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# GC and GC-–MS Analysis of Volatile Compounds From *Ballota nigra* subsp. *uncinata* Collected in Aeolian Islands, Sicily (Southern Italy)

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## Abstract

In the present study, the chemical composition of the essential oils from aerial parts of *Ballota nigra* subsp. *uncinata* (Bég.) Patzak collected in Sicily was evaluated by gas chromatography (GC) and GC-mass spectrometry. The main components of the oil were (*E*)-phytol (20.0%), α-pinene (9.0%), hexahydrofarnesyl acetone (5.7%), and α-selinene (5.1%). Cluster analysis of the essential oil compositions of all the taxa belonging to *B. nigra* s.l. group was performed.



## Keywords

*Ballota nigra* subsp. *uncinata*, Lamiaceae, Stachydeae, essential oil, (*E*)-Phytol

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*Ballota* L. (Lamiaceae) is a genus belonging to the tribe Stachydeae, subtribe Ballotae. The Plant List, which has been used to validate the scientific names of the species, includes more than 160 scientific plant names of species rank for the genus *Ballota*. Of these, only 30 are accepted species names. They are native to Macaronesia, Europe, Mediterranean to West Asia, Mauritania, Chad, and South Africa. *Ballota* species are perennial herbs characterized by flowers held in verticillasters and by an unpleasant aromatic foliage.[1]

Lamiaceae taxa have attracted interest by the researcher for the valuable biological properties of their extracts, such as antibacterial, antioxidant, and hypoglycemia.[2] Furthermore, Lamiaceae essential oils have shown a promising anti-inflammatory potential[3] and are widely used in aromatherapy for several minor clinical uses.[4] In this context, *Ballota* species have been used in folk medicine as antiulcer, antispasmodic, diuretic, choleretic, antihemorrhoidal, and sedative agents.[5] The antimicrobial activities[6-8] and the antioxidant activities[9] of *Ballota* species were recently reported as well as the antifungal activities of some flavonoids isolated from 2 species.[10,11] The water extract has been reported to have antinociceptive, antiinflammatory, and hepatoprotective activities.[12] In Europe, the polar extracts of the flowering aerial parts of *Ballota* are commonly used due to their neurosedative activity.[13,14] More recently, the general antioxidant activity,[15] the in vitro inhibition of LDL (low-density lipoprotein) peroxidation[16] and the antibacterial activity[17,18] of these plants have been published. Phytochemical investigations showed that labdane diterpenoids, flavonoids, and phenylpropanoids are the characteristic features of the genus, and recently the occurrence of non-volatile and volatile metabolites, the ethnopharmacological uses, and the biological properties of all the studied taxa of *Ballota* have been reviewed.[19]

As a continuation of our researches on *Ballota* species,[20-22] we decided to investigate the chemical composition of the essential oil *B. nigra* subsp. *uncinata*, collected in Sicily.

*Ballota nigra* subsp. *uncinata* (Bég.) Patzak (syn. *Ballota nigra* subsp. *ruderalis* (Sw.) Briq.) is present in the western part of North Africa, Southern Europe, Turkey, and Mediterranean Middle East.[23] Previous investigations on this taxon reported

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the occurrence of only 1 labdane diterpenoid, dehydrohispanolone,[24,25] the chemical composition of the essential oil of Turkish accession,[26] and its antilisterial activity against *L. monocytogenes*.[27]

## Results and Discussion

Hydrodistillation of *B. nigra* subsp. *uncinata* (**B.n.u.**) aerial parts gave a pale yellow oil. Overall, 49 compounds have been identified in **B.n.u.**, representing 96.6% of the total components. The components are listed in Table 1 according to their retention indices on an HP 5 MS column and are classified on the basis of their chemical structures into 9 classes.

