

COMPARATIVE STUDY OF ESSENTIAL OIL FROM TWO SPECIES OF MINT GROWN IN ORĂȘTIE

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Abstract

Fares Orăștie Company grows medicinal plants for processing and marketing as food supplements. Mint species represent a significant share of the herbs crops.

The purpose of this research is to analyze comparatively essential oil derived from 2 species of mint grown experimentally in Orăștie: *Mentha suaveolens* Ehrh. and *Mentha x rotundifolia* (L.) Huds. The essential mint oil from the 2 species was obtained by water steam distillation from the aerial parts of the plant. After obtaining the essential oil, it was analyzed by using gas chromatography with mass spectrometric detection (GC-MSD).

Rezumat

În cadrul societății Fares Orăștie se cultivă specii de plante medicinale în vederea prelucrării și comercializării sub formă de suplimente alimentare. O pondere importantă din culturile de plante medicinale revine speciilor de mentă.

Scopul prezentei cercetări este de a analiza comparativ uleiul volatil provenit de la 2 specii de mentă cultivate experimental la Orăștie: *Mentha suaveolens* Ehrh. și *Mentha x rotundifolia* (L.) Huds. Uleiul volatil de mentă de la cele 2 specii a fost obținut prin distilare cu vapori de apă din părțile aeriene de plantă. După obținerea uleiului volatil, acesta a fost analizat utilizând cromatografia de gaze cu detecție prin spectrometrie de masă (GC-MSD).

Keywords: Mentha, essential oil, gas chromatography.

Introduction

The interest for *Mentha* species is very high in the world and in Romania. The most studied taxon of *Mentha* in terms of composition is *Mentha x piperita* L.

Mentha suaveolens Ehrh. commonly known as "pineapple mint" is a species of mint found in southern and western Europe. It has often been mistakenly named *M. rotundifolia* in cultivation. The essential oil has a chemical composition that varies with the growing area, having piperitone oxide as a basic component, and the plant can appear in many morphological forms. Consequently, the taxonomic origin of this oil is not

conclusive, creating confusion between species [2, 10, 19]. *Mentha x rotundifolia* (L.) Huds also known as "apple mint" is a perennial herbaceous aromatic spontaneous plant. Numerous hybrids treated by various authors as varieties of *M. rotundifolia* are in fact today reckoned as synonyms for *M. x suaveolens*. It is a hybrid between *Mentha longifolia* and *Mentha suaveolens*. It is also often confused with *Mentha x villosa* Huds. [13, 14, 20]. The essential oil has piperitone oxide or dihydrocarvone as a basic component. [4, 5, 7, 12, 14].

Given the wide variety of chemotypes of *Mentha* and the different chemical contents reported by various authors, it can be said that there is a need for additional research to have a realistic picture of the chemical polymorphism of these species of *Mentha*.

Materials and Methods

Plant material

Mentha suaveolens Ehrh. and *Mentha x rotundifolia* (L.) Huds were planted in the experimental cultures of Orastie, using seedlings from Fares Orastie Company.

Harvesting the herbal product

To track the essential oil quality by year and harvest time, the 2 species of mint were grown and harvested in 2009, 2010 and 2011 in different harvesting periods, namely:

Harvest 1: at the beginning of flowering, when about 90% of the flowers are in a bud phase.

Harvest 2: during flowering, when about 50-60% of flowers are open.

Harvest 3: at the end of flowering, when about 90-100% of flowers are open.



Figure 1

Mentha suaveolens Ehrh. obtained in Orastie culture.



Figure 2. *Mentha x rotundifolia* (L.) Huds obtained in Orastie culture

Seven samples of *Mentha suaveolens* Ehrh. were obtained and 7 samples of *Mentha x rotundifolia* (L.) Huds, each harvested as follows:

2009 - One crop, during flowering

2010 - Three crops at the beginning, during and at the end of flowering.

2011 - Three crops at the beginning, during and at the end of flowering.

Drying the plant product

The 14 samples were dried in natural conditions, in special places within Fares Orastie Company. The drying took about 5 days. At the end of the drying, plant moisture was performed, the drying being considered completed when the plant had maximum 13% humidity.

