



## Differential impact of extracts from distinct *Sceletium tortuosum* chemotypes on central neurotransmitter concentrations in C57BL/6 mice

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### ABSTRACT

**Ethnopharmacology relevance:** *Sceletium tortuosum*, also known as kanna, or kougoed, has long been an integral part of the traditional medicinal practices of the San and Khoikhoi peoples of Southern Africa. Among the various *Sceletium* species, *S. tortuosum* is used for its mood-enhancing properties, attributed to the structurally related mesembrenine-type alkaloids found therein. While significant research has focused on mesembrace and mesembrenone, the therapeutic potential of extracts from plants that produce more of the so-called “minor alkaloids”, remains unexplored.

**Aim of the study:** To assess the CNS modulatory effects of two chemotypes of *S. tortuosum*, wild-collected from two different geographic locations in South Africa (Touwsrivier and De Rust), each featuring different alkaloid profiles and with elevated minor alkaloid concentrations.

**Materials and methods:** Extracts from these chemotypes, as well as a vehicle control and a commercial extract, were administered to four groups of mice for 35 days. Mice were then euthanised, and their frontal cortices, striata and hippocampi dissected. Serotonin, dopamine, noradrenaline, glutamate, and gamma-aminobutyric acid (GABA) concentrations were analysed using LC-MS.

**Results:** Both chemotypes, compared to both control and commercial extract exposure, robustly increased noradrenaline and decreased GABA concentrations in all regions of the mouse brain analysed.

**Conclusion:** This finding may support a mood-enhancing effect of *S. tortuosum* and indicates its potential to modulate anxiety and stress processing, attention, and alertness. Alkaloid profiling further suggests that the mesembrine alcohols and sceletium A4 may be important contributors in driving these neurochemical changes.

### 1. Introduction

Major depressive disorder (MDD) and generalised anxiety disorder (GAD) are among the most prevalent mental health disorders worldwide and are characterised by mood disturbances that affect emotional, cognitive and physical functioning (American Psychiatric Association DSM-5 Task Force, 2013). These conditions involve complex neurobiological mechanisms, including central dysregulation of serotonin, dopamine, noradrenaline, glutamate and gamma-aminobutyric acid (GABA) (Cosci and Chouinard, 2019). As such, both MDD and GAD are commonly treated with drugs that modulate the actions of these neurotransmitters, e.g., selective serotonin reuptake inhibitors (SSRIs), noradrenergic and serotonergic tricyclic antidepressants (TCAs), and GABAergic benzodiazepines (Ipser et al., 2006; Sharp and Collins,

2024). Nevertheless, MDD and GAD present significant public health challenges, often exhibiting treatment resistance, therapeutic non-adherence, and side effects such as weight gain, arrhythmia, anxiety, insomnia, and fatigue (Gaynes et al., 2020; Holvast et al., 2019; Nemerooff, 2007; Santomauro et al., 2021; Voineskos et al., 2020). Given these challenges, the exploration of novel drugs for the management of these conditions, is crucial.

In recent years, *S. tortuosum* has attracted widespread scientific interest due to its clinical potential in treating psychiatric disorders (Brendler et al., 2021; Gericke and Viljoen, 2008; Manganyi et al., 2021; Olatunji et al., 2022). In healthy, i.e. non-psychiatric individuals, the use of the plant may also assist in relieving stress, promoting sleep, and enhancing cognitive function. The medicinal properties of *S. tortuosum* are attributed to the presence of mesembrine-related alkaloids (Harvey

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et al., 2011) which may be classified into different types based on their core structures (Fig. 1) (Jeffs, 1981; Makolo et al., 2019; Smith et al., 1996). The mesembrine-type class, which contains the *cis*-3-a-dihydroindole core, includes mesembrine and mesembrenone (Krstensky, 2017). These are the most abundant alkaloids found in the plant and are also the only alkaloids that have been studied to date for their CNS-modulating activity (Faber et al., 2022; Smith et al., 1996). Mesembrine was the first *Sceletium* alkaloid to be isolated and structurally elucidated in 1957 by Bodendorf and Krieger (Bodendorf and Krieger, 1957; Jeffs, 1981; Krstensky, 2017).

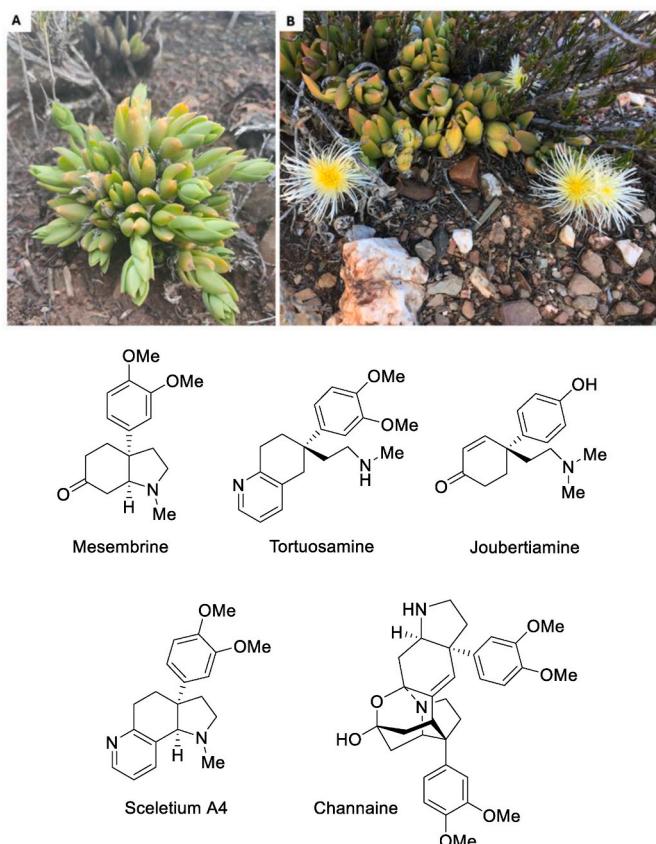
*Sceletium tortuosum* (L.) N. E. Br, is a member of the Aizoaceae family of the *Mesembryanthemaceae* subfamily and is one of eight species in the genus. These succulent plants are commonly known as kanna, kougoed, or sceletium. *S. tortuosum* is endemic to the Karoo and Namaqualand regions of Southern Africa (), and has a long history of traditional use among the indigenous people of the region, particularly the San and Khoikhoi (Nell et al., 2013). The plant is mainly chewed or smoked for its medicinal effects. The name kanna has a symbolic reference to the eland (*Taurotragus oryx*), an antelope referred to as the “trance animal” by the San (Smith et al., 1996). Kougoed, on the other hand, alludes to the traditional method in which the plant is used, being chewed after it has been dried and fermented; this is believed to intensify its effects (Faber et al., 2022; Harvey et al., 2011; Schultes, 2021). Kanna is prized for its mood-enhancing effects and for its ability to alleviate hunger and thirst. The early European settlers of the Cape region are recorded to have used it as a sedative tincture, and to relieve toothache when chewed. Other recorded uses of the plant include alleviating alcohol addiction and assisting with colic and sleep in infants. The plant is also

known for its intoxicating and euphoric effects (Faber et al., 2022; Olatunji et al., 2022; Smith et al., 1996). In many rural environments in the Karoo and Namaqualand, the traditional use of these plants continues, with ‘bossiedokters’ (or ‘bush doctors’), exploiting its psychoactive properties during spiritual and religious gatherings.

Phytochemical analyses have revealed chemotypic variation that is inherently associated with *S. tortuosum* plants growing in different geographic areas (Reddy et al., 2022; Shikanga et al., 2012; Zhao et al., 2018). These chemotypes have different alkaloid profiles, showing varying levels of mesembrine and mesembrenone, with some showing higher concentrations of the lesser known, or minor alkaloids. Using an *in silico* molecular docking approach, Reddy et al. (2022) showed that some of the phytochemicals that belong to the minor mesembrine alkaloid class, show greater binding to the 5-HT serotonin transporter (5I75), the GABA-A receptor (6D6T), and the acetylcholinesterase (AChE) enzyme (1QTI). However, this *in silico* study did not validate the biological actions. This was a key aim of this work in an *in-vivo* model system.

Extracts from *S. tortuosum*, as well as some of the isolated mesembrine-type of alkaloids, affect several central nervous system (CNS) targets which are implicated in both MDD and GAD, although studies of its effects in whole organism model systems, are few. For example, mesembrine is a selective reuptake inhibitor of serotonin, with only weak inhibition of noradrenaline and dopamine reuptake shown in *in vitro* assays (Gericke and Viljoen, 2008). In another study, an *S. tortuosum* extract was reported to inhibit serotonin transporter (SERT) action and phosphodiesterase 4 (PDE4) activity, the latter being an enzyme that metabolises cyclic adenosine monophosphate (cAMP), the primary second messenger of serotonin receptors (Harvey et al., 2011). Mesembrine seems to be the most potent inhibitor of SERT, while mesembrenone had both SERT and PDE4-inhibitory action at higher concentrations. The extract also showed an affinity for GABA-A,  $\mu$ -opioid,  $\delta_2$ -opioid and EP4 prostaglandin receptors (Harvey et al., 2011). In human astrocytes and mouse hippocampal neurons, an *S. tortuosum* extract down-regulated SERT expression in a similar manner to citalopram and upregulated the vesicular monoamine transporter-2 (VMAT-2) (Coetzee et al., 2016). However, the extract caused only mild inhibition of AChE and monoamine oxidase A (MAO-A), indicating that serotonin reuptake inhibition activity may be secondary to increased monoamine-release (Coetzee et al., 2016).

