AUTHENTICATION OF WILD *MENTHA AQUATICA*, FROM DANUBE DELTA, USING ^{1}H-NMR SPECTROSCOPY

Fulvia-Ancuța MANOLACHE[1], Cristina TODAȘCĂ[2], Valentin IONESCU[3], Enuța IORGA[4], Anamaria Andreea BELDIE[5], Mădălina Adriana MATEI[6]

In this study the authentication of the species Mentha aquatica L. was carried out and compared to other Romanian species, Mentha piperita L. and Mentha spicata L.

The main objectives were to develop a new method of measuring major compounds from essential oils, using ^{1}H-NMR spectroscopy and to use quantitative data for the differentiation of peppermint essential oil from different species. Markers for the identification and quantitative measurement of major compounds in Mentha aquatica were established, using NMR spectroscopy. Using chemometric methods (Principal Component Analysis - PCA) mint essential oil samples were differentiated according to variety.

Keywords: mentha, authentication, NMR spectroscopy.

1. Introduction

Essential oils and their separate components are widely used in medicine, food or cosmetic industries. The oil composition is determined mainly by the plant variety, although it can also be influenced by the extraction process. Some studies demonstrated that essential oils have antimicrobial effects. Mints (*Mentha* spp.) are one of the most popular essential oil crops, particularly in the Mediterranean area, where they are widely distributed. Many members of this

[1] PhD Student, National Research&Development Institute for Food Bioresources – IBA Bucharest, 6 Dinu Vintila St., Bucharest, Romania, e-mail: fulvia.manolache@yahoo.com

[2] Lecturer PhD Eng., University ''Politehnica'' of Bucharest, Faculty of Applied Chemistry and Material Science, ''C. Nenitescu'' Organic Chemistry Department, Bucharest, Romania, e-mail: cristina.todasca@upb.ro

[3] Senior Researcher, National Research&Development Institute for Food Bioresources – IBA Bucharest, 6 Dinu Vintila St., Bucharest, Romania, e-mail: valentin.ionescu@bioresurse.ro

[4] Senior Researcher, National Research&Development Institute for Food Bioresources – IBA Bucharest, 6 Dinu Vintila St., Bucharest, Romania, e-mail: enutai@yahoo.com

[5] Eng., National Research&Development Institute for Food Bioresources – IBA Bucharest, 6 Dinu Vintila St., Bucharest, Romania, beldie.andreea@gmail.com

[6] Eng., University ''Politehnica'' of Bucharest, Faculty of Applied Chemistry and Material Science, Bucharest, Romania, matei.madalinaa@yahoo.com

genus are cultivated for ornamental purposes, but above all for the beneficial properties of their essential oils.

Medicinal plants are widely used by human beings all over the world, having many beneficial properties such as carminative, stomachic, diuretic and antibacterial [1-2]. Belonging to the *Lamiaceae* family, the genus *Mentha* contains some of the most common aromatic medicinal plants. It has over 30 species and hybrids, growing well in temperate climate in America, Europe, Australia and Asia. External factors such as climate, region and altitude are known to influence the chemical composition of the plants.

Peppermint *(M. piperita),* water mint *(M. aquatica)* and spearmint *(M. spicata)*, are cultivated in many countries for their properties and used in food, pharmaceutical and cosmetic industry. *M. piperita* is a hybrid between the other two species: *M. spicata* and *M. aquatica* [3-4]. The characteristic flavour of peppermint is given especially by menthol and its isomers (isomenthol, neomenthol and neoisomenthol).

Essential oils of *Mentha* species have many uses thanks to their antioxidant compounds. For example, *M. spicata*'s antioxidant properties are given by the presence of active constituents like menthol, menthone, rosmarinic acid and carvon**e** [5]. In many countries around the world like Pakistan, USA, France or Romania, carvone represents the major compound found in harvested *M. spicata* [6].

