Chemical Composition of Aerial Parts Essential Oils from Six Endemic Malagasy *Helichrysum* Species

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Received: 22 January 2020; Accepted: 15 February 2020; Published: 18 February 2020

**Abstract:** The essential oils of six endemic Malagasy *Helichrysum* species were investigated by GC (RI), GC–MS and 13C NMR spectrometry. In total, 153 compounds were identified accounting for 90.8% to 99.9% of the total composition. The main constituents were α-pinene for *H. benthamii*, 1,8 cineole for *H. dubardii*, (E)-β-caryophyllene for *H. indatum*, and *H. bojerianum*. *H. diotoideus* essential oil was characterized by the presence of two lilac alcohols and four lilac acetates whereas *H. hirtum* essential oil exhibited an atypical composition with 7β-H-silphiperfol-5-ene, 7-epi-silbengorgiol, and 7-epi-silphiperfol-5-en-13-oic acid as major components.

**Keywords:** endemic *Helichrysum*; Madagascar; essential oil

1. Introduction

Madagascar, a big island in the Indian Ocean, is one of the countries in the world having particular hotspot biodiversity. Together with this biological richness, medicinal plants hold an important place in the everyday life of Malagasy people. The medicinal plants inventoried in Madagascar consist of 3245 species, of which 60% are endemic. Croton L. and *Helichrysum* Mill. are the most represented genera.

*Helichrysum* Mill. [1] is a large genus of the Asteraceae family and 112 species are known in Madagascar among which 46 are endemic [2]. Essential oils and extracts are obtained from the whole plant or from different parts of the plant and they are used in perfumery and aromatherapy. Biological activity or insecticidal activity have been reported [3]. In the literature, the chemical composition of essential oil (EO) from seven species has been studied [3–11]. *H. faradifani* and *H. gymnocephalum* are the most studied species and four papers reported their chemical compositions. Three different compositions are described for *H. faradifani* essential oil: (i) (E)-β-caryophyllene (34.6%) [6], (ii) β-himachalene (15%–32.8%) associated with α-fenchene (13.1%–27.3%) [5], and (iii) α-fenchene (32.3% and 35.5%) [3,10]. The chemical composition of *H. gymnocephalum* oil is homogeneous and characterized by the occurrence of 1,8-cineole (66.7% [7], 59.7% [6], 47.4% [4], and 17.4% [9]). monoterpenes hydrocarbons such as β-pinene (38.2%–40.5%) are dominant in *H. selaginifolium* oil [6,7]. Two chemical compositions are reported for *H. hypnoides* essential oil.
dominated by 1,8-cineole (51.5%) [11] or (E)-β-caryophyllene (34.0%) [6]. This last compound is also found as a major component in *H. cordifolium* oil (46.4%–55.6%) [6,11] and in *H. russillonii* oil (29.5%) [11]. Finally, *H. bracteiferum* oil exhibited a chemical composition with β-pinene/1,8-cineole/α-humulene as major components [6,8,11]. These literature data reported an important chemical variability characterized by the presence of monoterpenes such as α-fenchene, β-pinene, 1,8-cineole or sesquiterpene hydrocarbons: (E)-β-caryophyllene, β-himachalene and α-humulene.

In this study, we were interested in six species: *Helichrysum dubardii* R. Vig. and Humbert, *H. benthamii* R. Vig. and Humbert, *H. hirtum* Humbert, *H. indutum* Humbert, *H. bojerianum* DC., *H. diotoides* DC [11]. *Helichrysum dubardii* and *H. benthamii* consist of subshrub plants with ericoid growth form, the leaves are deltoid, erect and applied on the twigs. *H. dubardii* leaves have only a single midrib vein, glabrous on the upper side, silvery white on the lower side. The bractal appendages of the inflorescences are yellowish white. In *H. benthamii*, the leaves have one to three veins arising from the base, covered with a dense gray tomentum above, loose underneath. The bractal appendages of the flowers are sulfur yellow [12]. *H. hirtum* and *H. indutum* are subshrub plants, leaves evenly distributed on the stem while the flowers have white bractal appendages. The first species has twigs covered with glandular hairs interspersed with fine cottony hairs and glands; the leaves are sessile, oblong, and with five veins arising from the base. The second species is covered with homogeneous fine cottony hairs sometimes dotted with sessile glands [12]. *H. bojerianum* and *H. diotoides* are also subshrub plants, with leaves evenly distributed on the stem. The bractal appendages of the inflorescences are sulfur yellow. *H. bojerianum* is covered with an ashy white aranose tomentum. The leaves are elliptical, acute sessile, with three veins arising from the base while *H. diotoides* is covered with a grayish aranose tomentum. The leaves are deltoid and sessile.

The aim of this work was to study for the first time, the chemical composition of the leaf essential oil extracted from these six endemic species growing wild in the center of Madagascar: *H. dubardii*, *H. benthamii*, *H. hirtum*, *H. indutum*, *H. bojerianum*, and *H. diotoides*.

