Chemical Composition of Essential Oil from Flowers of Five Fragrant Dendrobium (Orchidaceae)

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Abstract: A detailed chemical composition of Dendrobium essential oil has been only reported for a few main species. This article is the first to evaluate the essential oil composition, obtained by steam distillation, of five Indian Dendrobium species: Dendrobium chrysotoxum Lindl., Dendrobium harveyanum Rchb.f., and Dendrobium wardianum R.Warner (section Dendrobium), Dendrobium amabile (Lour.) O’Brien, and Dendrobium chrysanthum Wall. ex Lindl. (section Densiflora). We investigate fresh flower essential oil obtained by steam distillation, by GC/FID and GC/MS. Several compounds are identified, with a peculiar distribution in the species: Saturated hydrocarbons (range 2.19–80.20%), organic acids (range 0.45–46.80%), esters (range 1.03–49.33%), and alcohols (range 0.12–22.81%). Organic acids are detected in higher concentrations in D. chrysanthum, D. wardianum, and D. harveyanum (46.80%, 26.89%, and 7.84%, respectively). This class is represented by palmitic acid (13.52%, 5.76, and 7.52%) linoleic acid (D. wardianum 17.54%), and (Z)-11-hexadecenoic acid (D. chrysanthum 29.22%). Esters are detected especially in species from section Dendrobium, with ethyl linolenate, methyl linolate, ethyl olate, and ethyl palmitate as the most abundant compounds. Alcohols are present in higher concentrations in D. chrysanthum (2.4-di-tert-butylphenol, 22.81%), D. chrysotoxum (1-octanol, and 2-phenylethanol, 2.80% and 2.36%), and D. wardianum (2-phenylethanol, 4.65%). Coumarin (95.59%) is the dominant compound in D. amabile (section Densiflora) and detected in lower concentrations (range 0.19–0.54%) in other samples. These volatile compounds may represent a particular feature of these plant species, playing a critical role in interacting with pollinators.

Keywords: Dendrobium; essential oil; steam distillation; mass spectrometry; pollinator

1. Introduction

The Orchidaceae family, with its huge number of species that evolved different pollination systems, is known for the variety and complexity of its floral scents, which according to Kaiser (1993), could potentially cover all the spectrum of fragrances occurring in nature [1]. Floral scent, which derives from the composition of volatile organic compounds emitted by the flowers’ tissues (floral VOCs), is fundamental for the defense against pathogens/herbivores and pollinator responses [2]. This trait, together with other characteristics of flowers, such as the color, the presence of nectar, and other peculiarities of the reproductive portions, contributes indeed to defining pollination syndromes [3]. The genus Dendrobium Sw., 1799 (Epidendroideae; Dendrobiinae), which accounts for about 1100 species distributed in Pacific Islands, Asia, and Australia, is one of the largest of the family [4]. As potted and cut flowers, Dendrobium species and hybrids are of great economic interest, being at the top ten among the most commercially traded orchid taxa [5]; several species are also
grown and sold for medicinal purposes [6,7]. A large number of taxa, the great morphological diversity, and the wide distribution range have contributed to taxonomic ambiguities that are currently under debate [4,8,9]. In the phylogenetic revision of the genus, Takamiya et al. (2014) considered the presence of papillae on the flower’s lip in entities belonging to different clades. They demonstrated that this character evolved as an adaptation to bee pollination by Dendrobium species [4]. As stated in previous studies, bee-pollinated orchid flowers exhibit papillose carpets, identified as osmophores, structures of accumulation of substances responsible for floral fragrances [10,11]. Takamiya et al. (2014) recorded odor-producing cells in all species of Section Densiflora and the majority of the Section Dendrobium, thus hypothesizing that this character has probably been acquired after the divergence between the Asian and the Australasian Superclades [4]. Despite the great number of studies aimed at optimizing in vitro propagation protocols (i.e., Marting and Madassery, 2006; Teixera da Silva et al., 2015; Calevo et al. 2020; and references therein) [12–14], and at characterizing anatomical and chemical traits (Carlsward et al., 1997; Xu et al., 2013; Devadas et al. 2016 and references therein) [15–17], the genus Dendrobium has been little investigated from the point of view of the reproductive biology, and even less is known about floral volatilome [18]. To the best of our knowledge, only a few authors had carried out characterizations of floral volatiles from Dendrobium species. Flath and Ohinata (1982) investigated the VOCs of D. superbum Rchb. f. (syn. D. anosmum Lindl.), which is pollinated by the melon fly (Dacus cucurbitae), finding a significant amount of 4-phenylbutan-2-one, whose structure is closely related to another known fly attractant [19]. Brodmann et al. (2009) worked on D. sinense Tang and F.T.Wang and reported that this species emits (Z)-11-eicosen-1-ol (a molecule present in the alarm pheromone of honeybees) to attract hornets for pollination [20]. Silva et al. (2015) recognized terpenes as the most abundant class of compounds in the floral volatiles of D. nobile Lindl. [21]. Julsrigival et al. (2013) found a prevalence of 2-pentadecanone in D. parishii Rchb.f. [22]. Robustelli della Cuna et al. (2017), instead, compared the essential oil of different portions of D. moschatum (Buch.-Ham.) Sw., including the inflorescence: They observed differences among the volatile compositions, and then hypothesized that compounds like ketones or long-chain methyl and ethyl esters play a role as pollinator attractants [23]. The few reports dedicated to reproductive biology have stated that there are various ways for which Dendrobium species attract pollinators: There are cases of shelter mimicry [24–28], nectar rewarding [18], chemical and visual attraction [29], rest and mating place offering, or generalized food deception strategies like a simulation of other co-flowering species occurring in the same habitat [30]. In this work, we aimed to characterize and compare the floral volatiles of five Dendrobiums belonging to sections Dendrobium and Densiflora of the Asian Superclade [4,9]. In particular, we characterized the volatile fractions of the inflorescences of D. chrysanthum Wall. ex Lindl. (Figure 1A), D. harveyanum Rchb. f. (Figure 1B) and D. wardianum R.Warner (Figure 1C) from section Dendrobium, Core subclade of Clade A, and D. chrysotoxum Lindl. (Figure 1D) and D. amabile (Lour.) O’Brien (Figure 1E) from Clade A and C, respectively, of section Densiflora (according to Takamiya et al. 2014) [4].
2. Results

