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Full Length Research Paper

Microwave- assisted Hydrodistillation of Essential Oils from *Echinophora platyloba DC*

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Microwave-assisted hydro distillation (MAHD) protocol, applied optimal extraction condition (650 W, 35 min) was used to extract an essential oil from the aerial part of *Echinophora platylabo DC,* which was collected from the Binaloud Mountain- Nishapor city. To evaluate the effect of microwave energy on the yield and composition of essential oil, MAHD method results were compared to those obtained by conventional hydro distillation (HD). The volatile compounds obtained by both methods were analyzed by Gas chromatography (GC) and GC-MS. It was indicated that, the yield of extracted essential oil using MAHD method is about 40% higher than regular HD method. In addition, the use of microwave irradiation not only influence the composition of the essential oil and the quantity of major compounds (trans-β- ocimene and γ –decalactone) but the extraction time in comparison to the HD method is shorter (35 min compared to 3 hours in HD). It was found that MAHD method is a good alternative for the extraction of essential oil in terms of the quality of the essential oil cost of the operation and saving energy. Thus we suggest that MAHD method can be considered as a green technology.

Key words: Microwave, essential oil, E-β- ocimene and γ–decalactone, *Echinophora platyloba DC.*

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds derived, which have made large contribution to human health and well being (El Astal *et al.,* 2005). Natural products have served as a major source of drug for centuries and due to side effect of chemical drugs, attention drifting to pharmaceutical plants has been increased (Clark, 1996).

Echinophora platyloba DC (local name is Khosharizeh) is a native plant commonly widespread in high latitude (1400 to 2000 m above sea level) of Northwest Iran. It is represented in the flora of Iran by four species including two endemics (Heywood, 1985; Mozaffarian, 1994, 1996). There are *E. sibthorpiana Guss., E. orientalis* and the two endemic species, *E. platyloba DC* and *E. cinerea (Boiss)* Hedge et Lamond (Mozaffarian, 1996). Therapeutically, this plant and its essential oils are used as antiseptic, antimicrobial and antifungal (Mazloomifar *et al.,* 2004; Asghari *et al.,* 2003; Avijgan *et al.,* 2005, 2006; Farsi *et al.,* 2006). The genus *E. chinophora* has been the subject of scant phytochemical and biological investigation. A number of papers are available reporting both bioassays and component analysis of *E. platyloba*, which was widespread in high latitude of northwest and central parts of Iran (Asghari *et al.,* 2003; Mazloomifar *et al.,* 2004; Hassanpouraghdam and Sepehri, 2009; Avijgan *et al.,* 2006). Recently, essential oil of this plant has been used as a natural preservative in dairy products industries (Mazloomifar *et al.,* 2004; Asghari *et al.,* 2003). This application is inspired by the long term traditional uses of *E. platyloba DC,* as flavoring materials in yoghourt and cheese and also to prevent the fungal

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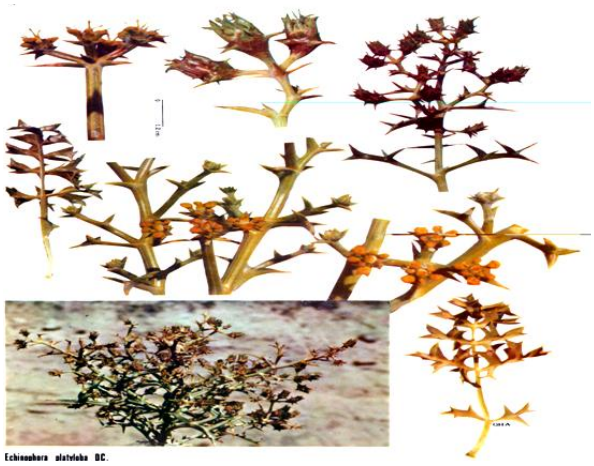

Figure 1. Photo of *E. platyloba*.

