



Green extraction techniques: Effect of extraction method on lipid contents of three medicinal plants of Apiaceae

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ABSTRACT

Fixed oils (lipids) in the fruits of Celery (*Apium graveolens* L.), Parsley (*Petroselinum crispum* Mill) and Fennel (*Foeniculum vulgare* L.), plant fruits were extracted by three different extraction methods viz. percolation, Ultrasonic assisted extraction (UAE) and Supercritical fluid extraction (SFE). The total yield of extracted lipids of the studied extraction methods were 9.80 g, 14.40 g and 8.75 g for Celery, 9.7 g, 11.39 g and 9.42 g for Parsley and 13.6 g, 17.9 g and 13.72 g for Fennel. Petroselinic acid (C18:1), the characteristic fatty acid of Apiaceae family, was evaluated using GC/MS. The results revealed that both UAE and SFE enhanced the extraction efficiency of the fatty acid of Celery, Parsley and Fennel. UAE gave the highest percentage yield (75.6%, 71.6%, 76.4%), whereas SFE gave (66.4%, 69.8%, 43.1%) compared to the percolation method (58.7%, 62.8%, 61.4%). Also, the total lipids (mono ..., di..., tri-glycerides, total free fatty acids and total fatty acid methyl esters) of the three different extraction methods of Celery, Parsley and Fennel fruits were evaluated using High Performance Thin Layer Chromatography (HPTLC). Ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SFE) not only enhanced the total lipid extraction but also saved time, reduced the solvents use and produced, ecologically, green technologies.

Keywords: Celery (*Apium graveolens* L.), Parsley (*Petroselinum crispum* Mill), Fennel (*Foeniculum vulgare* L.), Ultrasonic assisted extraction (UAE), Supercritical fluid extraction (SFE), total lipids, Petroselinic acid, GC/MS, HPTLC.

INTRODUCTION

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plants. Yet the extraction step remains often a neglected area, which over the years has received much less attention and research [1,2].

The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents. Thus, the major significant shortcomings of traditional extraction techniques is the lengthy extraction time that can be 8, 16, and 24 hours or more, which results in consumption of considerable time and heat energy [3,4]. The fact that one single plant can contain several secondary metabolites makes the need for the development of high performance and rapid extraction methods an absolute necessity [5]. Keeping in pace with such requirements, recent times has witnessed the use and growth of new extraction techniques with shortened extraction time, reduced solvent consumption, increased pollution prevention concern and with special care for thermo labile constituents. Novel extraction methods including microwave assisted extraction (MAE) [6], supercritical fluid extraction (SCFE) [7-9], accelerated solvent extraction (ASE) [10], subcritical water extraction (SWE) [11] and ultrasound assisted extraction (UAE) [12] have drawn significant research attention in the

last decade [13]. If these techniques are explored scientifically, they can provide an efficient extraction technology for ensuring the quality of herbal medicines worldwide.

The family Apiaceae is commonly known as the carrot family. It has approximately 2000 to 3000 species; out of these 174 grow in Mediterranean region. The most commonly cultivated members of the family are Celery (*Apium graveolens* L.), Parsley (*Petroselinum crispum* Mill) and Fennel (*Foeniculum vulgare* L.) [14]. Apiaceae represents one of the best-known plant families, widely distributed in temperate climate regions where they are often used as spices, vegetables, or drugs owing to the presence of useful secondary metabolites [15,16].

The genera of Apiaceae should be regarded as a useful source for the extraction of petroselinic acid, which represents an important oleo chemical raw material [17]. In addition to petroselinic acid, the Apiaceae taxa are also distinguishable by the occurrence of umbelliferose, and polyacetylenes, which are characteristic compounds in this family. They also contain several specific phenols, phenylpropanoids, terpenes, saponins and coumarins in fruits, leaves or roots [18]. Petroselinic acid (C18:1) is the predominant fatty acid constituent. It consisted of more than half of the oil. Linoleic acid is the second highest fatty acid component in all of the Apiaceae genera [19,20].

Celery (*Apium graveolens* L.) has been cultivated for the last 3000 years, notably in pharaonic Egypt, and was known in China in the fifth century BC [21,22]. Celery has been used as a food, and at various times both the whole plant and the seeds have been consumed as a medicine. The characteristic odor of celery essential oil is due to a series of phthalide derivatives [23].

