



Pharmacological actions of the South African medicinal and functional food plant *Sceletium tortuosum* and its principal alkaloids

Alan L. Harvey^{a,*}, Louise C. Young^a, Alvaro M. Viljoen^b, Nigel P. Gericke^c

^a Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G4 0RE, UK

^b Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

^c HG&H Pharmaceuticals (Pty) Ltd., Bryanston 2191, South Africa

ARTICLE INFO

Article history:

Received 1 March 2011

Received in revised form 14 June 2011

Accepted 11 July 2011

Available online 20 July 2011

Keywords:

Mesembrine

Mesembrenone

Phosphodiesterase

Serotonin transporter

Selective serotonin reuptake inhibitor

Anti-depressant

ABSTRACT

Ethnopharmacological relevance: The South African plant *Sceletium tortuosum* has been known for centuries for a variety of traditional uses, and, more recently, as a possible source of anti-anxiety or anti-depressant effects. A standardised extract Zembrin® was used to test for pharmacological activities that might be relevant to the ethnopharmacological uses, and three of the main alkaloids were also tested.

Materials and methods: A standardised ethanolic extract was prepared from dried plant material, along with the purified alkaloids mesembrine, mesembrenone and mesembrenol. These were tested on a panel of receptors, enzymes and other drug targets, and for cytotoxic effects on mammalian cells.

Results: The extract was a potent blocker in 5-HT transporter binding assays (IC_{50} 4.3 μ g/ml) and had powerful inhibitory effects on phosphodiesterase 4 (PDE4) (IC_{50} 8.5 μ g/ml), but not other phosphodiesterases. There were no cytotoxic effects. Mesembrine was the most active alkaloid against the 5-HT transporter (K_i 1.4 nM), while mesembrenone was active against the 5-HT transporter and PDE4 (IC_{50} 's < 1 μ M).

Conclusions: The activity of the *Sceletium tortuosum* extract on the 5-HT transporter and PDE4 may explain the clinical effects of preparations made from this plant. The activities relate to the presence of alkaloids, particularly mesembrine and mesembrenone.

© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Sceletium tortuosum is a succulent plant which is indigenous to South Africa and belongs to the family Mesembryanthemaceae (Smith et al., 1996; Klak et al., 2007). It has a long history of traditional use, and more recently, it has attracted attention for its possible utility in supporting and promoting a sense of well-being in healthy people, and treating people with anxiety, stress or depression (for review see Gericke and Viljoen, 2008). Eight species

Abbreviations: 5-HT, 5-hydroxytryptamine; n, adenosine receptor; AT, angiotensin receptor; BZD, benzodiazepine; B₂, bradykinin receptor; CGRP, calcitonin gene related peptide; CB, cannabinoid receptor; CCK, cholecystokinin; D₁, dopamine receptors; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; ET, endothelin; GAL, galanin; H₁, H₂, histamine receptors; IBMX, 3-isobutyl-1-methylxanthine; MC, melanocortin; MT, melatonin; M₁–M₅, muscarinic cholinergic receptors; NK, neurokinin; NPY, neuropeptide Y receptors; NT, neurotensin; n_H, slope factor from Hill plot; DOP, δ_2 opioid receptors; KOP, κ opioid receptors; MOP, μ opioid receptors (MOP); NOP, orexin ORL1 receptors; PCP, phencyclidine; PDE, phosphodiesterase; PDGF, platelet-derived growth factor; EP, prostaglandin; TP, thromboxane A₂ (TXA₂/PGH₂) receptors; PGI₂, prostacyclin; P2X, P2Y, ATP receptors; sst, somatostatin; GR, glucocorticoid receptors; VIP, vasoactive intestinal peptide.

* Corresponding author. Tel.: +44 141 553 4155; fax: +44 141 552 8376.

