

## Chemotaxonomic studies of mesembrine-type alkaloids in *Sceletium* plant species<sup>6</sup>

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Complementary medicines containing the succulent herb *Sceletium* are being sold without information regarding their phytochemical contents, which is essential for the quality control of medicines. Furthermore, several different *Sceletium* species exist and little has been reported on the alkaloidal identities and contents of the various species. We therefore conducted phytochemical investigations on six selected *Sceletium* specimens, identified on the basis of their venation pattern as either 'emarcidum' or 'tortuosum' type. The tortuosum type consisted of *S. tortuosum*, *S. expansum* and *S. strictum*, whereas the emarcidum type consisted of *S. emarcidum*, *S. exalatum* and *S. rigidum*. Analysis was conducted by high-performance liquid chromatography with UV and alkaloids were identified by online mass spectroscopy. *S. tortuosum* and *S. expansum* samples contained mesembrine together with mesembrenone, mesembranol and epimesembranol, although the latter two alkaloids were present in low concentrations in *S. expansum*. *S. strictum* contained mesembrenone, mesembrine and either 4'-*O*-demethylmesembrenone or 4'-*O*-demethylmesembrenol. The emarcidum type specimens showed a complete absence of the major alkaloid mesembrine, as well as the other alkaloids usually associated with these species. In only one of the species of the emarcidum type – *S. exalatum* – two peaks corresponded to either 4'-*O*-demethylmesembrenone or *O*-methyljoubertiamine and either 4'-*O*-demethylmesembrenol or N-demethylmesembrenol, respectively. This study clearly indicates that not all *Sceletium* species contain the mesembrine-type alkaloids usually associated with *Sceletium*. It is thus important to identify the correct *Sceletium* species to ensure correct alkaloidal content for the manufacture and quality control of products containing this plant material.

### Introduction<sup>11</sup>

The quality of medicines containing plant extracts or material is a major concern worldwide. The market for herbal products is lucrative as a result of increased consumer interest in phyto-pharmaceutical therapies. As a consequence, commercial interest in herbal plant medicines and the production of their dosage forms is undergoing great expansion. Such expansion, however, is associated with increased concerns relating to the quality of such products. A standardised or constant composition of the relevant components in herbal preparations is an important requirement, both from a regulatory point of view as well as for the consumer.<sup>1</sup> However, because of the multiplicity of endogenous compounds usually present in most plants, standardisation, identification and quality control of the contents of pharmacologically active components present in the plant are generally not achieved. The factors that affect the content of active constituents in herbal plants include the age of the plant, temperature, daylight exposure, atmosphere, sampling method, the presence of toxic residues in soil, rainfall, altitude, soil composition and microbial contamination.<sup>2</sup>

Different growth, harvest, drying and storage conditions also result in variation in quality of the raw plant materials.<sup>3</sup> It is also important to note that wild harvesting of medicinal plants is considered to be a problem as it causes loss of biodiversity, results in variation in the quality of the plant's medicinal constituents and contributes to improper plant identification.<sup>4</sup> Proper identification of medicinal plant species based on phytochemical content and associated therapeutic value is of primary importance for their commercial value and many of these plants are now cultivated in large numbers to keep up with the demands of the consumer. Proper identification is thus necessary in order to cultivate the correct species of the medicinal plant which will yield the requisite compound or constituents which are pharmacologically active.

Historically, *Sceletium* was used by the Khoisan of southern Africa and records of its use as a commercial material have been discussed and reported by Smith et al.<sup>5</sup> The use of *Sceletium* has been on the rise as a result of pharmacological claims that it has anti-anxiety and mood elevating properties. The fact that the use of *Sceletium* for the treatment of psychiatric disorders has been patented,<sup>6</sup> provides sufficient evidence of its importance as a commercially important medicinal plant.

Apart from the *Sceletium* species *S. expansum*, *S. strictum*, *S. tortuosum* (= *S. joubertii* = *S. namaquense*) and *S. varians* (= *S. subvelutinum*), which belong to the 'tortuosum' type and which have been reported to contain alkaloids, the alkaloidal content in 'emarcidum'-type *Sceletium* species, such as *S. emarcidum*, *S. exalatum* and *S. rigidum*, is currently unknown.