Despite the stem being the most commonly ingested portion of the plant as a common vegetable, the seeds of celery appears to have been used for medicinal purposes (Egypt and China) to treat; bronchitis, asthma, liver and spleen diseases and with hepatoprotective activity against many hepatotoxins [24,25]. It has also been reported to be diluted in beverages and drunk with wine to cool a hot temper [26].

Celery seed oil is comprised of both the oil component (fatty acids) and the volatile component (small molecular weight molecules); the fatty acids in celery seed oil include: Petroselinic, Linoleic acid, Palmitic acid and Oleic acid [27-30].

The chemical constituents include organic and inorganic compounds such as glycosides, steroids, Phenols, flavonoids, sodium, potassium, calcium and iron. The seeds also contain apiin, apigenin, caffeic acid and chlorogenic acid [31].

Fennel (*Foeniculum vulgare* Mill.) has been known as a medicinal and aromatic herb and its fruit is commonly used as a natural remedy against digestive disorders [32]. The dried, aromatic fruits are widely employed in culinary preparations for flavoring bread and pastry, in candies and in alcoholic liqueurs of French type, as well as in cosmetic and medicinal preparations [33].

Classes of constituents previously isolated from *Foeniculum vulgare* Mill. are *trans*-Anethole, fenchone, methyl chavicol, limonene, α -pinene, camphene, β -pinene, β -myrcene, α -phellandrene, 3-carene, camphor, *cis*-anethole [34].

Parsley (*Petroselinum crispum* Mill.) is cultivated throughout the world and used as a spice, salad and herbal remedy. Use of parsley in food has a long history going back to ancients, Greeks and Romans. It has been reported to have possible medicinal attributes as an antioxidative, antimicrobial, anticoagulant, antihyperlipidemic and antihepatotoxic [35].

Parsley seeds (*Petroselinum crispum* Mill) contain lipids with approximately 13-20% fatty oils with approximately 14% non-saponifiable substances, flavonoids including largely apiin, tannins, polysaccharides, traces of furanocumarins (bergapten) and organic acids: petroselinic acid (up to the level of 50% of the total fatty acids to 80%), oleic acid, linoleic acid, glycolic acid and palmitic acid [36,37]. The seeds of Parsley (*Petroselinum crispum* Mill) contain lipids with a very high level of petroselinic acid which is accompanied by fairly low levels of oleic acid [38].

The main aim of the present work is to evaluate the use of UAE and SFE in fixed oil extraction from Celery, Parsley and Fennel fruits and compare the extraction yield and fatty acids profile of the oil with traditional solvent extraction method (percolation).

EXPERIMENTAL SECTION**Plant Material:**

Celery (*Apium graveolens* L.), Parsley (*Petroselinum crispum*) and Fennel (*Foeniculum vulgare*) plant fruits were purchased from the local market from "Giza Company for Seeds and Herbs". The company depends mainly on exportation of raw plant materials to USA and Europe. This means that the standard of the materials quality is high. The plant fruits were authenticated by Professor Kamal Zayed, Botany Department, Cairo University. A voucher specimen was kept in the herbarium of the National Research Center of Egypt.

Methods of Extraction:

Fixed oils (lipids) of Celery (*Apium graveolens*), Parsley (*Petroselinum crispum*) and Fennel (*Foeniculum vulgare*) plant fruits were extracted by three different extraction methods for each *viz.* percolation, sonication and CO₂ as follows:

Conventional Extraction Method:

100 g of powdered fruits of each plant were, separately, percolated with chloroform/methanol (2:1) (2000 ml) and recycling for 4 days. After complete exhaustion, the chloroform/methanol extracts were evaporated under vacuum at 40°C.

Ultrasonic-Assisted Extraction:

100g of homogenous dried powdered fruits, of each plant, were mixed with 800 mL of chloroform/methanol (2:1) for 20 min at power 400 W (amplitude 0.5 and rotation 70 cycles) using an Ultrasonic Processor UP400S (400 watts, 24kHz, Hielscher) direct sonication, ultrasonic probe with a tip diameter of 20 mm, fitted into the flask and the tip was inserted at the half height of the extraction solvent. After extraction, the extract was centrifuged at 4000 rpm and the supernatant evaporated under reduced pressure.