There are also a few behavioural studies in which *S. tortuosum* extracts showed anti-depressant-like or anxiolytic-like activity in rats. For example, an acute dose of an *S. tortuosum* extract (tradename Zembrin®), was found to exhibit antidepressant-like effects in a rodent model of MDD, with results comparable to that of the SSRI, escitalopram (Gericke et al., 2022). In rats, Zembrin® resulted in electropharmacograms similar to those elicited by compounds associated with cognitive improvement, anti-depressant-like action and analgesia, an effect likely mediated via dopaminergic and glutamatergic activation (Dimpfel et al., 2016). In a study using an *ex vivo* rat hippocampus, Zembrin® also blunted  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated neurotransmission. Follow-up studies using the pure alkaloids found that only mesembranol and mesembrenol (both mesembrines with a hydroxyl substituent at C6) blunted this transmission (Dimpfel et al., 2018). With respect to anxiety, a low-dose *S. tortuosum* extract was found to mildly decrease restraint-induced self-soothing behaviour and corticosterone levels in rats (Smith, 2011). However, the same formulation also resulted in an inflammatory reaction and, strikingly, T helper 1 (Th1) cell-directed immunosuppression, potentially pointing to brain-immune interactions underlying its effects in this model. An anxiolytic-like effect of an enriched *S. tortuosum* fraction was also shown in chicks that exhibited lower distress vocalisations following administration of the extract (Carpenter et al., 2016). Moreover, the impact of *S. tortuosum* on anxiety processing has also been shown in a handful of clinical studies (Reay et al., 2020; Terburg et al., 2013), although continued investigation is vital in light of



**Fig. 1. Top.** Example of *S. tortuosum* growing in the wild (A) Non-flowering plant from the Touwsriver (photo credit: Hope Botes) (B) Flowering plant growing underneath other woody shrubs found in the De Rust (photo credit: NP Makunga). **Bottom.** Different skeleton types of *Sceletium* alkaloids with a representative member from each type: Mesembrine, Tortuosamine, Joubertiamine, Sceletium A4 and Channaine.

the conclusions of a recent systematic review (Gouhie et al., 2023). In terms of its procognitive value, extracts of *S. tortuosum* showed promising effects. For example, it was found that recreationally trained men and women showed improvement in performing complex reactive tasks that demanded a high cognitive load, albeit in the absence of any additional benefit to mood (Hoffman et al., 2020). Similarly, in a randomised placebo-controlled cross-over trial, Zembrin® significantly improved cognitive flexibility and executive function (Chiu et al., 2014). Moreover, positive changes in mood and sleep were also reported (Chiu et al., 2014).

Considering this summary, no studies have yet investigated the potential contribution of the minor, lesser-known alkaloids, and by implication, the effects of different *S. tortuosum* chemotypes, on the CNS-modulating activity of *S. tortuosum*. Therefore, the purpose of the present work was to explore the differential contribution of the major and minor alkaloids, as extracted from different *S. tortuosum* chemotypes on central neurotransmitter concentrations in stress-naïve C57BL/6 mice. To this end, two chemotypes that were characterised to contain a higher diversity, and quantity of minor alkaloids were used.

## 2. Materials and methods

### 2.1. Plant collections

Two different *S. tortuosum* chemotypes were collected from wild occurring populations during the southern hemisphere autumn (April 25, 2022). Herbaria collections, the South African National Biodiversity Institute-Botanical Database of Southern Africa (SANBI-BODATSA) that contains past and current plant data observational records, together with iNaturalist submissions were used to curate field trips for wild collection of *S. tortuosum* plants. Plant collections and species identifications were conducted by NP Makunga (PhD), a botanist at Stellenbosch University (SU), together with K Reddy (PhD, SU). From each individual plant, three cuttings (10–15 cm in length) of the above-ground succulent foliage were made. Twenty individuals were randomly sampled per population, and these were placed in brown paper until they were stored under refrigeration. Collections were conducted with permission from landowners as governed by the Cape Nature Permit (CN35-8720155). Voucher specimens were kept for both chemotypes and termed Scel\_KR\_1 and Scel\_KR\_6 for the Touwsrivier-and De Rust-growing plants, respectively. The voucher specimens were lodged in the Department of Botany and Zoology at Stellenbosch University. The Gerbaulet key (1996) and herbarium specimens housed in the Compton Herbarium (Kirstenbosch National Botanical Garden Research Centre) were also used to confirm the taxonomic identity of the plants. Additional confirmation of the species identity was possible with the consultation of Dr D Kirkwood (PhD), curator of the SU Botanical Garden, as a living collection of these chemotypes was established here. One of the chemotypes was collected from the Touwsrivier area of the Klein Karoo (33.593399 S, 19.871185 E) that is defined by Langeberg sandstone fynbos and shale renosterveld vegetation. Alluvial soils that are sandy to loamy with quartzitic rocks are characteristic of this region. The average annual rainfall is about 23 mm, occurring mostly during the winter months. The De Rust chemotype (33.526416 S, 22.482249 E) also grows in the succulent Karoo biome in an area that is defined to have karroid and renosterveld shrubland with alti-montane fynbos, and soils that are loamy and mesotrophic. In this part of the Klein Karoo, average rainfall of 433 mm per year has been recorded. The De Rust chemotype was found on private farmlands that occur at the foothills of the Swartberg Mountains. During the time of collection, average temperatures at both sites ranged from 10.9 to 23.9 °C. The work presented herein was guided by the Biodiversity Act of 2004 and the Bio-prospecting, Access and Benefit Sharing regulations of 2008 (Reference number: BABS/000522N).

### 2.1.1. Preparation of *Sceletium tortuosum* plant material

After harvesting, the *Sceletium* plant material was frozen and stored at –80 °C. For extraction, the plant material was completely thawed at room temperature and juiced with a Nutribullet 600 series blender. The juice was transferred to 50 mL tubes and centrifuged for 20 min at 10 000 revolutions per minute (rpm) (Thermo Scientific, Sorval RC 6+). The cellular debris was discarded and the supernatant transferred to clean tubes prior to freeze drying (Virtis Benchtop Pro, SP Scientific) to yield the final powder products.

### 2.1.2. Profiling of the alkaloids in the *Sceletium tortuosum* extracts

Profiling of the *S. tortuosum* alkaloids was performed according to the method published by Makunga et al. (2022), with modifications similar to the study of Reddy et al. (2022). A detailed methodology is provided in Section S4 of the supplementary information (SI). In short, samples were extracted into methanol and analysed using reverse-phase ultrahigh pressure liquid chromatography (UHPLC) with quadrupole time-of-flight (Q-TOF) mass spectrometric (MS) detection. The alkaloids were quantified using the integrated peak areas of extracted mass chromatograms with mesembrine utilised as the in-house standard (LGC standards Ltd.). A total of 12 alkaloids were detected in the samples. These were 1. mesembrine, 2. mesembrenone, 3. mesembrenol, 4. mesembranol, 5. 4'-O-demethylmesembrine, 6. 4'-O-demethylmesembrenone, 7.  $\Delta^7$ -mesembrenone, 8 epimesembranol, 9. epimesembrenol, 10. O-acetylmesembrenol, 11. sceletium alkaloid A4, and 12. dihydrojoubertiamine. Mesembrenone and mesembrenol were identified using in-house isolated standards. Mesembrine was identified using a commercial standard, while the remainder of the alkaloids were tentatively identified as outlined in Section S4 and according to (Reddy et al., 2022).

## 2.2. Animal study

### 2.2.1. Mice

A total of 46 C57BL/6 mice (32 female and 14 male), aged 10–18 weeks at the onset of the investigation (Theron et al., 2025), were obtained from the North-West University (NWU) vivarium (SAVC reg. no.: FR15/13458; AAALAC accreditation file: 1717; ethics approval no.: NWU-00753-22-A5). Experimental mice were randomised from at least twelve different breeding pairs without cage, sex or weight bias. Mice were assigned to the following groups at random: control (5% ethanol), commercial extract, Touwsrivier, and De Rust. Prior to the onset of experimentation, mice were raised and maintained in same-litter, same-sex rearing cages (maximum 6 mice per cage) until the required age. All cages were climate-controlled (35 (l) x 20 (w) x 13 (h) cm; Tecniplast S.P.A., Varese, Italy) and kept at 23 °C on a 12-h light-dark cycle (06h00/18h00). Throughout the course of the investigation, food and water were provided *ad lib* and cages cleaned weekly. Every day, mice were monitored for welfare and health status according to standard vivarium protocol. None of the mice, irrespective of exposure group, showed signs of deteriorating health throughout the course of study.

