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Volatile constituents of *Notobubon* and *Nanobubon* (Apiaceae, tribe Tordylieae)

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ABSTRACT

The essential oil composition and headspace volatiles from the aerial parts of the genera *Notobubon* and *Nanobubon* are reported for the first time. Eleven of the thirteen *Notobubon* and two of the three *Nanobubon* species were studied. Oil yields obtained by hydro-distillation ranged from 0.1 to 3.6% of dry weight. Forty-four compounds representing 63.8–96.8% of the total oil were identified from the essential oil of eight species of *Notobubon* and one species of *Nanobubon* by GC/MS-FID. *p*-Cymene was present in all samples of *Notobubon* but not detected in *Nanobubon*. GC/GC-ToF-MS headspace analysis of eleven species of *Notobubon* showed α-pinene (1–48%), β-pinene (2–31%), *p*-cymene (1–41%), sabinene (1–27%), α-thujene (1–22%), and in one species estragole (78–97%) as the main compounds, while in *Nanobubon* myrcene (1–37%), limonene (13–30%), 3-methyl-2-methylene-butanenitrile (0–27%), sabinene (1–26%) and α-pinene (1–25%) were identified as major constituents.

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Notobubon; *Nanobubon*; Apiaceae; Umbelliferae; essential oil composition; headspace volatiles

Introduction

The genus *Notobubon* consists of thirteen species, twelve of which are endemic to the Cape Floristic Region, with one (*Notobubon laevigatum* (Aiton) Magee) extending to the eastern parts of South Africa (1, 2). The genus can be distinguished from other African peucedanoid genera by the woody habit, persistent leaves, small fruits and in some species, the presence of additional vittae below the ribs (1, 3). The only available chemical data for the genus is that of *Notobubon galbanum* (L.) Magee. This species, known as 'blister bush', is of interest because it causes blistering of human skin after contact with the leaves (4). Campbell et al. (5) predictably reported phototoxic furanocoumarins such as xanthotoxin and psoralen and also reported *p*-cymene, *trans*-β-ocimene, and nonane-4-one as the major compounds of the volatile oil.

The three species of *Nanobubon* are endemic to the Cape fynbos region of South Africa (6, 7). They are closely related to *Notobubon* and share the persistent sclerophyllous leaves but differ in the much smaller habit, subterranean stems, and acicular leaf segments (3). Both genera were previously regarded as part of the genus *Peucedanum* but molecular systematics using nuclear ribosomal ITS (3) showed that they are unrelated to *Peucedanum* (tribe Selineae) and rather belong to the tribe Tordylieae, together with several other African genera (3, 8).

The aim of this paper is to present a first detailed account of the volatile compounds of both genera and their potential chemosystematic value.

Experimental

Plant material

Freshly collected samples as well as older air-dried plant material comprising stems, leaves, and fruits of eleven species of *Notobubon* and two species of *Nanobubon* were used for the study. Voucher specimens (Table 1) were deposited in the Herbarium of the University of Johannesburg (JRAU) and the Compton Herbarium (NBG) of the South African National Biodiversity Institute at Kirstenbosch National Botanical Garden.

Essential oil isolation

The essential oil of air-dried plant materials were isolated by hydro-distillation for 3 hours in a Clevenger-type apparatus. The oils were stored in tightly sealed 1.5-mL amber vials at 4°C prior to analyses.

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Table 1. Voucher specimens and percentage yield of essential oils from *Notobubon* and *Nanobubon* species.

Species	Voucher specimens	Analytical methods	Plant parts	Oil yield (%)
Nanobubon capillaceum (Thunb.) Magee	*Magee & Boatwright 36* (JRAU)	GC/GC-ToF-MS	Leaf	
	Sobiyi & Van Wyk 43 (JRAU)	GC/MS	Leaf & stem	0.1
Nanobubon strictum (Spreng.) Magee	*Magee et al. 58* (JRAU)	GC/GC-ToF-MS	Leaf & fruit	
Notobubon capense (Eckl. & Zeyh.) Magee	*Sobiyi & Magee 23* (NBG)	GC/MS; GC/GC-ToF-MS	Leaf & stem; fruit	1.3
	Sobiyi & Magee 23 (NBG)	GC/MS	Fruit	0.3
Notobubon collinum (Eckl. & Zeyh.) Magee	*Magee 1137* (NBG)	GC/GC-ToF-MS	Leaf	
Notobubon ferulaceum (Thunb.) Magee	*Magee, Boatwright & Manning 110* (JRAU)	GC/GC-ToF-MS	Leaf	
Notobubon galbaniopse (H. Wolff) Magee	*Magee et al. 55* (JRAU)	GC/MS; GC/GC-ToF-MS	Leaf & stem; fruit	0.2
Notobubon galbanum (L.) Magee	*Sobiyi & Van Wyk 27* plant A (JRAU), LS 1, F 2	GC/MS; GC/GC-ToF-MS	Leaf & stem; fruitFruit	0.8
	Sobiyi & Van Wyk 27 plant B (JRAU), F 3	GC/GC-ToF-MS		
	Sobiyi & Van Wyk 27 plant C (JRAU), LS 2, F 4	GC/MS; GC/GC-ToF-MS	Leaf & stem; fruit	1.2
	Magee & Boatwright 10 (JRAU), LS 3	GC/MS	Leaf & stem	0.8
	Sobiyi & Magee 18 (NBG), F 1	GC/GC-ToF-MS	Fruit	
Notobubon gummiferum (L.) Magee	*Magee et al. 61* (JRAU)	GC/MS; GC/GC-ToF-MS	Leaf & stem; leaf, fruit	0.6
Notobubon laevigatum (Aiton) Magee	*Magee & Boatwright 37* (JRAU)	GC/GC-ToF-MS	Fruit	
	Sobiyi & Van Wyk 45 (JRAU)	GC/MS	Leaf & stem	0.6
Notobubon pearsonii (Adamson) Magee	*Magee et al. 42* (JRAU)	GC/MS; GC/GC-ToF-MS	Leaf & stem; leaf	0.1
Notobubon pungens (Sond.) Magee	*Magee et al. 50* (JRAU)	GC/GC-ToF-MS	Leaf	
Notobubon striatum (Thunb.) Magee	*Sobiyi & Van Wyk 32* plant A (JRAU), LS 1; F 1	GC/MS; GC/GC-ToF-MS	Leaf & stem; fruit	1.5
	Sobiyi & Van Wyk 32 plant C (JRAU), LS 2	GC/MS	Leaf & stem	1.2
	Sobiyi & Van Wyk 32 plant C (JRAU), F 2	GC/MS	Fruit	3.6
Notobubon tenuifolium (Thunb.) Magee	*Magee & Van Wyk 60* (JRAU)	GC/GC-ToF-MS	Fruit	
	Magee et al. 44 (JRAU)	GC/MS	Leaf & stem	0.7