The main constituents of **B.n.u**. were found to be (*E*)-phytol (20.0%), *a*-pinene (9.0%), hexahydrofarnesyl acetone (5.7%), *a*-selinene (5.1%), 1-undecene (5.0%), and (*Z*)-caryophyllene (4.2%). Sesquiterpenes constituted the most abundant fraction of the oil (39.9%), with a prevalence of sesquiterpene hydrocarbons (30.7%) among which *a*-selinene (5.1%), (*Z*)-caryophyllene (4.2%), and germacrene D (3.7%) predominated. Among the 7 oxygen-containing sesquiterpenes (9.2%), viridiflorol (2.8%) was the most abundant. With regard to monoterpenes, 6 oxygen-containing monoterpenes accounted for 3.5% of the total oil and monoterpene hydrocarbon represented 12.9% with *a*-pinene (9.0%) as the main compound. Also, diterpene fraction was noteworthy (21.5%), with (*E*)-phytol (20.0%) being the main compound of the class and the oil. Hexahydrofarnesyl acetone (5.7%) and 1-undecene (5.0%) were, instead, the principal products among the carbonylic compounds (8.2%) and the hydrocarbons (7.7%), respectively.

Table 2 reports the main compounds of the essential oils of the different taxa of *B. nigra* sl. group studied so far.

Considering the compounds occurring in the oils with an abundance of more than 3% only, the comparison of our data with those reported in the literature (Table 2) allows to point out some interesting considerations.

Almost all the *B. nigra* species contain the sesquiterpenes caryophyllene and/or caryophyllene oxide among the main compounds with the exception of 2 cases: *B. nigra* 3 collected in Iran[30] and *B. nigra* subsp. *anatolica* 2 collected in Turkey.[26]

The *B. nigra* subsp. *uncinata*, we examined, contains on the contrary to the previously investigated *B. nigra* subsp. *uncinata*, also a relevant amount (9%) of monoterpene *a*-pinene exclusively contained in the *B. nigra* 3 collected in Iran.[30] A cluster statistical analysis (Figure 1) of these data, based on a comparison of the species according to the relative amounts of compounds, shows a certain resemblance of our sample with *B. nigra* subsp. *foetida* 4 collected in Serbia.[36] In fact, both species, apart from caryophyllene and germacrene D, are the only ones containing the diterpene phytol as the most abundant compound.

## Experimental

### *Plant Material*

Aerial parts (flowers, stems, and leaves) of *B. nigra* subsp. *uncinata* (Bég.) Patzak (**B.n.u**.) were collected at Lipari (38°29'51.652"N, 14°57'1.519"E), Aeolian Islands, Sicily (Italy), at the beginning of June 2018. The authentication was carried out by Mr Emanuele Schimmenti, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy. A voucher specimen (PAL MB-2018/86) was deposited in Department STEBICEF, University of Palermo.

### *Isolation of the Essential Oil*

The air-dried samples (200 g) were ground in a Waring blender and then subjected to a single hydrodistillation for 3 hours using *n*-hexane as the solvent, according to the standard procedure previously described.[39] The extracts were dried over anhydrous sodium sulfate and then stored in sealed vials, at −20°C, ready for the gas chromatography (GC) and GC-mass spectrometry (MS) analyses. The samples yielded 0.016% (**B.n.u**.) of yellow oils (w/w).

### *Gas Chromatography–Mass Spectrometry*

Analytical GC was carried out on a Perkin-Elmer Sigma 115 GC fitted with an HP-5 MS capillary column (30 m × 0.25 mm), 0.25 μm film thickness. Column temperature was initially kept at 40°C for 5 minutes, then gradually increased to 250°C at 2°C/min rate, held for 15 minutes and finally raised to 270°C at 10°C/min. Diluted samples (1/100 v/v, in *n*-pentane) of 1 μL were injected at 250°C, manually, and in the splitless mode. Flame ionization detection (FID) was performed at 280°C. GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica DB-5 capillary column (30 m × 0.25 mm), 0.33 μm film thickness, coupled to an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 eV; electron multiplier energy 2000 V. GC conditions were as given; transfer line temperature, 295°C. Analysis was also run by using a fused silica HP Innowax polyethyleneglycol capillary column (50 m × 0.20 mm, 0.20 μm film thickness). In both cases, helium was used as carrier gas (1 mL/min). Identification of compounds was carried out using NIST 11, Wiley 9, FFNSC 2, and Adams databases.[40] These identifications were confirmed by linear retention indices with those available in literature by the SciFinder database. Some of the compounds were also confirmed by comparison of mass spectra and retention times with standard compounds available in the laboratory. The retention indices were determined in relation to a homologous series of *n*-alkanes (C8–C30) injected under the same

