Isolation, chemical composition, antioxidant and antimicrobial potential of essential oil from *Mentha Arvensis* L. organically planted from Macedonia

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The volatile composition, antioxidant and antimicrobial activity of essential oils from flowers, leaves and whole plant of *Mentha Arvensis* L. organically planed from the territory of Macedonia were object of this study. The essential oils from dried and powdered plant materials were isolated by hydro-distillation using Clevenger-type apparatus. The composition of three essential oils was identified by GC-MS and quantified by GC-FID. Thirty-seven components were identified and quantified in the three essential oils isolated from the flowers, leaves and whole *Mentha* plant. 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 oils was determined against ABTS radical and total phenolic contet (TPC). The maximal values from both assays indicated the essential oil from flowers of *Mentha arvensis* L. as the oil with highest antioxidant activity. The antimicrobial activity of *Mentha* essential oils was determined against *Escherichia coli* and *Candida albicans*. Our results showed the highest antibacterial activity against *Escherichia coli* ATCC 25922 (29.3 mm) and the highest antifungal activity against *Candida albicans* ATCC 10231 (39.4 mm) inhibition zone for essential oil from the leaves of *Mentha arvensis* L.

Keywords: *Mentha arvensis* L. plant, essential oil, isolation, chemical composition, antioxidant activity, antimicrobial activity.

1. INTRODUCTION

Mint has been used as a medicinal and aromatic plant since ancient times, in both western and eastern cultures [1]. Many studies on the therapeutic values of mint and mint oils have been reported; these are stomachic, carminative, antispasmodic, stimulant, local anesthetic, anti-inflammatory, diuretic, anthelmentic, antibacterial, antifungal and antioxidant [2, 3].
Zeinali *et al.*, reported fifteen principal components in the oils of 12 variety of Iranian mints accessions. The oils obtained from Mint variety Mzin 9 and Mzin 10 contained the highest value of *p*-cymene with the levels of 48.9 and 48.6%. In the mint oils from variety Mzin 5 and Mzin 11 was quantified cis-carveol over 70%. Carvon oxide was the most dominant compound in Mzin 4 with the level of 52.5% [4]. The Johnson working group determined the anti-bacterial efficacy of chloroform, ethanol, ethyl acetate and water extracts of inter-nodal and leaves derived calli extracts from *Mentha arvensis* against *Salmonella typhi*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Bacillus subtilis* [5]. The chemical composition of *Mentha* oils from different varieties from the Marmara region in Turkey was published by Başer *et al.* Eleven Turkish oils contained mentofuran as the main constituent in the range from 21 to

48%. Elemol, β-caryophyllene and viridiflorol were sesquiterpenes present in higher concentration in comparison to other compounds [6]. The effect of *Sclerotinia sclerotiorum* on the disease development, growth, oil yield and biochemical changes in the plants of *Mentha arvensis* was studied [7]. The effect of different dates of planting for three menthol mints (*Mentha arvensis*) cultivars Saksham, Kushal and Kosi developed by Central Institute of Medicinal and Aromatic Plants, Lucknow, India on herb yield, oil yield and oil quality was object of study of Chauhan et al. According to their findings, the best period of planting was middle of February for cultivar Saksham and beginning of March for Kushal and Kosi variety with maximal level of menthol 89.39, 152.04 and 170.22% respectively [8]. Rajeswara Rao et al. stated that flowers, leaves and stems contributed 31.3%, 37.4% and 31.3% to biomass yield and produced essential oil yields of 1.50%, 1.56% and 0.06%, respectively. The flowering whole herb gave 0.59% oil yield [9]. The antimicrobial activity of the steam distilled essential oil of *Mentha arvensis* from India was object of study of working group of Ahmad et al. According to their findings, major isolates were monoterpenes, menthone and isomenthone, menthol and its acyl derivatives. *In vitro* bioactivity evaluation of the oil and its derivatives showed activity against a wide spectrum of human pathogenic fungi and bacteria tested. The activity of the isolated pure compounds and menthol derivatives was comparable to the bioactivity of original oil [10]. The aromatic attributes of plants from *Mentha arvensis* L. were related to the presence of essential oil rich in monoterpenes and sesquiterpenes and volatile constituents such as menthol, menthone, carvacol, pulgone and isomenthone, imparting characteristic mint flavor [11-13]. A field experiment conducted by Rajeswara Rao in the farm of Mr. Jayanthilal Sachdev, a progressive farmer on a red clay loam soil in the semi-arid tropical climate of South India, investigated the influence of planting cornmint (*Mentha arvensis* L. f. *piperascens* Malinvaud *ex* Holmes, family: Lamiaceae) in different months on its biomass and essential oil yields. In this climate, cornmint was harvested six-seven times in a period of 17-18 months. August (rainy), November (autumn), and December (winter) planted crop produced significantly superior total biomass and essential oil yields compared to September and January planted cornmint. The results indicated the feasibility of intercropping cornmint with tomato during the first harvest period. The quality of the essential oil with 73.0% menthol, 9.6% menthone, 4.0% isomenthone, and 4.0% menthyl acetate was found to be good and readily accepted in the market. This investigation in a farmer's field with 42.5–63.5 t:ha total biomass yield and 196.3-271.5 kg:ha total essential oil yield, clearly demonstrated the economic feasibility of cultivating cornmint in semi-arid tropical climate. It has also shown the possibility of planting the crop during different seasons and growing it as a biennial [14]. The effects of 25, 50, 75 and 100% of light intensity on aspects related to volatile synthesis in menthol mint were investigated. More precisely, fresh weight production, glandular trichome density and essential oil content showed a significant linear positive correlation with light intensity with over 75% of menthol [15]. The newest scope of interest was a novel process for isolation of major bio-polymers from *Mentha arvensis* distilled biomass and saccharification of the isolated cellulose to glucose by working group of Prakash [16].

