Abstract: The volatile constituents of the flowers of Caralluma europaea (Guss.) N.E.Br (Apocynaceae) from Lampedusa Island were analyzed by a headspace GC method. The analyses allowed the identification and quantification of 41 compounds. The main components were, among the monoterpenoids, terpinolene (23.3%), α-terpinene (19.1%) and linalool (18.4%), whereas, among the carbonylic compounds the major constituents were heptanal (2.0%), octanoic acid (2.4%) and hexanoic acid (1.7%). The presence of a nitrogen containing compound, indole (0.8%) and of a sulphur containing compound, dimethylsulphide (t), noteworthy. The compounds found in the flowers of C. europaea have been compared with data available in the literature as regard to their odor, presence in other sapromyiophilous taxa, possible role as semiochemicals, and presence in decaying organic matter. 89.3% of total constituents have been described in other sapromyiophilous taxa. Some of the compounds are present in several types of decaying organic matter (excrements, decomposing bodies, and spoiled fish, etc). Several volatiles found in C. europaea flowers are used as semiochemicals by Hymenoptera, Coleoptera, Diptera,
Lepidoptera and other insects. Sixteen volatiles, accounting for 32.4% of the total constituents, are described as attractants of some Diptera families, with a biology linked to decaying organic matter. Our data thus confirm that *C. europaea* floral bouquet falls within the sapromyiophilous pollination syndrome.

**Keywords:** *Caralluma europaea; Apteranthes europaea; Diptera; pollination; sapromyiophily; volatiles*

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**Introduction**

Headspace GC analysis is widely used to study the volatiles composition of flowers and over 1,700 volatile compounds have been identified so far, as reported in a review by Knudsen et al. [1] describing volatiles present in the flowers of 991 taxa of the Angiosperms and a few Gymosperms. The volatile composition of flowers plays a major role, besides other tasks, in attracting insects involved in pollination [2]. Most floral scent are bouquets composed of at least a few, but usually many components. Although the blend is often dominated by one or a few main components, this does not necessarily mean that said component(s) provide the most important signal to the pollinators but instead it is the total scent [2]. Plant species with similar pollinators show similarities, not only in the visual attractants but also in the floral scent composition, regardless of the phylogenetic relatedness of the species.

*Caralluma europaea* (Guss.) N.E.Br [= *Apteranthes europaea* (Guss.) Plowes] belongs to the family Apocynaceae, subfamily Asclepiadoideae. In this subfamily the pollination system is phenotypically high specialized, with pollen aggregated in pollinia [3] like Orchidaceae, and almost all the taxa are insect pollinated. However, while the volatile composition of the flowers of Orchidaceae has been investigated by several authors, relatively few studies have been carried out on the Apocynaceae [1]. Recently Jürgens et al. [4,5] analysed the chemical volatile composition of some Asclepiadoideae and discussed their possible role in the biology of pollination. The chemical composition of flowers of the genus *Caralluma* is little studied, the only available data being those reported by Jürgens et al. [4] for *Desmidorchis flava* (N.E.Br) Meve & Liede (= *Caralluma flava* N.E.Br) and *Apteranthes joannis* (Maire) Plowes (= *Caralluma joannis* Maire).

In the present paper we present the volatile composition of flowers of *C. europaea* as determined by headspace analysis and discuss the possible role in its pollination biology. *C. europaea*, like most Asclepiadoideae, is pollinated by Diptera and falls within the sapromyiophilous syndrome where insects are attracted by the color and odor of the flowers simulating breeding sites or food sources [4].

**Results and Discussion**

In the flowers of *C. europaea* we detected 41 compounds (Table 1) belonging to nine different classes. The analyses allowed the identification of monoterpenoids, sesquiterpenoids, alcohols, aldehydes, ketones, acids and derivatives, nitrogen- and sulphur bearing compounds, and phenols. More than two thirds of the identified compounds were isoprenoids. In fact monoterpenoids were the
main components, representing 77.0% of the compounds identified, accompanied by sesquiterpenoids (1.7%). Among these compounds, the monoterpane hydrocarbons were the most representative, amounting to 56.7%, with terpinolene (23.3%) and α-terpinene (19.1%) as the main components of this fraction. Linalool (18.4%) was the main oxygen containing monoterpane and represented almost the whole content of these compounds, being the remaining four compounds of this class present in lower amounts (0.2%-0.7%).