2. Results

Twelve oil samples obtained by hydrodistillation (yields: 0.11%–0.26%) of aerial parts of six *Helichrysum* species growing wild in Madagascar were analyzed by gas chromatography (GC) in combination with retention indices on two columns of different polarity, by gas chromatography coupled with mass spectroscopy (GC–MS) and by carbon-13 nuclear magnetic resonance (13C NMR). Due their complexity, *H. hirtum* and *H. dubardii* essential oils were also fractionated on silica gel column chromatography (CC). In total, 153 compounds were identified accounting for 90.8% to 99.9% of the total composition (Table 1).
Table 1. Chemical composition of six *Helichrysum* essential oil samples.

| No. | Samples                  | H. benthami | H. dubardii | H. ind | H. boj | H. dio | H. hirtum |
|-----|--------------------------|-------------|-------------|--------|--------|--------|-----------|
|     | Components               | RI₂ | RIₚ | Hbe1 | Hbe2 | Hbe3 | Hd1 | Hd2 | Hd3 | Hi | Hbo | Hi | Hh1 | Hh2 | Hh3 |
| 1   | α-Thujene                | 920 | 1014 | 0.2  | 0.2  | -    | 0.3 | 0.4 | 0.3 | -  | -   | -  | -    | -   | -   |
| 2   | α-Pinene                 | 929 | 1014 | 50.8 | 51.9 | 23.1 | 5.8 | 6.6 | 5.8 | 2.7 | tr  | tr  | 1.6  |     |     |
| 3   | α-Fenchene               | 941 | 1053 | 0.1  | 0.1  | tr   | -   | -   | -   | -  | -   | -  | -    | -   | -   |
| 4   | Camphene                 | 943 | 1063 | 0.3  | 0.2  | 0.1  | 0.3 | 0.3 | 0.3 | 0.3 | -   | -   | -    | -   | -   |
| 5   | Thuja-2,4(10)-diene      | 944 | 1125 | 0.7  | 0.4  | 0.2  | -   | -   | 0.1 | -  | -   | -  | -    | -   | -   |
| 6   | Oct-1-en-3-ol           | 961 | 1445 | -    | -    | -    | -   | -   | 0.2 | -  | -   | -  | -    | -   | -   |
| 7   | Sabinene                 | 964 | 1121 | 0.6  | 1.0  | 0.7  | 0.7 | 0.6 | 0.7 | -  | -   | 0.1 | -    | -   | -   |
| 8   | β-Pinene                 | 969 | 1110 | 0.6  | 0.7  | 0.6  | 2.8 | 2.1 | 2.8 | 0.8 | -   | -  | 0.1  | -   | -   |
| 9   | 1,8-dehydro Cineole    | 976 | 1192 | 0.1  | 0.1  | tr   | 0.2 | 0.1 | 0.2 | -  | -   | -  | -    | -   | -   |
| 10  | Octanal                  | 976 | 1281 | 0.1  | tr   | tr   | -   | -   | -   | -  | -   | -  | -    | -   | -   |
| 11  | Myrcene                  | 979 | 1159 | 0.1  | 0.1  | tr   | 0.6 | 0.4 | 0.6 | 1.3 | 0.1 | -  | 0.2  | -   | -   |
| 12  | α-Phellandrene           | 995 | 1164 | 0.2  | 0.1  | 0.1  | 0.1 | 0.2 | 0.2 | tr  | -   | -  | -    | -   | -   |
| 13  | α-Terpinene              | 1008 | 1179 | 0.2  | 0.2  | 0.2  | 1.2 | 1.3 | 1.2 | 0.2 | tr  | -  | -    | -   | -   |
| 14  | p-Cymene                 | 1010 | 1269 | 0.6  | 0.6  | 0.4  | 1.5 | 1.0 | 1.5 | 0.8 | 0.1 | tr | -    | -   | -   |
| 15  | Limonene                 | 1019 | 1200 | 1.6  | 0.4  | 1.1  | 0.7 | 0.4 | 0.7 | 5.4 | 1.2 | 0.4 | 0.1  | -   | -   |
| 16  | 1,8-Cineole              | 1021 | 1212 | 3.0  | 4.0  | 3.0  | 28.2 | 35.7 | 26.9 | 13.4 | 1.7 | 0.5 | 0.1  | -   | tr  |
| 17  | (Z)-β-Ocimene            | 1023 | 1232 | tr   | tr   | 0.1  | -   | -   | -   | 0.5 | 0.1 | -  | -    | -   | -   |
| 18  | (E)-β-Ocimene            | 1036 | 1249 | tr   | -    | tr   | -   | -   | tr  | 1.9 | 0.2 | -  | -    | -   | -   |
| 19  | γ-Terpinene              | 1047 | 1243 | 0.3  | 0.3  | 0.3  | 2.6 | 2.5 | 2.6 | 1.0 | 0.3 | 0.1 | -    | -   | -   |
|     | trans-Sabinene           |      |      |      |      |      |     |     |     |     |     |    |      |     |     |
| 20  | THF                      |      |      |      |      |      |     |     |     |     |     |    |      |     |     |
| 21  | cis-Linalool oxide       |      |      |      |      |      |     |     |     |     |     |    |      |     |     |
|     | TTHF                     |      |      |      |      |      |     |     |     |     |     |    |      |     |     |
| 22  | Nonan-2-one              | 1067 | 1388 | 0.3  | 0.2  | -    | -   | -   | -   | 0.1 | 0.1 | -  | -    | -   | -   |
| 23  | p-Cymenene               | 1070 | 1438 | 0.2  | 0.1  | -    | 0.1 | 0.1 | 0.1 | -  | 0.1 | -  | -    | -   | 0.1 |
| 24  | Terpinolene              | 1076 | 1283 | 0.2  | 0.1  | -    | 0.6 | 0.6 | 0.6 | 0.3 | 0.3 | -  | -    | -   | -   |
| 25  | Nonanal                  | 1079 | 1394 | 0.2  | 0.2  | -    | 0.1 | -   | 0.1 | -  | -   | -  | -    | -   | -   |
| 26  | Linalool                 | 1082 | 1544 | 0.2  | 0.1  | -    | 0.9 | 0.4 | 0.1 | 7.2 | 5.3 | 9.8 | -    | -   | 0.4 |
| 27  | Fenchyl alcohol          | 1099 | 1584 | -    | -    | -    | -   | -   | -   | 0.3 | 0.4 | 0.3 | -    | -   | -   |
| 28  | α-Campholenal            | 1101 | 1482 | 1.0  | 0.6  | 1.0  | -   | -   | tr  | -   | -  | -   | -    | -   | -   |
| No. | Compound                | Formula | Plant Family | Yield (g/100 kg) | Composition (w/w%) | Reference |
|-----|-------------------------|---------|--------------|------------------|--------------------|-----------|
| 29  | cis-p-Ment-2-en-1-ol    |         | Menta        | 1104             | 1482 0.1 0.1 0.1 0.2 0.2 0.2 - - - - - - |          |
| 30  | Camphor                |         |              | 1118             | 1515 - - - tr 0.1 tr - - - - - - |          |
| 31  | trans-Pinocarveol      |         |              | 1122             | 1653 1.4 1.0 1.8 0.3 0.6 - 0.2 - - - - |          |
| 32  | trans-Verbenol         |         |              | 1126             | 1677 0.6 0.3 0.2 0.1 - 0.1 - 0.1 - - - - |          |
| 33  | Camphene hydrate       |         |              | 1133             | 1592 - tr tr - - tr 0.1 - - - - - - |          |
| 34  | Pinocarvone            |         |              | 1135             | 1567 0.7 0.6 1.0 - 0.2 - - 0.1 0.1 - - - |          |
| 35  | p-Mentha-1,5-dien-8-ol |         |              | 1143             | 1714 1.2 - - - - - - - - - - - - |          |
| 36  | δ-Terpineol            |         |              | 1144             | 1670 - 0.7 0.9 0.4 0.5 0.4 0.2 0.1 tr - - - |          |
| 37  | Borneol                |         |              | 1146             | 1682 2.4 0.8 2.1 1.7 1.5 1.7 0.7 1.4 0.8 - - |          |
| 38  | Mentha-1,8-dien-4-ol   |         |              | 1156             | 1673 - - - - - - 0.1 0.1 0.1 0.1 - - - |          |
| 39  | p-Cymen-8-ol           |         |              | 1157             | 1848 0.2 0.2 0.2 - - - - - - - - - |          |
| 40  | Terpinen-4-ol          |         |              | 1160             | 1599 0.4 0.7 0.9 4.9 4.7 4.9 1.0 0.7 0.3 - - 0.6 |          |
| 41  | Myrtental              |         |              | 1167             | 1627 0.5 0.3 0.5 - - 0.1 0.1 0.1 - - - |          |
| 42  | α-Terpineol            |         |              | 1170             | 1694 0.4 0.5 0.5 4.3 4.0 4.3 1.8 2.5 1.5 - - |          |
| 43  | Myrtenol               |         |              | 1177             | 1790 - 0.4 0.3 - tr 0.2 0.2 - - - - |          |
| 44  | α-Campholenol *        |         |              | 1177             | 1791 0.9 - 0.4 0.2 - - - - - - - |          |
| 45  | Lilac alcohol A (2S,2'S,5'S) |  |              | 1179             | 1742 - - - - - - - - 1.7 - - - |          |
| 46  | Verbenone              |         |              | 1182             | 1703 0.1 0.2 0.3 - - tr - - - - - |          |
| 47  | cis-Piperitol          |         |              | 1184             | 1682 tr tr - - tr - - - - - - |          |
| 48  | Lilac alcohol B (2R,2'S,5'S) |  |              | 1188             | 1718 - - - - - - - - - - 3.6 - - - |          |
| 49  | trans-Cardaveol        |         |              | 1196             | 1834 0.3 0.1 0.2 - - tr 0.2 tr - - - |          |
| 50  | Nerol                  |         |              | 1208             | 1799 - - - - - - tr - tr tr - - - |          |
| 51  | Carvone                |         |              | 1212             | 1738 0.1 0.1 0.1 - - - - tr 1.3 - - - |          |
| 52  | Piperitone              |         |              | 1233             | 1730 - - - - - - - - tr - - - |          |
| 53  | Geraniol               |         |              | 1234             | 1835 - - - - - - - - 0.1 - 0.1 - - - |          |
| 54  | Isopiperitenone        |         |              | 1236             | 1859 - - - - - - - - - - 0.1 - - - |          |
| 55  | Bornyl acetate         |         |              | 1266             | 1577 - tr 0.5 0.3 0.5 0.5 tr - 0.2 - - - |          |
