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# VOLATILE COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM *MENTHA ARVENSIS* L. ORGANICALLY PLANTED FROM MACEDONIA

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## Introduction

The aromatic plant Mentha arvensis belonging to the family Lamiaceae and has been used as a medicinal and aromatic plant since ancient times, in both western and eastern cultures. Many studies on the therapeutic values of mint and mint oils have been reported (1,2); these are stomachic, carminative, antispasmodic, stimulant, local anesthetic, anti-inflammatory, diuretic, anthelmentic, antibacterial, antifungal and antioxidant [1,2].

The volatile profile, antioxidant and antimicrobial potential of essential oil from Macedonian *Mentha arvensis L.* was object of this study. The plant was organically produced from for the first time at south-east region of Macedonia (41º49`N, 21º 59`E) on the overlapping area of two climate types: the Mediterranean and Continental climate.

## Materials and Methods

**Oil isolation and analysis:** A sample of 250 g of dried leaves and stems was mixed with 500 mL of tap water in flask and water distilled for 2 h using a Clevenger-type apparatus. The oil content was measured based on mL oil per 100 g dry matter (mL/DM). The oil compositions were analyzed by GC (FID) and GC/MS. Gas chromatography was carried out with an Agilent HP 6890 gas chromatograph equipped with flame-ionization detector (FID) and quantitation was carried out by addition of pure standards as well as area normalization and neglecting response factors. The analysis was conducted using a HP-5 (5% Phenyl Methyl Siloxane) fused silica capillary column (30 m x 0.50 mm, film thickness 0.32 μm, J &W Scientific Inc., Rancho Cordova, CA). The operating conditions were as follow: injector and detector temperature: 250˚ C, carrier gas: helium; inlet pressure: 35.4 kPa. Oven temperature program was 50 - 220˚ C at the rate of 4˚ C/min. Quantitative data concerning the percentage contribution of each constituent were taken with this system. GC/MS analysis was carried out using an Agilent HP 6890 gas chromatograph fitted with the same column as described above, coupled to quadrupole 5973 MSD, which was operated at an ionization potential of 70 eV and electron multiplier energy 2000 V. The temperate program started at 50˚ C during the split injection and then programmed to 220˚ C with increment of 4˚ C/min. The oil components were identified by comparing their retention indices and mass spectra data with those of authentic samples and published data.

**Antioxidant assays:** The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity of methanol extract and essential oil under study. For this purpose 10 mL of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) solution was prepared from 39.23 mg of ABTS and 7.17 mg of potassium thiosuphate disolved in Nanopure water to volume. Trolox with different concentrations (0.1 – 10 mg/L) was dissolved in methanol and was used as standard for the preparation of the calibration curve (Fig. 1 and Table 1).

**Antimicrobial activity:** Antibacterial activity against *gram-positive bacterial strain*: ***Escherichia coli* (ATCC 25922)**, and against antifungal activity using: ***Candida albicans* (ATCC 10231)**. Each microorganism was suspended in Mueller Hinton (MH) broth and diluted approximately to 10E6 colony forming unit (cfu)/mL. The plates were incubated at 37˚ C and the diameters of the growth inhibition zones were measured after 24 h. Gentamicin (10 μg/well) was used as positive control. The negative control was performed with only sterile broth cultured 24 h with 10 μL of 70% ethanol.