### 2.2.2. Extract preparation and dose administration

The commercial *S. tortuosum* extract was purchased from GESLabs (Cape Town, South Africa) as a standardised nanoemulsion. Since crude plant extracts vary with respect to total alkaloid content, the extracts and commercial extract were standardised for the administered dose based on total alkaloid content (TAC). Thus, the same TAC was administered for each of the three extracts, although the formulas differed based on the profile of alkaloids they contained. The TAC of the respective extracts was analysed and reported as follows: (1) Commercial extract 0.7% TAC; (2) Touwsrivier 1.04 % TAC; De Rust 0.56 % TAC. Stock solutions of the Touwsrivier and De Rust extracts were prepared by solubilising 500 mg extract in 3 mL ethanol (EtOH) (Merck Life Science, Johannesburg, South Africa), followed by vortexing, brief

sonication (10 s), and filtration to remove any undissolved debris. These stock solutions were made fresh at the beginning of each week and stored at 4 °C for 1 week and then discarded. The TACs, irrespective of chemotype, were found to be stable in EtOH following 1 week storage at 4 °C (data presented in SI). Each day, a dilution from the stock solution was prepared to provide a dose of 10 mg/kg and 1.4 µg TAC per mouse in 5 % EtOH. Mice were administered 200 µL of the extract solution via oral gavage (Jones et al., 2016) at 08h00 each morning for 35 days.

### 2.2.3. Euthanasia and sampling

On exposure day 35 (no vehicle or extract administered), mice were euthanised by means of isoflurane inhalation, decapitated, and the whole brain removed on ice (Marx et al., 2024). The frontal cortices, hippocampi and striata were dissected, snap-frozen in liquid nitrogen, and stored at –80 °C until further analysis. These tissues were selected for their relevance to cognition, mood, and anxiety processing (Ionescu et al., 2013; Mann et al., 2012).

## 2.3. Neurotransmitter analysis

### 2.3.1. Sample preparation

Dissected brain samples (Section 2.2.3.) were thawed, weighed, and added to 250 µL of the preparation solution which consisted of formic acid (0.1 % v/v) in methanol and the internal standard (ethyl-4-hydroxy-2-quinolinecarboxylate; EHQC) at a concentration of 250 ng/mL. Samples were then homogenised by sonication (twice for 12 s, at an amplitude of 23 kHz; MSS150.CX4.5 Ultrasonic Disintegrator, MSE, Nuaillé, France). Sample mixtures were then left on ice for 20 min to complete protein precipitation and subsequently centrifuged at 20817 rcf for 25 min at 4 °C. Supernatants were then transferred to an amber sample vial for analysis.

### 2.3.2. LC-MS method

Dopamine, noradrenaline, serotonin, GABA, glutamate, EHQC, methanol, formic acid, and acetonitrile were obtained from Merck Life Science (Johannesburg, South Africa). Two µL of each sample was injected into an Ultivo Triple Quadrupole LC-MS/MS System, controlled by MassHunter software (Agilent Technologies, Santa Clara, CA 95051 US), consisting of a quaternary pump, column oven, autosampler and a triple quadrupole mass detector. A Kinetix C18 analytical HPLC column (Phenomenex, Torrance, CA, USA, 2.1 × 100 mm, particle Ø 2.6 µm, pore size 100 Å, surface area 200 m<sup>2</sup>/g) was used for chromatographic separation. The results were converted from ng/mL into ng/mg of the wet-weight brain tissue (Harvey et al., 2002; Viljoen et al., 2018).

### 2.3.3. Statistical data analysis

Results for the LC-Q-TOF-MS profiling analyses of alkaloids are expressed as an average and standard deviation of three independent determinations for the Touwsrivier and De Rust samples. Each alkaloid was quantified as mg/g, and results are expressed as a percentage of the total alkaloid content (TAC). All data sets from the animal study were first screened for normality and homogeneity of variance (Shapiro-Wilk and Levene's tests, respectively). Since some data sets violated the normality assumption, Kruskal-Wallis *H* tests were used to determine how the treatments influenced the measured parameters. Subsequent pairwise comparisons were performed using Dunn's procedure with Bonferroni adjustment. In all instances, *p* < 0.05 was considered statistically significant where *p* < 0.05 (\*), *p* < 0.01 (\*\*), *p* < 0.001 (\*\*\*)<sup>†</sup>, and *p* < 0.0001 (\*\*\*\*). All statistical analyses and graphs were generated using GraphPad Prism v10. Samples that were below the limit of detection, as well as outliers from each group, as determined by Grubbs' test, were removed from the analysis (indicated by the statistical descriptors and individual data points on graphs).

## 3. Results

### 3.1. Effects of *S. tortuosum* extracts on central neurotransmitter concentrations in C57BL/6 mice

Two novel chemotypes that contain a higher percentage of minor mesembrine alkaloids were chosen for the mouse bioassay. All other extracts that have previously been studied have contained mesembrine and the other major alkaloids at a greater concentration.

#### 3.1.1. Serotonin

Extract exposure had a significant main effect on the median striatal serotonin concentrations (*H*[3] = 19.40, *p* = 0.0002; Fig. 2B), with the commercial extract causing a significant serotonin decrease (0.24 ng/mg) compared to the Touwsrivier (1.33 ng/mg, *p* = 0.0003) and De Rust exposures (1.21 ng/mg, *p* = 0.0075). Notably, neither the Touwsrivier nor De Rust chemotypes affected the result compared to the control exposure. No group differences in serotonin concentrations were shown in the prefrontal cortex (*H*[3] = 4.306, *p* = 0.2303) or the hippocampus (*H*[3] = 3.354, *p* = 0.3402), two neuroanatomical regions often associated with serotonin dysregulation in patients with MDD (Frokjaer et al., 2009; Fujita et al., 2000) and GAD (Akimova et al., 2009).

#### 3.1.2. Dopamine

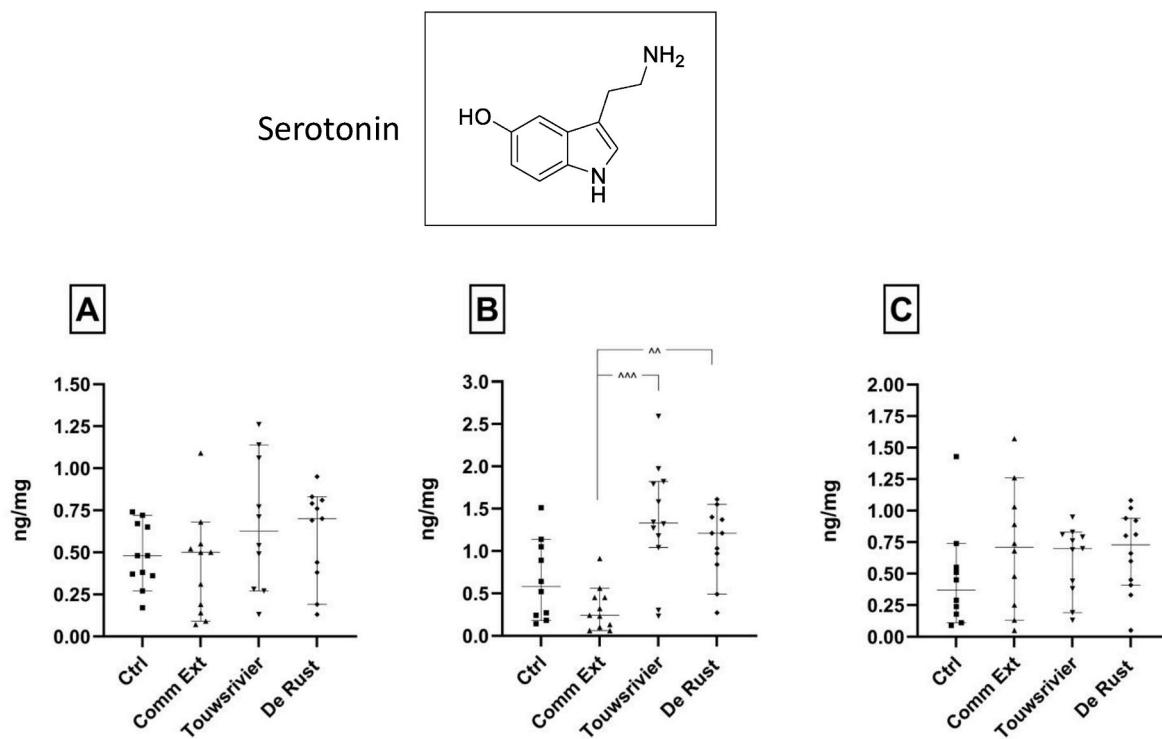
With respect to dopamine, exposure mainly affected the median hippocampal (*H*[3] = 21.60, *p* < 0.0001), but not prefrontal cortical (*H*[3] = 2.836, *p* = 0.4132) or striatal (*H*[3] = 0.9158, *p* = 0.8216) concentrations (Fig. 3). Strikingly, this result also highlights clear separation between the alkaloid composition of the Touwsrivier and De Rust chemotypes, since only the Touwsrivier (0.055 ng/mg), and not the De Rust (0.14 ng/mg) extract significantly decreased hippocampal dopamine concentrations compared to both the control (0.200 ng/mg, *p* < 0.0001) and commercial extract (0.175 ng/mg, *p* = 0.0061) exposures.

