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Microwave Assisted Extraction of L-fenchone from *Foeniculum vulgare* Mill. in Hexane

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ABSTRACT

Medicinal plants produce natural chemicals either pure or in the form of mixture of compounds which are diverse in nature. These compounds show activities which are very important for human health such as antioxidant activity, antimicrobial effect, anti-helminths activity, anti-inflammation activity, immune system activation, decrease of platelet aggregation, treatment of diabetes and modulation of hormone metabolism and anticancer property. Demand of these natural compounds is increasing therefore it is needed to develop the more effective methods for extraction of these compounds. *Foeniculum vulgare* Mill. is one of the most commonly used medicinal plants used in cure of many diseases. In present study the experimental design was developed for the extraction of compounds specially "L-fenchone", an essential part of fennel essential oils by Microwave Assisted Extraction (MAE) from seeds of *F. vulgare*. Extraction was done in hexane from different particle sizes (40 μ and 80 μ) of plant material at 600W of microwaves power in 1 and 5 mint. Gas chromatography mass spectroscopy (GCMS) was used for the quantification of L-fenchone. MAE was proved to be an efficient and rapid method of extraction from seeds of *F. vulgare*. Highest amount was extracted from 80 μ , which was 183.33 mg per gram of plant sample. MAE consumed lesser energy by taking lesser time (5 minutes) for extraction. It proved to be cost effective technique as consumed lesser solvents and samples.

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INTRODUCTION

Medicinal plants produce variety of natural compounds which are used in pharmaceutical industries to formulate multiple drugs. Importance of natural compounds is increasing day by day as these are easily available, less expensive, safe and don't have side effects. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. *Foeniculum vulgare* Mill. is one the best medicinal plant used in herbal industry to cure different

diseases. It is herbaceous plant which belongs to the family Apiaceae (Díaz-Maroto et al., 2006). It is native to Southern Europe and grows mostly all over Europe, Middle East, Argentina, Pakistan, China, India, Germany, Greece and Turkey (Goswami and Chatterjee, 2014). Fennel seeds have essential oil in rich amount. Due to its strong flavor and effect this is extracted from seeds often and used for medicinal purposes like as antifungal, aphrodisiac, anti-inflammatory, digestive, carminative, stimulant and antispasmodic. It is also used in

aromatherapy to relief the patients from pain (Lawless, 1995).

As plants contain variety of compounds, for the analysis of a specific compound we need to isolate it. Extraction is the very first step for the isolation and purification of chemical constituents of plants (Huie, 2002). There are number of methods given for isolation of chemicals constituents of plants such as maceration, decoction, infusion etc. Selection of particular method is dependent on type and properties of particular molecule, so when someone wants to isolate and purify the compound, one needs to select the best method for isolation of maximum amount of compound without any damage and it should be cost effective (Mueen, 2008).

Conventional methods consume more time to draw maximum amount of the particular compound (Mukherjee, 2002). But with passage of time scientists have realized the drawbacks of conventional techniques such as needed more time and energy consumption as well as solvents in large amount (Grigonis et al., 2005). Now a days, energy crises is one of the major problems specially in developing countries. Energy crises have encouraged the development of some advance techniques, which can reduce the time and material consumption as well the energy usage, such as Microwave Assisted Extraction (MAE).

Microwave assisted extraction (MAE) is a new technique developed in the late 1980s. It has greatly reduced the time for extraction and increased the yield of natural products of plant origins (Mandal et al., 2008). Microwaves are combination of electric and magnetic waves which oscillates in perpendicular direction to each other, and frequency range is 0.3-300 GHz. These electromagnetic waves have ability to penetrate into certain materials. These can interact with components which show polarity to produce the heat. This produced energy effect the molecules by phenomenon of ionic conduction and dipole rotation. Therefore, only selective materials are heated based on their dissipation factor and dielectric constant (Chan et al., 2011; Routray and Orsat, 2012).

There are some factors which affect the extraction process of microwave assisted extractor, such as type of solvent, solvent composition, power level, temperature, and time of extraction. Temperature and power levels are the most important parameters because each solvent has its own boiling point and solvents are heated in microwave extractor during extraction. These directly

affect the efficiency of microwaves (Eskilsson and Björklund, 2000; Nemes, 2012).

Demand of natural chemical compounds is increasing day by day especially in medical field. Yield of compounds depends on selected method of extraction due to different physiochemical properties of these. The presented work was done to extract L-Fenchone from *F. vulgare* by microwave assisted extraction to establish a cost-effective protocol for lesser consumption of time and energy.

