Phytochemical profile of three Ballota species essential oils and evaluation of the effects on human cancer cells

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ABSTRACT
Three Ballota species, Ballota undulata, Ballota saxatilis and Ballota nigra ssp. foetida, were investigated for their cytotoxicity against two human cancer cells, hepatoma HepG2 cell line and breast cancer MCF-7 cell line, and for their antioxidant activity. The chemical composition of essential oils was studied by GC and GC–MS. Sesquiterpenes were the main constituents. The most antiproliferative essential oil against HepG2 cells was B. undulata with a percentage of inhibition of 81.36 ± 3.54 at a concentration of 100 μg/mL, while against MCF-7 cells essential oil from B. saxatilis was the most active with a percentage of inhibition of 24.18 ± 1.13 at a concentration of 100 μg/mL. The antioxidant activity was investigated by DPPH test for all the oils. B. undulata showed the highest antiradical effect, with IC_{50} value of 529.7 ± 37.4 μg/mL.

1. Introduction
The genus Ballota includes perennial herbs of the Lamiaceae family widespread in Europe. Ballota species have been used in folk medicine as antiulcer, antispasmodic, diuretic,
choleretic, antihaemorrhoidal and sedative agent (Vural et al. 1996). *Ballota nigra* is used externally, in the treatment of wounds and burns (Pieroni 2000). The antimicrobial activities of *Ballota* species was reported (Didry et al. 1999) as well as the antifungal activities (Fraternale & Ricci 2014). *Ballota* species could be a good source of natural preservatives in foodstuffs for their antioxidant properties (Erdogan-Orhan et al. 2010).

*B. undulata* (Sieber ex Fresen.) Benth., *B. saxatilis* Sieber ex C.Presl and *B. nigra* ssp. *foetida* (Vis.) Hayek are three *Ballota* species well known in the traditional folk medicine (Pieroni 2000). The reactive oxygen species (ROS) such as superoxide anion radical, hydroxyl radical and hydrogen peroxide are well-known inducer of cellular and tissue pathogenesis leading to various diseases including cancer, neurodegenerative and cardiovascular diseases. Since ROS have been implicated in some of the illnesses and complaints against which these *Ballota* species are traditionally used, such as gastrointestinal tract disorders and inflammatory injury, (Halliwell & Gutteridge 1989) their antioxidant capacities need to be clarified. Until now, only polar extracts of *Ballota* species have been analysed for their antioxidant properties. It has been recently reported that polyphenols (e.g. verbascoside, forsythoside B, arenarioside and ballotetroside) from *B. nigra* inhibited LDL peroxidation (Seidel et al. 2000). In addition, the antioxidant properties of several isolated phenylpropanoid derivatives such as alyssonoside, lavandulifolioside and angoroside from *B. nigra* have also been determined (Daels-Rakotoarison et al. 2000). The methanol extracts of *Ballota pseudodictamnus* and *Ballota acutabulosa* have been reported to have antioxidant activities (Couladis et al. 2003). However, no information exists for the essential oils of the three *Ballota* species used in this study as regard antiproliferative activity. So, we now report the antioxidant activities as radical scavengers and the antiproliferative activity against two human cancer cells (hepatoma HepG2 cell line and breast cancer MCF-7 cell line) of essential oils of *B. undulata*, *B. saxatilis* and *B. nigra* ssp. *foetida*, along with their chemical composition.

2. Results and discussion

2.1. Chemical composition of the essential oils

The chemical composition of the essential oils from the three *Ballota* species (*B. undulata*, *B. saxatilis* and *B. nigra* ssp. *foetida*) was performed through analytical gas chromatography and GC–MS analysis (Table 1). Seventy-three compounds were identified in the oil from *B. nigra* ssp. *foetida* (*Bn*), that accounted for 95.1% of the total oil. The sesquiterpenes germacrene D (23.1%), (E)-β-caryophyllene (20.3%) and caryophyllene oxide (6.2%) were the main constituents, that together accounted for about the half of the total oil (49.6%). On the whole, sesquiterpenes (69.9%) and particularly sesquiterpene hydrocarbons (55.9%) prevailed over all the other components. Other fractions were carbonylic compounds (8.5%) and monoterpenes (7.7%). Our results are essentially in agreement with previous studies on the essential oils of this subspecies, reporting germacrene D, (E)-β-caryophyllene and caryophyllene oxide as main compounds of the oil but in different percentages (Bader et al. 2003; Fraternale et al. 2009; Fraternale & Ricci 2014). Differently, α-pinene and β-pinene were the main components identified in the oil of *B. nigra* L. from Iran analysed by Jamzad et al. (2011).

