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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.
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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

cultured in Dulbecco's modified Eagle's medium with 10% foetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C in a humidified 5% CO_2 environment. Human SERT, NAT and DAT clones were transfected in COS-7 cells using TransIT transfection reagent (Mirus Inc., Madison, WI), following the protocol supplied by the manufacturer. Subsequently, cells were dispensed into poly-D-lysine coated white 96-well plates at 50% confluence. The transfection efficacy was the same for all assays. Uptake inhibition assays were performed +40 h after transfection when cells were confluent. The medium was removed and the cells were washed twice with phosphate-buffered saline (NaCl 137 mM; KCl 2.7 mM; Na_2HPO_4 4.3 mM; KH_2PO_4 1.4 mM; pH 7.3) containing 0.5 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBSCM). After washing, cells were incubated for 30 min in PBSCM containing 50 nM [^{3}H]-5-HT (SERT assay) or 50 nM [^{3}H]-dopamine (NAT and DAT assays) and increasing concentrations of extracts. Uptake was terminated by washing twice with PBSCM. All washing steps were carried out with an automatic plate washer (ELx50 Microplate Strip Washer from Biotek). The amount of accumulated [^{3}H]-5-HT or [^{3}H]-dopamine was determined by solubilizing cells in scintillant (MicroScint-20) followed by direct counting of plates in a Packard TopCounter. Specific uptake was calculated by subtracting uptake values from control values (wells lacking extracts). Assays were carried out in triplicate and repeated four to six times.

2.3. In vivo models

2.3.1. Animals and drug administration

Male Wistar rats (220–300 g), male albino Swiss mice (25–30 g) and male C57BL/6J mice (19–27 g) were kept in a 12 h light/dark cycle at a constant temperature of 22 ± 1 °C with free access to food (standard laboratory pellets) and water prior to the experiments. The vehicle was Tween 80:water (1:10) in which the extracts were suspended and imipramine (hydrochloride, Polfa-Starogard, Poland) and desipramine (hydrochloride, Polfa-Starogard, Poland) were dissolved. All experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków and carried out in compliance with the EC Directive 86/609/EEC.

2.3.2. Tail suspension test in mice

The experiment was carried out according to a method adapted from Steru et al. (1985) with the use of C57BL/6J mice due to no effect of positive control (imipramine) in Albino Swiss mice under our laboratory conditions (Wesolowska et al., 2006). Briefly, male C57BL/6J mice were individually hung by the tail using adhesive tape placed approximately 1 cm from the tip of the tail attached to the edge of a tabletop and hanging 75 cm above the floor. The total duration of immobility was scored manually during a 6-min test session. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration. Resulting immobility were converted to percent of vehicle control.

Vehicle, imipramine (20 mg/kg) or plant extract (125, 250, 500 mg/kg) were administrated orally by gavages 30 min before the assay. Groups consisted of a minimum of eight mice each. Resulting immobility were converted to percent of vehicle control.

2.3.3. Forced swim test in mice (mFST)

Antidepressant-like effects of the extracts were also tested in the forced swim test according to a method with modifications adapted from Porsolt et al. (1977). In short: Mice were individually placed into separate 2000-ml glass beakers containing a column of 7 cm of water at 24 ± 1 °C. The total immobility time of the mice was assessed during the last 4 min of a 6-min observation period. Immobility was defined as the absence of movement except for movement in order to keep the head above water.

Vehicle, imipramine (20 mg/kg) or plant extract (125, 250, and 500 mg/kg) were administrated orally by gavages 30 min before the assay. Groups consisted of a minimum of nine mice each. Resulting immobility were converted to percent of vehicle control.

