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FATTY ACIDS OF SOME PLANTS OF THE GENUS *Calamintha*

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The genus *Calamintha* Miller (eng. *calamint*), accepted as a separate genus in this article, consists of 7 native species belonging to the Lamiaceae family, subfamily Nepetoideae and tribe Mentheae ("Saturejeae"). It is represented by 6 extremely polymorphic species in the area of the Balkan Peninsula [1, 2]. These are medium to large size erect herbaceous perennial or rarely annual plant species. Some of them (*C. glandulosa, C. vardarensis*) are robust, woody in the base, and have almost grey indumentums conspicuously rich in glandular hairs. The main stem produces several side-branches bearing verticillate blossoms and lilac, violet or almost white and spoted corollas. All parts of these plants, excluding the underground one, are strongly aromatic and possess a sweet and intrinsic scent (gr. *kalos-minthe*, meaning beautiful mint). Since ancient times the species of the *Calamintha* Miller genus has been traditionally used as an stomach tonic, and as an antiseptic, antipyretic, diaphoretic, expectorant, and sedative [3–6].

Different species of the genus *Calamintha s.l.* are native in meridional, submeridional, and temperate zones of Europe, Asia, and North Africa. Four species, *sensu* Silic, *C. sylvatica* Bromf., *C. grandiflora* (L.) Moench, *C. glandulosa* (Req.) Bentham, and *C. vardarensis* Silic are studied in this article. The essential oil of those species was previously investigated; the results showed that there are remarkable differences in the major constituents of their oil [7]. There is no previous phytochemical study in the literature concerning the chemical composition of the fatty acid.

Bearing in mind some new systematic studies, the total number of *Calamintha* species, as well as the taxonomical position of the same genus, is permanently fluctuating. According to the literature, the composition of essential oil, fatty acids, and the ratio of unsaturated/saturated fatty acids (U/S) could be useful as a chemotaxonomic indicator concerning the species of the genus. Since it was previously shown that the fatty acid composition of photosynthetic tissues can be a tool for chemotaxonomic studies, the aim of the present work was to study the possibility of using the fatty acid content from the herb of the genus *Calamintha* Miller as a taxonomic tool.

The plant material was collected in the blooming stage, from different localities of Serbia, Montenegro, and FYR Macedonia. Voucher specimens are deposited in the Herbarium collection of the Institute of Biology and Botanical Garden "Jevremovac", University of Belgrade (BEOU) (*C. glandulosa* BEOU No. 16014, *C. sylvatica* BEOU No. 16015, *C. grandiflora* BEOU No. 16131, and *C. vardarensis* BEOU No. 16140).

The plant material (100 g) was extracted by maceration in chloroform/methanol (2:1). After separation of solvents the residue was extracted four times more in the same way. The extracts were concentrated in a rotary evaporator at reduced pressure till a constant weight was achieved.

The chloroform extract and 12% ethanolic solution of NaOH were refluxed for 2 hours on the steam bath. Water (50 mL) was added to the reaction mixture and cooled to room temperature. The part of the extract that failed to react was separated by extraction with petroleum. The water–ethanolic solution of soaps was acidified with HCl (1:1) to pH 5–6 and extracted four times with 25 mL of petroleum. The combined organic phases were washed with a 10% sodium solution to pH 7. The yield of fatty acids after evaporation of the solvent under reduced pressure is shown in Table 1.

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TABLE 1. The Yield of Fatty Acids (%) and Fatty Acid Content in the Examined Species of the *Calamintha* Miller Genus

