Antifungal activity of the essential oils of wild-growing *Mentha piperita* L and *Mentha spicata* L from the Mariovo region, Republic of Macedonia

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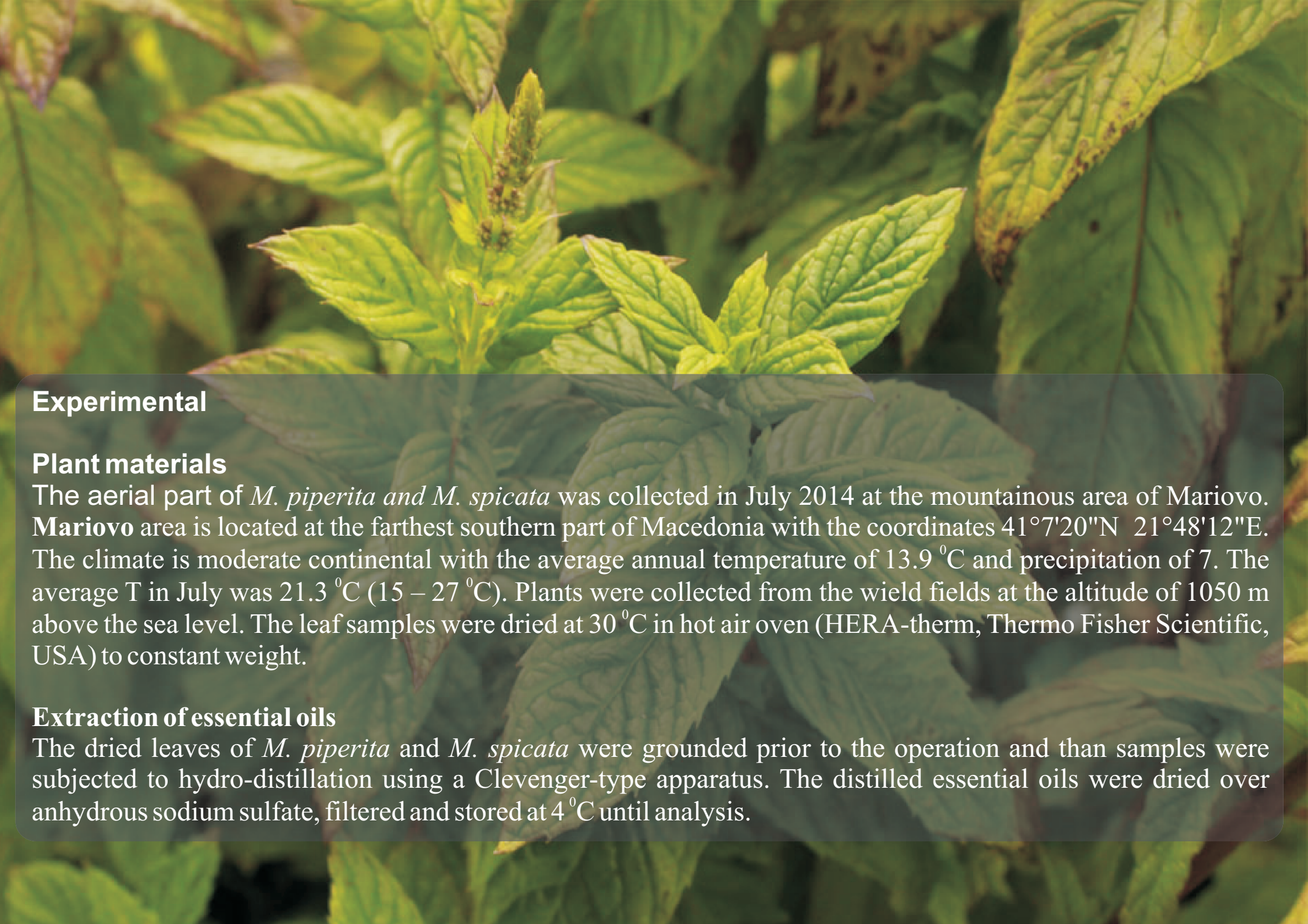
Mentha species grow on the whole territory of the Republic of Macedonia, mainly as wild plants. *M. piperita and M. spicata* are the most abundant species of the genus *Mentha* in the Republic of Macedonia. Many experiments have been conducted for chemical characterization of *M. piperita and M. spicata* essential oils in various parts of the world.

