



The chemotypic variation of *Sceletium tortuosum* alkaloids¹ and commercial product formulations

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ABSTRACT⁵

Commercial production and trade in *Sceletium tortuosum* and its products is on the rise due to an increase in consumer demand. Mesembrine-type alkaloids are the main active compounds responsible for the therapeutic properties of the plant. In this investigation, GC–MS was used to evaluate the variability in the mesembrine-type alkaloid content of 151 specimens of *S. tortuosum* growing wild in 31 localities in the south-western region of South Africa. In addition, the variability of the mesembrine-type alkaloid content of eight commercial products was determined. The total alkaloid content of wild plant material was found to vary between 0.11 and 1.99% of dry weight. Hierarchical cluster analysis and principal component analysis grouped the plants growing in the wild into five main chemotypes (A, B, C, D and E). Chemotype A is represented by samples devoid of mesembrine-type alkaloids, while chemotypes B, C and E are characterised by high levels of mesembrenol (64.96–95.55%), mesembrine (51.25–92.50%) and mesembrenone (50.86–72.51%), respectively. Chemotype D is an intermediate cluster representing specimens containing all four alkaloids in moderate amounts. Qualitative differences in the alkaloid profiles were observed between and within different populations of *S. tortuosum*, as well as among the investigated products. Mesembrine was identified as the main alkaloid constituent in most of the commercial products analysed. Mesembrenol, mesembrine and mesembrenone can be used as marker compounds for quality monitoring of *S. tortuosum* raw materials and products.

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1. Introduction¹⁰

Sceletium tortuosum (L.) N.E.Br (Mesembryanthemaceae), a succulent sub-shrub endemic to South Africa, is highly valued for its traditional uses as a masticatory and medicine. The species is used in the treatment of psychiatric conditions and neurodegenerative diseases (Gomes et al., 2009). It is mainly distributed in the Karroid areas of the Cape region of South Africa (Gerbaulet, 1996) and is locally referred to as “kanna” or “canna” (Khoi) and “kougoed” (Afrikaans) (Gericke and van Wyk, 1999). The use of *Sceletium* in South Africa was documented as early as 1685 (Smith et al., 1996) and trade in this plant as

Abbreviations: CNS, central nervous system; PCA, principle component analysis; PDE, phosphodiesterase; PLS, partial least squares; QC, quality control.

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a commodity took place in the Little Karoo, in the mid-nineteenth century (Digby, 2005). This area was previously known as “Cannaland” and is still referred to as “Kannaland”, the name derived from the *Sceletium* growing there. 1

The first commercial cultivation of *S. tortuosum* was initiated by Grassroots Natural Products in 1996, under contract to the phytomedicines programme of the South African pharmaceutical company known as Pharmacare Ltd. Commercial cultivation of *S. tortuosum* has expanded in recent years and is carried out in the Eastern Cape, Western Cape and Northern Cape provinces of South Africa, as well as in Namibia (www.Sceletium.org/cultivation.htm). Although South Africa is the main producer of *Sceletium* raw material and products, many companies (mainly internet-based), trading in *Sceletium* raw materials and manufactured products, have been established in various parts of the world including the USA, Canada, United Kingdom, Germany and China. In 2009, the South African company HG&H Pharmaceuticals Pty Ltd. was granted the country's first integrated export and bioprospecting permit, allowing it to export the first standardised extract of *S. tortuosum* under the trade mark Zembrin® (Patnala and Kanfer, 2009). 2

Traditionally, *S. tortuosum* was used as a masticatory drug for the relief of thirst and hunger, as a traditional medicine, and for spiritual purposes by San and Khoikhoi people inhabiting the Cape region of South Africa (Smith et al., 1996; Gericke and Viljoen, 2008). Dried plant material (aerial parts; leaves and twigs) is typically chewed and the resulting saliva and plant juices swallowed; it is also consumed in the form of teas, decoctions and tinctures and is sometimes smoked and used as snuff. In recent years, *Sceletium* has received a great deal of commercial interest due to its potential as an adaptogen for relieving stress in healthy people, and for treating a broad range of psychological, psychiatric and inflammatory conditions (Gericke, 2001). The number of websites advertising *Sceletium* raw materials and products in various formulations, including tablets, extracts, sprays, capsules and tinctures, is increasing rapidly (Patnala and Kanfer, 2009). 3

Quality control (QC) has been a great challenge in the development of herbal medicines due to the diversity and complexity of their secondary metabolites (Hu et al., 2006). Biomarkers or pharmacologically active compounds are generally used as standards in the quality control of traditional medicines (Li et al., 2007). Various techniques, including chemical profiling of secondary metabolites and metabolomics have been useful tools for the evaluation and QC of herbal materials and derived products. Chromatographic (HPLC, GC and HPTLC), spectrometric (MS and NMR) and vibrational spectroscopic techniques (MIR, NIR and Raman spectroscopy), have been applied in the quality assessment of phytomedicines and their raw materials (Hu et al., 2006; Li et al., 2007). The complexity of data (multivariate data) collected from analytical techniques makes classical statistical methods of data analysis challenging (Van der Kooy et al., 2008). Chemometric techniques such as principle component analysis (PCA) and partial least squares (PLS) analysis, have thus become important scientific tools for faster analysis of multivariate data. Multivariate data are complex experimental data arising from multiple variables measured for multiple numbers of observations (samples) and require multivariate projection methods, such as PCA and PLS, to simplify the complex data. 4