GC/MS analysis of the essential oil

GC/MS-FID analysis was performed with an Agilent 6890 N GC system coupled directly to a mass spectrometer (a 5973 MS) with an 24.79 psi autosampler. Column: HP-Innowax polyethylene glycol 60 µm × 250 µm ID × 0.25 µm film thickness. The temperature program started at 60°C (10 minutes), raised to 220°C at 4°C/min and held constant at 220°C (10 minutes), then raised to 250°C at 1°C/min. Inlet temperature was 250°C and the carrier gas was Helium (1.2 mL/min). The injection mode: split (200:1) and spectra were recorded at 70 eV, scanning from 35 to 550 *m/z*. Flame ionization detection (FID): 250°C. *n*-Alkanes (C_6–C_{22}) were used as reference points to calculate the relative retention indices. Component identifications were made by comparing retention indices and mass spectra. Library searches were carried out using Flavour®, Mass Finder®, and NIST® libraries (9).

Headspace analysis

The GC/GC system consisted of a Multi-Purpose Sampler (Gerstel) which was operating in the headspace mode. The system was equipped with a 1000-µL gas syringe and a tray for 20-mL vials (Gerstel). Samples were placed in headspace vials and analyzed using a Leco Pegasus© 4 GC/GC-ToF-MS system. Sample (0.5 g) was heated and agitated at the same time for 3 minutes at 80°C in the pre-heating module and 1000 µL headspace collected from each vial was injected into the GC/GC system with a 20:1 split ratio. The system consists of an Agilent© 7890 gas chromatograph with cryogenic thermal modulator and a secondary oven. A 30 m × 0.25 mm × 0.25 µm film thickness Stabilwax® GC capillary column was used as the first column for analysis, followed by a 0.790 m × 0.25 mm × 0.25 µm film thickness Rxi-5Sil–MS® GC capillary column. Helium was used as the carrier gas at a constant flow rate of 1.50 mL/min, with front inlet septum purge flow at 3 mL/min, purge valve time 60 seconds after the beginning of the run. The inlet temperature was 200°C throughout the analysis and the primary column was programmed with an initial oven temperature at 40°C for 1 minute, at the rate of 7°C/min and ramped to 220°C for 3 minutes. The secondary column temperature program was set to an initial temperature of 60°C for 0.50 minutes then ramped at 7°C/minutes to 240°C for 2 minutes. Both the front inlet and transfer line temperatures were constant at 200 and 225°C, respectively. The MS mass range was 45–550 *m/z* with an acquisition rate of 100 spectra/second. The ion source chamber was set to 230°C. The hot pulse time and modulation period were set at 0.60 and 1.5 seconds, respectively (Kamatou, unpublished).

Data processing and analysis

Data were processed automatically using LECO ChromaTOF® software version 4.50. The minimum signal to noise ratio (S/N) cut off >250 based on 'unique mass' was used after deconvolution of the mass spectra signal. Identification of peaks was based on the NIST Mass Spectral Library (NIST 11). Library similarity factors were reported on a scale ≥700 (high value is associated with better match) for both forward and reverse searches. Relative

abundance (% area) calculations were based on the ratio between the peak area of each compound and the sum of all integrated compounds.

Results and discussion

The percentage oil yield for stems and leaves of *Notobubon* and *Nanobubon* was variable (Table 1) and ranged from 0.1 to 1.5 (w/w%). Fruit materials gave higher yields (ca. 0.3 to 3.6 w/w%) than stems and leaves (ca. 0.1 to 1.5 w/w%). In particular, the fruits of *Notobubon striatum* (Thunb.) Magee yielded the highest percentage of oil.

The major compounds in the essential oil of eight species of *Notobubon* and one species of *Nanobubon* as analyzed by GC/MS-FID are presented in Table 2. Forty-four components of the oil were identified, representing approximately 64–97% of the essential oil. The major components in all or most species were *p*-cymene (0.4–77%), myrcene (1–37%), β-pinene (1–26%), sabinene (0.6–23%), (*E*)-β-ocimene (0.4–17%), and γ-terpinene (1–8%). Interestingly estragole (78–97%) was found only in *N. striatum*.