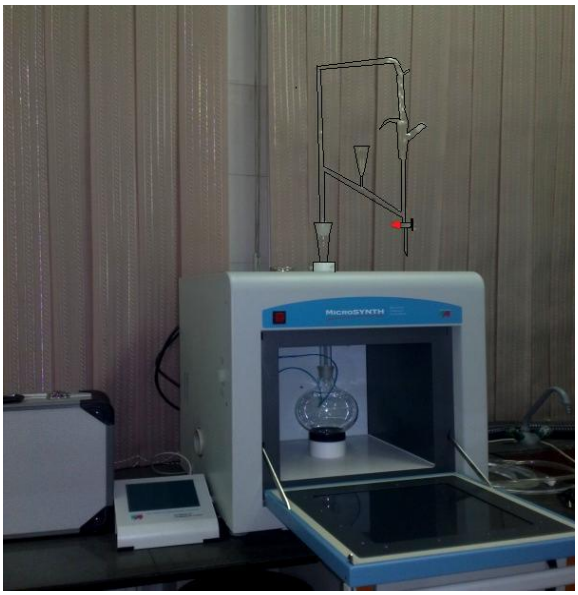

Figure 2. Adapted microwave distillation apparatus.

growth on some food like tomato paste and pickled cucumber in Iran (Sadrai *et al*., 2002). Essential oil of *E. platyloba DC* has been the subject of some studies conducted in the past (Mazloomifar *et al*., 2004; Asghari *et al.*, 2003) were usually extracted by Clevenger type apparatus. Asghari *et al.* reported that (E)-β- ocimene (67.9%), 2- furanone (6.2%) and myrcene (6%) were the main components of *E. platyloba* essential oil from Isfahan province in the central of Iran and Mazloomifar *et al.,* (2004) reported (E)-β- ocimene (49.9%), γ- decalactone (8.4%), α- pinene (6%) and linalool (5.6%) as the principle constituents of *E. platyloba* essential oil from Tehran province. The other report, (Z)-β- ocimene (38.9%) and α- phellandrene (24.2%) were the principle monoterpene hydrocarbon constituents of essential oil from Maragheh district in Northwest Iran reported (Hassanpouraghdam and Sepehri, 2009).

Microwave-assisted hydro distillation (MAHD), which combines microwave and traditional hydro distillation, has gained wide acceptance as a powerful tool for sample preparation of solid matrices and many microwave-assisted essential oil extractions from several plants and subsequent product analyses have been reported (Lucchesi *et al.,* 2007; Ferhat *et al.,* 2006; Deng *et al.,* 2006; Wang *et al*., 2006; Chemat *et al.*, 2006; Hemwimon *et al.,* 2007; Tigrine-Kordjani *et al.,* 2006; Lucchesi *et al.,* 2004). In contrast to traditional extraction techniques "steam distillation, hydro distillation and water distillation", it possesses more advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost and lower decomposing of the target species.

MATERIALS AND METHODS

Plant material

The *E. platyloba DC* (Figure 1) was collected from the Binaloud Mountain- Nishapor city at the altitude of 1400 m in April, 2010. A voucher specimen was deposited in the herbarium of the Faculty Sciences, Golestan University, Gorgan, Iran. The harvested materials from the flowering aerial parts were air- dried in shaded place at ambient temperature (25°C) and air circulation. The air-dried material was mixed and grounded into a homogeneous fine grade powder by disintegrator.

The aim of the present work to evaluate the feasibility of microwave- assisted extraction in Clevenger apparatus for extraction of essential oil from the aerial part of *E. platyloba*, which is harvest in Nishabor area. We investigated the effect of microwave on the extraction yield of compounds in essential oil under optimized condition of MAE, compared to hydro distillation using Clevenger-type apparatus. The operational parameter such as time of radiation, temperature and power were optimized using single factor experiments. We also reported the compounds isolated from essential oil by gas chromatography mass spectrometry (GC-MS) and GC. It is noticeable so far that there have not been any phytochemical studies on this plant by MAHD.

Microwave- assisted extraction

The microwave extractor (Microsynth.), equipped with Clevenger-type apparatus, temperature controller and time controller, was installed as shown in (Figure 2). 750 ml water was added to 50 gr of dried powered sample in the round-bottomed flask. A magnetic stirrer bar was used to enhance through mixing of the solvent (water) with the sample. The extraction was carried out at different extraction conditions: the microwave vessel was irradiated at a 20 to 45 min and irradiation power (400 to 700 W). The extracted pale-yellow colored essential oil was trapped with $CHCl_3$ as an organic collecting solvent. The oil was dried over anhydrous sodium

