Fast Isolation and Purification of the Antidepressant Alkaloids Mesembrine and Mesembrenone From Mesembryanthemum tortuosum

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Short Report

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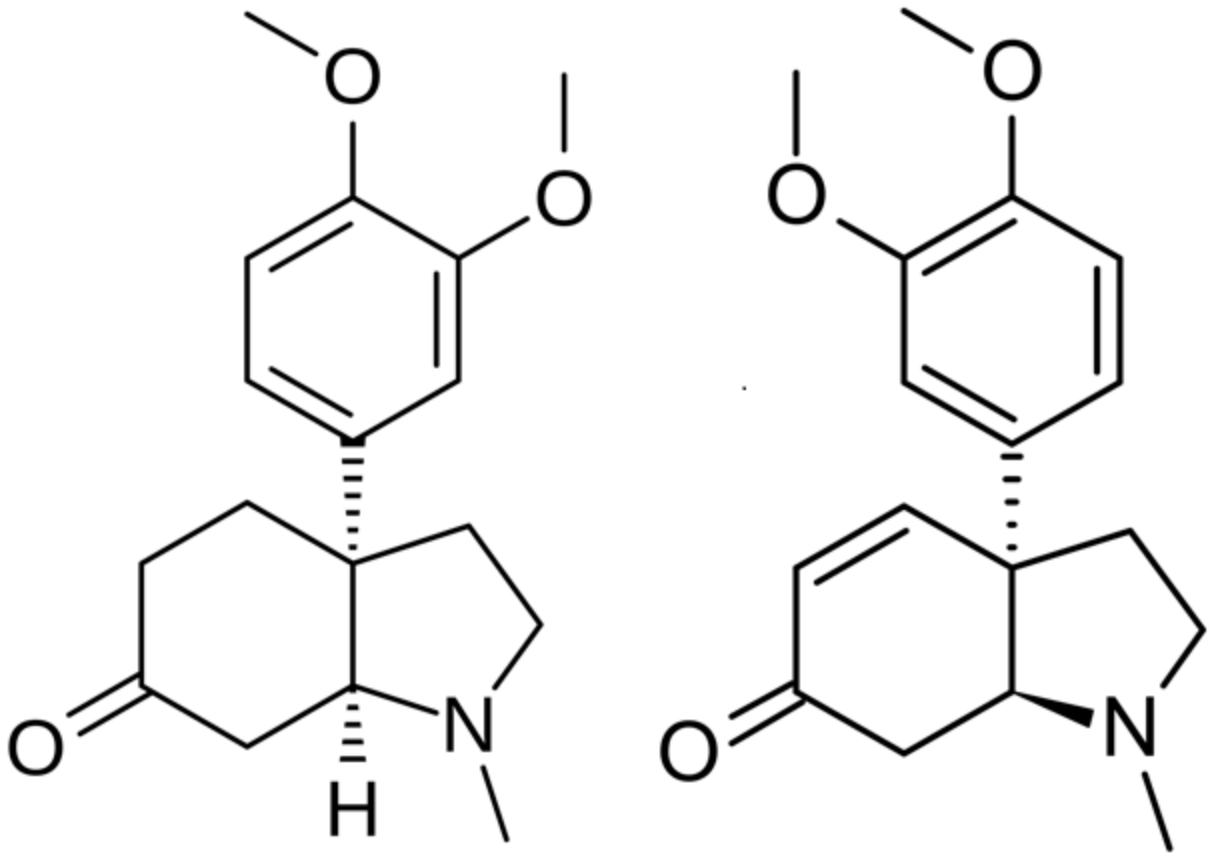
Abstract

