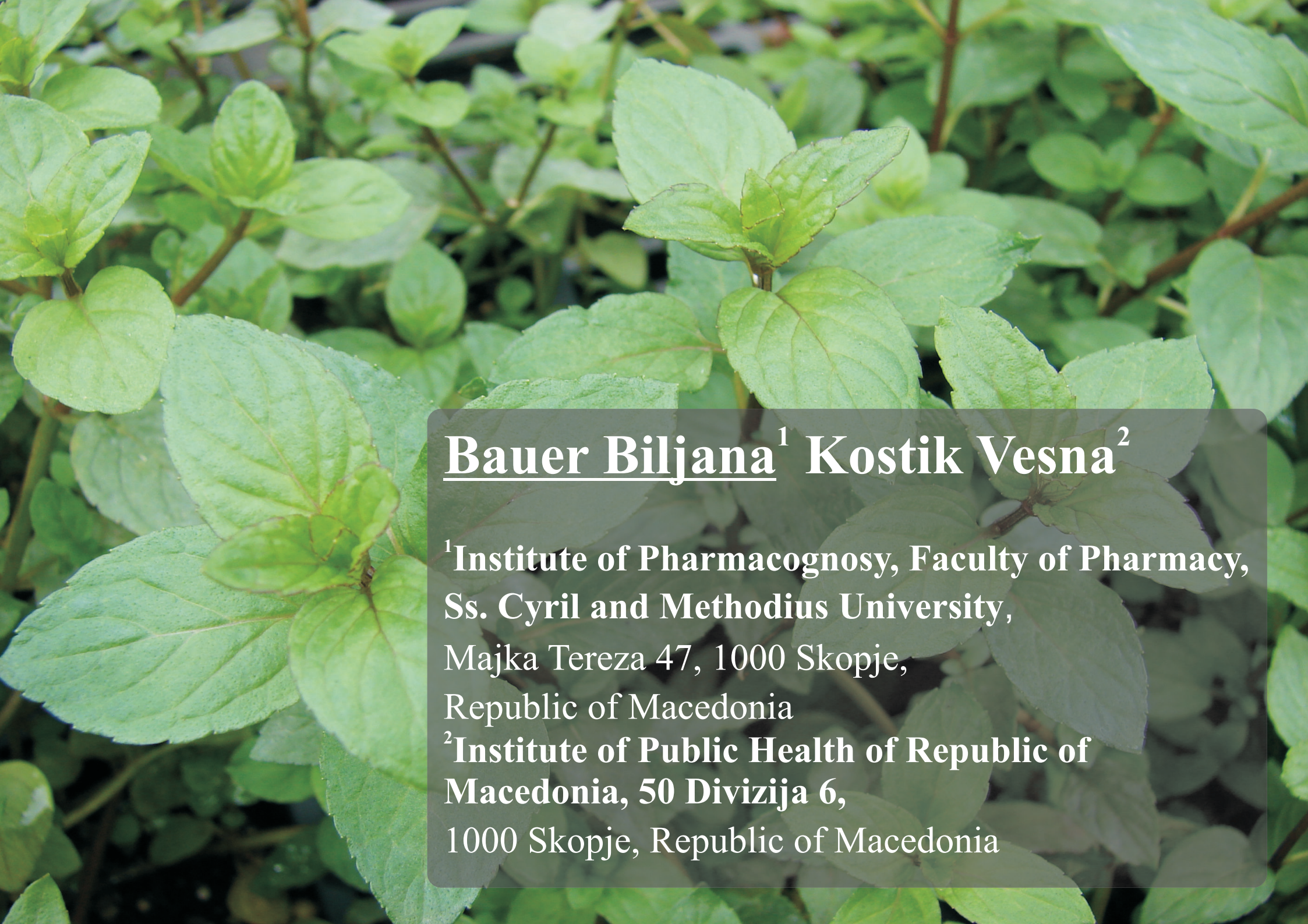
Chemical composition of essential oils of wild-growing *Mentha piperita* L and *Mentha spicata* L from the Mariovo region, Republic of Macedonia