The yields of *D. amabile*, *D. chrysanthum*, *D. chrysotoxum*, *D. harveyanum*, and *D. wardianum* essential oils obtained by steam distillation from fresh flowers were evaluated as 0.09%, 0.34%, 0.33%, 0.39%, and 0.33% (weight/dry weight basis), respectively. Table 1 shows the results of qualitative and quantitative oil analyses on the Elite-5MS column. The compounds are listed in order of their elution and are reported as percentages of the total essential oil. Differences in the qualitative and quantitative compositions of the obtained essential oils have been observed. As shown in the Venn's diagram (Figure 2), only palmitic acid was shared by all five taxa. On the other hand, 30 compounds were uniquely identified in *D. chrysotoxum*, and nine, eight, four, and three in *D. wardianum*, *D. harveyanum*, *D. chrysanthum*, and *D. amabile*, respectively. Furthermore, 21 compounds were found shared by *D. chrysotoxum* and *D. wardianum*. Below, the qualitative and quantitative description of essential oils for each taxon. The Pie chart (Figure 3) shows that the essential oils were different depending on the different species: It can be observed that the main constituents were compounds belonging to saturated hydrocarbons, acids, esters, coumarin, and alcohol classes.
Table 1. Essential oils composition of inflorescences from the five *Dendrobium* species.

