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 potential³ 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, choleric, antihemorrhoidal, and sedative agents.⁵ The antimicrobial activities⁶⁻⁸ and the antioxidant activities⁹ 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, anti-inflammatory, and hepatoprotective activities.¹² In Europe, the polar extracts of the flowering aerial parts of *Ballota* are commonly used due to their neuromodulatory activity.¹³⁻¹⁴ More recently, the general antioxidant activity¹⁵ the in vitro inhibition of LDL (low-density lipoprotein) peroxidation¹⁶ and the antibacterial activity¹⁷,¹⁸ 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
the occurrence of only 1 labdane diterpenoid, dehydrohispanolone,\textsuperscript{24,25} the chemical composition of the essential oil of Turkish accession,\textsuperscript{26} and its antilisterial activity against \textit{L. monocytogenes}.

### Results and Discussion

Hydrodistillation of \textit{B. nigra} subsp. \textit{uncinata} (\textit{B.n.u.}) aerial parts gave a pale yellow oil. Overall, 49 compounds have been identified in \textit{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 \textit{B.n.u.} were found to be (E)-phytol (20.0%), \textit{\alpha}-pinene (9.0%), hexahydrofarnesyl acetone (5.7%), \textit{\alpha}-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 \textit{\alpha}-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 monoterpane hydrocarbon represented 12.9% with \textit{\alpha}-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 \textit{B. nigra} s.l. 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 \textit{B. nigra} species contain the sesquiterpenes caryophyllene and/or caryophyllene oxide among the main compounds with the exception of 2 cases: \textit{B. nigra} 3 collected in Iran\textsuperscript{30} and \textit{B. nigra} subsp. \textit{anatolica} 2 collected in Turkey.\textsuperscript{26}

The \textit{B. nigra} subsp. \textit{uncinata}, we examined, contains on the contrary to the previously investigated \textit{B. nigra} subsp. \textit{uncinata}, also a relevant amount (9%) of monoterpane \textit{\alpha}-pinene exclusively contained in the \textit{B. nigra} 3 collected in Iran.\textsuperscript{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 \textit{B. nigra} subsp. \textit{foetida} 4 collected in Serbia.\textsuperscript{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 \textit{B. nigra} subsp. \textit{uncinata} (Bég.) Patzak (\textit{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 \textit{n}-hexane as the solvent, according to the standard procedure previously described.\textsuperscript{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% (\textit{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 \times 0.25 mm), \textit{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 \textit{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 \times 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 polyethylene glycol capillary column (50 m \times 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.\textsuperscript{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 \textit{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<sup>a</sup> | K<sup>b</sup> | Component | B.n.u.<sup>c</sup> | Ident.<sup>d</sup> |
|-------------|-------------|-----------|-----------------|------------------|
| 930         | 1014        | α-Thujene  | 1.7             | 1, 2             |
| 936         | 1073        | 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          |

Monoterpene hydrocarbons

| 1098        | 1553        | Linalool   | 0.8             | 1, 2, 3          |
| 1143        | 1532        | Camphor    | 0.4             | 1, 2, 3          |
| 1167        | 1719        | Bornecol   | 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*-Chrysantheryl acetate | 0.4 | 1, 2          |

Oxygenated monoterpenes

| 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)<sub>2</sub>-Caryophyllene | 4.2 | 1, 2, 3          |
| 1407        | 1538        | α-Gurjunene   | 0.5 | 1, 2          |
| 1418        | 1612        | (E)<sub>2</sub>-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        | β-Selinene   | 0.8 | 1, 2          |
| 1487        | 1731        | *α*-Selinene | 5.1 | 1, 2          |
| 1495        | 1740        | Valencene    | 2.1 | 1, 2          |
| 1521        | 1773        | γ-*epi*-α-Selinene | 1.1 | 1, 2          |
| 1526        | 1173        | *β*-Cadinene | 1.2 | 1, 2          |
| 1554        | 1856        | Germacrene B | t<sup>e</sup> | 1, 2          |

Sesquiterpene hydrocarbons

| 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

| 1836        | 1963        | Neophytadiene           | 0.5 | 1, 2          |
| 2054        | 2524        | Abietatriene            | 1.0 | 1, 2          |
| 2132        | 2625        | (E)-Phytol              | 20.0 | 1, 2          |
| 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        | Hexahydrofarneol acetone | 5.7 | 1, 2          |
| 1915        | 2387        | (E,E)-Farnesyl acetone  | 0.6 | 1, 2          |

Carboxylic compounds

| 1353        | 2186        | Eugenol                 | 1.5 | 1, 2, 3          |
| 1094        | 1095        | Phenol                  | 1.5 | 1, 2          |
| 2300        | 2300        | Tricosane               | 0.6 | 1, 2, 3          |

(Continued)
Table 1. Continued

| K<sup>a</sup> | K<sup>b</sup> | Component | B.n.u.<sup>c</sup> | Ident. <sup>d</sup> |
|-------|--------|-----------|--------|------|
| 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   |       |

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

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

| Taxa | Origin | Compounds | Ref |
|------|--------|-----------|-----|
| B. nigra | 1: Mazandaran, Iran | Caryophyllene oxide (7.9), *ep*-α-muuroolol (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-murolene (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), a-humulene (7.4/10.4), δ-cadinene (3.8/0), (E)-phytol (2.5/3.8) | 29 |
|       | 3: Golestan, Iran | β-Pinene (39.0), a-pinene (34.5), sabinene (7.7), a-phellandrene (4.1) | 30 |
| B. nigra subsp. anatolica | 1: Mazandaran, Iran | Germacrene D (18.1), nerolidol epoxyacetate (15.4), scareol oxide (12.1), linalyl acetate (11.5), β-caryophyllene (10.5), spathulanol (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-dimethyl 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-t-tert-octyl-p-benzoquinone (7.3), arachidic acid (6.0), tetracosane (4.5), heneicosane (4.4), heptacosane (4.3), 2-methyl-1-hexadecanol (3.3), octadecano (3.3), 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), a-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), a-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), a-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), spathulanol (4.2), caryophyllenol II (3.8), bicyclogermacrene (3.7) | 26 |
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 6.1 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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