| 56  | Lavandulyl acetate     |         |              | 1269             | 1605 - - - - - - - - - - 0.1 0.2 0.6 - - - |          |
| 57  | Myrtenyl acetate       |         |              | 1305             | 1678 0.1 tr 0.1 0.1 - 0.1 0.2 - - - - 0.2 |          |
| 58  | Piperitenone           |         |              | 1306             | 1910 - - - - - - - - - - 0.2 - - - |          |
| 59  | Lilac acetate A *      |         |              | 1318             | 1773 - - - - - - - - - 0.2 0.6 - - - |          |
|   | Plant name                  | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 | Value 9 | Value 10 |       |
|---|----------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|-------|
| 60| Lilac acetate B             | 1322    | 1744    | -       | -       | -       | -       | -       | -       | -       | -        | 2.3   |
| 61| 7α-H-Silphiperfol-5-ene     | 1324    | 1420    | -       | -       | -       | -       | -       | -       | -       | 0.1      | 0.3   |
| 62| Eugenol                    | 1325    | 2165    | 0.1     | 0.1     | 0.2     | -       | -       | tr      | -       | -        | -     |
| 63| Lilac acetate C             | 1328    | 1779    | -       | -       | -       | -       | -       | -       | -       | 0.3      | -     |
| 64| Lilac acetate D             | 1333    | 1773    | -       | -       | -       | -       | -       | -       | -       | 8.7      | -     |
| 65| 7β-H-Silphiperfol-5-ene     | 1343    | 1446    | -       | -       | -       | -       | -       | -       | -       | 9.1      | 1.8   |
| 66| α-Cubebeine                 | 1346    | 1453    | 0.1     | 0.2     | 0.3     | 1.2     | 0.6     | 1.2     | -       | -        | 0.3   |
| 67| Cloveone                    | 1363    | 1501    | 0.5     | 0.4     | -       | -       | -       | 0.2     | -       | 0.2      | 0.1   |
| 68| Cyclosativene               | 1367    | 1476    | 0.2     | 0.1     | 0.2     | -       | -       | tr      | -       | -        | 0.9   |
| 69| α-Ylangene                  | 1371    | 1484    | -       | -       | -       | -       | -       | -       | -       | 0.1      | -     |
| 70| α-Copaene                   | 1372    | 1486    | 5.4     | 6.2     | 8.5     | 2.3     | 1.8     | 2.3     | 0.8     | 2.0      | 1.1   |
| 71| β-Bourbonene                | 1380    | 1514    | 0.4     | 0.4     | 0.8     | 0.2     | 0.2     | 0.2     | -       | -        | -     |
| 72| β-Cubebeine                 | 1384    | 1532    | 0.2     | 0.2     | 0.4     | 0.3     | -       | -       | -       | -        | -     |
| 73| Sativene                    | 1388    | 1528    | 0.1     | 0.2     | 0.2     | -       | -       | -       | tr      | -        | 0.3   |
| 74| Ylanga-2,4(15)-diene        | 1397    | 1606    | 0.5     | 0.6     | 0.7     | -       | -       | -       | -       | -        | -     |
| 75| Italicene                   | 1400    | 1537    | -       | -       | -       | -       | -       | -       | -       | 1.6      | 1.5   |
| 76| Isocaryophyllene            | 1407    | 1570    | -       | 0.1     | 0.2     | -       | -       | -       | 0.2     | 0.2      | 0.1   |
| 77| cis-α-Acoradiene            | 1409    | 1562    | tr      | tr      | 0.4     | -       | -       | -       | -       | 0.2      | 0.3   |
| 78| (E)-β-Caryophyllene         | 1415    | 1590    | 1.5     | 2.3     | 5.2     | 2.7     | 3.4     | 2.7     | 33.1    | 16.1     | 15.0  |
| 79| β-Copaene                   | 1422    | 1585    | 0.1     | 0.1     | 0.2     | 0.2     | -       | 0.2     | -       | tr       | -     |
| 80| trans-α-Bergamotene         | 1429    | 1578    | 0.1     | 0.1     | 0.2     | -       | -       | tr      | 0.2     | 0.1      | -     |
| 81| α-Guaiene                   | 1433    | 1583    | -       | -       | 0.1     | -       | tr      | 0.3     | 0.3      | -        | 0.2   |
| 82|trans-Muurola-3,5-diene      | 1441    | 1746    | 0.1     | 0.1     | 0.2     | -       | -       | 0.2     | 0.1      | -        | -     |
| 83| Aromadendrene               | 1443    | 1585    | -       | -       | -       | -       | -       | -       | 0.2     | 0.2      | -     |
| 84| α-Humulene                  | 1448    | 1662    | 3.1     | 4.4     | 6.4     | 1.6     | 0.5     | 1.6     | 3.3     | 2.3      | 3.5   |
| 85| (E)-9-epi-Caryophyllene     | 1452    | 1637    | 0.2     | 0.1     | 0.1     | -       | -       | -       | 0.5     | 0.3      | -     |
| 86| α-Acoradiene                | 1457    | 1669    | tr      | tr      | 0.1     | -       | -       | -       | 0.1     | 0.1      | -     |
| 87| β-Acoradiene                | 1460    | 1689    | 0.1     | tr      | tr      | -       | 0.1     | -       | 0.2     | 0.1      | -     |
| 88| trans-Cadina-1(6),4-diene   | 1466    | 1654    | 0.1     | 0.1     | 0.2     | -       | -       | 1.5     | 0.6     | 0.2      | -     |
| No. | Compound                     | Retention Time (min) | Concentration (ppm) |
|-----|------------------------------|----------------------|---------------------|
| 89  | *ar*-Curcumene               | 1.4-1.5              | 0.3-0.5             | 2.9-3.8 |
| 90  | *γ*-Muurolene                | 1.0-1.4              | 0.5-3.8             |
| 91  | *γ*-Curcumene                | 1.0-1.3              | 3.8-0.6             |
| 92  | trans-*β*-Bergamotene        | 1.4-1.6              | 2.2-2.4             |
| 93  | Selina-4,11-diene            | 1.4-1.7              | 2.3-2.6             |
| 94  | *β*-Selinene                 | 1.4-1.6              | 2.3-1.9             |
| 95  | Aristolochene                | 1.4-1.7              | 2.3-2.6             |
| 96  | Germanrene D                 | 1.4-1.7              | 2.3-2.6             |
| 97  | *α*-Selinene                 | 1.4-1.7              | 2.3-2.6             |
| 98  | *epi*-Zonarene               | 1.4-1.7              | 2.3-2.6             |
| 99  | *α*-Muurolene                | 1.4-1.7              | 2.3-2.6             |
| 100 | Amorpha-4,7(11)-diene        | 1.4-1.7              | 2.3-2.6             |
| 101 | (E,E)-*α*-Farnesene          | 1.4-1.7              | 2.3-2.6             |
| 102 | *β*-Bisabolene *             | 1.4-1.7              | 2.3-2.6             |
| 103 | *α*-Bulnesene *             | 1.4-1.7              | 2.3-2.6             |
| 104 | *β*-Curcumene                | 1.4-1.7              | 2.3-2.6             |
| 105 | *γ*-Cadinene                 | 1.4-1.7              | 2.3-2.6             |
| 106 | *cis*-Calamene               | 1.4-1.7              | 2.3-2.6             |
| 107 | Presilphiperfolan-9-*α*-ol   | 1.4-1.7              | 2.3-2.6             |
| 108 | δ-Cadinene                   | 1.4-1.7              | 2.3-2.6             |
| 109 | Zonarene                     | 1.4-1.7              | 2.3-2.6             |
| 110 | trans-Cadina-1,4-diene       | 1.4-1.7              | 2.3-2.6             |
| 111 | *α*-Calacorene               | 1.4-1.7              | 2.3-2.6             |
| 112 | *α*-Cadinene                 | 1.4-1.7              | 2.3-2.6             |
| 113 | Selina-4(15),7(11)-diene     | 1.4-1.7              | 2.3-2.6             |
| 114 | (E)-*α*-Bisabolene           | 1.4-1.7              | 2.3-2.6             |
| 115 | Selina-3,7(11)-diene         | 1.4-1.7              | 2.3-2.6             |
| 116 | (E)-Nerolidol                | 1.4-1.7              | 2.3-2.6             |
| 117 | *β*-Calacorene               | 1.4-1.7              | 2.3-2.6             |
| 118 | Caryolan-8-ol                | 1.4-1.7              | 2.3-2.6             |
|   | Plant                        |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 146 | 14-hydroxy-β-Caryophyllene | 1648 | 2323 | - | - | - | - | - | - | - | - | - | - | - | - | 3.3 | 3.1 | 1.7 |
| 147 | α-Bisabolol | 1664 | 2211 | - | - | - | - | - | - | 0.1 | 0.1 | 0.4 | - | - | - | - |
| 148 | 14-hydroxy-α-Humulene | 1691 | 2448 | - | - | - | - | - | - | - | - | - | 2.9 | 3.1 | 1.4 |
| 149 | 7,14-anhydro-Amorpha-4,9-diene | 1744 | 2522 | - | - | - | - | - | - | 1.0 | - | - | - | - | - | - |
| 150 | Beyerene | 1922 | 2184 | - | - | - | - | - | 0.1 | 0.1 | 0.4 | - | - | - | - |
| 151 | Manool | 2034 | 2646 | 0.6 | tr | 0.9 | - | - | - | - | - | - | - | - | - |
| 152 | 7-epi-Silphiperfol-5-en-13-oic acid | NE | 2830 | - | - | - | - | - | - | - | - | - | 18.2 | 40.0 | 20.8 |
| 153 | Silphiperfol-5-en-13-oic acid | NE | 2927 | - | - | - | - | - | - | - | - | - | 4.0 | 10.6 | 4.8 |

