*https://doi.org/10.31925/farmacia.2022.3.9* **ORIGINAL ARTICLE**

# THE CHEMICAL COMPOSITION AND THE ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS OBTAINED FROM THREE VARIETIES OF *MENTHA x PIPERITA* F. *CITRATA*

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**Abstract**

The aim of the present study is represented by the investigation of the chemical composition and the *in vitro* antibacterial activity of essential oils obtained from three new varieties of cultivated *Mentha x piperita* f. *citrata* ("Orange", "Chocolate" and "Basil"). The chemical composition of the essential oils obtained by hydro-distillation of fresh and dry vegetal materials was analysed by a gas chromatography coupled with mass spectrometry method (GC-MS) and the antibacterial activity of essential oils was evaluated by the determination of the minimum inhibitory concentration (MIC). The results of the GC-MS analysis indicated linalool (28.07%; 58.23%) and linalyl acetate (10.75%; 38.49%) as major constituents in mint essential oils obtained from the "Orange" and "Basil" varieties, while levomenthol (31.06%; 32.58%) and isomenthone (24.94%; 28.33%) were majority in the mint "Chocolate" variety essential oil. All studied essential oils displayed antibacterial potential against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Salmonella enterica*) bacteria. The most effective activity was obtained for the mint "Chocolate" essential oil. Our results can provide the scientifically support for the beneficial use of these three varieties of mint as valuable raw materials for phytomedicines, cosmetics and food industry.

**Rezumat**

Scopul prezentului studiu este reprezentat de investigarea compoziției chimice și a activității antibacteriene *in vitro* a uleiurilor volatile, obținute de la trei noi varietăți cultivate de *Mentha x piperita* f. *citrata* („Orange", „Chocolate" și „Basil"). Compoziția chimică a uleiurilor volatile obținute prin hidrodistilare din material vegetal proaspăt și uscat, a fost analizată printr-o metodă gaz-cromatografică cuplată cu spectrometrie de masă (GC-MS), iar activitatea antibacteriană a uleiurilor volatile a fost evaluată prin determinarea concentrației minime inhibitorii (MIC). Rezultatele analizei GC-MS au indicat linaloolul (28,07%; 58,23%) și acetatul de linalil (10,75%; 38,49%) ca fiind constituenți majoritari în uleiurile volatile de mentă obținute din soiurile „Orange" și „Basil", în timp ce levomentolul (31,06%; 32,58%) și izomentona (24,94%; 28,33%) au fost majoritari în uleiul volatil din soiul de mentă „Chocolate". Toate uleiurile volatile studiate au prezentat potențial antibacterian atât împotriva bacteriilor Gram-pozitive (*Staphylococcus aureus*) cât și Gram-negative (*Escherichia coli, Salmonella enterica*). Cea mai intensă activitate a fost observată în cazul uleiului volatil din soiul „Chocolate". Rezultatele obținute pot oferi suport științific pentru utilizarea acestor trei soiuri de mentă ca materii prime valoroase pentru fitomedicină, cosmetică și industria alimentară.