It is obvious that many studies have already been published on the chemical composition and antimicrobal potential of essential oils from *Mentha*, but, till now, to the best of our knowledge, there are no published results for the organic production and quality of the essential oils from *Mentha arvensis* L. from the region of Macedonia. Furthermore, this is the first to be published on this plant's cultivation results in the South-East region of Macedonia.

Therefore, the main object of this study is to give an overview on the chemical composition and on the general quality of essential oils from Macedonian *Mentha arvensis*. Furthermore, the antioxidant potential of essential oils from the flowers, leaves and whole plant will be evaluated by application of total phenolic content (TPC) and ABTS assay. The last goal of this work is the estimation of the antimicrobial activity of three essential oils. More precisely, the antibacterial activity will be performed against *Escherichia coli* ATCC 25922 and the antifungal activity will be estimated against *Candida albicans* ATCC 10231.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

The plant of *Mentha arvensis* L. was organically produced from for the first time in the south-east region of Macedonia (41º49`N, 21º59`E) on the overlapping area of two climate types: the Mediterranean and Continental climate. The leaf samples were dried at 35ºC in a hot air oven (the smart oven air, Breville, USA) to constant weight.

The plant specimen was identified and authenticated by Prof. Dr. Ljupco Mihajlov, from Faculty of Agriculture, University "Goce Delcev"-Stip from Macedonia. Further authentication was made by comparison with authentic vouchers of *M. arvensis* (12099) deposited in the Herbarium of the Botany Department of the University of Natural Science, Skopje, Macedonia.

2.2. ESSENTIAL OIL SEPARATION

The three samples of 250 g of dried leaves, flowers as

well as whole powdered plant were mixed with 500 mL of tap water in flask and water distilled for 2 h using a Clevenger-type apparatus.
The dried flowers, leaves and whole plant of M. arvensis was grounded prior to the operation and than 100 g of samples were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored at 4ºC until analysis. The yields (g/kg) of the oils were calculated on a moisture free basis.

2.3. GAS CHROMATOGRAPHY ANALYSIS

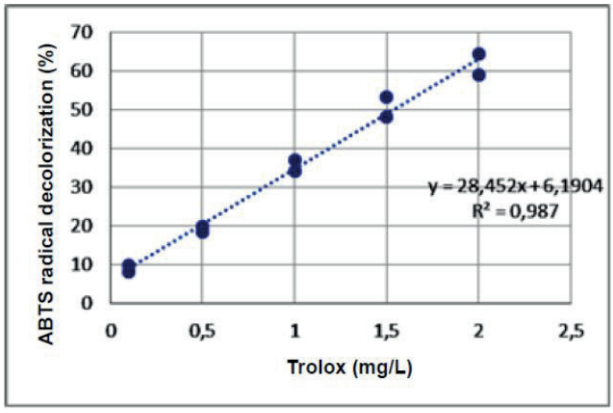
The oil compositions were analysed by GC-FID and GC/MS. Gas chromatography was carried out with an Agilent HP 6890 gas chromatograph equipped with flame-ionisation detector (FID) and quantitation was carried out by addition of pure standards as well as area normalisation and neglecting response factors. The analysis was conducted using a HP-5 (5% Phenyl Methyl Siloxane) fused silica capillary column (30 m × 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 ionisation 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.

2.4. ANALYTICAL STANDARDS

The pure standard of menthe oils composition namely 3-thujene (98.5%); α-pinene (99.5%); n-octan-3-ol (95%); sabinene (99%); β-pinene (98.5%); myrcene (95%); eucalyptol (99%); α-terpinene (95%); p-cymene (98%); limonene (99%); piperitol (99%); γ-terpinene (98.5%); α-terpinolene (99%); menthol (95%); menthofuran (99%); neomenthol (98.5%); α-terpineol (98%); isopulegon (99%); piperitone (98%); menthyl acetate (98%); geranyl acetate (95%) were supplied by *Fluka* (Buch, Switzerland) and *Sigma-Aldrich* (St. Louis, MO, USA). α-bourbonene (95%) *Chemical Sources Association* (Neptune, NJ 07753, USA); isopulegol (95%) *BOC Sciences* (Shirley, NY 11967, USA.); ledol (98%) *Santa Cruz Biotechnology* (Dallas, Texas 75220, USA); spathulenol (99%) *Shanghai Yuanye Bio-Technology Co.* (Shanghai, China); isomenthyl acetate (98.5%) *Simagchem Corporation* (Xiamen, China); γ-elemene (95%) *Alpha Chemistry* (Stony Brook, NY 11790, USA).

2.5. ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity of essential oil under study.
The chromophore ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) was dissolved in distilled water to 7 mM concentration and leaving the mixture to stand in dark at room temperature for 12-14 h before use. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting with 4.9 mM potassium persulphate solution. After forming of ABTS radical cation, the solution was diluted with water in a 1:9 v/v ratio (10 mL is quantitatively transferred into 100 mL calibrated flask and diluted) to 0.7 mM. The concentration of the resulting blue-green ABTS radical solution was adjusted to an absorbance of 0.80 ± 0.020 at 735 nm.
A 245 µL volume of reagent is pipetted into a quartz cuvette with subsequent addition of 100 µL of oil extract. The decrease in absorbance at 735 nm was measured after 30 min and incubation at 37°C. The estimation of the antiradical activity with TEAC assay was calculated using a calibration curve of 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 (Graphic).
Trolox equivalent antiradical capacity (TEAC) was expressed as percentage of decolarisation of ABTS radical cation ($ABTS^{\bullet+}$).
The total phenolic content (TPC) of essential oils was determined with Folin–Ciocalteau reagent. For each sample, 50 µL of diluted (1:50) oil were added to 750 µL water and 50 µL of Folin–Ciocalteu reagent. The solution with total volume of 850 µL was incubated in the dark for 5 min. Then, 150 µL of 20% sodium carbonate solution was added and samples were incubated in the dark for 1 h. Reference solution was prepared with distilled water instead oil extracts and



Graphic - Calibration curve with Trolox

treated with the Folin–Ciocalteu reagent in the same way as the assayed samples. The samples turned to a blue colour with different degrees, depending on the content of phenolic compounds in the samples. The absorbance at 750 nm was recorded against the absorbance of the reference solution. The measurements were performed in duplicate. The content of total phenolic compounds was calculated using a calibration curve of gallic acid (the linearity range: 1–10 mg/100 µL, $y = 0.0632x + 0.015$ ($R^2 = 0.9943$). The total level of electron-rich components (mainly known as total phenolic content) for each sample was determined in terms of gallic acid equivalents.