#### 3.1.3. Noradrenaline

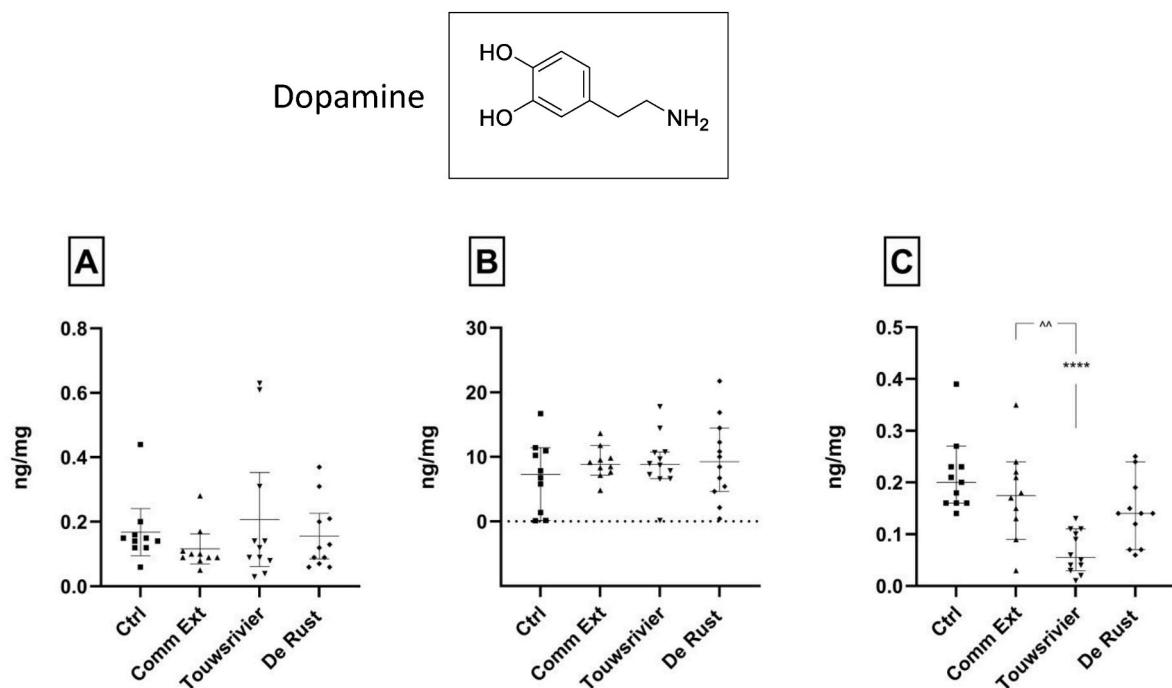
Compared to both control and commercial extract exposure, both the Touwsrivier and De Rust extracts significantly increased noradrenaline concentrations in most brain regions studied. In the prefrontal cortex (Fig. 4A), exposure had a significant main effect on the median noradrenaline concentrations (*H*[3] = 20.16, *p* = 0.0002). Both the Touwsrivier (17.98 ng/mg, *p* = 0.0009) and De Rust (18.42 ng/mg, *p* = 0.0023) chemotypes increased noradrenaline compared to the commercial extract (9.46 ng/mg), with Touwsrivier exposure trending towards the same compared to control exposure (11.5 ng/mg, *p* = 0.07). In the striatum (Fig. 4B), exposure also had a significant main effect on the median noradrenaline concentrations (*H*[3] = 27.95, *p* < 0.0001). Compared to control exposure (4.78 ng/mg), both the Touwsrivier (12.41 ng/mg, *p* = 0.0001) and De Rust (12.20 ng/mg, *p* = 0.0011) extracts robustly increased the measured noradrenaline concentrations. Both extracts also significantly increased noradrenaline levels compared to the commercial extract (5.01 ng/mg; Touwsrivier: *p* = 0.0016; De Rust: *p* = 0.0088). In the hippocampus (Fig. 4C), median noradrenaline concentrations also differed between the exposure groups (*H*[3] = 33.12, *p* < 0.0001). Touwsrivier (17.50 ng/mg) and De Rust (20.76 ng/mg) exposure resulted in significantly higher noradrenaline concentrations compared to both the control (11.52 ng/mg, Touwsrivier: *p* = 0.0013; De Rust: *p* < 0.0001) and the commercial extract (11.17 ng/mg, Touwsrivier: *p* = 0.0057; De Rust: *p* = 0.0001).

#### 3.1.4. Glutamate and GABA

In the prefrontal cortex (Fig. 5A), exposure to the extracts caused a significant main effect on glutamate concentrations (*H*[3] = 18.81, *p* = 0.0003). Here, all the extracts, including the commercial extract, caused a significant decrease in glutamate concentrations compared to the vehicle control (1592 ng/mg) (commercial extract: 1008 ng/mg, *p* = 0.0077; Touwsrivier: 1056 ng/mg, *p* = 0.0060; De Rust: 1008 ng/mg, *p*



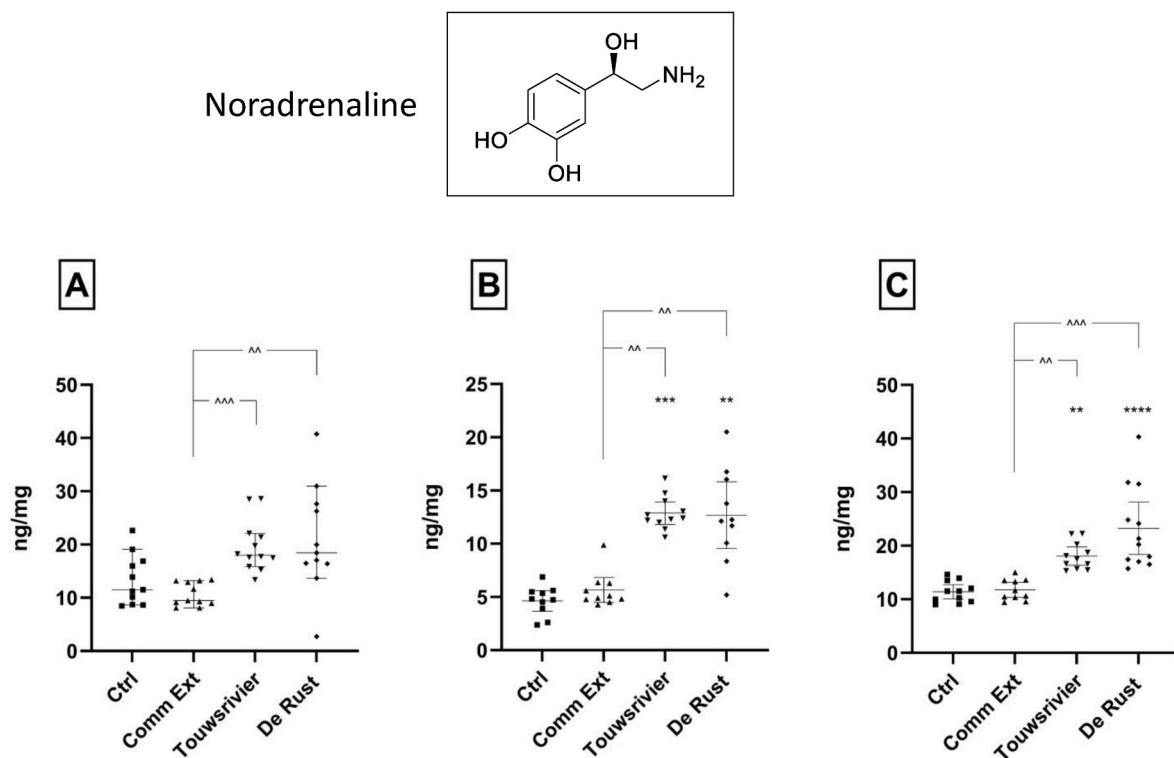
**Fig. 2.** Frontal-cortical (A), striatal (B), and hippocampal (C) serotonin concentrations of mice exposed to the vehicle control (Ctrl), the commercial extract, or the two ecotypes, Touwsrivier and De Rust. Data are represented as median with the 95 % confidence interval. Kruskal-Wallis analysis, followed by Dunn's post-hoc. \* indicates significant median differences compared to Ctrl. ^ indicates significant differences among groups.