**Table 1.** Percent Composition of the Essential Oils of Aerial Parts of *Ballota nigra* subsp. *uncinata* Arranged by Class.

| $K_i$[a] | $K_i$[b] | Component | B.n.u.[c] | Ident.[d] |
|---|---|---|---|---|
| 930 | 1014 | *α*-Thujene | 1.7 | 1, 2 |
| 936 | 1075 | α-Pinene | 9.0 | 1, 2, 3 |
| 973 | 1132 | Sabinene | 0.6 | 1, 2 |
| 978 | 1118 | *β*-Pinene | 1.1 | 1, 2, 3 |
| 1030 | 1203 | Limonene | 0.5 | 1, 2, 3 |
| | | Monterpene hydrocarbons | 12.9 | |
| 1098 | 1553 | Linalool | 0.8 | 1, 2, 3 |
| 1143 | 1532 | Camphor | 0.4 | 1, 2, 3 |
| 1167 | 1719 | Borneol | 0.9 | 1, 2, 3 |
| 1176 | 1611 | Terpinen-4-ol | 0.4 | 1, 2, 3 |
| 1187 | 1706 | *α*-Terpineol | 0.6 | 1, 2, 3 |
| 1262 | 1583 | *cis*-Chrysanthenyl acetate | 0.4 | 1, 2 |
| | | Oxygenated monoterpenes | 3.5 | |
| 1363 | 1492 | Cyclosativene | 2.1 | 1, 2 |
| 1377 | 1497 | *α*-Copaene | 1.8 | 1, 2 |
| 1385 | 1535 | *β*-Bourbonene | 0.7 | 1, 2 |
| 1404 | 1666 | (*Z*)-Caryophyllene | 4.2 | 1, 2, 3 |
| 1407 | 1538 | *α*-Gurjunene | 0.5 | 1, 2 |
| 1418 | 1612 | (*E*)-Caryophyllene | 2.2 | 1, 2, 3 |
| 1437 | 1628 | Aromadendrene | 2.6 | 1, 2 |
| 1463 | 1661 | *allo*-Aromadendrene | 0.6 | 1, 2 |
| 1475 | 1715 | *β*-Selinene | 2.0 | 1, 2 |
| 1477 | 1726 | Germacrene D | 3.7 | 1 ,2 |
| 1479 | 1698 | *δ*-Selinene | 0.8 | 1, 2 |
| 1487 | 1731 | *α*-Selinene | 5.1 | 1, 2 |
| 1495 | 1740 | Valencene | 2.1 | 1, 2 |
| 1521 | 1773 | 7-*epi*-*α*-Selinene | 1.1 | 1, 2 |
| 1526 | 1173 | *δ*-Cadinene | 1.2 | 1, 2 |
| 1554 | 1856 | Germacrene B | t[e] | 1, 2 |
| | | Sesquiterpene hydrocarbons | 30.7 | |
| 1527 | 2001 | Isocaryophyllene oxide | 0.5 | 1, 2 |
| 1581 | 2008 | Caryophyllene oxide | 1.1 | 1, 2, 3 |
| 1584 | 2176 | Longiborneol | 1.9 | 1, 2 |
| 1586 | 2099 | Globulol | 0.8 | 1, 2 |
| 1593 | 2104 | Viridiflorol | 2.8 | 1, 2 |
| 1636 | 2185 | *γ*-Eudesmol | 1.5 | 1, 2 |
| 1735 | 2355 | Eremophilone | 0.6 | 1, 2 |
| | | Oxygenated sesquiterpenes | 9.2 | |
| 1836 | 1963 | Neophytadiene | 0.5 | 1, 2 |
| 2054 | 2524 | Abietatriene | 1.0 | 1, 2 |
| 2132 | 2625 | (*E*)-Phytol | 20.0 | 1, 2 |
| | | Diterpenes | 21.5 | |
| 854 | 1209 | (*E*)−2-Hexenal | 0.6 | 1, 2 |
| 1102 | 1401 | Nonanal | 0.7 | 1, 2 |
| 1380 | 1835 | *β*-Damascenone | 0.6 | 1, 2 |
| 1845 | 2131 | Hexahydrofarnesyl acetone | 5.7 | 1, 2 |
| 1915 | 2387 | (*E,E*)-Farnesyl acetone | 0.6 | 1, 2 |
| | | Carbonylic compounds | 8.2 | |
| 1353 | 2186 | Eugenol | 1.5 | 1, 2, 3 |
| | | Phenol | 1.5 | |
| 1094 | 1095 | 1-Undecene | 5.0 | 1, 2 |
| 2300 | 2300 | Tricosane | 0.6 | 1, 2, 3 |

