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Comparison of the Microwave-Assisted Hydrodistillation with the Traditional Hydrodistillation Method in the Extraction of Essential Oils from *Ferulago angulata* (Schelcht.) Boiss

Jila Asghari[1*], Cheshmnaz Khamoie Touli[1], Mohsen Mazaheritehrani[1] and Mahnaz Aghdasi[2]

[1]Department of Chemistry, Science Faculty, Golestan University, P.O. Box: 49138-15739, Gorgan, Iran
[2]Department of Plant Physiology, Science Faculty, Golestan University, P.O. Box: 49138-15739, Gorgan, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. JS designed the study, wrote the protocol, and wrote the first draft of the manuscript. CKT managed the literature searches, analyses of the study performed the spectroscopy analysis and MM managed the experimental process and MA identified the species of plant. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The evaluation of the chemical constituents and the percentages of extracted volatile oils from the aerial parts of *Ferulago angulata* (Scheleht.) Boiss (Apiaceae) by using MAHD and HD.
Study Design: Extraction of essential oils from *F. angulata* through traditional hydrodistillation (HD) and microwave- assisted hydrodistillation (MAHD) methods.
Place and Duration of Study: Fresh *F. angulata* was collected from the Dalaho Mountain- Kermanshah province (west of Iran) in June 2010.
Methodology: The aerial parts of *F. angulata* was extracted through microwave-assisted hydrodistillation (MAHD) protocol and compared to the conventional hydrodistillation (HD)

**Corresponding author: E-mail: jasghari@gu.ac.ir;*

method.
Results: The optimal extraction conditions for the extraction of cis and trans-β- ocimene by MAHD was as follows: Irradiation power 600 and 650W for Z- and E-β- ocimene respectively at radiation time of 34 min. The essential oil was analyzed by GC and GC/Mass. The 28 and 18 components have been identified in MAHD and HD methods at 34 min and 4h respectively. The final amount of yield in the MAHD was more than the amount extracted by HD (93.54% compare to 75.8). The major components, which were found in aerial parts, were E- and Z-β- Ocimene by both methods.
Conclusion: We found yield/ efficiency, chemical composition, quality of the essential oils and cost the operation MAHD was superior in terms of saving energy and extraction time.

Keywords: Ferulago angulata; essential oil; microwave-assisted hydrodistillation (MAHD).

1. INTRODUCTION

Ferulago angulata (local name chavir in Iran) is a perennial shrub with 60-150 cm height (Mozaffarian, 1983; Zaargar, 1981), olialypetal, yellow colored flower, compound umbel, compound thin narrow leaves which grows at altitudes of 1900-3200 m (above sea level) (Javadnia, et al., 2006; Masomi et al., 2001). It is native species in western parts of Iran. Except for Iran it is distributed in the east of Turkey, North of Iraq, Greece, Serbia and Macedonia. Some studies have been also performed in these countries (Can, 2002; Husnu and Demiri, 2002). The genus *Ferulago* comprises thirty five species world wide of which seven species are found in Iran. *F. angulata* has two subspecies which includes subsp. *angulata* (schlecht.) and subsp. *carduchorum* (Boiss & Hausskn). It has been used medicinally as tonic, remedy of digestive pains, seductive, aphrodisiac properties, hemorrhoids (Taran, et al., 2010; Akhlaghi, 2008; Ghasempour, et al., 2007) and is good drug for ulcers, snake bits, spleen and headache (Javidnia, et al., 2006). Furthermore, *F. angulata* contains a strong preservative substance for diary and is effective for digestive system diseases (Zos, et al., 2000). The extracted essence is also useful for cosmetic industries and perfumery. There are some reports on the anti-oxidative and anti-diabetic effects of this plant. A number of papers are available reporting both bioassays and component analysis of *F. angulata* which was widespread in high latitude of North West and central parts of Iran (Amirghofran et al., 2009). Traditionally in Iran, it was being added to diary and oil ghee in order to give them a pleasant taste and prevents their decay.

