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# Aroma Profile and Essential Oil Composition of *Helichrysum* species

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Essential oils and volatile emission from leaves and flowers of three *Helichrysum* species (*H. arenarium*, *H. nudifolium*, *H. graveolens*) were analysed by GC-MS and SPME analysis. A total of 112 components were identified in the EOs representing 92.3-99.9% of the whole oil composition, while 109 constituents were identified in the spontaneous volatile emission accounted for 78.7-98.8% of the total volatile organic compounds (VOCs). Sesquiterpenes represented the main class of constituents in the EOs of the majority studied species. β-caryophyllene was the key compound of *H. arenarium* and *H. nudifolium* leaves, even though (*E*)-2-hexenal showed high percentage in *H. arenarium* leaves. *H. graveolens* EO showed a good amount of diterpenes (cembrene and beyerene), absent in the other species. SPME analyses were carried out for the first time to complete the chemotaxonomic investigation on the volatile organic constituents of these plants.

**Keywords:** *Helichrysum arenarium*, *H. nudifolium*, *H. graveolens*, *Asteraceae*, HS-SPME, GC-MS.

Genus *Helichrysum* Mill. (*Asteraceae*) consists of approximately 500 species distributed worldwide, of which 250 are indigenous to South Africa (including Namibia), while only 16 are spontaneous in Europe and in the Mediterranean areas, including Italy [1-5].

Three *Helichrysum* species (*H. arenarium*, *H. nudifolium*, *H. graveolens*) native from different geographical areas but acclimatised and grown in Italy in homogeneous conditions (CREA-Sanremo collection) were selected in this work to evaluate their aromatic profile and contribute to their ornamental or industrial applications. *Helichrysum arenarium* (L.) Moench, is a perennial herb, 50 cm height, that grows in dry sandy and semi-desert soils, dunes, steppes of a large geographic area including Scandinavia, Atlantic Europe, Balkans, European Russia, Siberia, Caucasus, Asia Minor, Central Asia, Mongolia and China. This species has greyish leaves, with stems that are covered with white trichomes and the colour of flowers varies from yellow to orange. *H. arenarium* is used to treat stomach pain, asthma, arthritis disorders, cystitis and jaundice and its inflorescences are traditionally used in Central Europe as antiseptic and spasmolytic remedies [6]. It is also used as repellent against brown house moths [7]. Many studies confirmed several biological properties of the species *H. arenarium* including antioxidant, hepatoprotective, antibacterial, antiviral, antifungal, anti-inflammatory and antiproliferative activities [8-11] together with psychotropic effects [12]. Essential oil from *H. arenarium* showed antioxidant and antimicrobial properties [13].

*Helichrysum nudifolium* (L.) Less is found in grasslands throughout Southern Africa, with solitary flowering stems which can be tall till 1.5 m. The inflorescence is flat-topped contracted head-like. This species has radical leaves nerved, linear or lanceolate, elliptic to ovate, thinly and thickly white-woolly [14, 15, 16]. The leaves of *H. nudifolium* are externally used for wounds and respiratory infections; moreover, the poultice of this plant is recommended for swellings and the roots are employed as antiemetic and antiseptic remedies [2b]. No papers on biological activity of *H. nudifolium* extracts are reported in the literature to confirm its folk uses.

*H. graveolens* (M. Bieb.) Sweet grows in high pastures, subalpine and alpine meadows of Crimea, Caucasus, Balkan Peninsula, Asia Minor, Armenia and Kurdistan. It is a greyish-green plant with yellow flowers and linear or linear lanceolate leaves, acute, often spinulose [16]. Flowers from *H. graveolens* are used in the folk medicine of Turkey for treatment of diabetes mellitus, wound, jaundice and as diuretic [17, 18, 19]. Antidiabetic and antioxidant effects together with its use to treat urolithiasis have been reported in the literature for *H. graveolens* flowers [19, 20- 21].

The chemical composition of the EOs and the spontaneous emission of volatiles (SPME) obtained from *H. arenarium* (*Ha*), *H. nudifolium* (*Hn*) and *H. graveolens* (*Hg*) flowers (*Fl*) and leaves (*Le*) were analysed by GC-FID and GC/MS analyses. The results are reported in Table 1 and 2, respectively. A total of 112 components were identified in the EOs representing 92.3-99.9% of the whole oil composition, while 109 compounds were identified by SPME analysis accounted for 78.7-98.8% of the total volatile organic compounds (VOCs).