E-mail address: a.l.harvey@strath.ac.uk (A.L. Harvey).

of *Sceletium* are generally recognised (*S. crassicaule*, *S. emarcidum*, *S. exalatum*, *S. expansum*, *S. rigidum*, *S. strictum*, *S. tortuosum* and *S. varians*), although Klak et al. (2007) suggested that the *Sceletium* group is more properly classified as: *Mesembryanthemum crassicaule*, *M. emarcidum*, *M. exalatum*, *M. expansum*, *M. archeri* (= *S. rigidum*), *M. ladismithiense* (= *S. strictum*), *M. tortuosum* and *M. varians*. The chemistry of various *Sceletium* species has been extensively studied, although the focus appears to have been exclusively on alkaloids: The Dictionary of Natural Products (2011) gives details of 31 different alkaloidal components from five sources of *Sceletium* (*S. joubertii*, *S. namaquense* [both now placed under synonymy with *S. tortuosum*], *S. strictum*, *S. subvelutium* [also now within the species concept of *S. tortuosum*], and *S. tortuosum*). *Sceletium tortuosum* is known to contain at least mesembrine, mesembrenol, mesembrenol, mesembrenone, alkaloid A4, chennaine and tortuosamine (Gericke and Viljoen, 2008).

Despite the interest in the possible use of *Sceletium* extracts or alkaloids in medicinal and dietary supplements, very little experimental pharmacological work has been published. Mesembrine, isolated from *Sceletium tortuosum*, was shown to be a potent inhibitor of 5-hydroxytryptamine (5-HT) reuptake (US Patent, 2001), and this was confirmed with synthetic (–)-mesembrine. Its IC_{50} against 5-HT uptake was 27 nM, with much weaker effects on

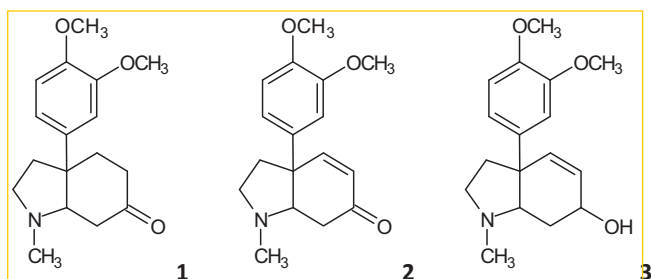


Fig. 1. Chemical structures of *Scelletium* alkaloids. 1, mesembrine; 2, mesembrenone; 3, mesembrenol.

noradrenaline uptake ($IC_{50} \sim 10 \mu M$) and no effect on dopamine uptake at $10 \mu M$ (Gericke and Viljoen, 2008). Mesembrenone has been tested for cytotoxic effects on three cell lines (Weniger et al., 1995). It was found to affect a human T cell lymphoma line (Molt4 cells) with an EC_{50} of $\sim 2 \mu M$ ($0.6 \mu g/ml$), but to have little effect on a hepatoma cell line (Hep G2) or on a mouse fibroblast line (LMTK cells; EC_{50} $10 \mu g/ml$). Mesembrine and synthetic analogues were shown to inhibit phosphodiesterase 4 (PDE4) activity (Napoleitano et al., 2001). Mesembrine itself was a very weak PDE4 inhibitor (IC_{50} $29 \mu M$) but some synthetic analogues were considerably more active, with IC_{50} s of 0.1 – $1 \mu M$.

There have also been a few published *in vivo* studies with *Scelletium tortuosum*. In animal studies, repeated oral doses of dry powdered plant material were shown to have no toxic effects in cats and dogs (Hirabayashi et al., 2002, 2004). The authors also reported that the plant material had beneficial effects in cats with signs of stress and dogs showing signs clinically diagnosed as dementia. The latter effects were reported to be confirmed in a more extensive study on dogs (Hirai et al., 2005). More recently, a *Scelletium* extract was tested in rats in an immobilisation stress model (Smith, 2011) where it was found to have positive effects on psychological stress. Human volunteers taking a *Scelletium* preparation reported anxiolytic effects (US Patent, 2001).

The present work was undertaken to conduct a broad pharmacological profiling of a proprietary standardised extract of *Scelletium tortuosum* known as Zembrin[®] and three main alkaloids, mesembrenol, mesembrenone, and mesembrine (Fig. 1).

