*Article*

# Terpenoid profiles of the essential oils from the underground parts of *Dianthus thunbergii* S.S. Hooper and *Hypoxis argentea* Harv ex Baker as affected by pre-distillation drying

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**Abstract**

The roots of *Dianthus thunbergii* and corms of *Hypoxis argentea* are commonly used in the Eastern Cape Province of South Africa for various medicinal purposes, although their effectiveness as fresh or dried forms is often a subject of debate. The compositions of the volatile oils from the underground parts of these plants were analyzed for the first time by gas chromatography-mass spectrometry (GC-MS). The yields of the essential oil fractions from fresh and oven-dried plant parts varied from 0.42-0.72%. The terpenoid composition of *D. thunbergii* oils were dominated by α-pinene and β-selinene, although overall terpenoid content decreased from 77.17% in fresh roots to 47.58% in the dried roots. *H. argentea* corm oils were dominated by alkanes, amides and amino acids, while total terpenoid content of the oils from fresh and dried corms were 10.85% and 3.45%, respectively. Generally, pre-distillation drying of the underground parts of both plants produced significant reductions in the terpenoid composition of the volatile oils, suggesting that drying may considerably reduce their medicinal potentials.

**Keywords** *Dianthus thunbergii*; *Hypoxis argentea*; roots; corms; terpenoids; GC-MS.

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## 1 Introduction

The roots of *Dianthus thunbergii* and corms of *Hypoxis argentea* are reportedly utilized traditionally for a variety of medicinal uses in the Eastern Cape Province of South Africa (Erasto et al., 2005; Oyedemi et al., 2009; Sagbo et al., 2022). Despite widespread traditional medicinal applications of members within their respective families, Caryophyllaceae and Hypoxidaceae, very few reports document the analysis of their

biologically active components. The *Dianthus* genus made up of over 300 species of annual to perennial herbs are distributed across warm temperate regions in Asia, Europe, Africa and North America. *D. thunbergii*, called 'wild pink' from the color of its flowers is among 16 species commonly found in the southern region of South Africa (Fassou et al., 2022). *Hypoxis* species, on the other hand, are generally geophytic herbs recognized by their bright yellow, star-shaped flowers and strap-like leaves arising directly from the underground rootstock (corm). About 41 species are concentrated in southern Africa, with a good number occurring in the eastern part of South Africa (Mofokeng et al., 2020).

The flowers of some members of the family of carnations (Caryophyllaceae) are known to contain compounds such as eugenol, β-caryophyllene and benzoic acid derivatives, which impart their characteristic fragrance (Zucker et al., 2002; Jurgens et al., 2003). Phytochemical studies of the corms of some popular members of the Hypoxidaceae in South Africa have revealed the presence of a nor-lignan glycoside, called Hypoxoside, as the most important chemical constituent (Nair et al., 2006; Najafian, 2016). Hypoxoside can be hydrolyzed by β-glucosidase to its aglycone, rooperol which is believed to be responsible for the therapeutic benefits of the *Hypoxis* species.

The chemical composition of plants' essential oils depends on various factors, including soil and climate of origin, genetic potential of plants and developmental stage of plant materials, as well as post-harvesting treatment conditions (Najafian, 2016). Plant parts not meant for immediate uses are commonly preserved by artificial drying (usually at temperatures ranging from 40-60ºC) to reduce their moisture contents (Singh et al., 2020). Removal of moisture from plant parts prior to use serves toinhibit microbial growth and limits certain biochemical changes within the plant tissue (Hossain et al., 2010; Kubra and Rao, 2012). Drying also facilitates the marketability of plant parts and aids in their storage and transportation (Rocha et al., 2011).