MATERIALS AND METHODS

The presented research work was done in Biochemistry Lab of Botany Department in Lahore College for Women University (LCWU). The experimental work of the research was done in four steps given below.

Preparation of Plant Material

Taxonomically identified seeds of *Foeniculum vulgare* Mill. were taken from National Agricultural Research Center (NARC), Islamabad. Seeds were crushed with help of grinder to form fine powdered plant material. Fennel seed powder was further separated into particles of three different sizes i.e., (40 μ and 80 μ) with the help of sieves of required size. It was done to optimize that which one could give the maximum yield. Then the plant material was stored in airtight jars for further experimental work.

Microwave Assisted Extraction of Compound

Extraction of the targeted compound was the second step of whole process, very crucial step, was done by a modern technique "Microwave Assisted Extraction". In this step, extraction of L-fenchone was carried on Microwave Assisted Extractor (Model MDS-6G). Parameters selected for this technique to optimize the yield of L-fenchone compound were time (1 and 5 min) at 600 W power level of microwave irradiation along with different particle sizes (40 μ and 80 μ) of plant material in hexane. The solvent was added to main inner vessel and placed in outer protective vessel. Temperature measuring tube was used to cover the vessel. Then, it was loaded to the master frame horizontally. Plant sample of 1 g with 20 ml solvent was taken in clean inner vessels. Inner vessels were inserted into the outer protective vessels. Vessel were covered carefully and loaded onto the frame of standard digestion. Master vessel was positioned on its specific place. Sample vessels were loaded according to

sequence to keep the balance of main frame. Then temperature, pressure and time were set to run the extractor. After that obtained extract was stored in vials properly covered and labeled, in Fridge at 4 °C.

Quantification of L-Fenchone

Quantification of compound was done with help of Gas chromatography and Mass Spectroscopy (quantitative analysis). GC-MS analysis of fenchone extracted from seeds of *F. vulgare* was done in Central lab of Lahore College for Women University (LCWU). DB-5 capillary column was used. Starting temperature was 70°C for 2 min then it was 150°C/min to 250°C for 4 min. Helium was the carrier gas with 1ml/min flow rate. Temperature for the injector was 250°C and transfer line temperature was set at 240°C and the ion source at 250°C. Dilution of extract was done by adding methylene chloride in it to prepare 0.5 mg/ml solution. Then 1µl of the prepared solution was injected in DB-5 column to run in system. The MS detection of the compound was done in the electron impact mode. The scanning was done under 45-400 amu range with an ionization energy of 70 eV as modified from (Najdoska et al., 2010). Standard was also run by following same conditions given above.

Analysis of Data

The data produced in result of experimental work was collected and analyzed. Analysis was done by considering many factors and parameters. The mean values of the data of different treatments were compared by applying one-way ANOVA with the help of a computer software i.e. SPSS. Duncan's New Multiple Range Test of post hoc test was applied at significant level of 5 %.

RESULTS AND DISCUSSION

Extraction is the very first step for the isolation and purification of chemical constituents of plants. There are different methods to extract compounds from plant material, MAE is one of the most advanced techniques. Microwave assisted extraction was done from the seeds of *F. vulgare* by using different particle sizes i.e., 40µ and 80µ in hexane. Time effect on extraction process was concerned mainly.

Effect of Time: Results suggested that there was a correlation between the amount of extract and time of irradiation. The maximum amount of extract was obtained at longer exposure to microwaves. The highest extracted amount was 183 mg after 5 min of irradiation. The lowest amount was 100 mg at 1 min of irradiation. It was significant difference between yields, shown that amount of extract had been increased by increasing the time period. Mandal et al. (2008) described that no hurdle was faced when extraction was done with MAE because it consumed lesser time and gave more yield. It was proved a fast technique for the purpose of extraction of compounds from plant samples.

Effect of Particle Size: Particle size of plant material have impact on the extraction of compounds. As the smallest the particle size, the greater the surface to volume ratio which help in deep penetration of the microwaves that ultimately allows more extraction of compounds. The presented results suggested that maximum amount was extracted 80µ particle size of powdered material *F. vulgare* seeds. Guan et al. (2007) also proved that particle size of the plant sample greatly influenced the productivity of essential oil from clove buds.