Among the most popular *Mentha* species, *M. aquatica* has a wide distribution across Europe, Asia and Africa. It usually grows in very damp ground and is often found on the banks of rivers and ponds. The main compound is menthofuran, consisting in more than 50% of the total essential oil. In this variety of water mint several important compounds have been extracted and identified, among them naningerin and viridiflorol with important anti-mutagenic effect. Some studies demonstrated that naringenin is important because it can exercise an effect on the central nervous system, due to the ability to cross the blood–brain barrier [7]. On the other hand, it has been shown that viridiflorol can inhibit acetylcholinesterase, an enzyme often linked with Alzheimer's disease [8].

In Romania, *Mentha* species grows in almost every type of region, but they vary according to environmental and climacteric conditions. Depending on this fact, there are over 8 species of this genus and more than 13 hybrids, including *M. piperita, M. aquatica, M. longifolia, M. spicata, M. arvensis, M. pulegium* etc.

Generally, both ^{1}H-NMR and ^{13}C-NMR are used to determine the structure of chemical compounds. Nevertheless, most of the studies carried on essential oils are based on the GC-MS technique [9-11].

^{1}H-NMR has a lot of advantages, such as its non-invasive nature and its conditions for work that allow the experiment to run without prior transformations (compared to GC-MS) [12]. This technique is also recommended for volatile

compounds, because it doesn't include heat and doesn't create any unwanted modifications on the substances profiles. Moreover, it is much faster than ^{13}C-NMR, offering an excellent alternative to the most popular methods currently used, thanks to its efficiency [13].

Even if GC-MS seems to be the most prevailed method, there are multiple reasons for which ^{1}H-NMR should be taken into consideration when it comes to essential oils and its substances. Now, there are only a few studies that choose this approach and in general, it is used only as a back-up, not as a main method for studying them [14-15].

2. Materials and methods

21 *Mentha* samples were analyzed, some cultivated in Romania (*Mentha piperita* and *Mentha spicata)*, some from the spontaneous flora of the Danube Delta (*Mentha aquatica*) and respectively some purchased from the main Romanian producers.

The essential oils extraction was realized through hydro distillation at a ratio of 1:10 (plant (g): water (mL)). The applied method, hydro distillation in a Clevenger apparatus, is the standard one, described in literature by Craveiro et al [16]. The volatile oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4° C until analysis.

The NMR analysis was conducted on a Bruker Ascend spectrometer 400 MHz, operating at 9.4 Tesla corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus. The spectrometer was controlled using TopSpin (version 3.2) Samples were analyzed in 5 mm NMR tubes (Wilmad 507). The NMR samples were prepared by dissolving the essential oil in $CDCl_3$ (2:8 v/v). The chemical shifts are reported in ppm and the quantitative measurement was performed using caffeine as internal standard. Each data set was collected under automation with the same parameter set using ICON-NMR software (version 4.7.5 Build 1). The average acquisition time of the ^{1}H-NMR spectra was approximately 2 minutes.

3. Results and Discussion

The profiles of the ^{1}H-NMR spectra of the main studied mint essential oil show important variations according with the *Mentha* variety.

Establishing the markers for major compounds

Identification of the major compounds from mint essential oil was made using standard compounds (carvone, menthol, menthone and menthofuran) purchased from Sigma Aldrich. For the identification of the corresponding signals for each major compound, a small quantity of standard compounds was added in