(Continued)

**Table 1.** Continued

| $K_i$[a] | $K_i$[b] | Component | B.n.u.[c] | Ident.[d] |
|---|---|---|---|---|
| 2500 | 2500 | Pentacosane | 0.7 | 1, 2, 3 |
| 2700 | 2700 | Heptacosane | 0.9 | 1, 2, 3 |
| 2900 | 2900 | Nonacosane | 0.5 | 1, 2 |
| | | Hydrocarbons | 7.7 | |
| 977 | 1452 | 1-Octen-3-ol | 0.7 | 1, 2 |
| 1002 | 1243 | 2-Pentylfuran | 0.7 | 1, 2 |
| | | **Others** | 1.4 | |
| | | **Total** | 96.6 | |

[a]HP-5 MS column.
[b]HP Innowax column.
[c]B.n.u.: *Ballota nigra* L. subsp. *uncinata* (Fiori et Beg.) Patzak.
[d]1: Retention index, 2: mass spectrum, 3: co-injection with authentic compound.
[e]t: Trace, <0.05%.

**Table 2.** Main Compounds (>3%) of the Essential Oils From the Aerial Parts of Taxa of *Ballota nigra* sl Group.

| Taxa | Origin | Compounds | Ref |
|---|---|---|---|
| *B. nigra* | 1: Mazandaran, Iran | Caryophyllene oxide (7.9), *epi-α*-muurolol (6.6), *δ*-cadinene (6.5), *α*-cadinol (6.3), *γ*-amorphene (4.3), *β*-bourbonene (4.1), 6,10,14-trimethyl-2-pentadecanone (4.0), (*E*)-caryophyllene (4.0), germacrene D (3.8), aromadendrene (3.4), *γ*-muurolene (3.2), germacrene D-4-ol (3.2), *α*-bisabolol (3.2), *α*-amorphene (3.0) | 28 |
| | 2: Jadovnik Mt., Serbia | *β*-Caryophyllene (35.4/39.1), germacrene D (27.4/35.7), *α*-humulene (7.4/10.4), *δ*-cadinene (3.8/0), (*E*)-phytol (2.5/3.8) | 29 |
| | 3: Golestan, Iran | *β*-Pinene (39.0), *α*-pinene (34.5), sabinene (7.7), *α*-phellandrene (4.1) | 30 |
| *B. nigra* L. subsp. *anatolica* | 1: Mazandaran, Iran | Germacrene D (18.1), nerolidol epoxyacetate (15.4), sclareol oxide (12.1), linalyl acetate (11.5), *β*-caryophyllene (10.5), spathulenol (9.0), linalool (5.2), longipinene epoxide (4.7) | 31 |