Mesembrine-type alkaloids (mesembrine, mesembrenone, mesembranol and mesembrenol) have been reported to be responsible for the psychoactive properties of *S. tortuosum* (Harvey et al., 2011). This has been attributed to their ability to act as selective serotonin re-uptake and phosphodiesterase-4 (PDE4) inhibitors (Gericke and Viljoen, 2008). The indigenous cultural relevance, potential health applications of *S. tortuosum* and the psychoactive alkaloids, as well as the increasing commercial interest in the species, prompted this investigation of the phytochemical variation of mesembrine-type alkaloids in wild populations of *S. tortuosum* plants and in manufactured products, to establish the range of chemotypes present in this species, and their provenances. 5

2. Materials and methods 6

2.1. Standards and solvents 7

Four alkaloid standards (mesembrine, mesembrenol, mesembranol and mesembrenone), were previously isolated from *S. tortuosum* using high-speed countercurrent chromatography (Shikanga et al., 2011). The identities and purities of the alkaloids were determined using a combination of TLC, HPLC, GC–MS and ^1H and ^{13}C (1 and 2D) NMR, as well as by comparing their spectral data with literature data. All the solvents used, including methanol (MeOH), dichloromethane (DCM), ammonia (NH_3 ; 25% w/w solution) and sulfuric acid (H_2SO_4 ; 98.08% w/w), were AR grade and were purchased from Merck™ (Germany). 8

2.2. Sampling 9

Aerial parts of wild *S. tortuosum* plants were collected from various localities in the south-western region of South Africa in August and November, 2009, at altitudes ranging from 232 to 803 m above sea level. Voucher specimens of all collections were deposited at the Department of Pharmaceutical Sciences, Tshwane University of Technology and are listed in Table 1. Commercial products containing *S. tortuosum* (Com1–8) were purchased from local health shops and directly from manufacturers. 10

2.3. Sample preparation 11

The plant materials were dried in an oven (Labotec LTD; Johannesburg, South Africa) at 30 °C for two weeks prior to extraction. Dry plant material (leaves) was pulverised to fine powder using a Retsch® MM 400 ball mill (Monitoring and 12

Table 1Alkaloid yield, percentage composition and voucher specimen numbers of *Sceletium tortuosum* specimens from different localities.

Area/Locality	Locality id	Voucher specimen no.	Chemotype	^a % Alkaloid yield	% Mesembrenol	% Mesembranol	% Mesembrine	% Mesembrenone
Lemoenshoek	L1	SCT001	A	0.28	nd	nd	nd	nd
		SCT002	A	0.11	nd	nd	nd	nd
		SCT003	A	0.80	nd	nd	nd	nd
Zoar	L2	SCT004	B	1.09	80.02	1.51	3.99	14.48
		SCT005	B	0.97	92.71	2.96	0.21	4.12
		SCT006	B	1.44	93.05	2.34	nd	4.61
		SCT007	B	1.46	68.00	3.68	1.01	27.31
Oudtshoorn hill (S side)	L3	SCT008	E	0.60	13.22	7.12	14.63	65.03
		SCT009	E	0.58	27.93	7.06	14.01	51.00
		SCT010	E	1.49	10.92	12.94	3.63	72.51
		SCT011	B	0.42	91.03	3.90	1.06	4.01
Oudtshoorn hill (N side)	L4	SCT012	A	0.82	nd	nd	nd	nd
		SCT013	A	0.54	nd	nd	nd	nd
		SCT014	A	0.42	nd	nd	nd	nd
		SCT015	A	0.34	nd	nd	nd	nd
Oudtshoorn (Beleza restaurant)	L5	SCT016	D	0.75	6.97	14.18	33.91	44.95
		SCT017	D	0.49	8.48	15.93	21.53	54.06
		SCT018	E	0.38	7.04	7.70	14.09	71.17
		SCT019	D	0.72	27.85	18.20	22.26	31.69
		SCT020	D	0.52	36.81	15.34	13.57	34.28
Oudtshoorn	L6	SCT021	D	0.82	5.93	16.91	40.47	36.69
		SCT022	D	0.36	6.83	24.14	29.39	39.64
		SCT023	D	0.38	9.31	24.37	34.76	31.57
		SCT024	D	1.22	8.23	36.85	8.95	45.97
		SCT025	E	0.70	3.97	13.56	13.78	68.69
		SCT026	E	0.55	9.30	13.05	19.89	57.77
Calitzdorp	L7	SCT027	C	0.48	0.30	5.56	92.50	1.64
		SCT028	C	0.65	1.81	11.14	80.01	7.04
		SCT029	C	0.66	0.64	14.44	70.11	14.81
		SCT030	C	0.77	nd	8.54	87.64	3.82
Calitzdorp	L8	SCT031	C	1.36	2.08	21.77	61.13	15.02
		SCT032	C	0.84	1.42	8.51	85.64	4.43
		SCT033	C	1.66	1.02	8.32	86.14	4.52
		SCT034	C	0.33	1.41	12.13	72.54	13.92
Calitzdorp	L9	SCT035	A	0.36	nd	nd	nd	nd
		SCT036	A	0.64	nd	nd	nd	nd
		SCT037	A	0.36	nd	nd	nd	nd
		SCT038	A	0.28	nd	nd	nd	nd
Calitzdorp	L10	SCT039	A	0.37	nd	nd	nd	nd
		SCT040	A	0.34	nd	nd	nd	nd
		SCT041	A	0.36	nd	nd	nd	nd
		SCT042	A	1.56	nd	nd	nd	nd
Uniondale	L11	SCT043	A	0.44	nd	nd	nd	nd
		SCT044	A	1.99	nd	nd	nd	nd
		SCT045	A	0.83	nd	nd	nd	nd
Ockertskraal	L12	SCT046	A	0.41	nd	nd	nd	nd
		SCT047	A	1.05	nd	nd	nd	nd
		SCT048	A	0.39	nd	nd	nd	nd
		SCT049	A	0.52	nd	nd	nd	nd
Cacadu	L13	SCT050	C	0.78	2.00	17.77	56.02	24.21
		SCT051	C	0.92	3.56	6.04	62.50	27.90
		SCT052	C	0.64	0.91	26.03	59.64	13.42
		SCT053	C	0.65	1.69	7.88	76.62	13.81
		SCT054	C	0.44	5.71	9.60	51.25	33.44
		SCT055	C	0.15	0.48	17.70	77.12	4.70
Toorwater	L14	SCT056	B	0.45	96.55	3.45	nd	nd
		SCT057	B	0.44	93.05	6.95	nd	nd
		SCT058	C	0.44	2.94	35.20	61.86	nd
		SCT059	C	1.02	6.15	2.21	91.64	nd
		SCT060	C	0.50	5.24	34.55	60.21	nd
		SCT061	B	0.61	94.22	5.78	nd	nd
		SCT062	C	0.88	0.51	23.91	75.58	nd
Toorwater	L15	SCT063	C	0.81	1.91	32.65	65.04	nd
		SCT064	C	0.42	8.43	27.25	64.32	nd
		SCT065	C	0.86	12.89	35.47	51.64	nd
		SCT066	D	0.37	6.93	37.03	34.00	22.04
		SCT067	B	0.53	64.94	1.96	3.10	30.00