2. Materials and methods

2.1. Extract and alkaloids

The above ground parts of a naturally occurring low-mesembrine chemotype of *Scelletium tortuosum* cultivated commercially by the company Niche Botanicals (Pty) Ltd., South Africa were harvested and air-dried before extraction. The plant:extract ratio was 2:1 by weight, with the solvent being 70% ethanol, 30% water by volume. The plant extract was spray-dried on a conventional inert carrier.

To isolate *Scelletium* alkaloids, 500 g of dried plant material of the low-mesembrine chemotype was extracted with 0.25 M sulfuric acid ($3 \times 6 L$), shaken and left to settle for 10 min before filtration. The process was repeated three times and the combined filtrates were made alkaline with 20% aqueous ammonia solution (750 ml) and the alkaloids were recovered with dichloromethane ($3 \times 875 ml$). The alkaloid-rich extract was subjected to classical column chromatography followed by high-speed countercurrent chromatography. The alkaloid content of the extract *Scelletium tortuosum* Zembrin[®] was 0.42% weight by weight. The relative amount of the three key mesembrine-alkaloids in the extract, quantified by HPLC analysis against validated analytical reference compounds, conform to the following profile: mesem-

brenone + mesembrenol > 70%, mesembrine < 20%. The purities of each of the isolated compounds were confirmed by GC–MS. This latter technique was also used to determine the molecular masses of 1–3. The structures of 1–3 were confirmed from the MS data and from published NMR data (Jeffs et al., 1970).