**Keywords**: *Mentha x piperita* f. *citrata,* lemon mint, GC-MS, antibacterial

## Introduction

The genus *Mentha* (*Lamiaceae*) includes 23 - 30 species and hybrids, which have hundreds of sub-species, varieties and cultivars, widely cultivated in temperate areas around the world, to be used in pharmaceutical, food and dermato-cosmetics industries [6-11]. *Mentha x piperita* f. *citrata* (Ehrh.) Briq. (lemon mint) is one of the most cultivated mint species, having a citrus fragrance. It has dark purple stems, oval and purple leaves and small pale purple flowers [9]. The *Mentha x piperita* f. *citrata* "Orange" variety is a species growing at about 50 cm, with ovate, green leaves, green or reddish stem and purple flowers grouped in false verticils. The "Orange" mint variety tends to be stronger in flavour than many other varieties of mint with notes of citrus and spice with lavender undertones. The *M. x piperita* f. *citrata* "Chocolate" variety can be considered a decorative plant, growing at about 60 cm, with ovate-lanceolate, serrate leaves, slightly hairy, with brown shades on the veins and on the inferior side of the leaf. The flowers are grouped in purple verticils. The plant has an intense peppermint flavour and it is used as flavouring in desserts and drinks [7]. The *M. x piperita* f. *citrata* "Basil" variety grows at about 45 - 60 cm, having elliptical or elliptic-lanceolate leaves, dark green in colour, with reddish tints. The flowers are grouped in pale purple verticils. The plant has a mint and sweet fragrance [9]. Previous studies pointed out antimicrobial, anti-inflammatory and antioxidant properties of the essential oils obtained from different varieties of *Mentha sp.* [5, 6, 9, 10, 12, 14, 19]. In general, agricultural crops of medicinal plants are made with improved varieties that have certain superior characteristics (high yield, important content in valuable active ingredients, fine aroma, resistance to drought, frost, diseases and pests, etc.) [10]. This study was aimed to investigate the chemical composition and the *in vitro* antibacterial activity of some essential oils obtained from three new varieties of *M. x piperita* f. *citrata*: “Orange”, “Chocolate” and “Basil”, acclimatized in the agro-climatic conditions of Transylvania, Romania, considering both their therapeutic and flavouring qualities.

## Materials and Methods

*Plant materials*. The aerial parts (*herba*) of the three mint varieties (“Orange”, “Chocolate” and “Basil”) were harvested from the cultivated experimental field (Blaj, Alba County, Romania, in 2018), where the plantation was set up by seedlings. The plant materials were identified by R. Vârban, PhD. and voucher specimen’s species are deposited at the Department of Botany – UASVM Cluj-Napoca, Romania (Vouchers number 30075-30077). The plant material was used to extract the essential oils, as follows: a part of the plant material was used in fresh form (*Menthae herba recens*), and the other part was used after drying in an Excalibur Dehydrator at 30°C for 24 - 48 h.

*Essential oil extraction*. Six essential oils were obtained by hydro-distillation of the plant materials according to the Romanian Pharmacopoeia [20]. Briefly, 50.0 g of plant material were placed in a round-bottomed flask containing 1000 mL of distilled water, which was connected to a Clevenger-type apparatus with tap water for cooling, for 4 h. The distilled oils were collected, then separated and dried over anhydrous sodium sulphate and stored in tightly closed dark vials at 4°C until the analyses were carried out [2- 4, 13, 20].

*Gas Chromatography – Mass Spectrometry analysis*

The fingerprint and composition of the essential oil samples were determined by applying a GC-MS technique, using a Shimadzu QP-2010 equipment (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a Combi-PAL AOC-5000 autosampler (CTC Analytics, Zwingen, Switzerland) and capillary column ZB - 5 ms, 30 m x 0.25 mm i.d. x 0.25 µm (Phenomenex, USA). For the separation of the volatile compounds, the column temperature was initially maintained for 2 min at 50°C, then increased to 160°C with a rate of 3°C min and finally increased to 250°C at a rate of 15°C min and kept for 10 min. Both injector and detector temperatures were set at 250°C. The MS detection was performed on a quadrupole mass spectrometer operating in full scan (40 - 500 m/z) electron impact (EI) at ionization energy of 70 eV. The volatile compounds were identified by comparing the obtained mass spectra with those from NIST27 and NIST147 spectra libraries and verified by comparison with retention indexes extracted from www.pherobase.com and www.flavornet.org (for columns with stationary phase similar to ZB - 5 ms). The results are expressed as a percentage from the total peak areas (100%), considering only the compounds with min. 85% similarity index [17].