The main compound in *N. galbanum* was identified as *p*-cymene (49–65%), thus confirming the only published essential oil data reported by Campbell et al. (5), who stated that this compound had the highest percentage abundance in the essential oil of the aerial parts in the species. Similarly, *p*-cymene occurred in all the studied species of *Notobubon* (0.4–76.9%). In contrast, *Nanobubon capillaceum* (Thunb.) Magee oil showed myrcene (37.3%) and (*E*)-β-ocimene (11.6%) as the major compounds. At least nine volatile compounds were shared with species of *Notobubon* but it is noteworthy that that *p*-cymene was absent. As such *p*-cymene may be considered a useful chemosystematic marker for *Notobubon* (or for *Nanobubon*, if its absence is considered to be the apomorphic state).

Estragole was present only in the leaves and fruits of *N. striatum*, where it occurred as the dominant compound (96.8% in the fruit and 77.8–86.7% in the leaves and stems). As such it is an interesting diagnostic character for this species and explains the distinct and strong anise scent unique to *N. striatum*. This phenylpropanoid is known to be a major constituent in many plants such as basil (*Ocimum basilicum* L.) of the Lamiaceae but also well-known spices of the Apiaceae such as chervil [*Anthriscus cerefolium* (L.) Hoffm.], anise (*Pimpinella anisum* L.) and fennel (*Foeniculum vulgare* L.) (10, 11). However, it is the first time this compound is recorded in the tribe Tordylieae. The main component in the essential oil of *Notobubon pearsonii* (Adamson) Magee was γ-himachalene (30.6%), a compound that has been identified in *Pimpinella anisum* (12, 13) and *Seseli bocconi* (14). It was found at a low concentration (only 3%) in *Notobubon gummiferum* (L.) Magee (Figure 1). Molecular analysis of nrITS indicated that the morphological placement of *N. pearsonii* in *Notobubon* was ambiguous (3). It is therefore also noteworthy that myrcene and γ-terpinene were absent in the leaf oil of *N. pearsonii* but present in leaf samples of all other species of *Notobubon* (Figure 2). Isolongifolene was most abundant in the essential oil of *Notobubon galbaniopse* (H. Wolff) Magee (19.3%), while terpinen-4-ol had the highest level in *Notobubon tenuifolium* (Thunb.) Magee (18.3%) (Figure 3). Two main

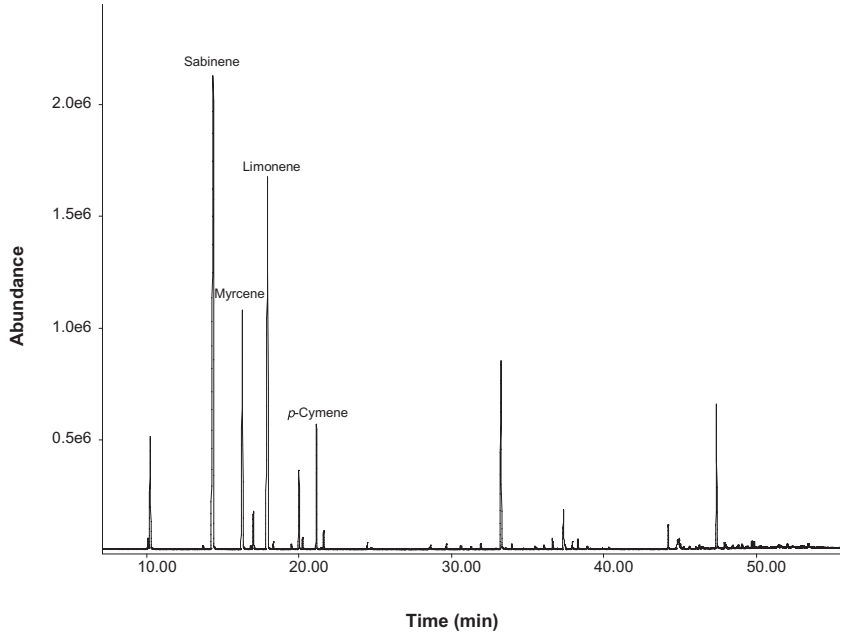

Figure 1. GC chromatogram of essential oil from leaves and stems of *Notobubon gummiferum*.

Table 2. Essential oil composition of *Nanobubon* and *Notobubon* species as determined by GC/MS-FID (% area ≥0.4; lower values are indicated as trace amounts (tr) but only when their presence was confirmed by headspace analysis.

