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Micromorphological traits and essential oil of *Micromeria longipedunculata* Bräuchler (*Lamiaceae*)

Research Article

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Abstract: *Micromeria longipedunculata* Bräuchler (syn. *M. parviflora* (Vis.) Rchb.) is an endemic Illyric-Balkan plant species from Bosnia and Herzegovina, Montenegro, and Albania. We investigated types and distribution of trichomes, pollen morphology, and chemical composition of essential oil (analysed by GC and GC-MS) in *M. longipedunculata*. Non-glandular trichomes, peltate trichomes, and two types of capitate trichomes (type 1 composed of one basal epidermal cell, and one head cell with subcuticular space; type 2 composed of one basal epidermal cell, two or three stalk cells, and one head cell with subcuticular space) were observed on leaves, bracteoles, the calyx, corolla, and the stem. The pollen grains had six apertures which were set in the equatorial pollen belt and showed medium reticulate ornamentation. A phytochemical analysis of essential oils from four different localities is characterized by similar chemical composition with spathulenol (23.7–39.5%), piperitone oxide (7.7–12.1%) and piperitone (7.3–8.9%) as the major compounds.

Keywords: *Micromeria longipedunculata • Micromeria parviflora • Trichomes • Pollen • Essential oil*

1. Introduction

The family of *Lamiaceae* Lindl. (syn. *Labiatae* Lindl., the mint family) has a world-wide distribution and comprises more than 7200 species across approximately 240 genera which are classified in seven subfamilies [1,2]. There are many aromatic species amongst them which produce considerable quantities of essential oils, and some of them have great economic importance.

The genus *Micromeria* Benth. is part of a complex group of genera in tribe *Mentheae* subtribe *Menthinae* [3], and includes 70–90 herbs, sub-shrubs and shrubs with a distribution range extending from the Himalayan region to the Macaronesian Archipelago, and from the Mediterranean to South Africa and Madagascar [4-6]. Twenty one *Micromeria* species have been described for Europe [4], eight species (*M. cristata* (Hampe) Griseb., *M. croatica* (Pers.) Schott., *M. dalmatica* Benth., *M. graeca* (L.) Benth. ex Reichenb. *M. juliana* (L.) Benth., *M. kerneri* Murb., *M. longipedunculata* Bräuchler (syn. *M. parviflora* (Vis.) Rchb.), *M. thymifolia* (Scop.) Fritsch.) for Montenegro [7,8], and five species (*M. croatica*, *M. kerneri*, *M. longipedunculata*, *M. pulegium* (Rochel.) Benth., and *M. thymifolia*) for Bosnia and Herzegovina [7].

M. longipedunculata is a dwarf shrub 5 to 25 cm in height, with ascending, simple or slightly branched,

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deflexed-puberulent stems. Leaves are 4–10 mm long and of varying shape (the lowest cordate-orbiculate to ovate, upper oblong to linear), glabrous or sparsely puberulent with a slightly revolute margin. Flowers are hermaphroditic, arranged from two to six in lax verticillasters, and exceed the subtending leaves. The calyx is tubular, glabrous or sparsely puberulent, 3.5–4 mm long, while the corolla is sympetalous, 2-lipped, purplish, 1–1.5 cm long [4,7]. It is an endemic and pioneer species found in open habitats in the crevices of dolomites and calcareous rocks, at altitudes of 50 to 1400 m [8]. The species' center of distribution is considered to be on the Montenegrinian mountains, from where it spread both north-westerly towards the Herzegovinian mountains and south-easterly towards the Albanian mountains. *M. longipedunculata* predominantly grows in the endemic plant community *Micromerio-Crepidetum pantocsekii* Ritter 1967 together with *Crepis pantocsekii* (Vis.) Latzel, *Asperula scutellaris* Vis., *Genista sericea* Wulfen, *Thymus bracteosus* Vis. ex Benth., *Polygala carniolica* A. Kern., and *Chrysanthemum chloroticum* Kern. et Murb. f. *simplex* [7,9]. Considering ethnobotany, there is no information about uses of *M. longipedunculata* in traditional medicine of Balcanic people or its use in other applications. The knowledge on biology of this rare and endemic species is essential for future protection and conservation measures. Additionally, the knowledge about chemical traits of poorly investigated endemic species like *M. longipedunculata* might lead to the discovery of potential phytotherapeutics. Up to date studies on *M. longipedunculata* have considered its ecology and syntaxonomy [8], biology and distribution [7], as well as taxonomical status [6,7], though little to no data on micromorphological traits are available.