From the Hierarchical analysis, HCA and the principal component analysis (PCA) three groups of EOs were obtained (Fig 1 and 2, respectively). One of these groups (I) was formed by *Ha* (flowers and leaves) and *Hn* flowers. The composition of EOs from *Ha* leaves and *Hn* flowers was dominated by sesquiterpene hydrocarbons (SH), whereas the *Ha* flower showed a similar amount of SH and non-terpene (NT) compounds. Monoterpenes were completely missing in *Hn* sample. The main constituents in group I were: β-caryophyllene, β-pinene, 1,8-cineole, pentadecanoic acid, 6,10,14-trimethyl-2 pentadecanone and bis-(2-methylpropylester)-1,2-benzendicarboxylic acid. *Hn* leaves are insert in group II because they were characterized by sesquiterpene hydrocarbons (SH) and non-terpenes (NT) by β-caryophyllene (43.9%) for SH and (*E*)-2-hexenal (41.0%) and (*Z*)-lanceol acetate (11.6%) for NT. Group III (*Hg* leaves and flowers) showed mainly sesquiterpene hydrocarbons (α-neo-clovene, *cis*-β-guaiene, *trans*-β-guaiene, selina-1(2)4-diene) and oxygenated sesquiterpenes (OS: γ-eudesmol, 7-epi-α-eudemol and valerianol). The diterpenes (DT)