Supercritical Fluid Extraction:

An applied separation system in the SFE mode was used for all the extractions. The extraction vessel was a 10 ml stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 200 bar and temperatures of 50 °C for a duration of 15 min, in static mode, followed by 3 hrs, in dynamic mode. Flow rate of CO₂ gas 1L/min.

Sample preparation for analysis:

0.5 gm of each sample extract was taken in 10 ml chloroform and 2 gm anhydrous sodium sulphate was added, vortexed.

Sample preparation for GC/MS:

50 mg of each extract was dissolved in 2ml of 1.5% sulfuric acid in methanol and heated for 3 hours at 90°C. Then, two ml of water and five ml of hexane, was added, vortexed. The upper layer was dehydrated over sodium sulfate anhydrous and inject to GC/MS for fatty acid methyl ester detection.

GC-MS Analysis:

The qualitative and quantitative determination of the major and minor constituents of vegetable oils are done by gas chromatography [39].

GC/MS apparatus and conditions

GC/MS was carried out using an HP5890 Series II Gas Chromatography, HP 5972 Mass Selective Detector and Agilent 6890 Series Auto sampler. A Supelco MDN-5S 30 m by 0.25mm capillary column with a 0.5 µm film thickness was used with helium as the carrier gas at a flow rate of 1.0 ml/min. The GC oven temperature was programmed at an initial temperature of 130°C for 1 minute, then heated up to 300°C at 5°C/min and held at 300°C for 5 minutes. Injector and detector temperatures were set at 250°C. Mass spectrometry was run in the electron impact (EI) at 70eV. The identification of the chemical constituents were determined by their GC retention times, interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database.

Reliability and accuracy of the analytical methods for the detection of fatty acids were ensured by the use of the certified reference matrix that consisted of a mixture of 37 FAME standards triglyceride standard, free F.A. standard and glycerol standard. The contents of the particular fatty acids are expressed as percentages of the sum of all of the fatty acids analyzed.

Sample preparation for HPTLC:

100 μ L of chloroform extract was taken and diluted to one ml, then subjected to HPTLC under the following conditions:

Stationary phase:

20 x 10 cm glass plates HPTLC silica gel 60 F₂₅₄ (Merck).

Sample application:

Apply 5 μ L of each tested sample as 6 mm band, 2 mm apart, 8 mm from the lower edge and 15 mm from left and right edges of the plate.

Temperature and humidity:

Record temperature and humidity in the lab. If the relative humidity exceeds 50% RH, condition the plate to about 30% RH using a suitable device.

Chromatography:

Developing solvent:

pet.ether/ether/formic acid (90/10/2).

Chamber:

Pour 12 ml of developing solvent in the right trough of chamber and 25 ml in the left one. Allow the chamber to saturate for 20 min.

Development:

Migration distance of developing solvent on the plate is 85 mm from lower edge of the plate.

Drying:

Dry the plate for 10 min.

Preparation of derivatizing reagents:**Copper sulphate reagent:**

20 gm of Copper sulphate pentahydrate + 200 ml methanol (at less than 20°C). Then, under cooling with ice, add 8 ml of sulfuric acid (98%) and 8 ml ortho-phosphoric acid (85%).

Derivatization with Copper sulphate reagent:

dip the plate into the tank of the immersion device charged with 200 ml of Copper sulphate reagent, by placing the plate in holder of immersion device (speed: 5, time: 5sec.), allow the plate to dry for 1 min inside the hood and heat in oven for 30 min at 140 °C.

-CAMAG Automatic TLC Scanner with CATS evaluation Software.

-Wave length scan at 420 nm.

-Scanning speed 20 mm/s.