Also, *M. longipedunculata* has been a problematic species taxonomically through history and its nomenclature status has only recently been resolved [6]. In Balkan literature *M. longipedunculata* is known under the name *M. parviflora* Rchb., with synonyms: *M. inodora* (Desf.) Benth., *Satureja parviflora* Vis. and *S. inodora* Host. [6]. According to Bräuchler et al. [6] the name *M. parviflora* is illegitimate and they proposed a new name *M. longipedunculata,* which refers to very long-peduncled cymes. Bräuchler et al. [6] gave the explanation that the name *M. parviflora* is illegitimate even when considering Art. 58.1 of the International Code of Botanical Nomenclature [10], since Reichenbach when transferring Visiani's illegitimate *Satureja parviflora* to *Micromeria*, cited *Satureja inodora* Host 1831, a legitimate name, in synonymy. It seems that both names might be based on duplicates of the same Tommasini collection from Mt Pastrovich in Montenegro. Though Visiani does not directly indicate Tommasini as the collector, in the introduction of his treatment he referred to Tommasini as having contributed part of the material for the study. All later researchers accepting this species under *Micromeria* referred to Reichenbach and Visiani, repeating their errors. The name *M. inodora* is occupied by a combination for *Thymus inodorus* Desf. since Bentham. As no published epithet is available, Bräuchler et al. [6] proposed the new name *M. longipedunculata*.

Glandular trichomes in the family *Lamiaceae* are the site of essential oil biosynthesis, secretion and accumulation and their structure has been studied by many authors [11-15]. The present taxonomy ascribes great value to the structure and distribution of trichomes, both secretory and non-secretory [16]. The essential oil composition of the genus *Micromeria* is variable. The major constituents of the oil of *M. cristata* were isoborneol (11.3%), borneol (8.5%), verbenone (8.2%) and 10-*epi-a*-cadinol (8.2%) [17]. The major constituents of the oil of *M. croatica* were caryophyllene oxide and β-caryophyllene [18] while pulegone and piperitone oxide were the main compounds in the essential oils of *M. albanica* (Griseb. ex K. Maly) Šilić and *M. thymifolia* [19].

Palynology gives valuable data about the origin, evolution and taxonomy of the species [20]. The pollen morphology of *Lamiaceae* has been investigated by several authors [21-25]. Pollen of *Micromeria marginata* (Sm.) Chater and *M. croatica* was investigated by Moon *et al.* [24] and Kremer *et al.* [18], respectively.

The aim of this study is to get insight into the types and distribution of glandular trichomes in *M. longipedunculata*, and to analyze the chemical composition of the essential oils. Additionally, a study on pollen morphology was done.

2. Experimental Procedures

2.1 Herbal material and extraction

Randomly selected samples of wild growing plants of *M. longipedunculata* were collected during the blooming period in July of 2011 at three localities in Montenegro and at one locality in Bosnia and Herzegovina (Table 1). Voucher specimens of herbal material from Montenegro were deposited in the Herbarium of the Faculty of Natural Sciences and Mathematics, University of Montenegro, Podgorica, Montenegro (TGU), while herbal material from Bosnia and Herzegovina were deposited in the Herbarium "Fran Kušan" (HFK-HR), Department of Pharmaceutical Botany with "Fran Kušan" Pharmaceutical Botanical Garden, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia.

The above ground parts of several dozen randomly selected plants were harvested from mature plants on

Locality	Voucher no.	Latitude	Longitude	Altidude (m)
Montenegro				
Cijevna Canyon	TGU-289702	42°25’44.6’’ N	19°28’53.5’’ E	161
Mount Krivošije*	TGU-289703	42°32’46.2’’ N	18°42’35.4’’ E	617
Nikšić	TGU-289704	42°46’02.7’’ N	18°57’24.0’’ E	608
Bosnia and Herzegovina				
Jazina	HFK-HR-1532	42°42’15.9’’ N	18°30’33.3’’ E	340

Table 1. Details on origin and collection data of investigated *M. longipedunculata* samples.