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.

2.6. STATISTICAL ANALYSIS

A one-way ANOVA was used to examine the level of every particular minor and major compound by considering the plant material from which the essential oil was obtained. The significance level of all statistical analyses was set at 0.05. The level of significance of differences between the percentages of monoterpenes, sesquiterpenes and esters was determined at 5% by a one-way ANOVA using Tukey's test. This treatment was carried out using SPSS v.16.0 software, IBM Corporation, USA.

The ANOVA results were classified using letters (different letters mean significant differences among results). The letters are a, b and c according to the decrease of the result values.

Multivariate analysis: Principal component analysis (PCA) was performed to gain an overview of how the samples were correlated to each other with regard to the equilibrium volatile headspace concentration. Correlation matrix was applied in multivariate analysis with Minitab software release 14 so that the data was autoscaled by variable to give the same weight to all components.

The principal component analysis was performed using the concentration of particular terpenes obtained from essential oils from flower, leaves and whole plant.

3. RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM THE FLOWERS, LEAVES AND WHOLE PLANT OF *MENTHA ARVENSIS* L.

The yield of essential oils was 9.4 g/kg, 11.8 g/kg and 7.9 g/kg for flowers, leaves and whole plant of M. arvensis, respectively. The values were significantly lower in comparison to the data published by the Chauhan working group when *Mentha arvensis* was planted on 18 February and comparable to the results of the yield with planting date of 20 December [8].

The chemical composition of essential oils from flowers, leaves and whole plant of *Mentha arvensis L.* was investigated. The identification of compounds was determined by GC-MS and quantification was determined by GC-FID. TIC chromatogram of essential oil obtained from whole plant was presented on Figure 1. Thirty-seven components were identified and quantified in the essential oils isolated from the flowers, leaves and whole plant from of *Mentha arvensis L*. (Tab. I).

As we can see from the Table I, the most dominant component in the three oils is mentol. The highest amount was detected in essential oil from the whole plant (52.53%). Generally speaking, the chemical composition of flowers, leaves and whole plant of *Mentha arvensis* L. had similar composition by three dominant oxiganated compounds: menthol, isomenthol and neomenthone [16]. Other monoterpens as: α-pinene, β-pienene, limonene, α-terpinene were in significantly lower level presented in essential oils.

The level of menthol in the three oils under study was significantly lower than the findings of Rajeswara Rao who quantified 75.2% and 74.5% menthol in essential oils of sole crop and intercrop respectively [17]. Furthermore, Upadhyay *et al.* found linalyl acetate and linalool as the most dominant compounds in essential oil obtained from Mentha arvensis L. (Ginger mint) with 39.72 and 34.57 relative percentage composition. Furthermore, 1.8-Cineole (eucalyptol) was quantified in the same essential oil with level of 10.04% [12]. The highest level of α-linalool and eucalyptol we quantified in essential oil obtained from the leaves of *Mentha arvensis* L. with amount of 0.38% and 5.4% respectively. However, Srivastava et al. identified and quantified very high level of menthol in essential oil obtained from *Mentha arvensis* L. cultivated on industrial scale in the Indo – Gangetic plains. The oil yield of the biggest part of the samples was in the range of 0.7 – 0.9% and menthol content was in the range between 66.7% and 77.4% [18]. The essential oil obtained from *Mentha arvensis* L. f. *piperascens Malinvaud* ex *Holmes*, planted in the farm of Mr Jayanthilal Sachdev indicated very high percentage of menthol with 73%, mentone 9.6%, isomenthone 4.0%, and methyl acetate 4.0%. All other identified and quantified compounds were below 4% [14]. Our findings indicated a significantly higher abundance of