*Antimicrobial activity of essential oils*

*Bacterial strains*. The following reference strains were used for testing: *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 13076. Each strain was grown in a test tube containing 10 mL sterile nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 24 h. The purity of the inoculum was confirmed by microscopic examination of the Gram-stained smear. A loopful of inoculum was transferred on selective medium: Baird-Parker agar base supplemented with Egg Yolk Tellurite Emulsion for *S. aureus*, TBX agar for *E. coli* and XLD agar for *S. enterica* (Oxoid Ltd., Basingstoke, Hampshire, England). Plates were incubated for 24 h at 37°C. Bacterial morphology was confirmed by optical microscopy. Several colonies were transferred in sterile saline solution (8.5 g/L), and adjusted to match the turbidity of McFarland 0.5 standard ($1.5 \times 10^8$ CFU *per* mL) [16].

*Determination of the minimum inhibitory concentration (MIC)*. The MIC was determined using the resazurin microtiter plate based antibacterial assay. Stock solutions of the essential oils were prepared in eight parts 50% v/v ethanol by mixing with one part Tween 80 [16]. Into the first well of a 96-well microtiter plate, 100 µL sterile nutrient broth and 100 µL sample were added. Serial 11-fold dilutions were performed by transferring 100 µL from well to well (on row). From the last well of the row, 100 µL was discarded. To each well, 10 µL of inoculum ($1.5 \times 10^8$ CFU/mL) was added. Gentamicin (0.04 mg/mL in saline solution) was used as a positive control. For the negative control, pure methanol was used. The microplates were incubated for 20 - 22 h at 37°C. To each well, 20 µL resazurin aqueous solution (0.2 mg/mL) was added. The microplates were incubated for 2 hours at 37°C. The concentration that completely inhibited bacterial growth (MIC) was the concentration at which the blue colour did not change into pink. Three replicates were performed for each sample [16, 21].

*Statistical analysis*

The MIC values are reported as means ± standard deviation (SD). ANOVA analysis of variance was used to compare the mean values, using SPSS 19.0 statistical analysis (IBM, New York, USA) and the

Tukey HSD test with a confidence interval of 95% or 99%. A p-value below 0.05 was considered statistically significant.

**Results and Discussion**

The present study was conducted in order to investigate the chemical composition and the antibacterial properties of the essential oils obtained by hydro-distillation from fresh and dried aerial parts harvested from three new varieties of *Mentha x piperita* f. *citrata* ("Orange", "Chocolate" and "Basil" cultivated on experimental fields. The chemical composition of essential oils, determined by the GC-MS technique, is shown in Table I, Table II and Table III, where the percentage composition and retention times of the constituents are given.

**Table I**

Chemical constituents of essential oils isolated from the *Mentha* x *piperita* f. *citrata* "Orange" variety