Eighty-five compounds were identified in *B. saxatilis* (*Bs*), accounting for the 95.0% of the oil. Linalool (11.2%), (E)-β-caryophyllene (8.8%), caryophyllene oxide (6.3%) and (E)-2-hexenal (5.6%) were the main compounds. The amounts of sesquiterpenes (24%) and monoterpenes
Table 1. Percent composition of the essential oils of aerial parts of B. nigra ssp. foetida, B. saxatilis and B. undulata arranged by class.

| Component                  | K\textsubscript{n} | K\textsubscript{s} | K\textsubscript{u} | Id |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Monoterpene hydrocarbons    |                     |                     |                     |    |                     |                     |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Thujene                   | 3.9                 | 8.4                 | 2.6                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Pinene                    | 0.1                 | t                   | 1.7                 |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| cis-Linalool oxide          | 0.5                 | 1.2                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Fenchone                    | 0.3                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| trans-Linalool oxide        | 0.2                 | 0.4                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Linalool                    | 2.0                 | 11.2                | 0.9                 |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Campholenal               | 0.3                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| trans-Pinocarveol           | 0.2                 | 0.3                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| cis-Verbenol                | 0.3                 | 0.1                 | 0.1                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Camphor                     | 0.7                 |                     |                     |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| trans-Verbenol              | 1.2                 | 0.3                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Borneol                     | 0.1                 | 0.5                 | 0.8                 |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| Terpinen-4-ol               | 0.3                 | 0.6                 | 1.2                 |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| trans-Verbenone             | 0.2                 | 0.3                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| β-Cyclotiral                | 0.2                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Pulegone                    | 0.2                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Geraniol                    | 0.1                 |                     |                     |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| Cumin alcohol               | 0.3                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Terpenyl acetate          | 1.3                 |                     |                     |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| Neryl acetate               | 0.7                 |                     |                     |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| Sesquiterpene hydrocarbons  |                     |                     |                     |    |                     |                     |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Cubebene                  | 0.2                 | 0.3                 | 0.5                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Cyclosativene               | t                   |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Ylangene                  | t                   |                     |                     |    | 0.7                 |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Copaene                   | 1.6                 | 0.4                 | 0.9                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Daucene                     |                     |                     |                     |    | 0.8                 |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Bourbonene                | 0.7                 | 1.7                 | 1.0                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| β-Elemene                   | 0.1                 | 0.8                 | 0.1                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Gurjunene                 | t                   | 0.4                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| (E)-β-Caryophyllene         | 20.3                | 8.8                 | 1.5                 |    | 1, 2, 3             |                     |                     |                     |                     |                     |                     |                     |                     |
| β-Gurjunene                 | t                   | 0.1                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| β-Cubebene                  | 0.5                 | 1.6                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Widdrene                    | 0.2                 | 1.0                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Aromadendrene               | 0.4                 | 0.3                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| γ-Elemene                   | t                   |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Bergamotene               | 0.6                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Humulene                  | 3.0                 |                     |                     |    | t                   |                     |                     |                     |                     |                     |                     |                     |                     |
| β-Chamigrene                | 1.4                 | t                   |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Amorphene                 | 0.1                 | 0.4                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Selinene                  | 0.3                 | t                   |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Germacrene D                | 23.1                |                     |                     |    | 16.0                |                     |                     |                     |                     |                     |                     |                     |                     |
| γ-Muurolene                 | 0.6                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Epizonarene                 | 0.1                 |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Selinene                  | 1.7                 | 0.2                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| Bicyclogermacrene           | 1.0                 |                     |                     |    | 10.4                |                     |                     |                     |                     |                     |                     |                     |                     |
| Viridiflorene               | t                   |                     |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| α-Cadinene                 | 0.3                 | 0.7                 |                     |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |
| γ-Cadinene                 | 1.3                 | 0.4                 | 1.1                 |    | 1, 2                |                     |                     |                     |                     |                     |                     |                     |                     |

(Continued)
| K<sub>a</sub> | K<sub>b</sub> | Component | Bn<sup>c</sup> | Bs<sup>c</sup> | Bu<sup>c</sup> | Id<sup>d</sup> |
|---|---|---|---|---|---|---|
| 1554 | 1856 | Germacrene B | 0.2 | 0.6 | 1, 2 |
| 1766 | 2256 | Cadalene | 0.2 | 0.2 | 1, 2 |
| 1773 | 2125 | Guaiazulene | 0.2 | 0.2 | 1, 2 |