Table 1 (continued) ¹

Area/Locality	Locality id	Voucher specimen no.	Chemotype	^a % Alkaloid yield	% Mesembrenol	% Mesembranol	% Mesembrine	% Mesembrenone ²
Steildrift	L16	SCT068	C	0.87	nd	25.70	74.30	nd
		SCT069	D	0.51	7.29	28.43	31.08	33.20
		SCT070	D	0.48	8.39	19.92	43.82	27.87
		SCT071	D	0.31	3.23	32.54	27.10	37.13
		SCT072	D	0.34	38.88	19.95	14.30	26.88
		SCT073	D	0.31	7.02	27.10	36.12	29.76
		SCT074	E	0.32	4.78	10.05	21.28	63.90
Steildrift	L17	SCT075	D	0.52	35.21	17.71	15.00	32.08
		SCT076	D	0.51	5.64	16.92	45.22	32.22
		SCT077	D	0.94	37.88	22.82	11.32	27.98
		SCT078	D	0.48	4.27	26.28	29.63	39.82
		SCT079	D	0.48	5.50	30.66	28.45	35.39
		SCT080	D	0.29	8.39	29.92	33.82	27.87
		SCT081	D	0.51	33.23	17.92	21.97	26.89
Van Wyksdorp	L18	SCT082	D	1.20	23.24	31.41	15.65	29.70
		SCT083	D	0.91	15.96	27.17	30.99	25.88
		SCT084	E	1.34	3.85	13.79	25.08	57.28
		SCT085	E	1.00	3.99	14.79	26.18	55.04
		SCT086	E	1.03	16.66	12.25	17.29	53.80
		SCT087	E	0.84	5.61	10.89	32.65	50.86
		SCT088	D	0.83	14.32	14.45	26.83	44.41
Van Wyksdorp	L19	SCT089	D	0.48	23.22	8.48	30.92	37.38
		SCT090	D	0.68	23.30	9.69	37.25	29.76
		SCT091	D	0.36	30.21	11.41	30.62	27.77
		SCT092	D	0.54	31.90	8.70	29.97	29.44
		SCT093	D	0.62	35.80	5.89	29.42	28.88
		SCT094	C	1.00	nd	31.85	68.16	nd
		SCT095	C	0.78	nd	22.22	64.82	12.96
Plathuis	L20	SCT096	C	1.03	nd	27.27	67.24	5.49
		SCT097	D	1.06	6.18	26.61	44.13	27.08
		SCT098	D	0.48	19.12	15.07	34.56	31.25
Steildrift	L21	SCT099	D	0.38	7.01	32.62	35.46	34.91
		SCT100	D	0.35	8.27	11.37	35.25	45.11
		SCT101	D	0.80	23.99	13.65	16.14	46.22
		SCT102	D	0.43	6.45	18.77	35.71	39.07
		SCT103	D	0.42	30.64	29.89	18.43	21.04
		SCT104	E	0.71	13.35	7.01	15.43	64.21
		SCT105	E	0.68	5.46	20.54	19.67	54.34
Steildrift	L22	SCT106	E	0.38	5.76	24.64	17.39	52.21
		SCT107	D	0.78	8.79	27.56	25.20	38.45
		SCT108	E	0.38	25.48	12.72	7.58	53.22
		SCT109	E	0.53	14.70	14.07	12.92	58.31
		SCT110	A	0.75	nd	nd	nd	nd
		SCT111	A	0.63	nd	nd	nd	nd
		SCT112	A	0.95	nd	nd	nd	nd
Ladismith	L23	SCT113	A	0.90	nd	nd	nd	nd
		SCT114	A	0.83	nd	nd	nd	nd
		SCT115	A	0.80	nd	nd	nd	nd
		SCT116	A	0.79	nd	nd	nd	nd
		SCT117	A	0.86	nd	nd	nd	nd
		SCT118	D	0.56	4.69	12.16	31.67	51.48
		SCT119	E	0.36	9.49	15.37	14.15	60.98
Volmoed	L24	SCT120	E	0.47	8.40	12.51	21.17	57.92
		SCT121	D	0.49	5.40	18.70	25.55	50.35
		SCT122	D	1.12	6.22	15.93	30.38	47.47
		SCT123	D	0.71	3.01	14.36	31.33	51.31
		SCT124	D	0.68	17.91	21.24	32.50	28.35
		SCT125	D	0.59	2.81	27.36	39.24	30.60
		SCT126	D	0.59	3.92	9.73	35.13	51.22
Volmoed	L26	SCT127	E	0.63	7.68	16.68	15.39	60.25
		SCT128	D	1.07	33.69	17.63	13.48	35.20
		SCT129	E	0.64	6.80	8.64	24.95	59.61
		SCT130	E	0.64	20.69	12.26	13.62	53.43
		SCT131	D	0.47	41.50	19.19	10.55	28.76
		SCT132	D	0.53	42.64	18.46	9.95	28.95
		SCT133	E	0.55	20.73	13.70	12.29	53.28
Volmoed	L28	SCT134	D	0.92	29.19	17.50	23.62	29.70
		SCT135	E	0.64	21.73	10.66	16.75	50.86

