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Sceletium tortuosum: A review on its phytochemistry, pharmacokinetics, biological and clinical activities

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ABSTRACT

Ethnopharmacological relevance: *Sceletium tortuosum* (L.) N.E.Br, the most sought after and widely researched species in the genus *Sceletium* is a succulent forb endemic to South Africa. Traditionally, this medicinal plant is mainly masticated or smoked and used for the relief of toothache, abdominal pain, and as a mood-elevator, analgesic, hypnotic, anxiolytic, thirst and hunger suppressant, and for its intoxicating/euphoric effects. *Sceletium tortuosum* is currently of widespread scientific interest due to its clinical potential in treating anxiety and depression, relieving stress in healthy individuals, and enhancing cognitive functions. These pharmacological actions are attributed to its phytochemical constituents referred to as mesembrine-type alkaloids.

Aim of the review: The aim of this review was to comprehensively summarize and critically evaluate recent research advances on the phytochemistry, pharmacokinetics, biological and clinical activities of the medicinal plant *S. tortuosum*. Additionally, current ongoing research and future perspectives are also discussed.

Methods: All relevant scientific articles, books, MSc and Ph.D. dissertations on botany, behavioral pharmacology, traditional uses, and phytochemistry of *S. tortuosum* were retrieved from different databases (including Science Direct, PubMed, Google Scholar, Scopus and Web of Science). For pharmacokinetics and pharmacological effects of *S. tortuosum*, the focus fell on relevant publications published between 2009 and 2021.

Results: Twenty-five alkaloids belonging to four structural classes viz: mesembrine, Sceletium A4, joubertiamine, and tortuosamine, have been identified from *S. tortuosum*, of which the mesembrine class is predominant. The crude extracts and commercially available standardized extracts of *S. tortuosum* have displayed a wide spectrum of biological activities (e.g. antimalarial, anti-oxidant, immunomodulatory, anti-HIV, neuroprotection, enhancement of cognitive function) in *in vitro* or *in vivo* studies. This plant has not yet been studied in a clinical population, but has potential for enhancing cognitive function, and managing anxiety and depression.

Conclusion: As an important South African medicinal plant, *S. tortuosum* has garnered many research advances on its phytochemistry and biological activities over the last decade. These scientific studies have shown that *S. tortuosum* has various bioactivities. The findings have further established the link between the phytochemistry and pharmacological application, and support the traditional use of *S. tortuosum* in the indigenous medicine of South Africa.

1. Introduction

A call to capitalize on African medicinal plants was recently made by the former president of Mauritius, Ameenah Gurib-Fakim, in the journal Nature. *Sceletium tortuosum* was singled out as an example of a medicinal plant native to the African continent that has been successfully commercialized (Gurib-Fakim, 2017). Given the above, and growing

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Abbreviations

5-HT	5-Hydroxytryptamine
17Bhsd	17β-Hydroxysteroid Dehydrogenase
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
BBB	Blood Brain Barrier
cAMP	Cyclic Adenosine Monophosphate
cAMP-CREB	Cyclic Adenosine Monophosphate-Response Element Binding Protein
CEMS	Capillary Electrophoresis Mass Spectrometry
CNS	Central Nervous System
DAT	Dopamine Transporter
DMSO	Dimethyl Sulfoxide
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EEG	Electroencephalogram
ESC	Escitalopram
FFT	Fast Fourier Transformation
fMRI	Functional Magnetic Resonance Imaging
FRL	Flinders Resistant Line
FSL	Flinders Sensitive Line
GABA	Gamma-Aminobutyric Acid
GC-MS	Gas Chromatography–Mass Spectrometry
HIV	Human Immunodeficiency Virus
HPA	Hypothalamic-Pituitary-Adrenal
HPTLC	High-Performance Thin-Layer Chromatographic
HRTOF	High-Resolution Time-Of-Flight
IP	Intraperitoneal
LCMS	Liquid Chromatography–Mass Spectrometry
LPS	Lipopolysaccharide
MA	Methamphetamine
MAOIs	Monoamine Oxidase Inhibitors
MDD	Major Depression Disorder
(MPP^+)	1-methyl-4-phenylpyridinium
MS	Mass Spectrometry
MTC	Maximum Tolerated Concentration
MTT	Methyl Blue Tetrazolium
NET	Noradrenaline Transporter
NMR	Nuclear Magnetic Resonance
PDA	Photo-Diode Array
PDE4	Phosphodiesterase-4
PR	Protease
RT	Reverse Transcriptase
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus
SERT	Serotonin Transporter
ST	Sceletium Tortuosum
SSRIs	Selective Serotonin Reuptake Inhibitors
SRI	Serotonin Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TRD	Treatment Resistant Depression
UHPLC	Ultra-High Performance Liquid Chromatography
UHPLC-MS	Ultra-High Performance Liquid Chromatography-Mass Spectrometry
UPC2-MS/MS	Ultra-Performance Convergence Chromatography-Tandem Mass Spectrometry
UPLC-MS-PDA	Ultra-Performance Liquid Chromatography-Mass Spectrometry- Photodiode Assay
UPLC-qTOF-MS^E	Ultra-Performance Liquid Chromatography (UPLC) coupled to Quadrupole-Time-of-Flight Mass Spectrometry (qTOF) with Collision Energy (MSE)
XTT	(2,3-Bis[2-Methoxy-4-Nitro-5-Sulfophenyl]−2H-Tetra-Zolium-5-Carboxanilide)

research interests on the biological activities of *S. tortuosum*, a need for a review on the recent research advances of *S. tortuosum* was identified.

Sceletium tortuosum (L.) N.E.Br. (Aizoaceae) commonly called "kanna" or "kougoed", is a succulent medicinal herb indigenous to South Africa (Carpenter et al., 2016). Aerial parts of the plant are commonly masticated or chewed, taken as tea or tincture, and occasionally smoked (Gericke and Viljoen, 2008). The plant is chewed for the relief of abdominal pain, toothache, and hunger, and fresh juice from the plant is given to induce sleep in young children (Gericke and Viljoen, 2008). It is currently used commercially to treat different central nervous system (CNS) related disorders, including stress, depression, and anxiety (Yin et al., 2019). Therefore, this plant species has been of specific scientific interest as a result of its potential in treating neurodegenerative diseases and neurological disorders (Van Wyk, and Gericke, 2000).

Mesembrine-type alkaloids, which include mesembrenone, mesembrine, mesembranol, and mesembrenol, are recognized as the major chemotaxonomic markers of the plant, and they account for its psychoactive properties (Shikanga et al., 2011; Yin et al., 2019). The psychoactive effects of mesembrine-type alkaloids and their capabilities in treating different CNS disorders have been attributed to their ability to act as phosphodiesterase-4 (PDE4) and serotonin reuptake inhibitors (SRIs), which are responsible for regulating intracellular messengers and synaptic and neuronal serotonin levels, respectively (Hoffman et al., 2020).

Owing to its pharmacological activities, S. *tortuosum* was first cultivated commercially in 1996 by Grassroots Natural Products under contract to the phytomedicine program of a South African pharmaceutical company, Pharmacare Ltd. In recent years, the commercial cultivation of *S. tortuosum* has expanded in different provinces of South Africa and Namibia. Global export and trade of *S. tortuosum* raw materials, products, and standardized extract (Zembrin®) are also well established. (Patnala and Kanfer, 2009a; Shikanga et al., 2012a). The chemical composition of the patented standardized extract, Zembrin®, should contain mesembrenone plus mesembrenol greater or equal to 60% and 20% or less of mesembrine.

Over two decades ago, Smith et al. (1996), published the first ethnopharmacological review on the genus *Sceletium* and more than a decade later, in 2008, Gericke and Viljoen (2008), published the second review focusing on the chemistry, pharmacology, clinical and veterinary applications of *Sceletium*, with much emphasis on *S. tortuosum.* Gericke (2018) provided a review of the historical uses, ethno-pharmacology and pre-clinical studies of Zembrin® and quite recently, a review was published on the biological and pharmaceutical properties of *S. tortuosum* (Manganyi et al., 2021). However, there have since been other important research studies conducted and published on *S. tortuosum*. Through this review, we aim to provide complete and comprehensive update on research advances on the ethnomedicinal use, phytochemistry, pharmacokinetics, biological and clinical activities of *S. tortuosum*. Current research projects on *S. tortuosum* and future perspectives of this important indigenous South African succulent medicinal herb are also discussed.

2. Methodology

A review of literature on all scientific articles published in English was conducted on different literature databases, including PubMed, Google Scholar, Web of Science, SciHub, and Scopus from 2009 to 2021. General botanical aspects and phytochemistry also included historical records published before 2009 using the above-mentioned databases. Information on the species was also collected from Ph.D. theses and MSc dissertations. The key search terms included; *Sceletium tortuosum*, mesembrine, kanna, kougoed, traditional uses, phytochemistry,

biological activities, pharmacological research, toxicity, and clinical study. The plant name and synonyms were confirmed from "World Flora Online", the updated version of "The Plant List". All chemical structures were drawn with ChemDraw.

3. Sceletium tortuosum

3.1. Taxonomy, description, and distribution

Sceletium tortuosum (L.) N.E.Br. (Aizoaceae) belongs to the genus *Sceletium*. According to the World Flora Online (WFO), there are currently 24 species, including intraspecific names reported as belonging to the genus, but only 20 correspond to accepted names (WFO, 2021). *Sceletium tortuosum* is the most sought after and commonly used species in the genus *Sceletium* (Shikanga et al., 2013). The taxonomic classification of *S. tortuosum* is given in Table 1. As an indication of complexity at the species level, homotypic and heterotypic synonyms (Table 2) have been used for *S. tortuosum* (CJB, 2012).