However, no earlier reports are available on the detailed chemical composition of the essential oils from *M. piperita,* and *M. spicata* native to the territory of the Republic of Macedonia. This motivated us to investigate the antifungal activity of the essential oils against several strains of plant pathogenic fungi was also investigated.



Experimental

Plant materials

The aerial part of *M. piperita and M. spicata* was collected in July 2014 at the mountainous area of Mariovo. **Mariovo** area is located at the farthest southern part of Macedonia with the coordinates 41°7'20"N 21°48'12"E. The climate is moderate continental with the average annual temperature of 13.9 ^{0}C and precipitation of 7. The average T in July was 21.3 ^{0}C (15 – 27 ^{0}C). Plants were collected from the wield fields at the altitude of 1050 m above the sea level. The leaf samples were dried at 30 ^{0}C in hot air oven (HERA-therm, Thermo Fisher Scientific, USA) to constant weight.

Extraction of essential oils

The dried leaves of *M. piperita* and *M. spicata* were grounded prior to the operation and than samples were subjected to hydro-distillation using a Clevenger-type apparatus. The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored at 4 ^{0}C until analysis.

Antifungal activity of essential oils

Mentha essential oils were individually tested against six strains of pathogenic fungi: *Alternaria alternata Alternaria solani, Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani* and *Rhizopus* solani.

Disc diffusion method

The antifungal activity of the *Mentha* essential oils and the principal components menthol, menthone, carvone, limonene and 1,8-cineole were determined by the disc diffusion method. Posaconazole was used as a positive reference for fungi, while the discs without samples were used as a negative control. Antifungal activity was assessed by measuring the diameter of the growth inhibition zone (IZ) in millimetres including disc diameter of 6 mm for the test organisms compared to controls.

Micro-dilution broth method

For minimum inhibitory concentration (MIC), a micro-dilution broth susceptibility assay was used. Microdilution broth test was performed in sabouraud dextrose broth (SDB, Oxoid). Essential oils were solubilized in dimethylsulfoxide (DSMO), and then diluted in culture media for use. Posaconazole was used as a reference compound for antifungal activities. MIC was calculated as the highest dilution (the lowest concentration of antifungal compound) showing complete inhibition of the test strains.

Antifungal activity

Table 1 Antifungal activity of essential oils from leaves of two *Mentha* species*

Tested fungal strain	*Mentha piperita*	*Mentha spicata*
Disc diffusion method		
Alternaria alternata	16 ± 1 A	20 ± 1 B
Alternaria solani	13 ± 1 A	26 ± 1 C
Aspergilus flavus	20 ± 2 A	25 ± 2 B
Aspergilus niger	28 ± 1 B	26 ± 2 B
Fusarium solani	19 ± 2 A	25 ± A
Rhizopus solani	30 ± 2 B	29 ± 2 AB
Minimum inhibitory concentration (MIC, µg mL^{-1})		
Alternaria alternata	138.6 ± 8.4 D	120.3 ± 6.5 D
Alternaria solani	154.5 ± 6.2 D	76.1 ± 5.2 A
Aspergilus flavus	115.4 ± 3.9 C	88.5 ± 3.9 C
Aspergilus niger	65.4 ± 3.1 A	80.5 ± 4.2 C
Fusarium solani	120.3 ± 4.2 C	97.3 ± 4.4 B
Rhizopus solani	50.6 ± 3.5 B	65.8 ± 3.8 B

*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) including disc diameter of 6 mm.

