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# Volatile Constituents of *Calamintha origanifolia* Boiss. Growing Wild in Lebanon

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The essential oil of aerial parts of *Calamintha origanifolia* Boiss. (Lamiaceae), growing wild in Lebanon, was obtained by hydrodistillation and was analysed by GC and GC-MS. 49 compounds, representing 92.2% of the oil, were identified. The major components, belonging to the class of oxygenated monoterpenes, were pulegone (22.5%), isomenthone (12.2%) and piperitenone (9.6%). The oil showed a slight antimicrobial activity against three bacterial strains.

**Keywords:** *Calamintha origanifolia*, essential oil, GC-MS, oxygenated monoterpenes, pulegone, isomenthone, piperitenone.

*Calamintha* (syn. *Cyclotrichium*) is a genus of about thirty species that belongs to the tribe Mentheae, subfamily Nepetoideae, family Lamiaceae. It is native to the northern temperate regions of Europe and Asia. According to Marin *et al.* [1], the genus *Calamintha* Miller is closely related to *Micromeria* Benth, *Satureja* L., *Clinopodium* L. and *Acinos* Miller, and for this reason the use of chemotaxonomic markers is essential to better differentiate these genera.

Many *Calamintha* species are used as spices in various culinary recipes because of their pleasant mint-like smell. Besides, they are known for different medicinal uses. Common calamint is used as diaphoretic, in syrups for coughs and colds and as an expectorant. The tea is used to help with gas and colic [2]. Externally, it is useful in poultices for bruises and as a strengthener and nerve soother. The essential oil shows different activities. The oil of *C. sylvatica* subsp. *ascendens* exerts significant sedating and antipyretic activities in the rat, due to the presence of the monoterpenes pulegone, menthone and eucalyptol [3]. Monoterpenes, particularly pulegone and isopulegone, are also reported to be the responsible of the strong antibacterial and antifungal activities showed by essential oils from different *Calamintha* species [4]. Due to its good antimicrobial activity, *C. officinalis* essential oil has been proved to be effective as preservative in two current formulations (cream and shampoo) [5].

*Calamintha origanifolia* Boiss. (syn. *Cyclotrichium origanifolium* (Labill.) Manden & Scheng.) is a strongly aromatic, suffruticose, much branched species wild growing in the Horsh Ehden reserve that is located on the northern part of the Lebanese western mountain range, just below Cornet As Sawda, the highest mountain peak in Lebanon. The Reserve represents a mountainous ecosystem on the elevated slopes of the northern Mt. Lebanon chain. In this paper, as a continuation of our studies on the essential oils from Lamiaceae growing wild in Lebanon [6], we report on the chemical composition of the essential oil of *Calamintha origanifolia* collected in the Lebanese Horsh Ehden reserve.