Sceletium tortuosum is a succulent plant (Fig. 1A). The generic name *Sceletium* is derived from the Latin word 'sceletus', referring to the leaf veins that persist as prominent skeleton-like structures in dry leaves (Fig. 1C) (Gericke and Viljoen, 2008; Schell, 2014). The specific name *tortuosum* implies 'twisted' or 'tortuous'. *Sceletium tortuosum* is a climbing or creeping plant with slender branches becoming thick and only slightly woody with age (Patnala and Kanfer, 2017).

The flowers are of medium size (20–30 mm diameter), ranging from white, yellow to pale pink, while the petals are white to pale yellow or pale pink (Fig. 1B). The calyx has four or five sepals, and fruits are 10–15 mm in diameter and open when wet. The fruit capsule contains several kidney-shaped seeds that are brown to black in colour (Gericke and Viljoen, 2008). The genus is distributed in the south-western parts of South Africa, from Namaqualand to Montagu through to Aberdeen, and commonly occurs in quartz patches (Fig. 2). It is usually found growing under shrubs in partial shade and has an affinity for arid environments (Chesselet, 2005). Although *S. tortuosum* shares numerous morphological traits with other species in the genus, it is usually distinguished from other species by comparing the flower, seed, fruit, and vegetative characteristics (Shikanga et al., 2013; Appleton, 2021). Only two species in the genus (*S. tortuosum* and *S. crassicaule*) produce the four important mesembrine-type alkaloids responsible for their psychoactive properties (Shikanga et al., 2013; Appleton, 2021).

3.2. Traditional use

Sceletium tortuosum is commonly known as 'kougoed' or 'kanna' and has been used by South African pastoralists and hunter-gatherers as a mood-altering substance from historic times (Gericke and Viljoen, 2008; Shikanga et al., 2011; Gericke, 2018; Appleton, 2021). The first known written account of the plant's use, referred to as Kanna, was in 1662 by Jan van Riebeeck. According to Watt and Breyer-Brandwijk (1962), *S. tortuosum* is used as a narcotic, and farmers in the Cape used it as a decoction or a tincture and as a sedative. The narcotic effect is claimed to be much more pronounced after fermentation (Smith et al., 1996; Chen and Viljoen, 2019a). The leaf is also chewed to relieve toothache and abdominal pain (Yin et al., 2019) and as a hunger and thirst suppressant during hunting trips (Gericke, 2020). The following was an early statement describing the clinical effects observed after *S. tortuosum* plant material was chewed: "Zwicky chewed 5 g of 'kougoed', the result being a bitter, astringent taste and an inclination to vomit, tingling of the tongue followed by weak and fairly persistent analgesia of the mouth, the pulse remaining normal." (Watt and Breyer-Brandwijk, 1962). The traditionally prepared dried *Sceletium* was often chewed and the liquid from it swallowed, but it has also been prepared into tablets, gel caps, teas, and tinctures. It is also used as a snuff and smoked (Meyer et al., 2015). In pregnant women, *S. tortuosum* is usually chewed to treat constipation, indigestion and nausea. An infusion of *S. tortuosum* is taken to contract the uterus, exorcize any residual after birth, and to relieve abdominal pain in women who have just given birth (Gericke, 2018). Additionally, oil extracted from the fried mix of *S. tortuosum* and the end portion of a sheep's tail is given to relieve colic in babies (Gericke and Viljoen, 2008; Gericke, 2018).

Table 1
Taxonomic classification of *S. tortuosum* (GBIF, 2019).

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Aizoaceae
Genus	Sceletium (Mesembryanthemum)
Species	*S. tortuosum* (L.) N.E. Br.

Table 2
Homotypic and heterotypic synonyms of *S. tortuosum* (CJB, 2012).

S. tortuosum (L.) N.E. Br.	Synonyms
	Homotypic
	Phyllobolus tortuosus (L.) Bittrich
	Mesembryanthemum tortuosum L.
	Pentacoilanthus tortuosus (L.) Rappa & Camarrone
	Heterotypic
	Sceletium boreale L. Bolus
	Sceletium compactum L. Bolus
	Tetracoilanthus concavus (Haw.) Rappa & Camarrone
	Sceletium concavum (Haw.) Schwantes
	Mesembryanthemum concavum Haw.
	Sceletium framesii L. Bolus
	Sceletium gracile L. Bolus
	Sceletium joubertii L. Bolus
	Sceletium namaquense L. Bolus var. *namaquense*
	Sceletium namaquense L. Bolus
	Sceletium namaquense var. *subglobosum* L. Bolus
	Sceletium ovatum L. Bolus
	Sceletium tugwelliae L. Bolus
	Mesembryanthemum aridum Moench

3.3. Phytochemistry

Zwicky (1914), performed general alkaloidal tests and reported that *S. tortuosum* contains alkaloids. According to Gericke and Viljoen (2008), Zwicky was the first to isolate and identify mesembrine and mesembrenine in *S. tortuosum*. However, the chemical structures were later corrected by Popelak and Lettenbauer (1967). While Snyckers et al. (1971) reported the structures of a partial racemic *Sceletium* alkaloid and tortuosamine, Jeffs et al. (1982) confirmed the structure of mesembrine and listed two additional unidentified ketonic alkaloids, which remained unidentified. Only in 1998 did Smith et al. (1998) identify mesembrenol, mesembrine and mesembrenone in fermented *S. tortuosum*. Patnala and Kanfer (2009a) investigated the transformation of alkaloids during fermentation. They reproduced the fermentation process of *S. tortuosum* and included the fermentation of pure mesembrine hydrochloride under controlled conditions, including exposure to sunlight and fermentation at ambient and elevated temperatures. They found that mesembrine transformed into mesembrenone, with the mesembrine concentration decreasing from 1.33% to 0.05%, and mesembrenone increasing from below the limit of detection to 0.11%. The pure mesembrine hydrochloride underwent the same transformation in aqueous solutions, but not in methanolic solutions or when kept in the dark. This transformation is not stoichiometric, but unfortunately, other chemical transformations or degradation products

Fig. 1. *Sceletium tortuosum* succulent leaf (A), flower (B) and skeletonized old leaf (C). Image: (Appleton, 2021); (N. Gericke and Viljoen, 2008).

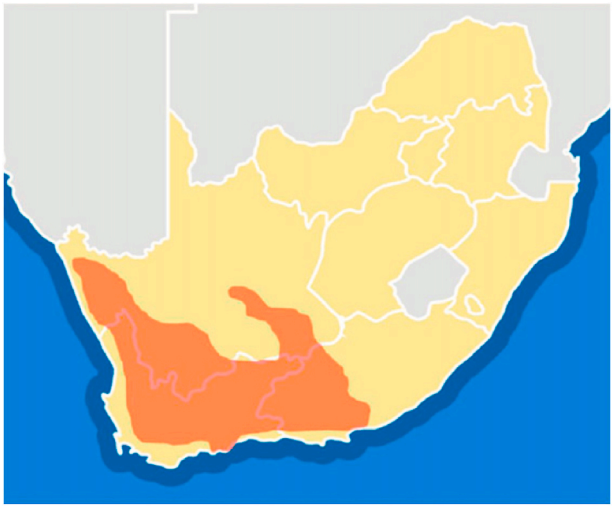

Fig. 2. Geographical distribution of *S. tortuosum* (orange zone) in South Africa (yellow). **Image:** https://sceletium.com/sceletium-botany/. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

that may have occurred during fermentation were not investigated.

More than 25 alkaloids belonging to four structural classes, viz. mesembrine, Sceletium A4, joubertiamine, and tortuosamine, have been identified from *Sceletium* species, of which mesembrine-types are predominant (Krstenansky, 2017; Patnala and Kanfer, 2017). In 2018, the chemical structure of a new alkaloid was reported. This compound, channaine, was tentatively identified in 1978, but the full structure elucidation was only recently published (Veale et al., 2018). Yin et al. (2019) identified two new alkaloids, sceletorines A and B, which are mesembrine-type mono-alkaloids. The structures and absolute configurations of the two compounds were determined by extensive spectroscopic data and electronic circular dichroism. The results of the study revealed that sceletorine B might be a precursor of channaine, which had been previously isolated and characterized by Veale et al. (2018). The chemical structures of the main alkaloids in *S. tortuosum* are shown in Fig. 3 (Krstenansky, 2017; Wen et al., 2020).

3.4. Isolation and chemical analysis of alkaloids

Shikanga et al. (2011) developed a countercurrent chromatographic method to isolate gram quantities of the four main alkaloids from *S. tortuosum* extracts. Patnala and Kanfer (2008) developed a capillary zone electrophoresis method to quantify mesembrine in *Sceletium* tablets. Shikanga et al. (2012b) developed a high-performance thin-layer chromatographic (HPTLC) method to quantify the main alkaloids in *S. tortuosum* raw materials and commercial products. The HPTLC method was validated, and the results obtained from the sample analysis correlated well with a gas chromatography-mass spectrometry (GC-MS) method. In the same year, the same research group published a validated method using Ultra-High Performance Liquid Chromatography (UHPLC) with a reversed-phase column and photodiode array (PDA) detection to quantify the four alkaloids in *S. tortuosum* (Shikanga et al., 2012c). Roscher et al. (2012) developed a nonaqueous chemical electrophoresis (NACE) mass spectrometry (MS) method for the separation and quantification of alkaloids in a *S. tortuosum* extract. Manda et al. (2015) developed and validated a sensitive UHPLC-MS method for the accurate quantification of mesembrenone and mesembrine in mouse plasma. Freund et al. (2018) developed a leaf spray MS technique in order to facilitate the rapid detection of plant metabolites without using chromatography. Fresh leaf material of *S. tortuosum* was analyzed, and the major mesembrine alkaloids were detected with the MS method. Lesiak et al. (2016) characterized *S. tortuosum* directly using real-time ionization coupled with high-resolution time-of-flight (HRTOF) mass spectrometry (MS). This method identified the material as *S. tortuosum,* but at the same time, it identified a scheduled substance (in this case, the stimulant ephedrine), which has been banned in herbal products and supplements.