2.3.4. Forced swim test in rats (rFST)

To investigate species differences of the antidepressant-like effect the extracts were tested in the forced swim test according to methods with modifications adapted from Porsolt et al. (1978). The method resembles the above described for mice but with some differences. In short, rats were individually placed into separate plastic cylinders (height 40 cm, diameter 18 cm) containing a column of 25 cm of water at 25 ± 1 °C. The rats learned in a pre-test of 15 min that they could not escape from the cylinder. In the test period, 24 h later, the total immobility time of the rats was assessed during a 5-min period observation period. Vehicle, imipramine (30 mg/kg) or plant extract (125, 250, and 500 mg/kg) were administrated orally by gavages 1, 5 and 24 h before the test. Groups consisted of a minimum of eight rats each. Resulting immobility were converted to percent of vehicle control.

2.3.5. Locomotor activity

In order to investigate whether changes in immobility were due to changes in motor activity, e.g. hyperactivity as described elsewhere (Porsolt et al., 1978), the spontaneous activity of animals treated with 500 mg/kg of the four extracts were recorded in photoresistor actometers illuminated by two light-beams, which were connected to a counter for the recording of light-beam interruptions.

Mice and rats were individually placed in the actometers and the number of light-beam crossings was counted during the first 10-min and during 30-min experimental sessions.

2.4. Statistical analysis

All the data are presented as the mean ± S.E.M. The results from the SERT, NAT and DAT uptake inhibition assays were evaluated by an unpaired *t*-test or by a one-way ANOVA followed by a Newman–Keuls multiple comparison test where applicable. The *in vivo* data were evaluated by a one-way ANOVA, followed by Dunnet's Multiple Comparison Test.

3. Results

3.1. Phytochemical fingerprints

TLC profiles were prepared in order to chemically characterize the extracts (Fig. 1). Flavonoids and cinnamic acid derivatives were detected as orange or bright white bands in all the extracts. All extracts contained several compounds detected with anisaldehyde–sulphuric acid, likely to be of terpenoid nature. Positive reactions with Dragendorf's reagent and cobalt thiocyanate were observed for *Boophone distica* and *Xysmalobium undulatum* indicating presence of alkaloids.

3.2. [^{3}H]-citalopram-binding assay

All four extracts inhibited the binding of [^{3}H]-citalopram (Fig. 2) with IC_{50} values (mg dry extract/ml) of 4.9 ± 1.3, 0.5 ± 1.5, 2.2 ± 1.4 and 1.1 ± 2.3 for AC, BD, MW and XU, respectively.