Microscopic analysis of the plant product

The microscopic analysis is a valuable method to establish the identity of medicinal plant products. We found this technique important as it enables us to establish preliminary criteria for distinguishing between these species based on the morphological details present on the upper and lower surface of leaves before using chromatographic analysis.

We believe these images obtained through stereomicroscopy can be useful for macroscopic analysis because they increase the morphological details seen with the naked eye. Another advantage of these images is that these analyses can also be applied with fragments of dry leaves, as it is the case of medicinal plant products.

The analyses were performed using the K-500L Motic Microscope (x 25).

The morphological details present on both surfaces of *Mentha* species leaves refer to the presence and density of tector hairs, presence and density of secretory glandular hairs, shape and density of veins and vascular islets separated from them.

Obtaining essential oil

The essential oil was obtained by using a distillation apparatus (according to European Pharmacopoeia 7th edition, method 8.2.12), the equivalent of the Clevenger system.

Analysis of the chemical composition of the essential oils obtained by gas chromatography

The mint essential oil obtained was analyzed qualitatively and semi-quantitatively using a gas chromatography system coupled with a mass spectrometer.

Operating parameters:

Gas chromatograph Agilent Technologies GC 7890A:

Oven program 60° C for 0 min then 3°C/min to 240°C for 0 min
Injection Volume 1 µL, Pressure 8.2317 psi, Total Flow 16 mL/min, Mode Split, Split Ratio 15:1, Split Flow 15 mL/min.

Column:

Agilent 19091S-433: 325°C: 30 m x 250 µm x 0.25 µm HP-5MS 5% Phenyl Methyl Silox: 831.62343.

Agilent Technologies 5975C mass spectrometer:

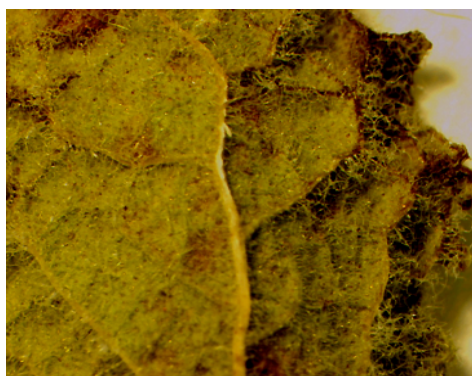
Low Mass 50.0, High Mass 550.0, Threshold 150, MS Source 230°C-250°C, MS Quad 150°C-200°C.

Results and Discussion

Microscopic analysis of the plant product

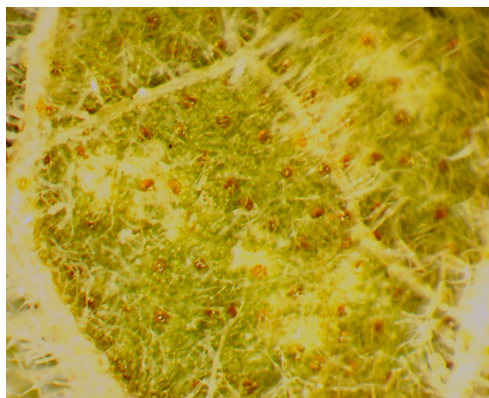
Mentha suaveolens Ehrh.

The presence of very dense trichomes on the upper epidermis is typical; they have no secretory tissues. On the lower surface one can see the veins, the tector hairs and a few glandular hairs, which appear as round reddish-brown circular areas.

**Figure 3***M. suaveolens* Lower epidermis (x 25)**Figure 4***M. suaveolens* Upper epidermis (x 25)

Mentha x rotundifolia (L.) Huds

On the lower side there are veins of different sizes, pale tector hairs and reddish-brown glandular hairs. On the upper side one can see the veins, the tector hairs, but the glandular hairs are missing.

**Figure 5***M. rotundifolia*
Lower epidermis (x 25)**Figure 6***M. Rotundifolia*
Upper epidermis (x 25)

In conclusion, we can say that the images obtained with the stereomicroscope for the mint leaves are of high scientific value and thus constitute a good criterion for identifying the *Mentha* species studied.

Species identity was confirmed by the Department of Pharmaceutical Botany of Iuliu Hatieganu University of Medicine and Pharmacy, Cluj Napoca, where the stereomicroscope analysis of these species of mint was performed.