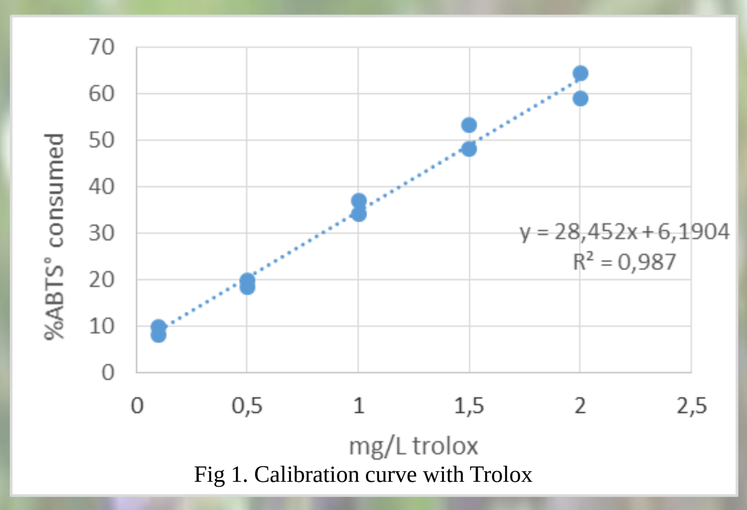


Fig 1. Calibration curve with Trolox

| Sample | A (abs) | SDV | mg TE/L oil |
|---|---|---|---|
| Mentha oil | **2,380** | **0,057** | **1,586** |