**Table 1:** Chemical composition of EOs from the three selected *Helichrysum* species and organs.

| | | | | Content (%)[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Entry* | Class | Components | *LRI* | *Ha* Fl | *Ha* Le | *Hn* Fl | *Hn* Le | *Hg* Fl | *Hg* Le |
| *1* | NT | (*E*)-2-Hexenal | 854 | | 1.8 | | 41.0 | | 0.6 |
| *2* | MH | α-Pinene | 939 | | 3.5 | | | tr | 0.9 |
| *3* | MH | Tricyclene | 926 | 2.0 | | | | | |
| *4* | MH | Camphene | 953 | tr | 0.3 | | | | tr |
| *5* | NT | 2-(*Z*)-Heptenal | 956 | | | | | 0.1 | |
| *6* | MH | β-Pinene | 980 | 7.4 | 9.5 | | | | 0.3 |
| *7* | MH | Myrcene | 991 | | 0.6 | | | | |
| *8* | NT | 6-Methyl-5-hepten-2-one | 985 | | | | | | 0.1 |
| *9* | NT | 2-Pentyl-furan | 995 | 0.3 | | | | 0.4 | 0.2 |
| *10* | NT | *trans*-2-(2-Pentenyl) furan | 1004 | | | | | | 0.1 |
| *11* | NT | *n*-Octanal | 1001 | 0.2 | 1.0 | | | 0.4 | |
| *12* | MH | α-Terpinene | 1018 | 0.2 | 0.4 | | | | 0.1 |
| *13* | MH | *p*-Cymene | 1026 | | 0.3 | | | | |
| *14* | MH | Limonene | 1031 | 2.0 | 5.2 | | | tr | |
| *15* | OM | 1,8-Cineole | 1033 | 2.4 | 8.3 | | | tr | 1.6 |
| *16* | MH | γ-Terpinene | 1062 | 0.7 | 2.7 | | | 0.2 | 0.2 |
| *17* | MH | Terpinolene | 1088 | 0.5 | 1.3 | | | tr | tr |
| *18* | NT | *n*-Nonanal | 1102 | 0.5 | 1.8 | 1.6 | | 1.7 | 0.1 |
| *19* | OM | Exo-fenchol | 1121 | tr | 0.3 | | | | tr |
| *20* | OM | Pinocampheol | 1173 | | | | | 0.3 | |
| *21* | OM | Borneol | 1165 | 0.3 | tr | | | | |
| *22* | OM | Terpinen-4-ol | 1177 | tr | 0.4 | | | | tr |
| *23* | OM | α-Terpineol | 1189 | | 1.6 | | | tr | |
| *24* | OM | γ-Terpineol | 1198 | 1.3 | | | | | |
| *25* | AC | Safranal | 1199 | | | | | 0.2 | |
| *26* | NT | *n*-Decanal | 1204 | 0.8 | 0.3 | 5.3 | | 1.2 | |
| *27* | NT | Butanoic acid, 3-methyl-3-hexen-1-yl-ester | 1199 | | 0.9 | | | | |
| *28* | NT | Hexyl-3-methylbutanoate | 1243 | | 0.9 | | | | |
| *29* | OM | Geraniol | 1255 | | | | | | 0.5 |
| *30* | NT | (*E*)-2-Decenal | 1261 | | | | | 0.2 | |
| *31* | OM | Perilla aldehyde | 1271 | | 0.5 | | | | tr |
| *32* | NT | 1-Dihydroedulan | 1295 | | | | | | 0.2 |
| *33* | NT | Undecanal | 1306 | | | 3.4 | | 0.2 | |
| *34* | NT | (2*E*, 4*Z*)-Decadienal | 1314 | | | | | 0.3 | |
| *35* | SH | α-Ylangene | 1372 | tr | 0.2 | | | 0.8 | |
| *36* | SH | α-Copaene | 1376 | 0.9 | 1.6 | | | 1.4 | 0.8 |
| *37* | SH | α-Duprezianene | 1388 | | | | | 1.2 | |
| *38* | NT | Tetradecane | 1399 | 0.2 | | | | 0.4 | |
| *39* | NT | Dodecanal | 1407 | | | 0.9 | | tr | |
| *40* | SH | β-Caryophyllene | 1418 | 27.5 | 46.0 | 27.0 | 43.9 | 1.9 | 1.9 |
| *41* | SH | *trans*-α-Bergamotene | 1439 | | | 8.6 | | | |
| *42* | SH | β-Gurjunene | 1432 | | | | | tr | 0.2 |
| *43* | SH | α-Guaiene | 1439 | | | | | 0.5 | |
| *44* | SH | α-Neo-clovene | 1445 | | | | | 3.2 | 3.5 |
| *45* | SH | *cis*-Muurola-3,5-diene | 1446 | | | | | | 1.8 |
| *46* | SH | (*E*)-β-Farnesene | 1458 | | 3.6 | | | | 1.2 |
| *47* | SH | β-Cadinene | 1474 | | | | | | 2.5 |
| *48* | SH | *trans*-Cadina-1(6),4-diene | 1470 | | | | | 1.8 | |
| *49* | SH | α-humulene | 1454 | 1.4 | 1.3 | 1.1 | | | |
| *50* | SH | 9-Epi-(*E*)-caryophyllene | 1467 | 2.2 | | | | | |
| *51* | SH | *cis*-Muurola-4(14), 5-diene | 1460 | | | | | 1.6 | 0.4 |
| *52* | SH | γ-Muurolene | 1477 | 1.2 | 0.9 | | | | 9.2 |
| *53* | SH | Viridiflorene | 1493 | | 0.3 | | | | |
| *54* | SH | *cis*-β-Guaiene | 1490 | | | | | 6.4 | 7.3 |
| *55* | SH | Valencene | 1491 | 0.3 | | | | 2.1 | |
| *56* | SH | δ-Amorphene | 1505 | 0.9 | | | | 2.0 | 1.4 |
| *57* | SH | Cadina-1(10),4-diene | 1527 | | | | | | 2.1 |
| *58* | OS | 10-Epi-cubebol | 1534 | | | | | 1.2 | |
| *59* | SH | Bicyclogermacrene | 1494 | | | 8.0 | tr | | |