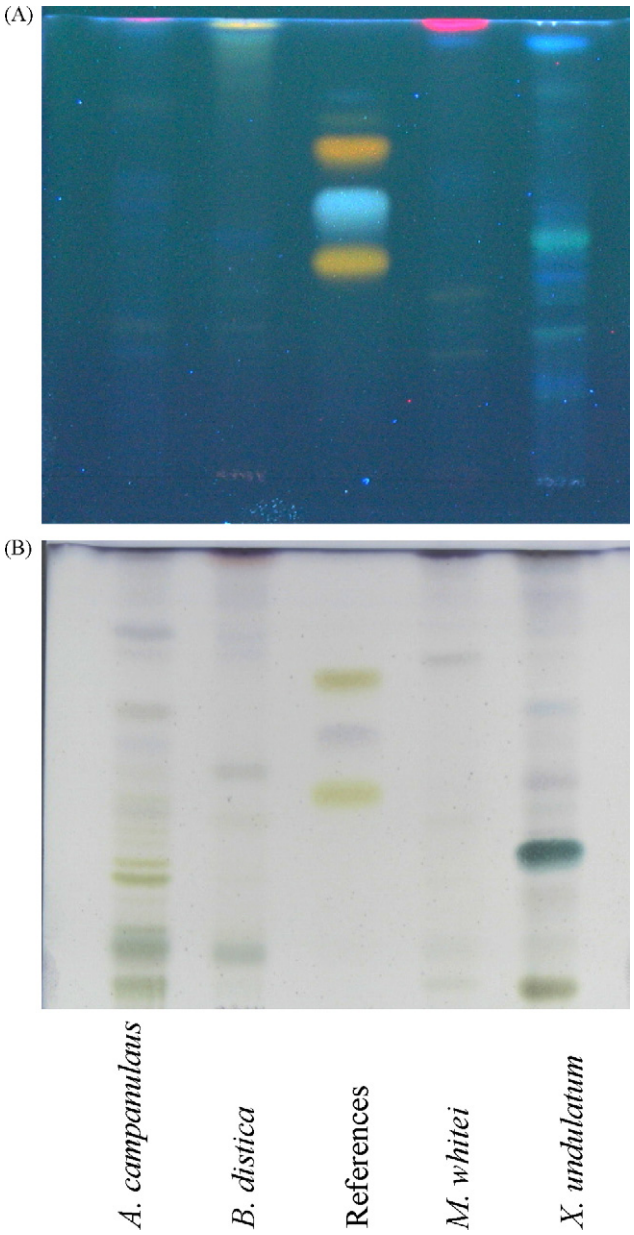

Fig. 1. TLC fingerprints of the ethanolic extracts on plates sprayed with natural product reagent (A) and PEG for detection of flavonoids, viewed under 365 nm; sprayed with anisaldehyde–sulphuric acid (B) for detection of terpenoids, saponins and propylpropanoids. References: rutin, chlorogenic acid:hyperoside.

3.3. SERT, NAT and DAT uptake inhibition assays

The results of the functional inhibition of SERT, NAT and DAT are presented in Table 2. The extract of AC (IC_{50} = 99.4 μg/ml) inhibited SERT significantly more than BD (IC_{50} = 423.8 μg/ml) and MW (IC_{50} = 283.0 μg/ml). AC (IC_{50} = 84.9 μg/ml) and BD (IC_{50} = 77.3 μg/ml) were equally potent inhibitors of NAT, while MW had no significant effect on this transporter. The uptake activity of DAT was not significantly affected by MW, but AC (IC_{50} = 76.2 μg/ml) inhibited DAT significantly more than BD (IC_{50} = 93.5 μg/ml). XU had no significant effect on SERT, NAT or DAT at the tested concentrations.

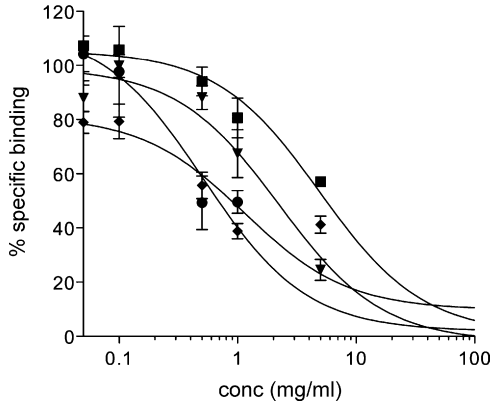

Fig. 2. Inhibition of binding of [^{3}H]-citalopram by extracts from *Agapanthus campanulatus* (squares), *Boophone distica* (circles), *Mondia whitei* (triangles) and *Xysmalobium undulatum* (diamonds). The crude ethanolic extracts displaced the specific binding of [^{3}H]-citalopram indicating affinity for the SERT. Data points are presented as mean ± S.E.M.

3.4. Antidepressant-like effects in tail suspension test

BD at a dose of 125 mg/kg (but not 250 or 500 mg/kg) significantly exhibited an antidepressant-like activity in the TST (Fig. 3). None of the other extracts had any significant effects.

3.5. Antidepressant-like effects in forced swim test

The results of the mFST are presented in Table 3. Extracts of AC exhibited an antidepressant-like activity in a dose dependant manner in mFST with the dose of 125 mg/kg showing no effect and the doses of 250 and 500 mg/kg producing increasing effects with increasing doses. The extracts of BD and the extracts of XU exhibited an antidepressant-like activity at doses of 250 and 500 mg/kg with the doses of 125 mg/kg having no effect. The extracts of MW did not show any antidepressant-like activity in the mFST at any dose.