| Monoterpene Hydrocarbon | | 56.7 | 56.4 | 26.9 | 17.3 | 16.5 | 17.5 | 15.2 | 2.4 | 0.5 | 2.1 | - | 0.1 |
| Oxygenated Monoterpene | | 13.9 | 10.8 | 13.6 | 42.1 | 48.3 | 40.0 | 25.8 | 14.2 | 31.7 | 0.1 | - | 1.3 |
| Sesquiterpene Hydrocarbon | | 16.9 | 21.4 | 34.2 | 24.3 | 21.3 | 26.0 | 50.2 | 70.2 | 47.9 | 22.2 | 4.2 | 36.4 |
| Oxygenated Sesquiterpene | | 10.3 | 9.4 | 16.4 | 6.5 | 5.5 | 9.9 | 6.4 | 6.6 | 10.7 | 68.8 | 89.6 | 62.1 |
| Diterpene | | 0.6 | - | 0.9 | 0.5 | 0.2 | 0.5 | - | - | - | - | - |
| Phenyl propanoid | | 0.1 | 0.1 | 0.2 | - | - | tr | - | - | - | 0.1 | - | - |
| Acyclic compound | | 0.6 | 0.4 | - | 0.1 | - | 0.1 | 0.2 | 0.1 | 0.1 | - | - |
| TOTAL | | 99.1 | 98.5 | 92.2 | 90.8 | 91.8 | 94.0 | 97.8 | 93.5 | 90.9 | 93.3 | 93.8 | 99.9 |