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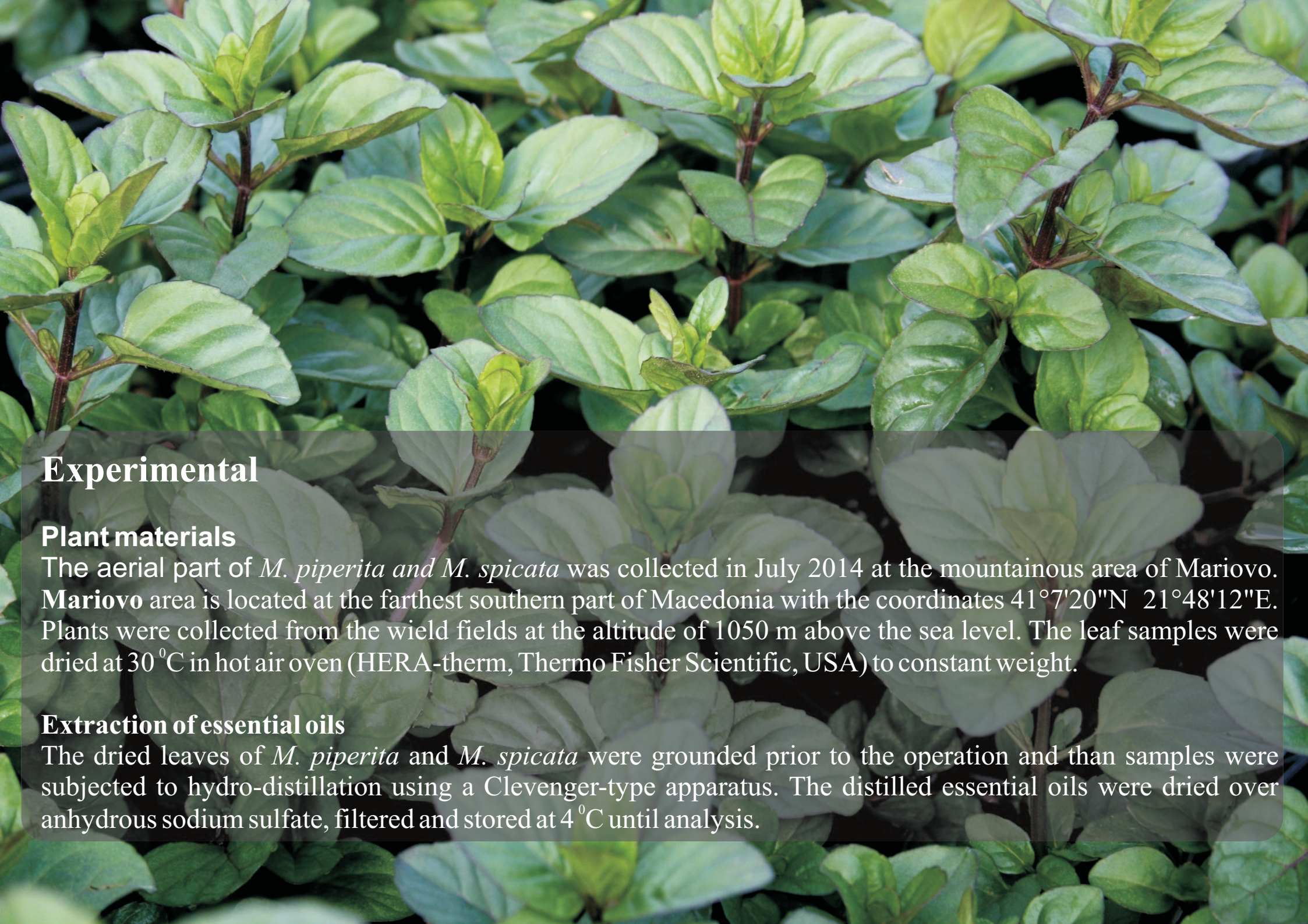
The genus *Mentha* (family Lamiaceae), comprising more than 25 species, grows widely throughout the temperate regions of the world. *Mentha arvensis*, *M. longifolia*, *M. piperita* and *M. spicata*, commonly known as menthol mint, wild mint, peppermint and spearmint, respectively, are frequently cultivated in many countries of Europe, East Asia, America and Australia for the production of essential oils. The essential oils and extracts from *Mentha* species have been in use since ancient times for the treatment of many digestive tract diseases, as well as, in cuisine.

M. spicata and *M. piperita* are the most abundant species of the genus *Mentha* which grow as wild crops mostly at the south parts of the Republic of Macedonia. The main goal of our study was evaluation of chemical composition of the essential oils obtained from the leaves of wild-growing *M. spicata* and *M. piperita* from the region of Mariovo, located at the farthest southern part of the Republic of Macedonia.



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. 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.

Chemical composition of essential oils

Gas chromatography

The essential oils were analysed using a gas chromatograph (2010, Shimadzu, Japan) equipped with flame ionization detector (FID), auto injector (AO 20i) and ZB-5 MS capillary column (30 m x 0.25 mm x 0.25 μm).

Gas chromatography–mass spectrometry (GC-MS)

GC-MS analysis of the essential oils was performed using a gas chromatograph (2010 plus, Shimadzu, Japan), equipped with a Shimadzu QP-2010 mass selective detector and AOC 5000 auto-sampler (Shimadzu). Compounds were separated on a ZB-5 MS capillary column (30 m x 0.25 mm x 0.25 μm).

