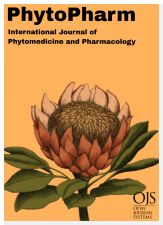

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Rapid Isolation and Purification of the Antidepressants Mesembrine and Mesembrenone from *Mesembryanthemum tortuosum*

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ABSTRACT
Mesembryanthemum tortuosum is a medicinal plant that has traditionally been used as a mood enhancer, stress reducer, analgesic, anxiolytic, and even a narcotic. Its primary activity is attributed to mesembrine-type alkaloids, which have shown pharmacological and clinical promise as antidepressants and anxiolytics. Due to the limited availability and high cost of pure reference compounds, the aim of this study was to develop a rapid purification method for mesembrine and mesembrenone from *M. tortuosum*. A simple acid-base partitioning of a dichloromethane extract was utilised as the initial partial purification step, directly followed by semi-preparative High-Performance Liquid Chromatography (HPLC) as a final purification step. This reduced the number of purification steps compared to previously reported methods. Analytical quantities of mesembrenone and mesembrine (chemical structures confirmed by Nuclear Magnetic Resonance spectroscopy and Mass Spectrometry) were purified to approximately 98% purity (as determined by HPLC). The entire purification process, from extraction to the isolation of pure compounds, was completed in a relatively short period of just four hours. Ultimately, a rapid and simple method to purify the mesembrine-type alkaloids, mesembrine and mesembrenone, was developed, yielding sufficient quantities for use in analytical studies. This provides a cost-effective approach to overcoming the scarcity of pure reference compounds for *M. tortuosum*.

1. Introduction

Mesembryanthemum tortuosum (L.) N.E.Br. (Mesembryanthemaceae), formerly known as *Sceletium tortuosum*, has garnered significant scientific interest due to its clinical potential in treating anxiety and depression. This specific pharmacological action is attributed to its active phytochemical constituents, known as mesembrine-type alkaloids (Maphanga et al., 2022; Olatunji et al., 2022; de Jong et al., 2026). Indigenous use of this species centered on its capacity to alleviate stress and pain, though it was also frequently documented as a traditional anxiolytic and, in specific contexts, a narcotic agent. It has also been employed for its euphoric and intoxicating effects, as a hunger suppressant, and to treat toothache and abdominal pain. The aerial parts of *M. tortuosum* are predominantly chewed, but they are also smoked, ingested as a tincture or tea, or used as a snuff (Gericke and Viljoen, 2008; Loria et al., 2014; Olatunji et al., 2022).

The major alkaloids in *M. tortuosum* are (−)-mesembrine and (+)-mesembrenone, with mesembranol and (+)-mesembrenol occurring at lower concentrations. Mesembrine is, however, the primary phytochemical constituent and is considered to be the main active component of *M. tortuosum* (Gericke and Viljoen, 2008; Shikanga et al., 2011; Krstenansky, 2017). These alkaloids have received considerable attention for the potential treatment of anxiety and depression (Olatunji et al., 2022; de Jong et al., 2026); they function in the brain via dual inhibition of phosphodiesterase-4 (PDE4) and serotonin (5-HT) reuptake, resulting in a synergistic therapeutic action (Gericke and Viljoen, 2008). Mesembrine has been shown to be a potent 5-hydroxytryptamine (5-HT) reuptake inhibitor, which is essential for the effective treatment of depression, whereas mesembrenone demonstrates greater activity against PDE4A and PDE4B than the other alkaloids (Harvey et al., 2011).

Due to the scarcity and high cost of pure reference standards, in-depth pre-clinical and clinical research in this field is severely hampered. There are currently very few suppliers, and those providing these alkaloids typically produce them synthet-

ically at a very high cost. Further complications arise from very long lead times, as the compounds are usually only synthesised on demand. The synthesis of mesembrine was first achieved in 1991, and while various improved methods of total synthesis have subsequently been reported, it remains a tedious, multistep process (Parkinson et al., 1991; Van Otterlo et al., 2018).

The purification of mesembrine alkaloids from *M. tortuosum* plant material has also been reported. Only one study has focussed solely on the purification of these alkaloids, detailing a method using gravity column chromatography (CC) to separate a crude extract into four fractions, followed by high-speed counter-current chromatography as a final purification step (Shikanga et al., 2011). Purification employing several CC steps has also been reported by other researchers, necessitated by the unavailability of reference compounds required for analytical, chemotaxonomic, pharmacological, pharmacokinetic, and toxicological studies (Patnala and Kanfer, 2010; Harvey et al., 2011; Patnala and Kanfer, 2013; Meyer et al., 2015; Manda et al., 2016).