When tested in the rFST no significant effects were observed at the low dose (125 mg/kg) or the high dose (500 mg/kg) of any extract. The extracts of BD and MW exhibited antidepressant-like effects at 250 mg/kg.

3.6. Effects on locomotor activity

Not one of the four extracts changed the spontaneous activity of the mice or rats.

Table 2
Inhibitory potencies of the four ethanolic plant extracts determined on human SERT, NAT and DAT

Drug	SERT IC_{50} (μg/ml)	NAT IC_{50} (μg/ml)	DAT IC_{50} (μg/ml)
AC	99.4 ± 14.6	84.9 ± 13.3	76.2 ± 1.5
BD	423.8 ± 51.8	77.3 ± 9.8	93.5 ± 11.5
MW	283.0 ± 38.6	n.d.	n.d.
XU	n.d.	n.d.	n.d.
Statistic analysis			
AC vs. BD	***	n.s.	*
AC vs. MW	***		
MW vs. BD	*		

Data are presented as mean ± S.E.M from a minimum of 4 experiments each performed in triplicate. Data were evaluated by an unpaired *t*-test or an ANOVA, followed by Newman–Keuls Multiple Comparison Test where applicable. n.d., not detectable in the tested concentrations. ***$p < 0.001$, *$p < 0.05$, n.s., not significant.

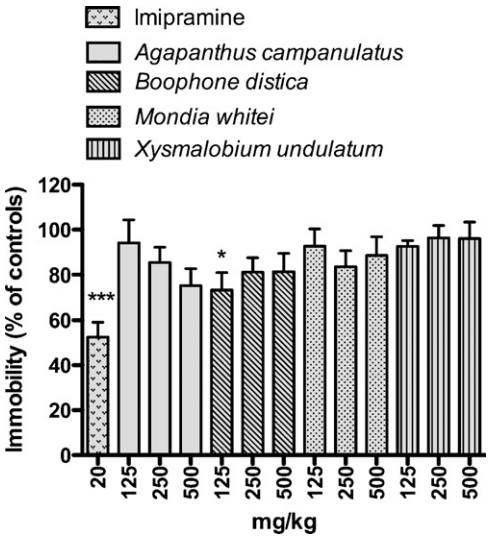

Fig. 3. Effects of *Agapanthus campanulatus*, *Boophone distica*, *Mondia whitei* and *Xysmalobium undulatum* in the tail suspension test in mice. Imipramine was the positive control. All doses were administrated orally 30 min before the test ($n \geq 8$). $^{***}p < 0.0001$, $^{*}p < 0.5$ vs. vehicle group.

4. Discussion

Agapanthus campanulatus (AC), *Boophone distica* (BD), *Mondia whitei* (MW) and *Xysmalobium undulatum* (XU) are used in traditional South African medicine to treat various mental illnesses that resemble the Western definition of depression.

The phytochemical investigation showed presence of alkaloids in *Boophone distica*. A false positive reaction with Dragendorf's reagent and cobalt thiocyanate was observed in *Xysmalobium undulatum* at $R_f = 0.15$, which was due to a reaction with the conjugated lactone in xysmalorin and uzarin (Fig. 4) (Pedersen et al., unpublished data) similar to the previously reported false positive reaction with digitoxin (Farnsworth et al., 1962). No alkaloids were detected in *Agapanthus campanulatus* and *Mondia whitei*. Alkaloids have previously showed affinity to the SERT (Sandager et al., 2005).

Flavonoids were detected in all four extracts (Fig. 1). Previous studies have shown antidepressant-like effect of flavonoids from *Hypericum perforatum* L. (Clusiaceae) in the forced swim test (Butterweck et al., 2000).