RESULTS AND DISCUSSION

Table 1: The total extracts yields/100 g:

	Percolation	Sonicator	CO2
Celery	9.8 g	14.4 g	8.75 g
Parsley	9.7 g	11.39 g	9.42 g
Fennel	13.6 g	17.9 g	13.72 g

GC/MS analysis of FAME

Table 2: Comparative Study of Fatty Acid Methyl Esters of the Prepared Extracts of Celery, Parsley and Fennel Fruits

Sample/Retention times (min)	12.59	12.72	16.60	17.30
Name	Palmitic acid	Myristicin	6-octadecenoic acid	Linoleic acid
Fennel				
Percolation	-	4.3	61.4	8.4
Sonication	-	6.5	76.4	8.7
SFE/CO ₂	13.6	3.5	43.1	9.1
Celery				
Percolation	4.1	7.9	58.7	16.1
Sonication	6.5	4.2	75.6	12.2
SFE/CO ₂	11.8	4	61.4	12.3
Parsley				
Percolation	2.5	4.7	62.8	10.1
Sonication	9.1	12.9	71.6	16.5
SFE/CO ₂	-	22.3	69.8	7.9

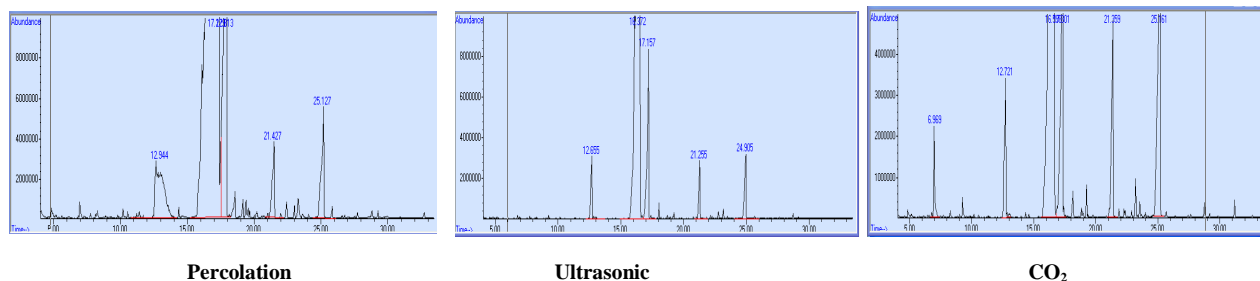


Figure 1: Comparison between total ion chromatograms of the fatty acid methyl ester prepared by percolation, ultrasound- assisted extraction and CO₂ of Celery fruits

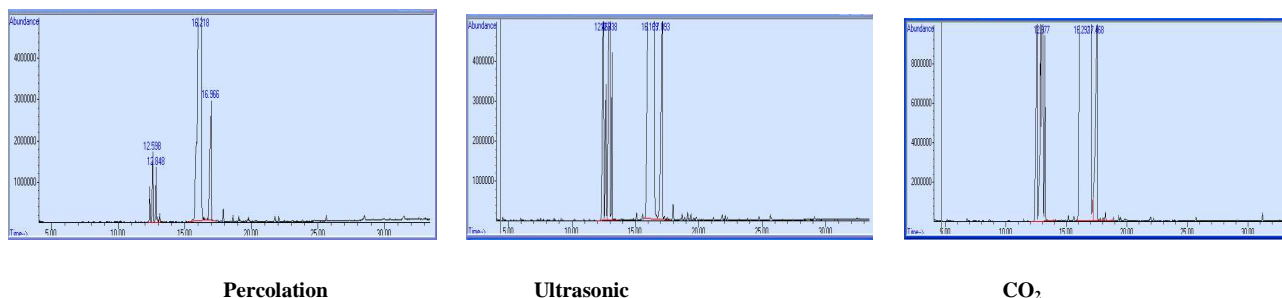


Figure 2: Comparison between total ion chromatograms of the fatty acid methyl ester prepared by percolation, ultrasound- assisted extraction and CO₂ of Parsley fruits

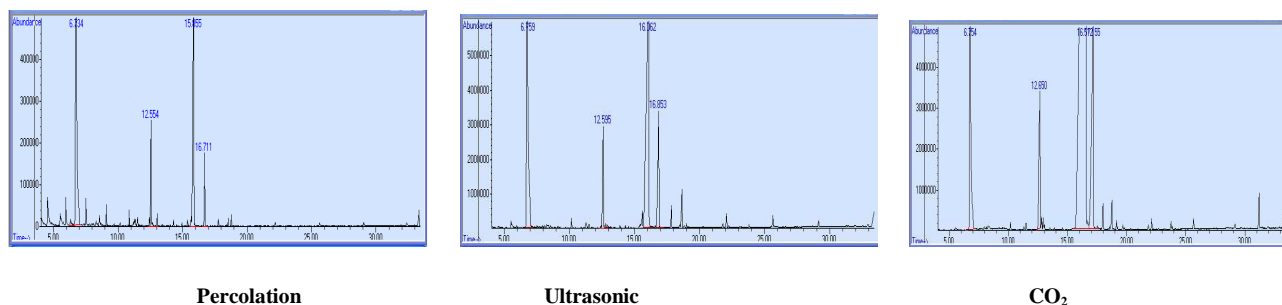


Figure 3: Comparison between total ion chromatograms of the fatty acid methyl ester prepared by percolation, ultrasound- assisted extraction and CO₂ of Fennel fruits