The difficulty in obtaining pure reference standards for the pharmacologically active phytochemicals of *M. tortuosum* therefore warrants a rapid and efficient method to purify the main alkaloids, mesembrine and mesembrenone. Here, we report on a rapid method using semi-preparative HPLC to directly purify mesembrine and mesembrenone from a crude extract of *M. tortuosum*, yielding high-purity compounds for analytical studies.

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade acetonitrile (ACN) was obtained from Thermo Fisher (Cape Town, South Africa) and CP grade dichloromethane (DCM) from MCL (Johannesburg, South Africa). Pure water was obtained from a Rephile direct pure UP Ultrapure & RO Lab water system (Boston, MA, USA). A synthetic mesembrine reference standard (98.6% purity – lot number 5888-078A8) was purchased from TLC Pharmaceutical standards (Ontario, Canada).

2.2. Plant material

Commercial cultivated *M. tortuosum* was obtained from GeoGreen Health (Klerksdorp, South Africa) which supplied fermented powder (TKP/240123/M) produced on 15/01/2024. According to the certificate of analysis, the sample contained 1.550 and 0.306% of mesembrine and mesembrenone, respectively.

2.3. General experimental procedures

An Ultimate 3000 semi-preparative HPLC system consisting of an HPG-3200BX Biocompatible binary semi-preparative pump, VWD-3100 variable wavelength detector and a Rheodyne manual injector with an 1 mL injection loop was used for separation and purification. The fractions were collected with a Fraction Collector model F. The system was operated with Chromeleon 7 software and a Fortis C18 column was used for separation (21.2 × 250 mm, 5 μm). An isocratic mobile phase consisting of 0.1% ammonia (A) and ACN (B) was used at a flow rate of 14 mL/min. 50% B was used for a total run time of 12 min with mesembrenone and mesembrine eluting at 7.0 and 9.0 min, respectively.

For determining the purity of mesembrenone and mesembrine a Shimadzu i-Nexera HPLC system equipped with a quaternary pump, autosampler, and a photodiode array detector (PDA) was used. The system was fitted with an GL sciences C18, 2.1 × 150 mm, 3 μm column with the column oven set to 40°C. The solvent system consisted of 0.1% ammonia (A) and ACN (B), and an isocratic system was employed in a 60% (A) 40% (B) ratio. The flow rate was 0.25 mL/min and 1 μL of each sample was injected. The PDA detector was set to 228 nm.

^{1}H spectra were recorded using a Bruker 600 MHz Avance NEO spectrometer that was equipped with a two-channel direct observation iProbe (Bruker, Ettlingen, Germany) using $CDCl_3$ as solvent. The spectrometer was operated with Topspin 4.4.0 (Bruker) and data were processed with MestReNova (version 6.0). All spectra were recorded with 16 scans at 25 °C and referenced to $CDCl_3$ at 7.26 ppm. MS data was generated on an Agilent Ultivo triple-quadrupole mass spectrometer (Santa Clara, California, United States) consisting of a 1260 Infinity II autosampler, 1200 quaternary pump, column oven and the Ultivo ESI TQ. Agilent Chemstation MassHunter Data Acquisition (version 1.2) and Quantitative Analysis (version 10.0) were used to control all instrument components and acquire, analyse, and store the data.

2.4. Extraction of alkaloids

A flow diagram of the process is provided in **Figure 1**. In short, DCM extraction was performed by adding 5 g of *M. tortuosum* plant material to 100 mL DCM and sonicating the solution for 15 min. The solution was filtered with Whatman® GF/C 55 mm filter paper (BN:1822055). The extraction procedure was repeated three times in total. The combined filtrates were liquid:liquid partitioned with 0.25 M sulfuric acid and separated from the DCM fraction (repeated three times). The pH of the combined acidified solution was adjusted to 9 with the addition of 20% (v/v) ammonia. Dichloromethane (3 × 50 mL) was added to the basified solution, and the solution was then shaken vigorously and allowed to separate. The final DCM layers were combined and evaporated to dryness.