Table 1. LRI, RI and percent composition of the volatile compounds of the flowers of *Caralluma europaea*.

| LRI<sup>a</sup> | Compound                  | %    | Ident.<sup>b</sup> | LRI<sup>a</sup> | Compound                  | %    | Ident.<sup>b</sup> |
|----------------|---------------------------|------|--------------------|----------------|---------------------------|------|--------------------|
| <800           | 2-Methylbutanal           | 1.3  | R<sub>s</sub>, MS   | 1097           | Linalool                  | 18.4 | R<sub>s</sub>, MS, P.C. |
| <800           | Dimethyl sulphide         | t    | R<sub>s</sub>, MS   | 1101           | Nonanal                   | 1.0  | R<sub>s</sub>, MS   |
| 866            | Hexanoyl                  | 1.1  | R<sub>s</sub>, MS   | 1144           | (Z)-Verbenol              | 0.4  | R<sub>s</sub>, MS   |
| 901            | Heptanal                  | 2.0  | R<sub>s</sub>, MS   | 1180           | Octanoic acid             | 2.4  | R<sub>s</sub>, MS, P.C. |
| 912            | Santolinatriene           | 2.2  | R<sub>s</sub>, MS   | 1193           | Myrtenal                  | 0.6  | R<sub>s</sub>, MS   |
| 927            | Tricyclene                | 1.2  | R<sub>s</sub>, MS   | 1194           | Myrtenol                  | 0.6  | R<sub>s</sub>, MS   |
| 938            | α-Pinene                  | 1.9  | R<sub>s</sub>, MS, P.C. | 1196          | 4-Ethylbenzaldehyde       | 0.2  | R<sub>s</sub>, MS   |
| 953            | Camphene                  | 2.5  | R<sub>s</sub>, MS, P.C. | 1206          | Verbenone                 | 0.7  | R<sub>s</sub>, MS   |
| 963            | Benzaldehyde              | 0.4  | R<sub>s</sub>, MS   | 1276           | 3-Ethyl-4-methyl-1H-pyrrole-2,5-dione | 0.5  | R<sub>s</sub>, MS   |
| 978            | β-Pinene                  | 3.8  | R<sub>s</sub>, MS, P.C. | 1278          | Nonanoic acid             | 1.2  | R<sub>s</sub>, MS, P.C. |
| 980            | Phenol                    | 0.8  | R<sub>s</sub>, MS   | 1290           | Indole                    | 0.8  | R<sub>s</sub>, MS   |
| 981            | Hexanoic acid             | 1.7  | R<sub>s</sub>, MS, P.C. | 1376          | Decanoic acid             | 0.8  | R<sub>s</sub>, MS   |
| 998            | Octanal                   | 0.8  | R<sub>s</sub>, MS   | 1377           | α-Copaene                 | 0.3  | R<sub>s</sub>, MS   |
| 1005           | α-Phellandrene            | 1.4  | R<sub>s</sub>, MS   | 1385           | β-Bourbonene              | 0.2  | R<sub>s</sub>, MS   |
| 1009           | Carene 3                  | 0.5  | R<sub>s</sub>, MS   | 1398           | α-Elemene                 | 0.3  | R<sub>s</sub>, MS   |
| 1013           | α-Terpinene               | 19.1 | R<sub>s</sub>, MS, P.C. | 1416          | (E)-Caryophyllene         | 0.6  | R<sub>s</sub>, MS   |
| 1025           | p-Cymene                  | 0.8  | R<sub>s</sub>, MS   | 1462           | Seychellene               | 0.8  | R<sub>s</sub>, MS   |
| 1037           | Benzyl alcohol            | 0.5  | R<sub>s</sub>, MS   | 1447           | 2:6-Di-tert-butyl-benzoquinone | 0.2  | R<sub>s</sub>, MS   |
| 1044           | Phenylacetaldehyde       | 0.4  | R<sub>s</sub>, MS   | 1519           | 1S-cis-Calamenene         | t    | R<sub>s</sub>, MS   |
| 1067           | Octanol                   | 0.8  | R<sub>s</sub>, MS   | 1541           | α-Calacorene              | 0.1  | R<sub>s</sub>, MS   |
| 1086           | Terpinolene               | 23.3 | R<sub>s</sub>, MS, P.C. |               |                           |      |                    |

Total compounds 96.6%

<sup>a</sup> LRI: linear retention indices; <sup>b</sup> Ident. R<sub>i</sub>: retention index; MS: mass spectrum, P.C.: same behaviour of the pure compound; t: trace amounts < 0.1%.

