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Full Length Research Paper

Chemical composition of essential oils from *Mentha aquatica* L. at different moments of the ontogenetic cycle

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This study investigates the chemical composition of essential oils extracted from *Mentha aquatica* plants. Aerial organs from *M. aquatica* were collected in three different stages: vegetative, flowering and senescence stage. The essential oil was extracted using the hydrodistillation method, with a Neo-Clevenger device. Essential oil samples obtained were analyzed with Agilent gas chromatography-mass spectrometry (GC-MS). Essential oils have varied in terms of quality, depending on the time when the investigations were performed. Substances (41) were identified, of which 20 are common in all samples collected in the three phenophases. The main substances identified were: menthofuran (51.26 to 58.59%), limonene (5.94 to 12.06%), trans-β-ocimene (5.59 to 6.10%), ledol (3.01 to 4.06%) and β-caryophyllene (2.923 to 3.557%).

Key words: *Mentha aquatica*, phenophases, essential oil, menthofuran.

INTRODUCTION

A constant concern of man over time was the study and use of medicinal plants in order to cure various diseases. *Mentha* is among the plants which have often been used for their therapeutic properties. Over 3,000 names exist for species of this genus, but most of them are synonyms (Tucker and Naczi, 2006). Six species and six hybrids of *Mentha* (including varieties) have been identified and described in the Romanian flora (Ciocârlan, 2009).

Mentha aquatica L. is a perennial plant of the Lamiaceae family, common in Europe, North Africa, and West Asia, and was recently introduced to America, Australia and Madeira. It grows on the banks of flowing waters, ponds and lakes, but prefers calcareous soils (Guşuleac, 1961). It is known for its allergenic, analgesic, antipyretic, antiseptic, antispasmodic, carminative, de-congestant, deodorant, diaphoretic, digestive, diuretic, antiemetic, insecticides, sedative, stimulatory and vermifuge actions (Gruenwald et al., 2002). The plant has aromatic properties due to the essential oil it contains. The essential oil is synthesized by the glandular hairs present on the surface of the aerial organs (Gershenzon et al., 1989). Secretory hairs from the genus *Mentha* may have unicellular or multicellular gland (Fahn, 1979). The information on the essential oil of *M. aquatica* is limited to the study of plants growing wild in nature. In the study realized by Jerkovic and Mastelic (2001) concerning *M. aquatica* from Croatia menthofuran, 1,8-cineole and trans-caryophyllene are presented as the main compounds. Similar research on *M. aquatica* was developed by Hefendehl and Murray (1971), Bozin et al. (2006), Getahun et al. (2008) and Sutour et al. (2011). The component which characterized the essential oils of *M. aquatica* analyzed was also menthofuran. Synthesis of this compound depends on environmental conditions: high temperatures throughout the day and night, low density of the photon flux and water stress (Burbott and Loomis, 1967; Clark and Menary, 1980).

In this paper, a comparative analysis was carried out on the chemical composition of essential oils obtained by

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hydrodistillation from *M. aquatica* species in three different phases of the ontogenetic cycle: vegetative, flowering and senescence stage, recording the main compounds variation during the cycle.

MATERIALS AND METHODS

Plant

The studies were performed on fresh plant material from *M. aquatica* collected in three different stages: vegetative, flowering and senescence. The material was gathered from Caraorman (Tulcea County), located in South-East Romania, a place with a low degree of human intervention. The soil on which these species grow is sandy and in rainfall conditions becomes flooded. The aerial organs of plants were harvested on sunny days in June, July and August of 2010. Plant material was determined by Dr. Ciprian Mânzu, taxonomist within the Faculty of Biology, Alexandru Ioan Cuza University, Iaşi.

Essential oil extraction

Essential oils were extracted from plants by hydrodistillation with water vapors, using a Neo-Clevenger type device. The crushed plant material was introduced in the flask of the device, 500 ml of water were added and it was adapted to steam distillation. The distillation was carried out for 3 to 4 h at moderate temperature.

Gas chromatography-mass spectrometry (GC-MS)

Separation of components was performed with gas chromatography (Agilent), with a mass spectrometric detector with quadrupole of DB 5 type, with a capillary column (30 m length and 0.25 mm diameter). We used helium as the carrier gas and the initial oven temperature was 500°C, and isothermic increased by 4°C min^{-1} to 280°C. Also, Kovats retention indices were used to confirm the exact position of peaks in the chromatogram, using a series of n-alkanes as references.