Determination of essential oil content

The quantities of essential oil determined by using the Clevenger method for the plant products analyzed are presented in Table I.

Table I
Results obtained considering essential oil content of the mint studied batches

Current no.	Batch	Essential oil content (mL/100g herbal product)
1	<i>Mentha suaveolens</i> , harvest 1, 2009	0.68
2	<i>Mentha suaveolens</i> , harvest 1, 2010	0.55
3	<i>Mentha suaveolens</i> , harvest 2, 2010	0.74
4	<i>Mentha suaveolens</i> , harvest 3, 2010	0.6
5	<i>Mentha suaveolens</i> , harvest 1, 2011	0.63
6	<i>Mentha suaveolens</i> , harvest 2, 2011	0.81
7	<i>Mentha suaveolens</i> , harvest 3, 2011	0.71
8	<i>Mentha rotundifolia</i> , harvest 1, 2009	0.96
9	<i>Mentha rotundifolia</i> , harvest 1, 2010	0.88
10	<i>Mentha rotundifolia</i> , harvest 2, 2010	1.04
11	<i>Mentha rotundifolia</i> , harvest 3, 2010	0.91
12	<i>Mentha rotundifolia</i> , harvest 1, 2011	0.97
13	<i>Mentha rotundifolia</i> , harvest 2, 2011	1.10
14	<i>Mentha rotundifolia</i> , harvest 3, 2011	1.02

For the analyzed mint species, the highest amount of essential oil was obtained for the plant products harvested during the flowering (when about 50-60% of flowers are open), and the lowest amount of essential oil was obtained for the plant products harvested at the beginning of flowering (when 90% of flowers are in bud stage).

Comparing the plant products harvested in different years, it was found that the highest percentage of essential oil for mint species was obtained in 2011.

Quantitative contents of herbs differ depending on the period they were collected, the geographical location and the natural or cultivated character of the source, or the plant density [8]. Also, the essential oil composition for herbs differs significantly, depending on the pedoclimatic factors, geographical origins, different regions of the plant and developmental stages [3, 6, 15].

GC-MSD analysis

The essential oil components of the two species of mint have been analyzed by gas chromatography coupled with mass spectrometry and the results are presented in tables II-VII.

To establish identity, the Wiley database containing 275,000 mass spectra was used; the retention indices calculated have been compared with a database created in-house that contains approximately 600 compounds commonly found in the composition of essential oils.

The Kovats retention index I (RI) was calculated using the formula:

$$I = 100 \left[\frac{\log X_i - \log X_z}{\log X_{(z+1)} - \log X_z} + z \right]$$

where X_i is the corrected retention time of the component to be analyzed, X_z and X_{z+1} are the corrected retention times of two n-alkanes that were eluted before and after the sample component, and z is the number of carbon atoms of the alkane eluted earlier.

Table II
Main components of *Mentha suaveolens* Ehrh. harvest 2009

Current no.	RT	Name of compound	Total %	RI
Harvest 1, 2009				
1	6.543	beta-pinene	1.497%	983
2	8.099	limonene	2.969%	1028
3	16.427	carvone	1.555%	1244
4	21.835	piperitenone oxide	73.773%	1373
5	22.990	mint furanone	3.314%	1400
6	25.309	beta-farnesene	1.017%	1458
7	26.215	germacrene-d	3.309%	1480
8	30.503	viridiflorol	1.455%	1590

RT – retention time; RI – retention index

The basic essential oil compound of *Mentha suaveolens* Ehrh. collected in 2009 is piperitone oxide, at the rate of 73.773%.

Table III
Main compounds found in *Mentha suaveolens* Ehrh., harvest 2010

Current no.	RT	Name of compound	Total %	RI
Harvest 1, 2010				
1	8.094	limonene	1.102%	1028
2	18.780	methylcamphor	2.782%	1300
3	21.632	piperitenone oxide	45.341%	1368
4	22.324	isopiperitenone	5.469%	1385
5	22.952	mint furanone	3.597%	1400
6	24.660	4-fluorocumene	28.161%	1442
7	25.306	beta-farnesene	1.456%	1458
8	30.500	viridiflorol	3.116%	1590