Mesembryanthemum tortuosum L., Aizoaceae, is a medicinal plant that has traditionally been used as mood elevator, stress reducer, analgesic, anxiolytic, and even a narcotic. The main activity is attributed to the mesembrine-type alkaloids, which have shown promise to provide pharmacologically and clinically antidepressant and anxiolytic effects. Due to the limited availability and cost of pure reference compounds of the main active chemical compounds of *M. tortuosum*, this study aimed to develop a fast purification method for mesembrine and mesembrenone from *M. tortuosum*. A simple acid-base extraction was used as the first semi-purification step, which was directly followed by semi-preparative HPLC purification as final step. This reduced the number of steps as previously reported. Analytical quantities of mesembrine and mesembrenone (chemical structures confirmed by NMR and MS) could be purified up to roughly 98% purity (as determined by HPLC) with the whole purification process from extraction to obtaining the pure compounds completed at the relatively short period of only four hours. A quick and simple method to purify the mesembrine-type alkaloids, mesembrine and mesembrenone, was developed which produced sufficient quantities for use in analytical and biological studies. This provides a cost-effective method to overcome the lack of pure reference compounds for *M. tortuosum*.

Introduction

Mesembryanthemum tortuosum L. Mesembryanthemaceae (Aizoaceae), also known as *Sceletium tortuosum* (L.) N.E.Br., has received significant scientific interest due to its clinical potential in treating anxiety and depression. This specific pharmacological action is attributed to its active chemical constituents referred to as mesembrine-type alkaloids (Olatunji et al. 2022; Maphanga et al. 2022). Traditionally, this medicinal plant has been used as mood elevator, stress reducer, analgesic, anxiolytic or even a narcotic. It has also been used for its euphoric/intoxicating effects, as a hunger suppressant or to treat toothache and abdominal pain. Aerial parts of *M. tortuosum* are mostly chewed, but it is also smoked, ingested as a tincture or tea and even used as a snuff (Gericke and Viljoen 2008; Loria et al. 2014; Olatunji et al. 2022).

The major alkaloids in *M. tortuosum* are (−)-mesembrine (**1**), and (+)-mesembrenone (**2**) with mesembranol, and (+)-mesembrenol being present at lower concentrations. Mesembrine is, however, the major chemical constituent and is considered to be the main active component of *M. tortuosum* (Gericke and Viljoen 2008; Shikanga et al. 2011; Krstenansky 2017). The mesembrine-type alkaloids have received a lot of attention for the possible treatment of anxiety and depression (Olatunji et al. 2022). The mesembrine-alkaloids function in the brain by dual inhibition of phosphodiesterase-4 (PDE4) and serotonin (5-HT) reuptake, which has 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 effective treatment of depression, whereas mesembrenone was found to have greater activity on PDE4A and PDE4B than the other alkaloids (Harvey et al. 2011).



Due to the scarcity and cost of pure reference standards of the mesembrine-type alkaloids, such as mesembrine and mesembrenone, in-depth pre-clinical and clinical research in this field is severely hampered. There are currently very few suppliers and those that do supply the alkaloids produce them synthetically at a very high cost. Further inconvenience is created by a very long lead time as the compounds are usually only synthesised on demand. The synthesis of mesembrine was first achieved in 1991 and subsequently, various improved methods of total synthesis have been reported, but it remains a tedious multistep process (Parkinson and Pinhey 1991; Van Otterlo and Green 2018).

Purification of mesembrine-type alkaloids from *M. tortuosum* plant material has also been reported. Only one study focussed solely on the purification of the alkaloids and reported on a method using gravity-fed 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 mainly due to the unavailability of reference compounds necessitating purification 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 quick and efficient method to purify the main alkaloids, mesembrine and mesembrenone. Here we report on a fast method using semi-preparative HPLC to directly purify

mesembrine and mesembrenone from a crude extract of *M. tortuosum* to yield highly pure compounds for analytical studies.

Materials and methods

HPLC grade acetonitrile (ACN) was obtained from Thermo Fisher (Cape Town, South Africa) and CP grade CH_2Cl_2 from MCL (Johannesburg, South Africa). Pure water was obtained from a Rephile direct pure UP Ultrapure & RO Lab water system (Boston, MA, USA). A mesembrine reference standard (98.6% purity, lot number 5888-078A8) was purchased from TLC Pharmaceutical standards (Ontario, Canada).

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.

