = broad, d= doublet, dd = doublet of doublets, m = multiplet, t = triplet

**Figure 3: The Suggested Chemical Structure of the Diglyceride in the Coriander Seed**

There is a presence of  $^1\text{H-NMR}$  signal in  $\delta_{\text{H}}$  3-4 ppm indicating the presence of methoxy group or terminal methyl ester ( $-\text{COOCH}_3$ ). It is believed that this sharp, singlet at  $\delta_{\text{H}}$  3.93 ppm could belong to *para*-anisaldehyde (Figure 4). The supporting evidence of the presence of this aldehyde could be seen at the chemical shifts at  $\delta_{\text{H}}$  9.95 ppm (s, 1H),  $\delta_{\text{H}}$  7.00,  $\delta_{\text{H}}$  7.85. This compound is naturally a minor constituent of the coriander [21]. More polar *Coriandrum* seed components were elucidated by ultrahigh resolution spectroscopic procedures [22].



**Figure 4: The  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ) Chemical Shifts of the Protons in *Para*-anisaldehyde**

## CONCLUSION

Based on the proton NMR data, it is concluded that the seed fraction could comprised of an aromatic aldehyde and a neutral lipid, dipetroselin (which is chemically known as either petroselinic acid diglyceride or glyceryl dipetroselinate or glycerol dipetroselinate). It is hoped that the other fractions could be continuously analysed in near future.

The coriander seeds extraction could provide more essential oils than leaves part. This might be due to the presence of the mericarp, outside of the seeds, which contain the oil glands. The coriander extraction could also be performed using other methods such as sub-water critical extraction or hydro distillation, in addition to the normal maceration. The solvents usage could also include various chemicals, from the non-polar to the polar solvents. *Coriandrum* seed products such as in a form of herbal tea, could be another alternative for the public in receiving coriander's healthy benefits as a superfood.

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