*Note: *- sample for pollen analysis*

a dry day and mixed to obtain a randomly selected sample. Samples were air-dried for ten days in a well-ventilated room at 60% relative air humidity and room temperature (22ºC), single-layered and protected from direct sunlight. Dried aerial parts (100 g) were subjected to hydrodistillation for 3 hours in Clevenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate.

For micromorphological investigation of trichomes samples (leaves, bracteoles, flowers, stems), seven plants per locality were fixed in FAA (formalin/96% ethanol/acetic acid/water – 5/70/5/20) and later transferred to 70% ethanol.

2.2 Micromorphological traits

For SEM-investigation, samples (leaf, bracteole, flower, stem) were transferred from 70% ethanol to 70% acetone, then dehydrated (70%, 90%, and 100% acetone) and subjected to critical point drying using CO_2 as the drying medium (CPD030; Baltec). After that, samples were sputter coated with gold (Sputter Coater, AGAR) and examined under the scanning electron microscope XL30 ESEM (FEI) with 20 kV acceleration voltages in high vacuum mode. The occurrence and frequency of the different trichome types were qualitatively assessed (– missing, ± rare, + present, ++ abundant). Pollen from several flowers per plant (seven plants from Krivošije population) was removed from anthers after the critical point drying and mixed to obtain random samples. The length of 30 pollen grains was measured. Common terminology was used in the description of micromorphology [26,29].

2.3 Gas chromatography (GC) and mass spectrometry (MS)

Gas chromatography (GC) analyses were performed on gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with flame ionization detector (FID), mass spectrometer (model 2100T; Varian Inc.) and capillary column VF-5ms (30 m × 0.25 mm i.d., coating thickness 0.25 µm; Varian Inc.). The chromatographic conditions were as follows: injector temperature was 250ºC, FID temperature was 300ºC, and helium was carrier gas at 1 mL·min^{-1}. VF-5ms column temperature was programmed at 60ºC isothermal for 3 min, and then increased to 246ºC at a rate of 3ºC min^{-1} and held isothermal for 25 min. The injected volume was 1 µL and the split ratio was 1:20. The mass spectrometry (MS) conditions were: ionization voltage 70 eV; ion source temperature 200ºC; mass scan range: 40–350 mass units. The analyses were carried out in duplicate.

The individual peaks were identified by comparison of their retention indices (relative to C8-C25 n-alkanes for capillary column VF-5ms) to those of authentic samples and literature [28], as well as by comparing their mass spectra with the Wiley 7 MS library (Wiley, New York, NY, USA) and NIST02 (Gaithersburg, MD, USA) mass spectral database. The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors). The component percentages were calculated as mean values from duplicate GC and GC-MS analyses.

3. Results and Discussion

3.1 Micromorphological traits

The type, occurrence and frequency of trichomes on all investigated plant parts (leaves, bracteoles, calyxes, corollas, and stems) of *M. longipedunculata* is shown in Table 2. Both non-glandular and glandular trichomes could be observed on the investigated parts (Figure 1). Non-glandular (NG) trichomes were bi-cellular to multicellular, unbranched, uniseriate and folded at different levels (Figure 1a, o). Parts of the surface of NG trichomes revealed a warty appearance due to the occurrence of cuticular micropapillae. The length of NG trichomes varied distinctly from very short hairs on the upper leaf surface (Figure 1d) to very long hairs on the edge of inner calyx side (Figure 1l). Moderately

Locality	Type	Leaf		Bract		Calyx		Corolla		Stem
		Adaxial	Abaxial	Adaxial	Abaxial	Outer	Inner	Outer	Inner	
Cijevna Canyon	attenuate*	+	+	–	+	+	+	–	–	+/++
	peltate	–	+	–	–	+	–	–	–	–
	capitate C1	±	+/++	±	±/+	+	±	–	±	+
	capitate C2	–	+	–	–/±	+	±	–	–	±/+
Mt Krivošije	attenuate	+	+	–	+	+	+	–	–	+
	peltate	–	+	–	–	+	–	–	–	–
	capitate C1	±	+/++	±	±/+	+	±	–	–/±	+
	capitate C2	–	+	–	–/±	+	±	–	–	±/+
Nikšić	attenuate	+	+	–	±/+	+	+	–	–	+
	peltate	–	+	–	–	+	–	–	–	+
	capitate C1	–/±	+	±	±	+	±	–	–/±	+
	capitate C2	–	+	–	–/±	+	–/±	–	–	±/+
Jazina	attenuate	+	+	–	+	+	+	–	–	+/++
	peltate	–	+	–	–	+	–	–	–	–
	capitate C1	±	+/++	±	±/+	+	±	–	±	+
	capitate C2	–	+/++	–/±	±/+	+/++	±	–	–	±/+