Order of elution and relative percentages of individual components are given on an apolar column (BP-1) excepted those with an asterisk (*) percentages on polar column (BP-20); RIs: retention indices measured on apolar and polar capillary columns respectively; percentages in bold: components identified by a combination of GC(RI), GC–MS and 13C NMR; 13C NMR (italic): compounds identified by 13C NMR in CC fractions; tr: trace level (<0.05%); * isomer not determined; NE: compound non-eluted on an apolar column BP-1; H. ind: H. indutum, H. boj: H. bojerianum, H. dio: H. dioitoides.
2.1. Helichrysum benthamii and H. dubardii Essential Oils

Two samples of *H. benthamii* (Hbe1 and Hbe2) produced a monoterpene hydrocarbon-rich oil characterized by the pre-eminence of α-pinene (50.8%–51.9%), associated with sesquiterpene hydrocarbons: α-copaene (5.4%–6.2%), α-humulene (3.1%–4.4%) and (E)-β-caryophyllene (1.5%–2.3%). The third sample of *H. benthamii* (Hbe3), also characterized by α-pinene (23.1%) as major compound, exhibited a slightly different chemical composition with percentages of sesquiterpene hydrocarbons more elevated: α-copaene (8.5%), α-humulene (6.4%) and (E)-β-caryophyllene (5.2%).