The effects of drying at high temperatures (>40ºC) on the composition of essential oils are generally two-fold. Drying may result in thermal degradation or breakdown of already existing bioactive compounds, causing reduction in their quantity or complete loss of certain components (Yi and Wetstein, 2011). On the other hand, high temperature application to plant materials may cause thermal destruction of cell walls and sub-cellular compartments, with liberation of higher amounts or numbers of compounds that were otherwise bonded to structural components of the cell (Jimenez-Monreal et al., 2009). In other instances, drying may provoke the heat-induced bio-conversion of certain compounds which then serve as precursors for the formation of other compounds (Tewari et al., 2019).

Terpenoids, constructed from two or more isoprenoid units (5-carbon building blocks), include hydrocarbons (terpenes) and their oxygenated, hydrogenated and dehydrogenated derivatives. They constitute one of the largest groups of natural compounds found in plants, and have been found to possess anti-inflammatory, antimicrobial, antiviral, anti-hyperglycemic and immunomodulatory activities (Wagner and Elmadfa, 2003). The stability of most terpenoids is, however, temperature-dependent, while others have an affinity for the water fraction of plant parts, enhancing their loss with water during the drying process (Hamrouni-Sellami et al., 2012). This study represents the first attempt to determine the composition of oils from the underground parts of *D. thunbergii* and *H. argentea*, as well as the effects of removal of moisture on the terpenoids as the major medicinal components. The underground parts of these plants have been studied because they are the parts often utilized for medicinal purposes in this region. In addition, these parts are most often believed to be the portions that have the capability to accumulate the highest levels of secondary metabolites with medicinal values.

## 2 Materials and Methods

### 2.1 Plant materials

Whole plant samples of *Dianthus thunbergii* SS Hooper and *Hypoxis argentea* Harv ex Baker, were collected from Alice, Eastern Cape, South Africa. They were identified and authenticated at the Giffen Herbarium, University of Fort Hare, South Africa, where voucher specimens (CRY-2502 and HYP-1230, respectively) were deposited. The roots of *D. thunbergii* and corms of *H. argentea* were separated from the rest of the plant and washed with clean tap water to remove soil residues. Fresh plant parts were immediately separated for hydro-distillation, while the remaining portions were oven-dried to constant weight at 30°C prior to distillation.

**2.2 Extraction of essential oils**

About 100 g each of the plant materials were separately subjected to hydro-distillation using a Clevenger-type apparatus fitted with a condenser and connected to a heat-resistant 5-L round bottom flask. The plant materials were heated in boiling water in the flask for about 3 hours, to produce a mixture of gases (oil vapor) which were conveyed with steam into the condenser, where they were cooled to below 30°C, producing two non-mixing liquid phases: a lower hydrosol portion and an upper layer consisting of the essential oil. The condensed liquids were gravity-fed into a separation funnel, where they were separated. The oils obtained were collected into small glass vials which were completely sealed before their analysis. The percentage yield of the essential oil fractions was calculated as weight of the oil divided by the weight of the plant material used.

**2.3 Gas chromatography-Mass spectrometry (GC-MS) analysis**

GC-MS analyses were performed using an Agilent 7890B GC system equipped with an Agilent 5977A mass selective detector (Chemetrix, Pty, Ltd, Agilent Technologies, DE, Germany) and a Zebron-5MS (cross-linked 5% phenylmethylpolysiloxane) column (ZB-5MS 30m x 0.25 mm x 0.25 µm). The following column and temperature conditions were used: GC grade helium was used as carrier gas at a flow rate of 2 ml/min and splitless 1 ml injections were used. The injector and source temperatures were both set at 280°C. Initial oven temperature was 70°C. This was then ramped at 15°C/min to 120°C, then ramped at 10°C/min to 180°C and then ramped at 20°C/min to 270°C and finally held at this temperature for 3 minutes. The data obtained were gathered with ChemStation. Identification of the components of essential oils was done by comparison of mass spectra obtained with those stored in the NIST11.L library.