**Oxygenated sesquiterpenes**

| 1564 | 2050 | (E)-Nerolidol | 0.3 | 0.5 | 1, 2, 3 |
| 1577 | 2148 | Spathulenol | t | 0.7 | 1.5 | 1, 2 |
| 1581 | 2008 | Caryophyllene oxide | 6.2 | 6.3 | 2.1 | 1, 2, 3 |
| 1593 | 2104 | Viridiflorol | 0.5 | 0.5 | 1, 2 |
| 1598 | 2108 | Guaiol | 0.2 | 0.2 | 1, 2 |
| 1615 | 2324 | Caryophylladienol II | 0.8 | t | 1, 2 |
| 1625 | 2127 | 10-epi-γ-Eudesmol | 2.2 | 2.2 | 1, 2 |
| 1632 | 2371 | (E)-Nerolidol | 0.3 | 0.5 | 1, 2, 3 |
| 1642 | 2185 | t-Cadinol | t | t | 0.4 | 1, 2 |
| 1640 | 2316 | Caryophylladienol I | 3.3 | 3.3 | 1, 2 |
| 1652 | 2253 | α-Cadinol | 0.5 | t | 0.4 | 1, 2 |
| 1693 | 2196 | Acorenone B | 1.3 | 1.3 | 1, 2 |

**Phenols**

| 1541 | 1687 | p-Vinyl anisole | 0.3 | 0.3 | 1, 2 |
| 1593 | 2198 | Thymol | 1.5 | 1.5 | 1, 2, 3 |
| 1598 | 2239 | Carvacrol | 1.3 | 1.3 | 1, 2, 3 |
| 1611 | 2180 | 4-Vinylguaiacol | 2.7 | 2.7 | 1, 2 |
| 1534 | 2188 | Eugenol | t | 0.5 | 1.7 | 1, 2, 3 |

**Carbonylic compounds**

| 854 | 1209 | (E)-2-Hexenal | 3.1 | 5.6 | t | 1, 2 |
| 963 | 1543 | Benzaldehyde | 0.3 | 0.6 | t | 1, 2, 3 |
| 978 | 1267 | Octan-3-one | 0.2 | 0.2 | 1, 2 |
| 1002 | 1295 | Octanal | 0.1 | 0.3 | 1, 2 |
| 1044 | 1663 | Phenylacetaldehyde | 0.7 | 0.7 | 1, 2 |
| 1102 | 1401 | Nonanal | 1.2 | 1.2 | 0.5 | 1, 2 |
| 1132 | 1543 | (E)-2-Nonenal | 0.2 | t | 0.1 | 1, 2 |
| 1204 | 1508 | Decanal | 0.2 | t | 0.3 | 1, 2 |
| 1302 | 1797 | p-Methoxyacetophenone | 0.5 | 0.5 | 1, 2, 3 |
| 1382 | 1838 | (E)-β-Damascenone | 0.2 | 0.5 | 0.9 | 1, 2 |
| 1452 | 1867 | (E)-Geranyl acetone | 0.8 | 0.8 | 1, 2 |
| 1484 | 1958 | (E)-β-Ionone | 0.5 | 0.9 | 1, 2, 3 |
| 1558 | 2025 | ψ-Ionone; Pseudoionone; (6E,8E)-Megastigmatrienone | 0.2 | 0.2 | 1, 2 |
| 1582 | 1845 | (E,E)-β-Ionone | 0.8 | 0.1 | 1, 2 |

**Hydrocarbons**

| 1845 | 2131 | Hexahydrofarnesyl acetone | 2.3 | 1.5 | 1.8 | 1, 2 |
| 1918 | 2389 | (E,E)-Farnesyl acetone | 0.5 | 0.5 | 1, 2 |

**Fatty acids and esters**

| 1385 | 2285 | Decanoic acid | 0.3 | 0.3 | 1, 2, 3 |
| 1566 | 2503 | Dodecanoic acid | 0.5 | 0.7 | 1, 2, 3 |
| 1569 | 2148 | cis-3-Hexenyl benzoate | t | 0.7 | 1, 2 |
| 1768 | 2713 | Tetradecanoic acid | 1.1 | 2.0 | 1, 2, 3 |
| 1928 | 2208 | Hexadecanoic acid methyl ester | 2.6 | 2.6 | 1, 2, 3 |
| 1958 | 2931 | Hexadecanoic acid | 2.6 | 4.9 | 4.5 | 1, 2, 3 |
| 2099 | 3157 | (Z,Z,Z)-9,12,15-Octadecatrienoic acid | 2.6 | 2.6 | 1, 2, 3 |
| 2115 | 3160 | (Z)-9-Octadecenoic acid | 2.3 | 2.3 | 1, 2, 3 |
| 2122 | 3160 | (Z,Z)-9,12-Octadecadienoic acid | 3.4 | 3.4 | 5.3 | 1, 2, 3 |