Phenol, indole and dimethyl sulphide were the only compounds of the benzenoid, nitrogen and sulphur bearing compounds noted, the former two being present in the same amount (0.8%), while the latter was detected only in traces. Acids and derivatives and aldehydes were present in quite similar amounts but the aldehydes are present with a greater number of compounds.
Thirty-three compounds, accounting for 89.3% of the total constituents, have been found in several sapromyiophilous taxa (Table 2 and references therein). Besides being present in sapromyiophilous taxa some of the compounds found are present in several types of decaying organic matter. As regards to excrements, heptanal, octanal, hexanol, nonanal, benzaldehyde, phenylacetaldehyde, phenol and indole have been found in dog faeces [6], hexanol, octanol and phenol in rabbit faeces [7], indole in faeces of lion, Canidae and Mustelidae [4]. In cow dung [8] nonanal is consistently present, while phenol, benzyl alchohol, benzaldehyde and indole were erratically sampled, and are important volatiles of the fresh dung of cattle [8]. Within animals α-pinene has been detected in human bodies in decomposition [9]; nonanal, octanal and benzaldehyde in spoiled fish [10]; heptanal, phenol, nonanal, decanal and nonanoic acid in smoked salmon [11]; nonanal in human bodies in decomposition and in humans, deer and dog bones [12]; 2-methylbutanal, heptanal, hexanoic acid, octanal, nonanal, decanal nonanoic acid and indole in cooked beef meats [13]; hexanoic acid, octanoic acid and nonanoic acid, indole and phenol in pig living quarters [14]. The presence of hexanol, heptanal, nonanal, phenylacetaldehyde, indole, dimethylsulphide, α-pinene, linalool, hexanoic acid, octanoic acid, nonanoic acid and decanoic acid has been reported in various types of cheese [15].

As regard to the presence in other sapromyiophilous taxa, traces of terpinolene are present in Desmidorchis flava and in Hoodia gordonii (Masson) Sweet (Apocynaceae) [4], in Arum maculatum L. (Araceae) where it accounts for 1-10% [16], and in the club-shaped organs of Sauromatum guttatum (Wallich) Schott. (Araceae). with 37.9% [17]. α-Terpinene and linalool are also present in the club-shaped organs of Sauromatum guttatum, where they account for 0.2% and 5.5%, respectively [17], while 0.8% of linalool has been found in the pistillate-stage spate of Peltandra virginica (L.) Kunth (Araceae) [18], and 1.5% in flowers of Zizyphus mauritiana Mill. (Rhamnaceae) [19]. Hexanoic acid and octanoic acid are present in Desmidorchis flava and, according to Jürgens et al. [4], are responsible for the typical urine odor. The frequent presence of hexanoic acid in the urine of various animals seems to be caused by bacterial activity [4].

Several volatile compounds found in C. europaea (Table 2) are used in Hymenoptera, Coleoptera, Diptera, Lepidoptera and others insects as semiochemicals (attractants, allomones, kairomones, pheromones); and one, (E)-caryophyllene, is a synomone for Hymenoptera [20]. Interestingly, 16 volatile compounds of C. europaea flowers, accounting for 32.4% of the total constituents, are described by El-Sayed [20] as attractants of some families of Diptera. In particular linalool, the third more abundant compound in C. europaea, is an attractant of the families Psilidae, Muscidae, Sarcophagidae and Tephritidae [20]. Some classes of compounds, like fatty acid derivatives, sulphur- and or nitrogen- compounds, alcohols, ketones and aldehydes found in the flowers of C. europaea are associated with Diptera and Coleoptera which feed or oviposit on decaying organic matter [21]. Recently Jürgens et al. [5] noted that most of the compounds found in 15 taxa of Apocynaceae are widespread floral scent compounds and that 84% of them are present in plants mainly pollinated by Hymenoptera and Lepidoptera, but also in 15 taxa of stapeliads with fetid floral odors [4]; the authors ascribe the presence of attractant of Lepidoptera and Hymenoptera to the phylogenetic relatedness of the plants involved.
Table 2. Volatile compounds of the flowers of *Caralluma europaea* arranged by class.