**3 Results**

The roots and corms of *D. thunbergii* and *H. argentea* are often used for medicinal purposes in the Eastern Cape Province of South Africa. This study represents the first attempt at cataloguing the chemical composition of the essential oils extracted from these plants. The yields of the essential oils from fresh and dried roots of *D. thunbergii* were 0.51% and 0.47%, respectively, while the fresh and dried corms of *H. argentea* yielded 0.72% and 0.41% oil, respectively. The composition of terpenoids and other groups of compounds in the essential oils from *D. thunbergii* are listed in Table 1, while those of *H. argentea* are provided in Table 2.

A total of 9 terpenoids were tentatively identified in the oils from the fresh roots of *D. thunbergii*. These included monoterpenes (α-pinene and β-pinene), monoterpenoids (cis-2-thujen-4-ol, Pinocarveol and verbenone), sesquiterpenes (β-selinene and γ-Muurolene), a sesquiterpenoid (Spathulenol) and a diterpenoid (3-keto-isosteviol). Of these, α-pinene, cis-2-Thujen-4-ol, and β-selinene also occurred in the oil from the dried roots, but were found at reduced percentages. The percentage value of verbenone was, however, increased in the oil obtained after drying. In addition, another monoterpenoid, cis-verbenol was only identified in *D. thunbergii* oil after the roots were dried. Thus, a total number of five terpenoids were identifiable in the essential oil from the roots of *D. thunbergii* after drying, indicating a considerable loss of these compounds due to heat treatment. Crucially, the most abundant compounds in the fresh root oil of *D. thunbergii* were α-pinene (38.22%) and β-selinene (23.95%). Similarly, these same compounds were most abundant in the

dried root oils, forming 12.82% and 19.87% of the total oil composition, respectively.

**Table 1** Composition of terpenoids and other classes of compounds in oils from fresh and dried roots of *Dianthus thunbergii*.

| *S/N* | *Name of compound* | *Molecular formula* | *Molecular weight (g mol$^{-1}$)* | **Fresh root oil** | | **Dried root oil** | |
|---|---|---|---|---|---|---|---|
| | | | | ***%A*** | ***$R_t$*** | ***%A*** | ***$R_t$*** |
| **1** | **α-pinene** | $C_{10}H_{16}$ | 136 | 38.22 | 4.01 | 12.82 | 4.01 |
| **2** | **Cis-2-Thujen-4-ol** | $C_{10}H_{16}O$ | 152 | 2.14 | 4.18 | 2.02 | 4.18 |
| **3** | **β-pinene** | $C_{10}H_{16}$ | 136 | 1.99 | 4.37 | – | – |
| **4** | **L-pinocarveol** | $C_{10}H_{16}O$ | 152 | 2.10 | 5.70 | – | – |
| **5** | **Cis-Verbenol** | $C_{10}H_{16}O$ | 152 | – | – | 3.02 | 5.71 |
| **6** | **Verbenone** | $C_{10}H_{14}O$ | 150 | 3.41 | 6.24 | 9.85 | 6.24 |
| **7** | **β-selinene** | $C_{15}H_{24}$ | 204 | 23.95 | 8.22 | 19.87 | 8.22 |
| **8** | **Spathulenol** | $C_{15}H_{24}O$ | 220 | 1.87 | 8.78 | – | – |
| **9** | **3-keto-isosteviol** | $C_{20}H_{28}O_4$ | 332 | 1.28 | 8.84 | – | – |
| **10** | **γ-Muurolene** | $C_{15}H_{24}$ | 204 | 2.21 | 9.13 | – | – |
| | ***Sub-total*** | | | **77.17** | | **47.58** | |
| | ***Alkanes*** | | | 20.89 | | 7.55 | |
| | ***Aldehydes*** | | | – | | 8.91 | |
| | ***Amino acids, amines and amides*** | | | – | | 4.99 | |
| | ***Fatty acid esters*** | | | 1.90 | | 14.78 | |
| | ***Ketones*** | | | – | | 3.25 | |
| | ***Others*** | | | – | | 12.54 | |
| | **TOTAL** | | | **99.9%** | | **99.60%** | |

%A = Peak area; $R_t$ = Retention times.