**(Continued)**
(25.8%) were almost equal and prevailed over the other fractions. Fatty acids and esters (14.5%), carboxylic compounds (12.6%) and phenols (6.3%) were well represented. These results are quite similar to those showed in two previous papers regarding plants collected from Turkey (Erdogan et al. 2014) and Jordan (Bader et al. 2003). Particularly, the oil from our sample has in common with the Turkish oil the abundance of caryophyllene and caryophyllene oxide. However, its composition is more similar to the oil previously described by Bader et al. (2003) that reports linalool as main compound. Differently, phenols, carboxylic compounds and fatty acids and esters that are well represented in our sample are scarce or totally absent in the oil from Jordan.

Ninety-one components were found in \( B. \text{undulata} \) \((B. u)\), that constituted the 91.7% of the oil, in which prevailed the two sesquiterpene hydrocarbons germacrene D (16.0%) and bicyclogermacrene (10.4%). As for \( Bn \), sesquiterpenes (46.6%) and particularly sesquiterpene hydrocarbons (39.3%) prevailed over the other fractions; fatty acids and esters (15.8%) were also well represented. The only previous report on the essential oil of this species collected in Jordan (Bader et al. 2003) shows similar results except from the presence of fatty acids and esters and hydrocarbons in our sample on respect to the Jordan sample.

### 2.2. Radical scavenging activity

The antioxidant potential of essential oils from \( Ballota \) species was determined by DPPH test (Menichini et al. 2013). The reduction of DPPH absorption is indicative of the capacity of the oils to scavenge free radicals, independently of any enzymatic activity. The scavenging effects of essential oils on DPPH were examined at different concentrations (0.025, 0.05, 0.1, 0.25, 0.5, 1 and 2 mg/ml). The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants. All essential oils were able to reduce the stable free radical DPPH to the yellow-coloured 1,1-diphenyl-2-picrylhydrazyl (Table 2). \( Bu \) showed the highest antiradical effect with IC\(_{50}\) value of 529.7 ± 37.4 μg/mL (Figure 1), while \( Bs \) and \( Bn \) showed lower activity (32.02 ± 3.40% and 48.22 ± 1.08% of inhibition at 2 mg/mL, respectively). The antioxidant activity of different \( Ballota \) species was previously

### Table 1. (Continued)

| Component          | Bu | Bs | Bn | Id |
|--------------------|----|----|----|----|
| Nonacosane         | 0.3| 1.3| 0.5| 1, 2, 3 |
| Hentriacontane     | 0.2| 0.6| 0.4| 1, 2, 3 |
| Others             | 4.9| 4.4| 5.0|    |
| (Z)-3-Hexenol      |    |    |    | 1, 2 |
| 1-Octen-3-ol       | 3.1| 1.2| 1.0| 1, 2 |
| 3-Octanol          | t  | t  | t  | 1, 2 |
| γ-Nonalactone      | 0.3| 0.4| 1, 2 |
| Mint furanone      | 0.2|    | 1, 2 |
| Dihydroactinidiole | 2.3| 3.4| 1, 2 |
| (Z)-Phytol         | 0.2|    | 1, 2 |
| (E)-Phytol         | 0.5| 0.6| 1, 2 |
| Others             | 95.1| 95.0| 91.7| |

aK\(_i\): Retention index on a HP-5MS column.
bK\(_i\): Retention index on a HP-Innowax column.
c\( Bn = Ballota \text{ nigra ssp. foetida}; Bs = Ballota saxatilis; Bu = Ballota undulata. \)
dIdentification: 1 = comparison of retention index; 2 = comparison of mass spectra with MS libraries; 3 = comparison with authentic compounds; t = trace, less than 0.05%.
investigated by Citoğlu and co-workers, among them *B. saxatilis* subsp. *saxatilis* exhibited remarkable antisu-peroxide anion formation (Citoğlu et al. 2004). The antioxidant activity determined for *B. nigra* as inhibition of Cu(2+)-induced LDL peroxidation was attributed to phenylpropanoid derivatives (Seidel et al. 2000). No antioxidant activity was previously demonstrated for *B. undulata*. Qazan demonstrated that *B. undulata* hydroalcoholic extract (70% EtoH) possesses active hypolipidaemic constituents when orally administered to the atherogenic rabbits (Qazan 2008).

