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EVALUATION OF THE ESSENTIAL OIL OF *FOENICULUM VULGARE* MILL (FENNEL) FRUITS EXTRACTED BY THREE DIFFERENT EXTRACTION METHODS BY GC/MS

Faiza M. Hammouda[1*], Mahmoud A. Saleh[2], Nahla S. Abdel-Azim[1], Khaled A. Shams[1], Shams I. Ismail[1], Abdelaaty A. Shahat[1] and Ibrahim A. Saleh[1]

[1]Department of Phytochemistry, National Research Centre (NRC), 12622 Dokki, Cairo, Egypt.
[2]Department of Chemistry, Texas Southern University, 3100 Cleburne Ave, Houston, TX 77004, USA.
***E-mail:** fmhammouda@yahoomail.com

Abstract
Background: Hydrodistillation (HD) and steam-distillation, or solvent extraction methods of essential oils have some disadvantages like thermal decomposition of extracts, its contamination with solvent or solvent residues and the pollution of residual vegetal material with solvent which can be also an environmental problem. Thus, new green techniques, such as supercritical fluid extraction and microwave assisted techniques, are potential solutions to overcome these disadvantages.
Materials and Methods: The aim of this study was to evaluate the essential oil of *Foeniculum vulgare* subsp. *Piperitum* fruits extracted by three different extraction methods *viz.* Supercritical fluid extraction (SFE) using CO_2, microwave-assisted extraction (MAE) and hydro-distillation (HD) using gas chromatography-mass spectrometry (GC/MS).
Results: The results revealed that both MAE and SFE enhanced the extraction efficiency of the interested components. MAE gave the highest yield of oil as well as higher percentage of Fenchone (28%), whereas SFE gave the highest percentage of anethol (72%).
Conclusion: Microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) not only enhanced the essential oil extraction but also saved time, reduced the solvents use and produced, ecologically, green technologies.

Keywords: *Foeniculum vulgare* subsp. *Piperitum*, microwave-assisted extraction (MAE), Supercritical fluid extraction (SFE), Essential Oils, Anethol, Fenchone, Limonene, Pinene, GC/MS.

Introduction

Fennel (*Foeniculum vulgare* Mill.), family Apiaceae (Umbelliferae) is an annual, biennial or perennial aromatic herb. It is native to southern Europe and the Mediterranean area (Pasrija Anubhuti, et al., 2011; Abdelaaty et al., 2012a).

Fennel is commercially cultivated in Egypt and grows wild in many areas of the Egyptian desert. It is represented in Egypt by two fennel types: sweet fennel, *Foeniculum vulgare* Mill. subsp. *vulgare*, and bitter fennel, *Foeniculum vulgare* subsp. *Piperitum* (Boulos, L. 2005). It is used as a spice and also as an important ingredient in various folklore medicines throughout the world. Several fennel parts are edible (bulbs, leaves, stalks, and fruits). Mature fruits (commonly known as seeds) and essential oil of fennel are used as flavoring agents in food products for appetizing, as digestive aid, liqueurs, bread, cheese and an ingredient of cosmetics and pharmaceutical products. Moreover fennel infusions are the classical decoction for nursing babies to prevent flatulence and colic spasms [Abdelaaty et al., 2012b; Mimica-Dukić et al., 2003; Perry et al., 2011; Bruyas-Bertholon et al., 2012]. The plant has been investigated extensively for several medicinal and therapeutic activities and has been reported for possessing carminative, antioxidant, antibacterial, antifungal and mosquito repellent properties [WHO, 2005; Damianova et al., 2004].

Fennel volatile oil is a mixture of many different constituents and the main ingredients are: anethole (40 - 70%), fenchone (1 - 20%) and estragole (2 - 9%). (Pasrija Anubhuti et al., 2011; Cosge et al., 2008; Raghavan S., 2006).
In the essential oil of sweet fennel, the fenchone content usually does not exceed 5%, whereas in the bitter types its content can be as high as 20%. In sweet fennel oil, the anethole content reaches 84-90%, whereas its proportion in bitter fennel is about 61- 70% (Lawrence, 1994).

Hydrodistillation (HD) and steam-distillation, or solvent extraction methods of essential oils have some disadvantages like thermal decomposition of extracts, its contamination with solvent or solvent residues. The pollution of residual vegetal material with solvent can be also an environmental problem (Árpád et al., 2011).

It is significant to develop more efficient methods for, standardization and quantification the extraction of the essential oil. Thus, new green techniques, such as supercritical fluid extraction, ultrasound and microwave assisted techniques, are potential solutions for energy consumption reduction (Lucchesi et al., 2004; Milojević et al., 2008) and/or for solving the environmental problems of residues (Lucchesi *et al.*, 2004). Nowadays, microwave-assisted hydrodistillation (MWHD) became a widely used method for obtaining the essential oils from different medicinal plants due to its advantages (e.g. more effective heating, shortened extraction time) in comparison with the classical HD. The heating process is based on the molecular motions of the polar molecules and ions inside the solvent and vegetal matrix; it is strongly influenced by the dielectric constants of the solid-liquid-vapors system, developed by process evolution. This heating way realizes a more homogeneous temperature distribution at plant powder suspension level (Kosar et al., 2007; Chemat et al., 2005).