Table 2. Occurrence and frequency of trichomes on aerial parts of Micromeria longipedunculata.

Note: trichomes: –, missing; ±, rare; +, present; ++, abundant; attenuate, non-glandular hairs.*

long NG trichomes were common on other investigated plant parts (Table 2). According to Payne's [26] plant hair terminology, they could be noted as attenuate hairs. These trichomes are also present in *M. croatica* [18], *M. fruticosa* (L.) Druce [11] and other *Lamiaceae* [16,29-31].

Glandular trichomes are common in *Lamiaceae* species, and their function is devoted to essential oil production and storage. Two main types of glandular trichomes could be distinguished in *M. longipedunculata*: peltate and capitate. The presence of peltate trichomes is common in *Lamiaceae.* The development and function of these trichomes was investigated by several authors [11,13,16,30]. Peltate trichomes consisted of a basal cell, a short unicellular stalk, and a multicellular head with a large subcuticular space (Figure 1a, k). In *M. longipedunculata* they occurred on the abaxial leaf side, on the outer side of the calyx, and on the stem.

Two types of capitate trichomes were observed on *M. longipedunculata*. The type one capitate trichome (C1) is composed of one basal epidermal cell and one elliptically formed head cell (Figure 1b). These trichomes were not upright but could be described as clinging to the surface. They were found on both the adaxial and abaxial side of leaf and bracteole, on both the outer and inner side of calyx, and on the stem (Table 2). Occasionally, these trichomes were also found on the inner side of the corolla (Figure 1m). The type two capitate trichome (C2) is composed of one basal epidermal cell, two or three stalk cells and a single celled head with a subcuticular space (Figure 1c). These trichomes were upright and they were found on the abaxial side of leaves and bracteoles, on the outer side of the calyx, and on the stem (Table 2). C2 trichomes seem to be more sensitive than C1 trichomes and they were collapsed earlier than C1 trichomes. Intact C2 trichomes are mainly present during the earlier stage of development, whereas they are collapsed on the older parts.

Husain et al. [12] described in detail only non-glandular trichomes on *M. longipedunculata* leaf samples from Mt Rumija (Montenegro), although unclear SEM-micrographs also showed peltate and capitate (probably C1 type) trichomes. Kremer *et al.* [18] found C1 trichomes on leaves, calyxes and the stem of *Micromeria croatica*. In, general C1 trichomes are not very common in *Lamiaceae,* and until now have only been reported from a few species. Trichomes comparable to C1 trichomes could be observed in micrographs of *Thymus capitatus* (L.) Hoffmanns, *Majorana syriaca* (L.) Rafin., and *Satureja thymbra* L. presented by Werker *et al.* [11], whereas the micrographs of *Micromeria fruticosa* (L.) Druce did not