| No. | Compounds | $R_T$ (min) | *Mentha* "Orange" | | | |
|---|---|---|---|---|---|---|
| | | | fresh **(%)** | | dry **(%)** | |
| 1. | α-Pinene (MH) | 7.946 | 0.19 | | 0.16 | |
| 2. | ß-Phellandrene (SH) | 9.318 | 0.20 | | 0.19 | |
| 3. | ß-Pinene (MH) | 9.494 | 0.38 | | 0.36 | |
| 4. | 1-Octen-3-ol (O) | 9.585 | 0.03 | | - | |
| 5. | ß-Myrcene (MH) | 9.916 | 0.94 | | 1.31 | |
| 6. | 3-Octanol (O) | 10.201 | 0.22 | | 0.19 | |
| 7. | *p*-Cymene (MH) | 11.237 | 0.15 | | - | |
| 8. | D-Limonene (MH) | 11.404 | 0.55 | | 0.50 | |
| 9. | Eucalyptol (OM) | 11.523 | **4.97** | | **4.38** | |
| 10. | β-*trans*-Ocimene (MH) | 11.658 | 0.25 | | 0.44 | |
| 11. | β-*cis*-Ocimene (MH) | 12.059 | 0.36 | | 0.75 | |
| 12. | 1,2-Oxolinalool (OM) | 12.987 | 0.13 | | - | |
| 13. | α-Terpinolene (MH) | 13.550 | 0.07 | | 0.20 | |
| 14. | *trans*-Linalool oxide (OM) | 13.609 | 0.09 | | - | |
| 15. | **Linalool** (OM) | 14.163 | **58.23** | | **55.36** | |
| 16. | 1-Octen-1-ol, acetate (O) | 14.410 | 1.64 | | 1.57 | |
| 17. | 3-Octanol, acetate (O) | 14.868 | 1.68 | | 1.56 | |
| 18. | Menthone (OM) | 16.287 | 0.91 | | - | |
| 19. | Menthol (OM) | 17.115 | 1.44 | | - | |
| 20. | **α-Terpineol** (OM) | 17.779 | **5.51** | | **3.83** | |
| 21. | *trans*-Carvyl acetate (OM) | 18.663 | 0.20 | | - | |
| 22. | *cis*-Geraniol (OM) | 18.846 | - | | 0.51 | |
| 23. | Carvone (OM) | 19.594 | 1.13 | | - | |
| 24. | **Linalyl acetate** (OM) | 19.792 | **14.04** | | **22.19** | |
| 25. | *trans*-Geranyl acetate (OM) | 21.024 | - | | 0.11 | |
| 26. | *cis*-Geranyl acetate (OM) | 24.378 | - | | 2.43 | |
| 27. | Lavandulyl acetate (OM) | 21.018 | 0.13 | | - | |
| 28. | α-Bourbonene (SH) | 24.627 | 0.06 | | - | |
| 29. | *cis*-Jasmone (MH) | 24.900 | 0.06 | | - | |
| 30. | Caryophyllene (SH) | 25.854 | 0.04 | | - | |
| 31. | (E)-ß-Farnesene (SH) | 26.920 | 0.08 | | 0.18 | |
| 32. | δ-Cadinene (SH) | 29.096 | - | | 0.06 | |
| 33. | Viridiflorene (SH) | 31.149 | 1.02 | | 1.10 | |
| 34. | Valencene (SH) | 32.108 | 0.39 | | 0.38 | |
| | | | **No.** | **%** | **No.** | **%** |
| | **Total identified** | | **29** | **95.09** | **22** | **97.76** |
| | MH | | 9 | 2.95 | 7 | 3.72 |
| | **OM** | | **11** | **86.78** | **7** | **88.81** |
| | SH | | 6 | 1.79 | 5 | 1.91 |
| | O | | 4 | 3.57 | 3 | 3.32 |

MH – monoterpene hydrocarbons, OM – oxygenated monoterpenes, SH – sesquiterpene hydrocarbons, O – Other; $R_T$ – retention time; "-" – Not detected

Based on these results, oxygenated mono-terpenes were found in highest amounts (> 80%, except "Basil" mint oil from fresh material < 60%), while mono-terpene hydrocarbons and sesquiterpene hydrocarbons were less present. In the "Orange" mint essential oils (Table I), 34 compounds (18 in both oils) were identified: 29 components in fresh mint oil, representing 95.09% of total compounds, and 22 in the oil obtained from dried aerial parts (97.76%). The main constituents in both oils were linalool (58.23% in fresh mint, 55.36% in dried mint oil) and linalyl acetate (14.04% in fresh mint, 22.19% in dried mint oil). Other important constituents were α-terpineol (5.51%; 3.83%) and eucalyptol (4.97%; 4.38%). Menthol (1.44%), carvone (1.13%) and menthone (0.91%) were determined in small amount in fresh mint and were absent in the dry material. The oxygenated monoterpenes were abundant in the two oils (86.78% in fresh mint; 88.81% in dried mint), while monoterpene hydrocarbons (2.95%; 3.72%) and sesquiterpene hydrocarbons (1.79%; 1.91%) were less represented. Only few studies conducted by other authors on mint "Orange" oil are known, assigning its' aroma to linalool (around 35%), the major constituent [5, 6, 9].