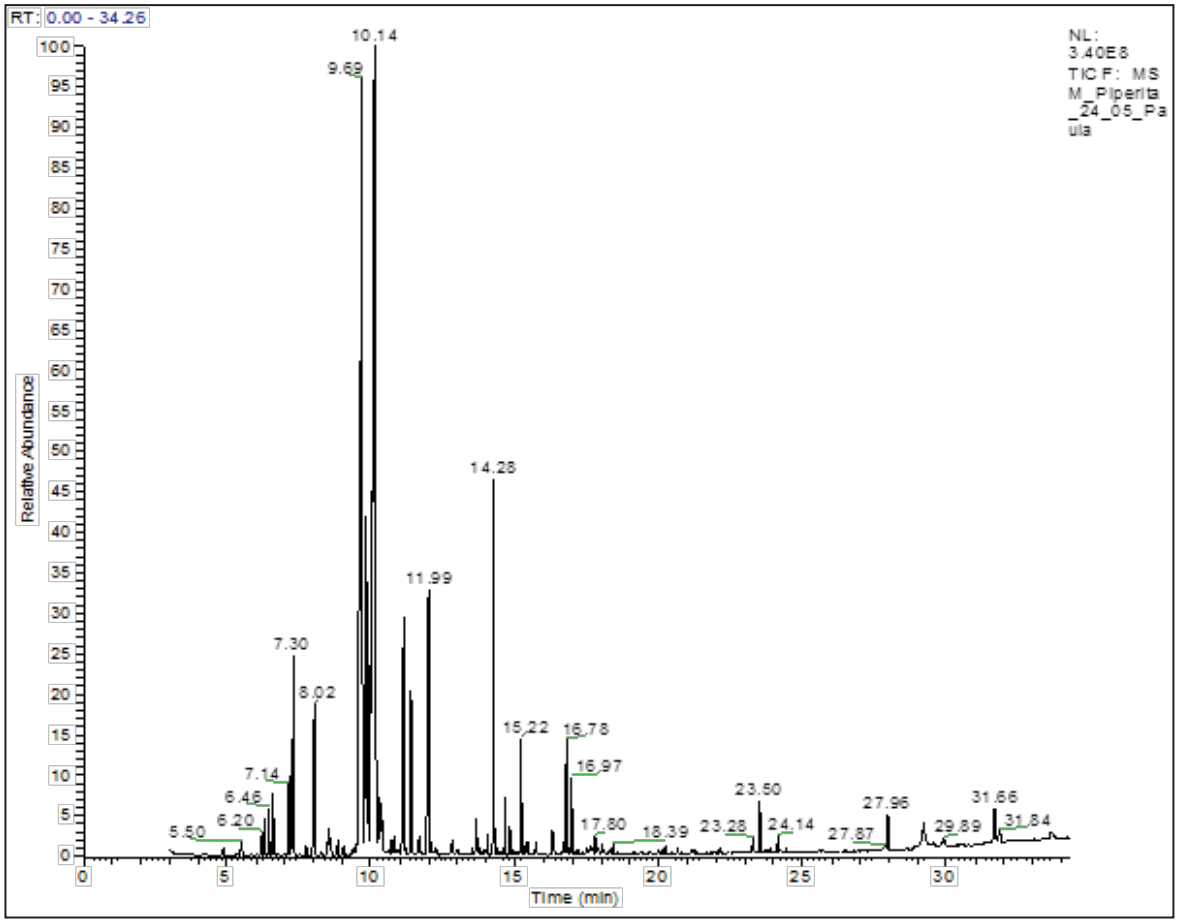


Figure 1 - TIC chromatogram of essential oil from whole plant of *Mentha arvensis* L.

isomenthone. The maximal quantity of isomenthone was detected in essential oil from the flowers (20.38%). The lower level was quantified in essential oil from leveas (15.97%) and the lowest amount was quantified in the essential oil from whole plant (8.42%) of Macedonian *Mentha arvensis*. According to terpenoid profile of essential oils from Mentha arvensis L. published by Phatak and Heble, the most dominant was menthol found in very wide range from 4.8% in callus cultures till 86.7% in the mother plant [19]. Our study showed the highest level of limonene in the essential oil from the flowers (2.07%), medium amount in the essential oil from the leaves (1.48%) and the lowest level in the essential oil from the whole plant (0.41%). The Mimica – Dukić research group investiagated the chemical composition of essential oils of some populations of *Mentha arvensis* L. grown on the territory of Serbia and Montenegro (two population from subsp. *Agrestis* (Sole) Briq.) and discovered pulegone with amount of 49% and 28% respectively. We discovered significantly lower level of isopulegone with maximal amount in essential oil from flowers (7.79%) The samples of *Austriatica* were rich source of menthofuran 11.5 - 29.9% which was similar to the level we quantified in the essential oil from the flowers (8.4%) [20]. The purpose of research group of Padila was to analyze and compare the essential oil quality of rain-winter crops (the planting material-producing crop) with the spring-summer crops (main crops) of *Mentha spicata* cv. MSS-5, *Mentha spicata* cv. *Ganga, Mentha citrata* cv. *Kiran*, *Mentha arvensis* cv. CIMAP-Saryu and *Mentha* × *piperita* cv. Kukrail. According to their findigs, the essential oil yield varied from 0.40% to 1.10% in the main cropping season, whereas it varied from 0.15% to 0.60% in the planting material-producing cropping season. The most abundant compounds were mentol (38.64 - 78.21%), carvone (≤ 0.10 - 57.78%), piperitenone oxide (67.31 - 80.60%), linalool (≤ 0.10 - 44.16%), iso-dihydrocarveol acetate (1.79 - 42.26%), linalyl acetate (22.34 - 48.10%), menthyl acetate (3.79 - 38.31%), cis-dihydrocarvone (≤ 0.10 - 24.37%) and menthone (≤ 0.10 - 24.30%) in different mint cultivars [21]. Comparing to our results, 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.

Verma *et al.*, publiched the results from examination of Menthol mint (*Mentha arvensis* L.) cultivar grown in Kumaon region for essential oil content and

Table I - Volatile components in essential oil from flowers, leaves and whole plant of *Mentha arvensis* L.