| Compounds         | Odour characteristic a | Sapromyiophilous taxa b | Semiochemicals c |
|-------------------|-------------------------|-------------------------|------------------|
|                   |                         | Apocynaceae [4]         |                  |
| Alcohols          |                         |                         |                  |
| Octanol           | Metallic; sulfur; burnt | *Hoodia currori* (Hook.) | x x x - -        |
|                   | matches; toasted bread; | Decne                   |                  |
|                   | herbal; fatty; floral;  | *Hoodia gordonii* (Masson) |                  |
|                   | woody; citrus; waxy;    | Sweet                   |                  |
|                   | moss; nut; mushroom     | *Huernia boleana* M.G. Gilbert |                  |
|                   |                         | (Apocynaceae) [4]       |                  |
|                   |                         | *Huernia keniensis* R.E. Fries |                  |
|                   |                         | (Apocynaceae) [4]       |                  |
|                   |                         | *Monolluma exagona* (Lavranos) |                  |
|                   |                         | Meve & Liede (Apocynaceae) [4] |                  |
|                   |                         | *Orbea semota* (N.E.Br) L.C. Leach subsp. orientalis Bruyns |                  |
|                   |                         | *Orbea variegata* (L.) L.C. Leach (Apocynaceae) [4] |                  |
|                   |                         | *Stapelia asterias* Masson (Apocynaceae) [4] |                  |
|                   |                         | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [22] |                  |
|                   | Berry; cherry; grapefruit; | *Duvalia corderoyi* N.E.Br (Apocynaceae) [4] | x x x x -        |
| Benzyl alcohol    | citrus; walnut; sweet   | *Hoodia currori* (Hook.) Decne |                  |
|                   |                         | *Hoodia gordonii* (Masson) Sweet |                  |
|                   |                         | *Monolluma exagona* (Lavranos) |                  |
|                   |                         | Meve & Liede (Apocynaceae) [4] |                  |
|                   |                         | *Orbea semota* (N.E.Br) L.C. Leach subsp. orientalis Bruyns |                  |
|                   |                         | *Orbea variegata* (L.) L.C. Leach (Apocynaceae) [4] |                  |
|                   |                         | *Stapelia asterias* Masson (Apocynaceae) [4] |                  |
|                   |                         | *Arum creticum* Boiss. & Heldr. (Araceae) [16-23] |                  |
|                   |                         | *Arum idaeum* Coust. & Gand. (Araceae) [23] |                  |
|                   |                         | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [17-22] |                  |
|                   | Hexanol                 | *Zizyphus mauritiana* Mill. (Rhamnaceae) [19 ] |                  |
|                   | Flowery; toasty; dry;   | *Hoodia currori* (Hook.) Decne (Apocynaceae) [4] | x x x x -        |
|                   | fruity; herbal; mild woody; | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [17] |                  |
|                   | sweet; green grass; leafy; resin |                  |                  |
Table 2. Cont.

| Aldehydes                  | Properties                      | Sources                                                                 |
|-----------------------------|---------------------------------|-------------------------------------------------------------------------|
| 2-Methylbutanal             | Citrus fruit; green; fatty; dry fish; pesticide; solvent; smoky; rancid; fruity; oily; woody; fruity; nutty; heavy; putty; soapy | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] Echidnopsis montana (R.A. Dyer & E.A. Bruce) P.R.O. Bally (Apocynaceae) [4] Hoodia gordonii (Masson) Sweet (Apocynaceae) [4] Huernia boleana M.G. Gilbert (Apocynaceae) [4] Huernia keniensis R.E. Fries (Apocynaceae) [4] Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4] Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns (Apocynaceae) [4] Orbea variegata (L.) L.C. Leach (Apocynaceae) [4] Stapelia asterias Masson (Apocynaceae) [4] Hydnora africana Thunb. (Hydnoraceae) [1] Zizyphus mauritiana Mill. (Rhamnaceae) [19] |
| Heptanal                   |                                | Desidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] Echidnopsis leachii Lavranos (Apocynaceae) [4] Echidnopsis montana (R.A. Dyer & E.A. Bruce) P.R.O. Bally (Apocynaceae) [4] Hoodia currori (Hook.) Decne (Apocynaceae) [4] Hoodia gordonii (Masson) Sweet (Apocynaceae) [4] Huernia boleana M.G. Gilbert (Apocynaceae) [4] Huernia keniensis R.E. Fries (Apocynaceae) [4] Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4] Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns (Apocynaceae) [4] Orbea variegata (L.) L.C. Leach (Apocynaceae) [4] Pseudolithos cubiformis (P.R.O. Bally) P.R.O. Bally (Apocynaceae) [4] Stapelia asterias Masson (Apocynaceae) [4] Arisaema erubescens (Wallich) Schott. (Araceae) [24] Arum creticum Boiss. & Heldr. (Araceae) [16-23] Arum idaeum Coust. & Gand. (Araceae) [24] Sauromatum guttatum (Wallich) Schott. (Araceae) [17] Hydnora africana Thunb. (Hydnoraceae) [1] Zizyphus mauritiana Mill (Rhamnaceae) [19] |
| Benzaldehyde               | Burnt sugar; almond; woody; cherry; sweet | Apteranthes joannis (Maire) Plowes (Apocynaceae) [4] Desidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] Echidnopsis leachii Lavranos (Apocynaceae) [4] Echidnopsis montana (R.A. Dyer & E.A. Bruce) P.R.O. Bally (Apocynaceae) [4] Hoodia currori (Hook.) Decne (Apocynaceae) [4] Hoodia gordonii (Masson) Sweet (Apocynaceae) [4] Huernia boleana M.G. Gilbert (Apocynaceae) [4] Huernia keniensis R.E. Fries (Apocynaceae) [4] Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4] Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns (Apocynaceae) [4] Orbea variegata (L.) L.C. Leach (Apocynaceae) [4] Pseudolithos cubiformis (P.R.O. Bally) P.R.O. Bally (Apocynaceae) [4] Stapelia asterias Masson (Apocynaceae) [4] Arisaema erubescens (Wallich) Schott. (Araceae) [24] Arum creticum Boiss. & Heldr. (Araceae) [16-23] Arum idaeum Coust. & Gand. (Araceae) [24] Sauromatum guttatum (Wallich) Schott. (Araceae) [17] Hydnora africana Thunb. (Hydnoraceae) [1] Zizyphus mauritiana Mill (Rhamnaceae) [19] |
| Octanal | Lemon; stew-like; boiled meat-like; rancid; soapy; citrus; green; flower; fruit; orange; honey; fatty; pungent; slightly fragment | Apteranthes joannis (Maire) Plowes (Apocynaceae) [4] | x | x | x | x | - |
| Phenylacetaldehyde | Apple; apricot; berry; cherry; chocolate; grape; grapefruit; honey; hyacinth; lemon; melon; orange; green; nutty; fruity; peach; peanut; vegetable; winelike; sweet; honey like; flower; daisy | Hoodia currori (Hook.) Deene (Apocynaceae) [4] | - | - | - | - | - |
| Nonanal | Gravy; green; tallowy; fruity; gas; chlorine; floral; waxy; sweet; melon; soapy; fatty; lavender; citrus fruit; apple; coconut; grape; grapefruit; lemon; lime; oily; orange; waxy; citrus; fatty; nutty; peach; rose; vegetable; meaty; fishy; slightly pungent; grass-like; animals | Arisaema ciliatum H. Li (Araceae) [4] | x | x | x | x | - |
| 4-Ethylbenzaldehyde | Fruity; anisic; minty; balsamic-sweet; nutty-almond | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4] | - | - | - | - | - |
Table 2. Cont.