Table 3
Effects in the mice forced swim test (mFST) shown as relative immobility in percent of vehicle control

Drug	Dose (mg/kg)	Relative immobility (% of vehicle control)
Controls		
Imipramine	20	74.2 ± 3.7***
Desipramine	20	54.4 ± 4.8***
AC	125	89.4 ± 5.5 n.s.
	250	74.4 ± 5.3***
	500	62.3 ± 5.5***
BD	125	101.1 ± 5.2 n.s.
	250	84.9 ± 3.7*
	500	83.3 ± 5.9*
MW	125	92.9 ± 4.8 n.s.
	250	95.3 ± 4.6 n.s.
	500	91.7 ± 6.1 n.s.
XU	125	83.7 ± 4.8 n.s.
	250	77.6 ± 6.0*
	500	67.9 ± 8.2 **

Data are presented as mean ± S.E.M. Data were evaluated by an ANOVA, followed by Dunnet's Multiple Comparison Test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s., not significant.

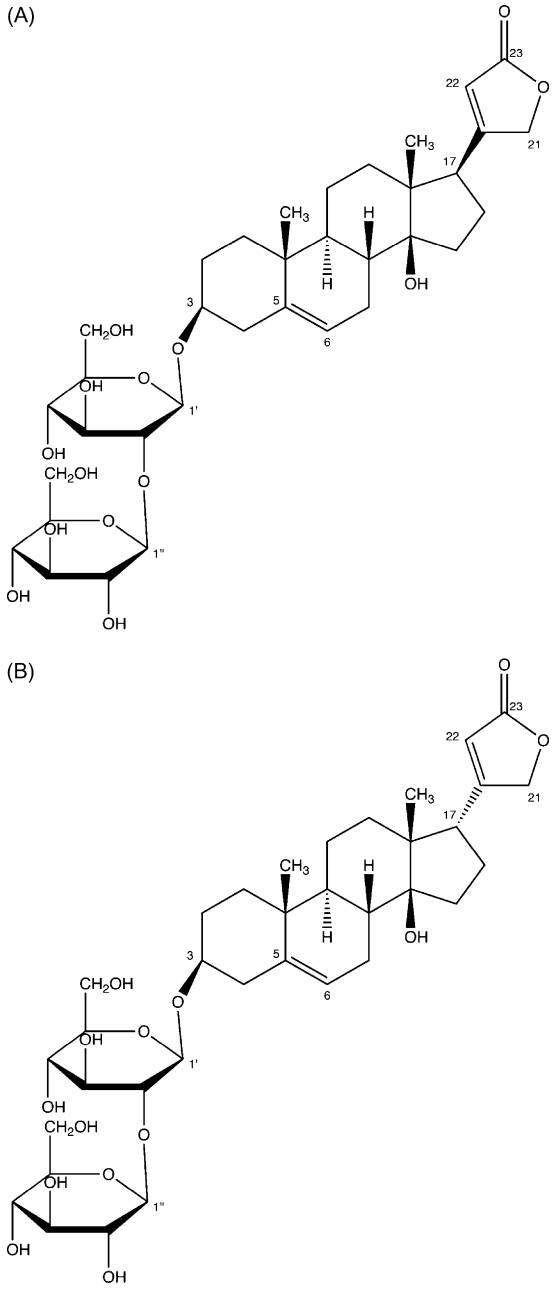

Fig. 4. Structures of (A) xysmalorin and (B) uzarin isolated from *Xysmalobium undulatum* resulting in a false positive reaction with Dragendorf's reagent.

Tannins can coagulate and precipitate proteins (Evans, 2001) and thus lead to false negative/positive results in the binding assay. However, the screenings for tannins were negative for all four extracts.

The screening in the [^{3}H]-citalopram-binding assay demonstrated presence of compounds with affinity for SERT in the crude ethanolic extracts. The different findings in previous studies (Nielsen et al., 2004) are probably due to the use of a hydroethanolic solvent for extraction versus pure ethanol as solvent in this study.