| *60* | SH | *trans*-β-Guaiene | 1500 | | | | | 5.6 | 1.8 |
| *61* | NT | Tridecanal | 1509 | | | 5.3 | | | |
| *62* | SH | *trans*-γ-Cadinene | 1513 | 1.0 | 0.7 | | | | 1.8 |
| *63* | SH | δ-Cadinene | 1524 | 3.2 | 1.7 | | | 2.3 | 3.8 |
| *64* | SH | α-Cadinene | 1538 | 0.2 | | | | | |
| *65* | SH | *trans*-Cadina-1(2)4-diene | 1533 | | | | | | 0.6 |
| *66* | SH | Selina-3,7-(11) diene | 1542 | tr | | | | 4.7 | 0.4 |
| *67* | SH | α-Calacorene | 1542 | tr | | | | 0.6 | |
| *68* | SH | β-Calacorene | 1563 | | | | | 1.2 | |
| *69* | OS | Caryophyllene alcohol | 1568 | 0.2 | | | | | |
| *70* | OS | Caryophyllene oxide | 1581 | 1.7 | 1.3 | 7.8 | tr | | |
| *71* | OS | Viridiflorol | 1590 | | | | | 0.6 | 0.7 |
| *72* | OS | Guaiol | 1595 | | | | | 0.6 | 1.2 |
| *73* | NT | Hexadecane | 1600 | 0.1 | | 1.5 | | | |
| *74* | OS | 5-Epi-7-epi-α-eudesmol | 1606 | | 0.2 | | | | |
| *75* | OS | 1,10-Di-epi-cubenol | 1614 | 0.7 | | | | 0.5 | |
| *76* | OS | Cadin-4-en-7-ol | 1637 | | | | | | 1.6 |
| *78* | OS | α-Acorenol | 1630 | | | 1.1 | | | |
| *79* | OS | 10-Epi-γ-eudesmol | 1619 | | | | | 0.4 | |
| *80* | OS | γ-Eudesmol | 1630 | | | | | 6.1 | 10.4 |
| *81* | OS | 1-Epi-cubenol | 1627 | 0.5 | 0.3 | | | | |
| *82* | OS | Hinesol | 1638 | | | | | | 4.7 |
| *83* | OS | Epi-α-cadinol | 1640 | | tr | | | 2.6 | |
| *84* | OS | α-Muurolol | 1645 | 1.1 | | | | | |
| *85* | OS | Valerianol | 1655 | | | | | 4.0 | 2.7 |
| *86* | OS | 7-Epi-α-eudesmol | 1605 | 0.4 | | | | 2.1 | 7.3 |
| *87* | OS | α-Cadinol | 1652 | 0.6 | | | | | |
| *88* | OS | Elemol acetate | 1680 | | | | | 0.2 | |
| *89* | NT | 1-Heptadecene | 1695 | 0.6 | | | | | |
| *90* | OS | β-Acorenone | 1685 | | | | | | 0.1 |
| *91* | NT | Davanol acetate | 1610 | | | | | | 0.1 |
| *92* | OS | Juniper camphor | 1691 | | | | | | 0.2 |
| *93* | NT | δ-Dodecalactone | 1671 | | | 7.5 | | | |
| *94* | NT | Pentadecanal | 1718 | 1.2 | | | | 0.8 | |
| *95* | NT | Tetradecanoic acid | 1766 | 1.7 | | | | 2.3 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *96* | OS | Hinesol acetate | 1783 | | | | | | 0.2 |
| *97* | OS | Nootkatone | 1999 | | | | | 0.7 | |
| *98* | NT | Octadecane | 1800 | | | 1.3 | | | |
| *99* | NT | Pentadecanoic acid, methyl ester | 1820 | 31.0 | | | | | |
| *100* | NT | 6,10,14-Trimethyl-2 pentadecanone | 1844 | | | 10.5 | | | |
| *101* | NT | Bis (2-methylpropylester)-1,2 benzenedicarboxylic acid | 1873 | | | 6.4 | | | |
| *102* | OS | Cedren-13-ol-acetate | 1788 | | | | | | 2.6 |
| *103* | NT | *n*-Nonadecane | 1900 | | | 0.3 | | | |
| *104* | DT | Totarene | 1922 | | | | | | 1.6 |
| *105* | NT | (*Z*)-Lanceol acetate | 1860 | | | | 11.6 | 0.7 | 0.3 |
| *106* | DT | Beyerene | 1924 | | | | | 0.7 | 12.7 |
| *107* | DT | Cembrene | 1929 | | | | | 25.7 | 0.3 |
| *108* | OS | 6-Acetate-α-chenopodiol | 1960 | | | | | 0.6 | 0.4 |
| *109* | SH | Bifloratriene | 1977 | | | | | | 1.0 |
| *110* | NT | *n*-Hexadecanoic acid | 1978 | | | | | | 0.2 |
| *111* | DT | Neocembrene | 1959 | | | | | 1.9 | |
| *112* | NT | 1-Eicosene | 2000 | | | 1.2 | 1.1 | 0.4 | |
| | | ***Total identified*** | | ***96.8*** | ***99.7*** | ***98.8*** | ***99.8*** | ***92.3*** | ***96.0*** |
| | | *Non-terpene derivatives* | | *36.6* | *6.7* | *45.2* | *53.7* | *7.1* | *1.6* |
| | | *Monoterpene hydrocarbons* | | *12.8* | *23.8* | | | *0.2* | *1.5* |
| | | *Oxygenated monoterpenes* | | *4.0* | *11.1* | | | *0.3* | *2.1* |
| | | *Sesquiterpene hydrocarbons* | | *38.8* | *56.3* | *44.7* | *43.9* | *36.1* | *43.1* |
| | | *Oxygenated sesquiterpenes* | | *5.2* | *1.8* | *8.9* | | *20.3* | *33.6* |
| | | *Diterpenes* | | | | | | *28.3* | *14.6* |