Current no.	RT	Name of compound	Total %	RI
Harvest 2, 2010				
1	6.544	beta-pinene	1.438%	983
2	8.096	limonene	2.413%	1028
3	18.783	methylcamphor	2.690%	1300
4	21.632	piperitenone oxide	65.325%	1368
5	22.960	mint furanone	6.546%	1400
6	24.586	4-fluorocumene	3.778%	1440
7	25.308	beta-farnesene	1.228%	1458
8	26.204	germacrene d	5.937%	1480
9	30.500	viridiflorol	3.462%	1590
Harvest 3, 2010				
1	6.537	1 octen 3 ol	1.215%	983
2	11.086	3-octenyl acetate	1.884%	1113
3	13.990	para-cymen	1.466%	1185
4	21.746	piperitenone oxide	67.220%	1371
5	22.924	cis jasmone	2.925%	1399
6	30.171	caryophyllene oxide	1.080%	1581
7	30.515	viridiflorol	7.427%	1590

RT – retention time; RI – retention index

The basic essential oil compound of *Mentha suaveolens* Ehrh. collected in 2010 is piperitone oxide.

Comparing the quality of the mint oil obtained in 2010 in the 3 different harvesting periods, it may be seen that the mint harvested at the end of flowering (when 90% of the flowers are open) contains a higher percentage of piperitone oxide in comparison with the mint harvested at the beginning and during flowering.

Table IV
Main compounds found in *Mentha suaveolens* Ehrh., harvest 2011

Current no.	RT	Name of compound	Total %	RI
Harvest 1, 2011				
1	6.542	beta-pinene	1.535%	983
2	8.100	limonene	3.622%	1028
3	21.833	piperitenone oxide	64.616%	1373
4	22.940	cis jasmone	2.697%	1399
5	25.319	beta-farnesene	2.046%	1458
6	26.239	germacrene-d	9.739%	1481
7	30.513	viridiflorol	3.854%	1590
Harvest 2, 2011				
1	6.543	beta-pinene	1.229%	980
2	8.097	limonene	1.944%	1025
3	11.088	piperidinone	1.262%	1032

Current no.	RT	Name of compound	Total %	RI
4	18.775	octanenitrile	1.203%	1293
5	21.798	piperitenone oxide	72.503%	1341
6	22.930	cis jasmone	2.124%	1392
7	30.509	viridiflorol	3.566%	1581
Harvest 3, 2011				
1	6.547	beta-pinene	1.819%	983
2	8.095	limonene	3.471%	1028
3	18.784	methylcamphor	9.221%	1300
4	21.580	piperitenone oxide	78.845%	1367
5	24.590	silane	6.645%	1440

RT – retention time; RI – retention index

The basic essential oil component of *Mentha suaveolens* Ehrh. collected in 2011 is piperitone oxide.

Comparing the quality of the mint oil obtained in 2011 in the 3 different harvesting periods, it may be seen that the mint harvested at the end of flowering (when 90% of the flowers are open) contains a higher percentage of piperitone oxide in comparison with the mint harvested at the beginning and during flowering.