		Na cap	No cap		No gap	No gal			No gum	No lae	No pea	No str			No ten
Compounds	RRI	LS	LS	F	LS	LS 1	LS 2	LS 3	LS	LS	LS	LS 1	LS 2	F	LS
α-pinene	1016	2.1	–	–	–	1.4	–	0.6	tr	5.8	1.1	–	–	–	0.6
α-thujene	1019	–	–	–	–	–	–	–	–	–	–	–	–	–	6.9
β-pinene	1104	1.4	–	–	0.6	9.9	tr	4.2	tr	1.5	25.9	–	–	–	3.3
sabinene	1117	0.6	–	–	–	–	4.0	–	22.9	21.5	–	9.0	5.4	–	17.0
myrcene	1159	37.3	1.1	–	1.8	1.7	1.5	2.5	12.1	1.1	–	1.1	0.6	–	2.4
α-phellandrene	1160	–	–	–	–	–	–	–	3.7	–	–	–	–	–	–
α-terpinene	1174	–	–	–	–	–	–	–	–	1.1	–	–	–	–	3.4
butyl 2-methylbutyrate	1191	–	–	–	–	–	–	–	–	–	–	–	–	–	1.3
limonene	1194	7.9	–	–	1.1	–	–	–	19.9	8.9	–	–	–	–	0.6
trans-3-caren-2-ol	1196	–	–	–	–	–	–	–	1.3	–	–	–	–	–	–
β-phellandrene	1203	–	–	–	–	–	–	–	–	–	–	–	–	–	2.4
isobutyl isovalerate	1206	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8
(*Z*)-β-ocimene	1232	6.1	0.8	–	–	1.6	0.4	4.8	–	1.5	–	0.4	–	–	1.0
(*E*)-β-ocimene	1250	11.0	2.3	–	–	3.3	0.9	17.0	–	5.1	–	0.9	–	–	0.4
γ-terpinene	1266	–	3.0	–	0.8	4.5	1.9	4.9	3.0	4.7	–	1.1	0.6	–	7.6
amyl isovalerate	1266	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7
p-cymene	1270	–	76.9	74.1	12.8	64.6	54.5	49.3	4.0	21.9	3.6	0.4	tr	tr	9.3
terpinolene	1281	–	–	–	–	–	–	–	0.7	0.7	–	–	–	–	1.7
isobutyl tiglate	1377	–	1.0	–	–	0.6	–	0.4	–	–	–	–	–	–	0.7
α-cubebene	1453	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6
3-methylbutyl ester	1490	–	1.4	–	0.5	–	–	–	–	–	–	–	–	–	–
6-undecanol	1492	–	0.5	–	–	–	–	–	–	–	–	–	–	–	–
β-bourbonene	1517	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7
isolongifolene	1527	–	–	–	19.3	–	–	–	7.0	–	–	–	–	–	–
β-cubebene	1540	–	–	–	–	1.3	–	–	–	1.0	–	–	–	–	–
trans p-menth-2-en-1-ol	1563	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0
β-caryophyllene	1596	4.4	–	–	3.3	–	–	–	9.1	–	–	–	–	–	–
terpinen-4-ol	1602	–	–	–	–	–	–	–	–	14.0	–	–	–	–	18.3
α-terpineol	1701	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–
cis p-menth-2-en-1-ol	1627	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0
estragole	1682	–	–	–	–	–	–	–	–	–	–	77.8	86.7	96.8	–
γ-himachalene	1704	–	–	–	–	–	–	–	2.6	–	30.6	–	–	–	–
bicyclogermacrene	1741	–	1.9	–	–	–	–	–	–	0.4	–	–	–	–	–
β-cadinene	1745	–	–	–	–	–	–	–	2.0	–	–	–	–	–	0.5
δ-cadinene	1763	–	–	–	1.2	–	–	–	–	–	–	–	–	–	–
caryophyllene oxide	2010	–	–	–	9.1	–	–	–	–	–	–	–	–	–	–
methyleugenol	2021	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–
β-guaiene	2112	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0
cycloisolongifolene	2168	–	–	–	–	1.4	–	–	–	–	16.7	–	–	–	–
carvacrol	2228	–	–	–	4.1	–	–	–	–	–	–	–	–	–	–
spathulenol	2114	3.4	–	–	–	–	–	–	–	0.8	–	–	–	–	–
(*E*)-nerolidol	2041	–	–	–	–	–	–	–	–	0.8	–	–	–	–	–
p-cresol	2088	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–
α-bisabolol	2223	–	–	–	–	–	–	–	–	1.2	–	–	–	–	–
Total (%)		74.2	88.9	74.1	66.3	90.3	63.8	83.7	88.3	92.5	77.9	90.7	93.3	96.8	83.2

Notes: Na cap, *Nanobubon capillaceum*; No cap, *Notobubon capense*; No gap, *Notobubon galbaniopse*; No gal, *Notobubon galbanum*; No gum, *Notobubon gummiferum*; No lae, *Notobubon laevigatum*; No pea, *Notobubon pearsonii*; No str, *Notobubon striatum*; No ten, *Notobubon tenuifolium*; LS, leaf & stem; F, fruit; RRI, Relative Retention Indices measured relative to *n*-alkanes C6–C22 on HP-Innowax column.

compounds, *p*-cymene (21.9%) and sabinene (21.5%) were recorded in *N. laevigatum*.

Headspace analysis of leaves and fruits of eleven species of *Notobubon* resulted in the identification of seventy-four components representing 74.2–99.7% of the total volatiles detected (Table 3). Apart from the larger number of volatiles identified, the main compounds were quite similar to those found in the essential oil. The main constituents identified in almost all the species of *Notobubon* included α-pinene (1–48%), β-pinene (2–31%), *p*-cymene (1–41%), sabinene (1–27%), and α-thujene (1–22%). This combination of constituents was also present in the essential oil samples and is commonly found in many members of the Apiaceae (15) and also in the genus *Peucedanum* (16). It therefore appears that essential oil has limited chemosystematic value at tribal and generic levels (11).