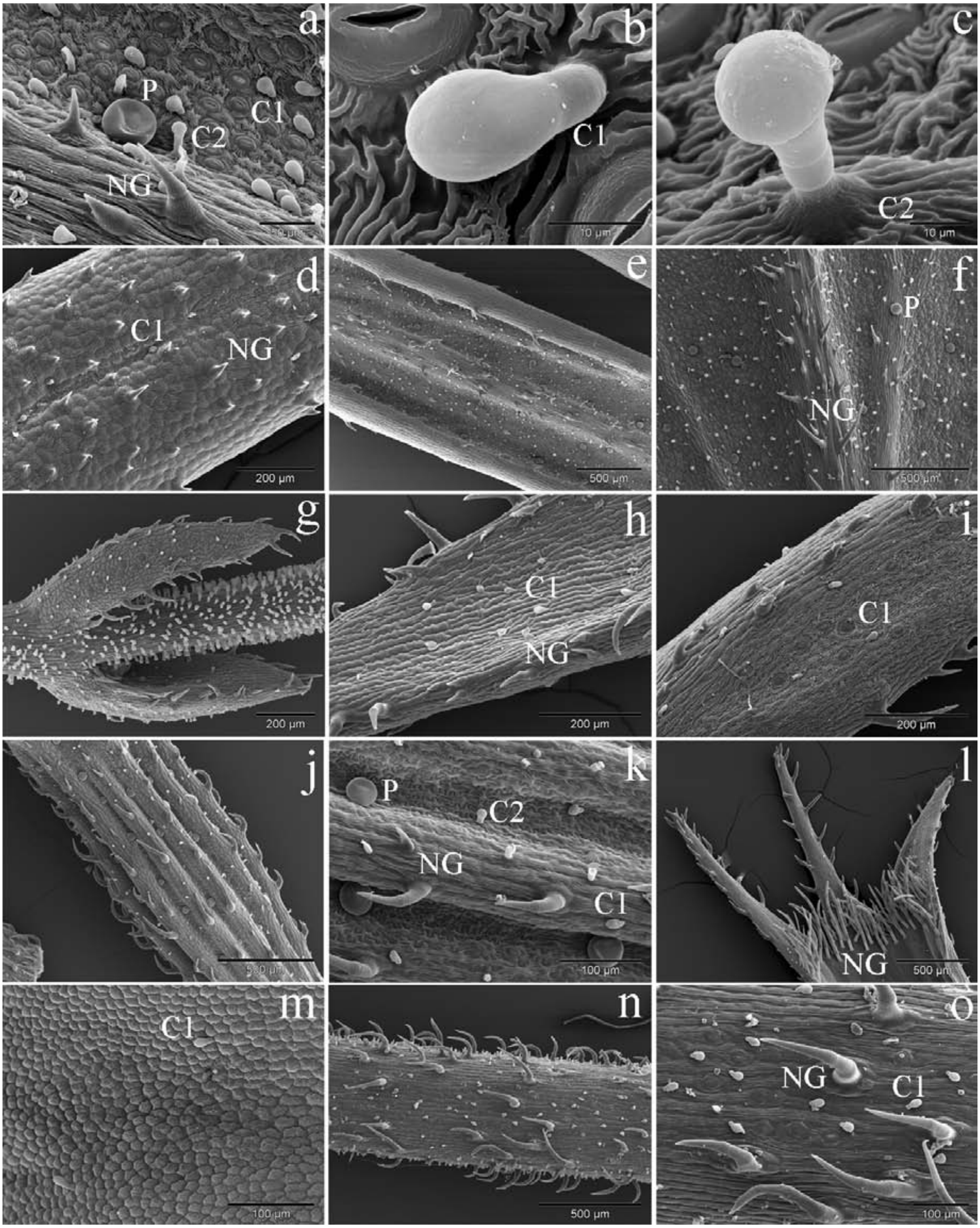

Figure 1. SEM micrographs with different types of trichomes on *M. longipedunculata*. Non-glandular trichomes (NG), peltate trichomes (P), type 1 capitate trichomes (C1) and type 2 capitate trichomes (C2) on the abaxial leaf side (a); C1 (b) and C2 (c) trichomes on the abaxial leaf side; distribution of the trichomes on the adaxial (d) and abaxial (e, f) leaf surface; distribution of the trichomes on the adaxial (h) and abaxial (i) bracteoles surface; outer side of calyx with NG, P, C1, and C2 trichomes (j, k); inner side of calyx with long NG trichomes (l); inner side of corolla with C1 trichomes (m); distribution of the trichomes on the stem (n, o).

show this hair type. C2 trichomes are more abundant in *Lamiaceae* and trichomes comparable to C2 trichomes could also be observed in some species of the genera *Salvia* L., and *Satureja* L. [11,29-31]. This hair type was also reported by Werker *et al.* [11] and Kremer *et al.* [18] for *Micromeria fruticosa* and *M. croatica*, respectively. Kremer *et al.* [18] observed C2 trichomes on the adaxial and abaxial leaf surface, on the calyx, and on the stem of *M. croatica*.