Table 1. Antioxidant activity of essential oil from whole plant

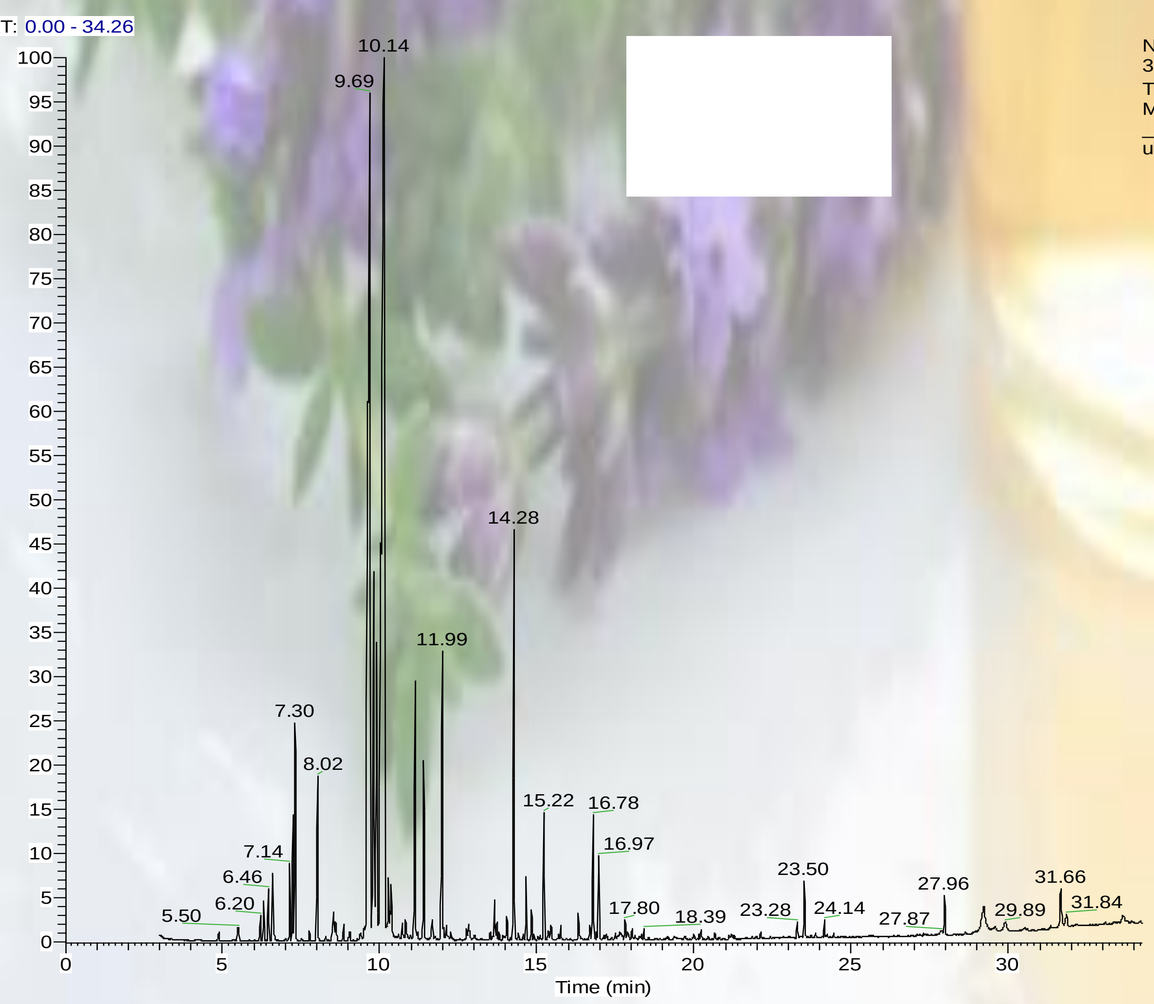


Fig 2. TIC Chromatogram of essential oil from Mentha arvensis L.

## Results and Discussion

Fifty-five components were identified and quantified in the three essential oils isolated from the flowers, leaves and whole Mentha plant ( Fig.2. and Table 2). The most abundant component in all three oils was menthol with 35.64%, 32.47% and 52.53% respectively. The second most dominant component in the three essential oils was isomenthone with 20.38%, 15.97% and 8.42% respectively. All other components were in quantity less than 8%.
The antioxidant activity of essential oil from whole Mentha plant was determined against ABTS radical with value of 1.58 TE mg/L of oil. The antimicrobial activity of essential oil isolated from whole Mentha plant was determined against Escherichia coli and Candida albicans. Our results showed significant antibacterial activity against Escherichia coli ATCC 25922 (24 mm) and significant antifungal activity against Candida albicans ATCC 10231 (32 mm).

Table 2. Volatile profile of three essential oils from Mentha arvensis L. analyzed by gas chromatography

| Apex RT | %Area | | | Component |
|---|---|---|---|---|
| | *Flowers* | *Leaves* | *Whole plant* | |
| 5,33 | 0,08 | | | 3-thujene |
| 5,49 | 1,22 | 0,95 | 0,38 | alpha-pinene |
| 6,19 | 0,51 | 0,7 | 0,16 | sabinene |
| 6,3 | 1,29 | 1,13 | 0,84 | beta-pinene |
| 6,44 | 0,18 | 0,17 | 0,2 | beta-myrcene |
| 6,57 | 0,18 | 0,31 | 0,3 | n-octan-3-ol |
| 7 | 0,05 | 0,09 | 0,05 | alpha-terpinene |
| 7,13 | 0,26 | 0,53 | 0,72 | cymene |
| 7,24 | 2,07 | 1,48 | 0,41 | limonene |
| 7,3 | 3,7 | 5,4 | 2,35 | 1,4 cineol (eucalyptol) |
| 7,76 | 0,14 | 0,38 | 0,3 | gamma-terpinene |
| 8 | 0,66 | 1,92 | 1,76 | 4-thujanol,cis |
| 8,27 | | 0,14 | 0,09 | cis-alpha-terpineol |
| 8,51 | 0,18 | 0,38 | 0,36 | alpha-linalool |
| 8,59 | 0,11 | 0,24 | 0,27 | cis-beta-terpinenol |
| 8,64 | 0,09 | | | isopenthyl alcohol,isovalerate |
| 9,05 | | 0,31 | | piperitol? |
| 9,48 | 0,15 | | | isopulegol(p-menth-8-en-3-ol) |
| 9,66 | **20,38** | **15,97** | **8,42** | **isomenthone (p-mentan-3-one)** |
| 9,8 | 8,4 | 4,08 | 2,58 | menthofuran |
| 9,88 | 3,08 | 5,24 | 4,47 | neomenthol (menthol,trans 1,3cis1,4) |
| 10,12 | **35,64** | **32,47** | **52,53** | **menthol** |
| 10,27 | 0,55 | | | neoisopulegol |
| 11,14 | 7,79 | 0,75 | 1,02 | cis-isopulegone |
| 11,4 | 0,52 | 0,62 | 1,03 | piperitone |
| 11,99 | 3,38 | 5,03 | 8,75 | isomenthyl acetate |
| 12,27 | | | 0,42 | menthyl acetate |
| 13,67 | | 0,55 | 0,57 | alpha-bourbonene |
| 14,17 | 0,89 | | | tetradecane |
| 14,26 | 3,77 | 3,5 | 3,6 | caryophyllene |
| 14,66 | 0,54 | 0,81 | 0,58 | alpha-farnesene |
| 15,24 | 1,7 | 2,59 | 2,14 | germacrene D |
| 15,46 | | 0,29 | | gamma-elemene |
| 15,68 | 1,08 | 0,92 | | naphthyl betene? |
| 16,7 | 0,12 | | 0,62 | spathulenol |
| 16,8 | 0,65 | | 1,05 | caryophylene oxide |
| 16,96 | | | 0,62 | ledol |
| 16,97 | 0,63 | 0,86 | 1,89 | viridiflorol |
| 21,08 | | 0,87 | | sebacic acid diethyl eter |
| 21,72 | | 3,71 | | palmitic acid |
| 23,52 | | | 0,92 | phytol |
| 23,9 | | 6,05 | | octadecenoic acid |
| 24,09 | | 1,03 | | stearic acid |
| 27,98 | | | 0,56 | diisooctylphtalate (contaminant) |
| 33,7 | | 0,54 | | glycerol tricaprilate |
| Total | **99,99** | **100,01** | **99,96** | |