mint essential oil. Fig. 1 presents ^{1}H-NMR spectrum of *Mentha aquatica* essential oil (red) and *Mentha aquatica* essential oil with menthofuran added (blue). By comparison of the two ^{1}H-NMR spectra, two signals have been selected as markers for menthofuran: at 2,6-2,7 ppm and 7 ppm (easily quantifiable and non-overlapping signals). In a similar way were established specific signals as markers for carvone, menthol and menthone.
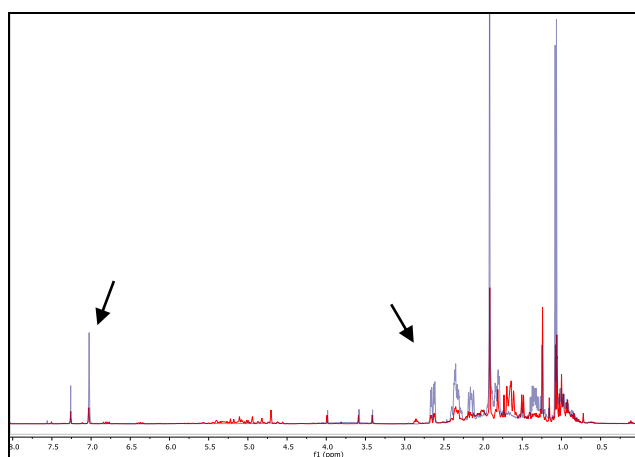

Fig. 1. Establishing marker's signal for menthofuran. ^{1}H-NMR spectrum of *Mentha aquatica* essential oil (red) and *Mentha aquatica* essential oil with added menthofuran (blue)

In Fig. 2 the ^{1}H-NMR spectrum of 3 different main varieties of essential mint oil is presented. The identified signals for the major compounds measurement are displayed.
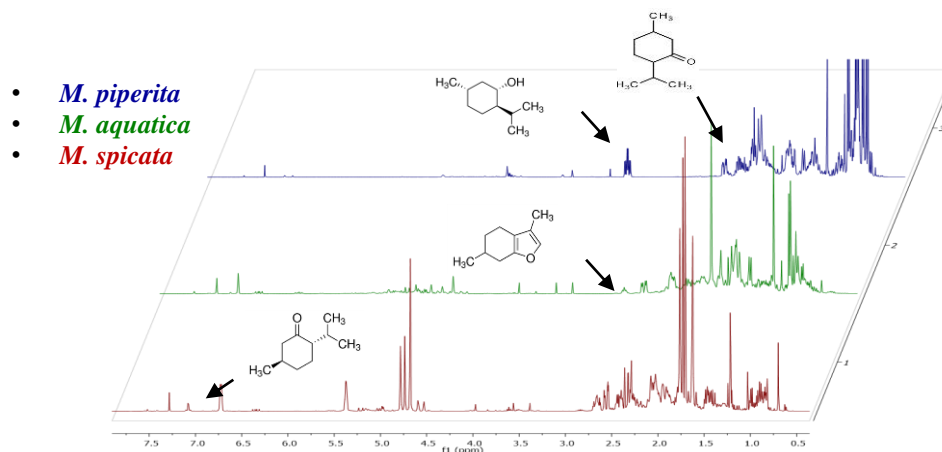

Fig. 2. Area 8-0.5 ppm of a ^{1}H- NMR spectra of mint oils from 3 main varieties harvested in Romania

It can be noticed that significant differences are visible in the NMR spectra for each variety.

Using the spectral attributions for the major compounds identified in mint essential oil, a screening of the analysed samples has been done. In Fig. 3 the quantitative results in terms of major components of different mint essential oils are displayed. Several aspects are evident: carvone is the main component of *Mentha spicata* essential oil, menthone and menthol for *Mentha piperita* and respectively menthofuran for *Mentha aquatica.*
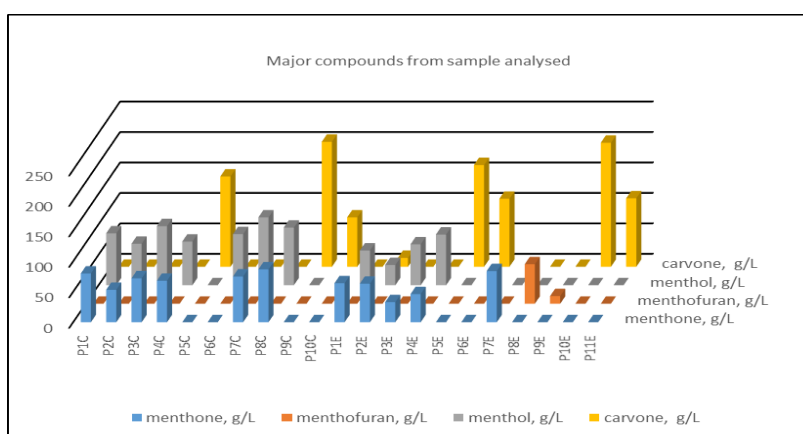