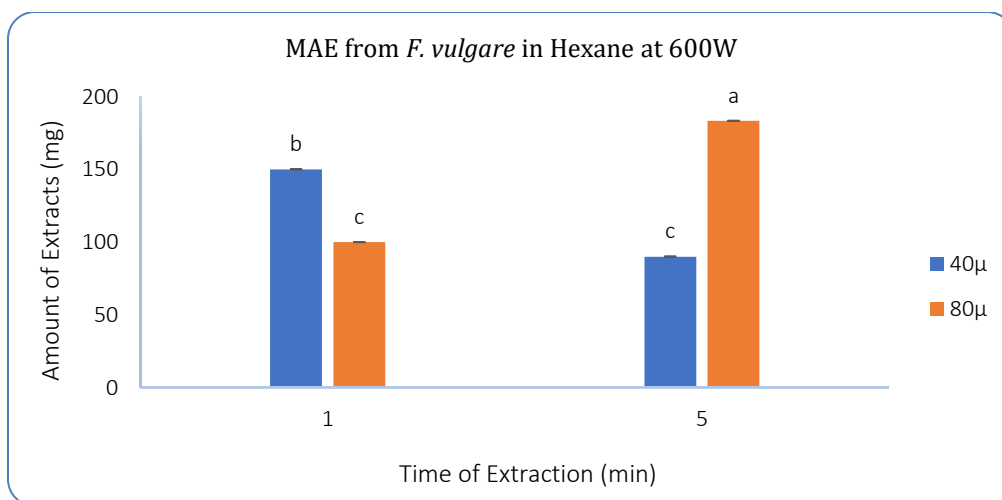


Figure 1. Effect of Time and Particle size on Microwave Assisted Extraction of compounds at 600W from *F. vulgare* Mill.

Quantification of L-Fenchone

Quantification of L-fenchone was done by using Gas Chromatography and Mass Spectroscopy. L-fenchone was detected at retention time of 15.550 as evident from the GCMS chromatogram of standard L-Fenchone. Samples for GCMS analysis were taken from MAE extracts (40 μ and 80 μ sizes of particles with hexane at 600 power level for 1 and 5 minutes of irradiation). The results have been shown in Table 1. Hexane extracted the highest amount of

L-fenchone from the particles with size of 80 μ at 5 minutes of irradiation. On the other hands lowest amount of L-fenchone was revealed by the extract taken from 40 μ particle size at 1 minute of irradiation. There was an increasing trend in amount of L-fenchone by increasing the particle size as well as the time of irradiation with MAE. Molecular formula of L-fenchone is C₁₀H₁₆O. Molecular weight is 152.237 g/mol.

Table 1. Amount of L-Fenchone with hexane in different particle sizes of *F. vulgare*.

Power Level (W)	Time (minutes)	Amount of L-Fenchone (%age) from <i>F. vulgare</i> with particle sizes	
		40 μ	80 μ
600	1	6.23 %	8.78 %
	5	7.93 %	9.35 %

L-Fenchone from 40 μ particle size of *F. vulgare*

When extraction from 40 μ particle size of fennel powder with hexane was done at 600 power level after 1min of irradiation, compounds were identified from Chromatogram shown in Fig. 2(a). These compounds were identified with the help of their retention time. Some compounds were extracted in minor concentration. The major identified compounds were D-limonene, L-fenchone, Cis-anethole, Palmitic Acid, 6-octadecenoic acid, Oleic acid, Stearic Acid and Phthalic acid. The

amount of L-fenchone was 6.23% among all the extracted compounds. When extraction from 40 μ particle size of fennel powder with hexane was done at 600 power level after 5min of irradiation, compounds were identified from Chromatogram shown in Fig.2(b). The major identified compounds were same as given above but the amounts were increased. The amount of L-fenchone was increased to 7.93% among all the identified compounds. The amount of L-fenchone was increased by increasing the timings of microwaves.

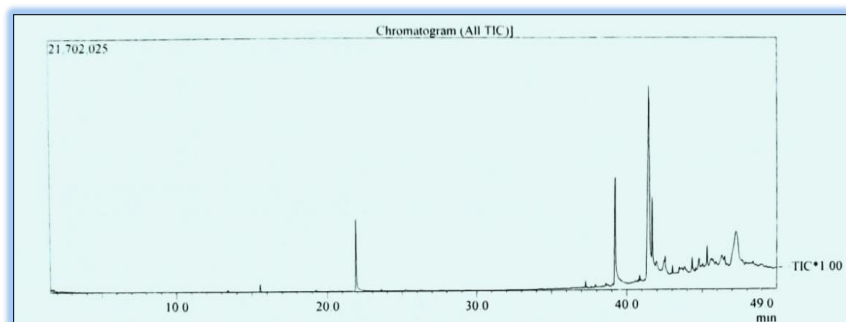


Figure 2(a). Chromatogram of 40 μ particle size of *F. vulgare* seeds after 1 minute.