**Evaluation of total lipids using High Performance Thin Layer Chromatography (HPTLC):
Documentation of derivatized plates (Copper sulphate reagent):**

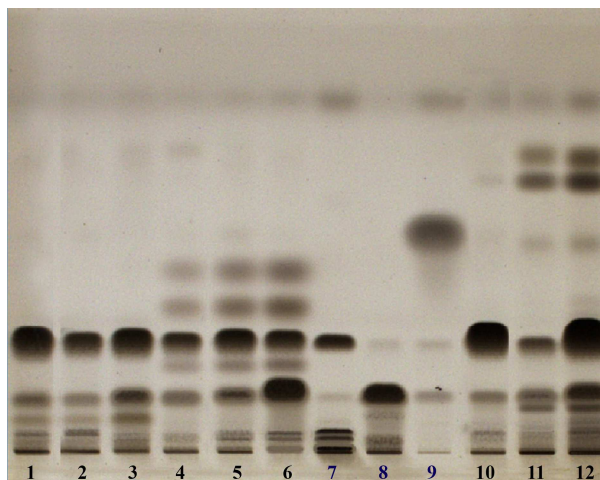


Figure 4: Derivatized Plate @ White R

- Track 1: Celery extracted by percolation
- Track 2: Celery extracted by sonication
- Track 3: Celery extracted by CO₂
- Track 4: Parsley extracted by percolation
- Track 5: Parsley extracted by sonication
- Track 6: Parsley extracted by CO₂
- Track 7: Standard Glycerides (mono ..., di..., Tri-glycerides)
- Track 8: Standard Free Fatty Acids
- Track 9: Standard Fatty Acid Methyl Esters (fames)
- Track 10: Fennel extracted by percolation
- Track 11: Fennel extracted by sonication
- Track 12: Fennel extracted by CO₂

Standard Solution Preparation:

Each standard solution was diluted to concentration of 5mg in one ml CHCl₃ and 5μl was injected.

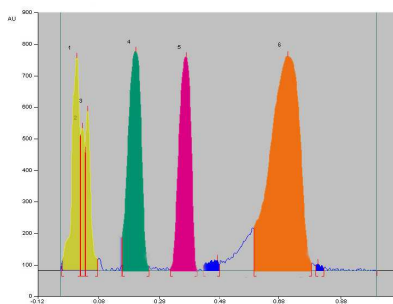


Figure 5: HPTLC Chromatogram for Standard samples

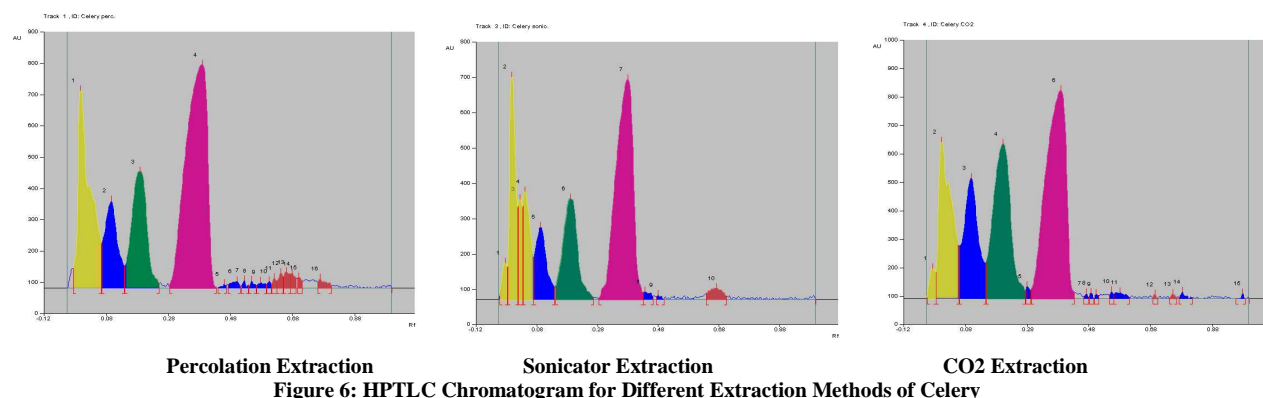
Peaks 1,2,3 represent mono.. & di-glycerides R_f range (-0.04 to 0.08).

