Chapter 7
Chemical Composition of *Hypoxis hemerocallidea* Fisch. & C.A. Mey from Eastern Cape, South Africa

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Abstract The present study describes the chemical composition of *Hypoxis hemerocallidea*. The essential oil was obtained by hydrodistilling the leaves and corms (fresh and dry) of *H. hemerocallidea*. The percentage yield of the oil was 2.0% in fresh leaves, 2.2% in dry leaves, 2.8% in fresh corms and 2.5% (v/w) in dry corms; the colour of the oils were pale yellow. The essential oil profile was determined by GC and GC-MS. Twenty two components out of the fifty-one components detected by the GC and GC-MS in the fresh leaves essential oil of *H. hemerocallidea* accounted for 97.3%, 27 components in the dry leaves oil accounted for 96.7%, 27 components in the fresh corms oil accounted for 95.8% and 26 components in the dry corms accounted for 93.0% of the total oil composition. The major compounds in the essential oils of *H. hemerocallidea* are sabinene (0.9–27.6%), linalool (15.3–25.4%), α-terpineol (3.5–13.8%), β-caryophyllene (2.2–11.5%), α-terpinolene (0.4–9.8%), β-terpineol (2.1–9.2%), terpinene-4-ol (6.6–8.6%), hexadecane (2.7–8.1%), *cis*-nerolidol (6.8–7.7%), myrcene (4.1–7.5%), β-phellandrene (1.3–7.5%), n-hexadecanoic acid (6.6–6.9%), γ-terpinene (2.6–6.5%), linoleic acid (3.2–6.5%), *trans*-β-ocimene (0.4–6.4%), δ-3-carene (0.5–6.4%), octadecane (2.0–6.4%), β-bourbonene (3.0–6.2%), α-ionone (1.5–5.3%), β-selinene (2.4–5.2%), α-caryophyllene (1.8–4.8%), *trans*-isolimonene (0.1–4.6%), limonene (1.1–4.3%), ethyl linoleate (1.5–4.3%) and δ-cadinene (3.2–4.2%). Non-terpenic groups such as aliphatic carboxylic acids, saturated hydrocarbons and aromatics were significantly present in essential oils of *H. hemerocallidea*. Additionally, other chemical groups such as esters, ketones, and alcohols were present in the oils but their percentages

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were low. This is the first time the essential oils of *H. hemerocallidea* from South Africa have been investigated and reported despite its use in traditional medicine.

Keywords *Hypoxis hemerocallidea* · Essential oil · Sabinene · Linalool · α-terpineol · β-caryophyllene

7.1 Introduction

Hypoxis is a well-known genus of the family Hypoxidaceae [1, 2] and its species are monocotyledons that are commonly distributed in the Southern Hemisphere including sub-Saharan Africa, America, south-east Asia and Australia [3, 4]. The genus *Hypoxis* is reported to have its centre of variation in South Africa [5, 6] where it occurs in open undisturbed grasslands [7]. There are 96 known species of *Hypoxis* in Africa, out of which 30 *Hypoxis* species are found in southern Africa [8], eleven species of which are used for medicinal purposes with *H. hemerocallidea* and *H. colchicifolia* reported to be the most popular [2, 9]. *Hypoxis* genus has generated pharmaceutical interest based on its use as a traditional medicine by indigenous people of eastern and southern Africa [10]. The *Hypoxis* corm has a catalogue of medicinal uses and also serves as a source of food; it is among the commonly prescribed medicines by traditional healers [11]. *Hypoxis* species have been used in South Africa as umuthi for hundreds of years by different tribes to treat various ailments [12]. In Zulu traditional medicine, *Hypoxis* roots or corms are used for treating infertility, urinary infections, intestinal parasites, heart weakness, nausea, cough, palpitations, nervous disorder and vomiting. An infusion made from the tuber of *H. colchicifolia* is taken as an emetic against fearful dreams. *H. rigula* and *H. hemerocallidea* leaves are used as ropes in KwaZulu-Natal. Corms from *H. obtuse* are used to make black polish which is then applied to the floors of the huts. In the midst of starvation the Xhosa and the Sotho people roast or boil the corms of some *Hypoxis* species so that they can eat them [9, 13, 14]. Extracts from the corms of *Hypoxis* are used as ingredients in a wide range of products such as anti-oxidants, anti-inflammatories, anti-diabetics and anti-convulsants [15]. Some of the species, such as *H. hemerocallidea*, *H. stellipilis*, *H. sobolifera* var. sobolifera [16] and *H. obtusa* [17, 18] to name a few, have been scientifically proven to contain hypoxoside, a phytochemical that has immune regulatory properties and its extracts are now widely used in the pharmaceutical industry [19].

