Chemical Composition of Mentha Longifolia Essential Oil from Albania Populations

Jonida Salihila, Aurel Nuro, Aida Dervishi, Dhimitër Peçi, Dorina Shëngjergji

***Abstract*— The essential oil of Mentha longifolia L. was analyzed from population of South, South-East and Central Albania. Mentha longifolia is easy to grow, with the same requirements as most mints. Found in most parts of Albania and easy to harvest, wild mint is a popular traditional medicine. Pulegon is the main compound of the plant responsible for most of its pharmacological effects followed by Menthone, Isomenthone, Menthol, 1,8-Cineole, Carvacrol, and Piperitenone. Based on the review of various studies, it can be saw that Mentha longifolia L. is a potential natural source for the development of new drugs. The areal parts of Mentha herbs were sampling in June, 2017. The air dried plant samples were subjected to European Pharmacopoeia apparatus (Clevenger type) for 4 hours to obtain Mentha essential oil. The chemical composition of the essential oils was analyzed using GC/FID technique. The oil of each Mentha longifolia L. essential oil samples were injected in a Varian 450 GC. VF-1ms capillary column (30 m x 0.33 mm x 0.25 um) were used for separation of compounds. Monoterpenes (especially oxygenated monoterpenes) were in higher percentage in Mentha longifolia essential oil for all areas. Terpenes that were found in higher percentage were: Cineole, Linalool, Menthol, Pulegon, Carvone, Piperthone, Thymol, beta-Caryophyllene. Their profile was not the same for all studied areas. Profile and leveles of Mentha longifolia L. samples from Central, South-East and South Albania was the same with other reported studies from Balkan and Mediterrean area.**

***Index Terms*— Mentha longifolia, Essential oils, Menthol, Linalool, Thymol, GC/FID.**

I. INTRODUCTION

Mentha longifolia is a species in the genus Mentha, family Lamiaceae, native to Europe, western and central Asia and northern and southern Africa. It is a very variable herbaceous perennial plant with a peppermint –scented aroma. Like many mints, it has a creeping rhizome, with erect to creeping stems 40–120 cm tall. The leaves are oblong-elliptical to lanceolate, 5–10 cm long and 1.5–3 cm broad, green to greyish-green above and white below. The flowers are 3–5 mm long, lilac, purplish, or white, produced in dense clusters on tall, branched, tapering spikes; flowering in mid to late summer. It spreads via rhizomes to form clonal colonies. *Mentha longifolia* is easy to grow, with the same requirements as most mints. Mints are fast growers but seem to be always on the move. With underground runners, they disappear and pop up wherever they find a suitable spot. Heavy feeders and water lovers, mints grow in semi-shade and full sun. Found in most parts of Albania and easy to harvest, wild mint is a popular traditional medicine [1]. It is mainly used for respiratory ailments but many other uses have also been recorded. It is mostly the leaves that are used, usually to make a tea that is drunk for coughs, colds, stomach cramps, asthma, flatulence, indigestion and headaches. Externally, wild mint has been used to treat wounds and swollen glands. Usage of *Mentha longifolia L.* in the treatment of throat irritation, mouth and sore throat is widespread. Studies have shown that plants of the genus *Mentha* possess significant antimicrobial activities, mainly due to the presence of oxygenated monoterpenes. The essential oil of the plant showed fungistatic and fungicidal activity. Menthol has been shown to be an antimicrobial and antifungal agent against ringworm and other fungal infestations of different kinds. Menthol is also effective against dental bacteria. Piperitenone oxide is the main integral that is attributed to the insecticidal activity of the plant [2],[3], [4].

