Essential oil composition and biological activity from Artemisia caerulescens subsp. densiflora (Viv.) Gamisans ex Kerguélen & Lambinon (Asteraceae), an endemic species in the habitat of La Maddalena Archipelago

Luigi Ornano,a,b Alessandro Venditti,e Mauro Ballero,b Cinzia Sanna,b Yuri Donno,c, Luana Quassinti,d Massimo Bramucci,d Luca A. Vitali,d Dezemona Petrelli,e Bruno Tirillini,f Fabrizio Papa,g Filippo Maggi,d and Armanodoriano Bianco,a,b

a Dipartimento di Chimica, Università degli studi di Roma “La Sapienza”, Rome, Italy; b Consorzio Interuniversitario Co.S.Me.Se., Dipartimento di Scienze Botaniche, Università di Cagliari, Cagliari, Italy; c Parco nazionale dell’ Arcipelago di La Maddalena, La Maddalena, Sardinia, Italy; d Scuola di Farmacia e dei Prodotti della Salute, Università di Camerino, Camerino, Italy; e Dipartimento di Biologia Ambientale, Università di Roma “La Sapienza”, Rome, Italy; f Dipartimento di Scienze Biomolecolari, Università di Urbino, Urbino, Italy; g Scuola di Scienze e Tecnologie, Università di Camerino, Camerino, Italy; h Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino, Camerino, Italy

ABSTRACT
The purpose of this study was to investigate the composition of the essential oil obtained from a population of Artemisia caerulescens subsp. densiflora growing in Razzoli, an island in the La Maddalena Archipelago (Sardinia, Italy). A. caerulescens sups. densiflora Viv. (Asteraceae), a wild herb, seldom studied in the Mediterranean, represents one of the many rare endemic species growing in North Sardinia. The essential oil composition was analysed by means of GC/MS analysis, which showed davana ethers as the major volatile components, accounting together for 17.5%, followed by (E)-nerolidol (4.5%), β-oplopenone (3.3%), cis-sabinene hydrate (5.2%) and terpinen-4-ol (4.7%). The oil was tested for antioxidant activity by means of DPPH test, inhibition of lipid oxidation test and hypochlorous acid test, which showed a quite interesting scavenger capacity. For the first time, we reported the cytotoxic activity of the essential oil of A. caerulescens subsp. densiflora, against three human tumour cell lines (A375, MDA-MB231 and HCT116), with IC50 values in the range 5.20–7.61 μg/mL, which deserved further studies to support its use as chemopreventive agent. Finally, the antimicrobial activity of the essential oil, displayed on a panel of human pathogens, was very low.