| | 2: Muğla, Turkey | Hexadecanoic acid (40.9), *β*-bisabolene (13.4), hexahydrofarnesyl acetone (7.9), 1-isobutyl-4-isopropyl-2,2-diemethyl succinate (6.6), *β*-eudesmol (3.5) | 26 |
| | 3: Western Turkey | 1-Hexacosanol (26.7), caryophyllene oxide (9.3), germacrene-D (9.3), *α*-selinene (8.7), *Z*-8-octadecen-1-ol acetate (7.1), 2,5-di-tertoctyl-p-benzoquinone (7.3), arachidic acid (6.0), tetracosane (4.5), heneicosane (4.4), heptacosane (4.3), 2-methyl-1-hexadecanol (3.3), octadecane (3.0), butyl phthalate (3.0) | 32 |
| *B. nigra* subsp. *foetida* | 1: Pisa, Italy | *β*-Caryophyllene (25.1), germacrene D (24.2), 1-octen-3-ol (7.3), (*E*)−2-hexenal (6.1), *α*-humulene (4.3), caryophyllene oxide (4.2) | 33 |
| | 2: Urbino, Italy | *β*-Caryophyllene (20.0), germacrene D (18.0), caryophyllene oxide (15.0), 1-octen-3-ol (6.8), (*E*)−2-hexenal (6.1), *α*-humulene (4.5), *β*-bourbonene (3.2) | 34 |
| | 3: Urbino, Italy | *β*-Caryophyllene (22.6), caryophyllene oxide (18.0), germacrene D (16.5), (*E*)−2-hexenal (6.5), 1-octen-3-ol (5.5) | 35 |
| | 4: Nis, Serbia | (*E*)-Phytol (56.9), germacrene D (10.0), *β*-caryophyllene (4.7), caryophyllene oxide (3.6), (*E*)-*β*-ionone (3.4) | 36 |
| | 5: Brac, Croatia | Germacrene D (23.1), *β*-caryophyllene (20.3), caryophyllene oxide (6.2), caryophylladienol I (3.3), (*E*)-2-hexenal (3.1), hexadecanoic acid (3.1), *α*-humulene (3.0) | 37 |
| *B. nigra* subsp. *kurdica* | Kurdistan, Iran | Caryophyllene oxide (39.4), *β*-caryophyllene (24.9), germacrene D (7.6), 1-undecene (4.2), isoaromadendrene epoxide (3.2) | 38 |
| *B. nigra* f. *uncinata* | Konya, Turkey | Caryophyllene oxide (21.2), hexadecanoic acid (19.9), *β*-caryophyllene (18.9), germacrene D (4.6), hexahydrofarnesyl acetone (4.4), spathulenol (4.2), caryolphyllenol II (3.8), bicyclogermacrene (3.7) | 26 |

