Short Communication
Forensic analysis of mesembrine alkaloids in Sceletium tortuosum by nonaqueous capillary electrophoresis mass spectrometry

The consumption of legal and illegal drugs follows an organic trend comparable to the current trend in food consumption. The investigation of such drugs is therefore of interest to characterize the active ingredients of plants and drug preparations. A new method of nonaqueous capillary electrophoresis coupled to mass spectrometry (NACE-MS) as a powerful tool for the separation of complex alkaloid mixtures in difficult matrices is presented in this study for the analysis of samples of Sceletium tortuosum and drug products called Kanna made thereof. The method was found to be suitable for the investigation of the alkaloid composition and relative quantification of the ingredients. It proved of value to separate a large number of isobaric compounds, most probably including diastereomers, double-bond isomers, and further structurally closely related compounds. A comparison of plant samples from different vendors, self-fermented samples, and products ready for consumption was made. The high separation power obtained allowed a better description of the chemotypic differences of plant samples as well as Kanna preparations compared to other methods presented in the literature so far. Thus, the use of the NACE-MS enables a new perspective on the alkaloid profile of Sceletium species.

Keywords: Kanna / Mesembrine alkaloids / Non aqueous capillary electrophoresis / Recreational drugs / Structural isomers DOI 10.1002/elps.201100683

A tea made of the leaves of Sceletium tortuosum, a plant endemic to South Africa, was traditionally used as an analgesic [1]. The drug Kanna, the “fermented” and dried material of the whole plant, was chewed or smoked more than 300 years ago by the Khoi (historically called Hottentotten) [1]. Analogous to the organic trend observed in food industries, the consumption of biogenic drugs such as Kanna as new “partydrugs” rises due to its anxiolytic and relaxing effects as well as euphoria in high doses as they are perceived as natural and harmless [2]. Due to this and Kanna’s broad availability as a herbal blend sold via the Internet, the characterization of its active ingredients is of interest both in terms of forensic toxicology but also of legislation issues [3]. Additionally, several Sceletium pharmaceutical products appear on the market [4]. The analysis of Kanna-alkaloids is difficult, as alkaloid standards are not commercially available and the complexity of the alkaloid mixture including a large number of diastereomers (see Fig. 1) requires a highly efficient separation method. In literature, the analysis of Kanna was achieved by GC [5, 6], HPLC [4], and CE [7]. The major alkaloids in S. tortuosum were found to be mesembrine, mesembranol, mesembrenone, and mesembranol [6, 8], though the chemotypic variation seems to be high [8].

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found to be 0.1–500 μM and the mean relative standard deviation for the repeatability of the effective electrophoretic mobility and the peak area were found to be 0.2% and 7.7%, respectively ($n = 5$).

*Sceletium tortuosum* plants were purchased from two different vendors (Vendor 1: Kakteen Haage, Erfurt, Germany; Vendor 2: Rühlemann’s Kräuter und Duftpflanzen, Hoerstedt, Germany), called *Sceletium* 1 and 2, respectively. For comparison, an *S. joubertii* plant was purchased from Vendor 1 as well. Additionally, two different commercially available Kanna powders were ordered from head shops via the Internet as test samples, called Kanna 1 and 2.

For the analysis of the fermentation process, whole plants (radix, leaf, and stem) of *Sceletium* 1, *Sceletium* 2, and *S. joubertii* were harvested, crushed, and homogenized. An aliquot of each was stored airtight in a transparent plastic container in the sun for 8 days and dried afterwards [5] in a SpeedVac Concentrator SAVANT SPD131DDA (Thermo SCIENTIFIC, Bremen, Germany) (RT, 1 mbar, 4 h). Another aliquot was dried directly. A total of 0.3 g of these samples as well as 0.6 g of Kanna 1 and 2 were extracted each with 1 mL methanol for 15 min in an ultrasonic bath. After centrifugation, 50 μL (100 μL in case of Kanna 2) of the methanolic extracts were mixed with 200 μL BGE and filled up to 1000 μL with methanol for injection (hydrodynamic injection with 50 mbar for 5 s). The CE separation was carried out applying 30 kV, resulting in a current of 16 μA.