Hypoxis hemerocallidea Fisch. & C.A. Mey (Hypoxidaceae) was previously known as *H. rooperi* ; however nowadays it is commonly known as the African potato, yellow star flower (English), inongwe (isiXhosa), ilabatheka, zifozonke (isiSwati) and Inkomfe (isiZulu). It is a stemless, geophytic, perennial herb with large corms (tubers) which are dark brown or black on the outside and bright yellow inside [20]; this type of *Hypoxis* species occurs in open grassland and woodland. It is widespread in South Africa in provinces like Eastern Cape, Free State, KwaZulu-Natal, Mpumalanga, Gauteng and Limpopo; *H. hemerocallidea* is also found in open

grass of Botswana and Lesotho and in savanna regions of Swaziland and Zimbabwe. Studies on the medicinal properties of *H. hemerocallidea* dated back to 1982 when unknowingly the corms were simultaneously studied for the first time in two countries by scientists in Italy and South Africa [21]. *H. hemerocallidea*, or African potato, is counted amongst the special indigenous medicinal species of commercial importance in southern Africa [6]. The corm of the *H. hemerocallidea* has been used in folk medicine to treat a variety of diseases, such as the common cold, flu, hypertension, adult-onset diabetes mellitus, psoriasis, urinary infections, testicular tumours, prostate hypertrophy and internal cancer, HIV/AIDS and some central nervous system disorders [22].

A hydroalcoholic extract of *H. rooperi* was patented with a long list of beneficial properties such as anti-inflammatory, antibiotic, antiarthritic, antiatherosclerotic, diuretic and stimulant of muscular and hormonal activities [23]. Some biomedical evidence suggests that *H. hemerocallidea* corm extract may be useful in the management of type 2 diabetes mellitus [9]. Previous studies have shown that crude aqueous and methanolic extracts of *H. hemerocallidea* exhibited good antibacterial activity against a number of bacteria strains including *Escherichia coli* and *Staphylococcus aureus* [13, 24]. The demand for *H. hemerocallidea* has intensified in recent years, following the isolation and elucidation of a phytosterol diglucoside, hypoxoside, which has various pharmacological activities [22, 25]. Bayley and Van Staden showed that the corms of *H. hemerocallidea* are the major site of biosynthesis of hypoxoside [26]. Some of the compounds isolated from *H. hemerocallidea* are desmosterol, β-sitosterol, campesterol, stigmastanol, stigmasterol and β-sitosterol glucoside [27–29]. The glucoside hypoxoside was first isolated and characterized from *H. obtusa* by Marini-Betolo et al. in 1982 four years before the findings by Drewes et al. (1984), and Vinesi et al., in 1990 [27, 28, 30]. Drewes et al., in 1984 reported the presence of hypoxoside in *H. acuminata*, *H. nitida*, *H. obtusa*, *H. rigidula*, and *H. latifolia* [27, 29]. The phytosterols including their main constituents, hypoxoside and its active derivative rooperol are now being used in fields of anti-oxidants, anti-inflammatories, anti-diabetes, anti-convulsants, inhibitors of drug marker substances, anti-cancerous and premalignant cancer cells [25]. Furthermore, the pharmacological properties of rooperol in studies conducted by several scientists have demonstrated its potency towards cancer, inflammation, and HIV [21, 26].

To the best of our knowledge no work has been reported on the essential oil chemical composition of *H. hemerocallidea*, Therefore, this study is aimed at extracting essential oils from both (fresh and dry) parts of *Hypoxis hemerocallidea* leaves and corms, to determine the chemical profile and then evaluate the medicinal potential of the essential oils. We therefore report the chemical composition of *H. hemerocallidea* essential oils from this study for the first time.