The main components of *H. dubardii* oil samples (Hd1-Hd3) were 1,8-cineole (26.9%–35.7%), followed by α-pinene (5.8%–6.6%), terpinen-4-ol (4.7%–4.9%) and α-terpineol (4.0%–4.3%). Sesquiterpene hydrocarbons were represented by α-murolene (3.2%–7.2%), γ-cadinene (1.6%–3.5%), δ-cadinene (1.8%–4.2%). It is noticeable that bayerene, a rare diterpene hydrocarbon was found in the three samples (0.2%–0.5%).

2.2. Helichrysum indutum, H. bojerianum and *H. diotoides* Essential Oils

*Helichrysum indutum, H. bojerianum* and *H. diotoides* (Hi, Hbo and Hdi samples) produced sesquiterpene-rich oils (47.9%–70.2%); (E)-β-caryophyllene, ar- and γ-curcumene, γ- and δ-cadinenes, aristolochene. Furthermore, among the monoterpenes, linalool was found in an appreciable amount in all samples (5.3%–9.8%) whereas 1,8-cineole was identified only in *H. indutum* EO (13.4%). It could be pointed out that *H. diotoides* oil is characterized by the presence of several lilac derivatives: lilac alcohol A (25.2%,5%) (1.7%), lilac alcohol B (2R,2S,5S) (3.6%) [13], lilac acetate A (0.6%), lilac acetate B (2.3%), lilac acetate C (0.3%), and lilac acetate D (8.7%).

2.3. Helichrysum hirtum Essential Oil

The chromatographic profile of *H. hirtum* oil samples (Hh1, Hh2 and Hh3) varied drastically from the others and was characterized by the presence of many oxygenated sesquiterpenes. In the process of analyzing the chemical composition of the essential oils, we noticed that several compounds remained undetermined, providing very unsatisfactory matching with commercial or in-house MS libraries. Then, the EO was fractionated by silica gel column chromatography (CC), using a gradient of solvents. These compounds could however be identified from the fraction of CC by applying our in-house $^{13}$C NMR computerized methodology [14,15].

We highlighted the identification of presilphiperfolane and silphiperfolane derivatives as major components in the three samples by comparison of their carbon chemical shifts values with those reported in the literature [16–22]: 7-epi-silphiperfol-5-en-13-oic acid (4.0%–20.8%) and silphiperfol-5-en-13-oic acid (4.0%–10.6%) (Table 2), 7-epi-subergorgiol (7.6%–14.8%), 7β-H-silphiperfol-5-ene (1.8%–14.8%), presilphiperfolan-9-α-ol (6.9%–8.0%), 7-epi-silphiperfolenal (1.4%–2.5%), 13-hydroxysilphiperfol-6-ene (1.2%–1.9%) and 7α-H-silphiperfol-5-ene (up to 0.3%). The presence of a compound including an acid group as major component is very unusual in essential oils.

We detailed the identification by $^{13}$C NMR of 7-epi-silphiperfol-5-en-13-oic acid (152) and silphiperfol-5-en-13-oic acid (153) in the sample Hh1 (Table 2). For these two compounds:
- all the expected signals were observed;
- the chemical shift variations between the reference spectrum (Marco et al. [16]) and the recorded spectrum of the sample Hh1 were low. Indeed, they were less than or equal to 0.08 ppm for at least 12 signals out of 15. Only the carbons of the acid function or near the acid function (i.e., C5, C6, C13) exhibited a higher chemical shift variation;
- a DEPT sequence confirmed the number of hydrogens linked to each carbon.

It should be point out that the chemical shift values of carbons, measured on spectra recorded using high field spectrometers, were given with two decimal places. Nevertheless, it occasionally arose that chemical shift values were given with only one decimal. In such a case, although it is not
mathematically correct, comparison of data given with one decimal and those given with two decimals unambiguously allowed identification of compounds.

Table 2. 13C NMR data (400 MHz, CDCl3) of compounds 152 and 153.

| 7-epi-silphiperfol-5-en-13-oic Acid | C | δC ppm 1 | δC ppm 2 | Δδ 4 |
|-------------------------------------|---|----------|----------|------|
| 1                                   | 52.4 | 52.45 | 0.05 |
| 2                                   | 30.4 | 30.41 | 0.01 |
| 3                                   | 37.3 | 37.27 | 0.03 |
| 4                                   | 57.6 | 57.66 | 0.06 |
| 5                                   | 154.8 | 155.15 | 0.35 |
| 6                                   | 136.9 | 136.74 | 0.16 |
| 7                                   | 47.8 | 47.81 | 0.01 |
| 8                                   | 65.3 | 65.37 | 0.07 |
| 9                                   | 42.5 | 42.52 | 0.02 |
| 10                                  | 35.9 | 35.96 | 0.06 |
| 11                                  | 34.6 | 34.63 | 0.03 |
| 12                                  | 19.7 | 19.69 | 0.01 |
| 13                                  | 171.0 | 171.16 | 0.16 |
| 14                                  | 14.8 | 14.83 | 0.03 |
| 15                                  | 19.2 | 19.23 | 0.03 |