CONTACT
Luigi Ornano luigi.ornano@uniroma1.it

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1. Introduction

The genus *Artemisia* (Asteraceae) includes a large number of species. It comprises 220 to more than 500 taxa (Tan et al. 1998) at the specific or subspecific levels which are distributed in five sections or sub-genera (Brown 2010), mostly arranged in the northern temperate region, ranging from 20 to 400, mainly found in Asia, Europe and North America. The genus displays a huge ecological plastic, with species occurring from sea level to high mountains and from arid zones to wet lands. *Artemisia* species are used for the treatment of diseases such as malaria, hepatitis, cancer, inflammation and infections by fungi, bacteria and viruses (Ling 1991; Willcox 2009). Essential oils have been largely employed for their antiseptic properties (bactericidal, virucidal and fungicidal). Their fragrance is used in embalmment, preservation of foods, and as microbicidal, analgesic, sedative, anti-inflammatory, spasmodytic and locally anaesthetic remedies (Bakkali et al. 2008). Iranian folk medicine used some *Artemisia* species for their various medicinal properties; in particular, local people used aerial parts of Artemisia species for their antiviral and spasmylic effects (Zargari 1996; Ramezani et al. 2004). Essential oils of different *Artemisia* species, have been used in the flavour industry and in medication (Gilemeister & Hoffmann 1961; Juteau et al. 2002). The genus *Artemisia* shows a wide range of morphological and phytochemical variability depending on different geographic origins of the samples. Polyploidy and cytotypes differ in external morphology, anatomy, fertility, phytochemistry and cytogenetics (Valles et al. 2011). The chemical composition of essential oils from the Artemisia genus has been extensively studied in several species from around the world (Abad et al. 2012). Abad et al. reported that *Artemisia* species display significant intraspecific variations in the terpene constituents of their essential oils as camphor, 1,8-cineole, cis-davanone, 4-terpineol, linalool, β-fenchyl alcohol and borneol; in particular, this report showed that the main constituents of the oils vary with changes in altitude (Jalali & Serehti 2007). *Artemisia. caerulescens* subsp. *densiflora* (Viv.) Gamisans ex Kerguélen & Lambinon (Asteraceae) is one of the endemic species of Sardinia and Corsica mainly found in Razzoli, along the coastal area and cliff of the granitic substrate. This plant is a small shrub, growing only 10–30 cm in height; the leaves are divided into linear segments with the upper ones progressively reduced, the heads grouped into a terminal ear and fruits are striated achenes. *A. caerulescens* subsp. *densiflora* is an aromatic plant that shows a very strong aromatic and characteristic scent. At present, *A. caerulescens* subsp. *densiflora* is considered an unexpected species (Pieri & Giusti 2009). In literature, there are few reports on the analysis of the essential oil of the Mediterranean population (Petretto et al. 2013). Interestingly, the variation in essential oil content between varieties and/or subspecies has been documented due to the existence of various chemotypes (or chemical races) into the species. The aim of this research was to investigate the chemical composition and the biological activity of the essential oil of *A. caerulescens* subsp. *densiflora* growing in the La Maddalena Islands. For this purpose, the antioxidant and antimicrobial activities and cytotoxicity on tumour cells of the essential oil were evaluated.

2. Results and discussion

2.1. Essential oil analysis

The composition of the essential oil hydrodistilled from *A. caerulescens* subsp. *densiflora* growing in Sardinia is reported in Table 1. A total of 73 volatile components were identified,
Table 1. Essential oil composition of Artemisia densiflora from Sardinia.