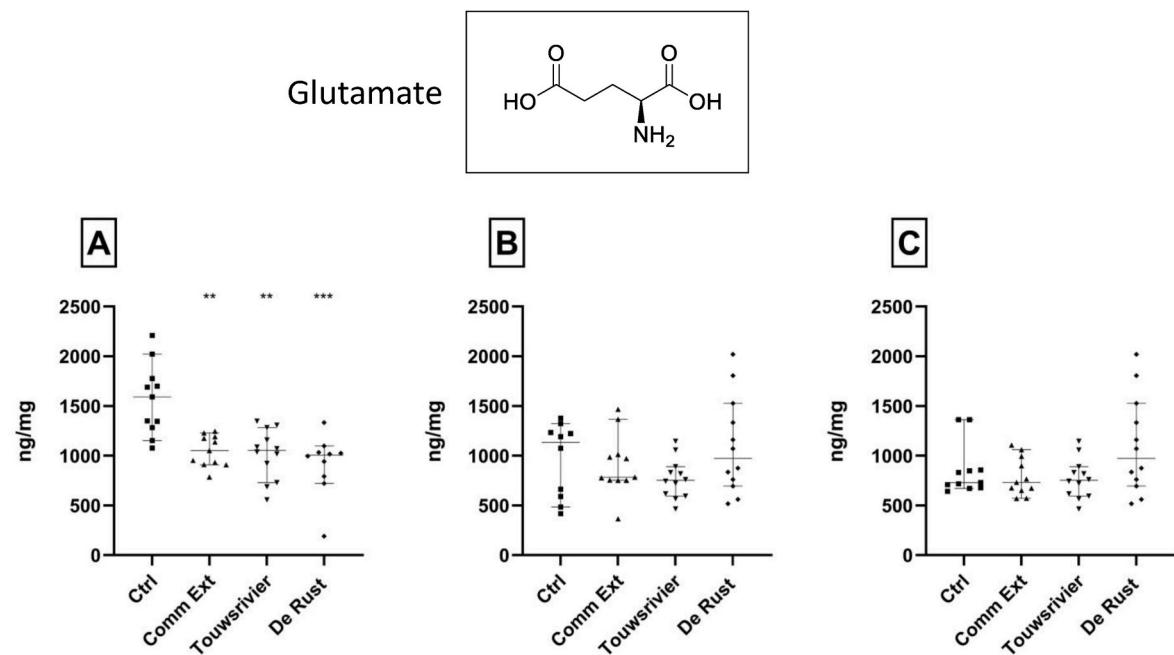
**Fig. 3.** Frontal-cortical (A), striatal (B), and hippocampal (C) dopamine concentrations of mice exposed to the vehicle control (Ctrl), the commercial extract, or the two ecotypes, Touwsrivier and De Rust. Data are represented as median with the 95 % confidence interval. Kruskal-Wallis analysis, followed by Dunn's post-hoc. \* indicates significant median differences compared to Ctrl. ^ indicates significant differences among groups.

= 0.0005). Since this effect was observed in all extracts, it may perhaps have resulted from the total alkaloid content which is the same overall. No group differences were observed in the striatum (Fig. 5B-H[3] = 3.495,  $p = 0.3215$ ) or the hippocampus (Fig. 5C-H[3] = 3.636,  $p = 0.3036$ ).

With respect to GABA, exposure to the extract influenced concentrations in all the brain regions analysed (Fig. 6A, prefrontal cortex:  $H[3] = 25.56$ ,  $p < 0.0001$ ; Fig. 6B, striatum:  $H[3] = 30.09$ ,  $p < 0.0001$ ; Fig. 6C, hippocampus:  $H[3] = 12.11$ ,  $p = 0.0070$ ). Also, the actions of the Touwsrivier and De Rust chemotypes once again showed divergence



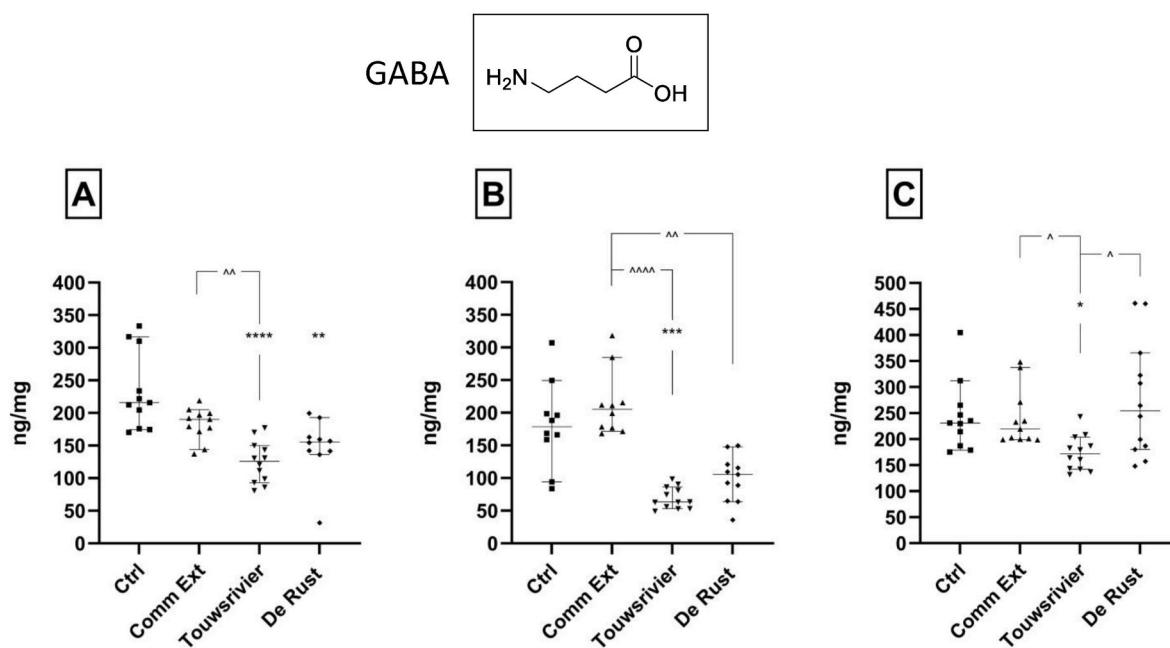
**Fig. 4.** Frontal-cortical (A), striatal (B), and hippocampal (C) noradrenaline concentrations of mice exposed to the vehicle control (Ctrl), the commercial extract, or the two ecotypes, Touwsrivier and De Rust. Data are represented as median with the 95 % confidence interval. Kruskal-Wallis analysis, followed by Dunn's post-hoc. \* indicates significant median differences compared to Ctrl. ^ indicates significant differences among groups.



**Fig. 5.** Frontal-cortical (A), striatal (B), and hippocampal (C) glutamate concentrations of mice exposed to the vehicle control (Ctrl), the commercial extract, or the two ecotypes, Touwsrivier and De Rust. Data are represented as median with the 95 % confidence interval. Kruskal-Wallis analysis, followed by Dunn's post-hoc. \* indicates significant median differences compared to Ctrl. ^ indicates significant differences among groups.

from that of the commercial extract, with both extracts decreasing GABA concentrations. In the prefrontal cortex (Fig. 6A), both the Touwsrivier ( $125.7 \text{ ng}/\text{mg}$ ,  $p < 0.0001$ ) and De Rust ( $155.6 \text{ ng}/\text{mg}$ ,  $p = 0.0078$ ) extracts decreased GABA concentrations compared to the control ( $215.7 \text{ ng}/\text{mg}$ ,  $p < 0.0001$ ), with the Touwsrivier extract also resulting in a

significant decrease compared to the commercial extract ( $190.1 \text{ ng}/\text{mg}$ ,  $p = 0.0076$ ). In the striatum (Fig. 6B) and the hippocampus (Fig. 6C), similar findings were observed. In the striatum, the Touwsrivier extract ( $63.39 \text{ ng}/\text{mg}$ ) decreased GABA concentrations compared to both control ( $178.4 \text{ ng}/\text{mg}$ ,  $p = 0.0007$ ), and commercial extracts ( $205.3 \text{ ng}/\text{mg}$ ,



**Fig. 6.** Frontal-cortical (A), striatal (B), and hippocampal (C) GABA concentrations of mice exposed to the vehicle control (Ctrl), the commercial extract, or the two ecotypes, Touwsrivier and De Rust. Data are represented as median with the 95 % confidence interval. Kruskal-Wallis analysis, followed by Dunn's post-hoc. \* indicates significant median differences compared to Ctrl. ^ indicates significant differences among groups.

$p < 0.0001$ ). GABA was also decreased in the De Rust exposed mice ( $105.6 \text{ ng}/\text{mg}$ ) compared to mice exposed to the commercial extract ( $p = 0.0045$ ) but not compared to control-exposed mice. Interestingly, hippocampal GABA was only decreased by the Touwsrivier chemotype ( $171.80 \text{ ng}/\text{mg}$ ) when compared to both control ( $230.90 \text{ ng}/\text{mg}$ ,  $p = 0.0386$ ) and commercial extract exposure ( $219.30 \text{ ng}/\text{mg}$ ,  $p = 0.0317$ ). Some diverging effect of the Touwsrivier and De Rust extracts on GABA concentrations is shown in that the hippocampal GABA concentrations of De Rust exposed mice ( $254.2 \text{ ng}/\text{mg}$ ,  $p = 0.0200$ ) were significantly higher, compared to that of Touwsrivier exposed mice.