Extraction of essential oil of *F. angulata* has conventionally been done through hydrodistillation and extraction by solvent. However, the high temperature and longer reaction times limited the use of this method. More recently by supercritical extraction method through carbon dioxide as a new method reported (Sodeifian et al., 2011). Subsequent product analyses and its major chemical compounds and their percentage have been reported (Adams, 2007; Ghasempour et al., 2007a, b; Hadjiakhoondi, 2005; Javidnia and Miri, 2006; Khanahmadi and Janfeshan, 2006; Khalighi-Sigaroodi; Rezazadeh, et al., 2003; Rustaiyan, et al., 2002; Sefidkon and Omidbaigi, 2004). But, search for more efficient extraction methods of essential oils from the plants notably those which are easy and safe have attracted much interest particularly from the viewpoints of green chemistry. Green chemistry approaches are significant due to the reduction of time, lowering of energy costs, high yield and environmental economic factors. In continuation of our former studies on using of microwave-assisted hydrodistillation (MAHD) (Asghari and Mazaheritehrani, 2010; Asghari et al., 2011; Ondruschka and Asghari, 2006; Jahanshahee et al., 2010) as a powerful tool for the extraction of several phytochemicals, in this work, much effort has been

focused on the extraction of essential oils from the flowering aerial parts of *F. angulata* which is harvested in Dalahoo Mountains (Kermanshah province) by using MAHD method. We investigated the effect of microwave on the extraction yield of compounds and the chemical constituents of the oils under optimized conditions and the results were compared with those obtained by the traditional hydrodistillation (HD) method using Clevenger-type apparatus. The operational parameter such as time of radiation, temperature and power were optimized using single factor experiments. The extracted oils using MAHD and HD were analyzed by GC and GC/MS to determine their chemical constituents. To the authors knowledge it is the first report on the extraction of volatile oils from the aerial parts of *F. angulata* by MAHD.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh *F. angulata* was collected from the Dalaho Mountain- Kermanshah province (west of Iran) in June 2010. A voucher specimen was deposited in the herbarium of the Faculty of Sciences, Golestan University, Gorgan, Iran. The harvested materials from the flowering aerial parts (Fig. 1) were air- dried in shaded place at ambient temperature (25ºC) and air circulation. The air- dried material was mixed and grounded into a homogeneous fine grade powder by disintegrator.

Fig. 1. The Scheme of *F. angulata*

2.2 Extraction Procedure

2.2.1 Microwave- assisted extraction

The microwave extractor (Microsynth.) was equipped with the Clevenger type apparatus, temperature controller and time controller (Fig. 2). In the MAE experiments, 750 mL water was added to 50 g of dried sample powder placed in balloon. A magnetic stirrer bar was placed into the vessel for through mixing of the solvent with the sample. The extraction was carried out with different extraction conditions: the microwave vessel was irradiated at a 20-45 min and irradiation power (400- 650 W) (Table 1). The extracted pale-yellow colored essential oil was trapped with 10 mL $CHCl_3$ as an organic collecting solvent. The essential oils were dried over anhydrous sodium sulphate and kept in refrigerator in dark glass vials at

4ºC until analysis. Essential oil content was obtained 3.8% as volume per weight (V/W) based on the dry weight of plant material.

Fig. 2. Adapted microwave distillation apparatus

2.2.2 Hydro distillation in a clevenger-type apparatus

100 g of the aerial parts of *F. angulata* powdered, mixed with 1200 mL of distilled water and the essential oils hydro-distilled in a Clevenger-type (Farmacupe) apparatus for 3h. The essential oils were dried over anhydrous Na_2SO_4 and stored at 4ºC in the dark. Essential oil yield was 1.7% based on the dry weight of the sample.

