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Effects of South African traditional medicine in animal models for depression

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ABSTRACT

Ethnopharmacological relevance: The four South African medicinal plants *Agapanthus campanulatus* (AC), *Boophone distica* (BD), *Mondia whitei* (MW) and *Xysmalobium undulatum* (XU) are used in traditional medicine to treat depression.
Aim: To evaluate the effect of ethanolic extracts of the plants in models for depression.
Materials and methods: The extracts were screened for affinity for the serotonin transporter (SERT) in the [^{3}H]-citalopram-binding assay. The inhibitory potency of the extracts towards the SERT, the noradrenalin transporter (NAT) and the dopamine transporter (DAT) were determined in a functional uptake inhibition assay. Antidepressant-like effects of the extracts were investigated using the tail suspension test (TST) and the forced swim test in both rats (rFST) and mice (mFST).
Results: All four plants showed affinity for SERT in the binding assay. AC and BD showed functional inhibition of SERT, NAT and DAT, MW affected SERT while XU showed no effect. BD showed significant effect in the TST and in the mFST/rFST, AC showed significant effect in mFST, MW showed significant effect in the rFST and XU showed significant effect in the mFST.
Conclusion: In this study we have demonstrated the antidepressant activity of four South African medicinal plants *in vitro* and *in vivo*, supporting their rational use in traditional medicine.

1. Introduction

Depression is a recurrent, life-threatening heterogeneous disorder with a diverse group of symptoms at the psychological, behavioural and physiological level. It is a serious disorder with an estimate of lifetime prevalence as high as 20% and a significant number of patients (30%) do not respond to current medical treatment (Charney et al., 2002; Cryan et al., 2002).

Several neurotransmitters are believed to be involved in the pathophysiology of depression including serotonin, noradrenalin and dopamine (Dailly et al., 2004; Moltzen and Bang-Andersen, 2006). The monoamine hypothesis is based on the assumption that depression is due to deficiency of one or another of these neurotransmitters (Rang et al., 2007) although many other factors are believed to be involved, including the hypothalamic-pituitary-adrenal axis (Hindmarch, 2002).

The four plants, *Agapanthus campanulatus* F.M. Leighton (Alliaceae), *Boophone distica* (L.f.) herb (Amarylliaceae), *Mondia whitei* (Hook.f.) Skeels (Asclepiadeace) and *Xysmalobium undulatum* (L.) Aiton.f. (Asclepiadeace) are used in southern Africa to treat mental illnesses related to depression (Table 1). In a preliminary screening of 34 plants used for treatment of depression, hydro-ethanolic extracts from various parts of the four plants showed affinity to the serotonin transporter (SERT) (Nielsen et al., 2004).

In the present study, ethanolic extracts from the four plants were screened for affinity to the SERT and for inhibitory effects on the SERT, the noradrenalin transporter (NAT) and the dopamine transporter (DAT). Furthermore, extracts were tested in animal models for depression to evaluate the antidepressant-like effects of the plants.

Abbreviations: AC, *Agapanthus campanulatus*; BD, *Boophone distica*; DAT, dopamine transporter; mFST, forced swim test in mice; MW, *Mondia whitei*; NAT, noradrenalin transporter; rFST, forced swim test in rats; SERT, serotonin transporter; TST, tail suspension test; XU, *Xysmalobium undulatum*.

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Table 1
Traditional uses of the four plants investigated in this report

Family *Species* Annotations	Voucher specimen	Traditional use, ethnobotanical information and known constituents
Alliaceae		
Agapanthus campanulatus F.M.Leight. (sometimes included in Agapanthaceae) Syn: *Agapanthus patens* F.M.Leight.	*Stafford 59 NU*	Used in the initiation of traditional healers (Hutchings et al., 1996). Various parts are used by the Sotho to treat people with a type of mental illness known as 'the spirit' (Laydevant, 1932). The Zulu are reported to use unidentified species of *Agapanthus* for inducing visions (*imibono*) and dreams (Sobiecki, 2002). Extracts exhibited SSRI activity (Nielsen et al., 2004)
Amaryllidaceae		
Boophone distica (L.f.) Herb. Syn: *Boophane distica* (L.f.) Herb.; *Boophane longepedicellata* Pax	*Stafford 53 NU*	Traditional healers and patients in South Africa drink bulb infusions to induce hallucinations for divinatory purposes, and also as a medicine to treat mental illness (Sobiecki, 2002). Weak decoctions of bulb scales given to sedate violent, psychotic patients (Van Wyk and Gericke, 2000). The alkaloids buphanidrine and buphanamine isolated from the bulb exhibit SSRI activity (Sandager et al., 2005)
Asclepiadaceae		
Mondia whitei (Hook. f.) Skeels Syn: *Chlorocodon whitei* Hook.f. (sometimes included in Periplocaceae)	*Stafford 43 NU*	The Zulu chew the roots to stimulate appetite (Bryant, 1966; Gerstner, 1941). Roots are used as an aphrodisiac in Zimbabwe (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985). The Shambala use root infusions to treat fits in children (Watt and Breyer-Brandwijk, 1962). Used by unspecified groups in South Africa to treat stress and tension in adults (Van Wyk and Gericke, 2000)
Xysmalobium undulatum (L.) Aiton.f.	*Stafford 47 NU*	Roots administered in the Transkei by Xhosa to treat hysteria (Hutchings et al., 1996). Extracts have exhibited weak CNS depressant and antidepressant activity (Hutchings et al., 1996). Leaf extracts exhibited SSRI activity (Nielsen et al., 2004)