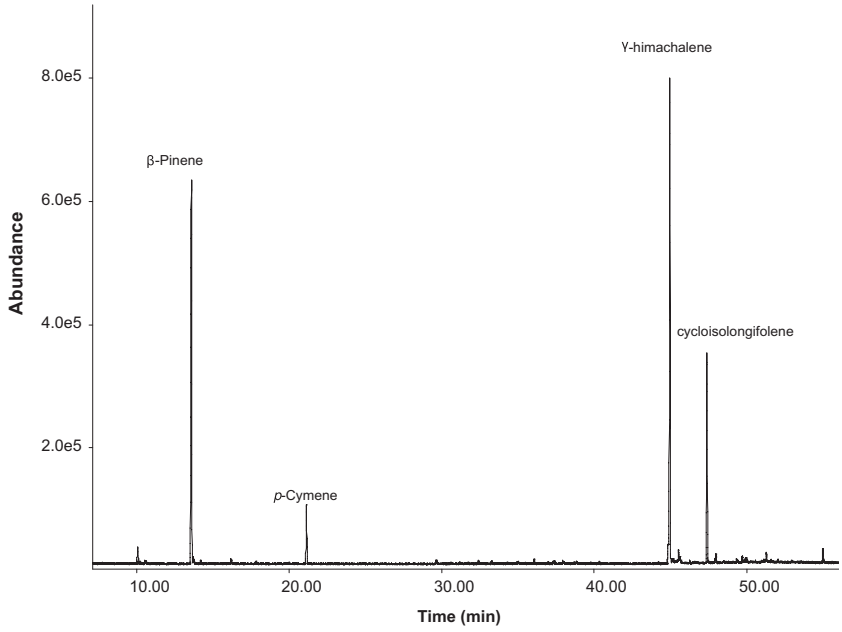

Figure 2. GC chromatogram of essential oil from leaves and stems of *Notobubon pearsonii*.

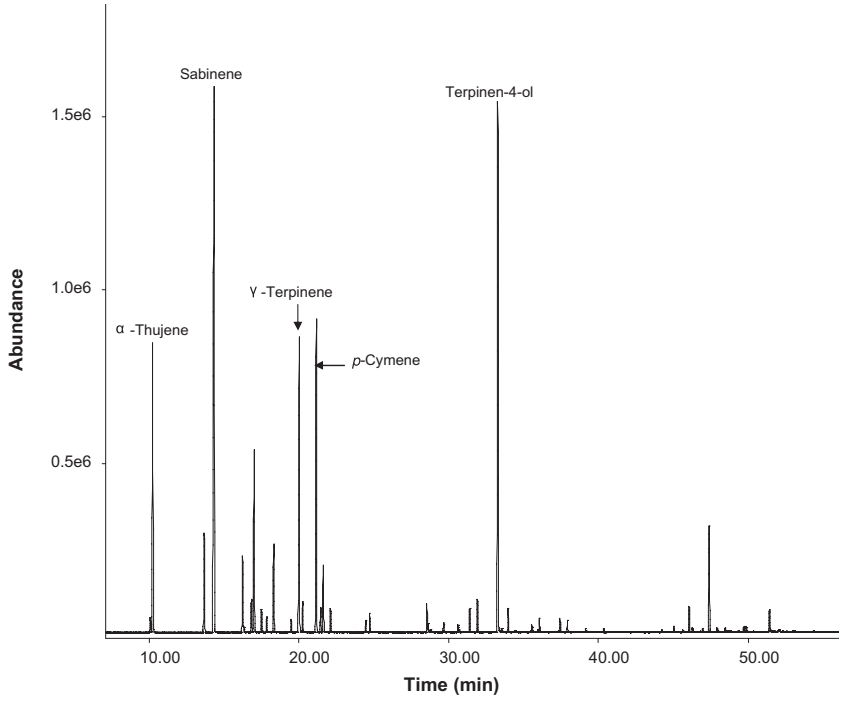

Figure 3. GC chromatogram of essential oil from leaves and stems of *Notobubon tenuifolium*.

Table 3. Headspace analysis (% area) of *Notobubon* and *Nanobubon* species.