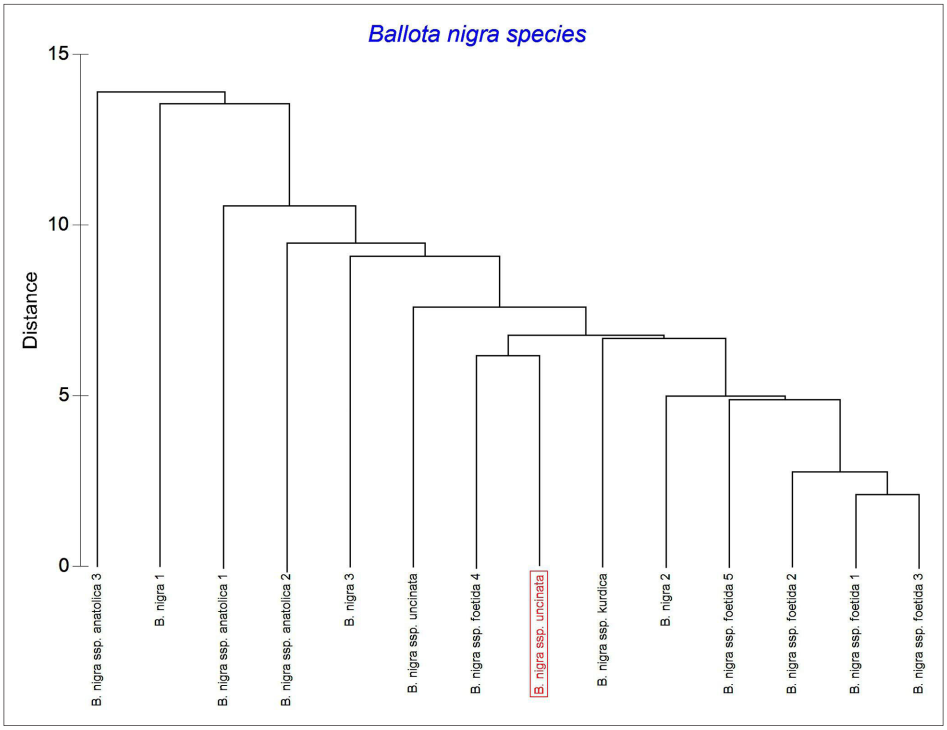


**Figure 1.** The dendrogram of the clusters obtained by statistical analysis.

operating conditions. Component relative concentrations were calculated based on GC peak areas without using correction factors.

### *Statistical Analysis*

It was carried out using the cluster method by Primer 6[41] using a matrix composed of the amount (%) of the 50 compounds occurring in 14 subspecies of *B. nigra* with abundance >3%.

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### References

1. World Checklist of Selected Plant Families (WCSP), Kew Science. Accessed 13 01 2020. http://apps.kew.org/wcsp/qsearch.do
2. Iauk L, Acquaviva R, Mastrojeni S, et al. Antibacterial, antioxidant and hypoglycaemic effects of *Thymus capitatus* (L.) Hoffmanns. et link leaves' fractions. *J Enzyme Inhib Med Chem*. 2015;30(3):360-365. doi:10.3109/14756366.2014.930453
3. Zuzarte M, Alves-Silva JM, Alves M, Cavaleiro C, Salgueiro L, Cruz MT. New insights on the anti-inflammatory potential and safety profile of *Thymus carnosus* and *Thymus camphoratus* essential oils and their main compounds. *J Ethnopharmacol*. 2018;225:10-17. doi:10.1016/j.jep.2018.06.025
4. Bagetta G, Cosentino M, Sakurada T. *Aromatherapy: basic mechanisms and evidence-based clinical use*. 2016. Boca Raton: CRC Press; 2016.
5. Citoğlu G, Tanker M, Sever B, Englert J, Anton R, Altanlar N. Antibacterial activities of diterpenoids isolated from *Ballota saxatilis* subsp. *saxatilis*. *Planta Med*. 1998;64(5):484-485. doi:10.1055/s-2006-957494
6. Çitoğlu GS, Yilmaz BS, Altanlar N. Antimicrobial activity of *Ballota* species growing in. Turkey. *J Fac Pharm Ankara*. 2003;32(2):93-97.
7. Dulger B, Dulger G. Antimicrobial activity of the leaves of *Ballota acetabulosa* on microorganisms isolated from urinary tract infection. *Turk J Pharm Sci*. 2012;9(3):257-262.