The aim of our work was to evaluate the essential oil of *Foeniculum vulgare* subsp. *Piperitum* fruits (bitter fennel), extracted by three different extraction methods *viz.* supercritical fluid extraction (SFE) using CO_2 as extractant, microwave-assisted extraction (MAE) and the classical method, Hydro-distillation (HD), with GC/MS**.**

Materials and methods
Plant Material

Wild bitter fennel (*Foeniculum vulgare* subsp. *Piperitum*) fruits were collected from Mediterranean Coastal Region (Matruh–El obayedd) during May 2011. The plant was identified by Prof. Dr. Kamal M. Zayed and Dr. Ibrahim Elgarf, Taxonomists, Cairo Univ., Faculty of Science, Botany Dept., Cairo, Egypt, to whom the authors are deeply indebted. The ripe fruits were ground into fine powder. A voucher specimen was kept in the herbarium of Botany Department.

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Extraction Methods
Hydrodistillation (HD)

100 g of ground fruits and 1000 mL distilled water were placed in a 2000 mL round-bottom flask and connected to a Clevenger-type apparatus to extract the essential oil with hydrodistillation for 3 hrs. The resulted essential oil was collected, dried with anhydrous sodium sulfate, and kept in a dark glass bottle at 4°C until analyzed by GC/MS (Shahat et al. 2011; Abdelaaty et al., 2012b).

Table 1: Extractable essential oils (in %0 through three methods

Method of extraction	Essentail oil %	Pinene	Fenchone	Limonene	Anethol
HD	0.98 %	3%	22%	3.5%	65%
CO_2 SFE	2.2%	0.8%	18%	1.2%	72%
MWHD	2.8%	1.5%	28%	2.2%	55%

Supercritical Fluid Extraction

An Applied Separation system in the SFE mode was used for extraction. The extraction vessel was a 10 mL stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 200 bar and temperature of 50 ºC for a duration of 15 min, in static mode, followed by 3 hrs, in dynamic mode with CO_2 gas flow rate 1L/min (Damianova et al., 2004).

Microwave Extraction

The essential oil was obtained by hydrodistillation for 60 min using a Clevenger-type apparatus placed in a modified microwave oven (MARS 240v/ 50Hz). During distillation, time, temperature, pressure and power were monitored and controlled with the "easy-control" software package of the system. Microwave power applied to the plant material was controlled by a shielded thermocouple inserted directly into the flask. The oven was operated for 10 min at 800 Watts up to 1000°C, and then kept at 1000°C for 50 min at 500 Watts followed by 5 min of ventilation. Essential oil percentages obtained from the experiments were calculated on moisture free basis. The essential oils obtained at different conditions were collected in amber colored vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen, and kept at 4°C until being analyzed (Kosar et al., 2007; Árpád et al., 2011).

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS analyses of the obtained essential oils were carried out using HP5890 Series II Gas Chromatograph, HP 5972 Mass Selective Detector and Agilent 6890 Series Autosampler (Agilent Technologies, USA). A supelco MDN-5S 30m by 0.25mm capillary column with a o.5 µm film thickness was used with helium as the carrier gas at a flow rate of 1.0ml/min. The GC oven temperature was programmed at an initial temperature of 40 ºC for 5 minutes, then heated up to 140 ºC at 5 ºC /min and held at 140 ºC for 5 min, then heated to 280 ºC at 9 ºC /min and held for 5 additional minutes. Injector and detector temperatures were set at 250 ºC. Mass spectrometry was run in the electron impact mode (EI) at 70eV. The identification of the chemical constituents of the essential oil was 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) data- base with those of authentic samples or published mass spectral data (Adams, 2007). The quantitative data were expressed as relative percentage of the oil constituents calculated from the GC peak areas without using correction factors, and each oil was analyzed three times.

Results

Evaluation of essential oils of different extraction techniques

We demonstrate our results on comparison of both the yield and composition of essential oil fractions obtained by traditional hydrodistillation and the newly implemented extraction methods (Table 1). In this work, Fennel has been extracted using three different extraction techniques; hydrodistillation, supercritical fluid extraction and microwave assisted extraction. The obtained oils have been analyzed by GC/MS. The following table summarizes the obtained essential oil percentages and the relative percent of the major constituents.

Discussion

Since the bitter fennel oil has been reported to contain not less than 60% anethol and 15% fenchone and not more than 6% estragol (European Pharmacopoeia, 1/2005:824; European Pharmacopoeia, 1/2005:825; Brand, 1993; Tóth, 1967; Trenkle K 1969, 1971 and 1972), with small amounts of alpha-pinene, limonene, p-cymene, beta-pinene, beta-myrcene and of a variety of other compounds. So, the results of the present work showed that the total oil extraction yield was highest by using microwave assisted extraction followed by supercritical fluid extraction and the lowest yield was obtained by the conventional hydrodistillation. The major oil component extracted by the three methods is anethol. The results revealed that both MAE and SFE have shown to enhance the extraction efficiency of the interested components. MAE gave the highest yield of oil as well as higher percentage of Fenchone (28%) and the relative concentration of anethol was lowest by microwave assisted extraction, despite the highest oil yield, compared with conventional HD method. Whereas, SFE gave the highest percentage of anethol (72%) than those detected in the extract prepared by traditional method (65%). Despite the fact that MWHD gave the higher extraction oil yield than both the traditional method and SFE, the percentage of anethol which is the most potent compound in fennel was still lower in its extract than those of the other two methods. So, further investigations should be carried out to reach the optimum conditions to get the maximum yield of of the extract.

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In conclusion, comparison of these extraction methods revealed that they extracted essential oil with similar qualitative characteristics, however slight quantitative differences. The innovative extraction techniques, SFE and MWHD, not only enhanced the extraction of essential oil but they also saved time, reduced the solvents use and produced, ecologically, green technologies.

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