STUDY REGARDING THE CHEMICAL COMPOSITION OF VOLATILE OILS OF *Mentha pulegium* L. FROM ROMANIA FLORA

ANDRO Anca Raluca, BOZ Irina, ZAMFIRACHE Maria-Magdalena

Abstract. In recent years a lot of techniques for determinations of the chemical composition of plants, specially the volatile compounds has developed very much due to the awareness that secondary metabolic products have numerous biological activities. Economic and medical interests, as well as taxonomic studies were the most powerful incentive for research on the chemical composition of plants. In the present study we analyzed the volatile oils obtained from aerial plant organs of *Mentha pulegium* L. in different phenophases. Volatile oils were obtained by hydrodistillation using Clevenger type apparatus, and for the identification of the chemical components have been use GC-MS. There were identified 20 chemical compounds, but only 13 are common to all 3 phenophases. The main compounds analysed were pulegone, menthone, germacrene D and isomenthone, compounds with important biological properties.

Keywords: *Mentha pulegium* L., volatil oil, medicinal plant, chemical compounds, phenophases.

Rezumat. Studii privind compoziţia chimică a uleiurilor volatile de *Mentha pulegium* L. din flora României. În ultimii ani s-au dezvoltat foarte multe tehnici de determinare a compoziţiei chimice a plantelor, în special a compuşilor volatili datorită conştientizării faptului că produşii de metabolism secundar prezintă numeroase activităţi biologice. Interesele economice, medicinale, precum şi studiile taxonomice au fost stimulatorii cei mai puternici pentru cercetări în privinţa compoziţiei chimice a plantelor. În această lucrare am analizat uleiurile volatile obţinute din organele vegetative aeriene ale speciei *Mentha pulegium* L. aflată în diferite fenofaze. Uleiurile volatile au fost obţinute prin hidrodistilare utilizând un dispozitiv de tip Clevenger, iar pentru identificarea compuşior chimici s-a utilizat GC-MS. S-au identificat un număr de 20 de compuşi chimici, însă doar 13 fiind comuni celor 3 fenofaze. Principalii compuşii analizaţi au fost pulegona, mentona, izomentona şi germacren D, compuşi cu importante proprietăţi biologice.

Cuvinte cheie: *Mentha pulegium* L., ulei volatil, plantă medicinală, compuşi chimici, fenofaze.

INTRODUCTION

Belonging to the Lamiaceae family, *Mentha pulegium* L. is a species widespread in Romania, preferring damp and marshy places (GUŞULEAC, 1961). On the surface of aerial organs, there are present secretory hairs, organs that produce volatile oil that provides aromatic properties to the plant. Due to these properties, it is often used in pharmaceutical, cosmetic and food industries.

In this study we analyzed the chemical composition of volatile oils from aerial organs of *Mentha pulegium* L. species in different vegetative phenophases. These data were correlated with data on specific environmental conditions of the taxon and compared with the data published in the specialized literature.

Recent research on the chemical composition of essential oil from *Mentha pulegium* L. were made by MAHBOUBI & HAGHI (2008) and DERWICH et al. (2010), who identified the presence of piperitone (38.0%), piperitenone (33.0%), α-terpineol (4.7%) and pulegone (2.3%) as most important compounds.

MATERIAL AND METHODS

Aerial organs from *Mentha pulegium* L. were collected in vegetative, flowering and senescence stages from Caraorman region (Tulcea District, Romania) in the summer of 2010. The species was determined by Ciprian Minzu, taxonomist within the Faculty of Biology, "Al. I. Cuza" University of Iaşi.

For the extraction of essential oils we used a Clevenger device, in accordance with the European Pharmacopoeia standards, the Laboratory of Plant Physiology, Faculty of Biology, "Al. I. Cuza" University of Iaşi. The plant material was crushed and placed in the balloon device and afterwards water was added. Distillation was carried out for 3 hours.