	LRI * (HP-5)	Flowers	Leaves	Whole plant	Component
1	926	0.08±0.01[b]	NI	NI	3-thujene
2	932	1.22±0.01[b]	0.95±0.08[c]	0.38±0.02[d]	α-pinene
3	970	0.51±0.07[b]	0.70±0.01[b]	0.16±0.04[c]	sabinene
4	974	1.29±0.02[b]	1.13±0.09[b]	0.84±0.01[c]	β-pinene
5	992	0.18±0.02[b]	0.17±0.06[b]	0.20±0.01[b]	β-myrcene
6	1003	0.18±0.07[c]	0.31±0.05[b]	0.30±0.02[b]	n-octan-3-ol
7	1017	0.05±0.01[b]	0.09±0.04[b]	0.05±0.01[b]	α-terpinene
8	1025	0.26±0.08[d]	0.53±0.11[c]	0.72±0.23[b]	*p*-cymene
9	1034	2.07±0.34[b]	1.48±0.09[b]	0.41±0.19[c]	limonene
10	1052	3.71±0.86[c]	5.40±0.11[b]	2.35±0.69[c]	1.8 cineol (eucalyptol)
11	1060	0.14±0.03[c]	0.38±0.09[b]	0.30±0.14[b]	γ-terpinene
12	1155	0.66±0.29[c]	1.92±0.21[b]	1.76±0.34[b]	4-thujanol.cis
13	1192	NI	0.14±0.02[b]	0.09±0.03[b]	cis-α-terpineol
14	1101	0.18±0.09[c]	0.38±0.11[b]	0.36±0.19[b]	α-linalool
15	1190	0.11±0.02[c]	0.24±0.08[b]	0.27±0.12[b]	cis-β-terpinenol
16	1210	0.09±0.02[b]	NI	NI	isopenthyl alcohol.isovalerate
17	1240	NI	0.31±0.03[b]	NI	piperitol
18	1244	0.15±0.07[b]	NI	NI	isopulegol(p-menth-8-en-3-ol)
19	1248	20.38±6.51[a]	15.97±4.09[b]	8.42±3.77[c]	isomenthone (p-mentan-3-one)
20	1251	8.40±2.11[a]	4.08±0.98[b]	2.58±0.09[c]	menthofuran
21	1262	3.08±0.31[c]	5.24±0.99[b]	4.47±1.15[b]	neomenthol (menthol.trans 1.3cis1.4)
22	1272	35.64±11.34[b]	32.47±14.01[b]	52.53±11.05[a]	menthol
23	1308	0.55±0.03[b]	NI	NI	neoisopulegol
24	1309	7.79±2.21[b]	0.75±0.01[c]	1.02±0.03[c]	cis-isopulegone
25	1355	0.52±0.02[c]	0.62±0.08[c]	1.03±0.03[b]	piperitone
26	1366	3.38±0.98[c]	5.03±1.07[c]	8.75±2.05[b]	isomenthyl acetate
27	1386	NI	NI	0.42±0.07[b]	menthyl acetate
28	1392	NI	0.55±0.21[b]	0.57±0.11[b]	α-bourbonene
29	1422	3.77±1.01[b]	3.50±0.88[b]	3.60±1.03[b]	caryophyllene
30	1459	0.54±0.08[b]	0.81±0.27[b]	0.58±0.33[b]	α-farnesene
31	1485	1.71±0.47[b]	2.59±0.86[b]	2.14±0.31[b]	Germacrene-D
32	1510	NI	0.29±0.08[b]	NI	γ-elemene
33	1522	0.12±0.05[c]	NI	0.67±0.16[b]	spathulenol
34	1464	0.65±0.28[c]	NI	1.05±0.67[b]	caryophylene oxide
35	1580	NI	NI	0.62±0.26[b]	ledol
36	1690	0.63±0.11[c]	0.86±0.21[c]	1.89±0.14[b]	viridiflorol
37	1809	NI	NI	0.92±0.33[b]	phytol

* Linear retention index identified by GC-MS equipped with HP 5 column.
* NI – not identified
* Each value is the mean±standard deviation of three independent measurements.
[a-d] Significant diference at p <0.05 among the amnout of the compounds in the three oils

composition at different stages of crop growth. In case of cultivars viz., 'Kukrail', 'CIM-Madhurus' and 'CIM-Indus', menthol content varied from 32.92% - 39.65%, 34.29% - 42.83% and 22.56% - 32.77%. Those levels were lower than our results for the same compound in the oil from whole plant of from *Menta arvensis* and simiar to the level in oil from leaves and flowers [22].

Regarding the chemical composition of the oils under study, a multivariate pattern recognition approach should be more effective in recognizing differences among the samples analysed. The results showed the comparison between the major chemical classes of volatile chemical compounds of three essential oils obtained from flowers, leaves and whole plant of *Mentha arvensis* L. organically produced from the territory of Macedonia (Fig. 2). PCA score plots were used to determine whether three different essential oils could be grouped into different classes (Fig. 3). To focus on the differences among the essential oils from *Mentha arvensis* L. and target volatile compounds, cluster observation and cluster variable dendrograms were constructed using the nearest neighbour (Fig. 4 and 5). Despite first two principal components, which

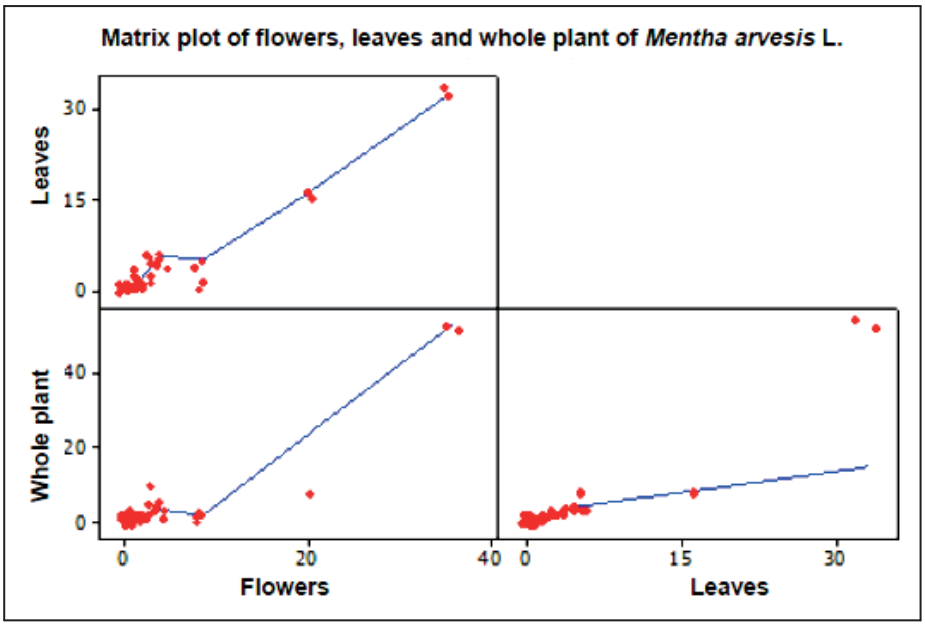


Figure 2 - Matrix plot of flowers, leaves and whole plant of *Mentha arvensis* L.

showed 100% of total variation as was presented in Figure 5, the remaining principal components didn't account for any variability and were probably unimportant.