| Ketones                      | Hexanoyic acid | Nonanoyic acid | Octanoyic acid |
|------------------------------|----------------|----------------|---------------|
| 3-Ethyl-4-methyl-1H-pyrrole-2,5-dione | -              | -              | -             |
| 2,6-Di-tert-butylbenzoquinone | -              | -              | -             |
| Nitrogen containing compounds| Butter; cheese; chocolate; grape; honey; jasmine; musty; floral; fatty; vanilla; animal; earthy; vegetable; wine-like; fishy; musty fecal; mothball-like | Apteranthes joannis (Maire) Plowes (Apocynaceae)[4] | Huernia keniensis R.E. Fries (Apocynaceae)[4] |
|                              |                | Hoodia gordonii (Masson) Sweet (Apocynaceae)[4] | Orbea variegata (L.) L.C. Leach (Apocynaceae)[4] |
|                              |                |                 | Sauromatum guttatum (Wallich) Schott. (Araceae) [17-22] |
|                              |                |                 | Zizyphus mauritiana Mill. (Rhamnaceae)[19] |
|                              |                |                 |                 |
| Acids and derivatives        | Fatty acid; cheese; fresh; moss; oily; body odour; rancid, pungent; sweet | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae)[4] | - |
|                              |                |                 |                 |
|                              | Green; fat; musty; sweaty; sour; cheese; waxy; goat | Apteranthes joannis (Maire) Plowes (Apocynaceae)[4] | Huernia keniensis R.E. Fries (Apocynaceae)[4] |
|                              |                |                 | Orbea variegata (L.) L.C. Leach (Apocynaceae)[4] |
|                              |                |                 | Stapelia asterias Masson (Apocynaceae)[4] |
|                              |                |                 | Sauromatum guttatum (Wallich) Schott. (Araceae) [17-22] |
|                              |                |                 | Zizyphus mauritiana Mill. (Rhamnaceae)[19] |
| Decanoic acid | Soapy; Fatty; citrus; warm; butter; fruit; grassy; cheese; milk; rancid | Apteranthes joannis (Maire) Plowes (Apocynaceae)[4] Desmidorchis flavata (N.E.Br) Meve & Liede (Apocynaceae)[4] Echidnopsis leachii Lavranos (Apocynaceae)[4] Hoodia currori (Hook.) Deene (Apocynaceae)[4] Hoodia gordonii (Masson) Sweet (Apocynaceae)[4] Huernia boleana M.G. Gilbert (Apocynaceae)[4] Huernia keniensis R.E. Fries (Apocynaceae)[4] Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns (Apocynaceae)[4] Orbea variegata (L.) L.C. Leach (Apocynaceae)[4] Stapelia asterias Masson (Apocynaceae)[4] Sauromatum guttatum (Wallich) Schott. (Araceae)[22] |
|--------------|---------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Monoterpenoids | | |
| Santolinatriene | - | - |
| Tricyclene | - | - |
| α-Pinene | Terpeny; fruity; sweet; green; woody; pine; citrus; lime; camphoraceous; turpentine | x x x x |
| | | | |
| Camphene | Sweet; fruity; camphoraceous; pine; oily; herbal; vanilla | x x x x |
| | | | |
| β-Pinene | Musty; green; sweet; pine; resin; turpentine; woody | x x x x |
| | | | |
| α-Phellandrene | Fruity; minty; herbaceous; citrus; lime; pepper; juniper; dill | x x x |
| | | | |
| Molecule                        | Description                                                                 | Origin                                                                 | Notes                              |
|--------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------|------------------------------------|
| Carene 3                       | Citrus fruit; orange peel; lemon                                             | *Apteranthes joannis* (Maire) Plowes (Apocynaceae) [4]                  | x x x x -                          |
|                                |                                                                            | *Echidnopsis leachii* Lavranos (Apocynaceae) [4]                        |                                    |
|                                |                                                                            | *Echidnopsis montana* (R.A. Dyer & E.A. Bruce) P.R.O. Bally (Apocynaceae) [4] |                                    |
|                                |                                                                            | *Hoodia currori* (Hook.) Deene (Apocynaceae) [4]                       |                                    |
|                                |                                                                            | *Hoodia gordonii* (Masson) Sweet (Apocynaceae) [4]                      |                                    |
|                                |                                                                            | *Huernia boleana* M.G. Gilbert (Apocynaceae) [4]                        |                                    |
|                                |                                                                            | *Huernia keniensis* R.E. Fries (Apocynaceae) [4]                       |                                    |
|                                |                                                                            | *Orbea semota* (N.E.Br) L.C. Leach subsp. orientalis Brunys (Apocynaceae) [4] |                                    |
|                                |                                                                            | *Orbea variegata* (L.) L.C. Leach (Apocynaceae) [4]                    |                                    |
|                                |                                                                            | *Peltandra virginica* (L.) Kunth. (Araceae) [18]                       |                                    |
|                                |                                                                            | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [17-22]              |                                    |
| α-Terpinene                    | Gasoline-like; ethereal; fruity; lemon; berry; sweet; vegetable; woody; pepper; medicinal; camphoraceous | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [17-22]              | - x x x -                          |
| p-Cymene                       | Lemon; fruity; fuel-like; sweet; herbal; spicy; citrus; solvent; gasoline   | *Apteranthes joannis* (Maire) Plowes (Apocynaceae) [4]                  | x x x x -                          |
|                                |                                                                            | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [17-22]              |                                    |
| Terpinolene                    | Woody; fruity; sweet; piney; slightly anisic; plastic                       | *Desmidorchis flava* (N.E.Br) Meve & Liede (Apocynaceae) [4]            | x x x x -                          |
|                                |                                                                            | *Hoodia gordonii* (Masson) Sweet (Apocynaceae) [4]                     |                                    |