**Table 1**: Essential oil composition of *Calamintha origanifolia* Boiss.

| I[a] | I[b] | Component | Method[c] | %[d] |
|---|---|---|---|---|
| 798 | | Hexanal | *I*,MS | 0.1 |
| 930 | | α-Thujene | *I*,MS | t |
| 963 | 1543 | Benzaldehyde | *I*,MS,Co-GC | 0.3 |
| 973 | 1132 | Sabinene | *I*,MS | 0.1 |
| 980 | 1118 | β-Pinene | *I*,MS,Co-GC | 0.3 |
| 1025 | 1280 | *p*-Cymene | *I*,MS,Co-GC | 0.2 |
| 1030 | 1203 | Limonene | *I*,MS,Co-GC | 0.6 |
| 1034 | 1213 | 1,8-Cineole | *I*,MS,Co-GC | 0.6 |
| 1111 | | *p*-Mentha-1,3,8-triene | *I*,MS | 0.7 |
| 1117 | 1152 | *trans*-*p*-Menth-2-en-1-ol | *I*,MS | 0.9 |
| 1125 | 1540 | Chrysanthenone | *I*,MS | 0.9 |
| 1138 | 1475 | Menthone | *I*,MS,Co-GC | 7.7 |
| 1145 | 1663 | *cis*-Verbenol | *I*,MS | 1.9 |
| 1163 | 1502 | Isomenthone | *I*,MS | 12.2 |
| 1175 | 1582 | Isopulegone[#] | *I*,MS | 5.8 |
| 1177 | 1755 | Dihydrocarveol | *I*,MS | 0.1 |
| 1182 | 1652 | Menthol | *I*,MS,Co-GC | 0.7 |
| 1233 | 1662 | Pulegone | *I*,MS,Co-GC | 22.5 |
| 1244 | 1750 | Carvone | *I*,MS | 1.5 |
| 1293 | 2198 | Thymol | *I*,MS,Co-GC | 0.8 |
| 1299 | 2239 | Carvacrol | *I*,MS,Co-GC | 1.1 |
| 1329 | 1949 | Piperitenone | *I*,MS | 9.6 |
| 1343 | 1748 | Piperitone | *I*,MS | 6.9 |
| 1353 | 2186 | Eugenol | *I*,MS,Co-GC | 0.2 |
| 1363 | | Piperitenone oxide | *I*,MS | 0.7 |
| 1372 | 1493 | α-Ylangene | *I*,MS | 0.2 |
| 1377 | 1497 | α-Copaene | *I*,MS | 0.1 |
| 1382 | | β Cubebene | *I*,MS | t |
| 1385 | 1535 | β-Bourbonene | *I*,MS | 0.1 |
| 1387 | 1600 | β-Elemene | *I*,MS | 0.2 |
| 1415 | 1612 | Caryophyllene | *I*,MS,Co-GC | 1.9 |
| 1451 | 1868 | Geranyl acetone | *I*,MS | 0.3 |
| 1452 | 1673 | (*E*)-β-Farnesene | *I*,MS | t |
| 1455 | 1689 | α-Humulene | *I*,MS | 0.1 |
| 1477 | 1726 | Germacrene D | *I*,MS | 0.1 |
| 1515 | 1776 | γ-Cadinene | *I*,MS | t |
| 1520 | 1839 | *cis*-Calamenene | *I*,MS | 0.2 |
| 1526 | | δ-Cadinene | *I*,MS | t |
| 1640 | 2187 | τ-Cadinol | *I*,MS | 4.0 |
| 1642 | 2209 | τ-Muurolol | *I*,MS | 0.7 |
| 1649 | 2255 | α-Cadinol | *I*,MS | 1.2 |
| 1835 | 2131 | Hexahydrofarnesylacetone | *I*,MS | 1.6 |
| 1957 | 2931 | Hexadecanoic acid | *I*,MS,Co-GC | 1.4 |
| 2500 | 2500 | Pentacosane | *I*,MS | 0.3 |
| 2600 | 2600 | Hexacosane | *I*,MS | 0.2 |
| 2700 | 2700 | Heptacosane | *I*,MS | 1.0 |
| 2800 | 2800 | Octacosane | *I*,MS | 0.1 |
| 2900 | 2900 | Nonacosane | *I*,MS | 1.3 |
| 3100 | 3100 | Hentriacontane | *I*,MS | 0.8 |
| | | **Total identified** | | **92.2** |

[a]: HP-5 MS column; [b]: HP Innowax; [c]: *I* is the retention index, MS = mass spectrum, Co-GC = co-injection with authentic compound; [d]: t = trace, less than 0.05%; [#]: correct isomer not identified.

Great variations occur in the volatile compounds from *Calamintha* genus, but the major components in the oils generally belong to the C-3 oxygenated *p*-menthanes such as pulegone, isomenthone, menthone, piperitone and piperitenone with their oxides [4a,4c-4f,5,7-9]. According to Baldovini *et al.* [8], three types of oils can be distinguished: in the first pulegone is the major component, associated with different compounds such as menthone and/or isomenthone, menthol and its isomers, piperitenone, piperitone and piperitenone oxides. The second type is characterized by the predominance of piperitone oxide and/or piperitenone oxide. Last type is distinguished by the presence of carvone and 1,8-cineole as main components [8 and references cited therein].

**Table 2**: Antimicronial activity of *Calamintha origanifolia* oil (**C**).

| Strain | MIC (MBC)µg/mL | |
|---|---|---|
| | **C** | **Ch** |
| *Bacillus subtilis* ATCC 6633 | 50 (100) | 12.5 |
| *Staphylococcus aureus* ATCC 25923 | 100 (>100) | 25 |
| *Staphylococcus epidermidis* ATCC 12228 | 25 (50) | 3.12 |
| *Streptococcus faecalis* ATCC 29212 | 100 | 25 |
| *Escherichia coli* ATCC 25922 | 50 (100) | 12.5 |
| *Klebsiella pneumoniae* ATCC 10031 | 100 | 50 |
| *Proteus vulgaris* ATCC 13315 | 100 (>100) | 25 |
| *Pseudomonas aeuriginosa* ATCC 27853 | >100 | 100 |