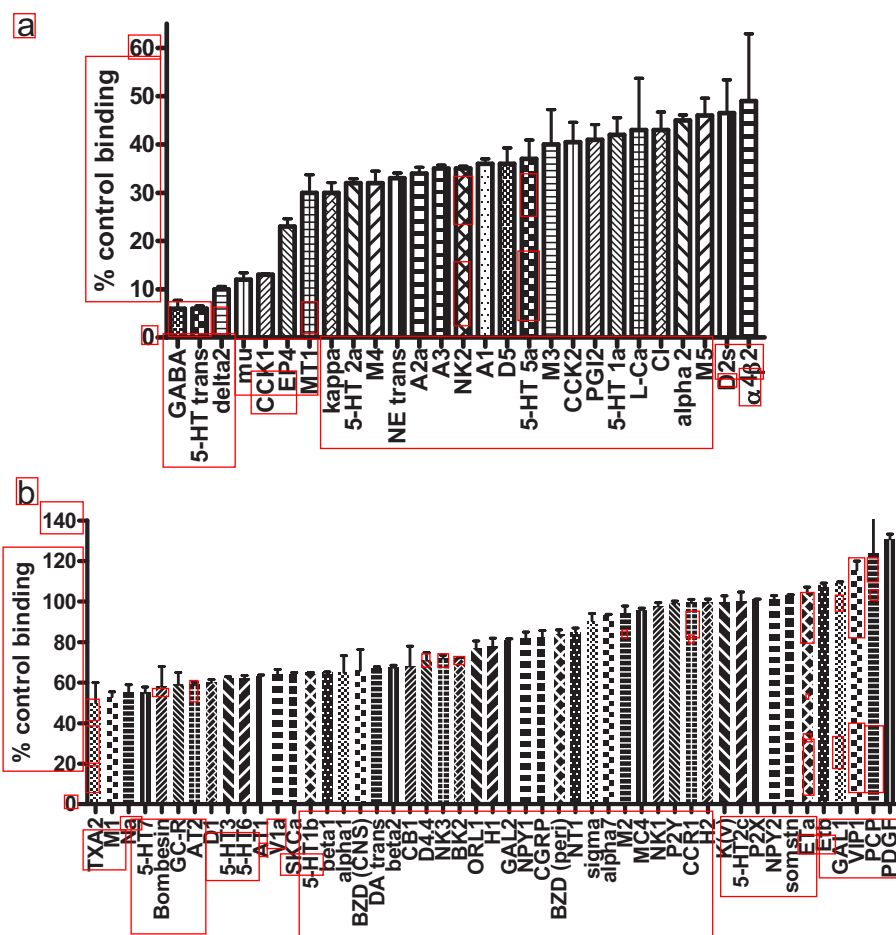
2.2. Radioligand binding assays

Extract and isolated compounds were tested on a panel of 77 radioligand binding assays (Cerep, France; http://www.cerep.fr/cerep/users/pages/catalog/p_ProfileCatalogue.asp?profile=2118): adenosine A_1 , A_{2A} , A_3 , α_1 adrenoceptors (non-selective); α_2 adrenoceptors (non-selective); β_1 adrenoceptors, β_2 adrenoceptors, angiotensin AT_1 receptors, AT_2 receptors, benzodiazepine (BZD) binding sites (central); BZD peripheral binding sites; bombesin receptors (non-selective); bradykinin B_2 receptors, CGRP receptors, cannabinoid CB_1 receptors, cholecystokinin CCK_A (CCK_1) receptors, CCK_B (CCK_2) receptors, dopamine D_1 receptors, D_{2S} receptors, D_3 receptors, $D_{4.4}$ receptors, D_5 receptors, endothelin ET_A receptors, ET_B receptors, GABA receptors (non-selective), galanin $GAL1$ receptors, $GAL2$ receptors, platelet-derived growth factor PDGF receptors, histamine H_1 receptors, H_2 receptors, melanocortin MC_4 receptors, melatonin MT_1 receptors, muscarinic cholinergic M_1 , M_2 receptors, M_3 receptors, M_4 receptors, M_5 receptors, neurokinin NK_1 receptors, NK_2 receptors, NK_3 receptors, neuropeptide Y_1 receptors, Y_2 receptors, neurotensin NT_1 receptors (NTS1), nicotinic cholinergic (neuronal) (α -BGTx-insensitive – $\alpha 4\beta 2$), nicotinic cholinergic (neuronal) (α -BGTx-sensitive – $\alpha 7$), δ_2 opioid receptors (DOP), κ opioid receptors (KOP), μ opioid receptors (MOP) (agonist site), orexin $ORL1$ receptors (NOP), NMDA receptors (phencyclidine PCP binding site), prostaglandin E EP_4 receptors, thromboxane A_2 (TP) receptors (TXA_2/PGH_2), prostacyclin (PGI_2), ATP $P2X$ receptors, $P2Y$ receptors, 5-HT $_1A$ receptors, 5-HT $_{1B}$ receptors, 5-HT $_{2A}$ receptors, 5-HT $_{2C}$ receptors, 5-HT $_3$ receptors, 5-HT $_{5A}$ receptors, 5-HT $_6$ receptors, 5-HT $_7$ receptors, σ receptors (non-selective), somatostatin (sst) (non-selective), glucocorticoid receptors (GR), vasoactive intestinal peptide VIP_1 receptors ($VPAC_1$), vasopressin V_{1a} receptors, Ca^{2+} channel (L, verapamil site) (phenylalkylamines), K^+ v channel, SK^+ Ca channel, Na^+ channel (site 2), Cl-channel (GABA-gated), noradrenaline transporter, dopamine transporter, and 5-HT transporter.

Experiments were performed in duplicate. The specific ligand binding to the receptors was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The results are expressed as a percent of control specific binding ((measured specific binding/control specific binding) $\times 100$) obtained in the presence of test materials. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (n_H) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting ($Y = D + [(A - D)/(1 + (C/C_{50})^{n_H})]$), where Y is the specific binding, D the minimum specific binding, A the maximum specific binding, C the compound concentration, $C_{50} = IC_{50}$, and n_H is the slope factor).

2.3. Phosphodiesterase activity assays

The following PDE enzymes were used along with corresponding reference compounds: PDE1 (bovine brain) with 8-methoxy-IBMX, PDE2 (differentiated U-937 cells) with EHNA, PDE3 (human platelets) with milrinone, PDE4 (U-937 cells) with rolipram, PDE4B and PDE4D, both human recombinant expressed in Sf9 cells, with rolipram, PDE5 (human platelets) with dipyridamole, PDE6 (bovine retina) with zaprinast, PDE7A human recombinant expressed in Sf9 cells, with BRL50481, PDE8A human recombinant