| N. | Componenta | RI calc.b | RI lit.c | %d | ID² |
|----|------------|-----------|----------|----|-----|
| 1  | Ethyl tiglate | 928 | 929 | 0.1 | RI,MS |
| 2  | 4-Methyl pent-2-enolide | 944 | 952 | 0.1 | RI,MS |
| 3  | α-Terpinene | 1009 | 1014 | 0.1 | RI,MS |
| 4  | p-Cymene | 1017 | 1020 | 0.1 | Std |
| 5  | Thuylol | 1018 | 0.1 | MS |
| 6  | 1,8-Cineole | 1022 | 1026 | 0.1 | Std |
| 7  | Santolina alcohol | 1031 | 1034 | tr | RI,MS |
| 8  | Lavender lactone | 1035 | 1034 | 0.1 | RI,MS |
| 9  | cis-Arbusculone | 1043 | 1046 | 1.9 | RI,MS |
| 10 | γ-Terpinene | 1050 | 1054 | 0.3 | Std |
| 11 | cis-Sabinene hydrate | 1058 | 1065 | 5.1 | RI,MS |
| 12 | trans-Arbusculone | 1063 | 1066 | 1.2 | RI,MS |
| 13 | Artemisia alcohol | 1078 | 1080 | tr | RI,MS |
| 14 | Terpinolene | 1079 | 1086 | 0.1 | Std |
| 15 | trans-Sabinene hydrate | 1089 | 1098 | 2.1 | RI,MS |
| 16 | Linalool | 1094 | 1095 | 0.3 | Std |
| 17 | Hotrienol | 1099 | 1101 | 0.1 | RI,MS |
| 18 | cis-p-Menth-2-en-1-ol | 1112 | 1118 | 0.6 | RI,MS |
| 19 | trans-Pinocarveol | 1128 | tr | 0.3 | Std |
| 20 | trans-p-Menth-2-en-1-ol | 1130 | 1136 | 0.3 | RI,MS |
| 21 | cis-Chrysanthenol | 1155 | 1160 | 0.2 | RI,MS |
| 22 | Terpinen-4-ol | 1167 | 1174 | 4.7 | Std |
| 23 | Dill ether | 1176 | 1184 | tr | RI,MS |
| 24 | p-Cymen-8-ol | 1178 | 1179 | tr | RI,MS |
| 25 | α-Terpineol | 1181 | 1186 | 0.2 | Std |
| 26 | cis-Piperitol | 1185 | 1195 | 0.1 | RI,MS |
| 27 | trans-Piperitol | 1198 | 1207 | 0.4 | RI,MS |
| 28 | trans-Carveol | 1202 | 1215 | tr | RI,MS |
| 29 | cis-Sabinene hydrate acetate | 1211 | 1219 | tr | RI,MS |
| 30 | Piperitone | 1245 | 1249 | tr | RI,MS |
| 31 | 2,4-Dimethylphenethyl alcohol | 1263 | 0.1 | MS |
| 32 | Terpinen-4-ol acetate | 1291 | 1299 | 0.1 | RI,MS |
| 33 | Teresantalol | 1294 | 1286 | 0.2 | RI,MS |
| 34 | Carvacrol | 1300 | 1298 | 0.1 | Std |
| 35 | Eugenol | 1351 | 1356 | 0.1 | Std |
| 36 | α-Copaene | 1361 | 1374 | 0.1 | RI,MS |
| 37 | (E)-methyl cinnamate | 1375 | 1376 | 0.1 | RI,MS |
| 38 | β-Elemene | 1379 | 1389 | 0.2 | RI,MS |
| 39 | (E)-Jasmone | 1384 | 1390 | 0.1 | RI,MS |
| 40 | (Z)-Jasmone | 1389 | 1392 | 1.0 | RI,MS |
| 41 | (E)-Caryophyllene | 1402 | 1417 | 0.3 | Std |
| 42 | α-Humulene | 1436 | 1452 | 0.1 | Std |
| 43 | allo-Aromadendrene | 1444 | 1458 | 0.8 | RI,MS |
| 44 | γ-Murolene | 1463 | 1478 | 0.1 | RI,MS |
| 45 | Germacrene D | 1465 | 1484 | 0.3 | RI,MS |
| 46 | β-Selinene | 1469 | 1489 | 0.6 | RI,MS |
| 47 | Bicyclogermacrene | 1480 | 1500 | 0.5 | RI,MS |
| 48 | Davana ether | 1486 | 1487 | 3.3 | RI,MS |
| 49 | Gymnomitrone | 1491 | 0.8 | MS |
| 50 | γ-Cadinene | 1498 | 1513 | 0.3 | RI,MS |
| 51 | Cubebol | 1500 | 1514 | 0.2 | RI,MS |
| 52 | Davana ether | 1507 | 1514 | 8.2 | RI,MS |
| 53 | β-Cadinene | 1510 | 1522 | 0.7 | RI,MS |
| 54 | Davana ether | 1526 | 6.0 | MS |
| 55 | α-Calacorene | 1532 | 1544 | 0.4 | RI,MS |
| 56 | cis-Cadinene ether | 1535 | 1552 | 0.3 | RI,MS |
| 57 | trans-Cadinene ether | 1540 | 1557 | 0.8 | RI,MS |
| 58 | (E)-Nerolidol | 1556 | 1561 | 4.5 | Std |
| 59 | Germacrene D-4-ol | 1560 | 1574 | 1.2 | RI,MS |
| 60 | Caryophyllene oxide | 1564 | 1582 | 0.8 | Std |
| 61 | Davanone isomer | 1570 | 1588 | 0.2 | RI,MS |
| 62 | Globulol | 1574 | 1590 | 0.3 | RI,MS |
| 63 | Viridiflorol | 1584 | 1592 | 0.4 | Std |