7.2 Materials and Methods

7.2.1 Plant Material

H. hemerocallidea plants were collected in the fields of Bathurst location near Grahamstown in the Eastern Cape Province. The plant samples were then sent to Rhodes University for identification; they were taxonomically identified by Mr. T. Dold and the voucher specimen was deposited in Selmar Schonland Herbarium Grahamstown (GRA) at Rhodes University; the collection number was PR/PL03.

7.2.2 Extraction of Essential Oils

600 g of fresh or dry (leaves and corms) of *H. hemerocallidea* were subjected to a hydro-distillation method for approximately 5 h using the Clevenger apparatus [31]. The extracted essential oils were then collected and stored in airtight amber glass bottles in a refrigerator at 4 °C until the time of analysis [32].

7.2.3 Analysis of Essential Oils

GC-FID was performed on a HP5890-II instrument, equipped with a DB-5MS (30 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column. Hydrogen was used as carrier gas adjusted to a linear velocity of 32 cm/s (measured at 100 °C). Split flow was adjusted to give a 20:1 ratio and septum sweep was a constant 10 mL/min. The oven was programmed as follows: 60–240 °C at 3 °C/min. The samples were injected using the splitless technique using 2 μL of oil in hexane (2:1000). Injector and detector were set at 250 °C. The GC was equipped with FID and connected to an electronic integrator HP 5896 Series II. The percentage yield of the samples was computed from the GC peak areas without using correction for response factors.

GC-MS was performed on a HP-6890 GC system equipped with a HP-5MS fused capillary column (30 m × 0.25 mm; 0.25 μm film thickness), coupled to a selective mass detector HP-5973. Helium (1 mL/min) was used as carrier gas; oven temperature program: 60–240 °C at 3 °C/min; splitless during 1.50 min; sample volume 2 μL of the oil solution in hexane (2:1000). Injector and detector temperature was 250 °C. EIMS: electron energy, 70 eV; ion source temperature and connection parts: 180 °C.

7.2.4 Identification of Essential Oils

Identification of compounds was done by matching their mass spectra and retention indices with those recorded in NIST08 library and by comparison of retention indices and mass spectra with literature values [33–35].

7.3 Results

7.3.1 Chemical Composition of Essential Oils

The essential oils extracted from the leaves and corms (fresh and dry) of *H. hemerocallidea* were pale yellow in color with an unpleasant odor; the percentage yields of the oils are as follows: 2.0% for fresh leaves, 2.2% for dry leaves, 2.8% for fresh corms and 2.5% for dry corms. Constituents identified in the leaves and corms (fresh and dry) of *H. hemerocallidea* together with their Kovat indices and percentage composition are listed in Table 7.1. A total of 51 components were identified in the fresh leaves GC-MS chromatogram of the essential oil of *H. hemerocallidea* with 22 components accounting for 97.3%, 27 components accounting for 96.7% in dry leaves, 27 components accounting for 95.8% in fresh corms and 26 components accounting for 93.0% in dry corms. The fresh leaves essential oil had the following main components: sabinene (27.6%), linalool (15.3%), terpinene-4-ol (8.6%), δ-3-carene (6.4%) and *trans*-β-ocimene (5.3%), while in dry leaves essential oil the main components were β-terpineol (9.2%), β-caryophyllene (11.5%), myrcene (7.5%), terpinen-4-ol (6.6%), γ-terpinene (6.5%), linoleic acid (6.5%), and β-selinene (5.2%). Hexadecane (8.1%), *cis*-nerolidol (7.7%), β-phellandrene (7.5%), *n*-hexadecanoic acid (6.9%), *trans*-β-ocimene (6.4%), octadecane (6.4%), β-bourbonene (6.2%) and α-terpinolene (5.1%) were the major components in the essential oil of fresh corms. In the essential oil of the dry corms, linalool (25.4%), α-terpineol (13.8%), α-terpinolene (9.8%), *cis*-nerolidol (6.8%) and *n*-hexadecanoic acid (6.6%) were the major components. The GC-MS analysis of the fresh leaves essential oil also showed that there was the presence of monoterpenes (55.6%), oxygenated monoterpenes (26.5%), sesquiterpenes (9.6%), aromatics (2.7%), esters (1.5%) and alcohols (1.4%), while in the essential oil of dry leaves monoterpenes (29.8%), oxygenated monoterpenes (23.1%), sesquiterpenes (28.7%), aromatics (4.0%), alcohols (0.3%), esters (4.3%) and carboxylic acids (6.5%) were found to be present. The essential oil of fresh corms was composed of monoterpenes (19.0%), sesquiterpenes (6.2%), oxygenated sesquiterpenes (7.7%), saturated hydrocarbons (22.4%), aromatics (28.3%), ketones (2.1%) and carboxylic acids (10.1%) while in the essential oil of dry corms the detected chemical classes of compounds were monoterpenes (10.7%), oxygenated monoterpenes (39.2%), oxygenated sesquiterpenes (6.8%), saturated hydrocarbons (10.4%), aromatics (13.1%),