		Na cap	Na stc		No cap	No col	No fer	No gap	No gal				No gum		No lae	No pea	No pun	No str		No ten
Compounds	RT (s)	L	L	F	F	L	L	F	F 1	F 2	F 3	F 4	L	F	F	L	L	F 1	F 2	F
octane	150, 0.70	1.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2-methyl butanal	158, 0.55	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–
2,4-dimethyl hexane	164, 0.68	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2-ethyl furan	176, 0.56	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
nonane	216, 0.78	0.3	–	–	–	–	–	–	0.7	–	–	0.2	–	–	–	1.2	–	–	–	–
2,4,6-trimethyl octane	220, 0.76	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2-methyl-3-buten-2-ol	226, 0.50	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
3-nonene	232, 0.78	2.8	–	–	–	–	–	0.5	–	–	–	2.0	–	–	–	–	–	–	–	–
cis-4-nonene	236, 0.77	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–
2-methyl butyrate isopropyl	266, 0.69	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
santolina triene	268, 0.80	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	0.2	–	–	–	–
3,7-dimethyl-1,3,7-octatriene	270, 0.80	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.0	–	–	–	–
hexanal	272, 0.58	0.1	0.1	–	–	0.2	0.1	–	0.1	–	–	–	0.1	–	–	0.1	1.1	–	0.1	–
α-pinene	276, 0.78	6.1	24.9	0.6	–	1.5	14.6	–	–	–	–	–	6.2	3.3	–	16.4	47.5	–	–	–
4-methyl-1,5-heptadiene	278, 0.74	1.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-thujene	280, 0.76	–	–	–	10.3	–	–	4.7	4.9	9.9	7.5	14.3	0.3	22.2	1.2	5.1	–	6.6	10.3	–
3-methyl nonane	282, 0.84	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–	–	–	–
3-methyl-2-isobutyl pyrazine	302, 0.75	–	–	–	–	–	–	–	–	14.0	–	–	–	–	–	–	–	–	–	24.5
camphene	312, 0.75	–	0.3	–	–	–	0.2	–	–	0.1	0.1	0.2	–	0.6	–	0.1	–	–	–	–
decane	312, 0.84	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–
2,4(10)-thujadiene	346, 0.78	–	–	–	–	–	–	–	–	5.4	–	–	–	–	–	19.5	–	13.2	–	–
3-carene	344, 0.78	–	–	9.1	–	–	–	–	–	–	–	–	–	–	–	5.6	–	–	–	–
β-pinene	346, 0.76	–	11.7	–	1.9	9.3	–	9.8	31.0	17.3	22.7	17.8	20.5	3.3	–	6.0	–	1.7	–	4.9
dimethyl sulfone	348, 0.79	–	–	–	3.6	–	–	–	–	–	–	–	–	–	–	–	–	0.8	–	–
2-methoxybenzyl alcohol	350, 0.72	–	–	–	–	–	–	–	–	4.1	–	–	–	3.0	–	–	–	1.7	–	–
sabinene	350, 0.75	2.5	26.0	0.9	15.6	–	10.6	0.6	3.9	0.9	2.5	3.6	22.6	6.5	11.2	–	26.8	6.4	21.9	–
3,4-dimethyl styrene	350, 0.79	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9	–	26.7
3-methyl-2-butenal	352, 0.53	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
α-terpinene	352, 0.74	–	–	–	–	–	–	–	–	2.5	–	–	–	12.3	–	–	–	–	–	–
2,7-dimethyl-2,6-octadien-1-ol	352, 0.77	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.8	–	–	–	–
2,7-dimethyl-3-octen-5-yne	354, 0.71	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9.3	–	–
α-phellandrene	354, 0.77	–	8.4	–	1.1	–	–	–	–	–	–	–	–	1.5	–	1.4	–	18.6	–	–
5-butyl, 1,3-cyclohexadiene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6.7
heptanal	364, 0.61	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
benzyl isobutyl ketone	366, 0.70	–	–	–	–	–	–	–	0.7	–	–	0.7	–	–	–	–	–	–	–	–
spiro[3.3]hepta-1,5-diene	370, 0.70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–	–
myrcene	380, 0.70	37.2	3.6	1.2	1.5	0.5	2.2	3.7	–	2.5	0.1	7.9	1.8	9.3	3.6	0.5	4.6	0.5	1.5	–
α-ocimene	386, 0.74	–	–	–	–	–	0.5	0.2	–	1.0	–	0.9	–	–	–	–	–	–	–	1.9
δ-carene	402, 0.72	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–
α-terpinene	402, 0.72	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–
terpinolene	402, 0.72	–	–	–	–	1.2	–	–	1.3	1.4	–	–	–	–	–	–	–	0.3	–	–
trans-3-caren-2-ol	406, 0.71	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	0.1	0.5
Z-3-undecene	410, 0.83	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–

(*Continued*)

Table 3. (*Continued*)