**Table II**

Chemical constituents of essential oils isolated from the *Mentha* x *piperita* f. *citrata* "Chocolate" variety

| No. | Compounds | $R_T$ (min) | *Mentha* "Chocolate" | | | |
|---|---|---|---|---|---|---|
| | | | fresh **(%)** | | dry **(%)** | |
| 1. | α-Thujene (MH) | 7.700 | 0.05 | | 0.05 | |
| 2. | α-Pinene (MH) | 7.946 | 0.84 | | 0.82 | |
| 3. | ß-Phellandrene (SH) | 9.320 | 0.64 | | 0.63 | |
| 4. | ß-Pinene (MH) | 9.499 | 1.25 | | 1.21 | |
| 5. | ß-Myrcene (MH) | 9.916 | 0.22 | | 0.23 | |
| 6. | 3-Octanol (O) | 10.197 | 0.31 | | 0.30 | |
| 7. | *p*-Cymene (MH) | 11.232 | 0.25 | | 0.04 | |
| 8. | D-Limonene (MH) | 11.401 | 1.61 | | 1.75 | |
| 9. | **Eucalyptol** (OM) | 11.527 | **8.80** | | **7.70** | |
| 10. | β-*trans*-Ocimene (MH) | 11.661 | 0.19 | | 0.14 | |
| 11. | γ-Terpinene (MH) | 12.511 | 0.40 | | 0.32 | |
| 12. | Sabinene hydrate (OM) | 12.977 | 1.15 | | 1.82 | |
| 13. | α-Terpinolene (MH) | 13.544 | - | | 0.10 | |
| 14. | Linalool (OM) | 14.113 | 0.47 | | 0.80 | |
| 15. | n-Amyl isovalerate (O) | 14.453 | 0.10 | | 0.07 | |
| 16. | 2-Methylbutyl valerate (O) | 14.450 | - | | 0.08 | |
| 17. | 3-Octanol, acetate (O) | 14.883 | 0.06 | | 0.07 | |
| 18. | **(+)-Isomenthone** (OM) | 16.309 | **28.33** | | **24.94** | |
| 19. | **Menthofuran** (OM) | 16.565 | **7.87** | | **9.68** | |
| 20. | (-)-; d-Isomenthone (OM) | 16.634 | 2.70 | | 2.46 | |
| 21. | Menthol (OM) | 16.822 | 2.94 | | - | |
| 22. | **Levomenthol** (OM) | 17.153 | **32.58** | | **31.06** | |
| 23. | α-Terpineol (OM) | 17.788 | 0.22 | | 0.16 | |
| 24. | *cis*-3-Hexenyl valerate (O) | 19.282 | - | | 0.04 | |
| 25. | Pulegone (OM) | 19.381 | 3.07 | | 2.96 | |
| 26. | D(+) Carvone (OM) | 19.599 | - | | 0.67 | |
| 27. | Linalool acetate (OM) | 19.780 | - | | 0.78 | |
| 28. | Isopiperitone (OM) | 19.964 | 0.29 | | 0.21 | |
| 29. | **(+)** Menthyl acetate (OM) | 20.646 | 0.06 | | 0.10 | |
| 30. | (-) Menthyl acetate (OM) | 21.274 | 1.63 | | 2.86 | |
| 31. | Caryophyllene (SH) | 25.863 | 0.84 | | 1.30 | |
| 32. | (E)-ß Farnesene (SH) | 26.918 | 0.10 | | 0.18 | |
| 33. | Viridiflorene (SH) | 31.154 | 0.15 | | 0.19 | |
| | | | **No.** | **%** | **No.** | **%** |
| | Total identified | | **28** | **97.12** | **32** | **93.72** |
| | MH | | 8 | 4.81 | 9 | 4.66 |
| | **OM** | | **13** | **90.11** | **14** | **86.20** |
| | SH | | 4 | 1.73 | 4 | 2.30 |
| | O | | 3 | 0.47 | 5 | 0.56 |