Chen and Viljoen (2019a) compared fermented and unfermented *S. tortuosum* samples using a validated UHPLC-MS method. Their results indicated that mesembrenol and mesembranol remained largely unchanged, whereas the concentration of mesembrine increased from between not detected and 1.6 μg/mL to 7.4–20.8 μg/mL, whereas mesembrenone decreased from 8.0 to 33.0 to 1.3–32.7 μg/mL. This result is in direct contrast with Patnala and Kanfer (2009a), Roscher et al. (2012), and Smith et al. (1998), who reported the exact opposite transformation occurring during fermentation. As mentioned before, Chen and Viljoen (2019a) concluded that the mesembrine levels declined or remained constant after fermentation. They were however the only researchers who complied with the following three criteria: (1) They compared fermented and unfermented material from the same plant and in triplicate using genetically consistent cultivated material. (2) They also used a validated method, which was not the case in one of the other studies. (3) Finally, the fermentation was done under controlled conditions, which was not the case for most of the other studies.

A typical chromatographic fingerprint of an ethanolic extract of the standardized extract of *S. tortuosum*, using UPLC-MS coupled to a (UPLC-MS-PDA) detector (Zhao et al., 2018), is shown in Fig. 3. This chemotype is commercially available as Zembrin®, and responsible for much of the clinical and *in vivo* animal studies describing the psychoactive actions of *S. tortuosum* (see Section 4.2.). The UPLC-MS-PDA chromatogram

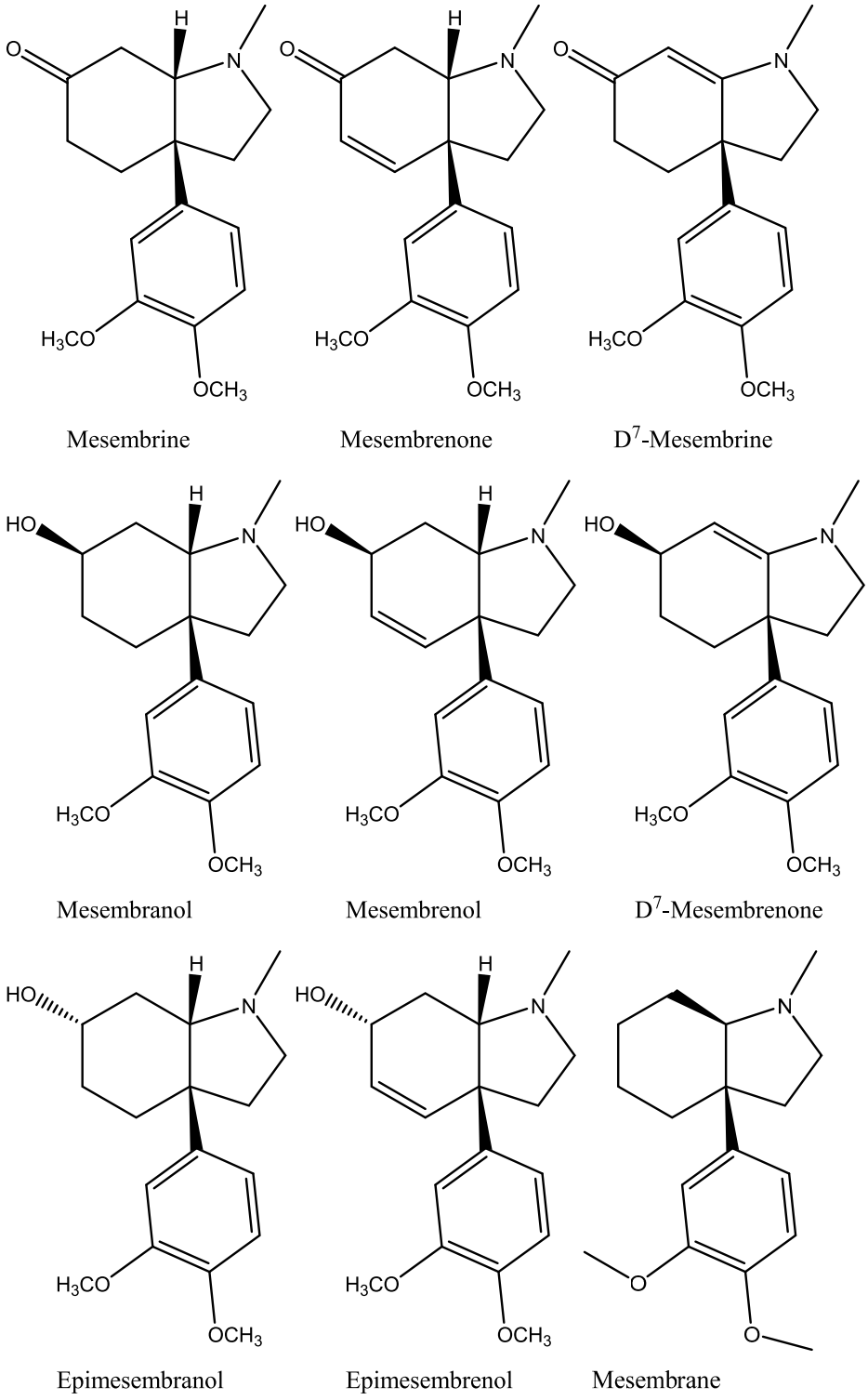

Fig. 3. Structures of the main alkaloid in *S. tortuosum* adapted from Krstenansky (2017); Wen et al. (2020).

displays four main peaks at retention times (Rt) 3.35, 3.62, 4.14, and 4.97 min, which was confirmed with the comparison with alkaloid standards to be mesembrenol, mesembranol, mesembrenone, and mesembrine, respectively. The total content of these four alkaloids was found to be 3.84 μg/mg of total plant extract. The ratio of the four main alkaloids in descending order was: mesembrenone (47.9%) mesembrenol (32%), mesembrine (13.3%), and mesembranol (6.8%).

3.5. Chemotaxonomy and metabonomic analysis

Roscher et al. (2012) conducted a forensic analysis of mesembrine alkaloids using capillary electrophoresis mass spectrometry (CEMS), and chemotypically compared several *S. tortuosum* extracts obtained from different vendors, self-fermented samples, and products ready for consumption. Patnala and Kanfer (2013) investigated the chemotaxonomic variation between various *Sceletium* species regarding their

mesembrine-type alkaloids and found pronounced variation. Their study indicated that not all *Sceletium* species contain the mesembrine-type alkaloids they therefore proposed a quality control measure to ensure that the correct *Sceletium* species are included in commercial products.

Shikanga et al. (2013) chemically distinguished between *S. tortuosum* and *S. crassicaule* using UHPLC and hyperspectral imaging combined with chemometric methods. The UHPLC analysis revealed that both species contain the psychoactive alkaloids used for quality control markers. Results from their hyperspectral image analysis revealed two distinctive clusters and they were able to correctly predict the species with an accuracy of >94%. Zhao et al. (2018) used nuclear magnetic resonance (NMR) and UHPLC-MS metabolomic techniques to explore the chemotypic variation of *S. tortuosum* collected from two provinces in South Africa. Two-dimensional NMR revealed the presence of pinitol and two new alkaloids, isobutylamine, and 2-methyl-butamine, which were used as marker compounds to differentiate the chemotypic geographic locations. Sandasi et al. (2018) reported on a non-destructive technique for the quality assessment of herbal tea blends from *S. tortuosum* and *Cyclopia genistoides* using hyperspectral imaging combined with chemometric analysis.

3.6. Permeation studies, pharmacokinetic and metabolite analysis

Shikanga et al. (2012d) evaluated the *in vitro* permeability of *S. tortuosum* alkaloids, viz. mesembranol, mesembrine, mesembrenone and mesembrenol, across porcine intestinal, sublingual, and buccal tissues in their pure form and in the form of three crude plant extracts (methanol, water, and an acid-base alkaloid-enriched extract). Caffeine was used as the positive control, and atenolol served as the negative control. In its pure form and in the form of crude extracts, the intestinal permeability of mesembrine was higher than that of the reference compound (caffeine), while mesembranol displayed permeability comparable to caffeine across intestinal tissue. However, mesembrenone and mesembrenol showed lower permeability than caffeine, but much higher permeability than the negative control. The results indicate good bioavailability of the alkaloids when the plant is chewed. Based on these permeability results across buccal and sublingual epithelial tissues, as well as the traditional way (mastication) of taking *S. tortuosum*, a study was conducted to formulate *S. tortuosum* extract into a chewing gum as a delivery system. It was shown that an extract from this medicinal plant was successfully incorporated into a medicated chewing gum preparation with sufficient pharmaceutical availability, as indicated by a dissolution experiment (Viljoen et al., 2021).

Meyer et al. (2015) conducted an in-depth investigation with GC-MS and liquid chromatography linked to mass spectroscopy (LC-MS) on the metabolism and toxicological detection of mesembrine and mesembrenone from *S. tortuosum*. They found that both alkaloids were O- and N-demethylated, dehydrated, and/or hydroxylated at different positions. They could identify most of these metabolites in both rat urine and human liver preparations.

4. Biological studies

4.1. In vitro studies

Harvey et al. (2011) tested the pharmacological effects of the ethanolic extracts of *S. tortuosum* along with the pure mesembrine, mesembrenone and mesembrenol. The plant extract and the purified alkaloids were tested individually on a panel of receptors, enzymes, other drug targets, and for cytotoxic activities on mammalian cells using radioligand binding, phosphodiesterase activity, cholinesterase activity, and cytotoxicity assays. The plant extract was reported to be a potent blocker in 5-HT transporter binding assays (with an IC_{50} value of 4.3 μg/mL) and exhibited high inhibitory effects on PDE4 with an IC_{50} value of 8.5 μg/mL. No cytotoxic effect was reported. Of the three alkaloids, mesembrine was the most active against the 5-HT transporter (K_i 1.4 nM), while mesembrenone was potent against the 5-HT transporter and PDE4 with IC_{50} values < 1 μM. The extract's activity on PDE4 and 5-HT transporter may explain the clinical effects of preparations from *S. tortuosum* in reversing depression, improving cognition, and alleviating anxiety.