[a]) Compounds present at less than 0.1% were excluded from the table. [b]) *LRI:* Linear retention index. Relative contents of the EO constituents expressed as percentages. [c]) Species and organs analyzed: *Ha* Fl: *H. arenarium* flowers; *Ha* Le: *H. arenarium* leaves; *Hn* Fl: *H. nudifolium* flowers; *Hn* Le: *H. nudifolium* leaves; *Hg* Fl: *H. graveolens* flowers; *Hg* Le: *H. graveolens* leaves.

**Table 2**: Spontaneous volatile emission of the three selected *Helichrysum* species (SPME analysis)

| | | | | Content (%)[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Entry* | Class | Components | *LRI* | *Ha* Fl | *Ha* Le | *Hn* Fl | *Hn* Le | *Hg* Fl | *Hg* Le |
| *1* | NT | Hexanal | 810 | | | tr | | 0.1 | |
| *2* | NT | 2-Octene | 815 | | | | | 0.1 | |
| *3* | NT | (*E*)-3-Hexen-1-ol | 858 | | | 0.9 | | | |
| *4* | NT | 1-Nonene | 860 | 2.5 | | | | | |
| *5* | NT | Hexanol | 870 | 0.4 | | 0.5 | | 0.5 | |
| *6* | NT | (*Z*)-3-Hexenol | 858 | | tr | | 15.0 | | |
| *7* | NT | Isopentyl acetate | 876 | | | 0.2 | | | |
| *8* | NT | 3-Nonene | 899 | 0.2 | | | | tr | |
| *9* | MH | Tricyclene | 934 | 0.2 | | | | tr | |
| *10* | MH | α-Pinene | 940 | 15.8 | 4.0 | 0.2 | 0.2 | 0.3 | 2.2 |
| *11* | MH | Camphene | 953 | 0.5 | | tr | | | |
| *12* | NT | *n*-Heptanol | 973 | 0.5 | | | 0.1 | tr | |
| *13* | MH | β-Pinene | 981 | 55.2 | 5.8 | | | tr | 0.2 |
| *14* | NT | 1-Octen-3-ol | 978 | | | 0.3 | | | |
| *15* | NT | 3-Octanone | 987 | | | | 6.6 | | |
| *16* | MH | Myrcene | 993 | 0.5 | | | | tr | 0.1 |
| *17* | NT | 3-Octanol | 994 | | | 0.1 | | | |
| *18* | MH | *p*-Mentha-1(7),8-diene | 1004 | 0.4 | | | | | |
| *19* | NT | Octanal | 1002 | | | 0.1 | | | |
| *20* | NT | (*E*)-3-Hexenol acetate | 1007 | 0.2 | | 0.8 | | tr | |
| *21* | NT | (*Z*)-3-Hexenol acetate | 1008 | | 49.6 | | 70.5 | | 0.1 |
| *22* | NT | *n*-Hexyl acetate | 1010 | tr | | 0.1 | | | |
| *23* | MH | α-Terpinene | 1018 | 0.4 | | | | 0.2 | |
| *24* | MH | Limonene | 1032 | 5.2 | | | | 0.2 | tr |
| *25* | OM | 1,8-Cineole | 1036 | 5.1 | 7.4 | tr | | 0.9 | |
| *26* | MH | (*Z*)-β-Ocimene | 1041 | | | | | 0.3 | |
| *27* | MH | (*E*)-β-Ocimene | 1053 | 0.7 | 0.3 | | | 0.5 | |
| *28* | MH | Terpinolene | 1089 | 0.9 | | | | 0.2 | |
| *29* | MH | γ-Terpinene | 1062 | 1.4 | 0.3 | 0.2 | | | |
| *30* | NT | Isopentyl isovalerate | 1106 | | | | 0.3 | | |
| *31* | OM | Linalool | 1102 | | 0.6 | | | | |
| *32* | NT | *n*-Nonanal | 1103 | Tr | | 0.4 | 0.8 | tr | tr |
| *33* | OM | Exo-fenchol | 1117 | 0.1 | | | | | |
| *34* | OM | *cis*-*p*-Menth-2-en-1-ol | 1122 | 0.2 | | | | | |
| *35* | OM | (*E*)-Myroxide | 1143 | 0.1 | | | | tr | |
| *36* | OM | Camphor | 1148 | | 1.2 | tr | | | |
| *37* | OM | Camphene hydrate | 1150 | 0.1 | | | | | |
| *38* | NT | 1,4-Dimethoxy benzene | 1163 | | | 0.8 | | | |
| *39* | OM | 4-Terpineol | 1180 | 0.5 | | | | tr | |
| *40* | OM | Borneol | 1169 | 0.3 | 0.6 | | | | |
| *41* | NT | (*Z*)-3-Hexenyl butyrate | 1191 | | 0.4 | | | | |
| *42* | OM | α-Terpineol | 1190 | 1.6 | | | | tr | |
| *43* | NT | Decanal | 1206 | 0.1 | | 0.2 | | tr | |
| *44* | OM | Dihydro carveol | 1196 | | 0.5 | | | | |
| *45* | NT | *n*-Decanal | 1206 | | 0.3 | | 0.3 | | 0.1 |
| *46* | NT | Butanoic acid, (*Z*)-3-methyl-3-hexenyl ester | 1231 | | 11.1 | | | | |
| *47* | NT | Hexyl-3-metyl butanoate | 1242 | tr | 1.6 | | | | |
| *48* | OM | Perilla aldeyde | 1280 | tr | 0.2 | | | tr | |
| *49* | OM | Isobornyl acetate | 1287 | | 3.0 | 0.1 | | tr | |
| *50* | SH | Presilphiperfol-7-ene | 1328 | | | | | 0.1 | |
| *51* | SH | δ-Elemene | 1340 | | | 1.8 | | | 0.2 |
| *52* | SH | 7-Epi-silphiperfol-5-ene | 1345 | tr | tr | | | 0.3 | |
| *53* | PP | Eugenol | 1361 | | 0.2 | | | | |
| *54* | SH | α-Ylangene | 1372 | tr | 0.2 | | | 4.4 | 3.7 |
| *55* | SH | α-Copaene | 1376 | 0.5 | 0.8 | tr | | 4.8 | |