MH – monoterpene hydrocarbons, OM – oxygenated monoterpenes, SH – sesquiterpene hydrocarbons, O – Other; $R_T$ – retention time; "-" – Not detected

A total of 33 compounds (Table II) were identified in the "Chocolate" mint essential oils (32 = 93.72% in the dried mint oil and 28 = 97.12% in the fresh mint oil, 27 being similar in both oils). The most abundant of the constituents were levomenthol (32.58% in fresh mint; 31.06% in dried mint oil), isomenthone (28.33%; 24.94%), eucalyptol (8.80%; 7.70%), menthofuran (9.68%; 7.87%). Other important constituents were: pulegone (3.07%; 2.96%), menthyl acetate (1.63%; 2.86%), D-limonen (1.61%; 1.75%), ß-pinene (1.25%; 1.21%), sabinene hydrate (1.15%; 1.82%). Good quality peppermint oil must contain 30 - 55% menthol and 14 - 32% menthone. Our results fit within these limits and are similar to those obtained by other authors [5, 6, 10, 14, 19]. The mint "Chocolate" essential oils contain mainly oxygenated monoterpenes (90.11% in fresh mint, 86.20% in dried mint oil), while monoterpene hydrocarbons (4.81%; 4.66%) as well as sesquiterpene hydrocarbons (1.73%; 2.30%) were present in low concentrations.

**Table III**

Chemical constituents of essential oils isolated from the *Mentha* x *piperita* f. *citrata* "Basil" variety

| No. | Compounds | $R_T$ (min) | *Mentha* "Basil" | | | |
|---|---|---|---|---|---|---|
| | | | fresh **(%)** | | dry **(%)** | |
| 1. | 2,5-diethyltetrahydrofuran (O) | 6.734 | 0.05 | | - | |
| 2. | α-Pinene (MH) | 7.943 | 0.34 | | 0.24 | |
| 3. | ß-Phellandrene (S) | 9.320 | 0.11 | | 0.25 | |
| 4. | ß-Pinene (MH) | 9.495 | 0.57 | | 0.55 | |
| 5. | ß-Myrcene (MH) | 9.914 | 1.11 | | 1.34 | |
| 6. | *p*-Cymene (MH) | 11.232 | 0.14 | | - | |
| 7. | D-Limonene (MH) | 11.402 | 0.57 | | 0.57 | |
| 8. | Eucalyptol (OM) | 11.523 | 2.45 | | **7.72** | |
| 9. | β-*trans*-Ocimene (MH) | 11.655 | 0.61 | | 0.71 | |
| 10. | β-*cis*-Ocimene (MH) | 12.059 | 0.57 | | 0.78 | |
| 11. | γ-Terpinene (MH) | 12.508 | - | | 0.08 | |
| 12. | α-Terpinolene (MH) | 13.551 | 0.15 | | 0.21 | |
| 13. | **Linalool** (OM) | 14.136 | **28.07** | | **31.14** | |
| 14. | Octen-1-ol, acetate (O) | 14.410 | 0.27 | | 0.48 | |
| 15. | 3-Octanol, acetate (O) | 14.868 | 0.16 | | 0.26 | |
| 16. | L-Menthone (OM) | 16.287 | - | | 0.47 | |
| 17. | (+)-Isomenthone (OM) | 16.288 | 0.06 | | 0.03 | |
| 18. | Levomenthol (OM) | 17.116 | 1 .19 | | 1.51 | |
| 19. | Terpinen-4-ol (OM) | 17.223 | 0.16 | | 0.09 | |
| 20. | **α-Terpineol** (OM) | 17.784 | **10.10** | | **3.16** | |
| 21. | Carveol (OM) | 18.664 | 0.03 | | - | |
| 22. | *cis*-Geraniol (OM) | 18.841 | 1.67 | | 0.37 | |
| 23. | Pulegone (OM) | 19.385 | - | | 0.08 | |
| 24. | L-Carvone (OM) | 19.602 | 0.17 | | 0.26 | |
| 25. | **Linalyl acetate** (OM) | 19.799 | **10.75** | | **38.49** | |
| 26. | α-Citral (OM) | 20.465 | 0.10 | | - | |
| 27. | Geranyl acetate (OM) | 24.379 | 4.68 | | 0.77 | |
| 28. | ß-Elemene (SH) | 24.807 | 0.38 | | - | |
| 29. | Menthyl acetate (OM) | 21.276 | - | | 0.11 | |
| 30. | Caryophyllene (SH) | 25.868 | 4.08 | | 2.06 | |
| 31. | (E)-ß-Farnesene (SH) | 26.914 | 0.60 | | 0.36 | |
| 32. | α-Caryophyllene (SH) | 27.066 | 0.42 | | 0.08 | |
| 33. | α-Cubebene (SH) | 27.910 | 0.61 | | - | |
| 34. | α-Muurolene (SH) | 28.466 | 0.08 | | - | |
| 35. | Δ-Cadinene (SH) | 29.094 | 0.38 | | 0.07 | |
| 36. | (-)-β-Elemene (SH) | 30.019 | **9.13** | | 1.33 | |
| 37. | Caryophyllene oxide (OS) | 30.881 | 0.60 | | - | |
| 38. | Viridiflorene (SH) | 31.155 | 0.49 | | 0.23 | |
| | | | **No.** | **%** | **No.** | **%** |
| | Total identified | | **34** | **80.85** | **30** | **93.80** |
| | MH | | 8 | 4.06 | 8 | 4.48 |
| | **OM** | | **12** | **59.43** | **13** | **84.20** |
| | SH | | 10 | 16.28 | 7 | 4.38 |
| | OS | | 1 | 0.60 | - | - |
| | O | | 3 | 0.48 | 2 | 0.74 |