Mentha longifolia is an herb with a wide range of pharmacological properties such as antimicrobial, gastrointestinal, and nervous system effects. Studies carried out on the chemical composition of the plant have shown that the main chemical compounds present in *M. longifolia* essential oil are monoterpenes, particularly oxygenated ones. Pulegone is the main compound of the plant responsible for most of its pharmacological effects followed by Menthone, Isomenthone, Menthol, 1,8-Cineole, Borneol, and Piperitenone. Moreover, the plant may dose-dependently exert toxic effects in different systems of the body. Based on the review of various studies, it can be concluded that *Mentha longifolia L.* is a potential natural source for the development of new drugs [5]. Essential oils derived from *Mentha* have valuable pharmacological properties that have been investigated by many scientists around the world. Due to their antimicrobial, insecticidal, antifungal, and antibacterial activities, essential oils have been intensely screened and applied in the fields of pharmacology, medical and clinical micro-biology, phytopathology and food preservation [6].

II. MATERIAL AND METHODS

A. Reactive and standards

Toluene of chromatographic grade was purchased from Merck (Darmstadt, Germany). A mixture of n-alkanes (Sigma Aldrich) from n-octane (C8) to eicosanes (C20) was used for calculation of Kovats indices (KI). Dried and ground plant material of *Mentha* (*Mentha longifolia* L.) was obtained from the Institute for Medicinal Plant Research "Dr Josif Pančić" (Belgrade, Serbia).

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B. *Sampling of Mentha longifolia L. from Albania*

Mentha longifolia samples were taken from population of South (2 sample from Tepelena and 3 sample from Vlora), South-East (two sample from Pogradeci, two sample from Korca and one sample from Kolonja) and Central Albania (three sample from Tirana and two sample from Elbasani). The *Mentha* herbs were sampling in June 2017. The sampling station of *Mentha longifolia* plants was shown in Figure 1. Areal parts (branches, leaves and flowers) of *Mentha longifolia L.* were used for this study. Material plants were air dried in shadow for saving their morphological characteristics.

Figure 1. The map of *Mentha* longlifolia L. sampling site, June 2017

C. *Isolation of Mentha essential oil*

Air dried plant material of *Mentha longifolia L.* were cut in small pieces (0.5 to 2 cm) before analyze. 50 g of plant material was subjected to hydrodistillation for 4 h, using a modified Clevenger-type apparatus to produce essential oil. 1 ml Toluene was added to the balloon for isolation of *Mentha* essential oils. The oil was dried by anhydrous sodium sulfate (Na_2SO_4) and kept sealed in dark glass vial at +4 ^oC until use. Diluted essential oil in Toluene was used for GC/FID analyses.

D. *Apparatus and chromatography*

Gas chromatographic analyses of *Mentha e*ssential oil were realized with a Varian 450 GC instrument equipped with a flame ionization detector and PTV detector. The temperature of PTV injector was 280^oC. 1 ul of *Mentha longifolia L.* essential oil diluted in Toluene was injected in splitless mode. A temperature for FID was held at 280°C. Nitrogen was used as carrier (1 ml/min) and make-up gas (25 ml/min). Hydrogen and air were flame detector gases with 30 ml/min and 300 ml/min, respectively. VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was used to isolate compounds of *Mentha longifolia L.* essential oil. The oven temperature was programmed as follows: 40°C (held for 2 minutes) to 150^oC (with 4°C/min), after that to 280 °C with 10°C/min and held for 2 minutes. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and literature [7], [8], [9], [10]. Chromatogram of the *Mentha* essential oil for sample site Tepelena, South Albania sample was shown in Figure 2.

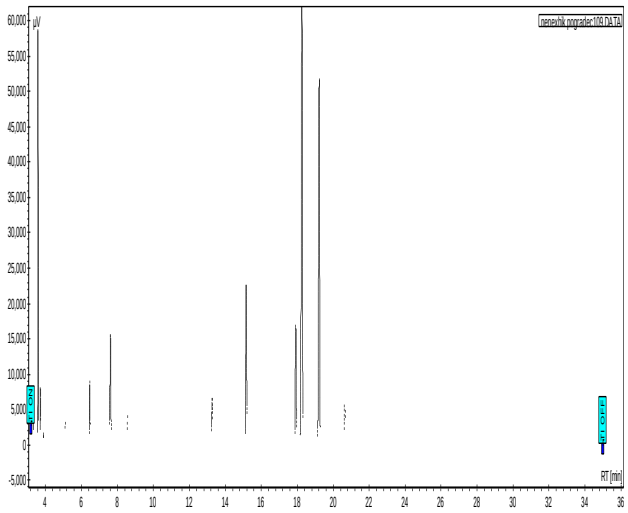

Figure 2. Chromatogram of *Mentha longifolia* essential oil for Tepelena sample, South Albania