Sixteen ethanolic extracts from *S. tortuosum*, prepared using different traditional methods, were evaluated by Setshedi et al. (2012) for antimalarial activities against *Plasmodium falciparum* using a plasmodium lactate dehydrogenase culture sensitivity assay. Chloroquine was used as the reference drug. Four extracts demonstrated potent antimalarial activities against *P. falciparum* with IC_{50} values in the range of 1.47 μg/mL and 7.32 μg/mL. The observed antimalarial activities of the four extracts were attributed to the presence of other compounds in the extract apart from mesembrine, which was shown to be inactive.

Kapewangolo et al. (2016) investigated the anti-retroviral (specifically human immunodeficiency virus, HIV) and free radical scavenging activities of crude ethanolic and ethyl acetate extracts of commercially available *S. tortuosum*. The inhibitory capabilities of the extract were tested against HIV-1 enzymes using protease (PR), reverse transcriptase (RT), and integrase assays. Acetyl pepstatin, doxorubicin, and sodium azide were used as positive controls for protease, reverse transcriptase, and integrase assays, respectively. The free radical scavenging activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay; ascorbic acid served as the control. Both extracts demonstrated anti-PR and anti-RT activities. In the HIV-1 PR inhibition test, IC_{50} values were <100.0 μg/mL for both extracts. Ethanol and ethyl acetate extracts recorded IC_{50} values of <50.0 and 121.7 ± 2.5 μg/mL, respectively in the HIV-1 RT inhibition test. The results of the free radical scavenging activities of the extracts showed a dose-dependent response and IC_{50} values of 49.0 ± 0.2 and 64.7 ± 3.1 μg/mL, which were obtained for the ethanol and the ethyl acetate extracts, respectively. The demonstrated antiradical activity of *S. tortuosum* extracts was attributed to the presence of alkaloids, phenols, flavonoids, and coumarins, as revealed by the phytochemical analysis of the extracts.

Using XTT (2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]−2H-tetrazolium-5-carboxanilide) and cytokinin measurement with magnetic bead panel assays, the immunomodulatory effects of Trimesemine™, a high content mesembrine (>70%) commercially available extract of *S. tortuosum* was evaluated on human monocyte viability, basally and in the presence of *Escherichia coli* lipopolysaccharide (LPS), an acute pro-inflammatory stimulus (Bennett and Smith, 2018). Trimesemine™ conferred cytoprotective effects to immune cells as it increased monocyte viability in the presence of acute immune challenge. Additionally, in the context of inflammatory signaling, Trimesemine™ showed high modulatory activities. The results also showed that Trimesemine™ demonstrated mild anti-inflammatory activities through up-regulation of monocyte IL-10 secretion. The most significant effect of *S. tortuosum* treatment was at a dose of 0.01 mg/mL. The findings of the study suggested that *S. tortuosum* may be valuable in offsetting systemic low-grade inflammation and cytokine-induced depression (Bennett and Smith, 2018).

In another study, Bennett et al. (2018) determined the cytoprotective and anti-inflammatory effects of two *S. tortuosum* extracts varying in alkaloidal composition (high mesembrine and high delta7-mesembrenone extracts) in delaying chronic disease progression. The extracts were evaluated on human astrocytes viability basally and in the presence of an acute pro-inflammatory stimulus (LPS) using the XTT assay and cytokine measurement. Additionally, the antioxidant capacities of the two extracts were determined using total DPPH and total phenolic content assays; ascorbic acid and gallic acid were used as standard references, respectively. The results of the study showed that the high mesembrine extract demonstrated anti-inflammatory and cytoprotective effects, while the polyphenols-rich delta7-mesembrenone extract showed potent antioxidant activity. Both extracts showed mild neuroprotective effects as indicated by inhibition of acetylcholinesterase and tyrosinase enzymes. The IC_{50} values for high mesembrine and high

delta7-mesembrenone extracts in the acetylcholinesterase inhibition assay were 0.299 ± 0.34 mg/mL and 0.983 ± 0.16 mg/mL, respectively. In the tyrosinase enzyme inhibition assay, IC_{50} values of 1.621 ± 0.75 mg/mL and 0.5908 ± 0.01 mg/mL, respectively were recorded for high mesembrine and high delta7-mesembrenone extracts. It was concluded that both extracts of *S. tortuosum* could be used either as a complementary therapy or preventive supplement to treat obesity and diabetes (Bennett et al., 2018).

Louw (2018) investigated the potential endocrine disruptive effects of a mesembrine-rich *S. tortuosum* extract (Trimesemine™). The investigation was conducted using human kidney cells transfected with 17βHSD, rat primary mixed testicular cell culture, and rat primary mixed ovarian cell culture. Effective dose (0.01 mg/mL) and high dose (0.5 mg/mL) of Trimesemine™ were used for the three-phased (human kidney cells transfected with 17βHSD, rat primary mixed testicular cell culture, and rat primary mixed ovarian cell culture) study. Analysis of steroid metabolites in the cell was done using ultra-performance convergence chromatography-tandem mass spectrometry (UPC2-MS/MS) and UHPLC-MS/MS. Trimesemine™ showed no disruptive properties at a lower (effective) dose, which is comparable to those recommended for consumer use. However, higher dose Trimesemine™ showed some adverse effects.

Manganyi et al. (2019) determined the antibacterial activity of 60 endophytic fungi isolated from *S. tortuosum* against different bacterial strains (*Escherichia coli* [ATCC 25922], *Enterococcus gallinarum* [ATCC 700425], *Bacillus cereus* [ATCC 10876], *E. coli* [O177], *Enterococcus faecalis* [S1299] and *Enterococcus faecium* [700,221]) using the agar disc diffusion assay. The study revealed that nine of the 60 endophytic fungi were effective as antibacterial agents against the test bacterial isolates. Fungi belonging to the *Fusarium* genus demonstrated the highest proportion of antibacterial activities with the zone of inhibition values that ranged from 2 mm to 9 mm. Unfortunately, they did not mention the zones of inhibition for the positive/negative controls used in the study.

In a more recent study, Luo et al. (2021) investigated the solvent extract fractions (petroleum ether and ethyl acetate fractions) of *S. tortuosum* for their neuroprotective activities. The pharmacologically active constituents of the solvent fractions responsible for the neuroprotective effects were further identified using UPLC coupled to quadrupole-time-of-flight mass spectrometry (qTOF) with collision energy (MSE) (UPLC-qTOF-MS^E). The neuroprotective effects of *S. tortuosum* extract fractions were evaluated in experimental neurotoxicity induced by 1-methyl-4-phenylpyridinium (MPP^+) in mouse N2a cells, and L-glutamate in PC12 cells using the MTT assay. Memantine and nimodipine were used as positive controls. The result of the neuroprotective screening showed that both ethyl acetate and petroleum ether fractions of *S. tortuosum* exhibited neuroprotective effects in PC12 and N2a cells injured by L-glutamate or MPP^+ respectively. Furthermore, identification of the pharmacologically active constituents using UPLC-qTOF-MS^E revealed the presence of delta7-mesembrenone, delta7-N-demethyl-mesembrenone, mesembrenone, 2-oxomesembrine, dihydrojoubertiamine, mesembrine, N-demethyl-N-formylmesembrenone, dihydrobuphanamine acetate, sceletenone, sceletium A4, N-methyldihydrojoubertinamine, 2-oxomesembranol, and other unidentified constituents. Additionally, it was reported that two sub-fractions each of ethyl acetate (E1 and E3) and petroleum ether (P5 and P6) were observed to show higher neuroprotective effects, with E1 and P5 being the most potent in comparison with other sub-fractions. An unidentified non-alkaloid and dihydrojoubertiamine were identified in the MS profile of E1 and P5 sub-fractions. Collectively, the results of the study imply that *S. tortuosum* has therapeutic potential in treating neurodegenerative diseases. Also, the results indicate that there are unidentified compounds in *S. tortuosum* with neuroprotective potential, and these require further isolation, identification and testing for their pharmaceutical activities.

4.2. In vivo studies

A number of mechanisms attributed to *S. tortuosum* have strong associations with anti-inflammatory, neuroprotective, and psychotropic actions, as highlighted in Table 3. Additionally, various *in vitro* and *in vivo* studies (summarized in Table 4) have shown that *S. tortuosum* has antidepressant, anxiolytic, antioxidant, antisteroidogenic, precognitive, and immunomodulatory effects, thus suggesting to be promising in treating psychiatric disorders such as depression and anxiety disorders.

In an *in vivo* model of psychological stress, Smith (2011) studied the effects of *S. tortuosum* extract using 90 male Wistar rats. The animals were given either placebo, 5 or 20 mg/kg of unfermented *S. tortuosum* extracts by daily oral gavage for 17 days. Half of the rats were subjected to repeated restraint stress that lasted for 1 h in the last three days of treatment. Using the elevated plus-maze, stress and/or chronic treatment-induced changes were evaluated in rats on the final day of restraint. The study reported that the lower dose of *S. tortuosum* extract (5 mg/kg/day) had positive effects in response to psychological stress in rats as it decreased restraint stress-induced self-soothing behaviour and exhibited a decrease in stress-induced corticosterone levels.