*Sceletium* (family: Aizoaceae) occurs in the Western, Eastern and Northern Cape Provinces of South Africa, most prominently in the Karoo regions. *Sceletium* also reportedly grows in the Namaqualand, Knersvlakte, Ceres Karoo and some parts of the Western Cape.<sup>7</sup> There are eight reported species of this genus<sup>8</sup>: *Sceletium crassicaule* (Haw.) L. Bolus, *Sceletium emarcidum* (Thunb.) L. Bolus ex H.J. Jaccobson, *Sceletium exalatum* Gerbaulet, *Sceletium expansum* (L.) L. Bolus [nt], *Sceletium rigidum* L. Bolus, *Sceletium strictum* L. Bolus, *Sceletium tortuosum* (L.) N.E.Br. [nt] and *Sceletium varians* (Haw.) Gerbaulet.

Attempts have been made to analyse *Sceletium tortuosum* and a monograph entitled *Monograph of Sceletium tortuosum*<sup>®</sup> describes the pharmacognostic features of the plant and also the thin-layer chromatography and

high-performance liquid chromatography (HPLC) profiles.<sup>9</sup> It is interesting to note that even though the presence of alkaloids in *Sceletium* has previously been well documented, the thin-layer chromatography profile of the *Sceletium* extract in the above monograph is matched with an unrelated reference compound – cineole. The HPLC profile was carried out on a methanolic extract of *Sceletium tortuosum* and indicates that the observed retention times (RTs) of 2.38 min, 3.15 min, 4.89 min and 7.84 min are major peaks, but no mention is made as to the identities of the substances resulting in those peaks. The monograph also mentions that the purity tests by assay are not yet available. Such reports have not contributed much in terms of accurately assessing alkaloidal profiles or estimating their content.

In view of the absence of comprehensive data describing the alkaloidal identity, content and composition found in the various *Sceletium* species, the present study was undertaken to investigate selected *Sceletium* plant specimens. We analysed them using HPLC with UV methods,<sup>10</sup> which were specifically developed to fingerprint the mesembrine-type alkaloid profiles and to quantitatively determine alkaloidal content using qualified reference substances. The online detection of the mesembrine-type alkaloids by electrospray ionisation mass spectroscopy (ESI-MS)<sup>11</sup> enabled the identification of specific alkaloids based on their *m/z* values, i.e. in accordance with their respective molecular weights (MWs).

It is important to note that two detection systems were used simultaneously – UV and MS – because the peaks detected by UV are not necessarily detected by MS and vice versa, as a result of differences in the specific conditions necessary for detection. In view of the mass specificity of MS detection, confirmation of the exact identity of an eluting compound is possible without the use of a reference standard, whereas a reference standard is necessary in UV detection. Some peaks detected by UV are hence labelled ‘unidentified’ because a corresponding reference standard was not available.

## Methodology<sup>4</sup>

### Collection of *Sceletium*<sup>5</sup>

*Sceletium* is found mainly in the northwestern Cape regions of southern Africa. Initial efforts to source the plant from its natural habitat in this region were unsuccessful because the plants are grown mostly in greenhouses. Hence, the availability of the plants depended on cultivators. Most of the samples were gratefully supplied as gifts by the *Sceletium* cultivators in their efforts to identify and ascertain the quality of their cultivated plant material. Plant specimens provided were not always flowering, so samples were cultivated and, upon flowering, were pressed to provide herbarium specimens for future reference. The specimens were deposited at the Selmar Schonland Herbarium in Grahamstown, South Africa (numbers SP01–SP06 for each of the plant species, respectively).