| *56* | SH | Isoledene | 1375 | | | | | | 1.9 |
| *57* | SH | β-Patchoulene | 1380 | | | | | 1.1 | |
| *58* | NT | (*Z*)-3-Hexenyl ester hexanoic acid | 1386 | | 0.8 | | | | |
| *59* | SH | Isolongifolene | 1385 | | | | | 0.3 | 0.2 |
| *60* | SH | β-Cubebene | 1389 | | | | | 0.3 | |
| *61* | NT | Hexyl *n*-hexanoate | 1388 | | 0.2 | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *62* | SH | Sativene | 1395 | | | | | 0.1 | |
| *63* | SH | β-Elemene | 1392 | tr | 0.2 | tr | | | |
| *64* | SH | Cyperene | 1398 | | | | | | 0.4 |
| *65* | SH | Isoitalicene | 1397 | | | | | 0.3 | |
| *66* | SH | Isocaryophyllene | 1405 | tr | | 1.2 | | | |
| *67* | PP | Methyl eugenol | 1407 | | 0.2 | | | | |
| *68* | NT | Dodecanal | 1409 | | | | 0.1 | | |
| *69* | SH | α-Gurjunene | 1409 | tr | | | | 6.1 | 4.0 |
| *70* | SH | β-Caryophyllene | 1418 | 1.0 | 1.0 | 79.4 | 2.5 | 6.0 | |
| *71* | SH | β-Gurjunene | 1432 | | | | | 1.4 | 9.6 |
| *72* | SH | β-Copaene | 1429 | 0.1 | 0.2 | | | | |
| *73* | SH | γ-Elemene | 1434 | | | 1.3 | | tr | 1.0 |
| *74* | SH | Aromadendrene | 1439 | | 0.4 | 0.2 | | | |
| *75* | SH | α-Guaiene | 1440 | tr | 0.1 | tr | | 1.2 | |
| *76* | SH | α-Neoclovene | 1454 | | | | | 10.6 | 11.6 |
| *77* | SH | α-Himachalene | 1450 | | tr | | | | |
| *78* | SH | α-Humulene | 1456 | | | 2.5 | | | |
| *79* | AC | Geranyl acetone | 1455 | tr | 0.4 | | 0.4 | | |
| *80* | SH | (*E*)-β-Farnesene | 1460 | 2.5 | 2.3 | | | | 10.3 |
| *81* | SH | *cis*-Muurola-4(14), 5-diene | 1463 | | | | | 4.5 | 1.7 |
| *82* | SH | Epizonarene | 1464 | | | | | 0.8 | |
| *83* | SH | Alloaromadendrene | 1462 | | | 0.1 | | 1.2 | |
| *84* | SH | γ-Himachalene | 1474 | | | | | tr | 0.3 |
| *85* | SH | *trans*-Cadina-1(6),4-diene | 1470 | tr | 0.1 | | | | |
| *86* | SH | γ-Muurolene | 1477 | 0.3 | 0.5 | tr | | 3.0 | 12.9 |
| *87* | SH | α-Muurolene | 1480 | | | | | | 3.2 |
| *88* | SH | Germacrene D | 1481 | | 1.4 | | | | |
| *89* | SH | β-Selinene | 1485 | tr | | | | 14.6 | 5.7 |
| *90* | SH | *cis*-β-Guaiene | 1489 | | | | | 11.0 | 5.5 |
| *91* | SH | δ-Selinene | 1492 | | | | | | 10.5 |
| *92* | SH | Valencene | 1493 | 0.1 | | | | 1.7 | 1.6 |
| *93* | SH | γ-Amorphene | 1494 | | 0.3 | | | | |
| *94* | SH | Bibyclogermacrene | 1495 | | | 5.5 | 0.3 | | |
| *95* | SH | *trans*-β-Guaiene | 1500 | 0.1 | 0.5 | tr | | | 1.2 |
| *96* | SH | δ-Amorphene | 1505 | tr | 0.1 | | | | 5.5 |
| *97* | SH | γ-Cadinene | 1513 | 0.2 | 0.1 | | | | 0.1 |
| *98* | SH | δ-Cadinene | 1523 | | 0.6 | | | | 1.1 |
| *99* | SH | *trans*-Cadina-1(2),4-diene | 1533 | tr | | | | 0.1 | |
| *100* | SH | Selina-3,7(11)-diene | 1542 | | | | | 0.5 | 1.2 |
| *101* | SH | α-Cadinene | 1537 | tr | | | | 0.7 | |
| *102* | OS | Spathulenol | 1568 | | | 0.2 | | | |
| *103* | OS | Prenopsan-8-ol | 1571 | | | | | | 0.1 |
| *104* | OS | Caryophyllene oxide | 1581 | | | 1.4 | | tr | |
| *105* | NT | Hexadecane | 1599 | | 0.1 | | | | |
| *106* | OS | 10-Epi-γ-eudesmol | 1627 | | | | | 0.1 | 0.2 |
| *107* | OS | Hinesol | 1638 | | | | | | 0.2 |
| *108* | OS | Caryophylla-4(14)8(15)-dien-5-ol | 1636 | | | 0.3 | | | |
| *109* | OS | (*Z*)-Lanceol acetate | 1856 | | | | | 0.2 | |
| | | ***Total identified*** | | ***97.8*** | ***97.6*** | ***98.8*** | ***97.0*** | ***78.7*** | ***96.6*** |
| | | *Non-terpene derivatives* | | *3.9* | *64.1* | *4.4* | *93.7* | *0.7* | *0.2* |
| | | *Monoterpene hydrocarbons* | | *81.2* | *10.4* | *0.4* | *0.2* | *1.7* | *2.5* |
| | | *Oxygenated Monoterpenes* | | *8.0* | *13.5* | *0.1* | | *0.9* | |
| | | *Sesquiterpene hydrocarbons* | | *4.8* | *8.8* | *92.0* | *2.8* | *75.1* | *93.4* |
| | | *Oxygenated Sesquiterpenes* | | | | *1.9* | | *0.3* | *0.5* |
| | | *Phenylpropanoids* | | | *0.4* | | | | |
| | | *Apocarotenoids* | | | *0.4* | | *0.4* | | |