| Compound          | RI   | RI  | D. chrysotoxum % | D. harvejanum % | D. wardianum % | D. amabile % | D. chrysanthum % | Identification   |
|-------------------|------|-----|------------------|------------------|----------------|--------------|------------------|------------------|
| Octane            | 800  | 800 | -                | 0.15             | -              | -            | -                | RI, NIST         |
| Hexanal           | 802  | 801 | 0.73             | 0.06             | 0.02           | -            | -                | RI, NIST         |
| 2-hexanol         | 804  | 808 | -                | 0.12             | -              | -            | -                | RI, NIST         |
| Diacetone alcohol | 841  | 841 | -                | -                | -              | -            | -                | 0.68 RI, NIST    |
| α-pinene          | 939  | 931 | 0.21             | -                | -              | -            | -                | RI, NIST         |
| Benzaldehyde      | 960  | 958 | 0.14             | -                | -              | -            | -                | RI, NIST         |
| β-pinene          | 979  | 973 | 0.03             | -                | -              | -            | -                | MS, NIST         |
| Caproic acid      | 1005 | 1003| 0.06             | -                | -              | -            | -                | RI, NIST         |
| α-terpinene       | 1017 | 1015| 0.10             | -                | -              | -            | -                | RI, NIST         |
| α-Cymene          | 1026 | 1023| 0.09             | -                | -              | -            | -                | RI, NIST         |
| Limonene          | 1029 | 1027| 0.17             | -                | -              | -            | -                | RI, NIST         |
| Benzyl alchol     | 1032 | 1035| 0.21             | -                | 0.52           | -            | -                | RI, NIST         |
| β-Isophorone      | 1042 | 1041| 0.51             | -                | -              | -            | -                | RI, NIST         |
| Phenylacetaldehyde| 1042 | 1043| 0.84             | -                | 0.06           | -            | -                | RI, NIST         |
| 2-octenal         | 1056 | 1058| -                | 0.13             | -              | -            | 0.06             | RI, NIST         |
| γ-Terpipene       | 1060 | 1059| 0.76             | -                | 0.04           | -            | -                | RI, NIST         |
| Unidentified      | 1065 | 1065| 2.89             | -                | -              | -            | -                | RI, NIST         |
| cis-sabinene hydrate | 1070 | 1067| 0.27             | -                | 0.04           | -            | -                | MS, NIST         |
| 1-octanol         | 1070 | 1074| 2.80             | -                | 0.41           | -            | -                | MS, NIST         |
| trans-sabinene hydrate | 1098 | 1098| 0.20             | -                | -              | -            | -                | RI, NIST         |
| Linalool          | 1097 | 1101| 0.34             | 0.08             | -              | -            | -                | RI, NIST         |
| Nonanal           | 1102 | 1105| 0.16             | -                | -              | -            | -                | RS, NIST         |
| 2-phenylethanol   | 1107 | 1115| 2.36             | -                | 4.65           | -            | -                | MS, NIST         |
| Methyl octanoate  | 1127 | 1127| 0.04             | -                | -              | -            | -                | RI, NIST         |
| cis-verbenol      | 1141 | 1142| 0.92             | -                | -              | -            | -                | RI, NIST         |
| trans-verbenol    | 1145 | 1148| 4.60             | -                | -              | -            | -                | RI, NIST         |
| Camphor           | 1150 | 1157| 0.12             | -                | -              | -            | -                | MS, NIST         |
| Nonenal           | 1162 | 1161| 0.41             | -                | 0.17           | -            | -                | RI, NIST         |
| α-phellandren-8-ol| 1170 | 1169| 2.15             | -                | -              | -            | -                | RI, NIST         |
| Terpinen-4-ol     | 1177 | 1179| 1.53             | -                | -              | -            | -                | RI, NIST         |
| Diethyl succinate | 1182 | 1184| 0.33             | -                | -              | -            | -                | RI, NIST         |
| p-cymen-8-ol      | 1183 | 1186| 0.29             | -                | -              | -            | -                | RI, NIST         |
| α-terpineol       | 1189 | 1192| 0.18             | -                | -              | -            | -                | 0.28 RI, NIST    |
| Compound                          | RI \(^b\) | RI \(^c\) | Section *Dendrobium* | Section *Densiflora* |
|----------------------------------|-----------|-----------|----------------------|----------------------|
|                                 | D. chrysotoxum | D. harvejanum | D. wardianum | D. amabile | D. chrysanthum |
| Ethyl octanoate                  | 1196      | 1199      | 0.20       | -         | -         | RI, NIST |
| Decanal                          | 1202      | 1206      | -         | -         | 0.04      | -         | RI, NIST |
| Verbenone                        | 1205      | 1210      | 0.20       | -         | -         | -         | MS, NIST |
| 2,4-nonandienal                  | 1212      | 1214      | -         | -         | 0.03      | -         | RI, NIST |
| 4-vinylphenol                    | 1224      | 1221      | -         | -         | 0.52      | 0.08      | RI, NIST |
| 3-phenyl-1-propanol              | 1232      | 1231      | -         | -         | 0.08      | -         | RI, NIST |
| Phenylactic acid ethyl ester     | 1247      | 1247      | 0.15       | -         | 0.72      | -         | RI, NIST |
| Nerol                            | 1254      | 1256      | 0.06       | -         | -         | -         | RI, NIST |
| 2,4-decadial (E,E)               | 1291      | 1295      | 0.40       | 0.39      | 0.39      | 0.16      | RI, NIST |
| 2-methoxy-4-vinyl-phenol         | 1315      | 1315      | -         | -         | 0.24      | -         | RI, NIST |
| 2,4-decadial (E,Z)               | 1319      | 1317      | 0.63       | 0.88      | 0.48      | 0.72      | RI, NIST |
| 2-nonenio acid-\(\gamma\)-lactone| 1345      | 1344      | 0.39       | -         | 0.49      | -         | RI, NIST |
| Capric acid                      | 1359      | 1359      | -         | 0.32      | -         | -         | RI, NIST |
| Eugenol                          | 1367      | 1366      | -         | -         | -         | 0.10      | RI, NIST |
| 1-tetradecene                    | 1390      | 1393      | -         | 0.07      | -         | 0.57      | MS, RI  |
| 3,4-dihydrocoumarin              | 1398      | 1399      | -         | -         | -         | 0.10      | RI, NIST |
| Coumarin                         | 1434      | 1436      | 0.71       | 0.19      | 0.54      | 95.49     | RI, NIST |
| 9-epi-(E)-caryophyllene          | 1466      | 1458      | -         | -         | 1.32      | -         | MS, NIST |
| Ethyl-cinnammate                 | 1467      | 1468      | -         | -         | 0.55      | -         | RI, NIST |
| 2,4-di-tert-butylphenol          | 1494      | 1489      | 0.25       | -         | 1.30      | -         | MS, NIST |
| \(\beta\)-selinene               | 1494      | 1489      | 0.25       | -         | 1.30      | -         | MS, NIST |
| 9-oxo-ethyl-nonanoate            | 1507      | 1510      | 1.28       | -         | -         | -         | RI, NIST |
| Lauric acid                      | 1566      | 1568      | 0.23       | -         | -         | -         | RI, NIST |
| Ethyl laurate                     | 1593      | 1596      | 0.15       | -         | -         | -         | RI, NIST |
| Unidentified                     | -         | 1658      | -         | 5.16      | -         | -         | -         |
| Pentadecan-2-one                 | 1667      | 1667      | -         | -         | 0.26      | -         | RI, NIST |
| Heptadecane                      | 1700      | 1700      | 0.31       | -         | 0.54      | -         | RI, NIST |
| Unidentified                     | -         | 1767      | 0.39       | -         | 3.04      | -         | -         |
| Myristic acid                    | 1780      | 1776      | -         | -         | 3.59      | -         | MS, NIST |
| 1-octadecene                     | 1790      | 1796      | 0.32       | -         | 0.41      | -         | MS, RI  |
| Methyl pentadecanoate            | 1820      | 1828      | 0.04       | -         | -         | -         | MS, NIST |
| Unidentified                     | -         | 1879      | 5.74       | -         | -         | -         | -         |
| Ethyl pentadecanoate             | 1890      | 1896      | 0.36       | -         | 0.19      | -         | MS, NIST |
Table 1. Cont.