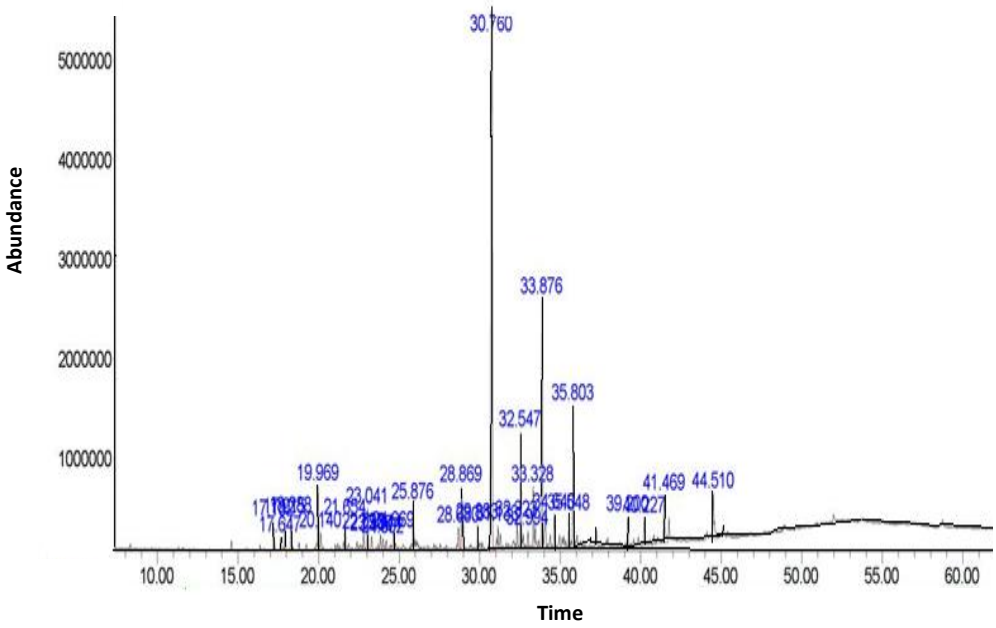

Figure 3. GC-Mass chromatogram of the essential oil extracted from *E. platyloba DC* by (MAHD).

sulphate and kept in dark glass vials, in refrigerator at 4°C until analysis. Essential oil content was calculated as volume per weight (V/W) based on the dry weight of plant material.

Gas chromatography (GC)

The analysis of the oil was carried out using a GC (Agilent technologies 7890 N) equipped with a FID detector and HB-5 fused capillary silica columns (30 m, 0.25 mm, 25 µm film thicknesses). The samples, dissolved in ethanol were injected (20 µL**)** 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 250 and 280°C respectively. The column temperature was set at 50°C for 2 min, and then programmed until 160°C at a rate of 10°C/min isothermal at this temperature for 3 min, and the finally increased at the rate of 5 ^{o}C/min to 280°C.

Gas chromatography- mass spectrometry analysis

GC-MS analysis was carried out on an Agilent technology (Avondale model), mass selective detector (MSD), and directly coupled to gas chromatograph. A HB-5 capillary column (30m, 0.25 mm, film thickness 0.25 µm) was employed, using helium carrier gas. The temperature programs were the same as described under GC.

RESULTS AND DISCUSSION

Microwave- assisted hydro distillation of the flowering aerial parts of *E. platyloba DC* gave oily pale yellow liquid with a yield of 0.4% (V/W) based on dry weight. They are separated by GC-MS, which revealed the presence of 24 compounds (96.4% of the total oil) under optimal condition (Table 3). The compounds were identified by comparing their retention time and kovats indexes with that of literature. The quantitative estimation of each peak was made by estimating area of the peak by computer attached by GC-MS instrument.

Optimization of microwave-assisted extraction condition of essential oil

Since different factors affect the MAE process, the optimization of the experimental conditions represents a critical step in the development of a MAE method (Pan *et al.*, 2008). Thus by MAE method, it is possible to reduce the time and cost for experimental investigations and improve the performance characteristics.

In the present study, in order to evaluate the microwave power effect on the yield of essential oil and target molecule extraction was carried out under different time and microwave power as summarized in Table 1. It was clear that, with increasing the microwave irradiation to 700 W, the yield of essential oil increased. However this power irradiation decreased the yield of Trans-β-ocimene (Table 2). This may be due to different materials having different appropriate microwave irradiation power and molecular interaction structure which can affect the

Table 1. Effect of microwave power on essential oil yield.