Acid	1 (0.53)	2 (0.63)	3 (0.31)	4 (0.30)	Acid	1 (0.53)	2 (0.63)	3 (0.31)	4 (0.30)
8:0	0.2	0.5	0.0	0.0	20:1	0.7	0.5	1.1	0.4
12:0	0.4	0.0	0.2	0.0	20:0	2.8	1.9	1.7	1.6
14:0	1.3	0.9	1.3	1.5	21:0	0.8	1.2	0.5	0.4
15:0	0.3	0.0	0.5	0.5	22;1	0.3	0.0	0.3	0.0
16:1 (7)	0.0	0.0	0.9	0.0	22:0	2.1	2.0	2.5	1.0
16:1 (9)	0.5	0.7	0.2	0.8	23:0	0.7	0.5	0.6	0.0
16:0	27.2	28.7	26.2	32.5	24:0	0.0	0.9	1.1	0.4
iso-17:0	0.3	0.0	0.5	0.3	25:0	0.0	1.1	0.2	0.3
17:0	0.7	0.7	0.6	0.9	26:0	0.4	0.3	0.9	0.0
18:2	17.1	13.0	13.8	15.6	Total, %	88.2	85.9	94.5	92.3
18:1	24.2	28.9	37.2	31.5	$\Sigma_{Unsat.}$	44.8	45.0	54.0	48.6
18:0	4.4	3.1	3.3	3.9	$\Sigma_{Sat.}$	43.8	40.9	40.5	43.7
19:0	0.2	0.5	0.2	0.5	U/S	1.0	1.1	1.3	1.1
18:3	1.9	1.9	0.5	0.2					

1 – *C. glandulosa* (Sutorine, Igalo); 2 – *C. sylvatica* (Bojanine Vode, Nis); 3 – *C. grandiflora* (Stojkova kuca, Sar-planina); 4 – *C. vardarensis* (Pestani, Galicica, Makedonija).
Data shown are relative area percentage, calculated from a flame ionization detector.

The fatty acids (0.3 g) and 1% methanolic solution of H_2SO_4 (38 mL) were refluxed for 1.5 hours on the steam bath. The reaction mixture was evaporated under reduced pressure. Water (30 mL) was added to the residue and the reaction mixture was extracted three times with 2% $NaHCO_3$ solution, then with water to pH 7. The organic phase was dried over anhydrous sodium sulfate and concentrated to 5 mL under reduced pressure.

The fatty acid methyl ester analysis was performed using GC and GC/MS. The constituents of the fatty acids were identified by comparison of their mass spectra with those from the MS library (Wiley 275.l) using a computer search and literature. For the purpose of the quantitative analysis the area percentage obtained by FID was used as the base.

The GC analysis of the methyl esters was carried out on a GC HP-5890 II apparatus, equipped with a split-splitless injector, attached to an HP-5 column (25 m × 0.32 mm, 0.52 μm film thickness) and fitted to FID. The carrier gas flow rate (H_2) was 1 mL/min, the split ratio 1:30, the injector temperature 250°C, and the detector temperature 300°C, while the column temperature was linearly programmed from 40–240°C (at the rate of 4°C/min). The same analytical conditions were employed for GC/MS analysis, where an HP G 1800C Series II GCD system was used. The transfer line was heated at 260°C. The mass spectra were acquired in the EI mode (70 eV), in the *m/z* range 40–400 (column HP-5MS 30 m × 0.25 mm, 0.25 μm film thickness). The results of the fatty acid analysis of the examined *Calamintha* species by GC and GC/MS are shown in Table 1.

Oleic (28.9, 37.2, 24.2, 31.5%), palmitic (28.7, 26.2, 27.2, 32.5%), and linoleic (13.0, 13.8, 17.1, 15.6%) acids have been the main constituents in the chloroform–methanolic extract of *C. sylvatica*, *C. grandiflora*, *C. glandulosa*, and *C. vardarensis*. The U/S index (unsaturated/saturated) is 1.1 for the *C. sylvatica*, 1.3 for *C. grandiflora,* 1.0 for *C. glandulosa*, and 1.1 for *C. vardarensis*. The average U/S value for the analyzed herb of *Micromeria* and *Satureja* genus (Lamiaceae) was 2.4 and 1.5, respectively [8].

Obviously, the taxonomy of the majority of the *Calamintha* species, as well as other closely related *Acinos*, *Clinopodium*, *Micromeria*, and *Satureja s.l.,* is still not clear enough. The aim of this work is to provide a more stable taxonomical position and more information on the differences between the chemotaxonomy of these taxa.

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