[a]) Compounds present at less than 0.1% were excluded from the table. [b]) *LRI:* Linear retention index. Relative contents of the volatile constituents expressed as percentages. [c]) Species and organs analysed- *Ha* Fl: *H. arenarium* flowers; *Ha* Le: *H. arenarium* leaves; *Hn* Fl: *H. nudifolium* flowers; *Hn* Le: *H. nudifolium* leaves; *Hg* Fl: *H. graveolens* flowers; *Hg* Le: *H. graveolens* leaves.

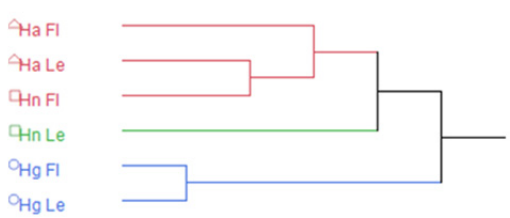


**Figure 1:** Dendrogram of the Hierarchical Cluster Analysis (HCA) of the EO composition of the three *Helichrysum* spp.

totarene, beyerene, cembrene, neocembrene, which were absent in *Ha* and *Hn*, contributed to the characterization of this group. β-caryophyllene represented a marker in the aroma of *H. arenarium* and *H. nudifolium*. High amount of sesquiterpenes chracterized the EO composition of the majority examined species except for *Hn* leaves.

These results agree with Maggio *et al*. [22] that reported the predominance of β-caryophyllene in the EOs from *Helichrysum stoechas*, *H. rupestre* and *H. italicum* cultivated in Italy. This

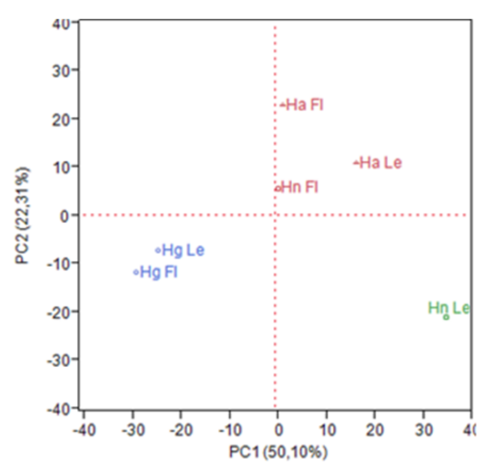


**Figure 2:** Principal Component Analysis (PCA) plot of the EO composition from the three examined *Helichrysum* spp..

compound, together with δ-cadinene, heneicosane and octadecane, was the main constituent of *H. arenarium* EO collected in Lithuania [7]. However aliphatic acids (34.6%) and their esters (28.5%)


**Figure 3:** Dendrogram of the Hierarchical Cluster Analysis (HCA) of volatiles emitted by the three *Helichrysum* spp..

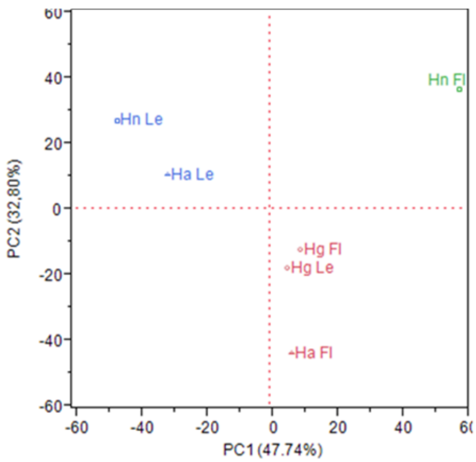


**Figure 4:** Principal Component Analysis (PCA) plot of volatile compounds spontaneously emitted by the three examined *Helichrysum* species.