| Compound a | RI b | RI c | Section Dendrobium | Section Densiflora | Identification d |
|------------|------|------|--------------------|--------------------|------------------|
|            | D. chrysotoxum | D. harvejanum | D. wardianum | D. amabile | D. chrysanthum |
| Heptadecan-2-one | 1902 | 1903 | 0.11 | - | - | - | RI, NIST |
| Methyl palmitate | 1927 | 1928 | 0.34 | - | 0.44 | - | RI, NIST |
| cis-9-hexadecenoic acid | 1942 | 1943 | - | - | - | - | RI, NIST |
| Z-11-Hexadecenoic acid | 1953 | 1953 | - | - | - | - | RI, NIST |
| Palmitic acid | 1958 | 1960 | 0.05 | 7.52 | 5.76 | 0.61 | 13.52 | RI, NIST |
| Neocembrene | 1960 | 1966 | 0.52 | - | 3.07 | - | - | MS, NIST |
| Ethyl palmitate | 1992 | 1997 | 3.05 | - | 0.99 | - | - | MS, NIST |
| Octadecan-1-ol | 2074 | 2071 | 0.17 | - | 0.60 | - | - | MS, NIST |
| Palmitic acid | 2000 | 2000 | - | 40.42 | - | - | 0.55 | RI, NIST |
| Unidentified | - | 2037 | - | 2.06 | - | - | - | - |
| Methyl linoleate | 2051 | 2068 | 7.48 | 2.50 | 13.17 | - | 1.03 | MS, NIST |
| 10-Heneicosene | 2060 | 2073 | - | - | - | 0.43 | - | MS, RI |
| Heneicosane | 2100 | 2100 | 1.01 | 2.92 | 1.66 | 0.25 | - | RI, NIST |
| Linoleic acid | 2144 | 2147 | 0.12 | - | 17.54 | - | - | RI, NIST |
| Ethyl linolenate | 2169 | 2171 | 26.98 | - | 32.24 | - | - | RI, NIST |
| Ethyl oleate | 2179 | 2181 | 5.39 | - | 0.72 | - | - | RI, NIST |
| Ethyl octadecanoate | 2193 | 2198 | 0.80 | - | 0.31 | - | - | RI, NIST |
| Docosane | 2200 | 2204 | 1.66 | 26.82 | - | 1.94 | 17.53 | RI, NIST |
| 9-Triacosane | 2279 | 2275 | 0.31 | - | - | - | - | MS, RI |
| Tricosane | 2300 | 2307 | 9.33 | - | - | - | - | RI, NIST |
| Tetracosane | 2400 | 2401 | 0.40 | 0.90 | - | - | - | MS, RI |
| 9-Pentacosene | 2474 | 2475 | 0.07 | - | - | - | - | RI, NIST |
| Pentacosane | 2500 | 2501 | 0.95 | 6.53 | - | - | 6.40 | RI, NIST |
| Hexacosane | 2600 | 2600 | - | 2.46 | - | - | - | RI, NIST |
| 9-Eptacosene | 2676 | 2676 | - | - | - | - | 1.15 | MS, RI |
| Heptacosane | 2700 | 2701 | 0.18 | - | - | - | - | RI, NIST |
| Aldehydes | 3.15 | 1.62 | 1.20 | 0.88 | 0.06 | - | - | - |
| Alcohols | 7.97 | 0.12 | 7.02 | 0.30 | 22.81 | - | - | - |
| Acids | 0.45 | 7.84 | 26.89 | 0.61 | 46.80 | - | - | - |
| Coumarin | 0.71 | 0.19 | 0.54 | 95.59 | - | - | 0.06 | RI, NIST |
| Esters | 46.59 | 2.50 | 49.33 | - | 1.03 | - | - | - |
| Ketones | 0.62 | 0.12 | 0.26 | - | 0.68 | - | - | - |
## Table 1. Cont.