2.3 Analysis Method

2.3.1 Gas chromatography (GC)

The analysis of the oil was carried out using a GC (Agilent Technologies 6890 N) equipped with a FID detector and HB-5 fused capillary silica columns (30 m long, 0.25 mm id and 0.25 µm film thickness). The samples, dissolved in ethanol were injected (20µL**)** in the split less mode into helium carrier gas with a constant flow rate of 1.1 mL/min. The injector and detector temperature were 250ºC and 280ºC respectively. The column temperature was set at 50ºC for 2 min, and then programmed until 160ºC at a rate of 10ºC/min isothermal at this temperature for 3 min, and then finally increased at the rate of 5ºC/min to 280ºC. Quantitative data of target compounds (E- and Z-β- ocimene) in the extracted product were determined from the appropriate calibration curve.

2.3.2 Gas chromatography- mass spectrometry analysis

GC-MS analysis was carried out using Agilent technology (Avondale model), mass selective detector (MSD) and directly coupled to gas chromatograph. A HB-5 capillary column (30 m long, 0.25 mm id and 0.25 µm film thickness) was employed, using helium carrier gas. The temperature programs were the same as described under GC. Retention indices were determined by using retention times of n-alkanes that had injected the oil under the same chromatographic conditions. The retention indices for all compounds were determined according to the Van Den Dool method using n-alkanes as standard (Van Den Dool and Kratz, 1963). The compounds were identified by comparison of retention indices with that

reported in the literature and by comparison of their mass spectra with Wiley library or with published mass spectra (Massada, 1976; Adams, 2001).

3. RESULTS AND DISCUSSION

Hydrodistillation of the flowering parts of *F. angulata* gave a pale yellow liquid with a yield of 3.8% and 1.7% based on dry weight by MAHD and HD, respectively. The gas chromatograms of the essential oils from both methods are presented in Figs. 7 and 8. Tables 2 and 3 were showing the identified components of the extracted oil by MAHD and HD which were 28 and 18 components respectively. The major constituents of the essential oil were found to be monoterpene and sesquiterpene hydrocarbons. The main component of monoterpene with their percentages including α- pinene (8.55%, 6.01%), Z-β- ocimene (33.91%, 26.78%), boronyl acetate (5.54%, 3.08%) and γ-tripinene (6.26%, 5.81%) by MAHD and HD methods respectively. Germacren-D as major sesquiterpene was also identified with their percentage (4.01%, 3.71%). Z-β- ocimene was the major constituent of the essence oil from both methods.

Gas chromatography- mass spectroscopy analysis of the extracted essential oils indicated that with increasing microwave power, level of the oil increases and compared with the thermal method great differences were observed. As is shown in Table 1, the extraction of essential oils was the highest rate at 70 min and power 650 w.

However, the maximum yields of target compounds in the extracted oil of *F. angulata* by using microwave radiation at different time and power were obtained at 34 min and power of 650w for cis β- ocimene and 600w for trans- β- ocimene (Figs. 3-6).

Table 1. The result of different factor on the extraction yield of essential oil

Microwave power (W)	Extraction time (min)	Yield (mg/g)
400	45	1.13
500	45	1.15
600	45	1.19
650	70	3.8

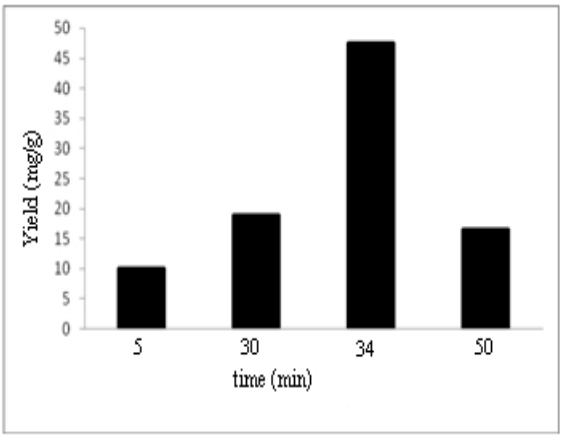

Fig. 3. Effect of microwave irradiated time on the extraction yield of Z-β- Ocimene from the flower of *F. angulata* at irradiation power 650 w