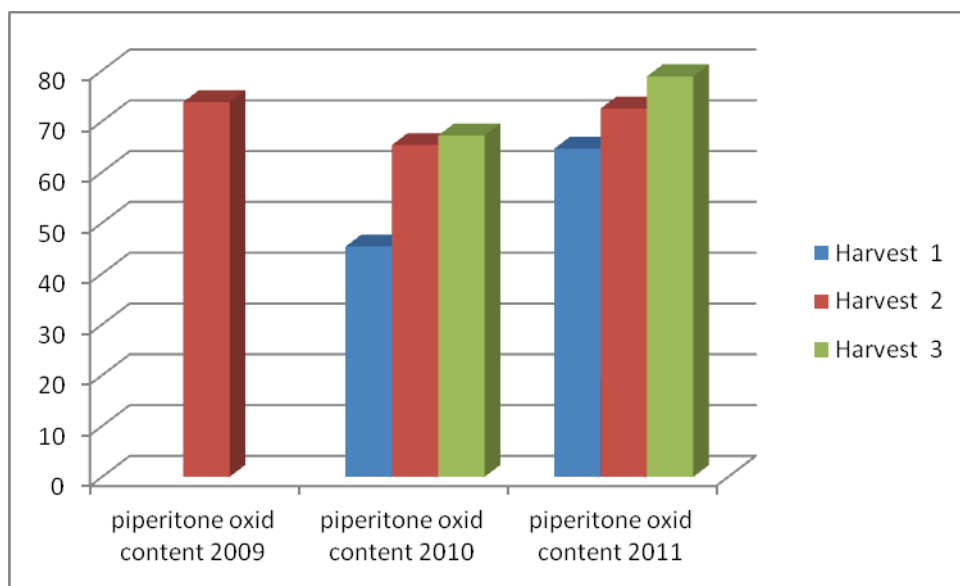


Figure 7

Piperitone oxide content in different harvests during 2009-2011.

Table VMain compounds of *Mentha rotundifolia*, harvest 1, 2009

Nr crt	RT	Denumire compus	% Total	RI
Harvest 1, 2009				
1	8.110	limonene	9.580%	1029
2	8.196	1,8-cineole	3.468%	1031
3	14.492	dihydrocarvone	2.712%	1198
4	16.640	carvone	66.662%	1249
5	16.912	piperitone	1.703%	1255

RT – retention time; RI – retention index

The basic essential oil compound of *Mentha rotundifolia* collected in 2009 is carvone.

Table VIMain compounds of *Mentha rotundifolia*, harvest 2010

Current no.	RT	Name of compound	% Total	RI
Harvest 1, 2010				
1	8.122	limonene	17.039%	1029
2	8.200	1,8-cineole	2.991%	1031
3	14.423	dihydrocarveol	6.530%	1196
4	14.522	dihydrocarvone	7.274%	1198
5	16.597	carvone	51.797%	1248
6	16.895	piperitone	1.535%	1255
7	20.008	dihydrocarvyl acetate	1.271%	1329
Harvest 2, 2010				
1	8.095	limonene	16.277%	1028
2	8.204	1,8-cineole	3.253%	1031
3	14.382	dihydrocarveol	2.217%	1195
4	14.487	dihydrocarvone	3.974%	1197
5	16.438	carvone	66.711%	1244
6	16.875	piperitone	1.353%	1254
7	18.784	methylcamphor	6.215%	1300
Harvest 3, 2010				
1	8.098	limonene	15.375%	1028
2	8.198	1,8-cineole	3.145%	1031
3	14.379	dihydrocarveol	1.669%	1195
4	14.485	dihydrocarvone	3.085%	1197
5	16.478	carvone	66.279%	1245
6	16.868	piperitone	1.582%	1254
7	18.784	methylcamphor	2.340%	1300
8	20.008	dihydrocarvyl acetate	1.037%	1329

RT – retention time; RI – retention index

The basic essential oil compound of *Mentha rotundifolia* collected in 2010 is carvone.

Comparing the quality of the mint oil obtained in 2010 in the 3 different harvesting periods, it may be seen that the mint harvested during flowering (when about 50-60% of the flowers are open) contains a higher percentage of carvone than the mint harvested during early flowering and slightly more carvone than the mint harvested at the end of flowering.

Table VII
Main compounds of *Mentha rotundifolia*, harvest 2011

Current no.	RT	Name of compound	% Total	RI
Harvest 1, 2011				
1	8.116	limonene	13.316%	1029
2	8.198	1,8-cineole	4.479%	1031
3	14.392	dihydrocarveol	1.269%	1195
4	14.492	dihydrocarvone	2.881%	1198
5	16.613	carvone	59.677%	1248
6	16.900	piperitone	1.349%	1255
7	21.563	piperitenone oxide	1.045%	1366
Harvest 2, 2011				
1	8.096	limonene	7.193%	1028
2	8.200	1,8-cineole	1.580%	1031
3	14.380	dihydrocarveol	1.520%	1195
4	14.485	dihydrocarvone	2.515%	1197
5	16.515	carvone	73.561%	1246
6	16.871	piperitone	1.726%	1254
7	21.563	piperitenone oxide	1.761%	1366
Harvest 3, 2011				
1	8.125	limonene	17.713%	1029
2	8.202	1,8-cineole	3.466%	1031
3	14.395	dihydrocarveol	1.895%	1195
4	14.495	dihydrocarvone	3.133%	1198
5	16.620	carvone	60.110%	1248
6	16.903	piperitone	1.632%	1255

RT – retention time; RI – retention index

The main essential oil compound of *Mentha rotundifolia* collected in 2011 is carvone.