Fig. 3 Graphical representation of the quantitative results, in terms of major components, of different mint essential oil

Principal Component Analysis – PCA

The quantitative results for the major components within mint essential oil have been used in multivariate analyses of data. The results obtained from principal component analysis for the covariance of the spectral data obtained, is displayed in Fig. 4.

A good separation of the samples according with mint variety is obtained. This offer the premise for a good authentication of *Mentha aquatica* samples against other varieties of mint samples.
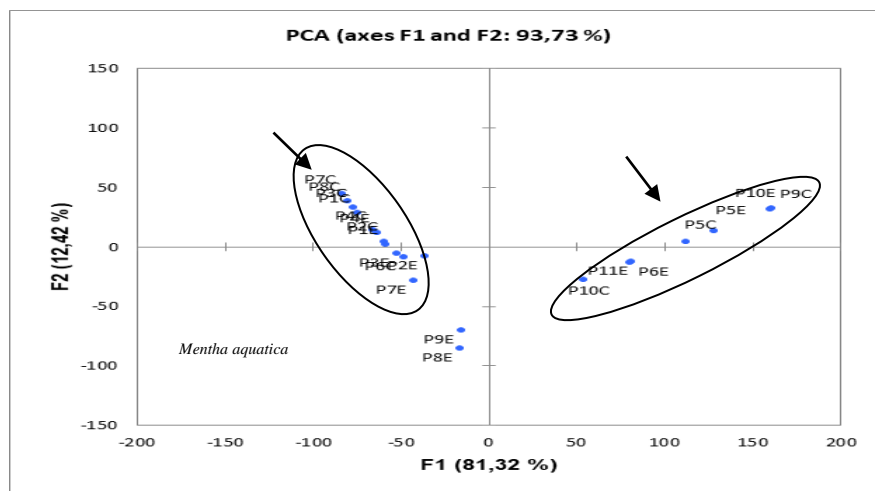
Fig. 4. Principal component scores PC1/PC2 for mint essential oils

4. Conclusions

We have analysed the most common types of mint from Romanian production. New methods for quantification of major compounds within mint essential oil have been developed, based on ^{1}H-NMR spectroscopy. It has been confirmed that there are compositional differences between mint species depending on the variety and geographic origin. Unlike other methods, NMR provides a short analysis time and a direct procedure (without prior sample processing) offering the sample's general profile, a very important tool combined with chemometric analysis, for authentication of essential oils.

Acknowledgements

R E F E R E N C E S

[1]. *E. Uribe, D. Marin, A. Vega-Galvez, I. Quispe-Fuentes, A. Rodriguez*, Assessment of vacuum-dried peppermint (*Mentha piperita* L.) as a source of natural antioxidants, Food Chemistry, **vol. 190,** 2016, pp. 559–565

[2]. *A. Sousa Barrosa, M.S. Moraisa, P. Travassos Ferreira, I. Gusmão Pinto Vieira, A. Aragão Craveiroc, R. Santos Fontenelle, J.E. Alencar de Menezes, F.W. Ferreira da Silva, H. Araújo de Sousaba*, Chemical composition and functional properties of essential oils from *Mentha* species, Industrial Crops, **vol. 76**, 2015, pp. 557-564

[3]. *M. Mehri, V. Sabaghi, F. Bagherzadeh-Kasmani, Mentha piperita* (peppermint) in growing Japanese quailsdiet: Performance, carcass attributes, morphology andmicrobial populations of intestine, Animal Feed Science and Technology, **vol. 207**, 2015, pp. 104–111