|                                |                                                                            | *Arum maculatum* L. (Araceae) [8-16]                                   |                                    |
|                                |                                                                            | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [22]                 |                                    |
| Linalool                       | Lemon; orange; citrus; floral; sweet; aniseed; lavender, muscat, parsley, fruity | *Peltandra virginica* (L.) Kunth. (Araceae) [18]                        | x x x x -                          |
|                                |                                                                            | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [17-22]              |                                    |
|                                |                                                                            | *Zizyphus mauritiana* Mill. (Rhamnaceae) [19]                         |                                    |
| (Z)-Verbenol                   | -                                                                          | *Zizyphus mauritiana* Mill. (Rhamnaceae) [19]                         |                                    |
| Myrtenal                       | Sweet; sharp; pleasant; spicy; cinnamon                                     | -                                                                      |                                    |
|                                |                                                                            | -                                                                      |                                    |
| Myrtenol                       | Medicinal; berry; medicinal; minty; woody; vanilla                          | *Sauromatum guttatum* (Wallich) Schott. (Araceae) [22]                 | x - x x -                          |
|                                |                                                                            | *Zizyphus mauritiana* Mill. (Rhamnaceae) [19]                         |                                    |
| Verbenone                      | Minty; spicy                                                               | *Arisaema tortuosum* (Wallich) Schott. (Araceae) [1-24]               | x x x x -                          |
| Sesquiterpenoids                  | Woody; earthy; spicy     | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] |
|-----------------------------------|--------------------------|----------------------------------------------------------|
|                                   |                          | Echidnopsis leachii Lavranos (Apocynaceae) [4]            |
|                                   |                          | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
|                                   |                          | Huernia keniensis R.E. Fries (Apocynaceae) [4]            |
|                                   |                          | Arum maculatum L. (Araceae) [8]                           |
|                                   |                          | Sauromatum guttatum (Wallich) Schott. (Araceae) [17-22]   |
| β-Bourbonene                      | Herbaceous               | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
|                                   |                          |                                                         |
| α-Elemene                         | -                        | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] |
|                                   |                          | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
|                                   |                          | Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4]|
|                                   |                          | Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns |
|                                   |                          | (Apocynaceae) [4]                                         |
|                                   |                          |                                                         |
| (E)-Caryophyllene                 | Musty; green; spicy; woody; terpene-like; fruity; sweet | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] |
|                                   |                          | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
|                                   |                          | Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4]|
|                                   |                          | Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns |
|                                   |                          | (Apocynaceae) [4]                                         |
|                                   |                          |                                                         |
| Seychellene                       | -                        | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] |
|                                   |                          | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
| 1S-cis-Calamenene                 | -                        | Desmidorchis flava (N.E.Br) Meve & Liede (Apocynaceae) [4] |
|                                   |                          | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
|                                   |                          | Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4]|
|                                   |                          | Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns |
|                                   |                          | (Apocynaceae) [4]                                         |
|                                   |                          |                                                         |
| α-Calacorene                      | Dry-woody                | Sauromatum guttatum (Wallich) Schott. (Araceae) [17]      |
| Phenols                           | Phenolic; medicinal odor | Duvalia corderoyi N.E.Br (Apocynaceae) [4]                |
|                                   |                          | Hoodia gordonii (Masson) Sweet (Apocynaceae) [4]          |
|                                   |                          | Hoodia currori (Hook.) Decne (Apocynaceae) [4]            |
|                                   |                          | Monolluma exagona (Lavranos) Meve & Liede (Apocynaceae) [4]|
|                                   |                          | Orbea semota (N.E.Br) L.C. Leach subsp. orientalis Bruyns |
|                                   |                          | (Apocynaceae) [4]                                         |
|                                   |                          |                                                         |
| Sulphur containing compounds      | Dimethyl sulphide        | Arisaema tortuosum (Wallich) Schott. (Araceae) [24]       |
|                                   | Asparagus; cabbage; corn; cowy; molasses; reminiscent of wild radish; sharp; sickly; sulfurous; vegetable; gasoline | |