Except for some monoterpene derivates namely neomenthone, isomenthyl acetate and menthol, the other target volatile compounds could be classified in one group in PC2, because the coefficients of

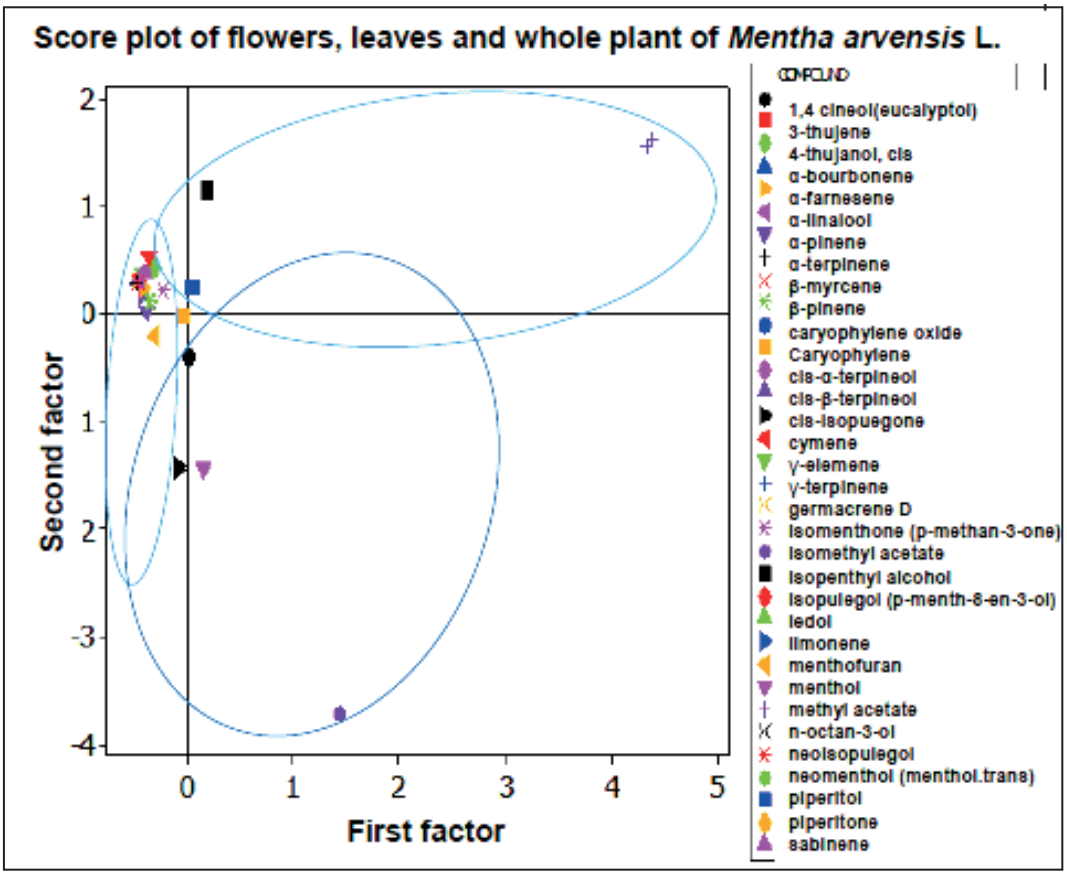


Figure 3 - PCA score plot of flowers, leaves and whole plant of *Mentha arvensis* L.

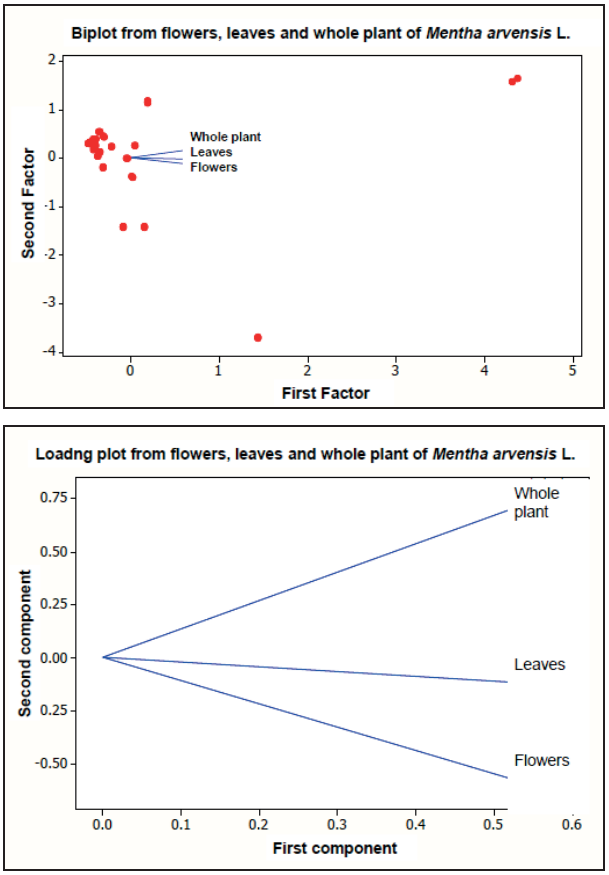


Figure 4 - Biplot and loading plot of flowers, leaves and whole plant of *Mentha arvensis* L.