8. Askun T, Tekwu EM, Satil F, Modanlioglu S, Aydeniz H. Preliminary antimycobacterial study on selected Turkish plants (Lamiaceae) against *Mycobacterium tuberculosis* and search for some phenolic constituents. *BMC Complement Altern Med*. 2013;13:365. doi:10.1186/1472-6882-13-365
9. Citoğlu GS, Çoban T, Sever B, İşcan M. Antioxidant properties of *Ballota* species growing in Turkey. *J Ethnopharmacol*. 2004;92(2-3):275-280. doi:10.1016/j.jep.2004.03.012
10. Çitoğlu GS, Sever B, Antus S, Baitz-Gács E, Altanlar N. Antifungal Flavonoids from *Ballota glandulosissima*. *Pharm Biol*. 2003;41(7):483-486. doi:10.1080/13880200308951339
11. Çitoğlu GS, Sever B, Antus S, Baitz-Gács E, Altanlar N. Antifungal Diterpenoids and Flavonoids from *Ballota inaequidens*. *Pharm Biol*. 2005;42(8):659-663. doi:10.1080/13880200490902626
12. Özbek H, Citoğlu GS, Dülger H, Uğraş S, Sever B. Hepatoprotective and anti-inflammatory activities of *Ballota glandulosissima*. *J Ethnopharmacol*. 2004;95(2-3):143-149. doi:10.1016/j.jep.2004.06.030
13. Racz-Kotilla G, Racz G, Jozsa J. Activity of some species belonging to Labiatiae on the central nervous systems of mice. *Herb Hung*. 1980;19:49-53.
14. Vural K, Ezer N, Erol K, Samin FP. Anxioloytic and antidepressant activities of some *Ballota* species. *Journal of Faculty of Pharmacy of Gazi University*. 1996;13(1):29-32.
15. Couladis M, Tzakou O, Verykokidou E, Harvala C. Screening of some Greek aromatic plants for antioxidant activity. *Phytother Res*. 2003;17(2):194-195. doi:10.1002/ptr.1261
16. Seidel V, Verholle M, Malard Y, et al. Phenylpropanoids from *Ballota nigra* L. inhibit in vitro LDL peroxidation. *Phytother Res*. 2000;14(2):93-98. doi:10.1002/(SICI)1099-1573(200003)14:2<93::AID-PTR558>3.0.CO;2-X
17. Didry N, Seidel V, Dubreuil L, Tillequin F, Bailleul F. Isolation and antibacterial activity of phenylpropanoid derivatives from *Ballota nigra*. *J Ethnopharmacol*. 1999;67(2):197-202. doi:10.1016/S0378-8741(99)00019-7
18. Dulger B, Kilcik MA. Antibacterial activity of *Ballota acetabulosa* against methicillin-resistant *Staphylococcus aureus*. *Asian J Chem*. 2011;23(1):416-418.
19. Rosselli S, Fontana G, Bruno M. A review of the phytochemistry, traditional uses, and biological activities of the Genus *Ballota* and *Otostegia*. *Planta Med*. 2019;85(11-12):869-910. doi:10.1055/a-0953-6165
20. Savona G, Bruno M, Piozzi F, Barbagallo C. Diterpenes from *Ballota* species. *Phytochemistry*. 1982;21(8):2132-2133. doi:10.1016/0031-9422(82)83067-7
21. Bruno M, Savona G, Pascual C, Rodríguez B. Preleosibirin, a prefuranic labdane diterpene from *ballota nigra* subsp. *foetida*. *Phytochemistry*. 1986;25(2):538-539. doi:10.1016/S0031-9422(00)85521-1
22. Bruno M, Bondí ML, Piozzi F, Arnold NA, Simmonds MSJ. Occurrence of 18-hydroxyballonigrine in *Ballota saxatilis* ssp. *saxatilis* from Lebanon. *Biochem Syst Ecol*. 2001;29(4):429-431. doi:10.1016/S0305-1978(00)00068-5
23. Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Accessed 13 01 2020. https://ww2.bgbm.org/EuroPlusMed/
24. Yilmaz BS, Çitoğlu GS. High performance liquid chromatographic analysis of some diterpenoids of the *Ballota* species. *FABAD J Pharm Sci*. 2003;28(1):13-17.
25. Çitoğlu GS, Yilmaz BS, Tarikahya B, Tipirdamaz R. Chemotaxonomy of *Ballota* species. *Chem Nat Compd*. 2005;41(3):299-302. doi:10.1007/s10600-005-0134-7