A much lower percentage content of terpenoids was identified in oil samples from the corms of *Hypoxis argentea*, irrespective of drying. The larger group of compounds in the essential oils of this plant included amides, amines and alkanes (Table 2). Similar to that observed for *D. thunbergii*, the relative concentration of terpenoids was higher in the oils from the fresh corms than those from the dried corms. The oil from the fresh corms of *H. argentea* contained up to eleven terpenoids, including a monoterpenoid (Citronellol), a diterpenoid (Phytol), sesquiterpenes (γ-gurjunene, cedrene, elixene, γ-elemene, δ-cadinene, eremophilene and alloaromadendrene) and sesquiterpenoids (Ledol and spathulenol). However, only five terpenoids were identified in the oil from the dried corms, which included monoterpenes (α-pinene and β-phellandrene), a monoterpenoid (verbenone) and sesquiterpenes (β-selinene and Elixene). Spathulenol (5.36%) was the most abundant terpenoid in the oil from the fresh corms, although it was not detectable in the dried corm oils.

**Table 2** Composition of terpenoids and other classes of compounds in oils from fresh and dried corms of *Hypoxis argentea*.

| *S/N* | *Name of compound* | *Chemical formula* | *Molecular weight ($gmol^{-1}$)* | *Fresh corm oil* | | *Dried corm oil* | |
|---|---|---|---|---|---|---|---|
| | | | | **%A** | $R_t$ | *%A* | $R_t$ |
| **1** | **α-pinene** | $C_{10}H_{16}$ | 136 | – | – | 1.74 | 4.01 |
| **2** | **γ-gurjunene** | $C_{15}H_{24}$ | 204 | 0.29 | 5.26 | – | – |
| **3** | **Phytol** | $C_{20}H_{40}O$ | 297 | 0.22 | 5.71 | – | – |
| **4** | **Citronellol** | $C_{10}H_{20}O$ | 156 | 0.15 | 5.795 | – | – |
| **5** | **Cedrene** | $C_{15}H_{24}$ | 204 | 0.80 | 5.798 | – | – |
| **6** | **Elixene** | $C_{15}H_{24}$ | 204 | 0.07 | 6.01 | 0.37 | 6.01 |
| **7** | **γ-Elemene** | $C_{15}H_{24}$ | 204 | 0.29 | 6.13 | – | – |
| **8** | **Verbenone** | $C_{10}H_{14}O$ | 150 | – | – | 0.27 | 6.24 |
| **9** | **δ-cadinene** | $C_{15}H_{24}$ | 204 | 0.44 | 6.68 | – | – |
| **10** | **Ledol** | $C_{15}H_{26}O$ | 222 | 0.29 | 6.93 | – | – |
| **11** | **Eremophilene** | $C_{15}H_{24}$ | 204 | 0.29 | 6.98 | – | – |
| **12** | **β-Selinene** | $C_{15}H_{24}$ | 204 | – | – | 0.80 | 8.22 |
| **13** | **Spathulenol** | $C_{15}H_{24}O$ | 220 | 5.36 | 8.79 | – | – |
| **14** | **Alloaromadendrene** | $C_{15}H_{24}$ | 204 | 2.65 | 8.84 | – | – |
| **15** | **β-Phellandrene** | $C_{10}H_{16}$ | 136 | **-** | – | 0.27 | 10.13 |
| | ***Subtotal*** | | | **10.85** | | **3.45** | |
| | ***Alkanes*** | | | 19.87 | | 74.76 | |
| | ***Aldehydes*** | | | – | | 1.73 | |
| | ***Amino acids, amines and amides*** | | | 47.24 | | 0.26 | |
| | ***Fatty acid esters*** | | | – | | 1.73 | |
| | ***Others*** | | | 12.45 | | 16.19 | |
| | **TOTAL** | | | **90.41%** | | **98.12%** | |

%A = Peak area; $R_t$ = Retention times.