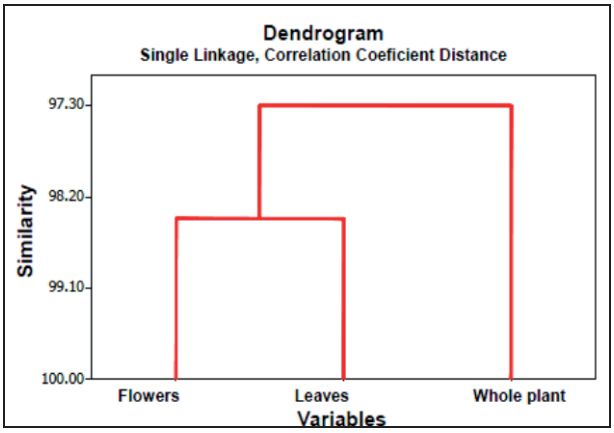


Figure 5 - Dendrogram of volatile components from flowers, pleaves and whole plant of *Mentha arvensis* L.

these volatile compounds were the same negative sign located in PC2 (Fig. 3). In most cases, the differentiation or closeness between the volatile flavour compounds directed in negative side of PC1 was dependent on their chemical classes. As shown in Figure 3, some of target volatile flavour compounds identified in three essential oils namely isomenthone, menthofuran and eucalyptol were classified with the same positive sign in PC while some other monoterpene hydrocarbons identified were placed in the negative side of PC (i.e. cis-isopulegone and α-farnecene). All other compounds identified in oils were classified in PC2. Biplot and loading plot significantly differentiated the essential oils from flowers, leaves and whole plant of *Mentha arvensis* L. (Fig. 4). The PCA analysis separated the essential oil from whole plant, leaves and flowers in the same positive side of PCA. Because the three samples have been processed with the same technology at the same season, quantitative differences in the essential oil compositions might be due to the different plant materials presented in Figure 5.

3.2. ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM *MENTHA ARVENSIS* L.

The antioxidant activity of essential oils from *Mentha* plant was determined by total phenolic content (TPC) and ABTS. As we can see from the Table II, there is no significant difference between the antioxidant activity of essential oils from flowers and the whole *Mentha* plant measured by ABTS radical, while the essential oil from the leaves indicated a lower antioxidant activity with a value of 1.42 TE mg/L of oil (Tab. II). On the other hand, total phenolic content showed almost equal values for essential oils from flowers and leaves and significantly lower value of essential oil from whole plant of *Mentha arvensis*. The standards for both antioxidant assays were Trolox and gallic acid. Due to the fact that the chemical structures of Trolox and gallic acid are significantly different, we can assume that the most abundant components in oils such as menthol, neomenthone, menthyl acetate, 1,8-cineole, limonene and caryophyllene reacted with standard chemicals in different ways. This might be the explanation for the slightly different but, comparable results from the two antioxidant assays [19, 23].

3.3. ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM *MENTHA ARVENSIS* L.

The antimicrobial activity of essential oils from *Mentha* plant was determined against *Escherichia coli* and *Candida albicans*. Our results showed the highest antibacterial activity of essential oil from leaves against *Escherichia coli* ATCC 25922 and the highest antifungal activity against *Candida albicans* ATCC 10231. On the contrary, the lowest antimicrobial activity was detected for the essential oil from the whole plant of *Mentha arvensis.*

The data presented in this study were in good correlation with those published in literature. The cornmint oil fraction and seven mint oil compounds [(-)-menthol, (-)-menthone, (+/-)-menthyl acetate, 1,8-cineole, limonene, b-pinene and b-caryophyllene] were investigated for their antimicrobial effects

Table II - Antioxidant and antimicrobial activity of essential oil of essential oil from flowers, leaves and whole plant of *Mentha arvensis* L.

Antioxidant activity			
Trolox equivalent antioxidant activity (TEAC)		**Total phenolic content (TPC)**	
Sample	A (abs) mg TE/L oil		mg GAE/100 µl oil
Mentha oil from flowers	1.61±0.03[a]		0.12±0.02[a]
Mentha oil from leaves	1.42±0.08[b]		0.11±0.01[a]
Mentha oil from whole plant	1.58±0.03[a]		0.09±0.01[b]
Antimicrobial activity			
*Inhibition zone***			
Mentha oil from flowers		*Mentha* oil from leaves	*Mentha* oil from whole plant
Gentamycin (10 µg/well)	4.1±0.1[b]	5.3±0.1[a]	3.2±0.1[c]
Bacteria			
Escherichia coli (ATCC 25922)	27.3±0.9[a]	29.3±0.5[b]	24.3±0.3[c]
Fungi			
Candida albicans (ATCC 10231)	37.4±0.6[a]	39.1±0.3[b]	32.4±0.9[c]

*Values are mean ± standard deviation (SD) of three different experiments. Mean values marked with the different letters in the same row represents significant difference at $p < 0.05$, by Tukey's test.
**Diameter of inhibition zone (mm)

against two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), five Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella* sp.) and the yeast *Candida albicans* using a modified agar dilution and agar diffusion method. Medium to high antimicrobial effects were found for both oils, the dementholised cornmint oil fraction, and the target-compounds against all Gram-positive bacteria, whereas against the Gram-negative bacteria and the yeast, one or more samples showed only weak or no activity [8]. However, our results proofed the statement that essential oil reached with hydrocarbons in pactricular monoterpenes and sesquiterpenes had significant antifungal potential [24].

4. CONCLUSIONS

We concluded that the region of South-east Macedonia had good potential for the production of high-quality Cornmint (*Mentha arvensis L*.) with an appreciable amount of menthol, isomenthone and isopulegone. Furthermore, antioxidant and antimicrobial activity of essential oils from flowers, leaves and the whole *Mentha* plant, as well as oil fractions enriched by menthol, isomenthole and isopulegone, can be interesting for further investigation for medicinal purposes.

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REFERENCES

[1] J.P. de Sousa Guedes, E.L. de Souza. Investigation of damage to *Escherichia coli*, *Listeria monocytogenes* and *Salmonella Enteritidis* exposed to *Mentha arvensis* L. and *M. piperita* L. essential oils in pineapple and mango juice by flow cytometry. Food Microbiol. *76*, 564-571 (2018).

[2] J. Bajeli, S. Tripathi, A. Kumar, A. Tripathi, R.K. Upadhyay. Organic manures a convincing source for quality production of Japanese mint (*Mentha arvensis* L.). Ind. Crops Prod. *83*, 603-606 (2016).

