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COMPARISON BETWEEN THE ESSENTIAL OIL AND SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF *MENTHA PIPERITA L.* CULTIVATED IN IRAN

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ABSTRACT

The chemical compositions of the essential oil and the volatile concentrate of *Mentha piperita L.* (Labiatae) obtained by supercritical carbon dioxide extraction (SFE) at 35°C and 100 atm were compared using GC/MS. Whereas twenty four components were identified in the essential oil, only seven compounds, including the main compounds of the peppermint oil were isolated by the SFE. The percent of major components of the oil and the extract were: Menthol (31.53 and 48.39), Menthone (23.37 and 26.68) and Isomenthone (11.11 and 6.85), respectively. From these results it may concluded that the SFE method supply a selective essential oil extract.

Keywords: *Mentha piperita*, Labiatae, SFE, Essential oil, Menthol, Menthone

INTRODUCTION

Herbs are commonly used in folk medicine, as food and beverage flavoring, and as fragrances in cosmetic products. In Iran, the genus *Mentha* (Labiatae) is represented by *M. aquatica, M. pulegium, M. spicata, M. longofolia* (with 7 varieties) and *M. mozaffariani,* where the latter one is endemic (1). The hybrid *Mentha piperita* have been cultivated in Iran.

Peppermint oil is an important raw material for flavouring confectionary, liquors, toothpaste and many other products (2).

Supercritical fluid extraction of essential oils from vegetable substances is in principle, an alternative process with respect to traditional extraction based on liquid solvents or distillation methods. SFE is developing rapidly because of reduced usage of organic solvents and increased throughput (via decreased extraction time and fewer sample handling steps). Carbon dioxide is probably the most widely used supercritical fluid because of its low critical temperature and pressure (31.1°C and 74.8 atm), which makes it an ideal solvent for extraction of thermally labile materials. Also because carbon dioxide is nontoxic, nonflammable, it is environmentally preferred over organic solvents. Attempts to extract peppermint oil by SFE have been described by several authors (3-5). In this study, the SFE of *Mentha piperita L.* cultivated in southwest of Iran, is reported. The results are compared with those obtained by conventional hydrodistillation.

MATERIAL AND METHODS

Plant material

The aerial parts of fully flowered *Mentha piperita L.* which were collected in July 2000 from Fadak Garden in Dezfool (Province of Khuzestan, southwest of Iran), wae identified by local taxonomist and the Research Institute of Forest and Rangelands (Tehran, Iran). A voucher specimen was deposited in the herbarium of Faculty of Pharmacy, Tehran Medical Sciences University (Tehran, Iran). The essential oil was prepared by hydrodistillation of the air–dried aerial parts, using Clevenger type apparatus. The volatile distillate was dried over anhydrous sodium sulphate and refrigerated till time of analysis. The yield of the oil was 2% w/w.

Reagents

Carbon dioxide of minimum purity 99.99 % was obtained from Sabalan Company (Tehran, Iran). HPLC grade dichloromethane was purchased from Aldrich.

Experimental apparatus

A suprex MPS/225 system (Pittsburg, PA) in the SFE mode was used for extraction. In this study, extraction was accomplished by the use of an 8 ml

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volume extraction vessel. The SFE extraction conditions were: Temperature, 35 °C and Pressure, 100 atm. A Duraflow manual variable restrictor was used in the SFE system to collect the extracted analytes. The supercritical carbon dioxide flow rate through the restrictor was approximately 0.35±0.05 ml/min. The oil was analyzed by GC/MS on a Hewlett–Packard 6890 gas chromatograph coupled with a mass detector (Hewlett–Packard, model 6973 HP). The column used for oil separation was a fused silica HP–5 column, 30 m x 0.25 i.d. x 0.32 µm film thickness. The mass spectra were obtained by electron ionization at 70 eV. The oven temperature program was 60 °C (3 min) isotherms, and then to 250 °C at 5 °C/min, the injector temperature was 250 °C. The carrier gas (Helium) flow rate was 1 ml/min. The sample (1 µl) was injected with a splite ratio of 1/10. The compounds were identified by comparison of their retention time and retention indices with authentic compounds and with data in the literatures (6), as well as their mass fragmentations with authentic or published data (7-8).