III. Results and Discussion

Essential oil of *Mentha longifolia L.* samples from South, South-East and Central Albania were analyzed using GC/FID technique. Averages of results for samples from the same areas were presented in this study. The data were in percent for the total of peaks except for the peak of Toluene that was the solvent used for dilution. For individual *Mentha longifolia* essential oil were found from 47 to 65 compounds. The peaks lower than 0.1% was not present in this study. The data present a total of 30 the main compounds that were found for all *Mentha* essential oil by the chromatographic method used. Their averages for the total were: 97.72% for Central Albania; 97.58% for South-East Albania and 98.52% for South Albania. Terpenes that were found in higher percentage were: Cineole, Linalool, Menthol, Pulegon, Carvone, Piperthone, Thymol, beta-Caryophyllene. Note that, their profile was not the same for all studied areas. Figure 3 shown percent of dedected compounds in analyzed Mentha essential oil samples. Profile of terpenes for samples of Central Albania was: Pulegon (40.43%) > Cineol (8.27%) > Thymol (6.47%) > Menthol (6.45%) > beta-Caryophyllene (6.01%). Linalool (1.33%) and Carvone (0.61%) was found in lower percentage for this area. Profile of terpenes for samples of South-East Albania was: Linalool (24.17%) > Pulegon (11.77%) > Cineol (8.6%) > Limonene (7.5%) > Thymol (6.28%) > Menthol (4.11%) > Piperithone (4.34%). Pulegone, Carvone (3.05%) and Menthol were found in lower percentage for this area compare with other areas. Profile of terpenes for samples of South Albania was: Pulegon (28.66%) > Carvone (20.33%) > Linalool (9.77%) > Menthol (6.49%) > Cineol (5.45%) > Menthon (3.47%) > Piperithone (3.43%). It was noted the higher percentage of Carvone for this area. These differences could be because of geographic and geologic factors for studied areas. Latitude and the position of the plants in the places where they are growing could affect in their composition. Soil composition could also affect in their composition. Monoterpenes were in higher percentage in *Mentha longifolia* essential oil for all areas. Their percentages were 86.43% in Central Albania, 89.71% in South-East Albania and 93.29% in South Albania samples. Aliphatic and monocyclic hydrocarbon monoterpenes were from 9.13% in South Albania to 19.18% in South-East Albania samples.

Table 1. Percent averages of main compounds in analyzed Mentha essential oil samples from South, South-East and Central Albania, 2017

	Central Albania	South-East Albania	South Albania
alfa-Thujene	0.32	0.15	0.16
alfa-Pinene	1.62	1.87	1.95
Camphene	0.95	1.27	0.52
beta-Pinene	2.19	1.20	0.96
Sabinene	0.32	0.14	0.10
d-3-Carene	0.84	0.67	0.69
Myrcene	3.05	2.19	2.69
alfa-Terpinene	1.18	3.55	0.12
Limonene	0.15	7.50	0.24
para-Cymene	0.67	2.55	0.18
1,8-Cineole	8.27	8.60	5.45
gama-Terpinene	0.17	0.39	0.28
Cis-Sabinenehydrat	0.47	0.25	1.42
Oktanol	0.43	0.58	1.36
Menthon	1.91	2.25	3.47
Isomenthone	1.43	0.38	1.89
Linalool	1.33	24.17	9.77
Borneol	0.23	0.18	0.24
Menthol	6.45	4.11	6.49
Neomenthol	1.90	0.13	0.14
Terpinen-4-ol	1.91	0.25	0.35
alfa-Terpineol	1.31	0.68	0.11
Pulegon	40.43	11.77	28.66
Carvone	0.61	3.05	20.33
Piperithone	1.59	4.34	3.43
Piperitenone	0.21	1.21	0.76
Thymol	6.47	6.28	1.53
beta-Caryophyllene	6.01	2.62	1.68
alfa-Humulene	0.75	2.01	0.66
d-Cadinene	4.55	3.24	2.89
Total	97.72	97.58	98.52
Total Monoterpenes	86.43	89.71	93.29
Hydrocarbon Monoterpene	11.26	19.18	9.13
Oxygenated Monoterpene	68.03	61.70	82.45
Aromatic Monoterpene	7.14	8.83	1.71
Sesquiterpene	11.31	7.87	5.23