2. Materials and methods

2.1. Preparation of extracts

Plants were collected in KwaZulu-Natal, South Africa. Voucher specimens are deposited in the University of KwaZulu-Natal Herbarium (Table 1). Plant material was dried at 50 °C for a maximum of 2 days.

Dried ground material was extracted three times with ethanol (1:10, w/v) for 60 min in an ultrasound bath. The extracts were then filtered under vacuum through filter paper (Whatman No. 1) and evaporated to dryness under reduced pressure at 40 °C finally giving 12.9%, 8.4%, 8.6%, and 6.7% yield for *Agapanthus campanulatus*, *Boophone distica*, *Mondia whitei* and *Xysmalobium undulatum*, respectively. The dry extracts were stored at 5 °C for not more than 2 weeks.

2.2. In vitro assays

2.2.1. Phytochemical fingerprints

Ethanolic extracts of the four plants were evaluated in a flavonoid and an alkaloid system (Wagner and Bladt, 1996). Extracts (50 μg) were applied to three Merck Silica gel 60_{F254} plate and eluted in ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26) (one flavonoid plate) or in toluene:ethyl acetate:diethylamine (70:20:10) (two alkaloid plates).

The flavonoid plate was sprayed with natural products spray (1% methanolic diphenylboric acid-β-ethylamino ester) followed by 5% ethanolic polyethylene glycol 4000. The plate was viewed under 365 nm. The plate was then sprayed with anisaldehyde–sulphuric acid and viewed under visual light. Photography was performed with a CAMAG Reprostar 3 system.

The two alkaloid plates were sprayed with Dragendorf's reagent and cobalt thiocyanate, respectively, and viewed under visual light. The ethanolic extracts were screened for tannin content using ferric chloride reagents (Wagner and Bladt, 1996).

2.2.2. [^{3}H]-citalopram-binding assay

The binding assay was carried out according to the previously published methods (Plenge et al., 1990; Nielsen et al., 2004). Whole rat brains, except cerebellum, were homogenized with an Ultra Turax homogenizer in 1:10 (w/v) buffer (Tris base 5 mM; NaCl 150 mM; EDTA 20 mM; pH 7.5). The homogenate was centrifuged at 16,000 × *g* for 10 min and the homogenized tissue pellet washed with 1:10 (w/v) of the same buffer. The supernatant was discarded; the pellet was suspended in buffer (Tris base 5 mM; EDTA 5 mM; pH 7.5), left for 20 min and centrifuged at 16,000 × *g* for 10 min. The supernatant was discarded and the pellet was suspended in 1:10 (w/v) buffer (Tris base 50 mM; NaCl 120 mM; KCl 5 mM; pH 7.5) and centrifuged at 16,000 × *g* for 10 min. The supernatant was discarded and the protein pellet finally suspended in 120 ml of the same buffer. The tissue homogenate was kept at −70 °C until use.

Twenty-five microliters of dilution of the plant extracts (12, 1.2, 0.12, 0.012 and 0.0012 mg/ml) in buffer (Tris base 50 mM; NaCl 120 mM; KCl 5 mM; pH 7.5) making a final concentration in the assay of 1, 0.1, 0.01, 0.001 and 0.0001 mg/ml were mixed with 50 μl [^{3}H]-citalopram (4 nM, final concentration in assay was 0.67 nM) and 225 μl tissue suspension in the listed order. For determination of non-specific binding, 25 μl 1.5 μM paroxetine were mixed with 50 μl [^{3}H]-citalopram and 225 μl tissue suspension. The total binding of [^{3}H]-citalopram was determined by mixing 25 μl buffer with 50 μl [^{3}H]-citalopram and 225 μl tissue suspension. All samples were left for equilibration for 2 h at room temperature. After incubation 5 ml of ice-cold buffer were added to the samples and the mixture poured directly onto glass fibre filters (Advantec GC50) under vacuum, and immediately washed once with 5 ml of ice-cold buffer. The amount of radioactivity was determined by conventional liquid scintillation counting using Ultimo Gold XR as scintillation fluid. Specific binding was calculated as total binding minus unspecific binding. All experiments were done in triplicate.

2.2.3. SERT, NAT and DAT uptake inhibition assays

The uptake inhibition assay was carried out as described in detail elsewhere (Kristensen et al., 2004). Briefly, COS-7 cells were