Ch: Chloramphenicol

The essential oil of *C. origanifolia* belongs to the first type, as pulegone (22.5%) is the most abundant component. In total, forty-nine constituents have been identified; representing 92.2% of the total oil; their retention indices and percentage composition are given in Table 1, where the components are listed in order of elution from a HP 5MS column. As reported in the literature for other *Calamintha* species [9], the oxygenated monoterpenes were the most abundant components of the oil, particularly those with *p*-menthane skeleton, and their content represented 59.7% of the oil. The most abundant compounds of this fraction were pulegone (22.5%), isomenthone (12.2%) and piperitenone (9.6%). The high content of isomenthone can be considered a characteristic of the present oil because this compound is reported in lower amounts in other *Calamintha* oils. Isomenthone was detected in a quite similar extent only in the oils of *C. grandiflora* (15.2%) [9b] and *C. sylvatica* ssp. *sylvatica* in the pre-blossom phase (13.4%) [9e]. The greatest amount of isomenthone was detected in the oil of *C. sylvatica* ssp. *ascendens* (36.8-43.3%) [9f]. Other ketones identified in the oil were chrysanthenone (0.9%), geranyl acetone (0.3%) and hexahydrofarnesyl acetone (1.6%). Also a few monoterpene hydrocarbons were present but they represented only 1.9% of the oil, ranging between 0.7% (*p*-mentha-1,3,8-triene) and traces (α-thujene). Twelve sesquiterpene hydrocarbons were detected. Caryophyllene represented the 1.9% of the oil whereas the other sesquiterpene hydrocarbons were present in low content, from traces to 0.2%. Three oxygen-containing sesquiterpenes were present and τ-cadinol (4.0%) was the major component of this fraction. In the oil were also identified three phenols that amounted to the 2.1%. Carvacrol (1.1%) and thymol (0.8%) were the most abundant while eugenol represented the 0.2% of the oil. Data obtained allow us to ascribe the oil of *Calamintha origanifolia* Boiss. growing wild in Lebanon to a type pulegone/isomenthone oil.

The MIC and MBC values of the essential oil against eight selected micro-organisms are reported in Table 2. The oil showed action mainly against *B. subtilis*, *S. epidermidis* and *E. coli*.

## Experimental

***Plant material:*** Aerial parts of *C. origanifolia* Boiss were collected at the full flowering stage from plants growing wild on rocky soil at Oyoun Ouvghanch, 2200 m a.s.l., in June 2005. The required authorizations for the plant collection were given by the Lebanese authorities to Apostolides Arnold. A voucher specimen (leg. & det. N. Arnold s. n., confirm. Th. Raus) was deposited in the Herbarium of the Botanischer Garten, Berlin Universität.

***Essential oil isolation:*** The oil from air-dried and ground aerial parts of plants was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in the *European Pharmacopoeia* [10]

The oil was dried over anhydrous sodium sulphate and stored under $N_2$ at +4°C in the dark until tested and analysed. The sample yielded 0.13% of yellow oil (w/w), with a pleasant smell of mint.

***GC analysis:*** Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with a HP-5 MS capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness). Helium was the carrier gas (1 mL $min^{-1}$). Column temperature was initially kept at 40°C for 5 min, then gradually increased to 250°C at 2°C $min^{-1}$, held for 15 min and finally raised to 270°C at 10°C $min^{-1}$. Diluted samples (1/100 v/v, in *n*-hexane) of 1 µL were injected manually at 250°C, and in the splitless mode. Flame ionization detection (FID) was performed at 280°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m x 0.20 mm i.d.; 0.20 µm film thickness).

***GC-MS analysis:*** GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica HP-1 capillary column (30 m x 0.25 mm i.d.; 0.33 µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. Gas chromatographic conditions were as reported above; transfer line temperature, 295°C.

***Qualitative and quantitative analyses:*** Most constituents were identified by gas chromatography by comparison of their retention indices (*I*) with either those of the literature [11,12] or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes ($C_8$-$C_{24}$) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature [11,13] and our home made library. Component relative concentrations were calculated based on GC peak areas without using correction factors.

***Antimicrobial activity:*** The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth dilution method as previously described [6e]. Eight bacteria species, selected as representative of the class of Gram positive and Gram negative, were tested: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (ATCC 13315) and *Pseudomonas aeruginosa* (ATCC 27853).