| 13C NMR data (400 MHz, CDCl3) of compounds 152 and 153. |
|----------------------------------------------------------|
| | C | δC ppm 1 | δC ppm 2 | Δδ 4 |
|-------------------------------------|---|----------|----------|------|
| 1                                   | 64.5 | 64.50 | 0.00 |
| 2                                   | 30.0 | 30.05 | 0.05 |
| 3                                   | 37.5 | 37.49 | 0.01 |
| 4                                   | 58.6 | 58.68 | 0.08 |
| 5                                   | 154.3 | 154.54 | 0.24 |
| 6                                   | 138.6 | 138.63 | 0.03 |
| 7                                   | 49.9 | 49.90 | 0.00 |
| 8                                   | 64.0 | 64.04 | 0.04 |
| 9                                   | 43.1 | 43.17 | 0.07 |
| 10                                  | 35.6 | 35.64 | 0.04 |
| 11                                  | 28.9 | 28.91 | 0.01 |
| 12                                  | 19.9 | 19.90 | 0.00 |
| 13                                  | 170.7 | 170.82 | 0.12 |
| 14                                  | 18.0 | 18.02 | 0.02 |
| 15                                  | 21.7 | 21.69 | 0.01 |

1 numbering according to Marco et al. [16]; 2 literature data; 3 experimental data; 4 differences between literature and experimental data.

3. Discussion

In a recent review, Rafidison et al., highlighted the actual state of Malagasy medicinal plants and particularly the pharmacological and ethnobotanical investigations. Croton and Helichrysum are the most cited genera. Even more, H. faradafini is present in the top 20 most cited species. Concerning essential oils, H. faradafini, H. bracteiferum, and H. gymnocephalum are actually the most produced in Madagascar and used as expectorant and as a preventative or curative remedy for treating coughs, colds, and bronchitis [2].

The studied oils reported several major components previously described in Malagasy Helichrysum EOs: (i) H. dubardi oil exhibited a close composition reported from H. bracteiferum oil and characterized by 1,8-cineole as major component (26.9%–35.7% vs. 27.3% respectively) [6,11]; (ii) H. indutum EO composition is dominated by (E)-β-caryophyllene which is also reported to be in similar amounts in H. faradifani [6], H. hypnoides [6] and H. russillonii [11] EOs (33.1% vs. 34.6%, 34.0% and
29.5% respectively). Thus, *H. dubardii* and *H. indutum* EOs, which exhibited a chemical profile close to *H. bracteiferum* and *H. faradani* respectively, were the good candidates for domestication.

*H. bojerianum* and *H. diotoides* EOs exhibited a different chemical composition. Even if, the percentage of (E)-β-caryophyllene was low in *H. bojerianum* and *H. diotoides* EOs (16.1% and 15.0% respectively), both oils can be classified as sesquiterpene hydrocarbon-rich oil (76.8% and 58.6% respectively). However, the *H. bojerianum* EO appeared original by the presence of six lilac derivatives (two alcohols and four acetates) at an appreciable ratio around 15%.

The composition of *H. benthamii* EO exhibited α-pinene as major component (23.1%–51.9%) while β-pinene was frequently reported as the major component [6–8,11]. However, percentages up to 20% have never been observed in the *Helichrysum* genus.

Finally, *H. hirtum* EO can be classified as sesquiterpene hydrocarbon-rich oil (91.0%) but the chemical composition differed drastically from the others by the (i) absence of monoterpenic hydrocarbons (only traces of α-pinene and 0.1% of p-cymene), (ii) a very low amount of oxygenated monoterpenes (1.3%), (iii) the presence of several sesquiterpenes exhibiting silphiperfolane and presilphiperfolane skeletons. To our knowledge, the presence of silphiperfolane and presilphiperfolane derivatives has never been reported in Helichrysum EOs but these skeletons were reported in asteraceae family (*Petasites, Matricaria, Sphaeranthus, Otanthus*) [23].

This original chemical composition can be an important feature for marketing. Taking into account that the wild populations of *H. hirtum* were distributed in a limited area (Tapia or *Uapaca bojeri* Forest, highlands of Madagascar—around Arivonimamo), over-exploitation has greatly increased the vulnerability of *H. hirtum* [24]. Therefore, the protection of *H. hirtum* populations should be a high priority now and domestication can be considered as an excellent alternative to supply the continuous market needs by producing high quality and stable raw material, and at the same time, alleviating the pressure on natural resources from overharvesting.

4. Materials and Methods

4.1. Plant Material

Aerial parts of five *Helichrysum* species were collected in September 2016 (dry season) at the region of Itasy, district of Arivonimamo (Figure 1): *H. dubardii* (Ambatobe, 19°14’42.05″ S, 47°00’25.09″ E), *H. benthamii* (Ambatobe, 19°14’42.05″ S, 047°00’25.09″ E), *H. bojerianum* (Near Mount of Tsiafakafokely, 2203 m above sea level, 19°16’24.7″ S, 047°12’52.7″ E), *H. diotoides* (South of Alakamisy kely, 19°15’10.6″ S, 47°06’39.3″ E), *H. hirtum* (West of Arivonimamo, 19°01’35.09″ S, 47°16’90.9″ E). Aerial parts of *H. indutum* were collected in September 2019 (dry season) at region Alaotra Mangoro, District of Maromizaha (19°16’48.9″ S, 047°02’05.2″ E) (Figure 1). Voucher specimens were deposited at TAN and CNARP herbaria under the accession Rakotonandrasana 1501 for *H. dubardii*, 1502 for *H. benthamii*, ST 1518 for *H. bojerianum*, ST 1508 for *H. diotoides*, ST 1519 for *H. hirtum* and, ST 1520 for *H. indutum*.