(Continued)
corresponding to 63.3% of the total composition. The major fraction of the oil was represented by oxygenated sesquiterpenes (32.0%), followed by oxygenated monoterpenes (14.9%), while monoterpene and sesquiterpene hydrocarbons occurred in small amounts (0.6 and 4.4%, respectively). The major volatile components of *A. caerulescens* subsp. *densiflora* were davana ethers, sesquiterpenoids containing two reduced furan rings, accounting together for 17.5% of the total composition. These components are formed from oxidation of davanone (Thomas & Dubini 1971) which is the main component of davana oil obtained from *A. pallens* (Coleman et al. 2007). Davanone is also found in other *Artemisia* species (Perez-Alonso et al. 2003; Ramezani et al. 2004; Sadeghpour et al. 2004; Vajs et al. 2004), being of chemotaxonomic value. Davanone has been reported to be odourless. Therefore, the odouriferous constituents of davana oil have been reported to be the davana ethers (Thomas & Dubini 1974). In particular, three out of four stereoisomers of davana ethers occurring in nature (Garneau et al. 2013) were detected in the essential oil of *A. caerulescens* subsp. *densiflora* investigated. Other oxygenated sesquiterpenes occurring in noteworthy amounts were (E)-nerolidol (4.5%) and β-oplopenone (3.3%). *cis*-Sabinene hydrate (5.1%) and terpinen-4-ol (4.7%) were the most abundant components among oxygenated monoterpenes. Among other components (11.3%), noteworthy was the presence of (Z)-methyl jasmonate (2.9%) and of *cis*– and *trans*-arbuscolone (1.9 and 1.2%, respectively, the latter reported as marker components of other *Artemisia* species (Lopes-Lutz et al. 2008). The essential oil from *A. caerulescens* subsp. *densiflora* growing in the archipelago of La Maddalena (Sardinia, Italy) was previously analysed for composition by Petretto et al. (2013). These authors found terpinen-4-ol (22%) as the major component, followed by *p*-cymene (7.6%) and α-terpineol (3.0%). Our results showed a significantly different composition, with the presence of davanone derivatives as the main characteristic of the essential oil. Also, the study of Biondi et al. (2000) reported a different composition with respect to our report, with α- and β-thujone and camphor as the major constituents (Biondi et al. 2000). Thus, it seems that *A. caerulescens*

| N. | Component       | RI calc. | RI lit. | % | ID  |
|----|----------------|----------|---------|---|-----|
| 64 | β-Oplopenone    | 1593     | 1607    | 3.3 | RI,MS |
| 65 | Muurola-4,10(14)-dien-1-beta-ol | 1614 | 1630 | 0.5 | RI,MS |
| 66 | epi-α-cadinol   | 1626     | 1638 | tr | RI,MS |
| 67 | epi-α-muurolol  | 1627     | 1640 | 1.1 | RI,MS |
| 68 | Desmethoxy encecalin | 1635 | 1646 | 0.2 | RI,MS |
| 69 | (Z)-methyl jasmonate | 1639 | 1648 | 2.9 | RI,MS |
| 70 | 2,4-Dimethylether-phloroacetophenone | 1658 | 1667 | 1.8 | RI,MS |
| 71 | Hexadec-(11E)-en-1-ol | 1765 |       | 0.1 | MS  |
| 72 | 2-Pentadecanone, 6,10,14-trimethyl- | 1836 | 1838 | 0.1 | RI,MS |
| 73 | Kaur-16-ene | 2002 | 2003 | 1.6 | RI,MS |

Table 1. (Continued).