together with linalool (1.7%), anethole (3.2%), carvacrol (3.6%) and α-muurolol (1.3%) were the major compounds in *H. arenarium* EO from Caucasus [8]. In a previous study from the literature aerial parts of *H. graveolens* grown in Turkey showed α-cubebene, β-caryophyllene, caryophyllene oxide and azulene-octahydro as main compounds [23] confirming the predominance of SH constituents, per other works [24, 25]. The EO of *H. arenarium* collected in Italy showed a similar composition of the same plant from Lithuania. Studies present in the literature showed the anti-inflammatory effects of β-caryophyllene and the strong antimicrobial properties of (*E*)-2-hexenal against pathogens confirming the use of these plants in the traditional medicine [26]. A different trend was found in the literature for *Helichrysum armenium* EO, another species growing in Iran, where limonene (21.2-29.2%) was the main constituent in all the aerial parts studied while in the present study was found in very low amount (0-5.2%) [27].

Monoterpenes were missing in *H. nudifolium* flowers and leaves but were present in low percentage in *H. graveolens*. Despite of several papers reported high number of monoterpenes in the EOs of several *Helichrysum* species (*H. gymnocephalum*, *H. dasyanthum*, *H. excisum*, *H. petiolare, H. italicum*), this class of constituents was absent also in other species like *H. felinum* [2a, 28-30].

In the SPME analysis of the *Helichrysum* spp. studied herein three groups can be observed by HCA and PCA statistical analysis (Fig 3 and 4): Group I with *Hn* and *Ha* leaves; Group II with only *Hn* flowers and Group III where *Hg* flowers and *leaves* had similar composition in comparison with *Ha* flowers. The leaves of *H. nudifolium* and *H. arenarium* showed a similar aroma profile with non-terpenes (NT) as predominant compounds. (*Z*)-3-hexenol acetate (49.6% to 70.5, respectively) were the main constituent in the volatiles of *H. arenarium* and *H. nudifolium* leaves. Monoterpene hydrocarbons as β-pinene (55.2%) and α-pinene (15.8%) were the main constituents in the volatiles from *H. arenarium* flowers. *H. nudifolium* flowers represented a single group where high amount of β-caryophyllene (79.4%) was evidenced. This compound was present in low percentage in the leaves of the same species as well as in the other species studied. *H. graveolens* flowers and leaves showed sesquiterpene hydrocarbons as major class of constituents. Within this class (*E*)-β-farnesene, γ-muurolene, δ-selinene exhibited high percentage in leaves, while β-selinene, *cis*-β-guaiene in the flowers. α-neoclovene was identified in both the plant organs. Diterpenes (DT), which were predominant in the EOs from *Hg* flowers and leaves, were absent in the spontaneous volatile emission of this plant. Since no studies are reported in the literature, this work represents the first contribution to the volatile emission of *H. graveolens*.

Since essential oils from several *Helichrysum* species have pharmaceutical and cosmetic uses, the knowledge of the EO composition and the aroma profile of the selected *Helichrysum* spp. can help to identify species and chemotypes useful for industrial or ornamental applications.

## Experimental

***Plant material:*** The studied specimens (*H. arenarium- Ha*, *H. nudifolium- Hn*, *H. graveolens*- *Hg*) were supplied by CREA- Unità di ricerca per la floricoltura e le specie ornamentali (Sanremo, Italy). The correct identifications were done by one of us (Claudio Cervelli) and the voucher specimens were deposited in the Herbarium of Giardini Botanici Hanbury, La Mortola, Ventimiglia (Imperia, Italy) (*Ha*: HMGB. e/ 9006.2015. 004; *Hn*: HMGBH. e/ 9006. 2015. 005; *Hg*: HMGBH. e/ 9006. 2015. 001).

***Essential Oil extraction:*** The aerial parts (leaves and flowers, separately) from the three *Helichrysum* species were subjected to hydrodistillation by Clevenger-type apparatus (3h). The essential oils (EOs) had very low yield (≤0.01%), so they were collected with *n*-hexane (HPLC grade) and stored at -4ºC in the dark until use.

***Solid Phase Micro Extraction analyses (SPME)*:** The analyses of the volatiles were performed using Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 µm) to establish the volatile organic compound (VOC) emission of flowers and leaves of each *Helichrysum* species in according with methodology described in our previous work [31].

***GC-FID and GC-MS analysis***: The GC-MS analyses were performed as described in our previous studies [32-33].

***Statistical analysis*:** JMP software package SAS (Institute, Cary, NC, USA) was used to statistical analysis. The hierarchical cluster analysis HCA was performed using Ward's method, with squared Euclidian distances as a measure of similarity. Compounds with contents below 1.0 % were excluded from statistical analysis.

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