[3] J.P. de Sousa Guedes, J.A. da Costa Medeiros, R.S. de Souza e Silva,J.M. Batista de Sousa, M.L. da Conceição, E.L. de Souza. The efficacy of Mentha arvensis L. and M. piperita L. essential oils in reducing pathogenic bacteria and maintaining quality characteristics in cashew, guava, mango, and pineapple juices. Int. J. Food Microbiol. *238*, 183-192 (2016)

[4] H. Zeinali, A. Arzani, K. Razmjoo, M.B. Rezaee. Evaluation of Oil Compositions of Iranian Mints (*Mentha ssp*.). J. Essent. Oil Res. *17*, 156-159 (2005).

[5] M. Johnson, E.G. Wesely, M.S. Kavitha, V. Uma. Antibacterial activity of leaves and internodal callus extracts of *Mentha arvensis* L. Asian pacific journal of tropical medicine. 196-200 (2011).

[6] K.H.C. Başer, M. Kürkçüoğlu, B. Demirci, T. Özek, G. Tarımcılar (2012). Essential oils of *Mentha species* from Marmara region of Turkey. J. Essent. Oil Res. *24*, 265-272 (2012).

[7] K. Perveen, A. Haseeb, P.K. Shukla. Effect of *Sclerotinia sclerotiorum* on the disease development, growth, oil yield and biochemical changes in plants of *Mentha arvensis*. Saudi Journal of Biological Sciences. *17*, 291-294 (2010)

[8] R.K. Chauhan, M. Anwar, S. Chand, D.D. Patra. Influence of different dates of planting on growth, herb, oil yield and quality of essential oil of menthol mint (Mentha arvensis) in the North Indian Plain. Archives of Agronomy and Soil Science *58*, 223-232 (2012).

[9] B.R. Rajeswara Rao, P.N. Kaul, G.R. Mallavarapu, S. Ramesh. Comparative Composition of Whole Herb, Flowers, Leaves and Stem Oils of Cornmint (*Mentha arvensis L*.f. piperascens Malinvaud ex Holmes). J. Essent. Oil Res. *12*, 357-359 (2000).

[10] A. Ahmad, M.P. Darokar, S. Tandon. Antimicrobial Activity of Isolates and Derivatives ex Indian *Mentha arvensis* Essential Oil. J. Ess. Oil Bearing Plants *14*(6), 796-804, (2011).

[11] S.K. Bose, R.K. Yadav, S. Mishra, RS. Sangwan, A.K. Singh, B. Mishra, A.K. Srivastava, N.S. Sangwan. Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in *Mentha arvensis* L. Plant. Physiol. Biochem. *66*, 150-158 (2013).

[12] R.K. Upadhyay, J.R. Bahl, R.S. Verma, R.C. Padalia, Amit Chauhan, D.D. Patra. New source of planting material for quality cultivation of menthol–mint (*Mentha arvensis* L.). Ind. Crops Prod. *59*, 184-188 (2014).

[13] P. Tiwari. Recent advances and challenges in trichome research and essential oil biosynthesis in *Mentha arvensis* L. Ind. Crops Prod. *82*, 141-148 (2016)

[14] 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*, 107-113 (1999)

[15] M.A.A. De Souza, L.A. dos Santos, D.M.C. de Brito, J.F. Rocha, R.N. Castro, M.S. Fernandes, S.R.J. de Souza. Influence of light intensity on glandular trichome density, gene expression and essential oil of menthol mint (*Mentha arvensis* L.) J. Essent. Oil Res. *28*(2), 138-145, (2016)

[16] O. Prakash, M. Naik, R. Katiyar, S. Naik, D. Kumar, D. Maji, A. Shukla, A.D. Nannaware, A. Kalra, P.K. Rout. Novel process for isolation of major bio-polymers from *Mentha arvensis* distilled biomass and saccharification of the isolated cellulose to glucose. Ind. Crops Prod. *199*, 1-8 (2018).

[17] B.R. Rajeswara Rao. Biomass yield, essential oil yield and essential oil composition of rose-scented geranium (*Pelargonium* species) as influenced by row spacings and intercropping with cornmint (*Mentha arvensis* L.f. *piperascens* Malinv. Ex Holmes). Ind Crop. Prod. *16*, 133-144, (2002).

[18] R.K. Srivastava, A.K. Singh, A. Kalra, V.K.S. Tomar, R.P. Bansal, D.D. Patra, S. Chand. A.A. Naqvi, S. Sharma, S. Kuma. Characteristics of menthol mint *Mentha arvensis* cultivated on industrial scale in the Indo-Gangetic plains. Ind. Crop Prod. *15*, 189-198 (2002).

[19] S.V. Phatak, M.R. Heble. Organogenesis and terpenoid synthesis in *Mentha arvensis*. Fitoterapia *73*, 32-39 (2002).

[20] N. Mimica-Dukic, O. Gasic, R. Jancic, G. Kite. Essential Oil Composition of Some Populations of *Mentha arvensis* L. in Serbia and Montenegro. J. Essent. Oil Res. *10*, 502-506 (1998).

[21] R.C. Padalia, R.S. Verma, A. Chauhan, P. Goswami. Quality evaluation of elite mint cultivars in two cropping seasons in subtropical conditions of Uttarakhand, India. J. Essent. Oil Res. *27*, 45-51 (2015).

[22] R.S. Verma, L. Rahman, R.K. Verma, A. Chauhan, A.K. Yadav, A. Singh. Essential Oil Composition of Menthol Mint (*Mentha arvensis*) and Peppermint (*Mentha piperita*) Cultivars at Different Stages of Plant Growth from Kumaon Region of Western Himalaya. J Med Aromatic Plants *1*, 13-18 (2010).

[23] H.A.E. Shaaban, A.H. El-Ghorab, T. Shibamoto, Bioactivity of essential oils and their volatile aroma components. Review. J. Essent. Oil Res. *24*(2), 203-212 (2012).

[24] A. Benabdallah, C. Rahmoune, M. Boumendjel, O. Aissi, C. Messaoud, Total phenolic content and antioxidant activity of six wild *Mentha species* (Lamiaceae) from northeast of Algeria. Asian Pac. J. Trop. Biomed. *6*(9), 760-766 (2016).

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