Compounds are listed in order of their elution from a HP-5MS column.

1 Linear retention index on HP-5MS column, experimentally determined using homologous series of C₈–C₃₀ alkanes.

2 Linear retention index taken from Adams (2007) and NIST 08 (2008).

3 Percentage values are means of three determinations with a RSD% in all cases below 10%.

4 Identification methods: std, based on comparison with authentic compounds; MS, based on comparison with WILEY, ADAMS and NIST 08 MS databases; RI, based on comparison of LRI with those reported in ADAMS, FFNSC 2 (2012) and NIST 08.

5 tr, % below 0.1%.
subsp. densiflora possesses a chemical polymorphism, with different chemotypes related to extrinsic and intrinsic factors of the species (Rosselli et al. 2012).

2.2. Antioxidant activity

The essential oil of A. caerulescens subsp. densiflora showed poor ability to scavenge free radical species (DPPH test systems) with respect to synthetic chemicals such as Trolox. In fact, more than 150 µL of essential oil was needed to equal the activity of 1 mg of Trolox. Of some interest was the inhibition of lipid oxidation (β-carotene-linoleate system) with respect to synthetic chemicals such as BHT. In this regard, 1 mg of BHT showed the same antioxidant power of about 28 µL of essential oil. Finally, the essential oil exhibited a quite interesting capacity to scavenge the hypochlorous acid with an activity only 13 times lower than that of Trolox. The correlation between antiradical/oxidant activity of essential oils and their chemical composition is not well understood. Some essential oils rich in phenolic compounds exhibited good free radical scavenging capacity, but also some essential oils that are phenolic free exhibited good antioxidant activity (Miguel 2010). The essential oil of A. caerulescens subsp. densiflora was rich in sesquiterpene hydrocarbons and oxygenated sesquiterpenes and poor in phenolic compounds (about 0.4%): we expect that this oil could be classified as a scarcely antiradical and antioxidant agent based on the actual relation between chemical composition and biological properties. The data resulting from the DPPH, β-carotene-linoleic acid and ClO− scavenging assay indicated that there is a different interaction of the oil components with the molecular mechanisms: the oil is inactive as antiradical but in same way active as antioxidant for the protection of unsaturated lipids and promising as ClO− scavenger (Table 2).

2.3. Cytotoxic activity

The cytotoxic activity of A. caerulescens subsp. densiflora essential oil was evaluated on human tumour cell lines: A375 human malignant melanoma cell line, MDA-MB-231 human breast adenocarcinoma cell line and HCT116 human colon carcinoma cell line by MTT assay. All cell lines were submitted to increasing concentrations of essential oil for 72 h. The results, collected in Table 3, showed that essential oil exhibited a significant cytotoxic activity against the human cancer cells examined and induced a concentration-dependent inhibitory effect in the dilutions ranging from 0.78 to 200 µg/mL. The IC50 values of the essential oil were 5.20, 5.49 and 7.61 µg/mL on A375, HCT116 and MDA-MB-231 cell lines, respectively. This is the first report of the cytotoxic activity of A. caerulescens subsp. densiflora essential oil. A compound or few compounds do not emerge from the composition of essential oil which can be responsible for the cytotoxic activity on human tumour cell lines. Terpinen-4-ol resulted active on melanoma as reported in the literature (Calcabrini et al. 2004), which potently induces cell cycle arrest, apoptosis and necrotic cell death (Greay et al. 2010). (E)-nerolidol

|                  | DPPH test | Linoleic test | Taurine test |
|------------------|-----------|---------------|--------------|
|                  | IC50 (mg/mL) | IC50 (mg/mL) | IC50 (mg/mL) |
| Essential oil    | 0.408 ± 0.053 | 0.380 ± 0.06 | 0.192 ± 0.03 |
| Trolox           | 0.003 ± 0.001 |              |              |
| BHT              |            | 0.013 ± 0.002 |              |
was toxic on HepG2 human hepatoma cell line (Ferreira et al. 2012). Their concentrations do not explain the high cytotoxic activity of the essential oil. The cytotoxic activity could be due to the synergism of the most abundant components or to synergism with the components not identified.