Loria et al. (2014) characterized the activities of mesembrine, ethanolic and alkaloid-enriched fraction extracts of *S. tortuosum* leaves on male Sprague-Dawley rats using several common rodent-based assays that model depression, nociception, anxiety, ataxia, and abuse liability. An ethanolic extract, alkaloid-enriched extract, or mesembrine were administered intraperitoneally (IP) to rats at different doses (5–100 mg/kg). Morphine (opioid analgesic), imipramine (tricyclic antidepressant), chlordiazepoxide (benzodiazepine anxiolytic), and muscimol (psychotogenic agent) were used as standard reference drugs for the different assays. Overall, ethanolic and alkaloid-enriched *S. tortuosum* fractions had antidepressant properties, but did not produce ataxia, while mesembrine showed analgesic properties without ataxia or abuse liabilities.

Carpenter et al. (2016) determined whether *S. tortuosum*-associated antidepressant and anti-anxiety activities in rat models would also be observed in an avian screening assay (chicken-anxiety model assay).

Table 3
Summary of currently available data relating to specific pharmacological effects of the most abundant alkaloids found in *S. tortuosum*, and psychotropic action, i.e. antidepressant, anxiolytic, anti-inflammatory etc.

Alkaloid	Pharmacological mechanisms	References
Mesembrine	SERT inhibition	Carpenter et al. (2016); Coetzee et al. (2016); Gericke and Viljoen (2008); Harvey et al. (2011); Krstenansky (2017)
	PDE-4 inhibition	Carpenter et al. (2016); Gericke and Viljoen (2008)
	Anti-inflammatory; cytoprotective	Bennett and Smith (2018)
	Upregulates VMAT-2	Coetzee et al. (2016); Krstenansky (2017)
	Mild inhibition of AChE	Coetzee et al. (2016)
	Mild inhibition of MAO-A	Coetzee et al. (2016)
	limited reuptake of NE and DA at high concentrations	Gericke and Viljoen (2008)
Mesembrenone	SERT inhibition	Carpenter et al. (2016); Harvey et al. (2011)
	PDE-4 inhibition	Harvey et al. (2011)
Mesembrenol	SERT inhibition	Carpenter et al. (2016); Harvey et al. (2011)
	PDE-4 inhibition	Carpenter et al. (2016)
Mesembranol	No data found	
Alkaloid enriched fraction (*in vivo*; behavioral)	Anxiolytic Antidepressant in FST	Carpenter et al. (2016) Loria et al. (2014)

Table 4
The psychotropic properties of *S. tortuosum. In vitro* and *in vivo* evidence.

Reference	Type of study	Sceletium preparation	Dose and route of administration	Main findings	Conclusion
Gericke and Viljoen (2008)	**Clinical case reports:** 1) Depressed woman	Not specifically stated. Tablets	50 mg daily oral (tablet). Withdrawn after 4 weeks	Improved mood Decreased general anxiety Insomnia improved (at onset) No symptoms of withdrawal after discontinuation	Effective anxiolytic and mood elevator
	2) Dysthymic woman with personality disorder	Not specifically stated. Tablets	50 mg daily oral (tablets). Doubled dose to 100 mg daily for exams (month later)	Mood lifted within 10 days More focused More engaged and less socially distant Decreased anxiety Less inclined to over-indulge in alcohol	Mood elevator, anxiolytic and more contained feeling
	3) Woman with MDD. Failed treatment with St. John's Wort	Not specifically stated. Tablets	100 mg daily oral (tablets)	Mood lifted within first day Hypersomnia improved Increase in energy 6 weeks treatment: fully recovered and maintained	Mood elevator
Harvey et al. (2011)	***In vitro* studies on mammalian cells** Receptors, enzymes and other targets	Zembrin® and Purified alkaloid extracts: Mesembrine Mesembrenone mesembrenol	N/A	Serotonin reuptake inhibition (inhibits serotonin transporter (SERT)) Potent inhibition of phosphodiesterase 4 (PDE4) and to lesser extent PDE3 At high doses, inhibition of GABA, δ_2-opioid, μ-opioid, cholecystokinin-1, melatonin-1, and EP4 prostaglandin receptors	Potent 5-HT reuptake inhibitor Potent PDE4 inhibitor
Loria et al. (2014)	***In vivo* animal study** Healthy Sprague Dawley rats	Alkaloid enriched fraction	5, 10, 20 mg/kg* 20 mg/kg	Reduced float time in FST. Ataxia in rotarod	Antidepressant with ataxia
	Behavioral tests: Conditioned Place Preference (CPP)*	Mesembrine	5, 10, 20 mg/kg* 20 mg/kg	Altered nociceptive responses approximate to morphine	Analgesic without abuse liability or ataxia
	Hotplate test Forced Swim Test (FST)	Alkaloid extract	25, 50, 100 mg/kg* 100 mg/kg		
	Elevated Plus Maze (EPM) Rotarod		Intraperitoneally	No psychoactive properties in EPM or CPP	No anxiolytic effects, no abuse liability
Schell (2014)	***In vivo* animal study** BALB/c mice (MDD model)	Direct acid extraction of mesembrine	10 mg/kg/day or 80 mg/kg/day	Decreased immobility in FST at low dose	Antidepressant
Carpenter et al. (2016)	***In vivo* animal study** **Chick anxiety-depression model** (distress vocalizations after social separation)	Alkaloid enriched fraction	10, 20, or 30 mg/kg (Experiment 1) 50, 75, or 100 mg/kg (Experiment 2)	No antidepressant effects (contradictory of literature in rodents) – could be due to altering effects of constituents on each other, narrower dose range for antidepressant effects, comorbid anxiety symptom overlaps, translational problems from rodents to avian model. Good anxiolytic effects	Anxiolytic
Bennett and Smith (2018)	***In vitro*: human monocytes** Immunomodulatory effects – cytokine release and mitochondrial viability	Lyophilised extract (Trimesamine®)	1.1 mg/mL or 1.0 mg/mL ± *E.coli* lipopolysaccharides (LPS)	Increased mitochondrial viability Upregulated anti-inflammatory cytokine IL-10 Prevented decreased mitochondrial viability (LPS-induced) Acute inflammatory response to LPS not negatively affected Best dose: 0.01 mg/mL	Cytoprotective against oxidative stress. Mild anti-inflammatory properties Beneficial for cytokine-induced depression and systemic low-grade inflammation
Terburg et al. (2013)	**Acute human studies:** pharmaco-fMRI on anxiety-related activity in amygdala. Healthy subjects. Perceptual-task and emotion-matching task	Zembrin®	Single 25 mg dose	Attenuated amygdala reactivity to fearful faces under low perceptual load conditions. Decreases amygdala-hypothalamus coupling	Anxiolytic potential by decreasing amygdala activity to unattended facial fear
Coetzee et al. (2016)	***In vitro* studies** Human astrocytes and mouse hippocampal cells: SERT; VMAT-2 MAO-A; AChE	Trimesamine™ High in Mesembrine	1, 0.1, 0.01, 0.001, 0.0001 mg/mL	Down-regulated SERT expression similar to citalopram. VMAT-2 upregulated significantly Relatively mild nhibition of AChE and MAO-A	SERT inhibition activity is a secondary function to monoamine-releasing activity of high-mesembrine extract
Dimpfel et al. (2016)	Acute clinical study in healthy subjects. Psychophysiological characterization of ST. EnkephaloVision method (EEG analysis during cognitive and emotional challenges)	Zembrin®	25 and 50 mg vs placebo	Significant increase in delta and theta spectral power in the frontal brain indicating positive effects on electrical activity of the brain during cognitive processing. Increased calmness and decreased	Positive action on cognitive and emotional processes in the brain

(*continued on next page*)

Table 4 (*continued*)

Reference	Type of study	Sceletium preparation	Dose and route of administration	Main findings	Conclusion
				depressive symptoms; increased memory and attention	
Chiu et al. (2014)	Clinical study in healthy subjects. Double-blind placebo-controlled croos-over design. CNS Vital Signs, Hamilton depression rating scale (HAM-D). Monitored side effects with treatment emergence adverse events scale	Zembrin®	25 mg capsule Once daily for 3 weeks vs placebo	Significantly improved cognitive set flexibility and executive function. Positive changes in mood and sleep. Well tolerated	Promising cognitive enhancing effects. Possible treatment of early Alzheimer's dementia.
Dimpfel et al. (2016b)	Clinical study in healthy subjects. EEG 1 h before and after administration of Zembrin. 6 Cognitive tests: d2-test, memory test, calculation performance test, reaction time test, number identifying test, number connection test. 3 Questionnaires (HAM-D, sleep questionnaire, Profile of Mood States.	Zembrin®	25 or 50 mg Daily for 6 weeks vs placebo	Increased delta and theta activity (50 mg). Increased alpha 1 spectral power. Improvement during performance of the arithmetic calculation test and number connection test. Decreased anxiety after 6 weeks. Significant activity on questionnaires, psychometry and quantitative EEG.	Improves some aspects of cognitive function, decreases anxiety, enhances mood
Maphanga et al. (2020)	***In vivo:* Zebrafish larvae** Anxiety study. Locomotor activity and reverse-thigmotaxis under continuous illumination and altered light/dark challenges.	Crude water extract 7 plants screened	Activity at 12.5 and 25 mg/L	Showed best anxiolytic properties of all plants screened.	Anxiolytic
Luo et al. (2021)	***In vitro:*** Neuroprotective effect of extract-fractions using UPLC-qTOF-MS with collision energy and MassFragment Software in mouse MPP^+-injured N2a cells and glutamate-injured PC12 cells.	Petroleum ether or ethyl acetate extracts	Screened	Polar and non-polar extracts have neuroprotective effects. Unidentified non-alkaloid found in two sub-fractions. Requires further investigation	Protection against experimentally induced neurotoxicity. Unidentified compounds could provide therapeutic moieties that can be used to treat neurodegenerative diseases.
Dimpfel et al. (2018)	***Ex vivo* and *in vitro*:** Tested excitability of rat hippocampal slices after 1 week Zembrin® treatment *ex vivo*. Alkaloids tested directly *in vitro*	Zembrin® Isolated alkaloids: mesembrine, mesembrenone, mesembrenol mesembranol	Zembrin® 5 and 10 mg/kg/ day orally for 1 week	All attenuated amplitude of population spike during electric stimulation (single shock and theta burst). Mesembrenol and mesembranol attenuated AMPA mediated transmission.	Potential treatment for epilepsy, especially mesembrenol and mesembranol (AMPA transmission attenuation)
Fountain (2016)	***In vivo* chick anxiety-depression model.** Distress vocalizations after social separation in stress-inducing box for 60 min	Fractions of *S. tortuosum* (20% tween and deionized water as vehicle)	5,10,30,50,75 or 100 mg/kg ip. 15 min prior to separation. 10 mg/kg imipramine as control	75 and 100 mg/kg attenuated distress vocalizations in anxiety phase – anxiolytic Imipramine, but not *S. tortuosum,* increased vocalizations in depression phase - antidepressant	Anxiolytic Not antidepressant
Gericke (2020)	***In vivo* rat model of depression** Flinders Sensitive Line rat study: acute dose response and chronic treatment study. Tested antidepressant properties using the forced swim test (FST) and open field test (OFT)	Zembrin®	Acute dose response: 5, 10, 25, 50 and 100 mg/kg; Escitalopram (ESC) 5, 10 and 20 mg/kg as reference drug. 15 day treatment: 5 mg/kg ESC, 50 mg/kg Zembrin®, and combination of Zembrin® 50 mg/kg and ESC 5 mg/kg	Acute dose response: Dose-dependent antidepressant effects with 25 and 50 mg/kg showing best reversal of immobility in the FST. 15 day treatment: Alone treatment with both ESC 5 mg/kg and Zembrin® 50 mg/kg showed no significant reversal of immobility or monoamines. Zembrin® alone increased BDNF. Combination treatment increased immobility and hippocampal serotonin levels	Acute dose-dependent antidepressant effects. No chronic antidepressant effects in alone treatment Combination with ESC may cause serotonin syndrome, or serotonin syndrome-like behaviour may be model-related. Potential chronic procognitive effects.