Separation and identification of chemical compounds of essential oils was performed using GC_MS (Gas Chromatography coupled with Mass Spectrometry) in the Research Center for Agri-food products, the Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine in Bucharest. Separated compounds were identified by retention time at a library of spectra, located in the device database.

RESULTS AND DISCUSSIONS

After analyzing the essential oil extracted from *Mentha pulegium* L. species throughout the vegetation period, there were identified 20 compounds. The number of identified compounds is 18 for the vegetative phenophase, 15 for flowering and 16 for fructification phenophase. Of the 20 compounds examined, 13 were common to the three samples, thus conferring the aromatic character of these oils. It was also noted that some compounds appear in only one or two phenophases.

Varying in percentage in the analysed volatile oils, in the first three positions, there are located the following compounds: pulegone (40.480%, 67.277%, 60.624%), menthone (18.348%, 15.366%, 20.866%), isomenthone (7.707%, 6.824%, 11.296%) and germacrene D (1.302%, 2.297%, 0.475%), as shown in chromatograms obtained from the analysis of volatile oil in the three vegetative stages, from diagrams (Figs. 1; 2; 3) and from Table 1.

Following the comparative analysis on the three samples of essential oil obtained from *Mentha pulegium* L., during the vegetation period there were observed percentage changes of chemical compounds in the three vegetative phenophases. Thus, it was observed that in the case of pulegone, the highest value was reached at flowering: 67.277%, but the lowest value was reached in the previous phase: 40.480%. For menthone the highest value was recorded at fructification (18.348%), while the lowest value was reached at flowering: 15.366%. Regarding content isomenthone it was noted that its evolution was similar as menthone, but smaller values were recorded, as shown in the aforementioned table.

Pulegone has antimicrobial properties but is can be also used as an agent in aromatherapy, while menthone has antibacterial, antiseptic and anticancer properties (ZEIGER, 1998). Isomenthone is a stereoisomer of menthone, present in many essential oils, being characteristic to peppermint essential oil.

Germacrene D is a hydrocarbon in sesquiterpenes class and is considered an intermediate in the biosynthesis of other sesquiterpenes (ADEWALE, 2009), which can be successfully used as a pesticide and pheromone (http://sun.ars-grin.gov). It also has numerous biological activities in nature (pheromone, antibacterial activity, antifungal) (CONNOLLY & HILL, 1991).

Table 1. Chemical composition of the volatile oils of *Mentha pulegium* L. species during vegetation period.
Tabel 1. Compoziția chimică a uleiurilor volatile de la specia *Mentha pulegium* L. pe parcursul perioadei de vegetație.

No.	Compounds	Vegetative phenophase (%)	Flowering phenophase (%)	Senescence phenophase (%)
1	α - Pinene	0.886	0.573	0.527
2	Sabinene	0.297	0.203	0.169
3	β - Pinene	0.742	0.507	0.453
4	3 - Octanon	0.111	0.170	0.169
5	Mircene	0.238	0.272	0.194
6	3 - Octanol	0.994	1.583	1.425
7	Limonene	1.625	0.685	0.469
8	Menthone	**18.348**	**15.366**	**20.866**
9	Izomenthon	**7.707**	**6.824**	**11.296**
10	Izo - Pulegone	0.882	1.807	1.656
11	Izo - Menthol	0.251	0.395	0.767
12	Pulegone	**40.480**	**67.277**	**60.624**
13	Piperitone	**-**	**-**	0.224
14	Methyl-acetate	0.095	-	-
15	Verbenon	-	0.413	0.512
16	α - Copaen	0.364	-	-
17	β - Cubeben	0.281	-	-
18	β - Cariophylene	0.545	1.629	0.176
19	α - Cariophylene	0.200	-	-
20	Germacrene D	1.302	2.297	0.475

Research on the chemical composition of volatile oil derived from *Mentha pulegium* L. species were also conducted by ZWAVING & SMITH (1971). They identified in the analyzed volatile oil the following compounds: limonene (11%), octyl-3-acetate (0.8%), octanol-3 (1%), menthone (8%), isomenthone (7%) and piperitone (70%). Pulegone, which is usually common in the oil of this plant, was not found.