Myrcene were found in higher percentage for all samples. Limonene and alfa-Terpinene were in higher percentage in South-East samples. The higher percentages were for oxygenated monoterpenes. These compounds are responsible for many health effects. Oxygenated monoterpenes percentages were respectively: 68.03% in Central Albania, 61.7% in South-East Albania and 82.45% in South Albania. Pulegon was in higher percentage for Central (40.43%) and South Albania (28.66%) samples. Linalool (24.17%) was the higher oxygenated terpene for South-East Albania and the Pulegon was the second with 11.77%. Linalool was found in higher percentage in South-East smaples (9.77%) but not in Central Albania (1.33%) samples. Carvone was found in higher percentage for South Albania samples with 20.33%. 1,8-Cineole was found for all samples from 5.45 to 8.6%. Total of Menthol, Menthon and related compounds were from 8.4 to 13.2%. Their percentage was higher in central Albania samples. Piperithone and Piperitenone were in higher concentration in South-East Albania samples. Thymol was aromatic terpene found in higher percentage from 1.53% to South Albania to 6.47% to Central Albania samples. Total of aromatic terpene were from 1.71% in South to 8.83% in South-East Albania samples. Sesquiterpenes were found from 5.23% (South Albania) to 11.31% (Central Albania). The higher percentage was for beta-Caryophyllene, the maximum was found in Central Albania (6.01%). Delta-Cadinene was the second sesquiterpene found in higher percentage. Profile and leveles of *Mentha longifolia L.* samples from central, South-East and South Albania was the same with other reported studies from Balkan and Mediterrean area [6].

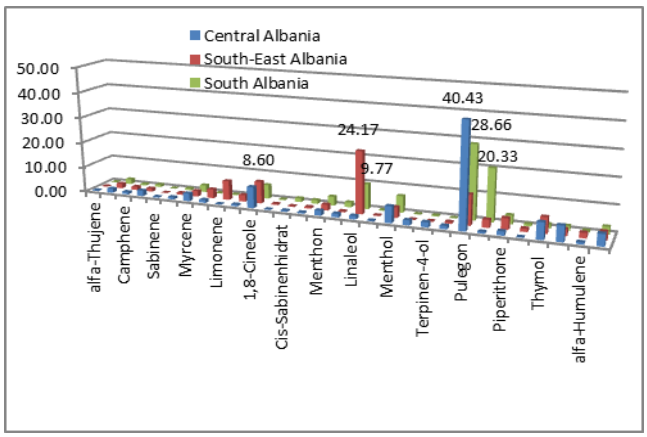

Figure 3. Percent average of main compounds in analyzed Mentha essential oil samples

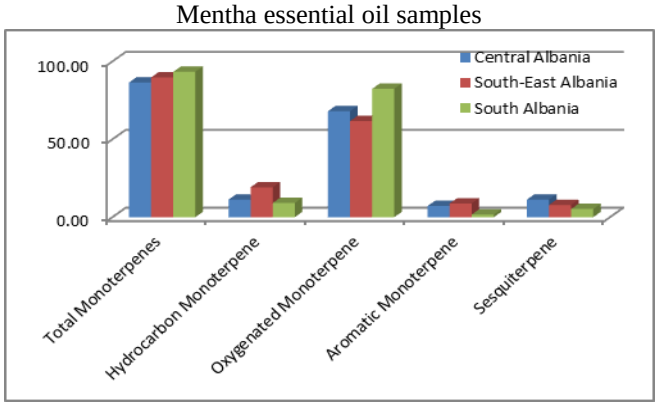

Figure 4. Distribution of terpenes in Mentha longifolia essential oil samples from South, South-East and Central Albania, June 2017