| Compound                     | RI<sup>a</sup> | RI<sup>b</sup> | D. chrysotoxum | Section Dendrobium | D. amabile | Section Densiflora | Identification<sup>d</sup> |
|------------------------------|---------------|---------------|----------------|-------------------|------------|--------------------|---------------------------|
| Saturated hydrocarbons       | 22.84         | 80.20         | 2.20           | 2.19              | 26.55      |                    |                           |
| Unsaturated hydrocarbons     | 0.69          | 0.07          | 0.41           | 0.43              | 1.72       |                    |                           |
| Terpenes                     | 2.04          | -             | 5.73           | -                 | -          |                    |                           |
| Oxygenated terpenes          | 8.31          | 0.11          | -              | -                 | 0.34       |                    |                           |
| Miscellanea                  | 0.48          | -             | 0.49           | -                 | -          |                    |                           |
| Unidentified                 | 6.13          | 7.22          | 5.92           | -                 | -          |                    |                           |

<sup>a</sup> Compounds are listed in order of elution from an Elite-5 column.  
<sup>b</sup> Retention Indices according to Adams [31], unless stated otherwise.  
<sup>c</sup> Retention index (mean) determined on an Elite-5 column using a homologous series of n-hydrocarbons.  
<sup>d</sup> Method of identification: MS, mass spectrum; NIST, comparison with library [32]; RI, retention indices in agreement with literature values.
Figure 2. Venn’s diagram shows both the number of compounds shared and unshared/peculiar among the five Dendrobium species. Percentages are referred to the total number of compounds found, not to the relative abundance.

Figure 3. Pie chart of distribution of the classes.

*Dendrobium amabile*: The dominant compound was coumarin, accounting for 95.59% of the total essential oil. Of its derivatives, 3,4-dihydrocoumarin has been detected but in lower amounts (0.10%). The second-largest class (2.19%) is represented by saturated hydrocarbons, particularly docosane (1.94%) and heneicosane (0.25%). Aldehydes (0.88%) are
represented by \((E,Z)-2,4\text{-decadienal}\) and \((E,E)-2,4\text{-decadienal}\) (0.72 and 0.16%). Unsaturated hydrocarbons are dominated by 10-heneicosene (0.43%).