26. Kaya A, Kürkçüoğlu M, Dinç M, Doğu S. Compositions of the essential oils of *Ballota nigra* subsp. *uncinata* and subsp. *anatolica* from Turkey. *Indian J Pharm Educ*. 2017;51(3s):s185-s189. doi:10.5530/ijper.51.3s.9
27. Sever Yilmaz B, Altanlar N, Saltan Çitoğlu G. Antilisterial activity of *Ballota* species growing in Turkey. *J Fac Pharm Ankara*. 2005;34(3):155-164.
28. Morteza-Semnani K, Saeedi M, Akbarzadeh M. The essential oil composition of *Ballota nigra*. *Chem Nat Compd*. 2007;43(6):722-723. doi:10.1007/s10600-007-0245-4
29. Vukovic N, Sukdolak S, Solujic S, Niciforovic N. Antimicrobial activity of the essential oil obtained from roots and chemical composition of the volatile constituents from the roots, stems, and leaves of *Ballota nigra* from Serbia. *J Med Food*. 2009;12(2):435-441. doi:10.1089/jmf.2008.0164
30. Jamzad M, Rustaiyan A, Jamzad Z, Masoudi S. Essential oil composition of *Salvia indica* L., *Thymus caucasicus* Wind. Ex Ronniger subsp. *grossheimii* (Ronniger) Jalas. and *Ballota nigra* L. three Labiatae species from Iran. *J Ess Oil Bearing Plants*. 2011;14(1):76-83. doi:10.1080/0972060X.2011.10643903
31. Kazemizadeh Z, Amini T, Nazari F, Habibi Z. Volatile constituents of *Ballota nigra* subsp. *anatolica* from Iran. *Chem Nat Compd*. 2009;45(5):737-738. doi:10.1007/s10600-009-9415-x
32. Ertaş A, Boğa M, Yeşil Y. Phytochemical profile and ABTS cation radical scavenging, cupric reducing antioxidant capacity and anticholinesterase activities of endemic *Ballota nigra* L. subsp. *anatolica* P. H. Davis from Turkey. *J Coast Life Med*. 2014;2(7):555-559.
33. Bader A, Caponi C, Cioni PL, Flamini G, Morelli I. Composition of the essential oil of *Ballota undulata*, *B. nigra* ssp. *foetida* and *B. saxatilis*. *Flavour Fragr J*. 2003;18(6):502-504. doi:10.1002/ffj.1257
34. Fraternale D, Bucchini A, Giamperi L, Ricci D. Essential oil composition and antimicrobial activity of *Ballota nigra* L. ssp. *foetida*. *Nat Prod Commun*. 2009;4(4):585-588. doi:10.1177/1934578X0900400429
35. Fraternale D, Ricci D. Essential oil composition and antifungal activity of aerial parts of *Ballota nigra* ssp *foetida* collected at flowering and fruiting times. *Nat Prod Commun*. 2014;9(7):1015-1018. doi:10.1177/1934578X1400900733
36. Đorđević AS, Jovanović OP, Zlatković BK, Stojanović GS. Chemical composition of *Ballota macedonica* Vandas and *Ballota nigra* L. ssp. *foetida* (Vis.) Hayek essential oils – the chemotaxonomic approach. *Chem Biodiv*. 2016;13(6):782-788.
37. Rigano D, Marrelli M, Formisano C, et al. Phytochemical profile of three *Ballota* species essential oils and evaluation of the effects on human cancer cells. *Nat Prod Res*. 2017;31(4):436-444. doi:10.1080/14786419.2016.1185722
38. Majdi M, Dastan D, Maroofi H. Chemical composition and antimicrobial activity of essential oils of *Ballota nigra* subsp. *kurdica* from Iran. *Jundishapur J Nat Pharm Prod*. 2017;12(3):e36314.

39. De Martino L, Bruno M, Formisano C, et al. Chemical composition and antimicrobial activity of the essential oils from two species of *Thymus* growing wild in Southern Italy. *Molecules*. 2009;14(11):4614-4624. doi:10.3390/molecules14114614
40. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy*. 4th ed. Allured Publishing. Carol Stream USA; 2007.
41 Clarke KR, Gorley RN; 2006. PRIMER (6.0). Plymouth UK: PRIMER-E.