### 3.2. Analysis of the alkaloids present in the extracts by LC-MS

The *S. tortuosum* extracts (Fig. 7) and commercial extract were analysed using a LC-Q-TOF-MS system and quantified based on peak area using mesembrenine as a reference standard. Due to limited availability, the commercially sourced extract was analysed only once, while the Touwsrivier and De Rust samples were each analysed in triplicate. The relatively low standard deviation (SD) of alkaloid concentrations in the De Rust and Touwsrivier samples (Fig. 8) supports the reliability of the commercial extract results, despite the single analysis. A total of 12 alkaloids, previously identified in *Scelidium* extracts (Reddy et al., 2022) were detected in the samples (Fig. 7). These alkaloids were mesembrenine (1), mesembrenone (2), mesembrenol (3), and mesembranol (4), while the detected minor alkaloids comprised 4'-O-demethylmesembrenine (5), 4'-O-demethylmesembrenone (6),  $\Delta^7$ -mesembrenone (7), epimesembranol (8), epimesembrenol (9), O-acetylmesembrenol (10), sceletium A4 (11), and dihydrojoubertiamine (12).

The commercial extract contained  $0.70 \text{ mg/g}$  total alkaloids. The Touwsrivier extract had a TAC of  $1.04 \text{ mg/g}$ , while the De Rust had a TAC of only  $0.56 \text{ mg/g}$ . Importantly, it should be reiterated that the doses administered to mice were adjusted so that each mouse received the same TAC in each dose. The specific alkaloids contained within the samples differed though, with the Touwsrivier extract being more similar to the commercial extract for the major alkaloids, i.e., being rich in mesembrenine ( $66.9$  vs  $69.4$  TAC %, respectively), but containing less mesembrenone ( $11.8$  vs.  $24.6$  TAC % respectively). In contrast, the De Rust extract was quite different, being enriched for mesembrenone over

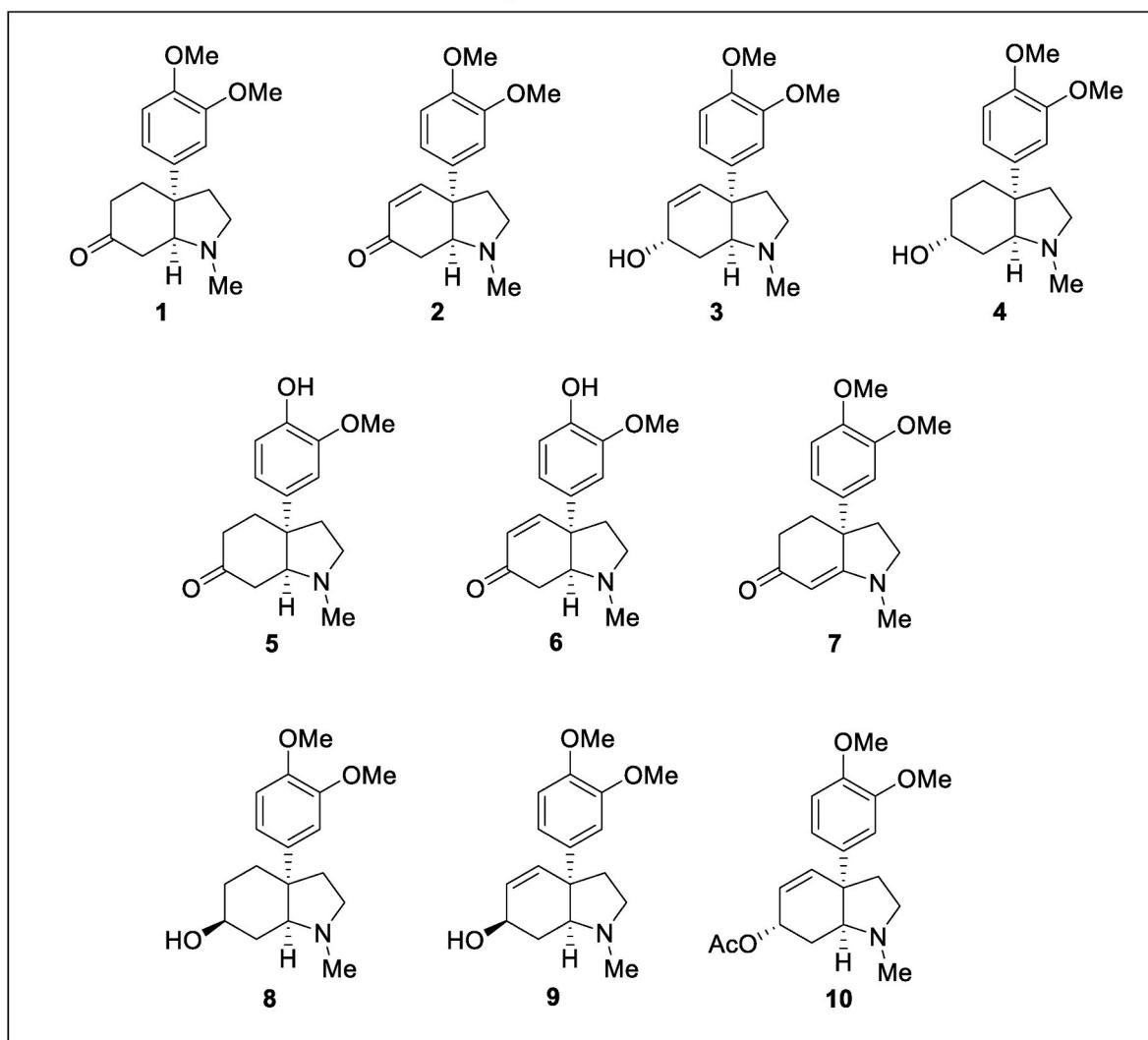
mesembrenine. Certain differences are evident though when comparing the two active extracts over the inactive sample as highlighted and boxed in Fig. 8. The two chemotypes which show activity, contain the mesembrenine alcohols, i.e., mesembrenol (3), mesembranol (4), eipmesembranol (8) and epimesembrenol (9) (mesembrenine-type alkaloids with a hydroxyl group at C-6), which are largely absent in the commercial extract (Fig. 8A and B), supporting a possible role of the mesembrenine alcohols in the observed activity. The sceletium A4 alkaloid (11a) and its isomer (11b) were also found to be elevated in the two chemotypes compared to the commercial extract also drawing attention to this class of alkaloid.

### 4. Discussion

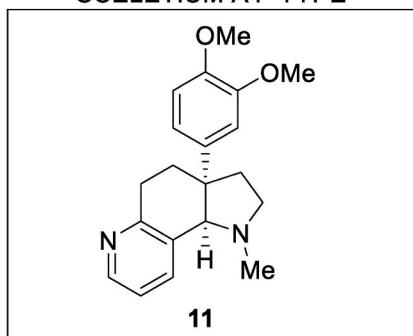
The main findings of this investigation are 1) compared to a commercial extract, the Touwsrivier and De Rust extracts distinctly modulate central neurotransmitter concentrations in a manner that could potentially be of benefit for stress- and anxiety-related conditions, and 2) that higher concentrations of the minor alkaloids, i.e., mesembrenine alcohols and sceletium A4, may be important for this action. The latter provides *in vivo* data to the study of Reddy et al. (2022) that indicated the potential effects of minor alkaloids in the CNS-modulating activity of *Scelidium* extracts.

Due to their sessile nature, plants exhibit high degrees of phenotypic plasticity in response to both biotic and abiotic stresses, governing the production of specialised metabolites (Li et al., 2024). Epigenetic regulation of specialised metabolism can arise naturally and may lead to heritable traits culminating in interpopulation-based chemotypic differences (Kooke and Keurentjes, 2015). Such epigenetic variation can thus lead to different metabolomic expressions of plants that belong to the same taxonomic lineage. Epigenetic changes that allow for plant fitness in their local geographic environments may ultimately lead to lasting population-based heritable changes, making each population show both qualitative and quantitative metabolomic differences based on site (Kaigongi et al., 2020). Therefore, the combination of geographic, environmental, and inherent genetic mechanisms exerts a combined influence on chemotypes causing temporally stable differences in chemical makeup (Katz et al., 2021). In this work, we