Interestingly, drying of the respective parts of both plants, led to an overall increase in the content, as well as total number of some other categories of compounds identified in the oils, as certain compounds were more abundant in dried plant parts compared to the fresh samples. For example, the content of alkanes in *H. argentea* oils was much higher in the dried corms (74.76%) than in the fresh corms (19.87%). An overlay of the total ion chromatograms showing comparison of peaks from the GC-MS analysis of oils from the respective fresh and dried plant materials of *D. thunbergii* and *H. argentea* is presented in Fig. 1.

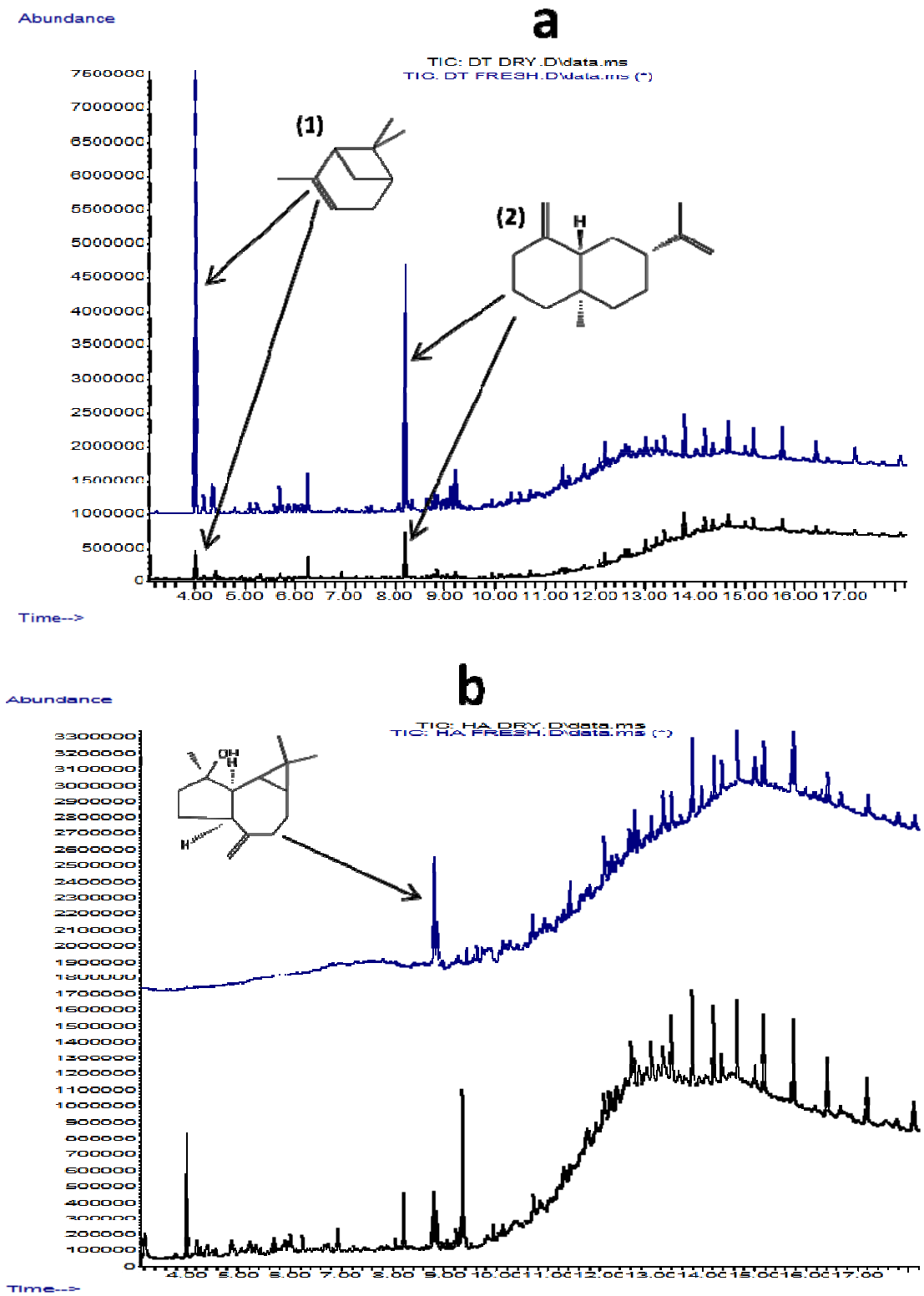


**Fig. 1** Overlay of Total ion chromatograms of the essential oils from (a) *D. thunbergii* roots, and (b) *Hypoxis argentea* corms. DTF = *D. thunbergii* fresh roots; DTD = *D. thunbergii* dried roots; HAF = *H. argentea* fresh roots; HAD = *H. argentea* dried roots. Compound structures from (a) above, Compound 1 is α-pinene, Compound 2 is β-selinene; Structure of compound in (b) is Spathulenol.

## 4 Discussion

The herbal industry commonly utilizes drying as an important post-harvesting processing strategy, although plants used for the extraction of essential oils are usually treated with an exception. There are, however, debates relating to the efficacy of essential oils when extracted from fresh or dried plant parts, in relation to their composition. This study, as part of more comprehensive attempts at validating the ethno-medicinal uses of *D. thunbergii* and *H. argentea* growing in the Eastern Cape, South Africa, has evaluated the changes in the composition of their respective oils, before and after drying at 30°C. Terpenoids are commonly the most abundant constituents of most essential oils, sometimes attaining up to 90% of total oil composition (Masyita et al., 2022). Consequently, the medicinal properties of essential oils are usually attributed to their content of the various terpenoids (Masyita *et al.