Table 7.1 Chemical constituents from the different parts of *H. hemerocallidea* essential oils

No.	Components	KI[a]	KI[b]	H.H.F.L	H.H.D.L	H.H.F.C	H.H.D.C	I.M
1	Butan-2-one	601	605	–	–	0.6	0.9	MS[c], RI[d]
2	2,4-Dimethylbenzene	822	825	–	–	0.5	0.7	MS[c], RI[d]
4	*m*-Xylene	866	906	–	–	0.2	0.2	MS[c], RI[d]
5	2-Ethylthiophene	874	877	0.4	3.2	–	–	MS[c], RI[d]
6	1,2,3-Trimethylcyclohexane	883	890	–	–	–	0.2	MS[c], RI[d]
7	*p*-Xylene	883	888	–	–	0.2	0.3	MS[c], RI[d]
8	Santolinatriene	908	909	–	–	–	0.2	MS[c], RI[d]
9	Cumene	926	912	–	–	0.2	1.1	MS[c], RI[d]
10	Camphene	953	954	–	1.4	–	–	MS[c], RI[d]
11	Sabinene	976	973	27.6	0.9	–	–	MS[c], RI[d]
12	β-Pinene	980	978	2.3	1.7	–	–	MS[c], RI[d]
13	*trans*-Isolimonene	983	989	1.5	4.6	–	–	MS[c], RI[d]
14	Myrcene	991	988	4.1	7.5	–	–	MS[c], RI[d]
15	1,3,5-Trimethylbenzene	994	992	–	–	2.4	2.8	MS[c], RI[d]
16	δ-3-Carene	1011	1004	6.4	0.5	–	–	MS[c], RI[d]
17	α-Terpinene	1018	1017	1.3	3.5	–	–	MS[c], RI[d]
18	1,2,4-Trimethylbenzene	1023	1021	–	–	2.9	0.4	MS[c], RI[d]
19	*o*-Cymene	1020	1022	–	–	3.7	0.6	MS[c], RI[d]
20	*p*-Cymene	1026	1028	2.3	–	2.9	–	MS[c], RI[d]
21	Limonene	1031	1029	4.3	1.1	–	–	MS[c], RI[d]
22	β-Phellandrene	1031	1030	–	1.3	7.5	–	MS[c], RI[d]
23	2-Phenylacetaldehyde	1043	1039	–	0.8	3.1	–	MS[c], RI[d]
24	*trans*-β-Ocimene	1050	1052	5.3	0.7	6.4	0.9	MS[c], RI[d]
25	*trans*-Decahydronaphthalene	1057	1062	–	–	2.6	1.0	MS[c], RI[d]
26	1,2-Diethylbenzene	1057	1055	–	–	2.9	2.2	MS[c], RI[d]
27	1-Methyl-3-propylbenzene	1058	1060	–	–	2.1	2.4	MS[c], RI[d]
28	4-Methyldecane	1059	1063	–	–	3.1	2.0	MS[c], RI[d]
29	γ-Terpinene	1062	1059	2.6	6.5	–	–	MS[c], RI[d]
30	*trans*-Sabinene hydrate	1068	1066	0.5	3.4	–	–	MS[c], RI[d]
31	*cis*-Linalool oxide	1074	1077	–	0.4	–	–	MS[c], RI[d]
32	*m*-Cymene	1082	1085	–	–	3.7	2.2	MS[c], RI[d]
33	α-Terpinolene	1088	1079	1.6	0.4	5.1	9.8	MS[c], RI[d]