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## References

[1] Marin PD, Grayera RJ, Veitcha NC, Kitea GC, Harborne JB. (**2001**) Acacetin glycosides as taxonomic markers in *Calamintha* and *Micromeria*. *Phytochemistry*, ***58***, 943-947.

[2] (a) Chevallier A. (**1996**) *The Encyclopedia of Medicinal Plants.* Dorling Kindersley, London; (b) Wren RC. (**1988**) *Potter's New Cyclopedia of Botanical Drugs and Preparation*. C.W. Daniel Company United, Saffron Walden, UK, 55.

[3] Ortiz de Urbina AV, Martin ML, Montero MJ, Moran A, San Roman L. (**1989**) Sedative and antipyretic activity of essential oil of *Calamintha sylvatica* subsp. *ascendens*. *Journal of Ethnopharmacology*, ***25***, 165-171.

[4] Castilho P, Liu K, Rodrigues AI, Feio S, Tomi F, Casanova J. (**2007**) Composition and antimicrobial activity of the essential oil of *Clinopodium ascendens* (Jordan) Sampaio from Madeira. *Flavour and Fragrance Journal*, **22**, 139-144; (b) Miladinovic D. (**2005**) Antimicrobial activity of some medicinal plants from Serbia. *Farmatsiya*, ***52***, 46-49; (c) Kitic D, Stojanovic G, Palic R, Randjelovic V. (**2005**) Chemical composition and microbial activity of the essential oil of *Calamintha nepeta* (L.) Savi ssp. *nepeta* var. *subisodonda* (Borb.) Hayek from Serbia. *Journal of Essential Oil Research*, ***17***, 701-703; (d) Flamini G, Cioni PL, Puleio R, Morelli I, Panizzi L. (**1999**) Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. *Phytotherapy Research*, ***13***, 349-351; (e) Petrucci S, Macianti F, Cioni PL, Flamini G, Morelli I, Macchioni G. (**1994**) *In vitro* antifungal activity of essential oil against some isolated *Microsporium canis* and *Microsporium gypseum*. *Planta Medica*, ***60***, 184-187; (f) Panizzi L, Flamini G, Cioni PL, Morelli I. (**1993**) Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. *Journal of Ethnopharmacology*, ***39***, 167-170.

[5] Nostro A, Cannatelli MA, Morelli I, Musolino AD, Scuderi F, Pizzimenti F, Alonzo V. (**2004**) Efficiency of *Calamintha officinalis* essential oil as preservative in two topical product types. *Journal of Applied Microbiology*, ***97***, 395-401.

[6] (a) Senatore F. Apostolides Arnold N, Piozzi F. (**2004**) Chemical composition of the essential oil of *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss. growing wild in Lebanon. *Journal of Chromatography A*, ***1052***, 237-240; (b) Senatore F. Formisano C, Apostolides Arnold N, Piozzi F. (**2005**) Essential oils from *Salvia* sp. (Lamiaceae). III. Composition and antimicrobial activity of the essential oil of *Salvia palaestina* Benth. growing wild in Lebanon. *Journal of Essential Oil Research*, ***17***, 419-421; (c) Senatore F, Apostolides Arnold N, Piozzi F. (**2005**) Composition of the essential oil of *Nepeta curviflora* Boiss. (Lamiaceae) from Lebanon. *Journal of Essential Oil Research*, ***17***, 268-270; (d) Grassia A, Senatore F, Apostolides Arnold N, Bruno M. Piozzi F, Rigano D, Formisano C. (**2006**) Chemical composition and antimicrobial activity of the essential oils from aerial parts of two *Marrubium* sp. (Lamiaceae) growing wild in Lebanon. *Polish Journal of Chemistry*, ***80***, 623-628; (e) Senatore F, Apostolides Arnold N, Piozzi F, Formisano C. (**2006)** Chemical composition of the essential oil of *Salvia microstegia* Boiss. et Balansa growing wild in Lebanon. *Journal of Chromatography A*, ***1108***, 276-278.