### 2.3. Antiproliferative activity on HepG2 and MCF-7 cells

The three *Ballota* species were evaluated for their in vitro antiproliferative properties against two human cancer cell lines: hepatoma HepG2 cell line and breast cancer MCF-7 cell line. The two human tumour cell lines were capable of attachment to form a homogeneous monolayer on the plastic substratum of the culture wells, as is ideal for the MTT assay. The MTT test is a simple bioassay used for primary screening of crude plant extracts and isolated compounds (Marrelli et al. 2013). For each cell line, there was a linear relationship between cell number and absorbance, measured at 550 nm in both control and drug-treated wells.
After 48 h of treatment, the antiproliferative activity was determined. The results on the growth of the human tumour cell lines are given in Table 3 and Table 4. The most antiproliferative essential oil against HepG2 cells was *B. undulata* (*Bu*) with a percentage of inhibition of $81.36 \pm 3.54$ at a concentration of 100 $\mu$g/mL (Figure 3) while against MCF-7

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**Figure 2.** Dose-depending effect of *Ballota* sp. on HepG2 cancer cells viability. (•) *B. undulata*; (■) *B. saxatilis*; (▲) *B. nigro* ssp. *foetida*.

| Concentration (μg/mL) | % Inhibition | % Inhibition | % Inhibition |
|-----------------------|--------------|--------------|--------------|
|                       | *B. undulata*| *B. saxatilis*| *B. nigro* ssp. *foetida*|
| 100                   | 81.36 ± 3.54$^a$ | 74.49 ± 2.56$^{a,b}$ | 70.04 ± 3.28$^b$ |
| 50                    | 41.21 ± 1.59$^{c}$ | 31.24 ± 1.08$^d$ | 28.54 ± 1.36$^{d,e}$ |
| 25                    | 22.88 ± 1.61$^e$ | 15.04 ± 0.69$^f$ | 16.46 ± 0.95$^{f,g}$ |
| 10                    | 7.35 ± 1.07$^{g}$ | 4.67 ± 0.65$^g$ | 3.16 ± 0.23$^g$ |
| 5                     | 2.19 ± 0.10$^g$ | 0$^g$ | 0$^g$ |
| 2.5                   | 0$^g$ | 0$^g$ | 0$^g$ |
| $IC_{50}$ values (μg/mL) | 54.75 ± 0.58 | 65.41 ± 0.82 | 69.92 ± 1.59 |

Notes: Data are expressed as mean ± S.E. ($n = 4$). Different letters (a-g) along and between columns indicate statistically significant differences at $P < 0.05$ (Tukey’s test).

**Table 3.** Antiproliferative activity against HepG2 cell line: % inhibition and IC$_{50}$ values.

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| Concentration (μg/mL) | % Inhibition | % Inhibition | % Inhibition |
|-----------------------|--------------|--------------|--------------|
|                       | *B. undulata*| *B. saxatilis*| *B. nigro* ssp. *foetida*|
| 100                   | 19.57 ± 2.49$^a$ | 24.18 ± 1.13$^a$ | 19.76 ± 1.80$^a$ |
| 50                    | 11.41 ± 1.44$^b$ | 12.93 ± 0.86$^b$ | 11.70 ± 1.63$^b$ |
| 25                    | 8.45 ± 0.90$^b$ | 9.04 ± 0.66$^b$ | 4.61 ± 0.23$^{b,c}$ |
| 10                    | 1.79 ± 0.46$^c$ | 2.47 ± 0.20$^c$ | 0$^c$ |
| 5                     | 0$^c$ | 0$^c$ | 0$^c$ |
| 2.5                   | 0$^c$ | 0$^c$ | 0$^c$ |
| $IC_{50}$ values (μg/mL) | > 100 | > 100 | > 100 |

Notes: Data are expressed as mean ± S.E. ($n = 4$). Different letters (a-c) along and between columns indicate statistically significant differences at $P < 0.05$ (Tukey’s test).
cells essential oil from *B. saxatilis* (*Bs*) was the most active with a percentage of inhibition of 24.18 ± 1.13 at a concentration of 100 μg/mL.

No previous study was conducted on the evaluation of antiproliferative activity on cancer cells of *Ballota* species. Cell type cytotoxic specificity is observed in some plant extracts. This specificity of plant extracts is likely due to the presence of different classes of compounds in the extract, as it has been documented in the case of known classes of compounds (Cragg et al. 1994). Previous studies showed that sesquiterpenes, particularly germacrene, the main compound identified in the *B. undulata* (*Bu*) essential oil, could be responsible, at least in part, for cytotoxic activity against different human cancer cell lines (Grecco Sdos et al. 2015).

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No potential conflict of interest was reported by the authors.
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