Recent studies on quantitative and qualitative analysis of essential oil from *Mentha pulegium* L. species were made by MAHBOUBI & HAGHI (2008). They identified the presence of piperitone (38.0%), piperitenone (33.0%), α-terpineol (4.7%) and pulegone (2.3%) as the most important compounds.

DERWICH et al. (2010) identified in the composition of essential oil from *Mentha pulegium* L. high values in terms of piperitone content (35.56%), piperitenone (21.18%), α-terpineol (10.89%) and pulegone (6.452%).

Environmental factors such as temperature, radiation and photoperiod play a foremost role in the quantity and quality of volatile oil (YAMAURA et al., 1989). The essential nutrient material for plant growth, water, mineral elements and nitrogen also play an important role in the chemical composition and quality of essential oil (RAJESWARA et al., 1990).

CONCLUSIONS

By correlating the obtained data we can determine the aromatic value of the studied species depending on the harvest period in the vegetation stage and the ontogenetic stage of the plant. The achieved results are used to identify wild plant populations with the highest degree of aromatic value.

ACKNOWLEDGEMENTS

This work was supported by the the European Social Fund in Romania, under the responsibility of the Managing Authority for the Sectoral Operational Programme for Human Resources Development 2007-2013 [grant POSDRU/88/1.5/S/47646] and by program "Developing the innovation capacity and improving the impact of research through post-doctoral programmes" POSDRU/89/1.5/S/49944.

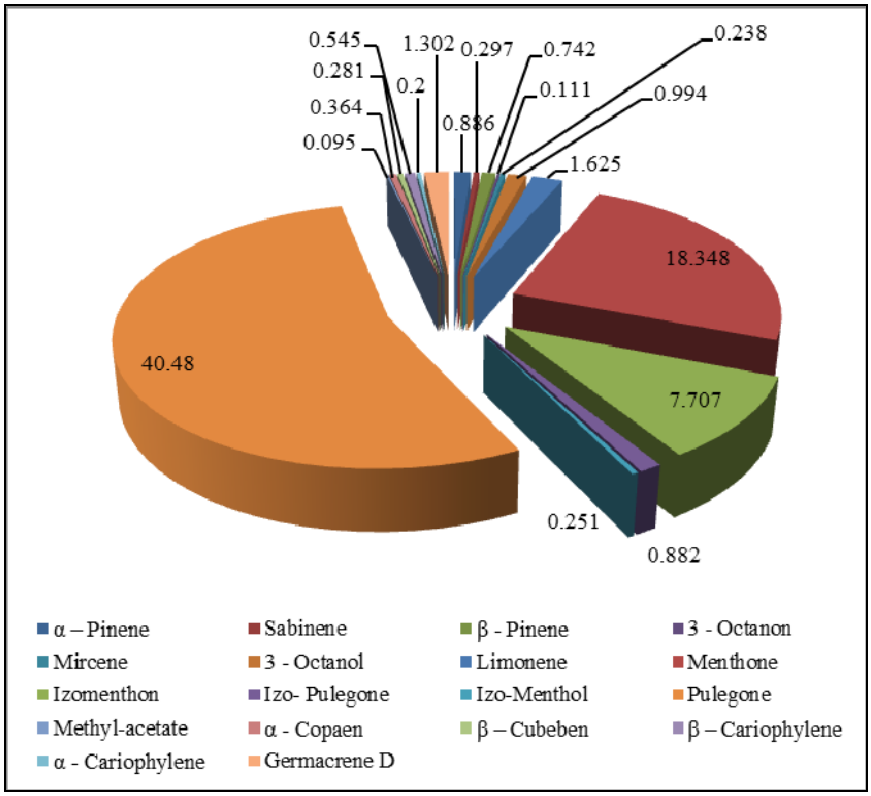

Figure 1. The chemical composition of essential oil of *Mentha pulegium* L. in the vegetative phenophase.
Figura 1. Compoziţia chimică a uleiului volatil de *Mentha pulegium* L. în fenofaza vegetativă.