In order to evaluate the fermentation process, the aliquots of the *Sceletium* samples were analyzed with NACE-MS. Figure 2 shows the results of the alkaloid composition of a fermented (Trace B) and a direct extract of *Sceletium* 2 (Trace A). The electropherogram of the unfermented plant material (A) shows six large peaks and one minor signal. These were tentatively identified via their $m/z$ values in comparison with literature data: Two (or three signals after fermentation) with $m/z = 292$ can be observed with time intervals of up to 4 min between these isobaric compounds ($t_R = 7.9, 12.0$, and 15.6 min). Two of them are most probably related to mesembranol and its diastereomer epimesembranol (compare Fig. 1). This impressively shows the separation capability of the NACE method as a similar aqueous CE-UV method by Patnala and Kanfer [7] could only partially separate two diastereomers with $m/z = 292$. The minor peak at about 12.3 min is related to $m/z = 278$, which was already isolated from *S. strictum* by preparative layer chromatography by Jeffs et al. in 1970 [10] and identified to be 4’-O-demethylmesembranol (see Fig. 1), but has not been observed in Kanna samples with other analytical techniques (GC, HPLC, CE) [4, 5, 7] yet. Here, it can easily be discriminated by the high selectivity provided by the NACE-MS method. One alkaloid with $m/z = 288$ ($t_R = 14.3$ min) is due to mesembrone or its double-bond isomer Δ⁷-mesembrone (see Fig. 1). No further attempt for the discrimination of these possible isobaric structures, for example, by MS/MS experiments was made and it remains unclear, if both isomers are present in the sample without being separated. In aqueous CE-UV, purified standards were well separated [7]. The largest signal in Fig. 2A ($t_R = 16$ min) reveals a mass of 290. Its intensity is eight times higher than that of any other signal in the electropherogram pointing to its identification as mesembrine in corroboration of the results of Smith et al. [5] However, as visible from Fig. 3, other samples reveal a higher intensity for another signal.
with \( m/z \) 290 at a different migration time. The last alkaloid peak is related to an alkaloid with \( m/z \) 276 (\( t_R = 18 \) min). Which of the tentative structures (see Fig. 1) belongs to this signal could not be identified yet. The signal labeled with \( m/z \) 269 is related to a sodium acetate cluster from the sample matrix, which was used as an internal standard for the normalization of the electropherograms.

Figure 2B shows the electropherogram of the fermented plant material. The alkaloid profile is almost the same as in the unfermented sample regarding the type of alkaloids present, but it shows differences regarding their relative intensities: Whereas the intensity of the large signal of \( m/z \) 290 remains almost constant, the intensities of three peaks \( m/z \) 292 (at 8.0 min), \( m/z \) 278, and \( m/z \) 288 were significantly increased upon fermentation. In case of \( m/z \) 292, this corroborates the observations by Smith et al. [5] and points to biochemical reactions during fermentation. A new peak with \( m/z \) 292 (\( t_R = 15.5 \) min) may be related to mesembranol isomers as described above, which may stem from biochemical reaction or from isomerization processes. A similar observation was impossible by Smith et al. [5] as their GC method was not capable of discriminating between these isomers.