Pollen grains were single (monad pollen) and isopolar with a more or less elliptical equatorial outline (Figure 2a). The polar view (Figure 2c) showed a circular shape with visible ends of the apertures. Pollen grains had six apertures (hexacolpate) which were set in the equatorial pollen belt (zonocolpate). Apertures were long, rather narrow (Figure 2a), with sharp edges and acute ends (Figure 2c). They are widest in the middle and gradually become narrower towards the poles. Membranes were ornamented (Figure 2c). The mesocolpium was relatively large (Figure 2a) while the apocolpium was quite small (Figure 2d). Pollen grain exine had medium reticulate ornamentation (Figure 2d) with unequal (hetrobrochate) reticulum meshes. The surface of the muri was smooth. The lumina were narrower or approximately the same width as the muri, and had an irregular shape with obtuse angles. They varied in size, and were smaller towards the apertures. The appearance of apertures and ornamentation of pollen of *Micromeria croatica* and *M. marginata* was described by Kremer *et al.* [18] and Moon *et al.* [24], respectively. According to micrographs presented by Kremer *et al.* [18], the lumina were somewhat smaller in *M. longipedunculata* pollen than in *M. croatica*.

According to Kremp's classification [33], pollen of *M. longipedunculata* belongs to small pollen (10–25 μm) with a polar axis of 17.92±0.63 μm and equatorial diameter of 20.51±1.07 μm. The minimum and maximum of the polar axis was 17.1 μm and 18.3 μm, respectively, while the minimum and maximum for the equatorial diameter was 19.4 μm and 22.1 μm, respectively. According to the *P/E* ratio (0.87), it has an oblate-spheroidal shape [34]. According to Kremer *et al.* [18], *M. croatica* pollen belongs to middle-large pollen, with a polar axis of 25.8 μm and equatorial diameter of 28.5 μm. *M. marginata* pollen also belongs to middle-large pollen with a polar axis of 26.6 μm and equatorial diameter of 35.2 μm [24]. *M. croatica* pollen has an oblate-spheroidal shape [18] while *M. marginata* pollen have oblate, suboblate or oblate-spheroidal shape [24].

3.2 Gas chromatography and gas chromatography – mass spectrometry

Samples of the essential oil of *M. longipedunculata* L. from four different localities were subjected to detailed GC and GC-MS analysis for the purpose of determining their possible mutual similarity and the variability in their chemical compositions. Considering the four oils together, a total of forty-nine different compounds were identified (Table 3): 43 from CC locality (82.6% of the total oil), 40 for MK (86.3% of the total oil), 42 for Ni localities (86.2% of the total oil), and 45 from the Ja locality (89.2% of the total oil). The total yields of oils

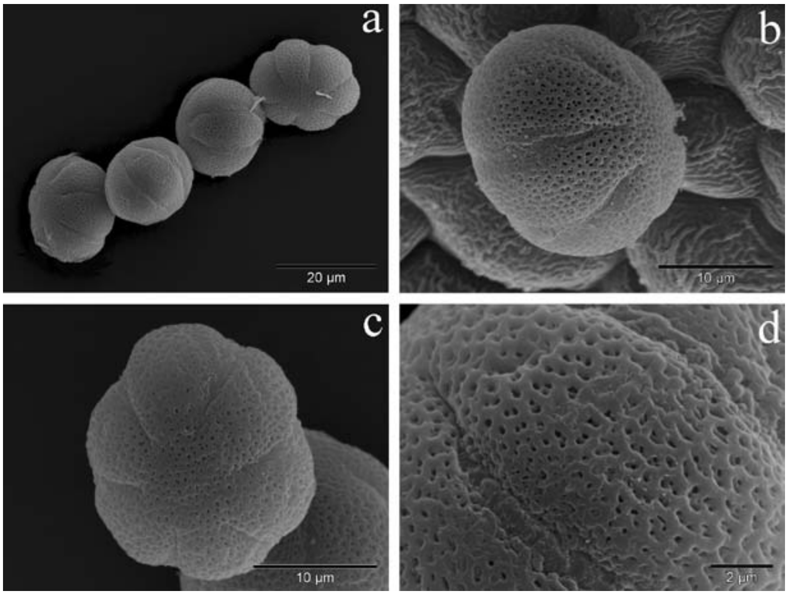

Figure 2. SEM micrographs of *M. longipedunculata* pollen after critical point drying. Equatorial (a, b) and polar (a, c) view; aperture and exine surface (d).