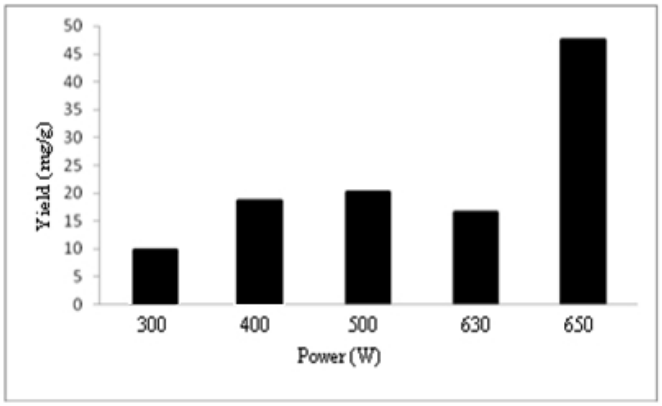

Fig. 4. Effect of microwave power on the extraction yield of Z-β-Ocimene from the flower of *F. angulata*

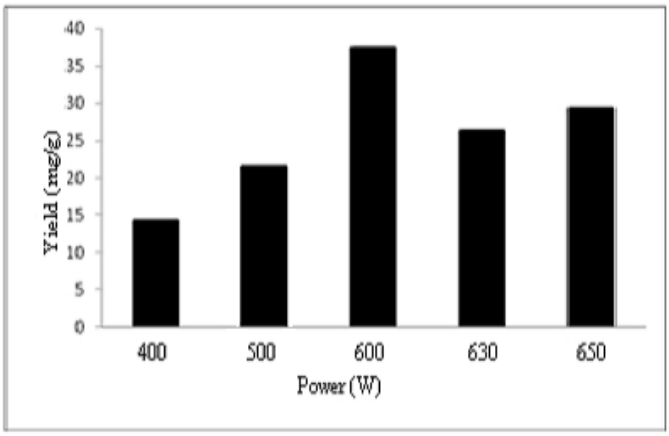

Fig. 5. Effect of microwave power on the extraction yield of E-β-Ocimene from the flower of *F. angulata*

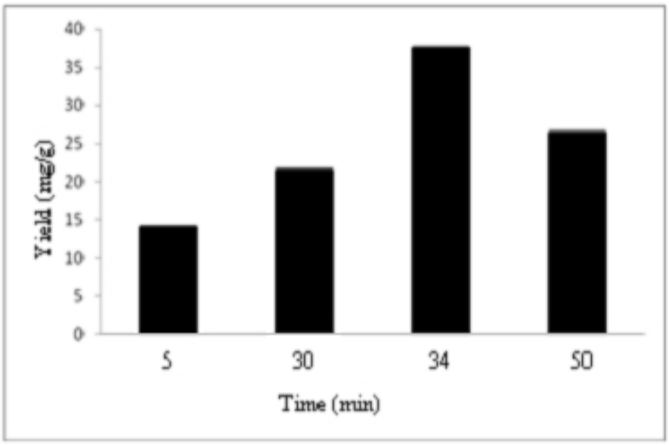

Fig. 6. Effect of microwave irradiated time on the extraction yield of E-β-Ocimene from the flower of *F. angulata*

Table 2. Chemical compositions of the essential oil of aerial parts of *F. angulata* from Dalahoo Mountain (Kermanshah province) extracted by microwave- assisted hydrodistillation (MAHD) method (650 W, 34 min)