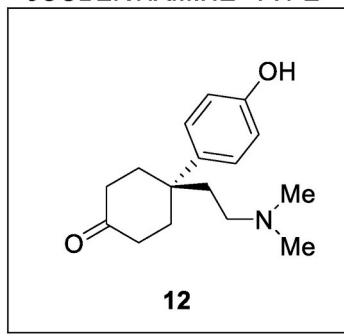
## MESEMBRINE-TYPE



## SCELETIUM A4 -TYPE



## JOUBERTIAMINE -TYPE



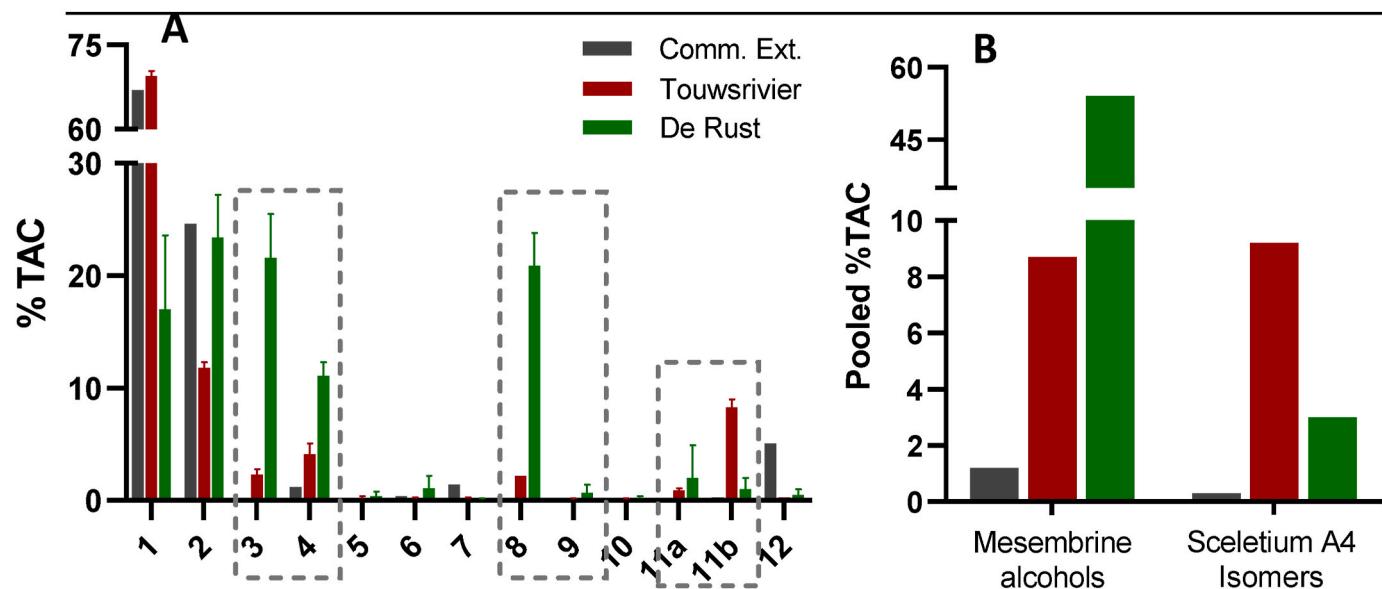
**Fig. 7.** Chemical structures of the alkaloids detected in the *S. tortuosum* extracts from three of the alkaloid class types. 1. mesembrine, 2. mesembrenone, 3. mesembrenol, 4. mesembranol, 5. 4'-O-demethylmesembrine, 6. 4'-O-demethylmesembrenone, 7.  $\Delta^7$ -mesembrenone, 8 epimesembranol, 9. epimesembrenol, 10. O-acetylmesembrenol, 11. sceletium alkaloid A4, 12. dihydrojoubertiamine.

investigated the neurobiological actions of two *S. tortuosum* chemotypes, selected for their unusual alkaloid profiles (Reddy et al., 2022). Notably, the chemotypes studied in this work have been maintained in cultivation and remain stable in their metabolic phenotypes.

The results from this investigation are striking in showing that both novel chemotypes, i.e., Touwsriver and De Rust, exerted markedly different effects on central neurotransmitter concentrations, compared

to a commercial extract. With respect to serotonin and dopamine, several important aspects for consideration should be highlighted. Firstly, given the role of striatal serotonergic action in the regulation of behavioural output (Balasubramani et al., 2015; Fischer and Ullsperger, 2017; Leisman and Melillo, 2013), i.e., promoting behavioural flexibility (Uddin, 2021), our findings regarding the effects of the Touwsrivier and De Rust extracts, compared to the commercial extract, point to a

No.	Alkaloid name	Comm. Ext.	Comm. Ext.	De Rust
			% TAC (Ave ± SD)	
1	mesembrine	66.9	69.4 ± 1.0	17.0 ± 6.6
2	mesembrenone	24.6	11.8 ± 0.5	23.4 ± 3.8
3	mesembrenol	0.0	2.3 ± 0.5	21.6 ± 3.9
4	mesembranol	1.2	4.1 ± 1.0	11.1 ± 1.2
5	O-demethylmesembrine	0.0	0.2 ± 0.2	0.4 ± 0.4
6	O-demethylmesembrenone	0.4	0.2 ± 0.1	1.1 ± 1.1
7	Δ <sup>7</sup> -mesembrenone	1.4	0.2 ± 0.1	0.1 ± 0.1
8	epimesembranol	0.0	2.2 ± 0.1	20.9 ± 1.6
9	epimesembrenol	0.0	0.1 ± 0.1	0.7 ± 0.2
10	O-acetylmesembrenol	0.1	0.1 ± 0.0	0.2 ± 0.0
11a	sceletium A4	0.0	0.9 ± 0.2	2.0 ± 2.9
11b	sceletium A4 isomer	0.3	8.3 ± 0.7	1.0 ± 0.4
12	dihydrojoubertiamine	5.1	0.3 ± 0.0	0.5 ± 0.1
TAC (mg/g)		0.70	1.04	0.56



**Fig. 8.** Summary of alkaloids detected in the *S. tortuosum* ecotypes and commercial extract by LC-MS. (A) The %TAC of each of the alkaloids detected. Boxed alkaloids showing increased levels of minor alkaloids in Touwsrivier/De Rust vs commercial extract. (B) Pooled TAC% of four mesembrine alcohols (red) and sceletium A4 and its isomer (blue).

potentially beneficial effect of both chemotypes at the level of executive processing that should be afforded attention in follow up work. That said, noting the parallel changes in hippocampal dopaminergic signalling elicited by the Touwsrivier extract, it is the potential impact of this result on stress and anxiety processing that may be more important. To explain, hippocampal dopamine signalling is a crucial facilitator of emotionally driven contextual learning, and thus the prediction of certain outcomes that may arise from specific, previously experienced scenarios (Kahnt and Tobler, 2016). Dopamine release in the hippocampus is closely regulated by the ventral tegmental area (VTA) and noradrenergic projections from the locus coeruleus (LC) (McNamara and Dupret, 2017). Overstimulation of hippocampal dopamine D1 receptors is associated with a dysregulated stress response (Liu et al., 2019; Valentini et al., 2011) and psychosis (Lodge and Grace, 2011), with decreased activation contributing to contextual memory impairment, especially

following aversive feedback (Broussard et al., 2016). Taking into consideration the mostly uniform dopamine concentrations of mice exposed to the control, commercial, and De Rust extracts, the effect of Touwsrivier is likely chemotype specific. In this respect, two possibilities may exist. Either the blunted hippocampal dopamine concentrations may impair contextual learning and memory (Broussard et al., 2016) (which may be of benefit in individuals exposed to severely stressful or traumatic events), or alternatively, may play a blunting role in the processing of anxiogenic stimuli (Liu et al., 2019). In fact, given that decreased striatal serotonin concentrations have been shown in rats showing high anxiety (Schwarting et al., 1998) the latter notion would agree with the increased striatal serotonin concentrations observed with Touwsriver and De Rust extract exposure. Both outcomes are worth investigating, considering the broad and drastic impact of the Touwsrivier (and De Rust) extracts on central noradrenaline concentrations

(see below), a key regulator of the mammalian stress response. Since both outcomes are equally relevant for neuropsychiatric illness, investigations with this focus must be a key priority for future *S. tortuosum* research.

In support of these observations, our data pertaining to the impact of the two novel chemotypes on noradrenaline concentrations deserve careful consideration. It should be reiterated that these chemotypes were studied here for the first time. The central noradrenergic system is a key regulator of the mammalian stress response and fear memory consolidation, with noradrenaline released from the locus coeruleus (LC) priming organisms to respond to potential threats (Pace and Myers, 2024). Noradrenaline also regulates mood, attention, wakefulness, and appetite (Brunello et al., 2003). The primary source of noradrenaline in the brain is the LC which projects to most cortical and subcortical regions of the forebrain, including the striatum (Zerbi et al., 2019) and hippocampus (Wagner-Altendorf et al., 2019). Apart from its direct noradrenergic influence over the afore processes, LC projections also regulate the release of other neurotransmitters, including dopamine, in these areas (McNamara and Dupret, 2017). While an in-depth review of the actions of noradrenaline falls beyond the scope of the present work, some aspects are worth considering. Noradrenergic regulation of fear memory consolidation, and thus its ability to influence the subsequent regulation of anxiety and stress in future similar contexts, is intricately related to its concentration according to an inverted U-shape manner (Chamberlain and Robbins, 2013; Holland et al., 2021; Rozendaal and Hermans, 2017). In this way, very low and high concentrations of noradrenaline, secreted at the time of a stressful event, will equally contribute to impaired memory retention (Baldi and Bucherelli, 2005). Against this background, impaired memory retention may either be detrimental (for example, where it results in sustained feelings of anxiety, even though it may no longer be warranted), or beneficial (in cases where memory over-consolidation may contribute to neuropsychiatric illness, e.g., post-traumatic stress disorder) (Izquierdo et al., 2016). Furthermore, associations between noradrenaline concentrations and changes in brain function are bidirectional. In other words, varying baseline concentrations of noradrenaline can determine the degree of stress and anxiety experienced, and also to what extent the same would respond to pharmacological treatment (Fitzgerald, 2013). Conversely, stressful and anxiogenic experiences contribute to increased secretion of noradrenaline (Blier and El Mansari, 2007), thus complicating an understanding of the temporal relationships between neuropsychological performance and noradrenaline secretion. Lastly, there is considerable inter-individual variance with respect to baseline noradrenaline concentrations in specific patient populations (i.e., what seems 'high' in some patients, may be relatively 'low' in others) and thus, diagnostic definitions of noradrenaline concentrations in neuropsychiatric illness remain elusive (Kalk et al., 2011).