MH – monoterpene hydrocarbons, OM – oxygenated monoterpenes, SH – sesquiterpene hydrocarbons, OS – oxygenated sesquiterpenes; O – Other; $R_T$ – retention time; "-" – Not detected

Thirty-eight different compounds (Table III) were identified in the essential oil obtained from "Basil" mint, 34 = 80.85% in fresh mint oil and 30 = 93.80% in dry mint oil. Of these, 26 were common for both oils. Linalool (28.07%; 31.14%) and linalyl acetate (10.75%; 38.49%) were found as major constituents; α-terpineol (10.10%; 3.16%), geranyl acetate (0.77%; 4.68%), caryophyllene (4.08%; 2.06%), eucalyptol (2.45%; 7.72%), (-)-β-elemene (9.13%; 1.33%) were also determined in important amounts. The essential oil obtained from the dried aerial parts was richer in linalyl acetate (38.39%) and linalool (31.14%) than the one obtained from the fresh aerial parts. Similar high amounts of linalyl acetate and linalool have been found in "Basil" mint essential oil grown in Estonia [6]. The dominant constituents of "Basil" mint oils were the oxygenated monoterpenes (59.43% for the fresh aerial parts oil and 84.20% for dried aerial parts oil), followed by sesquiterpenes (16.28%; 4.38) and monoterpenes (4.06%; 4.48%).

Overall, these results highlight that "Orange" mint and "Basil" mint varieties of *Mentha* x *piperita* f. *citrata* have the capacity to biosynthesize essential oils rich in linalool and linalyl acetate, while in the "Chocolate" variety essential oil, the major constituents were levomenthol and isomenthone. The chemical composition of essential oils obtained from dry and fresh vegetal materials is comparable. The differences between concentrations of compounds can be due to possible chemical transformations during the drying process: oxidation, hydrolysis, esterification, etc. Some compounds were lost, new compounds appeared, and in this way concentration values were slightly modified [1]. The antibacterial activity of the essential oils was performed using the broth microdilution method and the MICs values (µL/mL) are presented in Table IV. Gentamicin was used as positive control and methanol as negative control.