### Reagents and materials<sup>7</sup>

HPLC-grade methanol and acetonitrile (Romil Ltd., Cambridge, Great Britain) were used for sample preparation and for the mobile phase. Water was purified in a Milli-Q® system and Millex HV® hydrophilic PVDF 0.45-µm membrane filters (Millipore, Bedford, USA) were used during sample preparation. The reference substances – mesembrine, mesembrenone and  $\Delta^7$ mesembrenone – were extracted and isolated from *Sceletium* plant material. Mesembranol and epimesembranol were synthesised from mesembrine. These substances were qualified as reference substances by nuclear magnetic resonance (NMR) analysis (<sup>1</sup>H, <sup>13</sup>C and 2-D). Crystalline forms of mesembranol and mesembrine hydrochloride were further characterised by X-ray crystallography.<sup>11</sup>

### Instrumentation<sup>9</sup>

A Cole-Parmer ultrasonic bath (model 8845-30, Cole-Parmer Instrument Company, Chicago, IL, USA) was used for sonication for solvent extraction of samples. A Finnigan MAT LCQ ion trap mass spectrometer (Finnigan, San Jose, CA, USA) coupled to a SpectraSYSTEM P2000 pump connected to an AS1000 autosampler and UV1000 variable-

wavelength UV detector (Thermo Separation Products, Riviera Beach, FL, USA) were used for both qualitative and quantitative analyses. Separation of alkaloids was carried out on a Luna® C<sub>18</sub> (2) column (5 µm, 150 mm x 4.6 mm i.d., Phenomenex®, Torrence, CA, USA). An analytical balance (Type AG 135, Mettler Toledo, Switzerland) was used for weighing standards and samples. An electronic pipette (model 71050XET, Biohit PLC, Helsinki, Finland) was used to transfer standards and samples for dilutions. NMR analysis was performed on a Bruker Advance DRX 400 MHz NMR spectrometer (Rheinstetten, Germany).

### Identification of *Sceletium*<sup>12</sup>

The specimens were identified using the identification key of Gerbaulet.<sup>7</sup> The important taxonomic identification key is the leaf venation pattern which differs between *Sceletium* types. Based on the venation type (Figure 1), the species were classified as either ‘emarcidum’ or ‘tortuosum’ type.<sup>7</sup> Of the seven plant specimens examined, six were received as gifts, of which three were of the emarcidum type and the other three were of the tortuosum type. The emarcidum-type leaf was observed in the specimens *S. emarcidum*, *S. rigidum* and *S. exalatum*. The tortuosum-type leaf was observed in the specimens *S. tortuosum*, *S. expansum* and *S. strictum*. A validated HPLC method<sup>10</sup> was used for the quantitative determination of the relevant alkaloids and ESI-MS was carried out in conjunction with online UV detection.<sup>11</sup>

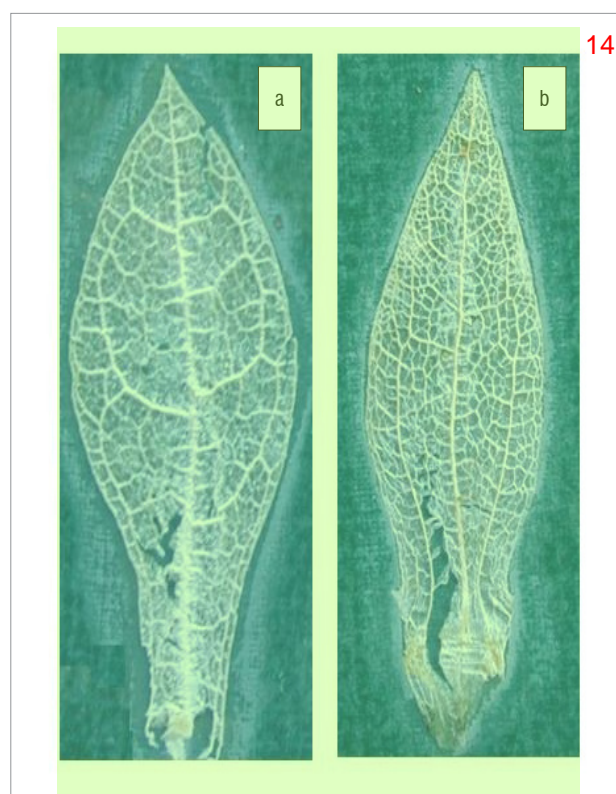
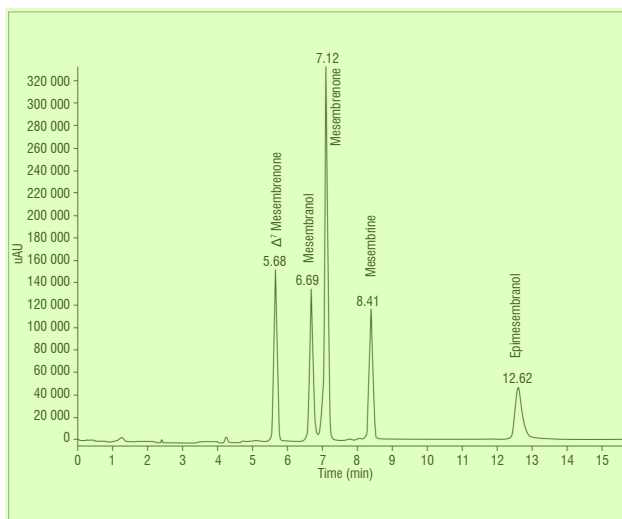