(continued)

Table 7.1 (continued)

No.	Components	KI[a]	KI[b]	H.H.F.L	H.H.D.L	H.H.F.C	H.H.D.C	I.M
34	4-Ethyl-1,2-dimethylbenzene	1093	1095	–	–	3.5	0.2	MS[c], RI[d]
35	Linalool	1098	1095	15.3	–	–	25.4	MS[c], RI[d]
36	β-Terpineol	1159	1163	2.1	9.2	–	–	MS[c], RI[d]
37	Ethyl linoleate	1159	1205	1.5	4.3	–	–	MS[c], RI[d]
38	Terpinen-4-ol	1177	1174	8.6	6.6	–	–	MS[c], RI[d]
39	α-Terpineol	1189	1188	–	3.5	–	13.8	MS[c], RI[d]
40	Tridecane	1299	1300	–	–	2.2	2.3	MS[c], RI[d]
41	β-Bourbonene	1384	1388	–	3.0	6.2	–	MS[c], RI[d]
42	β-Caryophyllene	1418	1419	2.2	11.5	–	–	MS[c], RI[d]
43	α-Ionone	1426	1426	–	–	1.5	5.3	MS[c], RI[d]
44	α-Caryophyllene	1454	1478	1.8	4.8	–	–	MS[c], RI[d]
45	β-Selinene	1485	1490	2.4	5.2	–	–	MS[c], RI[d]
46	δ-Cadinene	1524	1522	3.2	4.2	–	–	MS[c], RI[d]
47	*cis*-Nerolidol	1534	1565	–	–	7.7	6.8	MS[c], RI[d]
48	Hexadecane	1600	1600	–	–	8.1	2.7	MS[c], RI[d]
49	Octadecane	1800	1800	–	–	6.4	2.0	MS[c], RI[d]
50	*n*-Hexadecanoic acid	1984	1984	–	–	6.9	6.6	MS[c], RI[d]
51	Linoleic acid	2130	2134	–	6.5	3.2	–	MS[c], RI[d]

H.H.F.L—*H. hemerocallidea* fresh leaves; H.H.D.L—*H. hemerocallidea* dry leaves
H.H.F.C—*H. hemerocallidea* fresh corms; H.H.D.C—*H. hemerocallidea* dry corms
I.M—identification method
[a]KI: Kovat indices on HP-5MS capillary column
[b]KI: Literature Kovat indices [33–35]
[c]MS: Identification based on mass spectral data
[d]RI: Identification on the basis of NIST11 library and comparison with literature data

ketones (6.2%) and carboxylic acids (6.6%) as displayed in Table 7.2. Some of the major components identified in the essential oils of *H. hemerocallidea* are shown in Fig. 7.1.

7.4 Discussion

7.4.1 Chemical Composition of Essential Oils

The present study is the first report on essential oil composition of *H. hemerocallidea*. Monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes were the dominating groups in the oil profiles of the leaves and corms

Table 7.2 Chemical classes of compounds identified in the essential oils of *H. hemerocallidea*

Chemical classes of compounds	Fresh leaves (%)	Dry leaves (%)	Fresh corms (%)	Dry corms (%)
Terpenes				
Monoterpenes	55.6	29.8	19.0	10.7
Oxygenated monoterpenes	26.5	23.1	–	39.2
Sesquiterpenes	9.6	28.7	6.2	–
Oxygenated sesquiterpenes	–	–	7.7	6.8
Total	91.7	81.6	32.9	56.7
Non-terpenic compounds				
Saturated hydrocarbons	–	–	22.4	10.4
Aromatics	2.7	4.0	28.3	13.1
Alcohols	1.4	0.3	–	–
Ketones	–	–	2.1	6.2
Carboxylic acids	–	6.5	10.1	6.6
Esters	1.5	4.3	–	–
Total	5.6	15.1	62.9	36.3
Total of identified compounds	97.3	96.7	95.8	93.0