Component	R	CC	MK	Ni	Ja	Identification
Monoterpene hydrocarbons		**3.5**	**4.8**	**4.8**	**7.7**	
Camphene	962	-	0.2	-	0.1	RI, MS
β-Pinene	982	0.2	0.1	-	0.3	RI, MS, S
p-Cymene	1021	2.1	3.2	4.3	5.9	RI, MS
Limonene	1032	0.3	0.4	0.2	0.6	RI, MS, S
γ-Terpinene	1057	0.9	0.4	0.2	0.2	RI, MS
Linalool	1095	tr	0.5	0.1	0.6	RI, MS
Oxygenated monoterpenes		**22.3**	**28.2**	**24.2**	**31.8**	
β-Thujone	1121	-	0.1	-	-	RI, MS
trans-Pinocarveol	1147	-	0.2	0.4	0.6	RI, MS
Camphor	1151	-	-	-	0.1	RI, MS, S
Borneol	1176	0.3	0.8	0.1	0.8	RI, MS
Cymene-8-ol	1179	0.8	0.3	0.8	1.1	RI, MS
Terpinen-4-ol	1184	0.9	0.6	0.7	1.2	RI, MS
Myrtenol	1197	0.3	0.1	0.2	-	RI, MS
Verbenone	1204	0.2	0.1	0.2	0.9	RI, MS
Fenchyl acetate	1218	0.4	-	0.1	0.4	RI, MS
Pulegone	1234	1.9	1.8	2.3	6.9	RI, MS
Piperitone	1248	8.1	8.9	7.3	8.6	RI, MS
Linalyl acetate	1252	0.6	1.3	0.9	0.7	RI, MS
Bornyl acetate	1285	0.7	1.3	1.1	0.9	RI, MS
α-Terpenyl acetate	1349	0.4	0.6	0.4	0.7	RI, MS
Piperitone oxide	1364	7.7	12.1	9.7	8.9	RI, MS
Sesquiterpene hydrocarbons		**15.6**	**7.6**	**8.8**	**15.2**	
α-Cubebene	1345	0.5	0.2	0.2	0.3	RI, MS
α-Copaene	1377	0.8	-	0.1	0.4	RI, MS
β-Bourbonene	1383	0.6	0.7	0.3	0.6	RI, MS
α-Gurjunene	1407	0.8	-	0.2	0.3	RI, MS
E-Caryophyllene	1419	3.1	1.1	0.9	3.9	RI, MS, S
β-Copaene	1429	0.2	-	0.2	0.1	RI, MS
(Z)-β-Farnesene	1454	0.1	0.1	0.4	0.4	RI, MS
α-Humulene	1456	0.5	-	-	0.4	RI, MS
allo-Aromadendrene	1465	0.5	0.9	0.7	0.8	RI, MS
β-Chamigrene	1477	0.2	0.5	0.3	-	RI, MS
Germacrene D	1481	2.6	0.3	1.8	1.2	RI, MS
β-Bisabolene	1494	0.8	-	0.2	0.5	RI, MS
Bicyclogermacrene	1500	4.9	1.3	2.9	5.8	RI, MS
δ-Cadinene	1517	-	2.5	0.6	0.5	RI, MS

Table 3. Phytochemical composition (%) of essential oils of M. longipedunculata from Cijevna canyon (CC), Mount Krivošija (MK), Nikšić (Ni) and Jazina (Ja).

Note: R, retention time on capillary column VF5-ms; RI, identification using literature data [26]; MS, identification using database NIST02 and Wiley 7; S, identification by reference components; –, component is not identified; tr, traces (mean value below 0.1%).