		Na cap	Na stc		No cap	No col	No fer	No gap	No gal				No gum		No lae	No pea	No pun	No str		No ten
Compounds	RT (s)	L	L	F	F	L	L	F	F 1	F 2	F 3	F 4	L	F	F	L	L	F 1	F 2	F
1,8-cineole	414, 0.74	–	–	–	–	–	–	–	0.1	–	–	0.4	–	0.2	–	–	–	–	–	–
(*E*)-5-undecene	414, 0.82	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–
dimethyl styrene*	416, 0.74	–	–	14.1	–	3.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
cyclopentylethanol	418, 0.71	–	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	2.6	–
verbenene*	418, 0.72	14.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3-methyl-2-methylene-butanenitrile*	422, 0.73	–	–	26.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1,7-octadiene, 3,6-dimethylene	422, 0.73	–	–	–	–	12.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1,2-dimethyl-4-ethenyl benzene	424, 0.73	–	–	–	–	4.0	–	–	–	–	–	–	–	–	–	1.1	–	–	–	–
sylvestrene	426, 0.71	–	–	5.2	6.0	0.2	–	–	–	–	–	–	6.4	–	6.8	–	–	0.9	5.8	–
limonene	426, 0.72	30.3	17.3	13.1	–	33.9	31.3	–	–	–	–	–	19.6	–	–	9.1	10.8	–	–	–
β-phellandrene	428, 0.71	–	–	–	–	–	–	1.3	1.0	0.8	–	2.4	10.7	–	–	–	–	–	–	–
dimepiperate	432, 0.72	–	–	–	–	–	–	–	–	–	–	–	2.6	–	–	–	–	–	–	–
isobutyl angelate	434, 0.67	–	–	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–	–	1.0
Z -β- ocimene	–	–	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–	–	–	–	–
p-cymenene	438, 0.71	–	–	0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
m-cymene	448, 0.67	–	–	–	–	–	–	5.8	–	0.2	–	–	–	–	–	–	–	–	–	–
ɣ-terpinene	456, 0.71	–	1.8	0.1	–	5.5	0.8	–	–	–	15		1.6	4.1	35.7	3.1	–	0.7	1.0	–
o-cymene	458, 0.64	0.9	2.0	–	–	–	15.7	–	–	–	0.7	–	–	–	–	–	–	1.0	–	2.3
octanal	460, 0.62	–	–	–	–	6.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
p-cymene	460, 0.66	–	–	–	3.6	8.3	14.6	30.4	17.9	3.3	4.7	15.4	2.4	21.3	41.1	4.5	6.2	0.5	1.2	–
3-methyl-2-buten-1-ol	462, 0.67	–	–	–	–	–	–	–	–	–	–	5.9	–	–	–	–	–	–	–	1.7
unknown	464, 0.66	–	–	–	–	–	–	31.9	18.3	8.5	19.1	–	–	–	–	–	–	–	–	8.3
2, methyl, 1-hexen-4-yne, 3-ethylidene	466, 0.66	–	–	–	–	–	–	–	–	16.8	–	21.0	–	–	–	–	–	–	–	–
methyl acetophenone	468, 0.64	–	–	–	–	–	–	–	–	–	9.1	–	–	–	–	–	–	–	–	–
isobutyl tiglate	522, 0.64	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	0.2
perillene	558, 0.60	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
cis-thujone	588, 0.63	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–
cis-sabinene hydrate	590, 0.59	–	0.2	–	–	–	0.5	–	–	–	–	–	0.3	–	–	0.3	–	–	–	–
1-octen-1-ol, acetate	616, 0.63	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
trans-3-hexen-1-ol	616, 0.65	–	–	–	–	–	–	–	–	1.4	–	–	–	–	–	–	–	–	–	–
trans-sabinene hydrate	648, 0.57	–	–	–	–	–	0.3	–	–	–	–	–	–	–	– .	0.1	–	–	–	–
α-copaene	706, 0.79	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–
α-bourbonene	720, 0.78	–	0.3	–	–	0.1	–	–	–	–	–	–	0.1	–	–	0.3	–	–	–	–
methyl chavicol	736, 0.57	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–
anethole (*cis*/*trans*)	738, 0.56	–	–	–	26.0	–	–	–	–	–	–	–	–	–	–	–	–	5.3	7.1	–
estragole	738, 0.58	–	–	–	19.1	–	–	–	–	–	–	–	–	–	0.1	–	–	10.9	26.9	–
cryptone	740, 0.57	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–
β-caryophyllene	768, 0.76	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–	–	–	–	–
sesquisabinene	798, 0.73	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
bicyclogermacrene	848, 0.73	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total (%)		97.7	97.2	71.4	88.7	87.3	92.6	88.9	81.0	90.2	81.9	93.1	95.3	87.6	99.7	80.7	97.2	80.0	78.5	78.7

Notes: Na cap, *Nanobubon capillaceum*; Na stc, *Nanobubon strictum*; No cap, *Notobubon capense*; No col, *Notobubon collinum*; No fer, *Notobubon ferulaceum*; No gap, *Notobubon galbaniopse*; No gal, *Notobubon galbanum*; No gum, *Notobubon gummiferum*; No lae, *Notobubon laevigatum*; No pea, *Notobubon pearsonii*; No pun, *Notobubon pungens*; No str, *Notobubon striatum*; No ten, *Notobubon tenuifolium*; LS, leaf & stem; F, fruit; RT (retention times, in second) on the first and second columns; *: tentative identifications.

It is interesting to note that estragole occurs only in the large, shrubby form of *N. striatum* and not in the small, rhizomatous form [which has been reinstated as a distinct species (2), (Figures 4 and 5)]. These two taxa are morphologically very similar (except in their habits) and have hitherto been considered to be two forms of a single species (1, 2). Seventeen components were found in both the essential oil and headspace volatiles. *N. capillaceum* and *Nanobubon strictum* (Spreng.) Magee (Table 3) had twenty-seven components representing (71–98%) of the total volatiles detected (Figure 6). This is a first report on the volatile oil chemistry of the genus *Nanobubon*. The main compounds in the leaf samples of both species included myrcene (4–37%), limonene (13–30%), sabinene

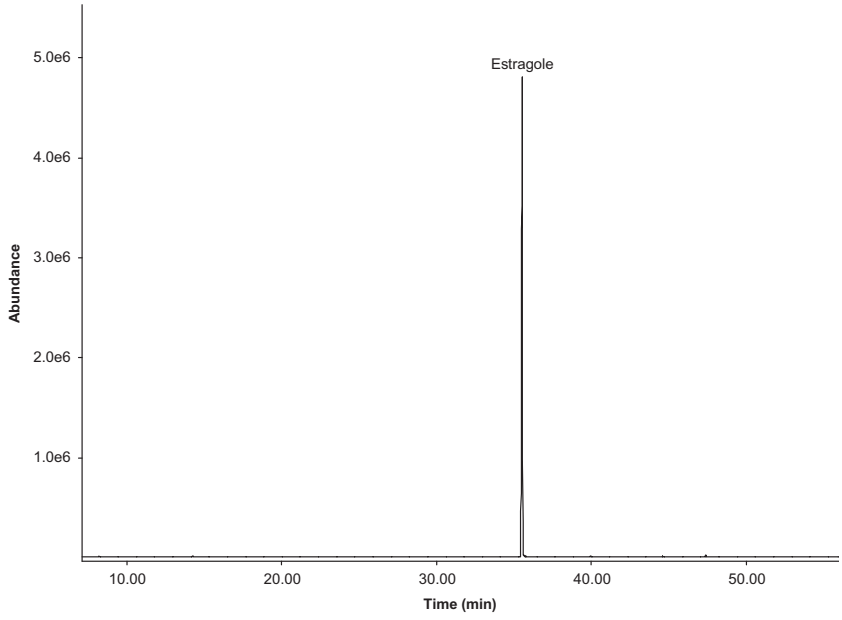

Figure 4. GC chromatogram of essential oil from the fruits of *Notobubon striatum*.