Four to six days old socially-raised male Silver Laced Wyandotte chicks were given alkaloid-rich *S. tortuosum* extract administered intraperitoneal at different doses. Imipramine was used as the reference control drug; a solution of 20% Tween 80 and deionized water served as the vehicle control. Alkaloid-rich *S. tortuosum* fraction at 75 and 100 mg/kg reduced distress vocalizations in the experimental chickens' anxiety phase, indicating the anxiolytic effects of *S. tortuosum* in the chicken-anxiety model.

Dimpfel et al. (2016) constructed electropharmacograms of Zembrin® after oral administration by gavage of three dosages of this standardized *S. tortuosum* extract to 17 adult Fischer rats. The resulting electropharmacogram of Zembrin® was compared to electropharmacograms for rolipram and citicoline as control drugs. The experimental animals were evaluated after 45 min of the pre-drug period and 5 h of recording after a rest period of 5 min. The data obtained were transmitted electronically and processed using Fast Fourier Transformation (FFT). Spectra power was calculated for eight frequency ranges, viz. alpha1, alpha2, theta, beta1a, beta1b, beta 2, and gamma power. In a dose-dependent manner, Zembrin® attenuated all frequency ranges to varying degrees. Relative to electropharmacograms of the

control drugs (rolipram and citicoline) and other botanical extracts, a similar electropharmacogram was obtained for Zembrin®. These results implied that Zembrin® has antidepressant, anxiolytic, and cognitive enhancing properties similar to that of control drugs. It also highlighted the putative involvement of the PDE-4 and phosphatidylcholine synthesis in said actions.

Using a chick anxiety-depression model, Fountain (2016) studied the effects of *S. tortuosum* in 4–6 days old socially-raised male Silver Laced Wyandotte chicks that were subjected to stress using a stress-inducing isolation chamber. Fifteen minutes before being placed in the stress-inducing chamber, the chicks received IP injections of either vehicle, imipramine, or *S. tortuosum* alkaloid enriched fraction doses (10, 20, 30, 50, 75, or 100 mg/kg). The chicks were thereafter placed in the stress-inducing chamber for 1 h and distress vocalizations were recorded during anxiety-like phase (first 3 min) and depression-like phase (30–60 min). Deionized water was used as the vehicle for imipramine while deionized water and 20% Tween served as the vehicle for *S. tortuosum*. The results of the study showed that two doses (75 and 100 mg/kg) of *S. tortuosum* reduced distress vocalization rates in the chicks in the anxiety phase. However, *S. tortuosum* did not show any effect on distress vocalization rates in both early depression and late depression phases. The results imply that *S. tortuosum* has more potential in alleviating acute stress behaviour than chronic stress behaviour.

Dimpfel et al. (2018) studied the effect of Zembrin® and four Zembrin® alkaloids (viz; mesembranol, mesembrine, mesembrenol and mesembrenone) on electric excitability of adult male Sprague-Dawlay rat's hippocampus using the hippocampus slice model. Zembrin® was tested *ex vivo,* while the four alkaloid constituents were tested directly *in vitro*. The experimental rats were orally administered with 5 mg/kg or 10 mg/kg of Zembrin® or control (placebo) daily for a week, and changes in the excitability of the hippocampus were checked the following day using *in vitro* slices. Similarly, the effects of the four Zembrin® alkaloids on population spike amplitudes were tested *in vitro* using two concentrations in the nanomolar range. The results of the study revealed that during the electric stimulation as single shock, as well as theta burst stimulation, Zembrin® *ex vivo* and Zembrin® alkaloids *in vitro* attenuated the amplitude of the population spike. The study further revealed that Zembrin® and two alkaloids (mesembranol and mesembrenol) acted by attenuation of α-amino-3-hydroxy-5-methyl-4 -isoxazolepropionic acid (AMPA) receptor-mediated transmission within the hippocampus. The attenuation of AMPA mediated transmission has been linked to effective complementary treatment of epileptic patients. The result, therefore, implies that Zembrin®, mesembrenol and mesembrenol may serve as potential therapeutic leads that can be used in the development of drugs for treating epilepsy.

In a more recent acute dose-ranging study in rats, varying doses of a standardized extract of *Sceletium*, Zembrin®, viz. 5, 10, 25, 50 or 100 mg/kg, were evaluated with respect to anti-depressant-like properties in a genetic rodent model of depression, the Flinders Sensitive Line (FSL) rat (Gericke, 2020). This dosage range was selected to coincide with the clinical literature described below. Zembrin® was compared to the serotonin reuptake inhibitor (SRI) antidepressant, escitalopram (ESC) oxalate (5, 10, or 20 mg/kg), both administered *via* oral gavage. Flinders Sensitive Line rats showed significant depressive-like behaviour, viz. decreased swimming and climbing (coping) behaviors, and significantly increased immobility (despair), *versus* Flinders Resistant Line (FRL) control rats. Escitalopram oxalate 5 mg/kg and Zembrin® at 25 mg/kg and 50 mg/kg showed significant dose-dependent reversal of immobility (anti-depressant effects) in FSL rats and variable effects on coping behaviors. Closer scrutiny found Zembrin® 50 mg/kg to be the most effective antidepressant dose, with equivalence to 5 mg/kg ESC in an associated chronic treatment study over 15 days (Gericke, 2020). However, both treatments alone did not show a significant antidepressant-like effect in the FST. The latter findings require confirmation and further study. That said, the combination of *S. tortuosum* 50 mg/kg and ESC at 5 mg/kg significantly increased hippocampal levels of serotonin, norepinephrine, as well as increased locomotor activity. Furthermore, treatment with Zembrin® (50 mg/kg alone) showed a significant increase in hippocampal brain-derived neurotrophic factor (BDNF). The above-mentioned monoaminergic and neurotrophin effects are positive indices in major depressive disorder treatment (Brand et al., 2015), while also indicating procognitive effects and improved neuroplasticity, which warrants further study. That an antidepressant response to Zembrin® could only be confirmed following acute, but not chronic treatment, may be due to the model used (viz. FSL rat), advocating further studies using other translational models.

In a recent study by Maphanga et al. (2020), seven medicinal plants reported in literature that are used in traditional medicine in treating anxiety and other central nervous system conditions were identified, selected and evaluated for their potential anxiolytic effects using an *in vivo* zebrafish assay. The plants used for the study are *Xysmalobium undulatum*, *Withania somnifera*, *Sutherlandia frutescens*, *Sceletium tortuosum, Piper methysticum*, *Mondia whitei* and *Melia azedarach*. Four different crude extracts (*viz*: distilled water, ethyl acetate, dichloromethane and methanol) of the plants were prepared, and the maximum tolerated concentration (MTC) of the extracts was determined before the anxiolytic activity assay. Anxiety was induced in 5-day-post-fertilization (dpf) zebrafish larvae using continuous illumination and alternating light-dark challenges. Determination of MTC values revealed that 13 of the 28 extracts tested were toxic, hence larvae were only treated with 15 non-toxic extracts at the dose that was determined in the MTC assay. Diazepam and dimethyl sulfoxide (DMSO) served as the positive and negative control, respectively. Reverse-thigmotaxis behaviour, which indicates anxiolytic activity, and general locomotor activity were analyzed in anxiety-induced zebrafish larvae. The results showed that *S. tortuosum* water extract of the 15 non-toxic extracts demonstrated the highest anxiolytic activity. Hence, *S. tortuosum* water extract was further evaluated for detailed behavioral effects. The result revealed that different tested concentrations of *S. tortuosum* water extract showed anxiolytic activity (reverse-thigmotaxis) on the zebrafish larvae when light-dark transitions and spontaneous locomotor activity were considered. Furthermore, during the 40 min of continuous illumination challenge, *S. tortuosum* water extract showed significant anxiolytic activity at two concentrations (12.5 and 25 mg/L; $P < 0.01$, $P < 0.05$ respectively) on the zebrafish larvae when compared with the control group (DMSO treated group). Collectively, the results indicate the therapeutic potentials of *S. tortuosum* in managing anxiety.