(continued on next page)

Table 1 (continued) 1

Area/Locality	Locality id	Voucher specimen no.	Chemotype	^a % Alkaloid yield	% Mesembrenol	% Mesembranol	% Mesembrine	% Mesembrenone
Volmoed	L29	SCT136	D	0.92	40.68	19.09	10.36	29.87
		SCT137	D	0.41	7.14	14.21	24.79	53.86
		SCT138	D	0.38	9.17	21.13	28.98	40.72
		SCT139	D	0.93	2.70	23.25	38.04	36.01
		SCT140	D	0.73	28.32	17.90	25.10	28.69
		SCT141	E	0.83	4.05	7.00	26.59	62.36
Volmoed	L30	SCT142	E	1.05	10.53	13.88	20.80	54.79
		SCT143	D	1.10	10.12	15.28	24.18	50.43
		SCT144	D	0.90	35.58	18.05	18.68	27.69
		SCT145	D	0.80	16.77	7.82	23.29	52.12
		SCT146	E	1.27	16.77	9.09	11.67	62.46
		SCT147	E	0.70	13.67	15.09	11.68	59.57
Volmoed	L31	SCT148	D	0.86	7.90	14.87	31.11	46.12
		SCT149	E	0.89	8.92	13.49	16.44	61.15
		SCT150	D	0.92	12.72	14.09	20.03	53.17
		SCT151	D	0.71	29.17	31.69	14.69	24.44

nd (not detected).

^a Alkaloid yield has been expressed as % mass of extract/dry weight of sample. 3

Control (Pty) Ltd; Haans, Germany) at a frequency of 30 Hz for 2 min. The powder was subsequently sieved using 500 µm mesh (Endcotts Filters LTD; London, UK). Alkaloids were extracted using acid-base extraction as described by Alali et al. (2008), with slight modifications. Sulfuric acid (0.5 M; 24.0 ml) was added to 2.0 g of each sample in a 25 ml centrifuge tube. After vortexing for 15 s (Vortex Gene vortex, Scientific Industries™; Florida, USA), the tubes were centrifuged (Juan BR4i; DJB Lab Care®; Buckinghamshire, UK) at 5000 rpm for 15 min, whereafter the supernatant from each tube was filtered into a matching set of new 25 ml tubes. The resulting filtrates were each neutralised with 6.0 ml of 20% NH₃. 4

A volume of 14.0 ml of DCM was added to the contents of each tube, which was vortexed for 15 s and then centrifuged for 15 min at 3000 rpm. The organic phase was filtered into clean 25 ml glass vials. Extraction with DCM was repeated and the two resulting filtrates were pooled and dried in a vacuum oven (Vismara Srl Scientific™; Milan, Italy) at 40 °C and 0.2 bar. The yield of each extract was expressed as a percentage of dry sample weight. Powdered material from capsules of each product was weighed (2.0 g) and extracted in the same way as described for the wild samples. 5