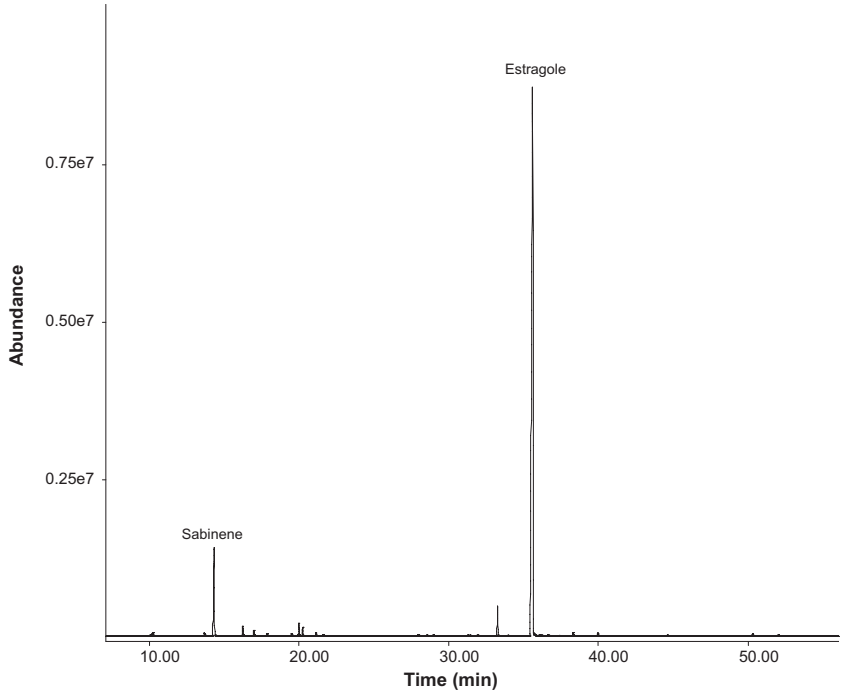

Figure 5. GC chromatogram of essential oil from leaves and stems of *Notobubon striatum*.

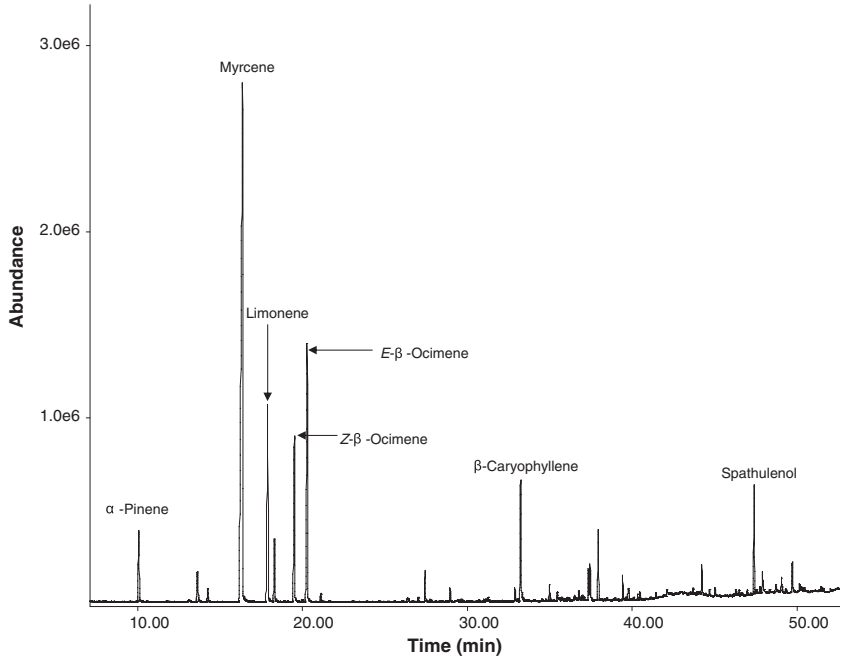

Figure 6. GC chromatogram of essential oil from leaves and stems of *Notobubon capillaceum*.

(1–26%), and α-pinene (1–25%), while the fruits of *N. strictum* uniquely had 3-methyl-2-methylene butane-nitrile as the main compound. In general, large quantitative differences (and even some apparent qualitative differences) were found between leaf and fruit samples. This aspect deserves further study, as it may be better to investigate specific plant parts for chemosystematic purposes.

More samples should be analyzed before definite conclusions can be made about possible generic differences but it is tempting to propose that the apparent absence of *p*-cymene can be regarded as a diagnostic character for *Nanobubon*. At species level, the overall pattern of chemical variation in *Notobubon* and *Nanobubon* did not reveal any obvious taxonomically useful chemical characters that can be used as synapomorphies to support the provisional phylogenies based on morphology (1) and molecular data (3, 17). There are nevertheless noteworthy differences between some of the species that may prove to be of diagnostic value.

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