Test	Microwave power (W)	Extraction time (min)	Yield (%)
1	300	45	0.1
2	400	45	0.12
3	500	45	0.15
4	600	45	0.2
5	650	45	0.4
6	700	45	0.45

Table 2. Effect of microwave power on the extraction yield of E-β-Ocemine from *E. platyloba*. extracted for 45 min.

Power (W)	Area (%)
500	21.26
600	43.49
650	58.63
700	35.78

extraction yield of Trans-β- ocimene. Hence, 650 W was chosen as the appropriate microwave irradiation power.

Extraction time is also a factor studied to increase the effectiveness of extraction of essential oil and Trans-β-ocimene. Studies were performed at different times for example 20, 30, 35 and 45 min. We shown as depicted in Table 1, with increasing the irradiation time from 20 to 45 min, the extraction of essential oil increased and reached its maximum at 45 min. However, the extraction yield of Trans-β- ocimene decreased with this irradiation time. A possible reason may be due to the decomposition of Trans-β- ocimene at long irradiation time. Thus 35 min considered as the appropriate irradiation time.

Identification and quantifications of the compounds

The percentage of essential oil components was evaluated from gas chromatography peak areas, without using correction factors. Identification of components in the volatile oil was based on a comparison of their mass spectra and retention time with those of authentic compounds and computer matching with Nist and Wiley library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature (Adams, 2001; Avijgan *et al*., 2006). Quantitative data of the target compounds that is, Z- and E- β- Ocimene in the extracted product were determined from the appropriate calibration curve. Results of chromatographic analysis of *E. platyloba* oil are presented in Table 3. In total 24 components (96.4% of total oil) were identified in the essential oil *E. platyloba DC*. The major components were γ-decalactone (43.9%), trans-β-ocimene (21.6%) and cis-β-ocimene (4.2%).

To evaluate the microwave effect in the extraction of essential oils, the results of MAHD, at optimal conditions, were compared with those of hydro distillation (HD) using the conventional Clevenger in terms of extraction time, extraction yield and efficiency. The yield of essential oil obtained from hydro distillation, for 3h, was 0.03% and the amounts of identified components recognized in the oil were 18 compounds (82.21% of the total oil). The yield of E- β- ocimene and γ–decalactone were found to be 8.87 and 20.67%, respectively.

Taking into account the chemical profile of the essential oil of wild- growing *E. platyloba* DC plants from Binaloud Mountain-Nishapor and reports of other scientists, it seems that there are significant qualitative and quantitative differences between the chemical profile of the essential oil (Asghari *et al*., 2003; Hassanpouraghdam and Sepehri, 2009; Mozloomifar *et al.,* 2004). These variation combinations are due to the diverse climatic and geographic differences of *E. platyloba* DC plant wild habitats in addition to different harvesting times and volatile oil extraction procedures.

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Table 3. The chemical compositions of the essential oil of *E. platyloba* aerial parts extracted by microwave- assisted hydro distillation (MAH) method (650 W, 35 min).

Entry	Compounds	Retention Index (RI)[a]	Area (%) MAH
1	α-Pinene	936	0.37
2	β-Pinene	978	0.77
3	β-myrcene	989	0.22
4	Limonene	1025	0.51
5	(Z)- β -ocimene	1034	4.23
6	(E)- β -ocimene	1046	21.56
7	γ-Terpinene	1061	0.33
8	Linalool	1095	0.54
9	Allo ocimene	1131	1.96
10	Cis-verbenol	1133	1.75
11	p-Cymene-8-ol	1185	0.5
12	α-Terpineol	1189	1.71
13	Cis-3-hexenyl 2-methyl butanoate	1227	1.41
14	Carvlacro	1278	0.38
15	Bornyl acetate	1291	1.35
16	Methyl eugenol	1404	3.01
17	Trans-caryophyllene	1418	0.83
18	γ -Decalactone	1466	43.96
19	2-furanone	1471	1.24
20	Cis-Nerolidol	1533	0.54
21	Cis-3-Hexenyl benzoate	1568	2.1
22	Spathulenol	1577	1.9
23	Caryophyllene oxide	1583	2.86
24	γ-Dodecalactone	1678	2.27
25	Total percentage		96.4

[a] Retention index to the normal alkane (C6 to C18) on HB-5 column.

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