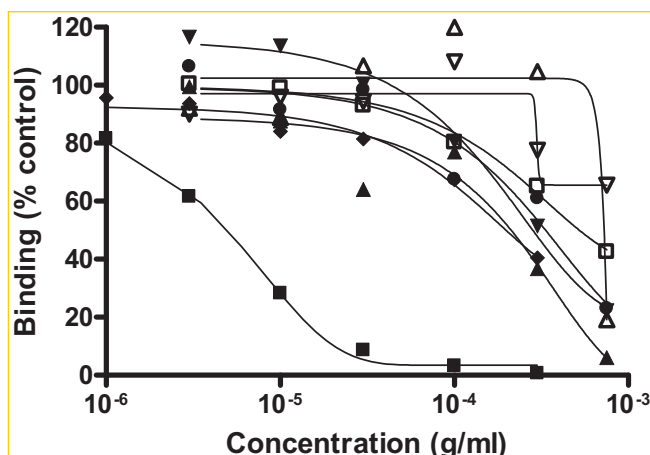


Fig. 3. Competition curves for the extract *Scelletium tortuosum* Zembrin® against the selected target sites. Filled square, 5-HT transporter; filled upward triangle, GABA-A receptors; filled downward triangle, μ -opioid receptors; filled diamond, δ_2 -opioid receptors; filled circle, EP4 receptors; open square, MT1 melatonin receptors; open upward triangle, CCK1 cholecystokinin receptors; open downward triangle, GABA-B receptors.

opioid receptors, and cholecystokinin-1 (or -A) receptors. Binding at the EP4 subtype of receptor for prostaglandin E2 was inhibited by 77%, but all others were inhibited by less than 70%. If an arbitrary threshold for “important” binding is taken at inhibition of 80% or greater, then the extract acts on the five sites listed above: namely, GABA receptors, 5-HT transporter, δ_2 -opioid receptors, μ -opioid receptors, and cholecystokinin-1 (or -A) receptors.

Based on the positive findings from the broad screening experiment, follow-up studies were performed to establish the concentration-dependency of the extract and its potency at key target sites: 5-HT transporter, GABA-A and GABA-B receptors, δ_2 -opioid receptors, μ -opioid receptors, cholecystokinin-1 (or -A) receptors, EP4 prostaglandin receptors and melatonin-1 receptors (Fig. 3). The extract had potent, concentration-dependent effects at the 5-HT transporter. The effects on the other sites were generally concentration-dependent but much higher concentrations of extract were needed to cause inhibition of binding. The calculated IC_{50} values and the Hill coefficients are shown in Table 1.

3.2. Effects on phosphodiesterase and cholinesterase activities

The extract *Scelletium tortuosum* Zembrin® was tested at 750 μ g/ml on activity of a panel of phosphodiesterases (Fig. 4). PDE4 activity was completely inhibited, and that of PDE3 was reduced by 88% (range 87–89%). The other enzymes were less inhibited: PDE1 by 27% (range 23–32%), PDE2 by 33% (range 30–36%), PDE5 by 29% (range 28–30%), PDE6 by 29% (range 27–31%), PDE7A by 34% (range 31–37%), PDE8A by 4% (range 2–6%), PDE10A by 49% (range 48–50%), and PDE11A by 50% (range 46–54%). In addition,

Table 1

Summary of analyses of the concentration–response curves with the extract *Scelletium tortuosum* Zembrin®.

Assay	IC_{50} (μ g/ml)	Hill coefficient
5-HT transporter	4.3	1.1
GABA-A	148	0.9
μ -Opioid	213	1.0
δ_2 -Opioid	236	0.9
EP4	293	1.0
MT1	536	0.8
CCK1	676	14.1
GABA-B	>750	Nd