Figure 1: *Sceletium* dried leaves showing the venation pattern of (a) emarcidum-type leaves and (b) tortuosum-type leaves.

### Preparation of standards and samples<sup>16</sup>

Standard methanolic stock solutions (1 mg/mL) of  $\Delta^7$ mesembrenone, mesembranol, mesembrenone, mesembrine hydrochloride and epimesembranol were prepared. A working stock solution was prepared and diluted to obtain a concentration of 100 µg/mL of each alkaloid. Standard solutions comprising a set of nine calibrators in the concentration range of 400–30 000 ng/mL were used for calibration. The HPLC-UV chromatogram of the reference standard alkaloids is shown in Figure 2.



**Figure 2:** A chromatogram derived from high-performance liquid chromatography with UV of standard alkaloids found in *Sceletium*.

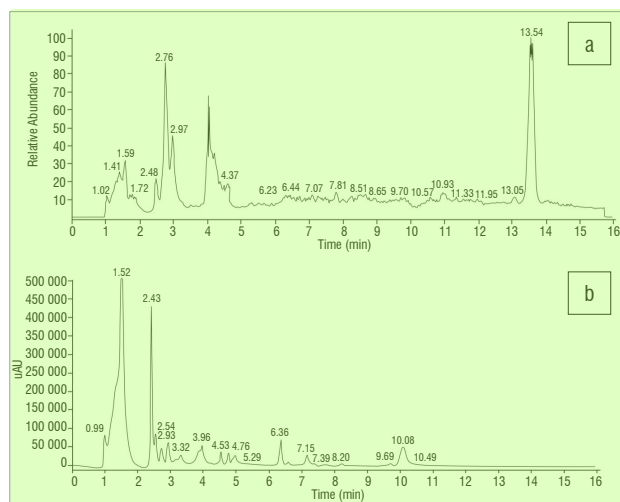
The MS ionisation formed  $[M+H]^+$  ions with  $m/z$  values of 288 ( $\Delta^7$ mesembrenone), 292 (mesembranol), 288 (mesembrenone), 290 (mesembrine) and 292 (epimesembranol) (Figure 3).

The *Sceletium* samples were obtained from cuttings of cultivated plants and dried at 80 °C prior to processing for analysis. Samples were extracted with methanol under sonication to obtain a final concentration of ~150 mg/mL plant material. The solutions were filtered through 0.45- $\mu$ m PVDF membrane filters prior to chromatography.