2.4. GC–MS analysis 6

The dried alkaloid extracts were re-suspended in methanol at a concentration of 10.0 mg/ml and analysed using GC–MS. 7 The chromatographic system was an Agilent 6890N gas chromatograph coupled to a model 5973 mass spectrometer. Splitless injection (2 µl) was applied with an auto-injector and an auto-sampler at 24.0 psi and an inlet temperature of 250 °C. The initial oven temperature of 259 °C was held for 2 min, then increased to 262 °C at a rate of 20 °C/min and held constant for 6 min. An HP-5MS 5% phenyl methyl siloxane column (30 m × 250 µm i.d. × 0.25 µm film thickness) was used to achieve the desired separation. The flow of helium (carrier gas) was maintained at 1.4 ml/min. Spectra were recorded after electron impact ionisation at 70 eV (35–550 *m/z*). A transfer line temperature of 280 °C was maintained. Four psychoactive alkaloids namely mesembrenol, mesembranol, mesembrine, and mesembrenone, were identified in the extracts within an 8 min run. Retention times were used as the primary criterion for peak identification in the extracts and the mixtures were also spiked with the reference standards. However, comparison of the mass spectra of the peaks in the extracts with those of authentic standards and data from the NIST® Library (Version 2004) proved to be useful for further confirmation. The percentage of each alkaloid in an extract was expressed as a percentage of the total composition of the four alkaloids in an extract.

2.5. Multivariate data analysis 8

Cluster and classification analysis were performed by chemometric computations using SIMCA-P+® version 12.0 software (Umetrics AB., Malmo, Sweden). Unsupervised principal component analysis (PCA) was performed to provide an overview of the clustering patterns of the samples (observations). Hierarchical cluster analysis was done through construction of a dendrogram and scatter scores plot to evaluate grouping and trends. 9

3. Results and discussion 10

3.1. Variation of the total alkaloid yields of the extracts of wild *S. tortuosum* samples and commercial products 11

This study indicates substantial variation in the total alkaloid yield and composition of *S. tortuosum* samples collected from different localities. The total yield of the extracts and the relative percentage of each alkaloid in the extracts are presented in 12

Table 1. Total yields of the samples were highly variable, ranging from 0.11 to 1.99% of dry plant weight (dw). None of the target alkaloids were observed in samples obtained from Lemoenshoek (L1), the northern side of Oudtshoorn Hill (L4), Calitzdorp (L9, L10), Uniondale (L11) and Ockertskraal (L12). However, samples collected from the majority of the localities (25 populations) contained some or all of the targeted alkaloids. Samples from Calitzdorp (L8; 0.83–1.66% dw) exhibited the highest alkaloid yields, while those collected from Steildrift (L12; 0.20–0.44%) and Lemoenshoek (L1; 0.11–0.39%) yielded the least. The yields of the extracts obtained from the commercial products were also variable (0.10–0.35%). Alkaloid yields of wild samples were higher than those of commercial products because excipients were added to the products, and this affected the amount of plant material in 2.0 g of the sample. Results on variation of the total alkaloid yield are congruent with a published report (Gericke and van Wyk, 1999) indicating that the yield of alkaloids in *S. tortuosum* is highly variable, ranging from 0.05 to 2.30% dw.

3.2. Variation of mesembrine-type alkaloids in wild *S. tortuosum* samples

Mesembrine and mesembrenol were found to display the highest variability (nd–92.50% and nd–96.55% respectively) in comparison to mesembrenone (nd–72.51%) and mesembranol (nd–41.8%). Variations in the profiles of the target alkaloids were observed between plants obtained from the same population and between different populations. For instance, samples collected on the south side of Oudtshoorn Hill (L3), were found to produce all four alkaloids, while those growing on the northern side of the hill (L4) were devoid of the compounds. This could imply that variations in weather patterns, such as precipitation, could influence the alkaloid profiles of the plants, since the northern side of the hill is the leeward side, while the southern side is the windward side. A similar trend was also observed for samples obtained from the various localities in Volmoed area (L24–L31), which lie to the south of the Oudtshoorn Hills. All the samples from this region contained the four alkaloids, although in variable ratios. Of the specimens collected from four localities in Calitzdorp (L7–L10), the alkaloids were only detected in those collected from L7 and L8. In these samples, mesembrine was the main constituent (61.01–92.50%). Samples collected from two localities in Toorwater L14 and L15, showed intra-population variability of the alkaloid profiles. In most samples from both localities, mesembrine was the major alkaloid, while mesembrenol was the main constituent of the remainder.

3.3. Chemotypes of *S. tortuosum* samples harvested from the wild

The results listed in Table 1 are indicative of the existence of large numerical ranges in the relative amounts of the alkaloid (0.10–96.55%) in the data set. Subsequently, the data were log transformed in order to produce normalised data to prevent over-expression of large variance variables at the expense of small variance variables (Eriksson et al., 2006). A three component PCA model was constructed for the GC–MS data, which explained 98.3% of the total variation in the X matrix ($R^2X(\text{cum}) = 0.983$). The goodness of prediction of the model was 87.5% ($Q^2(\text{cum}) = 0.875$) ($N = 151$, $K = 4$), where N represents the number of observations (samples), while K represents the number of variables (alkaloids) in the data set. According to Eriksson et al. (2006), a good model is characterised by R^2 and Q^2 values above 0.5, with the difference between the two values less than 0.2. This model is thus appropriate for the discriminate analysis, since the R^2 and Q^2 values of the model were close to 1 and the difference between them was only 0.108.