It follows that the noradrenaline-related findings of the present work are informative for a few reasons. Firstly, that both the Touwsrivier and De Rust extracts robustly increased noradrenaline concentrations in all the brain areas measured, likely points to their direct action on the primary source of noradrenaline secretion, the LC, or alternatively, potent noradrenalin-releasing or reuptake ability. This broad noradrenergic effect elegantly supports the traditional use of *S. tortuosum* as a mood-enhancing (Brunello et al., 2003) and appetite suppressing (Miller, 2019) substance. It also paves the way for continued study into its potential usefulness to modulate anxiety and stress, wakefulness and attention. Secondly, considering the region-specific changes noted with respect to serotonin and dopamine concentrations, we argue that the changes in serotonin and dopamine potentially arose as a function of interactions between implicit neurocognitive processes (e.g., the baseline state of anxiety and alertness of mice) and elevations in noradrenaline concentrations. In fact, if the Touwsrivier and De Rust extracts had direct effects on serotonin synthesis or release, similar patterns of reduced or elevated neurotransmitter concentrations could have been expected throughout the brain. Such a notion would be informing,

especially considering that some standardised extracts of *S. tortuosum*, have shown direct SERT inhibitory action in prior studies (Harvey et al., 2011). If so, the present findings would support the view that the minor alkaloids contained in the Touwsrivier and De Rust extracts impact central neurotransmission distinctly compared to the major alkaloids that have thus far mostly been characterised. That said, since the present work was not focused on behavioural outcomes, future studies are needed to explore these hypotheses.

Against this background, our results pertaining to GABA and glutamate concentrations also provide some direction for thought. Glutamate and  $\gamma$ -aminobutyric acid (GABA) are the two major excitatory and inhibitory amino acid neurotransmitters of the mammalian brain, respectively (Watkins and Jane, 2006; Zhou and Danbolt, 2014). Both neurotransmitters are ubiquitously found throughout the brain, with consensus regarding their neurotransmitter functions only reached in 1960 (Andersen and Schousboe, 2023). Considering the widespread distribution of glutamate in the brain, the general lack of a significant impact on glutamate levels was expected to align with past research (Andersen and Schousboe, 2023). However, noting the significant decline in frontal-cortical glutamate concentrations noted as a function of exposure to all the *S. tortuosum* formulations, the present result is in support of its proposed anxiolytic action (Modi et al., 2014). On the other end of the excitatory balance, GABA receptor agonists, such as the benzodiazepines, are some of the most effective and widely used anxiolytics. GABA secretion is regulated by several extrinsic (i.e., stress or drug exposure (Maguire, 2018);) and intrinsic factors (e.g., neurotransmitters, including serotonin, dopamine and noradrenaline (Azizi, 2022; Lawrence, 2008)). Within this context, any potential impact of *S. tortuosum* on anxiety, could reasonably be associated with increased, rather than decreased GABA. While our data are therefore somewhat surprising, a few key points of discussion should be highlighted. Firstly, the acute anxiolytic actions of GABA agonists are predominantly related to its blunting of amygdalar activation (Kaufmann et al., 2003), a brain region not studied here. Secondly, the impact of GABAergic modulation on anxiety processing is multifactorial, with stress itself known to either increase (Kaufmann et al., 2003) or decrease (Dolfen et al., 2021) regional GABA concentrations. Nevertheless, while the functional relevance of this finding must be investigated at a behavioural level, our results are consistent with the known reciprocal relationship between GABA and noradrenaline, whereby GABAergic stimulation leads to decreased noradrenalin release (Breton-Provencher and Sur, 2019). In this way, GABA is able to blunt arousal and attention via its modulation of noradrenalin release from the LC, with lower GABAergic activation in specific contexts, being of benefit (Breton-Provencher and Sur, 2019).

The neurobiological actions of the Touwsrivier and De Rust extracts clearly distinguish these chemotypes from the commercial extract and elegantly imply unique chemical compositions. Interestingly, the commercial and Touwsrivier extracts had similar alkaloid profiles, being both high in mesembrenone and low in mesembrenone, while the De Rust extract was quite different, being higher in mesembrenone and low in mesembrene. Thus, these major alkaloid profiles likely do not underpin the differences in neurobiological output reported. Rather, as we also alluded to earlier, differences in some of the minor alkaloids are proposed to be important, such as the higher concentrations of mesembrene alcohols (OH group at C6), as well as sceletium A4 and its isomer in both of the active chemotypes. The sceletium A4 isomer may be even more important as its levels are elevated in the Touwsrivier chemotype (Fig. 8A and B), which tended to exert even greater neurochemical changes in mice, than the De Rust extract. In support of this, a previous study showed that only the mesembrene alcohols (in contrast to the mesembrenes, i.e., mesembrene and mesembrenone) attenuated AMPA-mediated transmission of electrical activity in the *ex vivo* hippocampus of a rat (Dimpfel et al., 2018). Dihydrojoubertiamine, of the joubertiamine class of alkaloids, does not appear to be important, with elevated levels found in the commercial extract only. Nevertheless, the commercial extract was purchased as a nanoemulsion, while our extracts

were freshly prepared and made up as ethanolic solutions which were diluted into water for administration. Thus, bioavailability may not be comparable between the different formulations. There is also the possibility of another, yet unidentified compound or alkaloid in the extracts being responsible for the activity, which should be investigated in future. Nonetheless, given the robust and distinct effects of the Touwsrivier and De Rust chemotypes on noradrenaline and GABA concentrations, as well as the apparent differential effects of the various alkaloids, especially the mesembrine alcohols and sceletium A4 isomers, it will be important to investigate the psychobiological activity and properties of these alkaloids going forward. Considering the clinical relevance of manipulating the secretion of both noradrenaline and GABA in mood- and anxiety disorders, it would be valuable to explore the relevance of these results in studies of anxiety, attention and wakefulness.

## 5. Conclusions

The data presented here suggest that *S. tortuosum* chemotypes with elevated levels of minor alkaloids can have a unique impact on brain neurotransmitter concentrations, that is directly related to elevated concentrations of these minor alkaloids being produced in the plants. It appears that the contribution to the CNS-modulating effects of some minor alkaloids, especially the mesembrine alcohols and the sceletium A4 isomers, may be particularly important and warrant further investigation. Further, based on the concentrations of neurochemicals analysed in mouse brain tissue after chronic exposure, the Touwsrivier and De Rust extracts appear to target the noradrenergic and GABAergic systems specifically, with some lesser, downstream serotonergic and dopaminergic impact that may also be at play in distinct brain areas. Future work would have to explore the behavioural impact of the present findings in models of anxiety- and depressive-like behaviour, especially given the drastic impact of the two novel chemotypes on especially limbic noradrenergic signalling. In fact, our findings may have a clinically relevant impact on the overall health outcomes in patients, especially considering that these products are cultivated, and may be used for personal benefit, in the absence of regulatory approval. Any insight into the potentially diverse psychobiological actions of different *S. tortuosum* chemotypes, may thus contribute to better use and therapeutic outcomes. Collectively, our results point to a likely impact of the Touwsrivier and De Rust extracts on stress and anxiety processing and support further investigations into the role that the individual alkaloids, especially some of the minor alkaloids, play in the CNS-modulating effects of *S. tortuosum*.

## CRediT authorship contribution statement

**Catherine H. Kaschula:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Anna-Mart Engelbrecht:** Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Nokwanda P. Makunga:** Writing – review & editing, Methodology, Conceptualization. **Magriet Muller:** Writing – original draft, Validation, Formal analysis, Data curation. **André de Villiers:** Writing – review & editing, Data curation. **Kamano Mochoele Dube:** Writing – original draft. **Sarel Brand:** Formal analysis, Data curation. **Jo-Anne Stroebel:** Methodology, Investigation, Data curation. **Willem AL. van Otterlo:** Writing – review & editing. **De Wet Wolmarans:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization.

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## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: CHK, AME, NPM are directors of Phyenti, a Stellenbosch University spin-out company. CHK, AME, NPM, WvO, AdV are shareholders of Phyenti. KMD is current employee of Phyenti. MM was a previous employee of Phyenti. The Sceletium research is licensed from Stellenbosch University to Phyenti for commercialisation. DW, JS and SB have no conflict of interest.

## Appendix A Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2025.119974>.

## Abbreviations

AChE: acetylcholinesterase; AMPA:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS: central nervous system; EHQC: ethyl-4-hydroxy-2-quinolinecarboxylate; GABA: gamma-aminobutyric acid; GAD: generalised anxiety disorder; LC: locus coeruleus; LC-MS: liquid chromatography-mass spectrometry; MAO-A: monoamine oxidase A; MDD: Major depressive disorder; PDE4: phosphodiesterase 4 SERT: serotonin transporter; SSRI: selective serotonin reuptake inhibitors; TAC: total alkaloid content; TCA: noradrenergic and serotonergic tricyclic antidepressants; VMAT-2: vesicular monoamine transporter-2; VTA: ventral tegmental area (VTA).

## Data availability

Data will be made available on request.

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