2.4. Antimicrobial activity

Antimicrobial activity of the essential oil was determined against two representative species belonging to the Gram-positive bacteria (Staphylococcus aureus and Enterococcus faecalis), two Gram-negative species (Pseudomonas aeruginosa and Escherichia coli) and one representative fungus, Candida albicans. Results of the test are summarised in Table 4. The oil inhibited the growth of all microbes with the exception of P. aeruginosa. The yeast C. albicans was the most susceptible micro-organism. Nevertheless, the overall activity was very low (average size of the inhibition zone diameter 8.6 mm ± 1.1 mm) and made us judge that further analysis and testing was not required. Overall composition analysis of the oil was suggestive of terpinen-4-ol as being the component more probably responsible for the observed activity (Bonikowski et al. 2015).

3. Conclusion

The essential oil composition evaluated in A. caerulescens subsp. densiflora growing in the La Maddalena Archipelago suggests the existence of a worthy chemical polymorphism among population coming from different areas. Having found 73% of revealed compounds, our analysis shows that the rest of the compounds were unrecognisable, notwithstanding our

| Table 3. In vitro cytotoxic activity of A. caerulescens subsp. densiflora essential oil. |
|-------------------------------------|---------------------------------|------------------|
| Cell line (IC50 µg/mL)a             | A375b                           | MDA-MB 231c      | HCT116d          |
| Essential oil                      | 5.20                            | 7.61             | 5.49             |
| 95% CI                             | 4.74–6.08                       | 6.90–8.38        | 4.68–6.42        |
| Positive control                   |                                 |                  |
| Cisplatin                          | 0.49                            | 2.85             | 2.68             |
| 95% CI                             | 0.41–0.54                       | 2.16–3.11        | 2.23–3.05        |

| Table 4. Antimicrobial activity of A. caerulescens subsp. densiflora essential oil. |
|-------------------------------------|---------------------------------|------------------|
| Inhibition zone diameter (mm ± SD)  | S. aureus ATCC 29213            | E. faecalis ATCC 29212 | E. coli ATCC 25922 | P. aeruginosa ATCC 27853 | C. albicans ATCC 24433 |
| Essential oil                       | 8.0 ± 0.0                       | 7.0 ± 0.5        | 8.0 ± 0.8         | 6.0 ± 0.0                | 10.2 ± 0.8             |
| Reference antimicrobial             | 22.8 ± 0.5a                     | 20.3 ± 0.5a      | 28.0 ± 2.0a       | 28.7 ± 2.9a             | 17.0 ± 4.4a            |

|aIC50 = The concentration of compound that affords a 50% reduction in cell growth (after 72 h of incubation). |
|bHuman malignant melanoma cell line. |
|cHuman breast adenocarcinoma cell line. |
|dHuman colon carcinoma cell line. |
|eConfidence interval. |
extremely high performing database. Therefore, we can hypothesise that the remainder of
the contents are extremely rare molecules. In comparison to the composition of the oils
from the same population (Biondi et al. 2000; Pieroni & Giusti 2009; Petretto et al. 2013),
our sample showed a higher content of davana ethers (17.5%) missing in that report, and an
unexpected higher content of sesquiterpenes. Moreover, other marker components detected
by Petretto et al. (2013) such as their major constituent terpinen-4-ol, ρ-cymene and α-terpi-
neol showed a significantly different level content. For the first time, we report the cytotoxic
activity of the essential oil of A. caerulescens subsp. densiflora, which showed a significant
activity inhibition on the growth of three human cancer cells.

Disclosure statement
No potential conflict of interest was reported by the authors.

ORCID
Alessandro Venditti http://orcid.org/0000-0003-1492-6739

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