The hierarchical cluster analysis (dendrogram) of *S. tortuosum* samples, based on the four identified alkaloids, using two principle components (PCs) is illustrated in Fig. 1. Five chemotypes (A, B, C, D and E) could be identified from the data set. The dendrogram was further expressed as a two PCA scores plot (Fig. 2.) in order to visualise the clustering pattern of the samples. Table 2, shows the range of the percentage composition of each alkaloid in the different chemotypes. None of the target alkaloids were detected in samples constituting Chemotype A, which were obtained from Lemoenshoek (L1), the

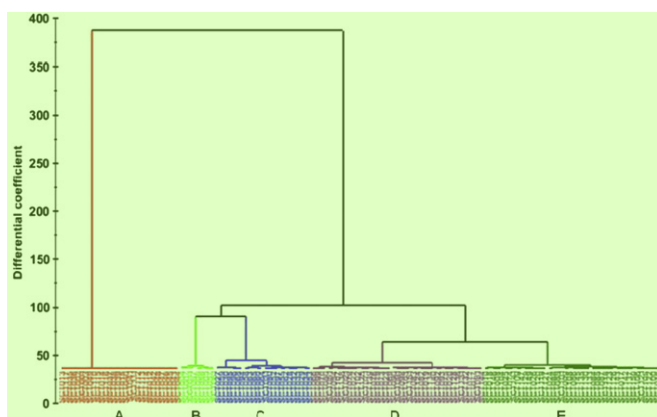


Fig. 1. Hierarchical cluster analysis (dendrogram) based on GC–MS data of the alkaloid extracts of *Sceletium tortuosum* specimens ($N = 151$) from 31 populations.

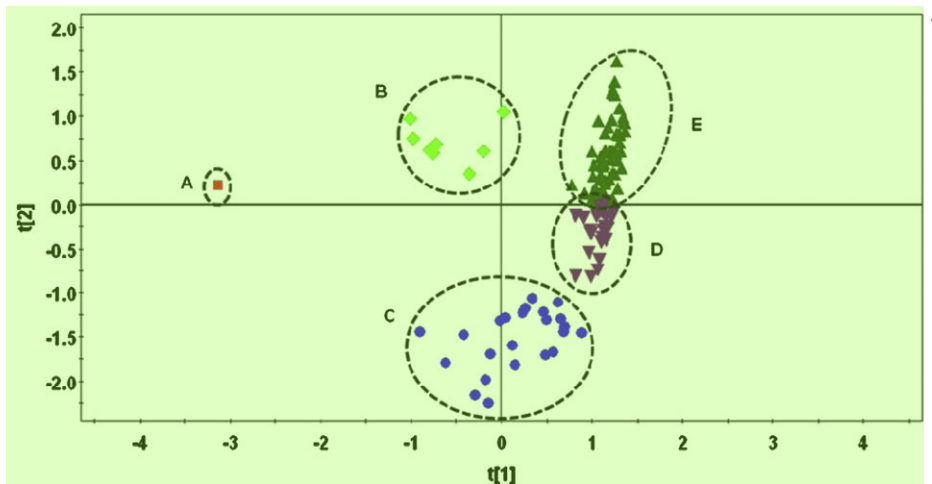


Fig. 2. Principal component analysis scores plot showing differentiation between the chemotypes of *Sceletium tortuosum* samples, based on mesembrine-type alkaloid content ($N = 151$).

northern side of Oudtshoorn Hill (L4), Calitzdorp (L9 and L10), Uniondale (L11) and Ockertskraal (L12). Mesembrenol, ranging from 64.94 to 96.55% was the major constituent of specimens constituting Chemotype B. The mesembranol ($\leq 6.95\%$) and mesembrine ($\leq 3.99\%$) content of the samples in this cluster were relatively very low, although a few samples contained as much as 27.34% of mesembrenone. Specimens comprising Chemotype C were observed to have high levels of mesembrine ($\geq 51.25\%$). In the majority of these samples, the relative amounts of mesembrenol and mesembrenone were either low or undetected. Chemotype D is placed specifically as an intermediate cluster, since the specimens contained all four alkaloids in moderate amounts. Samples in Chemotype D were observed to contain up to 42.64% mesembrenol, 37.03% mesembranol, 48.13% mesembrine and 54.06% mesembrenone. In addition to having relatively low amounts of mesembrenol, mesembranol and mesembrine, mesembrenone was the main constituent of specimens in Chemotype E ($\geq 50.86\%$). Chemotypes D and E displayed some overlap, resulting from specimens containing all four alkaloids in variable amounts, but with high mesembrenone levels ($\geq 50.86\%$). Samples SCT118 and SCT121 (L24), SCT123 (L25), SCT126 (L26), SCT137 (L28), SCT143 (L29) and SCT150 (L31) from Volmoed area constituted the samples that occurred in the overlapping region.