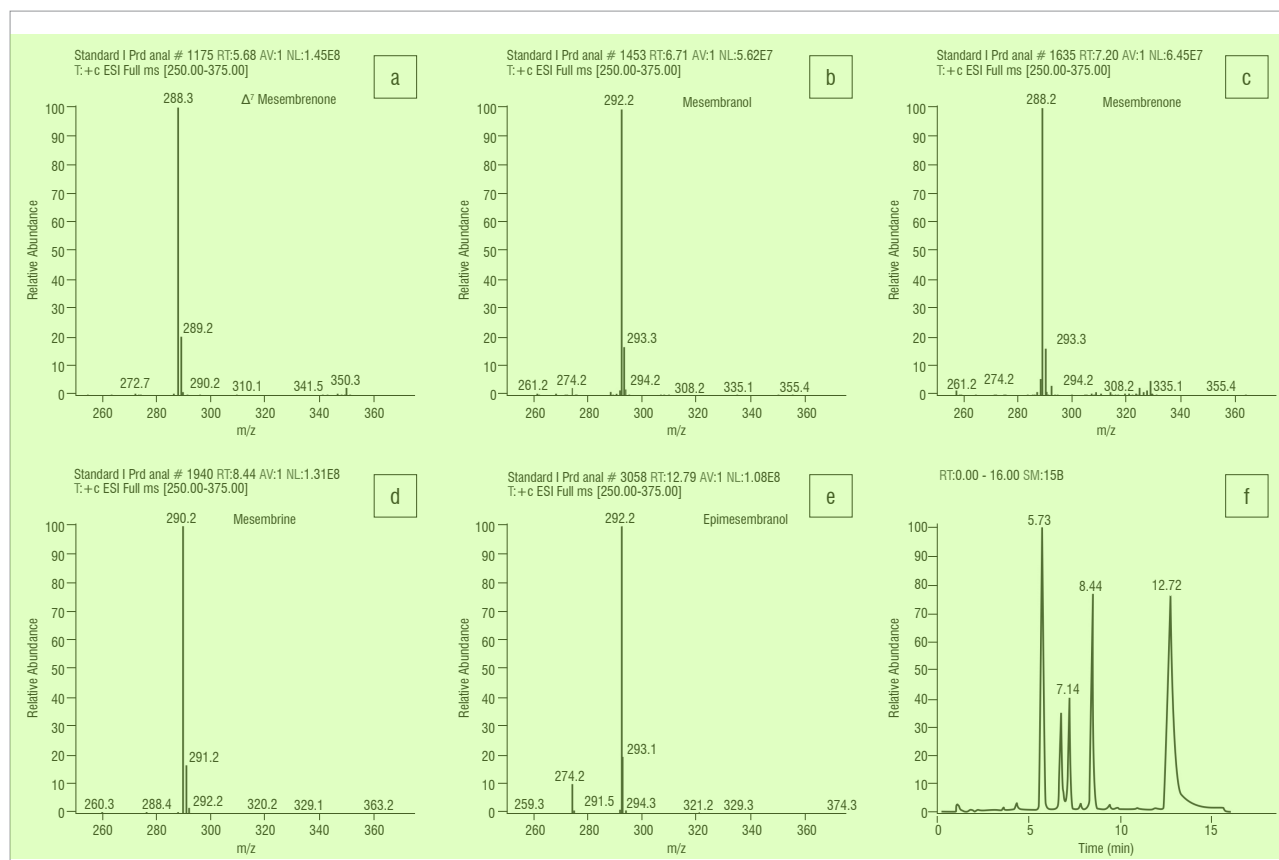
## Results

### *Emarcidum*-type *Sceletium* species

The HPLC-UV profile of the *S. emarcidum* specimen showed three peaks eluting at RTs of 6.36 min, 7.15 min and 10.08 min, which were close but not identical to the RTs of the reference standards (Figure 4). The MS profile showed one major peak at 13.54 min (Figure 4) which had an  $m/z$  of 360 which did not correspond to the  $m/z$  ratios of any of the *Sceletium* alkaloid reference standards.

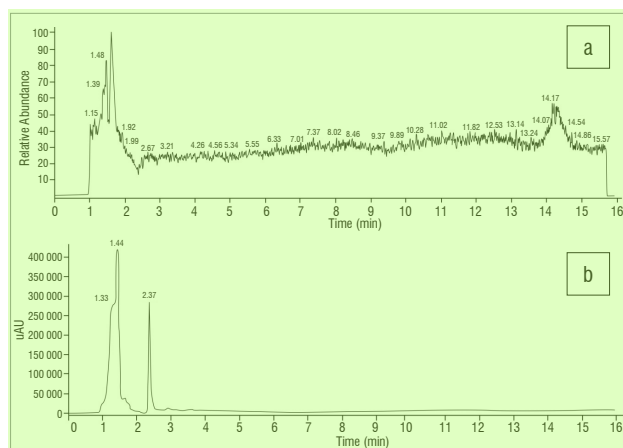


**Figure 4:** Chromatograms of *Sceletium emarcidum* derived from (a) electrospray ionisation mass spectroscopy and (b) high-performance liquid chromatography with UV.