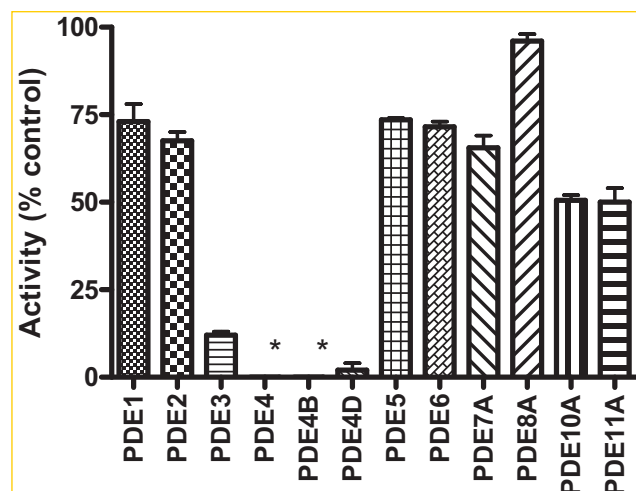


Fig. 4. Effects of the extract *Scelletium tortuosum* Zembrin® (750 μ g/ml) on different phosphodiesterases.

the extract was tested on two subtypes of PDE4: 4B and 4D. Extract at 750 μ g/ml abolished the activity of both subtypes of PDE4.

Follow-up studies were performed to establish the concentration-dependency of the extract's effects on PDE3 and PDE4. Both were inhibited in a concentration-dependent manner, although the extract was more potent against PDE4 (IC_{50} 8.5 μ g/ml) than against PDE3 (IC_{50} 274 μ g/ml).

When tested at concentrations up to 300 μ g/ml on activity of cholinesterases, the extract reduced activity of acetylcholinesterase by 7% and that of butyrylcholinesterase by 25%.

3.3. Effect of extract *Scelletium tortuosum* (Zembrin®) and isolated alkaloids on proliferation of mammalian cells

Extract (0.1–100 μ g/ml) and the alkaloids mesembrine, mesembrenol and mesembrenone (0.1–100 μ M) had no effect on the growth or viability of HS27 and HepG2 cells following 24 h exposure (data not shown).

3.4. Effects of isolated alkaloids on 5-HT transporter and other binding sites and on PDE4 activity

Mesembrenol, mesembrenone and mesembrine were tested at 3 μ M in the binding and enzymatic assays in which the *Scelletium* extract had previously been shown to have activity. The results are summarised in Fig. 5. All three alkaloids blocked binding to the 5-HT transporter, but had little effect on binding at GABA-A and GABA-B receptors, δ_2 -opioid receptors, μ -opioid receptors, cholecystokinin-1 (or -A) receptors, EP4 prostaglandin receptors and melatonin-1 receptors. Only mesembrenone had potent effects at PDE4A and 4B.

The three alkaloids were also tested at concentrations up to 300 μ M on activity of acetyl- and butyryl-cholinesterase. None of the compounds reduced activity of acetylcholinesterase by more than 5–10%. Butyrylcholinesterase activity was not affected by 300 μ M mesembrenone or mesembrenol, but it was reduced by 38% by 300 μ M mesembrine.

The three alkaloids were studied for their effects across a broad range of concentrations on binding to the 5-HT transporter and on activity of PDE4B. In all cases, concentration-dependent inhibition was found. The results are summarised in Table 2. In the 5-HT transporter assay, mesembrine was the most active compound, being 20 times more potent than mesembrenone and 87 times more active than mesembrenol. In the PDE4B assay, the most potent compound

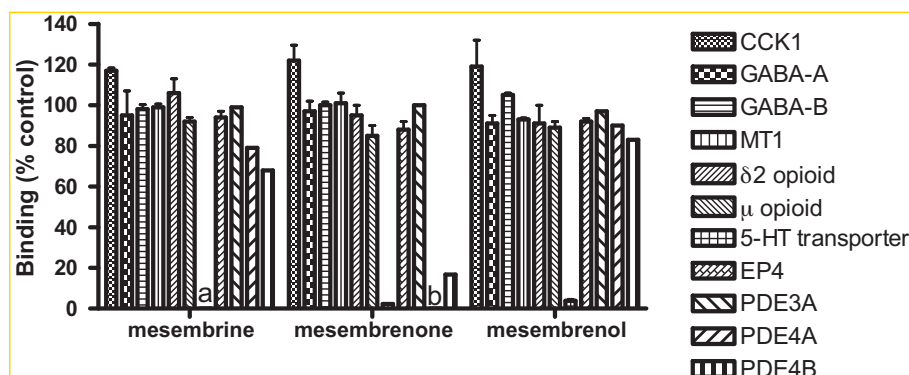


Fig. 5. Effects of mesembrine, mesembrenone and mesembrenol (all at $3 \mu\text{M}$) in binding and PDE assays. Values are mean of 2 separate assays with bars indicating the upper range. a, 100% block at 5-HT transporter; b, 100% block at PDE4A.