Representative total ion chromatograms of Chemotypes B, C, D and E, and the chemical structures of the four alkaloids are depicted in Fig. 3. It is important to note that the observed chemotypes were not distinguished by their geographical locations. The different chemotypes were distinguished based on three mesembrine-type alkaloids (mesembrenol, mesembrine and mesembrenone). These alkaloids therefore, have great potential for use as marker compounds in the quality control of *S. tortuosum* raw material and products.

3.4. Variation of mesembrine-type alkaloids in *S. tortuosum* products

Extracts of all the commercial products contained the four alkaloids. However, as was observed for the wild samples, differences in the yields and alkaloid profiles of the products were also evident. The relative percentage composition of each compound in the extracts of the selected products is illustrated in Fig. 4. Mesembrenol (0.20–59.91%) was found to display the highest variability in the products, while mesembrine (42.26–82.94%) was the main constituent of most of the products (Com1, 3, 4, 6, 7 and 8). High levels of mesembrenone were observed in products Com2 (43.32%) and 6 (41.14%), while mesembrenol was the major constituent in product Com5 (59.91%). Higher percentages of mesembrine were also observed in products Com2 (30.40%) and 6 (42.25%). Initial reports by Van Wyk and Gericke (2003) indicated that mesembrine is the major active constituent of *S. tortuosum*. Many commercial producers of this plant may therefore have selected plants with high mesembrine content for propagation and processing. However, *in vitro* investigation has shown that both mesembrenol and mesembrenone exhibit psychoactive properties (Harvey et al., 2011).

Table 2

Range in the percentage composition of mesembrine-type alkaloids in the different chemotypes.

Chemotype	% Alkaloid yield	% Mesembrenol	% Mesembranol	% Mesembrine	% Mesembrenone
A	0.11–0.80	nd	nd	nd	nd
B	0.42–1.46	64.94–96.55	1.51–6.95	nd–3.99	nd–27.34
C	0.42–1.03	nd–12.89	2.51–35.50	51.25–92.50	nd–33.40
D	0.31–1.22	2.23–42.64	7.84–37.03	8.95–48.13	24.44–54.06
E	0.32–1.49	3.85–27.93	7.00–24.64	3.63–32.65	50.86–72.51

nd (not detected).

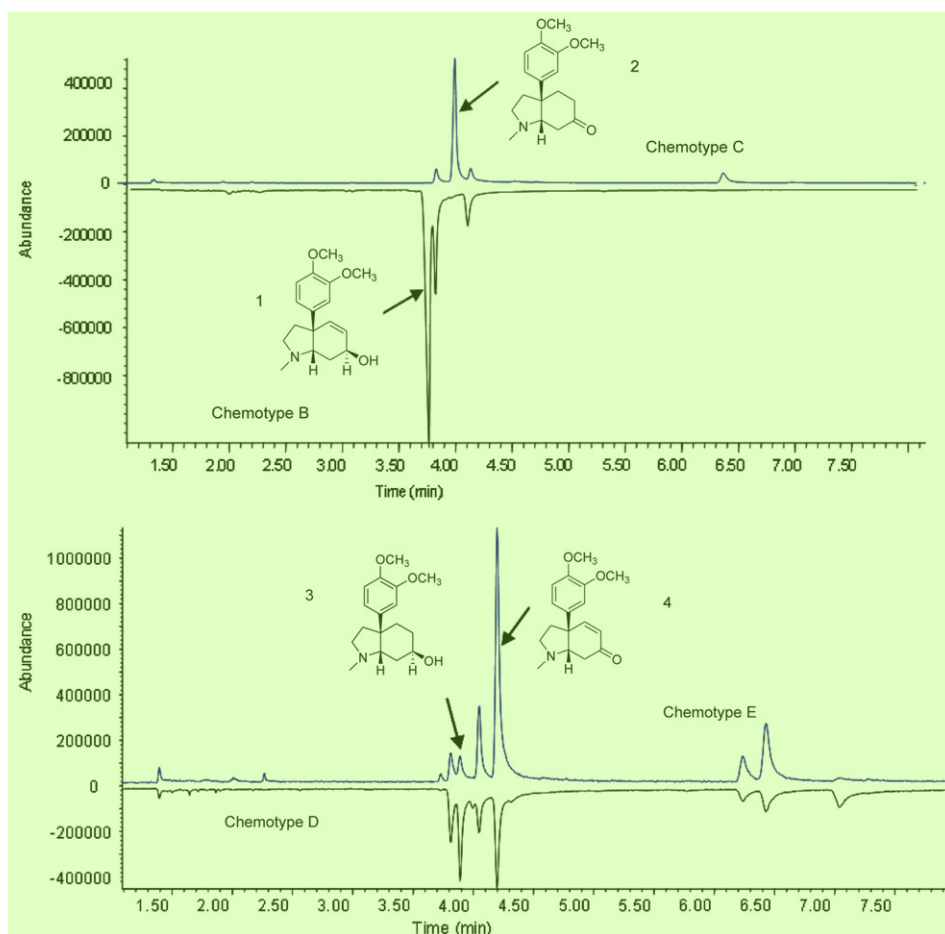


Fig. 3. Total ion chromatograms of *Scelletium tortuosum* extracts representing each of the different chemotypes and the chemical structures of the four alkaloids (1) mesembrenol; (2) mesembrine; (3) mesembranol and (4) mesembrenone.