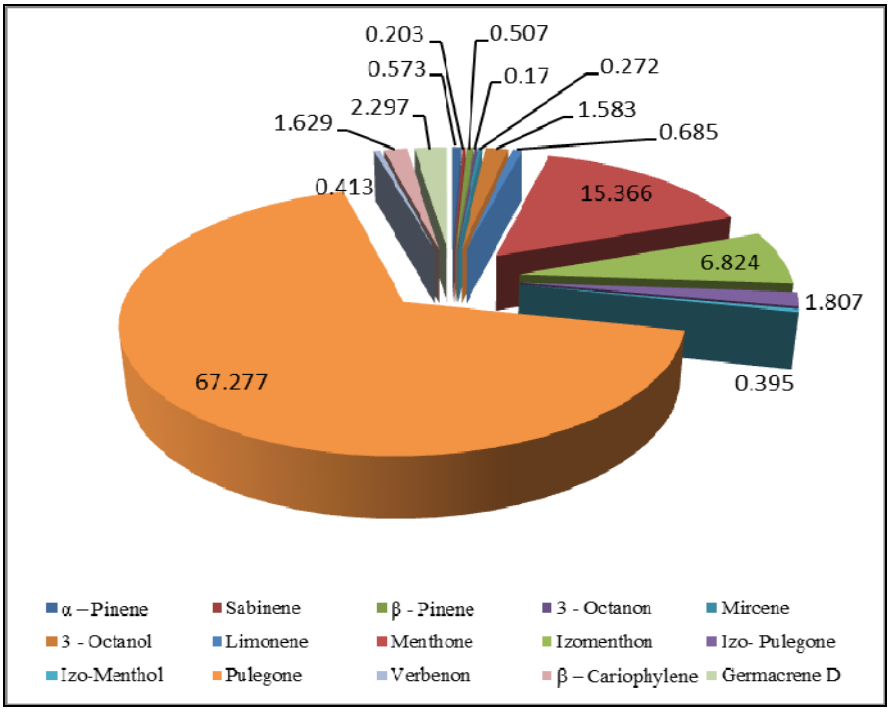

Figure 2. The chemical composition of essential oil of *Mentha pulegium* L. in the flowering phenophase.
Figura 2. Compoziţia chimică a uleiului volatil de *Mentha pulegium* L. în fenofaza de înflorire.