Comparing the quality of the mint oil obtained in 2011 in the 3 different harvesting periods, it may be seen that the mint harvested during flowering (when about 50-60% of the flowers are open) contains a higher

percentage of carvone in comparison with the mint harvested at the beginning and end of flowering.

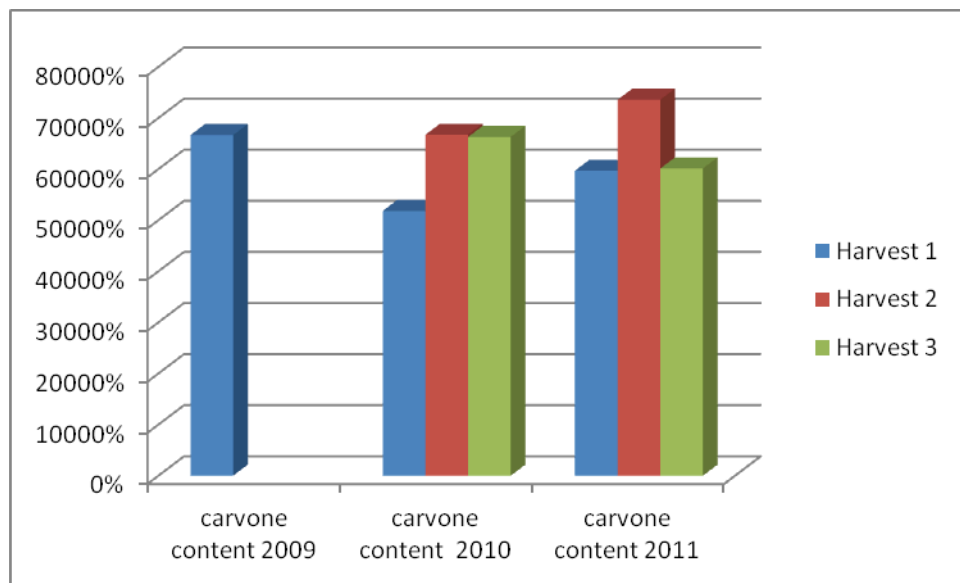


Figure 8

Carvone content in different harvests during 2009-2011

Conclusions

Considering the chromatographic analysis it was seen that the 2 species of mint - *Mentha suaveolens* and *Mentha rotundifolia* have a different chemical composition. *Mentha suaveolens* has piperitone oxide as its basic component, while *Mentha rotundifolia* has carvone as its main component.

Taking into consideration the fact that there are confusions regarding these two species of mint, from the essential oil analysis performed in this paper one can clearly distinguish the two species of mint, distinction also supported by the microscopic analysis conducted.

Comparing the quality of *Mentha suaveolens* essential oil obtained in the 3 different harvesting periods in 2010 and 2011, one may see that the pineapple mint harvested at the end of flowering (when 90% of the flowers are open) contains a higher percentage of piperitone oxide in comparison with the mint harvested at the beginning and during flowering.

Comparing the quality of *Mentha x rotundifolia* (L.) Huds oil obtained in the 3 different harvesting periods in 2010 and 2011, one may see that the

apple mint harvested during flowering (when about 50-60% of the flowers are open) contains a higher percentage of carvone in comparison with the mint harvested at the beginning and end of flowering.

The recent researches in medicine and pharmacy have focused their attention in developing new drugs and biomaterials based on essential oils or plant extracts, this field being a new future direction for treating different pathologies [9]. *Mentha suaveolens* has shown antiseptic, antimicrobial and antifungal effects in non-clinical studies [1, 11, 16, 17]. *Mentha suaveolens* obtained in Orastie culture will be considered for future research in pharmaceuticals with antiseptic, antimicrobial effect.

Mentha x rotundifolia (L.) Huds (with a different essential oil composition) has shown inhibitory effects on the central nervous system in rodents [18]. *Mentha rotundifolia* obtained in Orastie culture will therefore be considered for future research in relaxing and soothing products intended for aromatherapy.

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