Peak 4 represents free fatty acids R_f range (0.15 to 0.24).

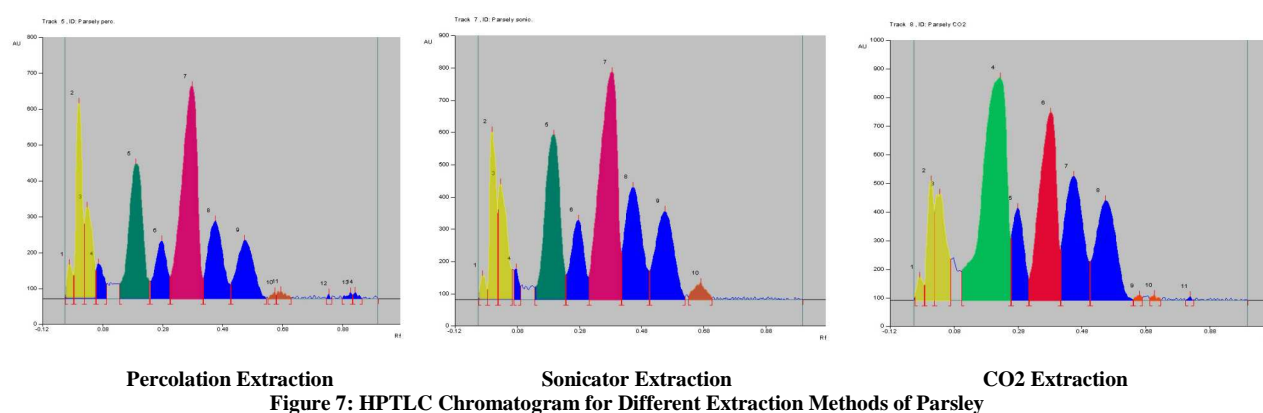
peak 5 represents tri-glycerides R_f range (0.32 to 0.41).

Peak 6 represents fatty acid methyl esters R_f range (0.57 to 0.76).

Comparative Study of Three Apiaceae Extraction Methods Celery Results:



Parsley Results:



Fennel Results:

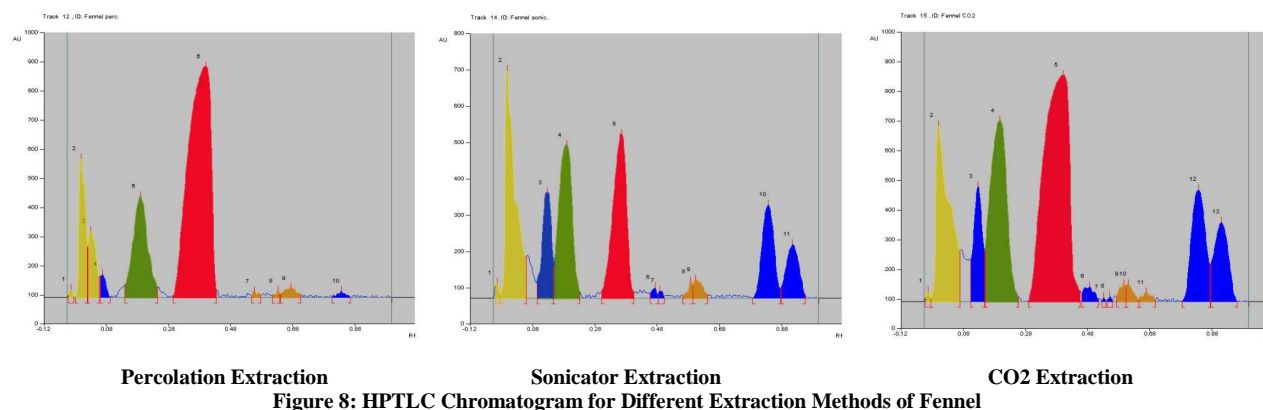


Table 3: Individual Compounds Percent of Celery, Parsley and Fennel Fruits Obtained by Different Extraction Techniques