The essential oils were obtained by hydrodistillation of fresh aerial parts (around 500–1000 g) over 3 h. Yields were calculated from fresh material: *H. dubardii*, 0.13%–0.26%; *H. benthamii*, 0.12%–0.21%; *H. bojerianum*, 0.26%; *H. diotoides*, 0.21%; *H. hirtum*, 0.11%–0.18% and *H. indutum* 0.19%.

4.2. Gas Chromatography (GC) Analysis

GC analyses were performed on a Clarus 500 FID gas chromatograph (PerkinElmer, Courtaboeuf, France) equipped with two fused silica gel capillary columns (50 m, 0.22 mm, film thickness 0.25 m), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min, injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: hydrogen (1.0 mL/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors. Retention indices (RIs) were determined relative to the retention times of a series of n-alkanes with linear interpolation (Target Compounds’ software of PerkinElmer).
4.3. Mass Spectrometry

The EOs were analyzed with a PerkinElmer TurboMass detector (quadrupole, PerkinElmer, Courtabœuf, France), directly coupled to a PerkinElmer Autosystem XL (PerkinElmer), equipped with a fused silica gel capillary column (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane). Carrier gas, helium at 0.8 mL/min; split: 1/75; injection volume: 0.5 μL; injector temperature: 250 °C; oven temperature programmed from 60 to 220 °C at 2 °C/min and then held isothermal (20 min); ion source temperature: 250 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired over the mass range 40–400 Da.

Figure 1. Collection maps of the six *Helichrysum* species.
4.4. NMR Analysis

$^{13}$C NMR analyses were performed on an AVANCE 400 Fourier Transform spectrometer (Bruker, Wissembourg, France) operating at 100.623 MHz for $^{13}$C, equipped with a 5 mm probe, in CDCl$_3$, with all shifts referred to internal tetramethyldisilane (TMS). $^{13}$C NMR spectra were recorded with the following parameters: pulse width (PW): 4 $\mu$s (flip angle 45°); acquisition time: 2.73 s for 128 K data table with a spectral width (SW) of 220,000 Hz (220 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt. The number of accumulated scans ranged from 2000–3000 for each sample (around 40 mg of oil in 0.5 mL of CDCl$_3$). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier Transformation.

4.5. Identification of Individual Components

Identification of the components was based: (i) on comparison of their GC retention indices (RIs) on polar and apolar columns, determined relative to the retention times of a series of n-alkanes with linear interpolation (“Target Compounds” software of PerkinElmer), with those of authentic compounds and (ii) on comparison of the signals in the $^{13}$C NMR spectra of EOs with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software [13–15]. In the investigated samples, individual components were identified by NMR at contents as low as 0.5%. Several compounds were identified by comparison of $^{13}$C NMR chemical shifts with those reported in the literature, for instance 7-epi silphiperfol-5-en-13-oic acid and silphiperfol-5-en-13-oic acid [16], beyerene [17], $\alpha$-terpineol [18], 7-epi-silphiperfolanal and 7-episilphiperfoliol [19], 13-hydroxysilphiperfol-6-ene [20], pogostol [21], 14-hydroxy-$\alpha$-humulene [22], and lilac alcohol B [13].

4.6. Essential Oil Fractionation

$H.$ dubardii oil sample Hd1 (1.0 g) was submitted to flash chromatography (silica gel: 200–500 $\mu$m). Four fractions (FHD1-FHD4) were eluted with a mixture of solvents of increasing polarity with pentane:diethyl ether (P:E) 100:0 to 0:100: FHD1 (P:E 100:0; 231.4 mg), FHD2 (P:E 98:2; 289.1 mg), FHD3 (P:E 95:5; 163.2 mg), and FHD4 (P:E 0:100; 218.4 mg). All fractions of chromatography were analyzed by GC (RI), GC–MS and $^{13}$C NMR.

An $H.$ hirtum oil sample Hh1 (1.0 g) was also submitted to flash chromatography (silica gel: 200–500 $\mu$m). Six fractions (FHh1–FHh6) were eluted with a mixture of solvents of increasing polarity P:E 100:0 to 0:100: FHh1 (P:E 100:0; 143.1 mg), FHh2 (P:E 98:2; 23.0 mg), FHh3 (P:E 95:5; 156.5 mg), FHh4 (P:E 90:10; 524.2 mg), FHh5 (P:E 80:20, 126.0 mg) and FHh6 (P:E 0:100, 18.0 mg). All fractions of chromatography were analyzed by GC (RI), GC–MS, and $^{13}$C NMR.

5. Conclusions

This study provides useful scientific data to promote in situ conservation and to select chemical profiles for eventual production. Our results confirmed that Malagasy Helichrysum EOs exhibited an important chemical variability and these data are useful for projects of biodiversity conservation.

Author Contributions: Conceptualization, D.J.R.R.; Botanical data and mapping S.R.R.; Chemical analysis, D.J.R.R, G.B, M.P., and A.B.; writing—original draft preparation, D.J.R.R. and F.T.; writing—review M.P. and A.B.; editing, M.P.; supervision, F.T.; project administration, C.A., P.A.R.R., and D.J.R.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Delphin J.R. Rabehaja thanks the University of Corsica for a financial support as Associated Professor, October–November 2019.

Conflicts of Interest: The authors declare no conflict of interest.

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