Entry	Compounds	Retention Index (RI) [a]	Area (%)
1	α-Thujene	931	0.63
2	α-Pinene	937	8.55
3	Camphene	950	1.51
4	Sabinene	973	1.11
5	β-Pinene	980	1.31
6	β-myrcene	991	3.91
7	*o*- ocimene	1018	5.08
8	**(Z)- β -ocimene**	**1031**	**33.91**
9	(E)- β -ocimene	1040	2.26
10	γ-Terpinene	1052	6.26
11	α-Terpinolene	1063	1.32
12	Linalool	1085	0.38
13	Allo ocimene	1120	0.89
14	cis-Verbenol	1132	1.1
15	Trans- verbenol	1135	4.05
16	*p*-mentha-1,5-dien-8-ol	1151	0.89
17	terpinen-4-ol	1168	0.4
18	α-Terpineol	1178	0.69
19	Bornyl acetate	1275	5.54
20	Pinocarveyl acetate	1297	0.49
21	α-copaene	1374	0.53
22	methyl eugenol	1383	1.08
23	β- Bourbonene	1385	0.79
24	γ-Curcumene	1478	1.54
25	Germacrene- D	1487	4.01
26	*m*-cymene	1496	2.26
27	Bicyclogermacrene	1501	0.73
28	(+) - Spathulenol	1576	2.32
29	Total percentage		93.54

[a] *retention index to the normal alkane (C6-C18) 0n HB-5 column*

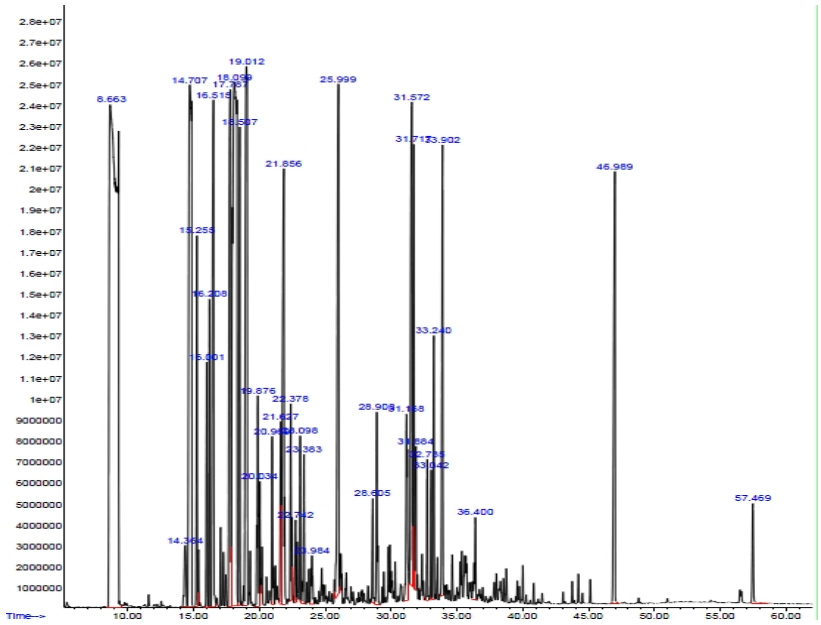

Fig. 7. GC- Mass chromatogram of the essential oil extracted from *F. angulata* by MAHD

The results of extracted products by distillation method in 4 hours were shown in Table 3.

Table 3. Chemical compositions of the essential oil of aerial parts of *F. angulata* extracted by hydrodistillation method

Entry	Compounds	Retention Index (RI) [a]	Area (%)
1	toluene	792	-
2	α-Pinene	935	6.01
3	Camphene	950	8.83
4	Sabinene	973	0.69
5	β-Pinene	980	0.87
6	β-Myrcene	991	2.67
7	*o*- Ocimene	1018	3.67
8	*p*-Cymene	1025	5.75
9	**(Z)- β -Ocimene**	**1031**	**26.78**
10	(E)- β -Ocimene	1040	4.84
11	γ-Terpinene	1052	5.81
12	α-Terpinolene	1063	0.73
13	Allo ocimene	1120	0.69
14	cis-Verbenol	1132	0.78
15	*p*-Mentha-1,5-dien-8-ol	1151	0.89
16	Bornyl acetate	1275	3.08
17	Methyl eugenol	1374	0.54
18	Germacrene- D	1487	3.17
19	Total percentage		75.8