2.5. Purification

The final DCM extract was redissolved in HPLC eluent to a concentration of 50 mg/mL. The solution was filtered prior to injection into the chromatograph, which was operated at room temperature. Fraction collection times was set to 7.0–8.5 and 9.0–10.5 min with a total run time of 12 min.

3. Results and Discussion

The method developed and described herein offers a rapid approach to purifying the biologically significant alkaloids, mesembrine and mesembrenone.

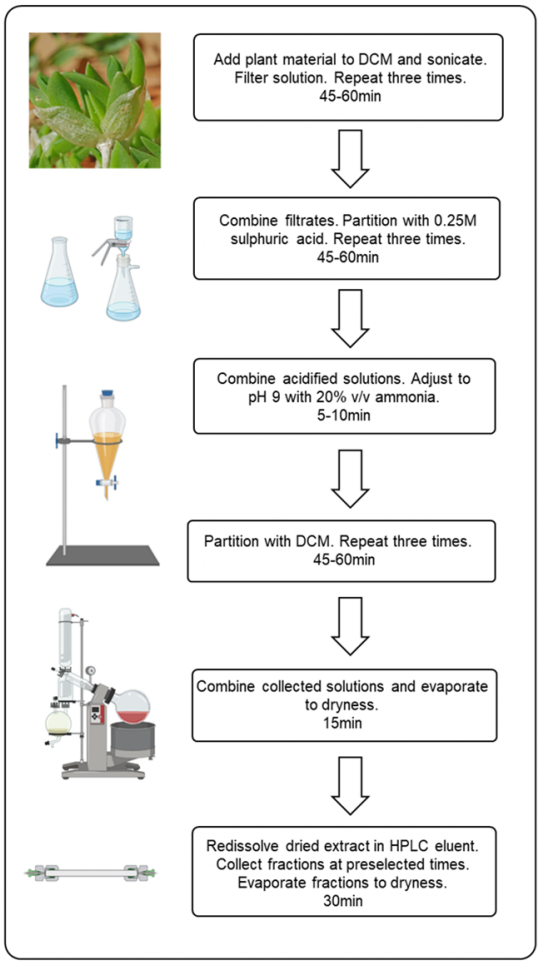

Figure 1: Flow diagram of the acid-base extraction process for *Mesembryanthemum tortuosum* alkaloids.

The most time-consuming phase remains the acid-base extraction and partial purification of the alkaloids. This process, which was repeated three times at each stage, took approximately two to three hours in total. This duration can be reduced by performing each step only once; however, this may lead to a reduction in total yield. Drying the final DCM extract yielded 176 mg (3.56% yield).

According to the supplier guidelines for the Fortis column, the loading capacity ranges from 10–200 mg, depending on several factors such as the retention of the target compounds (longer R_t = higher capacity), the complexity of the sample (higher capacity for simpler mixtures), and sample solubility. Therefore, the final DCM extract was reconstituted to a concentration of 50 mg/mL for injection. However, due to the limited solubility, a filtration step was required which removed approximately 40% of the extract. This resulted in an injectable concentration of only 30.1 mg/mL per run (determined by separately filtering and drying 1 mL of the solution).

The purification step is efficient, with a total run time of 12 min per 1 mL injection. The two target compounds were collected at 7.0–8.5 min (mesembrenone) and 9.0–10.5 min (mesembrine). Drying these two fractions yielded 3.4 mg and 8.4 mg for mesembrenone and mesembrine, respectively. This represents recoveries of 80% and 39.5% (theoretical yields, based on the certificate of analysis provided with the *M. tortuosum* sample, are 4.25 mg and 21.25 mg per 1 mL injected). The purity of the compounds, based on HPLC analysis, was 97.2% and 98.9% for mesembrenone and mesembrine, respectively (**Figure 2**). Furthermore, ^{1}H NMR, MS, and UV analyses confirmed that the chemical structures and identities corresponded closely with literature (spectra available as supplementary data)(Jeffs et al., 1970; Patnala and Kanfer, 2010; Meyer et al., 2015; Krstenansky, 2017).

The purchased authentic mesembrine standard was spiked with the purified mesembrine and analysed by HPLC. The observation of a single peak provided further confirmation of its identity.