was mesembrenone, which was 17 times more potent than mesembrine and 34 times more active than mesembrenol.

4. Discussion

There is interest in the possible use of *Sceletium* preparations in functional foods, beverages and supplements for promoting health and wellness in healthy people, in people experiencing stress, and for treating people with a variety of psychological and psychiatric disorders including anxiety and depression (Gericke and Viljoen, 2008). Despite the long documented history of the plant being used as a masticatory, health tea, and as a herbal medicine, there are few reports on its pharmacological activity (Smith et al., 1996). Its claimed effectiveness in cases of depression has been linked to inhibitory effects on 5-HT reuptake (Gericke and Viljoen, 2008), although such effects have not been extensively studied. The present results demonstrate that the proprietary standardised extract of *Sceletium tortuosum* known as Zembrin® does have potent inhibitory effects on the 5-HT transporter. Considering that a plant extract will have several hundred components, it is perhaps surprising that the *Sceletium* extract affected so few sites in the extensive panel of receptors, ion channels, transporters and enzymes used in this study. Potent inhibitory activity was also found against PDE4 and, to a lesser extent, PDE3, but not to other PDEs. There were also significant reductions in binding of radioligands at a few receptors: GABA receptors, δ_2 opioid receptors, μ -opioid receptors, cholecystinin-1 (or -A) receptors, EP4 prostaglandin receptors and melatonin-1 receptors. However, the concentrations needed were 30–150 times greater than those needed with the 5-HT transporter assays. Consequently, clinical effects of extract *Sceletium tortuosum* (Zembrin®) are likely to stem from their inhibitory effects on 5-HT uptake processes and PDE4 activity. Selective serotonin reuptake inhibitors are well-known as antidepressants, and PDE4 inhibitors have also attracted considerable attention as potential antidepressants, although the pharmaceutical compounds tested clinically have had dose-limiting side effects of nausea and emesis (Kanes et al., 2007). There is substantial experimental evidence from animal models

that PDE4 inhibitors can reverse depression, improve cognition and alleviate anxiety (O'Donnell and Zhang, 2004; Rutten et al., 2006). There is also a positive finding in an animal test of a PDE4 inhibitor relating to schizophrenia (Kanes et al., 2007), and there is evidence for a synergistic effect of PDE4 inhibition combined with monoamine-uptake inhibitors (Fujimaki et al., 1999). Therefore, the finding that the extract *Sceletium tortuosum* Zembrin® was a powerful inhibitor of both 5-HT uptake and PDE4 activity could be highly significant in terms of its health application in humans. Recently, the therapeutic advantages of dual 5-HT uptake inhibition and PDE4 inhibition have been discussed (Cashman et al., 2009), and include the possibility of using a lower dose to achieve enhanced efficacy with a reduced side-effect profile.

In order to establish the most likely active components of extract *Sceletium tortuosum* (Zembrin®), the activities of the isolated three main alkaloids present in the extract (mesembrenol, mesembrenone and mesembrine) were studied on the 5-HT transporter, and on PDE4. All three were potently active in the 5-HT transporter binding assay (K_i 's 1–60 nM) and against PDE4B activity (IC_{50} 's 0.5–16 μM). Mesembrenone is the closest to being a “dual-acting” 5-HT uptake and PDE4 inhibitor because the difference between concentrations for 50% effect on the two assays was 17 times, whereas it was 258 times for mesembrenol and 5500 for mesembrine. Conversely, mesembrine was the compound showing most selectivity for the 5-HT transporter over PDE4B.

Acknowledgement

We thank HL Hall & Sons Ltd. for financial support.