RESULTS

Analyses conducted on the three essential oils of *M. aquatica* extracted during the ontogenetic cycle have revealed the presence of 41 chemical compounds, of which only 20 were common, while 13 were identified only in one sample, and 8 were found in two samples (Table 1).

For the sample corresponding to the vegetative phenophase, 30 chemical compounds were identified, representing 99.21% of the total. Among the other compounds, the following had greater percentage: menthofuran (58.59%), limonene (9.91%), trans-β-ocimene (5.59%), ledol (3.29%) and β-caryophyllene (3.55%) (Figure 1). In the sample of essential oil extracted from the plants in the flowering phenophase, the presence of 28 chemicals was reported, summing 99.14%. The dominant compounds in this phase were: menthofuran (51.26%), limonene (12.06%), trans- β-ocimene (8.10%) and ledol (3.01%). For the sample extracted in senescence stage, 31 chemical compounds have been identified, representing 91.22%. For the following compounds, high percentage values were recorded: menthofuran (51.54%), limonene (5.94%), trans-β-ocimene (6.21%), cis-β-ocimene (4.62%) and ledol (4.06%).

Of the 23 chemical compounds of essential oil common to the three samples, only 5 have registered high percentage values, providing the common character of these essential oils.

DISCUSSION

Comparative analysis of the three samples of essential oil extracted from *M. aquatica* during the ontogenetic cycle led to the observation that some of the compounds mentioned earlier recorded significant percentage changes. Concerning menthofuran, the highest value (58.59%) was recorded in the vegetative phenophase decreasing towards the phase of senescence, when the percentage was 51.54%. The share of menthofuran corresponding to the flowering phenophase sample was close to that of the sample in senescence stage. The high content of menthofuran offers an intense smell to *M. aquatica* plants (Murray and Lincoln, 1972). Similar variations were observed for β-caryophyllene, but values were lower. For limonene, the highest value was reached during flowering (12.06%), while the lowest value was recorded at senescence (5.94%). Similar developments were observed for trans-ocimene and β-mircene compounds as well. Other compounds with a small share in essential oils such as α-pinene, β-pinene, and γ-terpinene, suffered a quantitative decrease throughout the ontogenetic cycle.

Analysis undertaken by Falahian et al. (2002) indicates larger amounts of α-terpinene (25.2%), piperitenone oxide (19.2%), 1,8-cineole (15.1%), menthofuran (6.9%), acetate geranyle (6.8%) and sabinene (6.4%) during vegetative growth and menthofuran (45.1%), 1,8-cineole (26.6%), menthol (8.6%) and β-caryophyllene (6.1%) during flowering phenophase. The results correspond to those obtained by Getahun et al. (2008) and Sutour et al. (2011), who found that menthofuran is the main compound of the essential oil of *M. aquatica* and are different from those obtained by Akbar et al. (2006) who concluded that the main substances are β-caryophyllene (22.4%), viridiflorol (11.3%) and 1,8-cineole (10.9%). Similar results, with several qualitative and quantitative differences, were obtained by Bozin et al. (2006). The authors mention menthofuran (19.0 to 24.5%) as the main compound of volatile oil in all the 3 phenophases, followed by 1,8 cineole (11.8 to 20.2%), E-caryophyllene (6.7 to 16.6%) and viridiflorol (7.6 to 9.0%).

In the essential oil obtained in this study, menthofuran was noticed to be the main compound (58.59 to 51.26%), followed by limonene (12.06 to 5.94%), trans-β-ocimene (8.10 to 5.59%) and ledol (3.01 to 4.06%).

Table 1. Chemical composition of essential oils from *M. aquatica* L. species during the ontogenetic cycle.