* Odor characteristics are taken from the following sources: [15,20,26-29]. * Sapromyiophilous taxa are taken from references quoted at the apices of reported taxa. *Semiochemicals: (A: Attractant; Al: Allomone; K: Kairomone; P: Pheromone; Sy: Synomone) [20].
The volatiles found give the floral bouquet of *C. europaea* sulfur, mushroom, woody, sweet, fish, smoky, rancid, woody, pungent, fecal, cheese and other odors (Table 1 and references therein). In the literature there are several reports about the pollinators of the genus *Caralluma* indicating that this genus is pollinated by flies [30,31,32,33] but there are few data on the volatiles of the flowers.

Flowers in taxa of the genus *Caralluma* show decaying organic matter odors, like *C. arachnoidea* with scents of rotting fruits and are pollinated by small Drosophilidae or Milichiidae, while the floral odor of *Desmidorchis flava* can be described as reminiscent of decaying urine or pungent and urinous and Coleoptera (Dermestidae) have been recorded as flower visitors, although it has not been determined whether they really act as pollinators [4]. Meve and Heneidak [3] state that *C. europaea* flowers have an odor of excrement without reporting any chemical analysis. Interstingly, 23 compounds found in *C. europaea* are also present in at least one fetid Asclepiadoideae [4], while 18 compounds are absent.

The analysis of the volatile composition of *C. europaea* (Fig.1) combined with that of the taxa studied by Jürgens *et al.* [4] shows that twelve taxa share similar volatile composition and that *C. europaea* has similarity to the volatiles present in *Hoodia gordonii*, *Desmidorchis flava* and *Orbea semota* spp. *orientalis*.

**Figure 1.** Cluster analysis based on a binary presence-absence matrix of the volatile profile of 15 taxa [4] and *C. europaea* using Euclidean distances between taxa. Unidenitified compounds were omitted from the analysis. By comparing our data with those of the 15 taxa studied by Jürgens [4] it is clear that *C. europaea* falls within the group of other three species: *Hoodia gordonii*, *Desmidorchis flava* and *Orbea semota* spp. *orientalis*.
The cluster analysis of latter group (Figure 2) confirms the similarity in volatiles of the four taxa.

**Figure 2.** Cluster analysis based on a binary presence-absence matrix of the volatile profile of *Hoodia gordonii*, *Desmidorchis flava* and *Orbea semota* spp. *orientalis* [4] and *C. europaea* using Euclidean distances between taxa showing the close relationship of volatiles composition among the taxa.