**Dendrobium chrysanthum:** The main bulk of constituents is represented by acids accounting for 46.80% of the total essential oil, from which \((Z)-11\text{-hexadecenoic acid}\) (29.22%), palmitic acid (13.52%), and \((Z)-9\text{-hexadecenoic acid}\) (4.06%) are the most abundant compounds. The second-largest class is featured by saturated hydrocarbons (26.55%) from which docosane (17.53%), pentacosane (6.40%), and tetracosane (2.07%) are the most abundant compounds. Alcohols (22.81%) are dominated by 2,4-di-tert-butylphenol (22.81%). Unsaturated hydrocarbons (1.72%) are represented by 9-heptacosene (1.15%) and 1-tetradecene (0.57%). Esters are represented by methyl linoleate (1.03%).

**Dendrobium chrysotoxum:** The main bulk of constituents is represented by esters (46.59%), from which ethyl linolenate (26.98%), methyl linoleate (7.48%), ethyl oleate (5.39%), ethyl palmitate (3.05%), and 9-oxo-nonanoic acid, ethyl ester (9.28%) are the most abundant compounds. The second-largest class is represented by saturated hydrocarbons, accounting for 22.84% of the total essential oil, from which heneicosane (10.01%), tricosane (9.33%), and docosane (1.66%) are the most abundant compounds. Oxygenated terpenes (8.31%) are dominated by \(\text{trans-}\)-verbenol (4.60%), followed by terpinen-4-ol (1.53%) and cis-verbenol (0.92%). Alcohols, accounting for 7.97% of the total essential oil, are featured by 1-octanol (2.80%), 2-phenylethanol (2.36%), and \(\alpha\)-phellandren-8-ol (2.15%). Aldehydes (3.15%) are represented by phyllocladuldehyde (0.84%), hexanal (0.73%), \((E,Z)-2,4\text{-decadienal}\) (0.48%) and \((E,E)-2,4\text{-decadienal}\) (0.40%). Terpenes (2.04%) are featured by \(\gamma\)-terpinene (0.76%) and neocembrene (0.52%).

**Dendrobium harveyanum:** The main bulk of constituents is represented by saturated hydrocarbons, accounting for 80.20% of the total essential oil, from which eicosane (40.42%), docosane (26.82%), pentacosane (6.53%) heneicosane (2.92%), and hexacosane (2.46%) are the most abundant compounds. The second-largest class is characterized by acids accounting for 7.84% of the total essential oil. The dominant compound of this class appears to be palmitic acid (7.52%). Aldehydes (1.62%) are represented by \((E,Z)-2,4\text{-decadienal}\) (0.88%) followed by \((E,E)-2,4\text{-decadienal}\) (0.39%).

**Dendrobium wardianum:** The main bulk of constituents is represented by esters (49.33%) from which ethyl linolenate (32.24%), methyl linoleate (13.17%), ethyl palmitate (0.99%), phenylacetic acid ethyl ester (0.72%), ethyl oleate (0.72%) and ethyl cinnamate (0.55%) are the most abundant compounds. The second-largest class is characterized by acids accounting for 26.89% of the total essential oil, from which linoleic acid (17.54%), palmitic acid (5.76%), and myristic acid (3.59%) are the most representative compounds. Alcohols, accounting for 7.02% of the total essential oil are featured by 2-phenylethanol (4.65%), octadecan-1-ol (0.60%), 4-vinylphenol (0.52%), benzyl alcohol (0.52%) and 2-methoxy-4-vinyl phenol (0.24%). Terpenes (5.73%) are characterized by neocembrene (3.07%), 9-\(\text{epi-}\)-(\(E\))-caryophyllene (1.32%) and \(\beta\)-selinene (1.30%). Saturated hydrocarbons, accounting for 2.20% of the total essential oil, are represented by heneicosane (1.66%) and heptadecane (0.54%). Aldehydes (1.20%) are featured by \((E,Z)-2,4\text{-decadienal}\) and \((E,E)-2,4\text{-decadienal}\) (0.48 and 0.39%).

### 3. Discussion

Little is known about the pollinators of the studied species, but as argued by Dobson (2006) and Witjes et al. (2011), it is possible to reconstruct the pollinator community behind a certain species by analyzing the volatile composition of flowers [33,34]. While research is still needed to identify pollinators, our analyses constitute a first contribution for the study of compounds possibly involved in plant-animal interactions. However, other functions of floral volatiles, that may play a crucial role in herbivory avoidance and as defensive molecules against pathogens, cannot be excluded [35,36]. Differences in the floral scents of related taxa could play a role in reproductive isolation by influencing pollinator’s behavior and choices [37–40]. Indeed, in some cases, a simple change in the amount of one floral VOC has been linked with strong reproductive isolation, as seen in *Silene dioica* (L.) Clairv. and *S. latifolia* Poir. [41]. However, this ethological type of isolation seems to be more or less...
pivotal depending on the specialization of both the plants and pollinators considered, highlighting the need to carry out additional detailed behavioral experiments to understand plant-pollinator interactions [3].