**Table IV**

Antibacterial activity of *Mentha* x *piperita* f. *citrata* varieties essential oils (MIC, µL/mL)

| **Essential oil from *Mentha* varieties** | ***E. coli* (Gram-negative)** | ***S. enterica* (Gram-negative)** | ***S. aureus* (Gram-positive)** |
|---|---|---|---|
| "Orange" fresh | $2.45 \pm 0.00^{a}$ | $3.35 \pm 0.73^{a}$ | $2.45 \pm 0.00^{a}$ |
| "Orange" dry | $\mathbf{1.17 \pm 0.00^{c}}$ | $2.45 \pm 0.00^{b}$ | $\mathbf{1.17 \pm 0.00^{c}}$ |
| "Chocolate" fresh | $\mathbf{1.17 \pm 0.00^{c}}$ | $\mathbf{1.17 \pm 0.00^{d}}$ | $\mathbf{1.60 \pm 0.73^{b}}$ |
| "Chocolate" dry | $\mathbf{1.60 \pm 0.73^{b}}$ | $\mathbf{1.60 \pm 0.73^{c}}$ | $\mathbf{1.17 \pm 0.00^{c}}$ |
| "Basil" fresh | $2.45 \pm 0.00^{a}$ | $3.35 \pm 0.73^{a}$ | $2.45 \pm 0.00^{a}$ |
| "Basil"dry | $\mathbf{1.60 \pm 0.73^{b}}$ | $2.45 \pm 0.00^{b}$ | $2.45 \pm 0.00^{a}$ |
| Significant differences | $p < 0.05$ | $p < 0.01$ | $p < 0.05$ |

Values are expressed as mean of three replicates ± SD. The means with different letters (a, b, c, d) within a column indicate significant differences ($p < 0.05$) using Tukey's Honestly Significant Differences (HSD) test with a confidence interval of 95% or 99%.

All tested essential oils displayed *in vitro* antimicrobial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli, Salmonella enterica*) bacteria. The highest efficacy was recorded for fresh and dry mint "Chocolate" essential oils and this potential may be related to their predominant constituents, menthol and isomenthone. Also, the mint "Orange" oil obtained from the dried aerial parts showed high antibacterial activity against *E. coli* and *S. aureus*, while the mint "Basil" oil (from dried aerial parts) was particularly active towards *E. coli*. Similar studies were reported on "Orange" and "Chocolate" mint essential oil's ability to inhibit *S. aureus* [20]. The essential oils are chemical combinations of a large variety of compounds, most of them terpenes and the antibacterial activity is the result of a complex mechanism, with several targets in the bacterial cell. The antimicrobial potential of peppermint oil described previously is attributed to monoterpenes compounds (e.g. menthol, linalool, linalyl acetate). Due to the lipophilic properties of the volatile monoterpenes, they have the ability to penetrate into the bacterial membrane structure and to determine the alteration of the permeability and modifications in substances and ion transport process [15, 18, 19]. The essential oils are widely applied for their antibacterial properties in the pharmaceutical, cosmetic and food industry.

## Conclusions

In the present study, the essential oils obtained from three varieties of *Mentha x piperita* f. *citrata*: "Orange", "Chocolate" and "Basil", grown on experimental lands in Transylvania, Romania, were analysed. Linalool and linalyl acetate were the major compounds identified in "Orange" and "Basil" essential oils, while levomenthol and isomenthone were found in the largest amount in the "Chocolate" mint oils. All the studied samples showed good antimicrobial activity against *E. coli*, *S. aureus* and *S. enterica*. The obtained results highlight the complex chemical composition and antimicrobial efficacy of essential oils isolated from three new varieties of mint acclimatized and cultivated in our country, and it suggests their potential use in medicinal, cosmetic and food industry.

## Conflict of interest

The authors declare no conflict of interest.

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