Component	R	CC	MK	Ni	Ja	Identification
Oxygenated sesquiterpenes		**39.7**	**42.5**	**45.2**	**30.3**	
Spathulenol	1577	33.1	35.9	39.5	23.7	RI, MS
Caryophyllene oxide	1581	2.3	1.2	4.3	1.3	RI, MS, S
γ-Eudesmol	1632	0.6	1.2	0.9	tr	RI, MS
α-Cadinol	1655	0.4	1.6	0.8	0.6	RI, MS
α-Bisabolol	1688	1.7	2.4	1.4	2.9	RI, MS
Bisabolol oxide	1748	1.6	0.2	0.3	1.8	RI, MS
Phenolic compounds		**0.7**	**0.6**	**1.0**	**0.9**	
Carvacrol	1298	0.5	0.6	1.0	0.9	RI, MS, S
Eugenol	1370	0.2	-	-	tr	RI, MS, S
Hydrocarbons		**0.8**	**2.6**	**2.2**	**3.3**	
Docosane	2200	-	0.1	0.3	0.7	RI, MS, S
Tricosane	2300	0.2	0.3	0.5	0.8	RI, MS, S
Tetracosane	2400	0.1	0.4	0.9	0.5	RI, MS, S
Pentacosane	2500	0.2	0.3	0.2	0.7	RI, MS, S
Hexacosane	2600	0.2	1.5	-	0.6	RI, MS, S
Heptacosane	2700	0.1	-	0.3	-	RI, MS, S
Total identified (%)		**82.6**	**86.3**	**86.2**	**89.2**	
Yield (%)		**0.2**	**0.2**	**0.3**	**0.2**	

continued **Table 3.** Phytochemical composition (%) of essential oils of M. longipedunculata from Cijevna canyon (CC), Mount Krivošija (MK), Nikšić (Ni) and Jazina (Ja).

Note: R, retention time on capillary column VF5-ms; RI, identification using literature data [26]; MS, identification using database NIST02 and Wiley 7; S, identification by reference components; –, component is not identified; tr, traces (mean value below 0.1%).

were 0.2% for three localities (CC, MK and Ja) and 0.3% for Ni locality (Table 3). Slavkovska et al. [19] classified species *M. longipedunculata* by the amount of essential oils (<0.5) in sections of *Eumicromeria* together with *M. croatica, M. juliana* and *M. cristata.*

The components of all investigated oils are listed in order of their elution from the VF-5ms column and are classified on the basis of their chemical structures in seven classes (Table 3). The main fraction of *M. longipedunculata* from all localities was constituted by sesquiterpenes (45.5–55.3%). Among these, oxygenated sesquiterpenes (30.3–45.2%) prevailed on sesquiterpene hydrocarbons (7.6–15.6%). Our results showed that the major compound in all investigated essential oils was oxygenated sesquiterpene spathulenol (23.7–39.5%). Similarly to our results, spathulenol was also identified as the major compound in the oil of *M. longipedunculata* from Serbia and Montenegro [19,35].

Also, oxygenated monoterpenes piperitone oxide (7.7–12.1%) and piperitone (7.3–8.9%) were the important constituents of the all investigated essential oil of *M. longipedunculata.* According to Slavkovska *et al.* [19], these components are characteristic of *M. thymifolia.*

In the monoterpene hydrocarbons fraction *p*-cymene (2.1–5.9%) was predominant, while linalool was identified as the minor compound (Table 3). Also Slavkovska *et al.* [19] reported the occurrence of linalool as one of the dominant compounds in the composition of essential oil of *M. longipedunculata* from Crijevna canyon, but in the present study linalool was represented in trace amounts from the same locality (Table 3). Groups of phenolic compounds and hydrocarbons of investigated populations represented 1.5–4.2% of the total oils.

4. Conclusions

In this study micromorphological traits and essential oil content of *Micromeria longipedunculata*, an endemic Illyric-Balkan species were investigated. Micromorphological investigations revealed the presence of attenuate non-glandular trichomes, peltate trichomes and two types of glandular trichomes. Investigations of pollen showed that pollen of *M. longipedunculata* belongs to small pollen and has oblate-spheroidal shape with six apertures (hexacolpate, zonocolpate) and medium reticulate ornamentation. A qualitative comparison of the oil constituents of the four investigated populations of *M. longipedunculata* with those of other *Micromeria* species reported in the literature showed variance in composition. The qualitative composition in the essential oils investigated in four *M. longipedunculata* populations was always similar, with spathulenol, piperitone oxide and piperitone as the major compounds. The present study contributes to the knowledge about micromorphological traits and essential oil contents on the genus *Micromeria*, and especially on endemic *M. longipedunculata*.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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