Table 2 Antifungal activity of pure substances*

Tested fungal strain	Menthol	Menthone	Carvone	1,8-cineol	Limonene	Posaconazole
Disc diffusion method						
Alternaria alternata	26 ± 2 B	17 ± 1 B	21 ± 1A	12 ± 1 A	10 ± 0 A	28 ± 2 C
Alternaria solani	23 ± 2 A	18 ± 1 B	14 ± 2 B	11 ± 1 A	10 ± 0 C	26 ± 2 B
Aspergilus flavus	18 ± 1 B	20 ± 1 A	21 ± 1 A	13 ± 1 A	11 ± 1 A	36 ± 2 C
Aspergilus niger	30 ± 2 A	25 ± 1 AB	26 ± 1 C	14 ± 1 AB	12 ± 1 A	37 ± 1 C
Fusarium solani	29 ± 1 B	23 ± 1 AB	29 ± 2 AB	15 ± 0 A	12 ± 1 B	36 ± 2 B
Rhizopus solani	31 ± 1 A	29 ± 2 A	28 ± 2 A	15 ± 1 C	13 ± 1 A	32 ± 1 C
Minimum inhibitory concentration (MIC, µg mL^{-1})						
Alternaria alternata	76.8 ± 3.5 A	120.6 ± 4.8 C	98.7 ± 5.6 A	180.5 ± 3.5 A	210.8 ± 8.1 B	60.5 ± 2.4 A
Alternaria solani	99.5 ± 1.8 A	127.8 ± 2.6 C	148.6 ± 3.2 B	195.2 ± 4.1 C	220.6 ± 7.3 C	71.4 ± 1.7 A
Aspergilus flavus	115.3 ± 3.8 B	114.5 ± 3.4 BC	102.6 ± 6.9 C	150.8 ± 5.2 D	170.3 ± 5.5 D	10.4 ± 1.3 B
Aspergilus niger	75.4 ± 2.4 C	95.6 ± 4.1 C	80.5 ± 4.5 A	143.4 ± 7.5 B	168.3 ± 6.2 B	10.2 ± 1.8 C
Fusarium solani	65.8 ± 1.8 B	105.4 ± 6.2 D	69.3 ± 3.9 D	130.8 ± 2.8 A	155.6 ± 4.8 C	10.5 ± 2.7 D
Rhizopus solani	36.4 ± 2.6 C	58.3 ± 1.9 B	70.5 ± 5.5 A	129.5 ± 4.2 B	149.5 ± 4.3 A	12.5 ± 2.9 A

*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) including disc diameter of 6 mm

The antifungal activity of the essential oils isolated by hydro-distillation from the leaves of wild growing *Mentha piperita* and *Mentha spicata* (Lamiaceae) at the region of Mariovo, Republic of Macedonia was tested by disc diffusion method and the micro-dilution broth method (MIC) against six plant pathogenic fungi: *Alternaria alternate Alternaria solani, Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani* and *Rhizopus* solani.

The results from the disc diffusion method followed by MIC indicated that M. *spicata* essential oil showed maximum antifungal activity with larger inhibition zone (20 – 29 mm) and smallest MIC values (65.8 – 120.3 µg mL-1) against all the strains tested. *M. piperita* essential oil exhibited good antifungal activity with inhibition zone of 19 and 20 mm and MIC values of 120.3 and 115.4 µg mL^{-1}, respectively against *Fusarium solani* and *Aspergilus flavus* and excellent antifungal activity with inhibition zone of 28 and 30 mm and MIC values of 65.4 and 50.6 µg mL^{-1}, respectively against *Aspergilus niger* and *Rhizopus solani*.

Menthol, menthone, carvone, limonene and 1,8 - cineole, the major constituents of *M. piperita* and *M. spicata,* were also tested for their potential antifungal activity. Posaconazole a triazole antifungal drug was used as a positive reference drug for fungi. Menthol exhibited excellent antifungal activity against *Alternaria solani*, *Alternaria alternata*, *Aspergilus niger* and *Rhizopus solani* (inhibition zone 23 – 31 mm, MIC 36.4 – 76.8 µg mL^{-1}), which was close to the antifungal activity of standard drug for the strains *Alternaria alternata, Alternaria solani* and *Rhizopus solani*. Menthone and carvone showed good antifungal activity against *Aspergilus flavus*, *Aspergilus niger* and *Rhizopus solani* (inhibition zone 20 – 29 mm, MIC 58.3 – 114.5 µg mL^{-1}). Carvone exhibited better antifungal activity for *Fusarium solani* than menthone. Contrary to these findings limonene and 1,8-cineol exhibited lower antifungal activity for all tested strains (inhibition zone 10-15 mm, MIC 69.3 – 220.6 µg mL^{-1}).

Conclusion

The results of the present study indicate that essential oils of *M. piperita* and *M. spicata* possess very good antifungal potential against the plant pathogenic fungi. This is due to the main components of the essential oils: menthol, menthone and carvone. The investigated essential oils of Mentha species may be used for the preservation of the processed foods as well as for pharmaceuticals