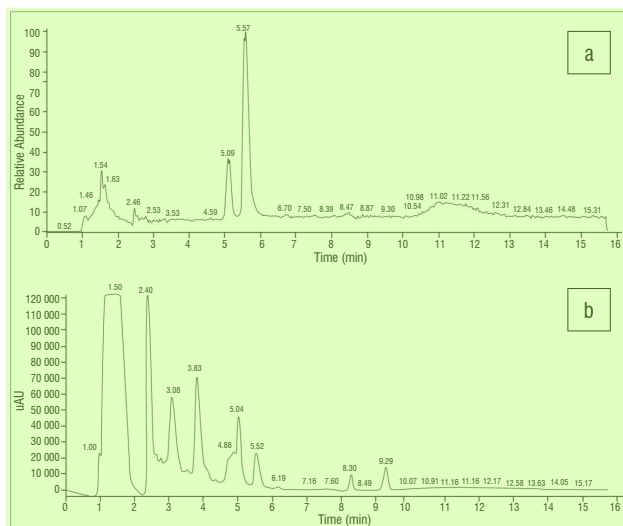
**Figure 3:** The  $[M+H]^+$  ions of (a)  $\Delta^7$ mesembrenone, (b) mesembranol, (c) mesembrenone, (d) mesembrine and (e) epimesembranol. (f) A mass spectrometry chromatogram of standard reference alkaloids:  $\Delta^7$ mesembrenone (5.73 min), mesembranol (6.71 min), mesembrenone (7.14 min), mesembrine (8.44 min) and epimesembranol (12.72 min).

Interestingly, the *S. rigidum* sample was devoid of any peaks, regardless of detection method (Figure 5).



**Figure 5:** Chromatograms of *Sceletium rigidum* derived from (a) electrospray ionisation mass spectroscopy and (b) high-performance liquid chromatography with UV.

The HPLC-UV profile of the *S. exaltatum* sample was complex, with peaks observed at RTs of 5.04 min, 5.52 min, 8.30 min and 9.29 min. These peaks did not correspond to any of the previously identified alkaloids with respect to the RTs of the alkaloid reference standard peaks (Figure 6). The MS profile of the sample showed two prominent ionised  $[M+H]^+$  peaks at 5.09 min and 5.57 min (Figure 6) with  $m/z$  values of 274 and 276. Whereas these peaks and the related  $m/z$  values did not correspond to any of the available *Sceletium* reference standard alkaloids used in this study, the  $m/z$  values did correspond with the molecular ions of 4'-*O*-demethyl-mesembrenone or *O*-methyl-joubertiamine (MWs=273) and 4'-*O*-demethyl-mesembrenol or *N*-demethyl-mesembrenol (MWs=275).<sup>12</sup>

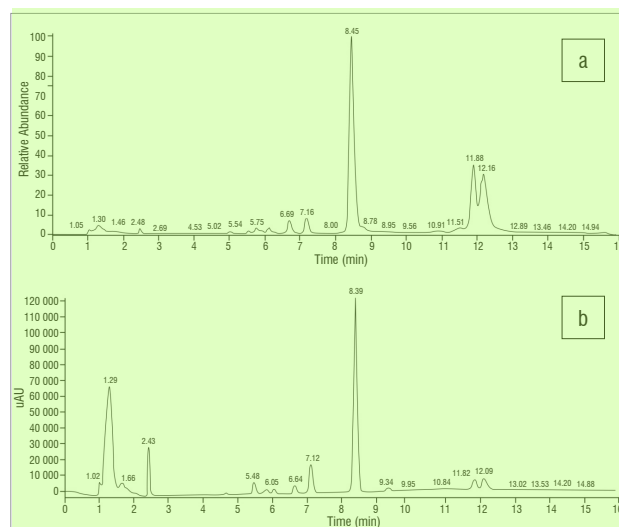


**Figure 6:** Chromatograms of *Sceletium exaltatum* derived from (a) electrospray ionisation mass spectroscopy and (b) high-performance liquid chromatography with UV.