*, 2022). As previous investigations have tended to focus on the chemical composition of the aerial and floral parts of plants from the *Dianthus* and *Hypoxis* species, the present study has investigated, for the first time, the composition of the essential oils from the underground parts from *D. thunbergii* and *H. argentea*, which are the parts of the plants utilized for medicinal purposes.

Terpenoids were indeed the major components of *D. thunbergii* oils, the most predominant ones being α-pinene and β-selinene, irrespective of drying of the plant's roots, although the overall percentages were significantly reduced in the dried roots. This observation concurs with those of other researchers (Raghavan et al., 1997; Omidbaigi et al., 2004; Sefidkon et al., 2006; Rocha et al., 2011), who reported that different drying methods, with different exposure temperatures, had significant effects on the yield and chemical composition of oils extracted from dried plant material. The abundance of α-pinene and β-selinene in the roots of *D. thunbergii*is contrary to previous reports which indicated that the major components of the essential oils from the aerial parts of other carnation varieties such as *Dianthus caryophyllus* were eugenol and β-caryophyllene (Ibrahim, 2016; Kirillov et al., 2017). These compounds are known to be responsible for the characteristic fragrance of the floral parts of these plants (Popova et al., 2020). The roots of *D. thunbergii* produces no such fragrance and this may thus be attributed to the absence of such compounds as eugenol in the root oils as determined in this study. Variation in the essential oil components among different species may also be due to the differences in their inherent physiology, climate and geographical location. For instance, Kirillo et al. (2017) discovered much lower amounts of eugenol (0.9%) and caryophyllene (0.2%) in the oils from the aerial parts of *Dianthusacicularis*growing wild in Khazakstan, while observing methyl ketones - 2-pentadecanone and 2-tridecanone as the main constituents.