In this work, the relative composition in floral VOCs of the five *Dendrobium* species was qualitatively studied. The highest number of species-specific compounds were recorded for entities from section *Dendrobium*. Palmitic acid was the only compound shared by all the five taxa examined. This molecule is frequently found in the volatilome of several plant species (Orchidaceae included) [23,35,42], and also in other organisms; we observed that it was relatively abundant in *D. chrysanthum* (13%), followed by *D. harveyanum* (7.52%) and *D. wardianum* (5.76%), while in the remaining two species it was less represented.

The scent recognized for both *D. chrysotoxum* and *D. wardianum* could be due to the high presence of esters in floral VOCs that we detected during our analyses. Esters are produced by the reaction of alcohols with organic acids; they typically have fruity smells and are indeed among the molecules responsible for the odors of many fruits [43]. High content of volatile esters has been linked with the strong flavor of the “snow chrysanthemum” cultivar of *Coreopsis* by Kim et al. (2020) [44]. In *D. moschatum*, a putative role as semiochemicals involved in pollinator attraction has been hypothesized for methyl and ethyl esters by Robustelli della Cuna et al. (2017) [23]. According to da Silva et al. (1999) and Cseke et al. (2007), terpenes are more abundant in flower VOCs of species pollinated by food-seeking bees [45,46]. As shown in Table 1, *D. wardianum* had the highest level (5.73%) of terpenes in the essential oil, followed by *D. chrysotoxum* (2.04%), but this class of compounds was not the predominant one in these two species. Conversely, oxygenated terpenes have been detected only in *D. chrysotoxum* (8.31%), while they were present in lower percentages in *D. harveyanum* and *D. chrysanthum*. Therefore, due to their ester and terpenoid contents, and considering similar results obtained by Flath and Ohinata (1982) for *D. superbum*, we cannot exclude that *D. chrysotoxum* and *D. wardianum* could rely on the action of frugivorous flies or bees, or other animals for their pollination [19].

It is noteworthy that the VOCs spectrum of *D. amabile*, a scented orchid, was almost entirely dominated by coumarin, a compound having a sweet smell that resembles vanilla. On the contrary, this compound was present only in very small percentages in all the other *Dendrobiums* considered. As previously stated by Robustelli della Cuna et al. 2017, coumarin was abundant, although less represented in respect to *D. amabile*, also in VOCs from inflorescences and leaves of *D. moschatum* [23]. In this species, authors hypothesized a phytoalexin-like defensive role for coumarin. In the future, a possible role of coumarin in plant-pollinator interactions should be investigated. Interestingly, *D. chrysanthum* showed a distinctive floral volatile composition compared to the other species. Indeed, this entity displayed the highest amounts of acids (accounting for 46.8% of the total essential oil), together with a good representation of alcohols (22.8%) if compared to the other species considered. Among acids, the most representative one (29.2%) was Z-11-Hexadecenoic acid, a known sex pheromone in moths [47]. Considering the relatively high content of this compound, we can again hypothesize its possible role as pollinator (putatively, moth) attractant. Concerning alcohols, 2,4-di-tert-butylphenol was relatively abundant in *D. chrysanthum*. This molecule was also present in traces in *D. amabile*. Zhang et al. (2017) and Huang et al. (2018) recorded the occurrence of this alcohol in flowers of *D. moniliforme* (L.) Sw. and rhizomes of *Gastrodia elata* Blume, respectively [48,49]. This compound, known for its toxicity, exerts several bioactivities and has insecticidal, nematocidal, antibacterial, and antifungal properties (Zhao et al., 2020 and references therein) [50]. Therefore, a defensive role for 2,4-di-tert-butylphenol in *D. chrysanthum* cannot be excluded. Finally, it is interesting to notice that among the *Dendrobium* and *Densiflora* sections, three self-incompatible species, *D. amabile* and *D. harveyanum*, and *D. chrysanthum*, respectively, showed a reduced spectrum of volatiles [51,52]. It is tempting to hypothesize that this has a role in pollination biology; indeed, discouraging pollinators from pollinating more flowers of the same plant and inducing pollinators to visit different individuals, would result in a higher fruit set.
4. Conclusions