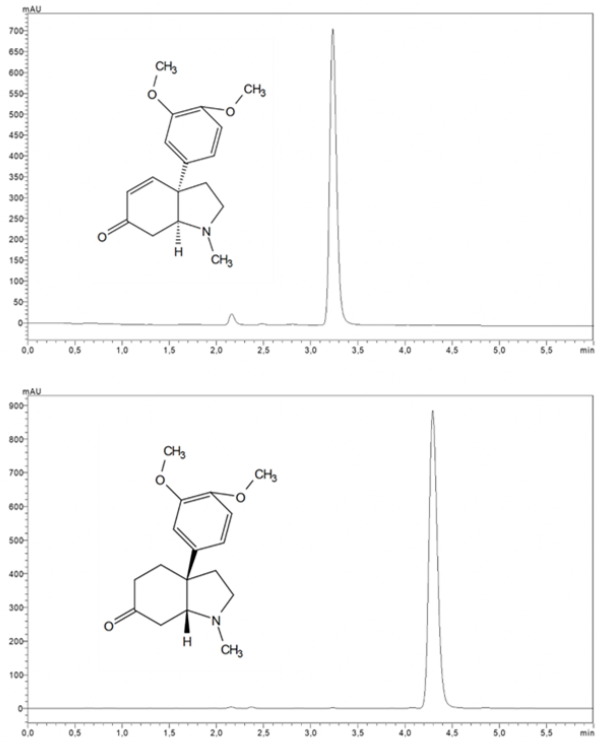

Figure 2: HPLC chromatograms and chemical structures of purified mesembrenone and mesembrine.

Mesembrenone: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.89 (dd, J = 8.0 and 2.0 Hz; H-6′), 6.85 (d, J = 2.0 Hz, H-2′), 6.84 (d, J = 8.0 Hz, H-5′), 6.72 (dd, J = 10.2 and 1.8 Hz, H-4), 6.11 (d, J = 10.2, H-5), 3.88 (s, 3H, 3′-OCH_3), 3.87 (s, 3H, 4′-OCH_3), 3.32 (m, 1H), 2.68 (m, 1H), 2.42–2.61 (m, 5H), 2.32 (s, 3H, NCH_3), 2.18–2.25 (m, 1H). UV λ_{max} 225 and 279 nm; MS (+H) 288.3.

Mesembrine: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.91 (dd, J = 8.4 and 1.8 Hz, H-6′, 1H), 6.89 (d, J = 1.8 Hz, H-2′, 1H), 6.87 (d, J = 8.4 Hz, H-5′, 1H), 3.88 (s, 3H, 3′-OCH_3, 3H), 3.86 (s, 3H, 4′-OCH_3, 3H), 3.12–3.16 (m, 1H), 2.95 (m, 1H), 2.59 (m, 2H), 2.32–2.45 (m, 2H) 2.33 (s, N-Me, 3H), 2.04–2.26 (m, 5H). UV λ_{max} 229 and 280 nm; MS (+H) 290.3.

4. Conclusions

Depending on the specific requirements of a study, a single 1 mL injection on the semi-preparative HPLC will yield sufficient purified material for analytical studies. With a run time of only 12 minutes, multiple injections can yield relatively large quantities (e.g. hundreds of milligrams) within a matter of hours. By employing a larger column and injector loop, gram quantities could potentially be purified using this method. Our protocol can also be adapted to focus on other mesembrine-type alkaloids by making minor adjustments to parameters such as solvent ratios and flow rates. The most critical factor, however, is acquiring a reliable source of plant material with the optimum alkaloid profile. Suppliers offer plant material or standardised extracts with specific alkaloid compositions (e.g. high-mesembrenone and low-mesembrine, or vice versa, or high levels of Δ^7-mesembrenone). Depending on the focus of a research project, the most suitable plant material can be sourced and the required alkaloid purified by making slight modifications to our method. Furthermore, the method can be adapted for use with a standard analytical HPLC by reducing the pressure limit on the pump (typically to 200 bar), which allows the user to increase the maximum flow rate to 10 mL/min. By optimising the solvent ratio and using a flow rate of 10 mL/min, our method could potentially be transitioned to an analytical HPLC, which is far more commonly available than a semi-preparative system.

CRediT authorship contribution statement

S van Niekerk: Investigation, data curation, writing – review & editing, visualization. **D Malan**: Investigation, writing – review & editing. **J Hamman**: Conceptualization, resources, writing – review & editing, supervision. **F van der Kooy**: Conceptualization, investigation, resources, writing – original draft, supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors have not used AI or AI-assisted technologies in the preparation of this manuscript.

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Data availability

Data and reference compounds are available from the corresponding author upon reasonable request.

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