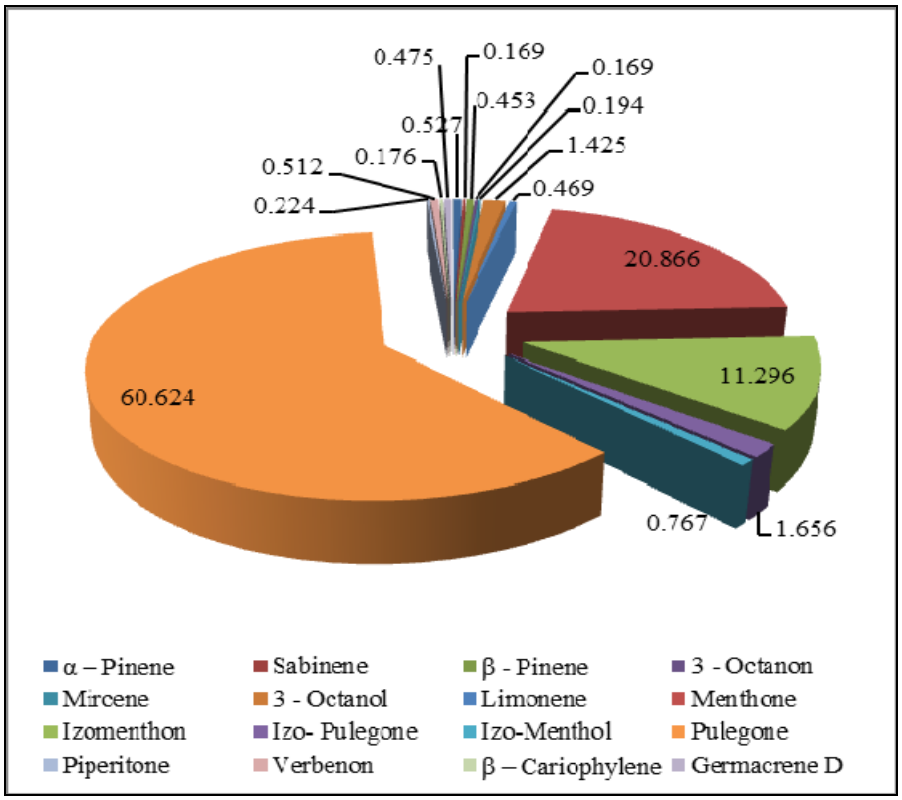

Figure 3. The chemical composition of essential oil of *Mentha pulegium* L. in the senescence phenophase.
Figura 3. Compoziția chimică a uleiului volatil de *Mentha pulegium* L. în fenofaza de fructificare.

REFERENCES

ADEWALE M. A. 2009. *Germacrenes A–E and related compounds: thermal, photochemical and acid induced transannular cyclizations.* Tetrahedron. Edit. Elsevier, Oxford. **65**(8): 1533-1552.

CONNOLLY J. D. & HILL R. A. 1991. *Dictionary of terpenoids*. Edit. Chapman & Hall, London: 214-285.

DERWICH E., BENZIANE Z., TAOUIL R., SENHAJI O., TOUZANI M. 2010. *Comparative Essential oil Composition of Leaves of Mentha rotundifolia and Mentha pulegium a Traditional Herbal Medicine in Morocco*, American-Eurasian Journal of Sustainable Agriculture. American-Eurasian Network for Scientific Information (AENSI), Jordan. **4**(1): 47-54.

GUŞULEAC M. 1961. *Labiate*. In: Flora Republicii Populare Române. Edit. Academiei R.P.R. Bucureşti. **8**: 82-394.

MAHBOUBI M. & HAGHI G. 2008. *Antimicrobial activity and chemical composition of Mentha pulegium L. essential oil*, Journal of Ethnopharmacology. Edit. Elsevier, Kashan. **119**: 325-327.

RAJESWARA R., BHASKARUNI R., KAKARAPARTHI P. SASTRY. 1990. *Variation in Yields and Quality of Geranium, under Varied Climatic and Fertility Conditions.* J. Ess. Oil Res., Edit. Elsevier. Oxford. **2**:73-79.

YAMAURA T., TANAKA S., TOBANA M. 1989. *Light Dependent Formation of Glandular Trichomes and Monoterpenes in Thyme Seedlings.* Phytochemistry. Edit. Elsevier, Oxford. **28**: 741-744.

ZWAVING J. H. & SMITH D. 1971. *Composition of the essential oil of Austrian Mentha pulegium*. Phytochemistry. Pergamon Press, England. **10**: 1951-1953.

ZEIGER. 1998. *Review of Toxicological Literature*. National Institute of Enviromental Health Sciences. P.O.BOX, Research Triangle Parck, North Carolina, 27709: 1-13.

***. http://sun.ars-grin.gov - Germacrene D (accesed November 15, 2009).

Andro Anca Raluca, Boz Irina, Zamfirache Maria-Magdalena
Department of Plant Biology, Faculty of Biology, "Al. I. Cuza" University of Iaşi,
No. 20 A "Carol I", Boulevard 700505, Iaşi, Romania
E-mail: anca_andro@yahoo.com
E-mail: irina_berciu@yahoo.com
E-mail: magda@uaic.ro

Received: March 31, 2011
Accepted: July 15, 2011