[a] *retention index to the normal alkane (C6-C18) HB-5 column*

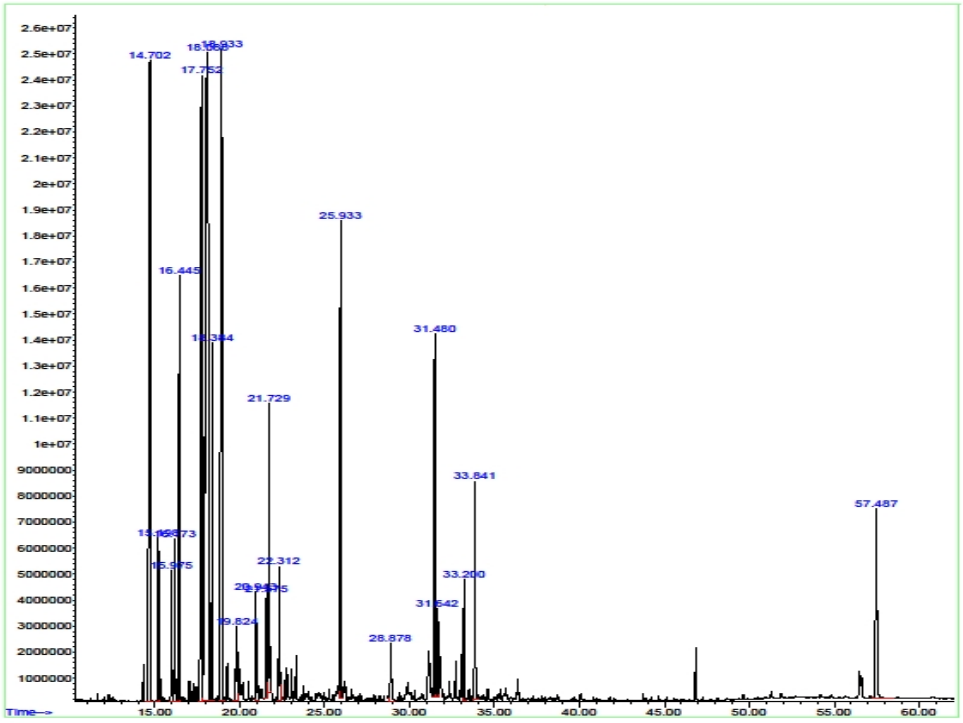

Fig. 8. GC- Mass chromatogram of the essential oil extracted from *F. angulata* by HD

Compared to the results of previous research work which was carried out in Kermanshah province on the same oil by Clevenger showed that although the major components are the same but the percentage of Z-β- Ocimene in the extracted oil in the present work is greater [33.91% as compared to 14.41% (Sodeifian et al., 2011)]. The reason is due to microwave radiation effect that can penetrate into biological materials and creates heat by interaction with polar molecules. Because, water inside the inner cells of plant tissues can absorb microwave energy and leads to over-heating thus, damage into the cells spread. This facilitates removal of active compounds, and ultimately improves their recovery significantly and increases the efficiency of the extraction process. In addition, migration of ions in solution leads to increased penetration of solvent into the plant tissue and facilitate the release of intracellular compounds. Therefore, the ripples of energy largely depend on the dielectric properties of the solvent and the plant tissue.

4. CONCLUSION

The extraction of essential oil from *F. angulata* (Schlecht) Boiss by microwave method under optimal conditions provided another example of the advantages of utilizing microwave irradiation at a heat source in isolating essential oils. Much shorter extraction times used in the process, give high yield of oil and Z- β-ocimene. We found MAHD as an alternative method for the extraction of plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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