(i.e. fresh and dry) of *H. hemerocallidea* contributing in a total of 10.7–55.6%, 23.1–39.2%, 6.2–28.7% and 6.8–7.7% respectively. These figures were largely due to sabinene (0.9–27.6%), myrcene (4.1–7.5%), δ-3-carene (0.5–6.4), *trans*-isolimonene (1.5–4.6%), limonene (1.1–4.3%), β-phellandrene (1.3–7.5%), *trans*-β-ocimene (0.7–6.4), γ-terpinene (2.6–6.5%), α-terpinolene (0.4–9.8%), linalool (15.3–25.4%), β-terpineol (2.1–9.2%), terpinen-4-ol (6.6–8.6%), α-terpineol (3.5–13.8%), β-bourbonene (3.0–6.2%), β-caryophyllene (2.2–11.5%), α-caryophyllene (1.8–4.8%), β-selinene (2.4–5.2%), δ-cadinene (3.2–4.2%) and *cis*-nerolidol (6.8–7.7%). Non-terpinic aliphatic carboxylic acids, saturated hydrocarbons and aromatics accounted for 6.5–10.1%, 10.4–22.4% and 2.7–28.3% respectively, with the main representatives being α-ionone (1.5–5.3%), hexadecane (2.7–8.1%), octadecane (2.0–6.4%), *n*-hexadecanoic acid (6.6–6.9%) and linoleic acid (3.2–6.5%). Additionally, there were also other chemical groups which were present in the essential oil of *H. hemerocallidea* such as esters, ketones and alcohols, their percentages being 1.5–4.3, 2.1–6.2 and 0.3–1.4% respectively.

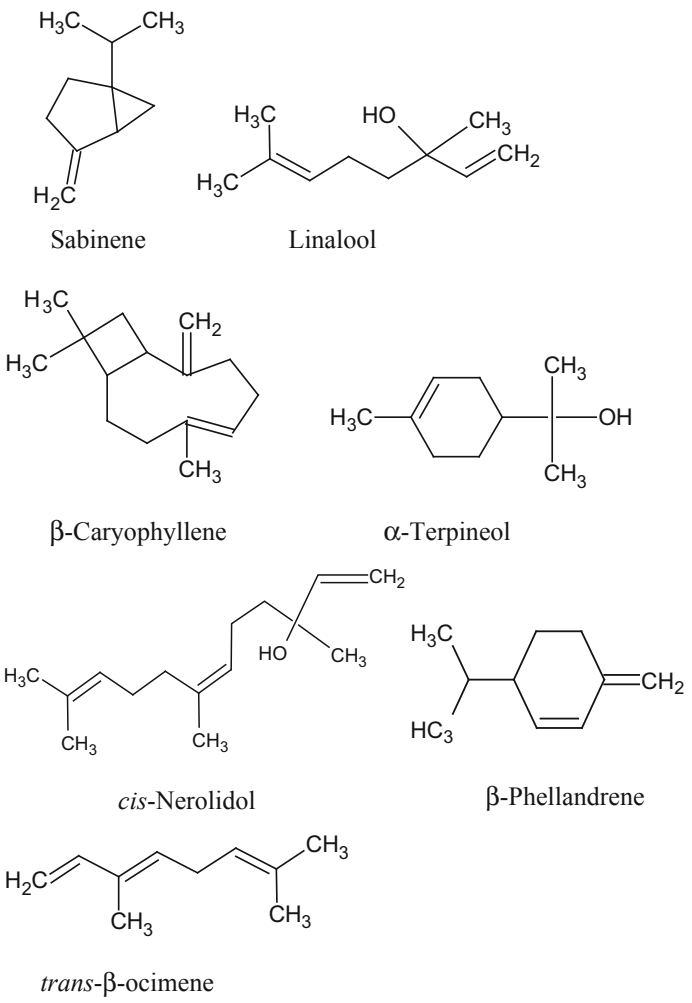

Fig. 7.1 Some of the major components identified in the essential oils of *H. hemerocallidea*

7.5 Conclusion

The findings of this study show that the *H. hemerocallidea* fresh/dry leaves and corms contain essential oils which varied in yields and chemical composition. The most abundant components identified in the essential oils were sabinene, linalool, α-terpineol and β-caryophyllene. The essential oils also had monoterpenes, monoterpenoids and sesquiterpenes as the most dominant chemical classes of compounds.

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