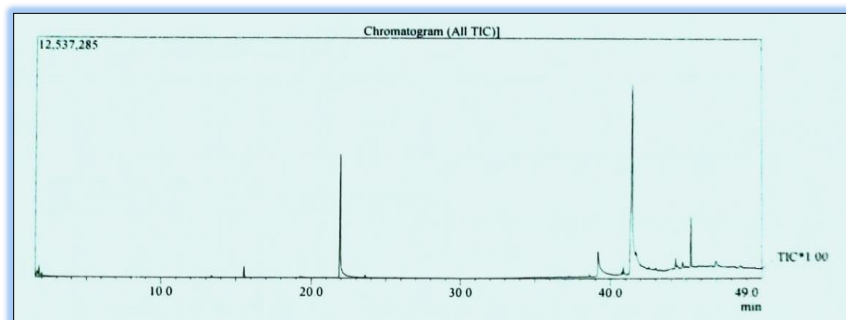


Figure 2(b). Chromatogram of 40 μ particle size of *F. vulgare* seeds after 5 minutes.

L-Fenchone from 80 μ particle size of *F. vulgare*

The extraction was done from 80 μ particle size of *F. vulgare* seeds. The concerned power level was 600 for 1 and 5 minutes of irradiation. Hexane was used as solvent. Chromatogram (Fig. 3(a)) obtained at 1 min of irradiation displays the compounds extracted in sample. The major identified compounds were hexane, l-fenchone, D-limonene, Palmitic Acid, Oleic acid, Stearic Acid and

Phthalic acid. Retention time of these compounds was used for identification. The amount of L-fenchone was 8.78% in sample among all the solvents.

Chromatogram (Fig. 3(b)) obtained at 5min of irradiation displays the compounds extracted in sample. All the compounds were same as mentioned above although the amounts were increased by increasing the time period of exposure to microwaves.

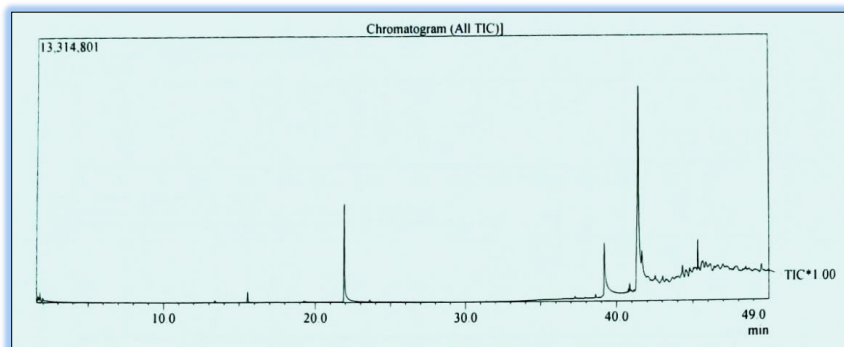


Figure 3 (a). Chromatogram of 80 μ particle size of *F. vulgare* seeds after 1 minute.

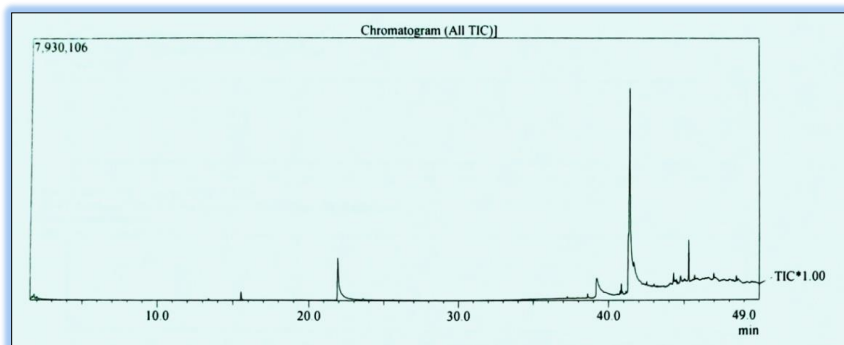


Figure 3(b). Chromatogram of 80 μ particle size of *F. vulgare* seeds after 5 minutes.

CONCLUSIONS

It is concluded from the presented work that MAE is more efficient technique for the extraction of targeted compounds such as L-fenchone from the seeds of *F. vulgare*. It consumes lesser amount of both samples and solvents and saves time too. It reduces the consumption of energy and due to this it has become therapeutically valuable as energy crisis is the world's largest issue. Pharmaceutical companies can use this cost-effective technique for the production of drugs from medicinal plants.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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