The major terpenoid constituents of *D. thunbergii* oils observed in the present study, α-pinene and β-selinene, have been reported to possess a variety of medicinal properties, including anti-microbial and anti-cancer properties (Deba et al., 2008; Salehi et al., 2019). α-pinene was identified in the aerial parts of another *Dianthus* species, *D.acicularis*, howbeit to much lesser concentrations (1.1%) than that observed in this study. α-pinene, along with its structural isomer, β-pinene which were both identified in this study have also been reported to be important monoterpene hydrocarbons in the essential oils from *D.caryophyllus* (Ibrahim, 2016). We found no previous report of identification of β-selinene in other *Dianthus* species, although essential oils from other plant species have been reported to be rich in this compound (Raal et al., 2008). It may be worth noting, therefore, that the roots *D. thunbergii* could potentially be a promising source of these low-molecular weight hydrocarbons–α-pinene and β-selinene–for pharmaceutical and food-related industries, where their use is in high demand.

The percentage composition of terpenoids in oils from *H. argentea* corms was much lesser than those found in the roots of *D. thunbergii.* Studies by Ahmad et al. (2014) on terpene composition in different plant tissues have also observed that the content of terpenes in the leaves is usually higher than those found in the stems and the underground tissues. Previous reports supporting these observations have adduced that the

higher content of terpenes in the aerial parts of plants may be related to their greater involvement in photosynthetic activities, as well as a higher likelihood of exposure to oxidative stress, compared to the underground parts (Opitz et al., 2008; Ninkuu et al., 2021). The corms of *H. argentea* possess much higher moisture contents than the roots of *D. thunbergii*, and this may also play a role in determining the release of some compounds into the essential oils during hydrodistillation.

To the best of our knowledge, there are no data on the composition of volatiles from the underground parts of species from the Hypoxidaceae. Previous reports on the phytochemistry of the rootstocks of some *Hypoxis* species have focused mainly on the identification of non-volatile constituents, such as the norlignandiglucoside, hypoxoside, and the group of phytosterols, including β-sitosterols, sterol, monoterpene glycosides, stanols, and stigmastanols (Laporta et al., 2007). As there are no literature data relating to terpenoid profiles of other *Hypoxis* species, we were not able to compare the accumulative trends of terpenoids among different species in the genus. It is, however, interesting to note that the volatile constituents of the corms of *H. argentea* oils, as observed in the present study, were dominated by amino acids, amines, amides and alkanes.

Generally, the data from the GC-MS analysis of the two plants consistently showed that drying caused considerable reduction in the amounts and numbers of most terpenoids identified. During the drying process, many compounds that have affinity for the water fraction are dragged to the surface of the plant materials by the evaporating water and may be lost. In addition, many terpenoids react readily with air and are lost to heat sources (Ashafa et al., 2008; Irving et al., 2023). This observation agrees with reports from other studies (Capecka et al., 2005; Alavi et al., 2010), who suggested that heat processing may indeed produce an increase in the content and/or biological activities of secondary metabolites in herbs and essential oils. A number of reasons have been adduced for such a heat-induced increase in the content of compounds in plant extracts and essential oils. The application of heat or the drying of herbs, in some cases, results in the thermal destruction of cell walls and sub-cellular compartments, with the liberation of higher numbers of compounds that were otherwise bonded to structural components of the cell (Jimenez-Monreal et al., 2009). In addition, an increase in the content and/or biological activity of herbal constituents may be a result of the formation of novel compounds due to heat-induced bio-conversion of certain compounds which serve as precursors for the formation of other compounds (Singh et al., 2020).

## 5 Conclusion

The major terpenoid constituents of *Dianthus thunbergii* oil were α-pinene and β-selinene, while those in *H. argentea* oils were Spathulenol and Alloaromadendrene, although their respective concentrations were lower in the dried root oil than the fresh root oil. The content of terpenoids as a percentage of total oil composition in *H. argentea* oils was, however, much lower than in *D. thunbergii* oils. The data generated in this study suggest the possibility of considerable loss of inherent bioactive properties of the plant materials after drying. This information must, therefore, be taken into consideration during preparation of these plants for medicinal use. Further, future efforts to isolate specific bioactive compounds from the essential oils of these plants will yield better results when utilizing the fresh, rather than dried plant material.

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