IV. Conclusions

Essential oil of *Mentha Longifolia L.* samples from South, South-East and Central Albania were analyzed using GC/FID technique. Gas chromatography technique is recommended by the literature. Averages of results for samples from the same areas were presented in this study. The data present a total of 30 main compounds that were found for all *Mentha* oils by the chromatographic method used. Terpenes that were found in higher percentage were: Cineole, Linalool, Menthol, Pulegon, Carvone, Piperthone, Thymol, beta-Caryophyllene. Terpene profile was not the same for all studied areas. Profiles of terpenes for studied samples of Central Albania were: Pulegon > Cineol > Thymol > Menthol > beta-Caryophyllene; in South-East samples was: Linalool > Pulegon > Cineol > Limonene > Thymol > Menthol > Piperithone and in South Albania profile was: Pulegon > Carvone > Linalool >

Menthol > Cineol > Menthon > Piperithone. *Mentha longifolia L.* essential oil has high percentage of the oxygenated monoterpenes. The geologic (soil composition) and geographical position (particulary the latitude) are important factors for the differences found between the analyzed *Mentha* samples. Profile of *Mentha longifolia L.* samples from Central, South-East and South Albania were comparable with other reported studies from Balkan and Mediterrean area. The work would be complete if it continues for several years.

REFERENCES

[1] Wolfgang K., Susanne H. & Andreas H., Medicinal and Aromatic Plants in Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania. A study of the collection of and trade in medicinal and aromatic plants (MAPs), relevant legislation and the potential of MAP use for financing nature conservation and protected areas" WWF Deutschland / TRAFFIC Europe-Germany. (2003)

[2] Mkaddem M., Bouajila J., Ennajar M., Lebrihi A., Mathieu F., Romdhane M. Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and viridis) essential oils. J Food Sci. 2009;74:M, pp.358–63.

[3] Hajlaoui H, Snoussi M, Ben Jannet H, Mighri Z, Bakhrouf A. Comparison of chemical composition and antimicrobial activities of *Mentha longifolia* L. ssp. *longifolia* essential oil from two Tunisian localities (Gabes and Sidi Bouzid) Ann Microbiol. 2008;58:pp. 513–20.

[4] Sarac N. & Ugur A. Antimicrobial activities and usage in folkloric medicine of some *Lamiaceae* species growing in Mugla, Turkey. Eurasian J Bio Sci. 2007;4: pp.28–37.

[5] Mikaili P., Mojaverrostami S., Moloudizargari M., Aghajanshakeri Sh., Pharmacological and therapeutic effects of *Mentha Longifolia* L. and its main constituent, menthol. Ancient Science of Life; 2013, Vol. 33(2): 131–138. doi: 10.4103/0257-7941.139059

[6] Daferera D.J., Ziogas B.N., Polissiou M.G., GC–MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*, *Jour-nal of Agricultural Food Chemistry*, 2000, ***48***, 2576–2581.

[7] Adams R. P., Identification of essential oil components by gas chromatography/mass spectroscopy, *Allured Publishing Cor-poration*, Carol Stream: Illinois, USA.1995.

[8] David F., Scanlan F., Sandra P., Szelewski M., Analysis of essential oil compounds using retention time locked methods and retention time databases, Application, *Agilent Technologies*, 2010, 5988-6530EN.

[9] Konig W.A., Bulow N., Saritas Y., Identification of sesquiterpene hydrocarbons by gas phase analytical methods, *Flavour Fragr. J. 1999, Vol. 14*, pp.367-378.

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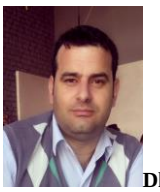

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