**Experimental**

**General**

Flowers of *C. europaea* were collected in Lampedusa Island (Italy, 35°29’28” and 35°21’39” N - 12°30’54” and 12°37’55” E) in April 2009 from plants growing in the “Guitgia” area at an altitude of 20 m a.s.l. Clones of the plants are cultivated at the Botanical Garden of Palermo and a voucher specimen (N° PAL/MS/1119) was deposited in the Herbarium, Orto Botanico, Palermo, Italy. The flowers were removed from the plants with a single stroke of a razor blade, the cut surface was sealed with a drop of metacrylate (Attak®) to avoid the release of volatile compounds due to the cutting, placed in 20 mL autosampler vials with cripto caps and stored at –10 °C. The direct headspace analyses were performed after equilibrating the vials in a heated block. Headspace conditions were: equilibration time 35 min at 105 °C, pressurization time 1.0 min and loop fill time 1.0 min. The chemical composition was determined by using a HP 7694E headspace sampler coupled to a gas chromatograph interfaced with a HP 6890 GC SYSTEM flame ionization detector. Components were separated using two fused-silica capillary columns connected in series by press-fit: first column Carbowax EASYSEP connected to the detector, 30 m × 0.53 mm i.d., 1 µm film thickness and the second CP Sil 5CB connected to the injector; 25 m × 0.53 mm i.d., 5 µm film thickness. GLC conditions were: oven temperature 40°C with 8 min initial hold and then two ramps: the first from 40 °C to 150 °C at 2 °C/min and the second from 150 °C to 210 °C at 35 °C/min (6 min). The injector
was maintained at 250 °C (splitless mode) and He was used as carrier gas (5 mL/min). Most constituents were identified by comparison of their retention indices (Ri) with either those of the literature [34,35] or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8-C18) under the same operating conditions. Further identification was made by comparison of their mass spectra with either those stored in NIST 98 library or with mass spectra from the literature [34,36] and a home-made library. Pure commercial essential oil components used as standards for GC-FID analyses were obtained from Aldrich and Fluka. The comparison of the volatile composition of *C. europaea* with that of other taxa was performed by cluster analysis based on a binary presence-absence matrix of the volatile profile using Euclidean distances among taxa [37].

**Figure 3.** The scent of *C. europaea* flowers attracts different families of Diptera. The photo shows *Musca autumnalis* (Muscidae) inserting its proboscis in the anther slit of the flower of *C. europaea* in search of food resource. Linalool, the third more abundant compound of *C. europaea* flower volatiles, is an attractant of Muscidae (Photo by P. Zito).

**Conclusions**

The volatile compounds found in the flowers of *C. europaea* seems to be a very attractive spectrum for the flies involved in pollination of this species. Flies are almost ubiquitous insects, occurring also in unfavourable habitats where other insects may be rare. Enabled by a highly sensitive olfaction system, flies are attracted to odors over long distances. *C. europaea* grows at the base of others plants or rocks or camouflaged in its environment, and flies may be the most abundant insects and therefore the sapromyiophilous syndrome reflects the adaptation of this species to this environment. Recently Pisciotta et al. [33] found that *C. europaea* in Lampedusa Island is pollinated by eight species of Diptera belonging to five families: Calliphoridae, Milichiidae, Muscidae (Figure 3), Sarcophagidae, and Trixoscelididae. It is interesting to note that all the Diptera families involved in pollination have a
biology linked to decaying organic matters and therefore C. europaea falls within the sapromyiophilous pollination syndrome. According to Dobson [21] a single fly species may visit distinct flowers for different purposes (i.e. food versus oviposition) and therefore pollinate flowers. In particular flies prefer yellow in the presence of sweet scents, which signal food sources, and brown-purple in the presence of odor of excrements, which indicate egg-laying sites. Flowers of C. europaea are brown-purple with yellow stripes, they contain compounds with both sweet odors and compounds found in excrements, and in this way they may mimic both food resources and oviposition sites thus augmenting the spectrum of potential pollinators.

Acknowledgements

The headspace GC determination was carried out in the Dipartimento di Ingegneria Chimica ed Alimentare, Università degli Studi di Salerno. The assistance of its staff is gratefully appreciated. We would like to thank the Director G. Nicolini and the staff of Riserva Naturale Orientata Isola di Lampedusa for their assistance and logistical support. We also thank Silvestro Pisciotta, PhD student at University of Palermo, for the useful discussions on the pollination syndrome in Apocynaceae.

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Sample Availability: The samples used for testing the behaviour of the pure compounds were all purchased from Aldrich (Milan) and are available from the authors.

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