References

- Cashman, J.R., Voelker, T., Johnson, R., Janowsky, A., 2009. Stereoselective inhibition of serotonin re-uptake and phosphodiesterase by dual inhibitors as potential agents for depression. *Bioorganic and Medicinal Chemistry* 17, 337–343.
- 2011. Dictionary of Natural Products on DVD. Version 19.2. CRC Press, USA.
- Fujimaki, K., Morinobu, S., Duman, R.S., 1999. Administration of a cAMP phosphodiesterase 4 inhibitor enhances antidepressant-induction of BDNF mRNA in rat hippocampus. *Neuropsychopharmacology* 22, 42–51.
- Gericke, N., Viljoen, A.M., 2008. Sceletium—a review update. *Journal of Ethnopharmacology* 119, 653–663.
- Hirabayashi, M., Ichikawa, K., Fukushima, R., Uchino, T., Shimada, K., 2002. Clinical application of South African tea on dementia dog. *Japanese Journal of Small Animals Practice* 21, 109–112.
- Hirabayashi, M., Ichikawa, K., Yoshi, A., Uchino, T., Shimada, K., 2004. Clinical effects of South African tea for cat. *Japanese Journal of Small Animals Practice* 23, 85–88.
- Hirai, M., Ichikawa, K., Yoshi, A., Shimada, K., Uchino, T., 2005. Clinical effects of South African tea for dementia animal. *Japanese Journal of Small Animals Practice* 24, 27–31.
- Jeffer, P.W., Hawks, R.L., Farrier, D.S., 1970. Structures of the mesembranols and the absolute configuration of mesembrine and related alkaloids. *Journal of the American Chemical Society* 91, 3831–3839.

Table 2

Summary of analyses of the concentration–response curves of the three alkaloids on binding to the 5-HT transporter and on activity of PDE4B.

Compound	5-HT transporter		PDE4B	
	K_i (nM)	n_H	IC_{50} (nM)	n_H
Mesembrine	1.4	1.0	7800	1.3
Mesembrenone	27	1.0	470	0.8
Mesembrenol	62	1.1	16,000	1.0

n_H , Hill coefficient.

- Kanes, S.J., Tokarczyk, J., Siegel, S.J., Bilker, W., Abel, T., Kelly, M.P., 2007. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. *Neuroscience* 144, 239–246.
- Klak, C., Bruyns, P.V., Hedderson, T.A.J., 2007. A phylogeny and new classification for Mesembryanthemoideae (Aizoaceae). *Taxon* 56, 737–756.
- Napoletano, M., Fraire, C., Santangelo, F., Moriggi, E., 2001. Mesembrine is an inhibitor of PDE4 that follows structure-activity relationship of rolipram. *CPS: medicchem/0103001*.
- O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cytotoxicity. *European Journal of Biochemistry* 267, 5421–5426.
- O'Donnell, J.M., Zhang, H.T., 2004. Antidepressant effects of inhibitors of cAMP phosphodiesterase (PDE4). *Trends in Pharmacological Sciences* 25, 158–163.
- Rutten, K., Prickaerts, J., Blokland, A., 2006. Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. *Neurobiology of Learning and Memory* 85, 132–138.
- Smith, C., 2011. The effects of *Sceletium tortuosum* in an *in vivo* model of psychological stress. *Journal of Ethnopharmacology* 133, 31–36.
- Smith, M.T., Couch, N.R., Gericke, N., Hirst, M., 1996. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: a review. *Journal of Ethnopharmacology* 50, 119–130.
- US Patent, 2001. Pharmaceutical compositions containing mesembrine and related compounds. *United States Patent* 6,288,104.
- Weniger, B., Italiano, L., Beck, J.-P., Bastida, J., Bergoñon, S., Codina, C., Lobstein, A., Anton, R., 1995. Cytotoxic activity of Amaryllidaceae alkaloids. *Planta Medica* 61, 77–79.
- Zhou, M., Diwu, Z., Panchuk-Voloshina, N., Haugland, R.P., 1997. A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases. *Analytical Biochemistry* 253, 162–168.