The chemotypic differences have not been described sufficiently in the literature, and qualitative and quantitative aspects of the alkaloid composition in Sceletium products are still poorly explored in general [8]. In this study, two samples
of \( S. \) tortuosum as well as \( S. \) jouberti (now considered as a part of \( S. \) tortuosum [6, 8], though the differentiation of \( Sceletium \) species still seems to be under discussion) and two Kanna samples from two different head shops were included. In order to show the differences in the alkaloid profiles present due to cultivation conditions, age, as well as turnover and degradation [5], all samples were treated identically. The results are given in Fig. 3. Traces C and D show the electropherograms of \( Sceletium \) 1 and 2, respectively. The alkaloid composition is clearly very similar in both samples, but there are obvious differences concerning relative and absolute intensities of the alkaloids. While the alkaloid related to \( m/z \) 290 (mesembrine or isobaric compounds) in Trace C is the dominant alkaloid, it is only the fourth intensive analyte in Trace D, in which \( m/z \) 292 (possibly mesembranol) and \( m/z \) 276 (possibly 4' -O-demethylmesembrine, 4' -O-demethylmesembranol, or \( \Delta^2 \) 4' -O-demethylmesembranol) have much higher intensities. These and minor differences show that the expression of the different alkaloids strongly depends on the cultivation conditions or the age/alkaloid turnover in the plants. Very similar results were obtained for the two Kanna powders, Kanna 1 (Trace A) and Kanna 2 (Trace B), with high similarities regarding the type of analytes. However, Kanna 2 shows two additional peaks (\( m/z \) 290, \( t_B = 8 \) min; \( m/z \) 278, \( t_B = 12.4 \) min) compared to Kanna 1. In contrast, the relative intensities of the alkaloids in these electropherograms are significantly different, like, for example, for the alkaloid with \( m/z \) 290 (mesembrine, mesembranol, or isomers thereof, \( t_B = 15.7 \) min). Similar observations were made for the self-fermented plant samples (Traces C and D). One major difference between the self-fermented and the commercial samples is interesting to note: The relative intensity of the sodium acetate cluster peak is significantly larger in the commercial powders than in the self-fermented samples, clearly pointing to differences in the manufacturing or fermentation of the \( Sceletium \) samples, for example, allowing different amounts of soil left in the plant samples upon fermentation.

Trace E shows the alkaloid profile of \( S. \) jouberti, which seems to be significantly different from all other samples. Some alkaloids described previously were detected here as well but there are clear differences, for example, the two signals for \( m/z \) 276 (\( t_B = 11.5 \) min) and \( m/z \) 278 (\( t_B = 11.9 \) min). The appearance of five peaks with \( m/z \) 276 and even three for \( m/z \) 278 is interesting to note and points to a large number of isobaric compounds with close structural similarity, probably even comprising diastereomers. It is not possible to conclude whether these differences stem from cultivation conditions or from species-related differences.

Altogether, the application of the modified NACE-MS method [9] is successful for both the relative quantification of the alkaloids in Kanna and the comparison of samples of different origin and \( Sceletium \) species. In comparison to existing methods for the analysis of \( S. \) tortuosum [4–8], this NACE-MS method is able to separate numerous alkaloids including a large number of isobaric structures with high resolution including diastereomers (e.g., the detection of isobaric compounds of \( m/z \) 278, 276, 292, and especially 290 (the mass of the active ingredient mesembrine) giving rise to a better view on the diversity and enormous complexity of the alkaloid composition of \( Sceletium \) species.

The results of this study show a large variation of relative alkaloid concentrations in \( Sceletium \) plants and in Kanna formulations, most probably related to different cultivation conditions, age, or storing/processing of the drug. Also the fermentation process itself is prone to largely influence the alkaloid composition depending, for example, on the amount of soil or microbial effects. The chemotypic differences have not yet been fully addressed in literature [8] and would need a large number of samples to be studied for a full comprehension. With the method presented here and its high separation power, the discrimination of samples is greatly enhanced and by that, it will enable a new perspective on the \( Sceletium \) species.

The results presented here and results obtained with very similar NACE-MS methods [9, 10] reveal that the high separation capabilities of NACE will give rise to the in-depth profiling of alkaloids in biogenic drugs important for a large number of fields, including biology, pharmacology, and ethnopharmacology, as well as forensic science. For the future, the applicability of the NACE-MS method with regard to other biogenic drugs and biological alkaloids will be investigated as well as the unambiguous identification of the detected alkaloids via high-resolution MS and MS\(^n\)-experiments or by preparative CE with NMR-spectroscopy.

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References


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