[7] (a) Adzet T, Passet J. (**1972**) Chemotaxonomy of the *Satureia calamintha* genus. *Rivista Italiana Essenze, Profumi, Piante Officinali, Aromi, Saponi, Cosmetici, Aerosol*, ***54***, 482-486; (b) De Pooter HL, De Buyck LF, Schamp NM. (**1986**) The volatiles of *Calamintha nepeta* subsp. *glandulosa*. *Phytochemistry*, ***25***, 691-694; (c) Souleles C, Argyriadou N, Philianos S. (**1987**) Constituents of the essential oil of *Calamintha nepeta*. *Journal of Natural Products*, ***50***, 510-511; (d) Sarer E, Pancali SS. (**1998**) Composition of the essential oil from *Calamintha nepeta* (L.) Savi ssp. *glandulosa* (Req.) P. W. Ball. *Flavour and Fragrance Journal*, ***13***, 31-32; (e) Kokkalou E, Stefanou E. (**1990**) The volatile oil of *Calamintha nepeta* (L.) Savi Subsp. *glandulosa* (Req.) P. W. Ball, endemic to Greece. *Flavour and Fragrance Journal*, ***5***, 23-26 ; (f) Akgül A, De Pooter HL, De Buyck LF. (**1991**) The essential oils of *Calamintha nepeta* subsp. *glandulosa* and *Ziziphora clinopodioides* from Turkey. *Journal of Essential Oil Research*, ***3***, 7-10; (g) Kirimer N, Başer KHC, Özek T, Kurkcuoglu M. (**1992**) Composition of the essential oil of *Calamintha nepeta* subsp. *glandulosa*. *Journal of Essential Oil Research*, ***4***, 189-190; (h) Velasco-Negueruela A, Perez-Alonso MJ, Esteban JL, Garcia Vallejo MC, Zygadlo JA, Guzman CA, Ariza-Espinar L. (**1996**) Essential oils of *Calamintha nepeta* (L.) Savi and *Mentha suaveolens* Ehrh., grown in Cordoba, Argentina. *Journal of Essential Oil Research*, ***8***, 81-84; (i) Fraternale D, Giamperi L, Ricci D, Manunta A. (**1998**) Composition of essential oil as a taxonomic marker for *Calamintha nepeta* (L.) Savi ssp. *nepeta*. *Journal of Essential Oil Research*, ***10***, 568-570; (j) Mastelič J, Milos M, Kustrak D, Radonic A. (**1998**) The essential oil and glycosidically bound volatile compounds of *Calamintha nepeta* (L.) Savi. *Croatica Chemica Acta*, ***71***, 147-154.

[8] Baldovini N, Ristorcelli D, Tomi F, Casanova J. (**2000**) Infraspecific variabilità of the essential oil of *Calamintha nepeta* from Corsica (France). *Fragrance Journal*, ***15***, 50-54.

[9] (a) De Pooter HL, Goetghebeur P, Schamp N. (**1987**) Variability in composition of the essential oil of *Calamintha nepeta*. *Phytochemistry*, ***26***, 3355-3356; (b) Souleles C, Argyriadou N. (**1990**) The volatile constituents of *Calamintha grandiflora*. *Planta Medica*, ***56***, 234-235; (c) Tucker A, Maciarello MJ. (**1991**) The essential oil of *Calaminta arkansana* (Nuttl.) Shinners. *Journal of Essential Oil Research*, ***3***, 125-126; (d) Baser KHC, Özek T, Kurkcuoglu M, Tümen G, Duman H. (**1997**) Essential oils of *Calaminta pamphylica* Boiss. et Heldr. subsp. *pamphylica* and subsp. *davisii* (Quezel et Contandr.) Davis. *Journal of Essential Oil Research*, ***9***, 371-373; (e) Kitic D, Palic R, Ristic M, Sojanovic G, Jovanovic T. (**2001**) The volatile constituents of *Calamintha sylvatica* Bromf. subsp. *sylvatica*. *Flavour and Fragrance Journal*, ***16***, 257-258; (f) Hidalgo PJ, Ubera JL, Santos JA, LaFont F, Castellanos C, Palomino A, Roman M. (**2002**) Essential oils in *Calamintha sylvatica* Bromf. ssp. *ascendens* (Jordan) P.W. Ball: Wild and cultivated productions and antifungal activity. *Journal of Essential Oil Research, **14***, 68-71; (g) Nickavar B, Mojab F. (**2005**) Hydrodistilled volatile constituents of *Calamintha officinalis* Moench. from Iran. *Journal of Essential Oil Bearing Plants*, ***8***, 23-27.

[10] *European Pharmacopoeia*, 5th edition. (**2004**) Council of Europe: Strasbourg Cedex, France 2.8.12, 217-218.

[11] Jennings W, Shibamoto T. (**1980**) *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography.* Academic Press, New York.

[12] Davies NW. (**1990**) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *Journal of Chromatography*, ***503***, 1-24.

[13] Adams RP. (**2001**) *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy.* Allured Publishing, Carol Stream IL.