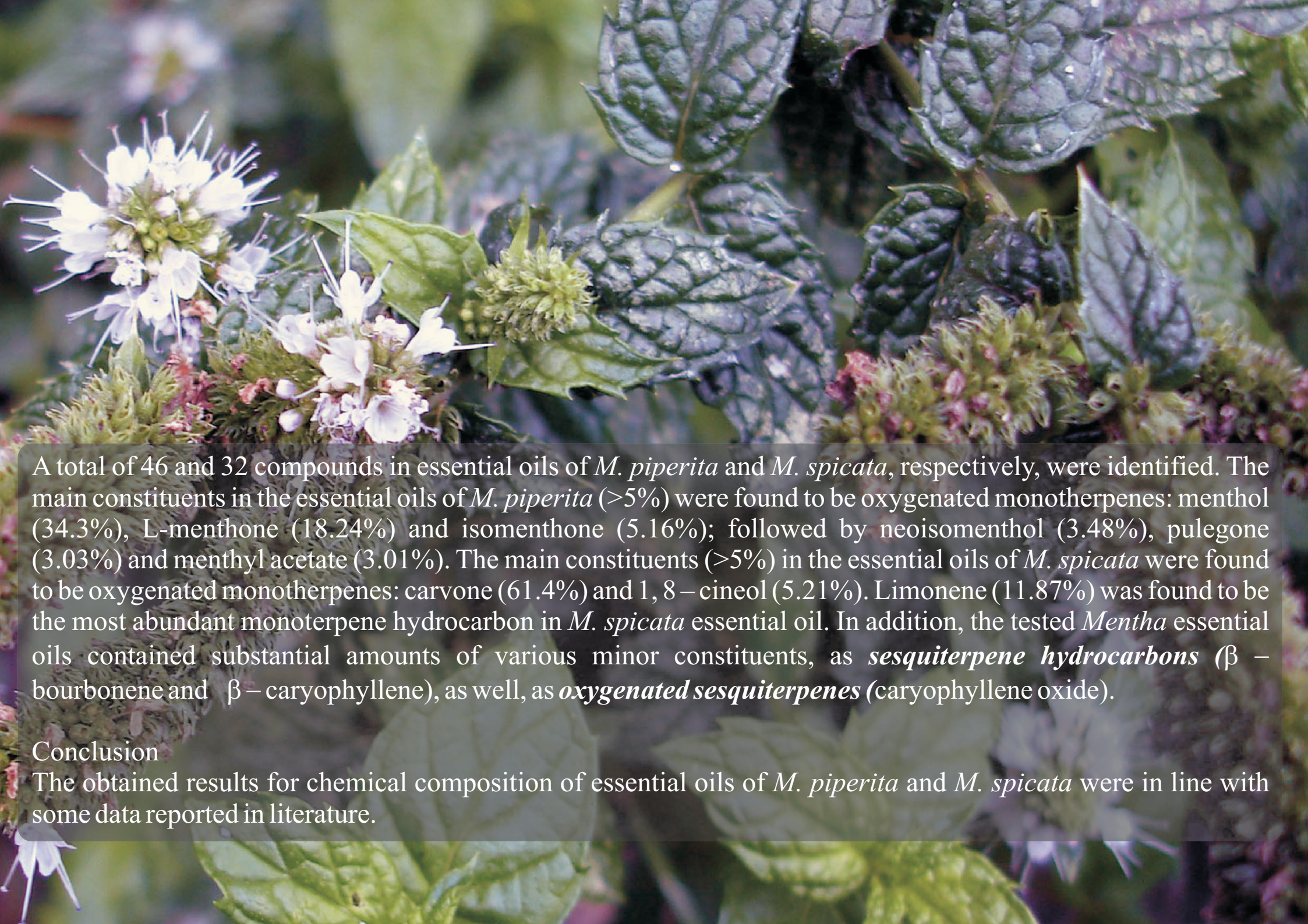
Compound identification

The constituents of the oil were identified by using standard reference compounds and also by matching the mass spectra fragmentation pattern with NIST Mass Spectra Library stored in the GC–MS database.