### *Tortuosum*-type *Sceletium* species

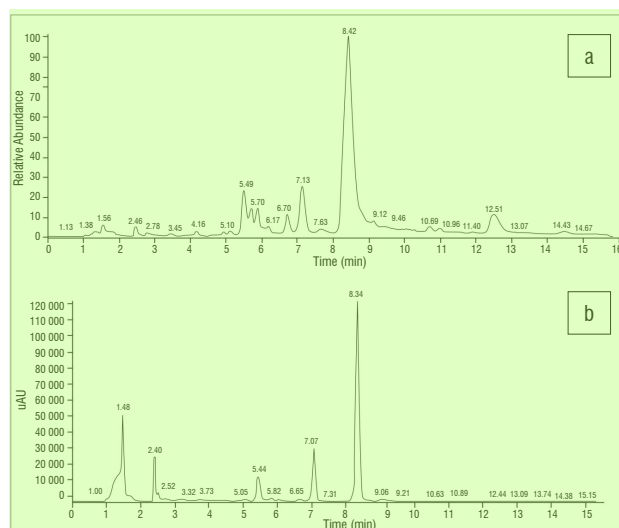
The HPLC-UV profile of the *Sceletium tortuosum* sample (Figure 7) showed peaks at RTs of 5.48 min (unidentified), 6.05 min (unidentified), 6.64 min (mesembranol), 7.12 min (mesembrenone), 8.39 min (mesembrine), 11.82 min (unidentified) and 12.09 min (epimesembranol). The assays (% w/w) of these alkaloids showed them to be present at 0.18%, 0.16%, 2.20% and 0.41%, respectively. The MS profile based on the  $m/z$  values of these alkaloids (Figure 7)

showed peaks eluting at 5.75 min ( $\Delta^7$ mesembrenone), 6.69 min (mesembrenone), 7.16 min (mesembranol), 8.45 min (mesembrine) and 12.16 min (epimesembranol). An unidentified peak at a  $m/z$  of 334 eluted at a RT of 11.88 min.



**Figure 7:** Chromatograms of *Sceletium tortuosum* derived from (a) electrospray ionisation mass spectroscopy and (b) high-performance liquid chromatography with UV.

The HPLC-UV profile of the *S. expansum* sample (Figure 8) showed three peaks at RTs of 5.44 min (unidentified), 7.07 min (mesembrenone) and 8.34 min (mesembrine). The alkaloids mesembrenone and mesembrine were assayed by HPLC-UV and found to be present at 0.03% and 0.30%, respectively. Mesembranol and epimesembranol were below their limits of detection using HPLC-UV; however, the presence of these alkaloids was confirmed by MS detection. The LC-MS profile showed peaks at 5.49 min, 6.70 min, 7.13 min, 8.42 min and 12.51 min (Figure 8) which corresponded to  $m/z$  values of 274, 292 (mesembranol), 288 (mesembrenone), 290 (mesembrine) and 292 (epimesembranol). The ionised peak at 5.49 min was also found in *S. strictum*, which had a  $m/z$  of 274, corresponding to 4'-*O*-demethyl-mesembrenone or *O*-methyl-joubertiamine (MWs=273).

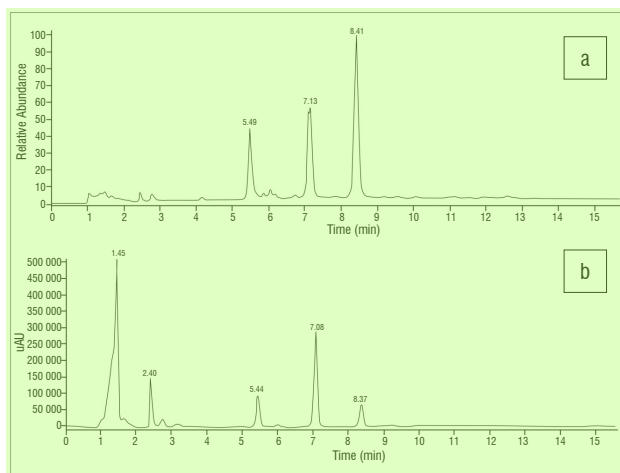


**Figure 8:** Chromatograms of *Sceletium expansum* derived from (a) electrospray ionisation mass spectroscopy and (b) high-performance liquid chromatography with UV.