The psychoactive properties of these alkaloids in the treatment of CNS ailments have been attributed to their ability to act as selective serotonin re-uptake and phosphodiesterase (PDE4) inhibitors (Gericke and Viljoen, 2008). Both selective serotonin re-uptake inhibitors and PDE4 inhibitors are commonly used as antidepressants (Kanes et al., 2007). *In vitro* studies on the aqueous ethanol extract (70%) of *S. tortuosum* and three mesembrine-alkaloids (mesembrine, mesembrenone and

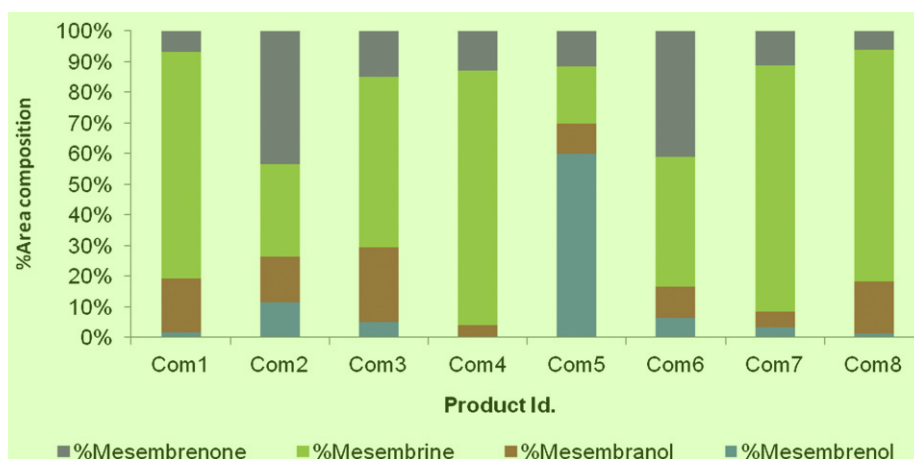


Fig. 4. Phytochemical variation of mesembrine-type alkaloids in commercial products of *Scelletium tortuosum* as determined by GC–MS.

mesembrenol) indicated that the extract (high mesembrenone and mesembrenol content) was a potent blocker in 5-HT transporter-binding assays and had powerful inhibitory effects on PDE4 (Harvey et al., 2011). In addition, both mesembrine and mesembrenol were observed to be active against the 5-HT transporter, although the activity of mesembrine was higher than that of mesembrenol. Mesembrenone was nevertheless observed to be active against both 5-HT transporter and PDE4 enzymes.

In vivo experimental evidence has also suggested that PDE4 inhibitors can reverse depression, improve cognition and alleviate anxiety (O'Donnell and Zhang, 2004). *S. tortuosum* plants with relatively high mesembrenone content, and isolated mesembrenone, can thus act as dual serotonin re-uptake inhibitors and PDE4 inhibitors, while plant material with relatively high amounts of mesembrine, as well as pure mesembrine, can act as selective serotonin re-uptake inhibitors. The therapeutic advantages of dual inhibition of serotonin re-uptake and PDE4 inhibition include the possibility of using a lower dose of plant material containing both mesembrenone and mesembrine (Chemotypes D and E) to achieve the desired effect (Cashman et al., 2009). For propagation, it is necessary to select the plant belonging to the appropriate chemotype to ensure consistency in the alkaloid content and maximum effect at lower doses.

These results are consistent with a previous report indicating variability in the distribution of alkaloids in *Sceletium* plants cultivated in different places (Herre, 1971). Although the author reported that alkaloids were not produced by *S. tortuosum* plants cultivated in Germany, they were produced by those cultivated in the USA. Due to the variability of alkaloids in this species, harvesting of *Sceletium* samples from the wild for propagation purposes could be challenging, since inconsistencies in the alkaloid profiles are evident, even for plants growing within the same locality. Factors such as geography, soil-type, altitude, precipitation, genetics, growth season, time of harvesting, storage conditions and method of processing are known to affect the yield and composition of secondary metabolites produced by a plant (Laasonen et al., 2002). These factors may cause inconsistencies in the chemical profiles of specimens of the same species growing in different localities. The chemical profiles of *S. tortuosum* samples collected from different localities could also be influenced by the prevailing environmental conditions. However, variations observed between plants growing in the same locality indicate that the differences may be attributed to genetic variability and may not necessarily depend on geographical and environmental factors alone.

4. Conclusions

Intra-specific variations of the profiles of the four alkaloids within and between different populations of *S. tortuosum* were observed. Chemotypic variation in the phytochemical profiles, based on mesembrine-type alkaloid composition, of *S. tortuosum* resulted in the identification of five chemotypes. Individual chemotypes identified were not restricted to particular geographical areas. The relative contributions of mesembrenol, mesembrine and mesembrenone, are useful for quality assurance of *S. tortuosum* raw materials and products, and for assigning the source raw plant material to one of the five identified chemotypes.

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