In conclusion, this is the first study reporting the floral volatile components of *D. amabile*, *D. chrysanthum*, *D. chrysotoxum*, *D. harveyanum*, and *D. wardianum*. Our results can put the basis for the investigation of *Dendrobium*’s pollination biology and plant-herbivore interactions, but further studies to find the pollinators and understand their behaviors are required for deciphering the role of the compounds detected in these five *Dendrobium* species. Considering the present results, studies on the fingerprint of the essential oils of other *Dendrobium* sections (i.e., *Calcarifera*, *Crumenata*, *Fugacia*, *Latouria*, and *Spatulata*) are in progress in our lab.

5. Materials and Methods

5.1. Plant Material

All examined species were provided by specialized sellers, in details: *D. wardianum* (Buchanan-Hamilton) Swartz, *D. chrysotoxum* (Lindley) and *D. harveyanum* (Rchb.f.) from Orchid’s and more, (Ismaning, Germany), *D. amabile* (O’Brien) and *D. chrysanthum* (Wallich ex Lindley) from Kopf Orchideen und Floristik, (Deggendorf, Germany). Plants were identified according to Dressler (2016) [53], and cultivated under intermediate greenhouse conditions at the University of Turin, Italy, for two years before analyses. Plants were grown in intermediate conditions in a greenhouse during winter months and outside from April to October using a bark well-drained potting medium. Samples were collected at the flowering stage and stored at $-20^\circ C$ until extraction. Before extraction, the flowers were brought back to room temperature and subjected to steam distillation.

5.2. Isolation of the Essential Oil

Flowers (*D. amabile* 25 g, *D. chrysanthum* 5.53 g, *D. chrysotoxum* 5.87 g, *D. harveyanum* 6.14 g, *D. wardianum* 6.26 g), to which octyl octanoate (98%, Sigma-Aldrich, Inc., St. Louis, MO, USA) was added as internal standard, were steam distilled with odor-free water for 3 h. The distillate was extracted with methylene chloride ($3 \times 100$ mL) (Merck, Darmstadt, Germany), dried over anhydrous sodium sulfate (Sigma-Aldrich, Inc., St. Louis, MO, USA), and concentrated at first with a rotary evaporator and subsequently using a gentle stream of $N_2$ for successive GC/FID and GC/MS analyses [23,36].

5.3. GC-FID Analysis

The analyses were carried out using a Hewlett Packard model 5980 GC, equipped with Elite-5MS (5% phenyl methyl polysiloxane) capillary column of (30 m × 0.32 mm i.d.) and film 0.32 µm thick. The carrier gas was He at a flow of 1 mL/min. One µL aliquots of essential oil were manually injected in splitless mode. The oven temperature program included an initial isotherm of 40 °C for 5 min, followed by a temperature ramp to 260 °C at 4 °C/min, and a final isotherm at this temperature for 10 min. Injector and detector temperatures were set at 250 and 280 °C, respectively. The relative amount of each component was calculated based on the corresponding FID peak area without response factor correction.

5.4. GC-MS Analysis

The analyses were carried out using a GC Model 6890 N, coupled to a benchtop MS Agilent 5973 Network, equipped with the same capillary column and following the same chromatographic conditions used for the GC/FID analyses. The carrier gas was He at a constant flow of 1.0 mL/min. The essential oils were diluted before analysis, and 1.0 µL was manually injected into the GC system with a split ratio of 30:1. The ion source temperature was set at 200 °C, while the transfer line was at 300 °C. The acquisition range was 40–500 amu in electron-impact (EI) positive ionization mode using an ionization voltage of 70 eV.
5.5. Identification and Quantification of the Essential Oil Components

The identification of the volatile oil components was performed by their retention indices (RI) and their mass spectra [31], and by comparison with a NIST database mass spectral library, as well as with literature data [32,54]. Retention indices were calculated by Elite-5MS capillary columns using an n-alkane series (C₆–C₃₅) (Sigma-Aldrich, Inc., St. Louis, MO, USA) under the same GC conditions as for samples. The relative amount of each component of the oil was expressed as percent peak area relative to total peak area from GC/FID analyses of the whole extracts. The quantitative data were obtained from GC/FID analyses by an internal standard method and assuming an equal response factor for all detected compounds.

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