The HPLC-UV profile of *S. strictum* (Figure 9) showed three peaks at RTs of 5.44 min (unidentified), 7.08 min (mesembrenone) and 8.37 min (mesembrine). The alkaloids mesembrenone and mesembrine assayed



by HPLC-UV detection were found to be present in the sample at 0.76% and 0.68%, respectively. The LC-MS profile (Figure 9) showed peaks eluting at 5.49 min, 7.13 min (mesembrinone) and 8.41 min (mesembrine), which corresponded to  $m/z$  values of 274, 288 and 290, respectively. The ionised peak with  $m/z$  of 274 corresponded to 4'-*O*-demethyl-mesmbrenone or *O*-methyl-joubertiamine (MWs=273). Neither mesembranol nor epimesembranol were detected by UV or MS methods. The peak at a RT of 5.49 min with a  $m/z$  of 274 corresponded to 4'-*O*-demethyl-mesmbrenone or *O*-methyl-joubertiamine (MWs=273), as for that observed in *S. expansum*.



**Figure 9:** Chromatograms of *Sceletium strictum* derived from (a) electrospray ionisation mass spectroscopy and (b) high-performance liquid chromatography with UV.

## Conclusions

*Sceletium* plant constituents are complex in nature and the alkaloids present are structurally closely related. Some of the closely related alkaloidal structures, such as mesembrinone or  $\Delta^7$ mesembrinone and the epimers mesembranol or epimesembranol have the same molecular weights but differ slightly in their chemical structure, such as the position of a double bond (mesembrinone or  $\Delta^7$ mesembrinone) or configuration (mesembranol or epimesembranol). We have shown the identity, alkaloidal content and composition of various *Sceletium* plant species. The 'tortuosum'-type *Sceletium* species contained specific alkaloids which differed in content and composition between species. *S. tortuosum* contained mesembrine as the major alkaloid along with other minor alkaloids,  $\Delta^7$ mesembrinone, mesembrinone and mesembranol. Interestingly, the 'emarcidum'-type specimens showed a complete absence of mesembrine as well as other alkaloids usually found in the tortuosum-type species. In only one of the species of the emarcidum type (*S. exalatum*), two peaks eluted at RTs of 5.09 min and 5.57 min whose  $m/z$  ratios corresponded to either 4'-*O*-demethyl-mesmbrenone or *O*-methyl-joubertiamine (MWs=273) and 4'-*O*-demethyl-mesembrenol or N-demethyl-mesembrenol (MWs=275), respectively. Species of the tortuosum type, *S. expansum* and *S. strictum* also showed peaks corresponding to a  $m/z$  of 274, but eluting at a RT of 5.49 min, which corresponded to 4'-*O*-demethyl-mesmbrenone or *O*-methyl-joubertiamine (MWs=273). The peak eluting at a RT of 5.49 min seems to be associated only with tortuosum-type species, whereas the peak eluting at a RT of 5.09 min seems to be associated with emarcidum-type species. Furthermore, it appears that both of the individual compounds in each pair are present in one or other of the particular species. The tortuosum-type species *S. tortuosum* and *S. expansum* revealed the presence of mesembrine together with mesembrinone, mesembranol and epimesembranol, although the latter two were present in concentrations below their limits of quantification in *S. expansum*. *S. strictum*, on the other hand, was found to contain measurable concentrations of mesembrinone and mesembrine and either 4'-*O*-demethyl-mesembrinone or 4'-*O*-demethyl-mesembrenol, based on the  $m/z$  and RTs measured.

The development of an appropriate analytical method for chemotaxonomic studies of *Sceletium* presents a formidable challenge in view of the fact that the plant contains a complex mixture of closely related compounds. Hence, a prerequisite for the appropriate chemotaxonomic profiling of species used as medicinal plants is the availability of relevant reference compounds. An appropriate analytical method that has the required specificity for fingerprinting, as well as the ability to assay plant material for constituents with the required accuracy and precision, are also necessary. The data presented here will facilitate proper identification of *Sceletium* species based on a chemotaxonomic approach.

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## Authors' contributions

S.P. performed the laboratory work; I.K. supervised the research; and both authors contributed to the writing of the manuscript.

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