Supercritical fluid extraction (SFE)

Exactly 2.0000 g of powdered plant material was weighted and filled into the extraction vessel. The plant was then extracted with supercritical carbon dioxide under the above conditions. The extracted analytes were collected in 3 ml dichloromethane in a 5 ml volumetric flask. The final volume of the extract was adjusted to 5 ml with dichloromethane.

RESULTS AND DISCUSSIONS

The constituents of the hydrodistillation essential oil and the supercritical fluid extract are shown in Table 1.

Twenty four compounds which represented about 99.69% of the total composition of the essential oil were identified. The major constituents were: menthol (31.53%), menthone (23.37%), iso-menthone (11.11%), 1,8-Cineol (10.67%), pulegone (4.90%), menthyl acetate (3.52%), β-caryophyllene (2.65%) and germacrene–D (2.32%).

Table 1. Constituents of the essential oil and the supercritical fluid extraction of *Mentha piperita L.*

Peak No	Compound	Essential oil	SFE	Retention indice
1	α-Pinene	1.79	---	935
2	Sabinene	0.43	---	970
3	β–Pinene	1.79	---	974
4	β–Myrcene	0.18	---	983
5	α–Terpinene	0.37	---	1013
6	1,8 Cineol	10.67	6.32	1023
7	γ–Terpinene	0.65	---	1055
8	Sabinene hydrate	0.46	---	1060
9	Linalool	0.38	---	1087
10	Menthone	22.37	26.68	1139
11	Isomenthone	11.11	6.85	1148
12	Menthol	31.53	48.39	1171
13	P-Menthan-3-ol	0.34	---	1175
14	α–Terpineol	1.02	---	1178
15	Pulegone	4.90	---	1235
16	Piperitone	0.77	---	1241
17	Menthyl acetate	3.52	2.52	1301
18	β–Bourbonene	0.39	---	1386
19	β–Caryophyllene	2.65	4.05	1424
20	β–Farnesene	0.46	---	1448
21	Germacrene–D	2.32	5.19	1480
22	Germacrene–B	0.27	---	1492
23	Caryophyllene oxide	0.17	---	1575
24	Viridiflorol	0.84	---	1580

In a previous work, pressure about 90 bar and temperature within the limits of 40 °C have been recommended for the extraction of essential oils with supercritical carbon dioxide, since their solubility are sufficient at this conditions, but solubility of fatty oils, resins and waxes present in the plant material are negligible (9). The operating conditions in present study were: T=35 °C and

P=100 atm. By employing these conditions, SFE extracted only 7 compounds, representing 99.99% of the total extract compositions. While these components were also appeared in the hydrodistillation essential oil, the percentages of some of them were higher: Menthol (48.39%), Menthone (26.85%), β–Caryophyllene (4.05%) and Germacrene–D (5.19%).

Barton et al used a single stage precipitation process and found optimum extraction conditions of 10-100 bar and 34 °C, which corresponds to carbon dioxide densities of ~0.7 g/ml (3). They concluded that peppermint steam distilled oil and the SFE oil possessed a very similar composition. The SFE process of peppermint essential oil involving the fractional separation of the extract in two separators in series was studied by Reverchon et al (4). They found that the fractional separation yielded peppermint oil that did not contain any undesirable materials, although, an extraction at P 90 bar and T 50°C was optimum for minimizing the coextraction of unwanted compounds. Aleksovski et al (5) studied the extraction of peppermint oil with supercritical carbon dioxide at 9-12 Mpa and 25-50 °C. Rate of extraction was dependent on the extraction pressure and temperature and on the amount of carbon dioxide passed through the extractor. Using analytical GC and GC/MS technique, they compared these extracts with the peppermint oil obtained by hydrodistillation. The main components were menthol, menthone, menthyl acetate and menthofuran. They concluded that only minor differences in the essential oil composition between the extracts and the distillate were found and the extraction condition had no effect on the oil composition (5).

In the present investigation, whereas the content of monoterpene hydrocarbons in the essential oil was 5.21%, SFE product did not contain any of these hydrocarbon compounds obtained by hydro-distillation.

Menthane structures constituted the highest proportion of both the distilled oil and the SFE extract: 88.25 and 90.67%, respectively.

The above results indicate that the SFE process produced a selective extract containing only the compounds responsible in the peppermint oil specific aroma.

This feature can be considered as a commercial advantage of this process (10), because of the quality of the aromatic profile of the extracts.

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