[4]. *M. Mehri, V. Sabaghi, F. Bagherzadeh-Kasmani*, *Mentha piperita* (peppermint) in growing Japanese quails'diet: Serum biochemistry, meat quality, humoral immunity, Animal Feed Science and Technology, **vol. 206**, 2015, pp. 57–66

[5]. *F. Brahmi, D. Hauchard, N. Guendouze, K. Madani, M. Kiendrebeogo, L. Kamaju, C. Stévigny, M. Chibane, P. Duez*, Phenolic composition, in vitro antioxidant effects and tyrosinase inhibitory activity of three Algerian *Mentha* species: *M. spicata* (L.), *M. pulegium* (L.) and *M. rotundifolia* (L.) Huds (*Lamiaceae*), Industrial Crops and Products, **vol. 74**, 2015, pp. 722-730

[6]. *A.I. Hussain, Anwar, M. Shahid,* Chemical Composition, and Antioxidant and Antimicrobial Activities of Essential Oil of Spearmint (*Mentha spicata* L.) from Pakistan, Journal of Essential Oil Research, **vol. 22**, 2010, pp. 78-84

[7]. *G.I. Stafford, M.E. Pedersen, J. van Staden, A.K. Jäger*, 2008, Review on plants with CNS-effects used in traditional South African medicine against mental diseases, Journal of Ethnopharmacology, **vol. 119**, pp. 513–537

[8]. *A.K. Jäger, J.P. Almqvist, S.A.K. Vangsøe, G.I. Stafford, A. Adsersen, J. van Staden,* Compounds from *Mentha aquatica* with affinity to the GABA-benzodiazepine receptor, South African Journal of Botany, **vol. 73**, 2007**,** pp. 518–521

[9]. *T.K. Tiên Do, F. Hajdi-Minaglou, S. Antoniotti, X. Fernandez,* Authenticity of essential oils, Trends in analytical chemistry, **vol. 66**, 2015, pp. 46-157

[10]. *S. Sutour, P. Bradesi, F. Tomi, J. Casanova,* Chemical composition of the essential oil from Corsican *Mentha aquatica* - Combined Analysis by GC(RI), GC-MS and ^{13}C NMR Spectroscopy, Natural Product Communications, **vol. 6**, 2011, pp. 1479-1482

[11]. *G.I. Stafford, J.P. Almqvista, S.A.K. Vangsøea, H.T. Olsena, S.B. Christensen, A. Adsersen, A.K. Jäger, J. van Staden*, South African Journal of Botany, **vol. 74**, 2008, pp. 389

[12]. *N.A. Chira, M.C. Todasca, A. Nicolescu, A. Rosu, M. Nicolae, S.I. Rosca,* Evaluation of the Computational Methods for Determining Vegetable Oils Composition using ^{1}H-NMR Spectroscopy, REV. CHIM Bucharest, **vol. 62**, 2011, pp. 42-46

[13]. *C.I. Cerceau, L.C.A. Barbosa, C.A. Filomeno, E.S. Alvarenga, A.J. Demuner, P.H. Fidencio,* Chemical composition and functional properties of essential oils from *Mentha* species, Talanta, **vol. 150**, 2016, pp. 97-103

[14]. *K.S.C. Tang, I. Konczak, J. Zhao,* Identification and quantification of phenolics in Australian native mint (*Mentha australis* R. Br.), Food Chemistry, **vol. 192**, 2016, pp. 698-705

[15]. *H.T. Olsen, G.I. Stafford, J. van Staden, S.B. Christensen, A.K. Jäger,* Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L., Journal of Ethnopharmacology, **vol. 117**, 2008, pp. 500-502

[16]. *A.A. Craveiro, F.J.A. Matos, J.W. de Alencar,* A simple and inexpensive steam generator for essential oils extraction, Journal of Chemical Education, no. *53*, **vol. 10**, 1976, pp. 652