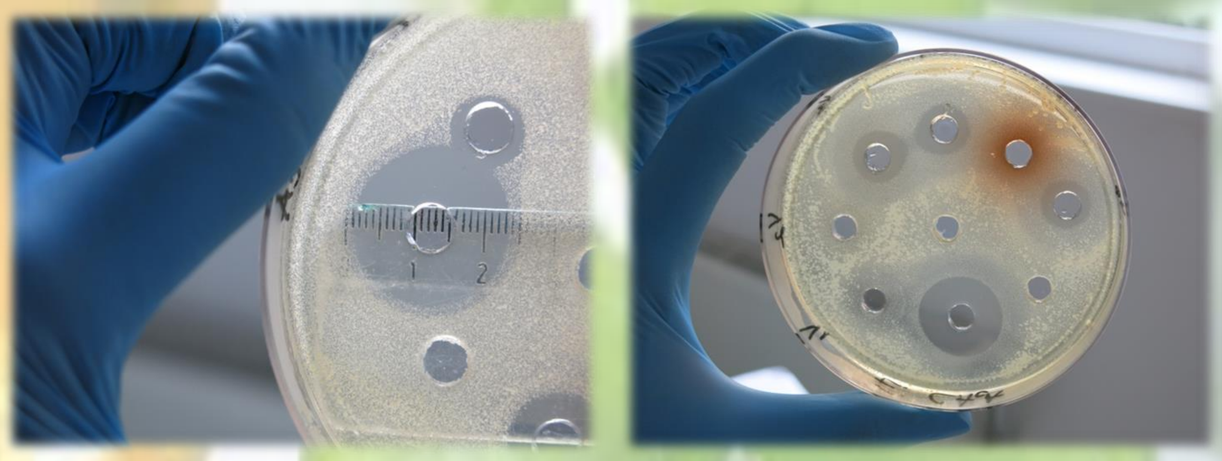


Fig 3. Antimicrobial activity of essential oil from Mentha arvensis L.

## Conclusion

We concluded that the region of South-east Macedonia had good potential for production of high-quality Cornmint (Mentha arvensis L.) with appreciable amount of menthol and menthone. Furthermore, antioxidant, antibacterial, and antifungal activity of essential oil from Mentha plant can be interesting for further investigation for medicinal purposes.

**References:**

1. A. Tiwari, Recent advances and callenges in trichome research and essential oil biosynthesis in *Mentha arvensis L.*, *Ind. Crop. Prod.* **82** (2016) 141-148.

2. B. R. Rajeswara Rao, Biomass and essential oil yields of cormint (*Mentha arvensis* L. f. *piperascens* Malinvaud ex *Holmes*) planted in different months in semi-arid tropical climate, *Ind. Crop. Prod.* **10** (1999) 107-113.