An Ultimate 3000 semi-preparative high-performance liquid chromatography (HPLC) system consisting of an HPG-3200BX Biocompatible binary semi-preparative pump, VWD-3100 variable wavelength detector and a Rheodyne manual injector with a 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 acetonitrile (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 was used. The system was fitted with a GL sciences C-18, 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 DAD detector was set to 228 nm. NMR spectra were recorded on a Bruker 600 Avance II NMR at 600MHz for ^1H NMR using $CDCl_3$ as solvent. MS data was generated on an Agilent Ultivo TQ system using the same analytical setup as described for HPLC.

A flow diagram of the process is provided in Fig. S1. In short, CH_2Cl_2 extraction was performed by adding 5 g of *M. tortuosum* plant material to 100 ml CH_2Cl_2 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 0.25M sulfuric acid and separated from the CH_2Cl_2 fraction (repeated three times). The pH of the combined acidified solution was adjusted to 9 with the addition of 20% (v/v) ammonia. CH_2Cl_2 was added to the basified solution, the solution was then shaken vigorously and allowed to separate (3 × 50 ml). The final CH_2Cl_2 solutions were combined and evaporated to dryness.

The final CH_2Cl_2 extract was redissolved in HPLC eluent to a concentration of 50 mg/ml. The solution was filtered before injection into the chromatograph, which was operated at room temperature. Fraction collection times were set to 7.0-8.5 and 9.0-10.5 min with a total run time of 12 min.

Results and Discussion

The method developed and described here offers a fast way to purify the important alkaloids mesembrine and mesembrenone. The most time-consuming step remains the acid-based extraction and semi-purification of the alkaloids. This step which was repeated three times for each stage took approximately two-three hours in total. This time can be reduced by only repeating each step once, but this can lead to some loss in total yield. Drying the final CH_2Cl_2 extract yielded 176 mg (3.56% yield).

According to the supplier of the Fortis column the loading capacity on the column is between 10–200 mg depending on several factors such as how well retained the target compounds are (longer R_t = higher capacity), the complexity of the sample (higher capacity for simple mixtures), and the solubility of the sample. Therefore, the crude extract was reconstituted to provide a 50 mg/ml solution for injection. However, due to the limited solubility of the CH_2Cl_2 extract, a filtering step was required which removed approximately 40% of the extract resulting in only 30.1 mg/ml that could be injected per run (determined by separately filtering and drying 1 ml of the solution).

The purification step is quick, with a total run time of 12 min per 1 ml injection with collection of the two target compounds at times 7.0-8.5 min (mesembrenone) and 9.0-10.5 min (mesembrine). Drying of these two fractions yielded 3.4 and 8.4 mg for mesembrenone and mesembrine, respectively. This represents a yield of 80% and 39.5%, respectively (the theoretical yields based on the certificate of analysis provided with the *M. tortuosum* sample are 4.25 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 (Fig. S2), with ^{1}H NMR, MS and UV analysis confirming the chemical structure and identity which corresponded well with literature (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. Only one peak was observed which provided further confirmation of its identity. Chemical data of the two purified compounds (i.e. mesembrenone and mesembrine) matched well with the literature (spectra available in the Supplementary Information).

*Mesembrine (***1***)*: ^{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.

*Mesembrenone (***2***)*: ^{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'-

OCH3), 3.87 (s, 3H, 4'-OCH3), 3.32 (m, 1H), 2.68 (m, 1H), 2.42–2.61 (m, 5H), 2.32 (s, 3H, NCH3), 2.18–2.25 (m, 1H). UV λ_{max} 225 and 279nm; MS (+ H) 288.3.

Depending on the specific requirements of a study, a single injection of 1 ml on the semi-preparative HPLC will yield enough purified material for analytical studies such as chemotaxonomic and/or metabolomics analysis. With a run time of only 12 min, multiple injections will yield relatively large quantities (i.e. 100's of milligrams) within a matter of hours and with the use of a larger column and injector loop, gram quantities could be purified using this method.

Declarations

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Author contributions

SEN: Investigation, data curation, writing - review & editing, visualization. DM: Investigation, writing - review & editing. JH: Conceptualization, resources, writing, review & editing, supervision. FK: Conceptualization, investigation, resources, writing original draft, supervision. All authors have read and agreed to the published version of the manuscript.

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Availability of data and material

Not applicable

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