4.3. Randomized controlled trials (Human clinical studies)

In a pharmaco-functional magnetic resonance imaging (fMRI) study, Terburg et al. (2013) examined the effects of a single dose of Zembrin® (25 mg) on anxiety-related amygdala activity and its connected neurocircuitry in the human brain. The study employed a double-blind, placebo-controlled, cross-over design using 16 healthy participants. The participant's brains were scanned during performance in a perceptual-load and an emotion-matching task and exposure to fearful faces. Following a single 25 mg dose, Zembrin® decreased amygdala reactivity to fearful faces under low perceptual load conditions and also decoupled amygdala–hypothalamus connectivity as revealed by follow-up connectivity analysis on the emotion-matching task. Although this study was underpowered, which also did not assess efficacy in anxious patients *per se*, the results indicate some support for the anxiolytic properties of *S. tortuosum* standardized extract (Zembrin®) in humans.

The neurocognitive effect, safety, and tolerability of Zembrin® were assessed in 21 cognitively healthy subjects (45–65 years old) using a randomized, double-blind placebo-controlled cross-over study design (Chiu et al., 2014). Subjects received either 25 mg Zembrin® capsule or placebo once daily for three weeks. At baseline and regular intervals, changes in nine cognitive domains were measured through a computerized battery of validated neuropsychological tests. Side effects from

treatments were also monitored. In comparison to the placebo group, 25 mg daily dosage of Zembrin® enhanced cognitive set flexibility and executive function in subjects. Zembrin® was well tolerated in subjects, and positive changes in mood and sleep were observed. This study suggests possible application in the treatment of early Alzheimer's dementia.

In a quantitative electroencephalogram (EEG) source density combined with eye movement study by Dimpfel et al. (2016), the psychophysiological characterization of 25 mg and 50 mg of Zembrin® was investigated relative to placebo in 60 healthy subjects. The subjects were exposed to several mental tasks and emotional audio-visual video clips before and 2 h after taking 25 mg or 50 mg of Zembrin® capsule or placebo. Furthermore, the effect of Zembrin® was tested using EnkephaloVision. In comparison to the placebo, intake of either 25 mg or 50 mg Zembrin® resulted in an increase in power of frontal brain wave frequencies. An increase in theta, delta, alpha1, and alpha2 spectral power in the frontal brain, as revealed in the study, has been linked to enhanced attention and memory.

In a follow-up study by Dimpfel et al. (2017), the effects of 25 mg or 50 mg of Zembrin® on brain electrical activity were measured when taken daily for 6 weeks relative to placebo in 60 healthy male and female subjects (50–80 years old). The effects of Zembrin® were measured on three levels of evidence including a questionnaire, psychometric (cognitive) testing, and quantitative EEG measurements during psychometric tests. EEG was recorded bi-polarly from 12 surface electrodes, before and after 1 h of Zembrin® intake. As revealed by quantitative EEG, in the frontotemporal region (part of the brain recognized for memory retrieval), there was an increase of delta activity during performance of the d2-test in the presence of Zembrin®, higher theta was also observed in this part of the brain, and an increase of alpha1 spectral power. Psychometric testing revealed improvement from both dosages during the performance of an arithmetic calculation test and the number connection test. The results of the study implied that Zembrin® lessened anxiety, improved some areas of cognitive function, and may have enhanced mood in healthy people (Dimpfel et al., 2017).

Reay et al. (2020) investigated the anxiolytic properties of a single 25 mg dose of *S. tortuosum* standardized extract (Zembrin®) on induced anxiety/stress response in 20 healthy young volunteers. The study was a 2-part research design that employed a placebo-controlled and double-blind between-subject experimental approach. A feeling of anxiety/stress was induced in subjects through a 20-min multitasking framework in study 1, and a 5-min simulated public speaking assay in study 2. Subjective experiences of mood, pre-stress induction, and post-stress induction were measured in the first part of the study. In the second part of the study, subjective experiences of anxiety, heart rate, and galvanic skin response at baseline, pre-stress, during-stress, and post-stress inductions were measured. There was no treatment effect in the first part of the study, and the lack of observable effect was attributed to a mild stressor, which disallowed observable treatment effects in subjective self-report measures. In the second study, however, Zembrin® caused a reduction in subjective anxiety levels. It was concluded that a single 25 mg dose of Zembrin® could ameliorate subjective and physiological indicators of anxiety/stress in healthy volunteers. This study suggests possible application in the treatment of stress and anxiety-related conditions.

Since earlier studies have investigated the effect of *S. tortuosum* extracts in mostly middle-aged and older adults, Hoffman et al. (2020) investigated the effects of eight days of 25 mg of *S. tortuosum* extracts supplementation in 60 recreationally trained college-aged (20–35 years) men and women. The effect of the extract was assessed on changes in reactive agility, visual tracking, and mood. Subjects were divided into groups and given either placebo or *S. tortuosum* extract. Multiple object tracking, visual tracking speed, and reactive performance were assessed. Fatigue and focus in subjects were also assessed. The assessments were done a day before and after supplementation. Compared with placebo, *S. tortuosum* extract significantly improved complex reactive tasks that required subjects to respond to repeated visual stimuli with a cognitive load. However, supplementation with the extract on the subjects had no benefits on mood. Previous studies that reported improved mood with *S. tortuosum* extract supplementation were conducted in older adults who may be more sensitive to cognitive enhancement from 5-HT and PDE4 inhibition.

All human clinical trials on *S. tortuosum* to date have been exploratory or pilot clinical studies in small numbers of healthy subjects. The efficacy of *S. tortuosum* in a clinical population is not yet known.

4.4. Toxicity and safety studies

Like any other drug, medicinal plants have to be assessed for toxicity before their use as therapeutic agents. A few toxicological studies have been conducted on the standardized extract of *S. tortuosum* (Zembrin®) *in vivo* (using Wistar rats and in human subjects). No severe adverse effects were reported in these studies. Thus, *S. tortuosum* extract could be considered a safe therapeutic agent.

Murbach et al. (2014) assessed the toxicological safety of Zembrin® in a 14-day repeated oral toxicity study in specific pathogen-free male and female Crl Wistar rats using five different daily doses (0, 250, 750, 2500, and 5000 mg/kg body weight [bw]/day). Additionally, a 90-day subchronic repeated oral toxicity assessment was conducted using lower doses of Zembrin® (0, 100, 300, 450, and 600 mg/kg bw/day). Different parameters including rearing behaviour, locomotion, spatial parameters, and turning behaviour were evaluated in the final study week. Neither mortality, nor treatment adverse effects, was observed in experimental animals in the 14- and 90-day studies.

In a randomized, double-blind placebo-controlled parallel-group study involving healthy adult volunteers, Nell et al. (2013) investigated the safety and toxicity of two doses of Zembrin® (8 mg and 28 mg equivalent to 16 mg and 50 mg of dry plant material, respectively), taken once daily for three months. The variables studied were assessed using physical examination, vital signs, 12-lead electrocardiogram, different laboratory assessments (urinalysis, biochemistry, and hematology), and recording of adverse events. The subjects were grouped randomly to receive either one of the two doses of Zembrin® or placebo, taken daily for three months. Both doses of Zembrin® were well tolerated in subjects. There were no observed differences between the three treatments regarding physical examination, vital signs, body weight, and 12-lead electrocardiogram. Additionally, no significant changes were observed in biochemical and hematology parameters. The adverse effects most often reported were headache, followed by abdominal pain, and upper respiratory tract infections, which were reported prevalently in the placebo group.

5. Current research and future perspectives

There is considerable interest in *S. tortuosum* as a psychoactive agent. Considering its evident application and apparent efficacy in various neuropsychiatric conditions, compounds from *S. tortuosum*, most likely mesembrine alkaloids or their metabolites, are presumed to cross the blood-brain barrier (BBB). Nevertheless, assessment of brain penetrance of the various extracts and the constituent mesembrine alkaloids is needed.

It is challenging to deliver biologically active molecules into the CNS since access to the brain from the systemic blood circulation is greatly restricted by the blood-brain barrier. Typically, less than 1% of the bioavailable drug dose in the blood reaches the brain (Sonvico et al., 2018). This is also applicable to the delivery of biologically active molecules from *S. tortuosum* to the brain. Fortunately, the olfactory region in the nose provides a platform for direct nose-to-brain delivery of molecules and thereby avoiding the blood-brain barrier (Bahadur and Pathak, 2012). Planned research will explore the rate and extent of delivery of mesembrine alkaloids in *S. tortuosum* extract across excised sheep olfactory and respiratory nasal epithelial tissues. This study will

indicate if the active molecules from *S. tortuosum* extract can be delivered rapidly and directly into the brain *via* the nasal route of administration and thereby giving immediate relief (e.g. rapid treatment of anxiety and depression symptoms). After the proof of concept by these *ex vivo* membrane permeation studies, the delivery of *S. tortuosum* biologically active molecules into the brain *via* intranasal administration should be investigated through *in vivo* studies.

The efficacy of bio-enhancers to improve the delivery of the active compounds of *S. tortuosum* across the blood-brain barrier should be investigated in future studies. This could lead to the administration of lower doses of *S. tortusoum* extract but still obtain the desired pharmacological activity. Also, the potential of alternative dosage forms such as chewing gums for systemic delivery of the active moieties of *S. tortuosum* should be investigated through *in vivo* studies.