Class of comp	R _f Range	Individual Compounds % of Extraction Methods								
		Percolation			Ultrasonic			CO2		
		Celery	Parsley	Fennel	Celery	Parsley	Fennel	Celery	Parsley	Fennel
Mono. & Diglyc.	(-0.04 to 0.08)	22.32	21.72	17.43	32.19	16.81	23.64	25.41	12.67	15.26
Triglyc.	(0.32 to 0.41)	44.96	31.6	62.41	42.65	29.4	23.16	37.95	19.49	37.69
Total Free F. A.	(0.15 to 0.24)	14.65	17.79	17.56	16.27	18.03	21.4	24.45	33.69	19.28
Total Fames	(0.57 to 0.76)	4%	10.33	2.01	1.72	13.74	2.48	0.77	13.05	1.94

The goal of this work is to compare classical (traditional) extraction techniques of plants with unconventional methods from three Apiaceae plants with respect of amount of extracted material and chemical composition of the extracts.

Table 1 showed the mass yield (g of extract/100 g of sample) obtained by three different techniques in the best conditions. The results of different extraction methods employed being presented.

The total oil yield percent of Celery, Parsley and Fennel plant fruits, obtained by percolation method, were 9.8, 9.7 and 13.6 respectively, showing the highest oil content for 4 days. Whereas, that obtained by the new methods, (14.4, 11.39, 17.9 for UAE within 20 min and 8.75, 9.42, 13.72 for SFE for 3 hours extraction time, for once) showed that these techniques, can improve the extraction yield at shorter reaction times and at low or moderate costs. To some extent, SFE showed to be inconvenient technique for extraction of lipids, depending on yield comparison.

The effect of UAE and SFE on the major components of the lipid constituents of the three Apiaceae plants has been evaluated by using HPTLC. The present study indicates that ultrasound assisted extraction can be used as a desirable alternative to conventional oil extraction techniques. The major advantage of these methods is the reduced time of extraction and energy consumption costs, when is compared to conventional methods. It allows also for better retention and availability of desirable nutraceuticals, such as free fatty acids (FFA) in the extracted oil where the percent of FFA is very highly improved by these techniques. This can be a new step to produce nutritional vegetable oils with higher nutrition value.

Table 3 showed the comparison of the amount of individual compounds in the extract obtained from every extraction technique.

According to the available literature, the fruits of Apiaceae contain approximately 20% fatty oil. Petroselinic acid (C18:1) is a characteristic fatty acid of this family. This acid is of interest because of its antimicrobial activity and because its oxidation gives Lauric acid (C12:0), a very important fatty acid used in the soap, cosmetic, medical and perfume industries. Petroselinic acid and oleic acid are always combined in Apiaceae oils. In our study, GC/MS showed that petroselinic acid is the major fatty acid. UAE and SFE gave very similar profile as shown by lipids obtained from percolation (**Table2**).

Also, using these innovative techniques offer a better control over the extraction conditions and allow the extraction to be performed in shorter times and in a more selective way. Ultrasonic radiation is a powerful aid to accelerate various steps of the analytical process. This energy is of great help in the pre-treatment of solid samples as it facilitates and accelerates operations such as the extraction of organic and inorganic compounds, homogenization and various others [40].

Vegetable oils are mainly constituted by triacylglycerol (95 - 98%) and complex mixtures of minor compounds (2-5%) of a wide range of chemical nature which was shown by the scanner and HPTLC results. These minor constituents show a broad qualitative and quantitative composition depending on the vegetable species from which they are obtained. Agronomic and climatic conditions, fruit or seed quality, oil extraction system and refining procedures can cause variation in the content and composition of the constituents of vegetable oil.

CONCLUSION

The three extraction techniques were qualitatively the same, but the GC/MS analysis, HPTLC and the scanner showed quantitative difference. Any way the new techniques, ultrasonic assisted extraction (UAE) and supercritical carbon dioxide fluid extraction (SFE), are applicable, reproducible. They saved time, reduced the solvents use and produced, ecologically, green technologies.

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