S/N	Component	Retention time (min)	Vegetative (%)	Flowering (%)	Senescence (%)
1	α-Pinene	13.025	1.57	1.32	0.96
2	Sabinene	14.775	1.18	1.63	1.22
3	β-Pinene	14.949	2.78	2.53	1.95
4	β-Mircene	15.540	1.81	2.96	2.16
5	α-Terpinene	16.483	0.13	-	-
6	Cimol	17.135	0.68	0.39	-
7	o-Cimene	17.163	-	-	0.36
8	Limonene	17.435	9.91	12.06	5.94
9	trans-β-Ocimene	17.801	5.59	8.10	6.21
10	cis-β-Ocimene	18.261	1.46	2.28	4.62
11	Ocimene	18.275	-	-	1.66
12	γ-Terpinene	18.791	0.41	0.37	0.28
13	Terpinolene	20.236	0.13	0.16	0.09
14	2-methyl-butyl-2-methyl-butaneat	20.921	0.34	0.34	-
15	Amil Isovalerate	20.982	-	-	0.32
16	Amil Valerate	21.179	-	-	0.07
17	Dimethyl-Octatrien	22.197	0.15	0.20	-
18	α-Ocimene	22.249	-	-	0.14
19	Isomenthone	23.506	-	1.78	-
20	Menthone	23.511	-	-	0.27
21	Menthofuran	24.257	58.59	51.26	51.54
22	Pulegone	27.607	0.21	1.83	0.30
23	Geranyl acetate	29.924	0.43	1.30	1.49
24	β-Elemene	34.602	0.20	-	-
25	α- Gurjunene	35.662	0.38	0.23	0.28
26	β-Caryophyllene	36.099	3.55	2.92	3.11
27	β-Farnesene	37.248	0.72	0.32	0.32
28	α-Caryophyllene	37.300	-	0.19	0.20
29	β-Cubebene	38.172	-	2.03	-
30	Germacrene D	38.224	1.45	-	1.09
31	Elixene	38.613	1.08	0.60	0.66
32	β-Cadinene	39.265	0.37	0.23	0.25
33	Elemol	39.903	1.03	0.28	0.62
34	Spathulenol	40.537	0.19	-	-
35	Germacrene D-4-ol	40.593	-	0.54	0.68
36	Caryophyllene Oxide	40.795	0.16	0.12	-
37	Ledol	41.155	3.29	3.01	4.06
38	Globulol	41.273	-	-	0.07
39	γ-Eudesmol	41.653	0.30	-	-
40	α-Cadinol	42.155	0.78	0.16	0.10
41	t-Murolol	42.198	0.34	-	0.20
-	Other compounds	-	0.79	0.86	8.78

The analyses of this study indicate a number of differences from previous studies, which may be due to different environmental conditions (brightness, temperature and rainfall may have a critical role in plant development and thus in the biosynthesis of essential oils) as well as depending on the ontogenetic stage of the plant (harvesting time).

Conclusion

Conclusively, it was observed that only 20 compounds are common to all ontogenetic stages out of the 41 chemicals identified when analyzing the essential oils from *M. aquatica* during the ontogenetic cycle. Menthofuran, a compound specific to essential oils from *M.*

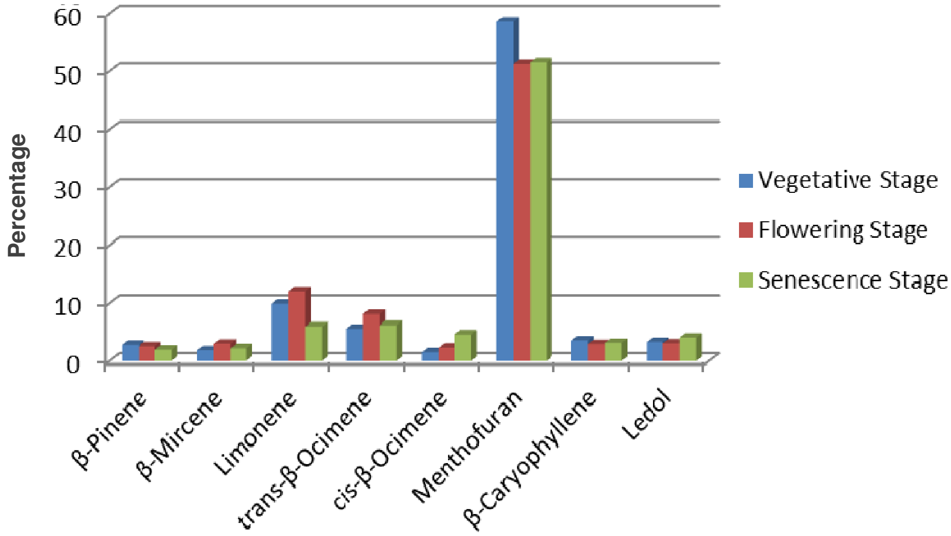

Figure 1. Evolution of the main chemical compounds from *M. aquatica* L. essential oil during the ontogenetic cycle.

aquatica, had a large share in all analyzed stages (51.26 to 58.59%). Beyond menthofuran, significant shares were recorded by limonene (5.94 to 12.06%) and trans-β-ocimene (5.59 to 8.10%) in all stages and in the senescence stage by cis-β-ocimene (4.62%) and ledol (4.06%).

The results of this study can be useful in the research of ontogenesis conditions in medicinal and aromatic plants as well as for their future therapeutic use.

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