Standardized extracts of *S. tortuosum* currently exists (e.g. Zembrin®) with chemical profiles that are as consistent as is possible with natural production. These extracts have been extensively used in *in vitro*, *in vivo* and in clinical trials and therefore they should be used in future studies so that results can be compared. There are 25 known alkaloid compounds, and it is likely that novel biologically active compounds will still be discovered. Little is known about the combined effect of these compounds, which in some cases make up a large proportion of the extract. Therefore, the development of universal minimum standards for *S. tortuosum* extract composition with respect to selected biologically active compounds will contribute to the improvement of the quality of products when proof can be given of compliance to these minimum standards. It will also allow direct comparison of results between studies.

Following from the above and given the different pharmacological properties of the various constituents of *S. tortuosum* (Table 4), any given extract of *S. tortuosum* will show variable pharmacological properties in accordance with its specific chemotype, i.e. the alkaloid content and relative alkaloid composition of the extract. Indeed, as noted earlier, mesembrine is the most active 5-HT transport inhibitor, while mesembrenone is both a potent 5-HT transporter and PDE4 inhibitor. Given the inherent high mesembrine content in Trimesemine™ (>70% mesembrine), it is more potently antioxidant and anti-inflammatory in action. On the other hand, Zembrin® contains much lower levels of mesembrine but is more prominent with regard to mesembrenone, possibly affording it more pronounced effects on mood, cognition, and well-being. Taking this further, these commercial extracts, by virtue of their differences in chemotype, may offer differences in clinical utility, e.g. anxiolytic, antidepressant, anti-addictive properties, etc. This is a valuable consideration worth exploring in controlled clinical studies.

We have already highlighted the possible benefit of *S. tortuosum* in treating depression, which coincides with its purported mechanisms of action described in Table 3. Current evidence in animal studies for antidepressant effects are mainly based on acute challenge, which indeed is considered to translate to potential clinical efficacy. However, given that the clinical management of depression requires chronic and not acute treatment for sustained efficacy, for more translational relevance pre-clinical studies need to be undertaken using validated translational models, such as the FSL rat, using a chronic treatment regimen followed by appropriate bio-marker analysis. We have also noted earlier that differences in acute versus chronic antidepressant response, for example to Zembrin®, may be dependent on the model used, thereby advocating further studies on *S. tortuosum* using other translational animal models such as the chronic mild stress model. Furthermore, while the current clinical findings with *S. tortuosum* have been performed in healthy subjects, controlled clinical studies in a clinical population are now urgently required.

The actions of *S. tortuosum* on serotonin and GABA (Harvey et al., 2011) may allow it credible utility in treating anxiety states (Brand et al., 2015). Indeed, the Koi-San of Africa have long since used *S. tortuosum* plant extracts to promote a sense of calm and relieve stress, reduce anxiety, improve mood, and enhance concentration (Dimpfel et al., 2016). Clinical studies have concurred, showing *S. tortuosum* (25 mg per day) to relieve anxiety and stress (Nell et al., 2013; Terburg et al., 2013). A single dose of Zembrin® was found to reduce psychological stress (Dimpfel et al., 2016). As with depression, clinical studies with *S. tortuosum* have to date been performed in healthy subjects, again highlighting the need for controlled clinical studies in anxiety disorders including general anxiety disorder, social anxiety disorder, post-traumatic stress disorder, and others.

The prevalence of major depression grows every year and the rate of depression-related morbidity and mortality is alarming (WHO, 2017). Inadequate pharmacotherapy and increasing prevalence of resistance to current antidepressants represent a severe related health burden (WHO, 2017). Old generation groups of antidepressants such as tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRIs) remain the primary treatment for the disorder (Brand et al., 2015). Although the majority of the patient diagnosed with MDD respond well to clinical antidepressant treatment, 10–30% of patients do not improve and go on to display further symptoms of depression (Al-Harbi, 2012). Multiple mechanisms are implicated in the pathophysiology of MDD (Brand et al., 2015). Therefore, a strategy of using antidepressants with dual-or triple action as first-line treatment, or to augment first-line treatment with agents with multiple modes of action (Dupuy et al., 2011), will allow the simultaneous targeting of deficits in central brain monoamines, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, neuroinflammation, impaired neuroplasticity, genetic predisposition, and environmental influences, among others (Brand and Harvey, 2017). Indeed, different symptoms of depression may be targeted by specifically addressing specific pathological processes in other regions of the brain, and in this way improve long-term outcomes. By virtue of its multi-mode of action (Table 3), *S. tortuosum* is indeed attractive as a possible augmentation strategy in treatment-resistant depression (TRD). As noted earlier, the presence of different chemotypes of *S. tortuosum* are relevant here. Assessment in specific translational animal models of TRD (Brand and Harvey, 2017; Mncube et al., 2021) are recommended as proof of concept before clinical assessment. It also needs to be borne in mind that commercial availability of complementary medicines often sees herbal preparations being used without medical supervision, even in combination with prescribed treatments (Ravindran and Da Silva, 2013). This poses a serious risk for possible drug interactions and toxicity, e.g. serotonin syndrome when SSRIs are co-administered with for instance Saint John's wort (Sarris, 2018). Given the serotonergic actions of ST (Table 4) (Harvey et al., 2011), and similar supportive evidence from our laboratory (Gericke, 2020), such studies need to be undertaken. These real-world issues, plus that plant constituents may have interacting effects with one another, warrant dedicated research into the pharmacokinetic and pharmacological profiles of herbal medicines (Sarris, 2018). Such studies need to be carried out with *S. tortuosum*.

Methamphetamine (MA) is the world's third most abused illicit substance, after cannabis and opioids (United Nations, 2020). The consequences of MA abuse are dire, including high rates of morbidity and mortality (Herbeck et al., 2015), yet there is currently no approved treatment for MA addiction (Olsen and Liu, 2016; Shahidi et al., 2019). Its primary psychoactive and addictive effects are mediated through an increase in mesolimbic dopamine levels (Kish, 2008), through inhibition of the dopamine transporter (DAT) (Lüscher, 2015). Moreover, MA also increases other monoamines such as noradrenaline and serotonin (Kish, 2008), through inhibition of the noradrenaline transporter (NET) and serotonin transporter (SERT); (Lüscher, 2015), leading to feelings of alertness, energy, and euphoria (Panenka et al., 2013). Methamphetamine also influences inflammatory pathways, *via* the cyclic adenosine monophosphate (cAMP)- phosphodiesterase 4 (PDE4)- cAMP-response element-binding protein (CREB) signal cascade (or cAMP-PDE4-CREB signal cascade); (Olsen and Liu, 2016), leading to a pro-inflammatory state. By displaying dual inhibitory effects on SERT and PDE4, as well as its anti-inflammatory (Loria et al., 2014), *S. tortuosum* may be an

interesting candidate with potential in treating MA addiction. Indeed, *S. tortuosum* has shown concentration-dependent activity at neuronal receptors known to be involved in addiction, e.g. mu- and delta-opioid receptors (Harvey et al., 2011; Loria et al., 2014; Carpenter et al., 2016). Pre-clinical animal models of addiction may be especially suitable for addressing this question (Kalivas et al., 2006). These models can be specially adapted to examine *S. tortuosum* co-administered with MA, specifically addressing the development of addiction, or administered post MA exposure (viz. during MA withdrawal), which more explores the treatment of symptom manifestation of MA addiction. A recent animal study considered whether Zembrin® could modify the behavioral and neurochemical profile of MA (Postma, 2021). The authors concluded that in this context and model, the monoaminergic actions of Zembrin® as described earlier, acts as a stimulant during MA exposure. This is not unlike the pro-dopaminergic actions of anti-addictive agents like bupropion (Postma, 2021), and hints at its possible utility as an anti-addictive agent. However, further investigation into the mechanism of action of Zembrin® during addictive states is needed, and whether it is capable of reversing psychotogenic drug-induced addictive-like behaviour.

Recently/currently, chronic day-to-day stress has been magnified during the COVID 19 pandemic, which has negatively impacted millions of people globally (Jansen van Vuren et al., 2021). Statistics revealed a seven-fold increase in major depressive disorder (MDD) in the general population during the pandemic (Bueno-Notivol et al., 2021). Evidently, these distressing statistics highlight the severity of this global disorder (Bueno-Notivol et al., 2021). The relationship between severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection and psychiatric diseases is of great concern, with an evident link between corona virus infections and various central and peripheral nervous system manifestations (Jansen van Vuren et al., 2021). Unmitigated neuro-inflammation has been noted to underlie not only the severe respiratory complications of the disease but is also present in a range of neuro-psychiatric illnesses (Brand et al., 2015). Several neurological and psychiatric disorders are characterized by immune-inflammatory states, while treatments for these disorders have distinct anti-inflammatory properties and effects (Jansen van Vuren et al., 2021). With inflammation being a common contributing factor in SARS-CoV-2, as well as psychiatric disorders (Jansen van Vuren et al., 2021), *S. tortuosum* may be of interest as it has antidepressant as well as mild anti-inflammatory properties, without hindering an adequate immune response to acute immune challenges. It also exerts antiviral effects, although current evidence has focused on its potential against HIV (Bennett and Smith, 2018; Kapewangolo et al., 2016).

6. Concluding statement

The last decade has seen a dramatic increase in the number of research outputs regarding *S. tortuosum,* which emphasizes the growing scientific interest in this medicinal herb. For example; Science Direct lists only 8 studies that were published between 1967 and 2001, 16 articles during 2002–2010 and an astonishing 87 articles were published since 2011 until now. Of note, the quality of research seemed to have improved in the sense that many recent studies have been conducted using standardized extracts that were investigated in *in vivo* animal or human trials. Standardization of herbal treatments is of critical importance to ensure consistent efficacy and safety. However, there is still a need to conduct well designed and sufficiently powered clinical trials using participants suffering from the various diseases as listed in this review in order to firmly establish a risk:benefit ratio for standardized formulations of *S. tortuosum*.

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