Table 1. Content and chemical composition of the essential oils from leaves of two *Mentha* species

		Composition (%)	
Monoterpene hydrocarbons		***M. piperita***	***M. spicata***
Components	**RI**		
α – Pinene	928	2.03 ± 0.23	0.06 ± 0.02
Camphene	950	0.22 ± 0.07	-
β – Pinene	971	2.03 ± 0.43	0.04 ± 0.02
β – Myrecene	989	0.34 ± 0.03	-
p – Cymene	1020	0.41 ± 0.09	-
Limonene	1024	4.54 ± 0.22	11.87 ± 0.45
Oxygenated monoterpenes			
1,8 – Cineol	1028	1.15 ± 0.20	5.21± 0.52
Cis- Sabinene hydrate	1068	-	0.12 ± 0.03
Linalool	1088	0.98 ± 0.24	1.14 ± 0.42
Isopulegol	1140	0.44 ± 0.14	0.01 ± 0.04
L –Menthone	1149	18.24 ± 1.9	2.10 ± 0.24
Isomenthone	1159	5.16 ± 1.1	-
Borneol	1163	-	2.46 ± 0.24
Menthol	1170	34.3 ± 1.5	0.76 ± 0.15
Terpinene -4 -ol	1174	1.82 ± 0.23	0.32 ± 0.09
Neoisomenthol	1184	3.48 ± 0.76	-
α-Terpineol	1186	2.45 ± 0.44	1.05 ± 0.23
Dihydrocarveol	1193	-	1.95 ± 0.22
γ-Terpineol	1195	2.15 ± 0.18	-
cis- Dihydrocarvone	1198	-	2.12 ± 0.34
trans- Dihydrocarvone	1200	-	0.19 ± 0.06
trans – Carveol	1214	-	0.29 ± 0.05
cis – Carveol	1227	0.11± 0.03	1.95 ± 0.09
Pulegone	1235	3.03 ± 0.18	-
Carvone	1240	0.65 ± 0.11	61.4 ± 1.80
Carvon oxide	1242	-	0.18 ± 0.03
Bornyl acetate	1286	-	0.30 ± 0.04
Menthyl acetate	1298	3.01 ± 0.18	-
Isopulegyl acetate	1260	0.22 ± 0.02	-
Piperitenone	1340	0.12 ± 0.01	-
cis-Carvyl acetate	1365	-	0.18 ± 0.03

Sesquiterpene hydrocarbons			
α-Ylangene	1369	0.28 ± 0.03	-
α-Copaene	1375	0.35 ± 0.07	-
β – Bourbonene	1384	1.45 ± 0.12	0.82 ± 0.15
β – Elemene	1388	0.88 ± 0.11	-
cis – Jasmone	1390	0.22 ± 0.04	-
Longifolene	1406	0.35 ± 0.02	-
β – Caryophyllene	1418	1.18 ± 0.22	1.15 ± 0.12
β – Cubebene	1420	0.55 ± 0.05	-
Thujopsene	1423	0.28 ± 0.02	-
Aromadendrene	1438	0.58 ± 0.04	-
α-Caryophyllene	1452	0.55 ± 0.03	0.56 ± 0.04
γ – Muurolene	1477	0.88 ± 0.04	0.22 ± 0.01
Germacrene D	1482	0.51 ± 0.03	0.71 ± 0.06
Ledene	1494	0.48 ± 0.08	-
α – Muurolene	1497	0.68 ± 0.06	-
Cuparene	1503	0.11 ± 0.02	-
Amorphene	1438	0.38 ± 0.04	-
γ-Cadinene	1510	-	0.15 ± 0.02
δ-Cadinene	1521	0.98 ± 0.13	-
α-Cadinene	1537	0.11 ± 0.02	-
Calamenene	1539	-	0.33 ± 0.02
α-Calacorene	1544	0.09± 0.01	–
Oxygenated sesquiterpenes			
Spathulenol	1575	0.39 ± 0.04	0.11 ± 0.02
Cariophyllene oxide	1580	1.05 ± 0.14	0.82± 0.05
α-Cedrol	1593	0.11 ± 0.02	-
1,10-di-epi-cubenol	1612	-	0.19 ± 0.03
α-Muurolol	1642	-	0.72 ± 0.23
β – Eudesmol	1649	0.12 ± 0.03	-
Total		99.44	99.48
Essental oil content (g/kg)		12.4 ± 1.05	12.2 ± 0.98



A total of 46 and 32 compounds in essential oils of *M. piperita* and *M. spicata*, respectively, were identified. The main constituents in the essential oils of *M. piperita* (>5%) were found to be oxygenated monotherpenes: menthol (34.3%), L-menthone (18.24%) and isomenthone (5.16%); followed by neoisomenthol (3.48%), pulegone (3.03%) and menthyl acetate (3.01%). The main constituents (>5%) in the essential oils of *M. spicata* were found to be oxygenated monotherpenes: carvone (61.4%) and 1, 8 – cineol (5.21%). Limonene (11.87%) was found to be the most abundant monoterpene hydrocarbon in *M. spicata* essential oil. In addition, the tested *Mentha* essential oils contained substantial amounts of various minor constituents, as ***sesquiterpene hydrocarbons (***β – bourbonene and β – caryophyllene), as well, as ***oxygenated sesquiterpenes (***caryophyllene oxide).

Conclusion

The obtained results for chemical composition of essential oils of *M. piperita* and *M. spicata* were in line with some data reported in literature.