To evaluate the effect of the binding to SERT, the extracts were tested for functional inhibition of SERT. The functional characterization of the extracts of AC and BD demonstrated inhibition of SERT,

NAT and DAT. This triple uptake inhibitor action might be an advantage in the clinical treatment of mental diseases due to enhanced antiobesity activity (Tizzano et al., 2008). It is generally being recognized that a balanced modulation of several targets might provide a superior therapeutic effect and side effect profile compared to the action of a selective ligand (Wermuth, 2004).

The extracts were tested in widely used and well-established screening paradigms for detecting compounds with antidepressant activity in animals: the TST and FST. The extract of BD exhibited antidepressant-like effects in the TST at the lowest concentration. The higher concentrations caused a loss of the effect, which could indicate a bell-shaped dose–response curve similar to that of citalopram in certain mouse strains (Crowley et al., 2006). This may indicate that even lower doses of BD are more active than the one tested. None of the other extracts had a significant effect in the TST.

The FST was conducted in both mice and rats to evaluate possible variation in antidepressant-like responses in different animal species. Similar results across species were found for BD but the results from the other extracts did not correlate with the findings in the mFST. The extracts of AC and XU exhibited a clear dose-dependent effect in the mouse but did not show any effect in the rat. On the other hand, the extract of MW had a significant effect (250 mg/kg) in the rat but no effect was observed in the mouse.

The inconsistent findings between the TST and the FST together with the mismatch between the rat and the mouse models illustrate the distinct differences between the two species. These inter-strain and interspecies variations in the FST have previously been reported (Bai et al., 2001; Cryan et al., 2002) and show the difficulties involved in testing compounds in animal models for depression. The FST has not traditionally been viewed as a consistently sensitive model for detecting SSRI activity. These antidepressants are generally reported to show effect in the TST (Cryan et al., 2005). The data in the present study suggest that strain and species comparisons might be needed to prevent false negative screening of compounds in various tasks.

In the FST, rats were administrated three times (24 h; 5 h and 1 h before test) to prevent false positive/negative results (Porsolt et al., 1978) whereas mice only received one dose 30 min prior to testing. The difference in response in the two FST might be explained by these differences in dosage regime even though one dose exposure in mice previous has proven to generate a stable immobility by acute pre-treatment with antidepressant agents (Cryan et al., 2002) (Table 4).

Table 4
Effects in the rat forced swim test (rFST) shown as relative immobility in percent of vehicle control

Drug	Dose (mg/kg)	Relative immobility (% of vehicle control)
Controls		
Imipramine	30	60.7 ± 4.4 ***
Desipramine	30	48.4 ± 5.2 ***
AC	125	101.7 ± 5.1 n.s.
	250	91.2 ± 5.9 n.s.
	500	102.6 ± 6.5 n.s.
BD	125	95.2 ± 7.2 n.s.
	250	74.2 ± 6.4 ***
	500	105.0 ± 4.9 n.s.
MW	125	83.9 ± 9.6 n.s.
	250	69.9 ± 5.3 ***
	500	111.6 ± 8.4 n.s.
XU	125	98.9 ± 6.1 n.s.
	250	85.8 ± 5.0 n.s.
	500	107.7 ± 9.6 n.s.

Data are presented as mean ± S.E.M. Data were evaluated by an ANOVA, followed by Dunnet's Multiple Comparison Test. ***$p < 0.001$, n.s., not significant.

Mice treated with XU developed a distinct behavioural pattern after some time. They tended to move close to the ground in hypolocomotion and in a circular movement with frequently spontaneous rotating Straub tail, tics, tremor—a phenotype generally consistent with 'serotonin syndrome'-like behaviour involved in SERT knockout mice (Fox et al., 2007; Kalueff et al., 2007). The extract of XU showed affinity to the SERT but did not have any effect at the tested concentrations at either the SERT, NAT or DAT, indicating a different mechanism of action.

The findings in this study demonstrate the potential use of the four medicinal plants to treat mental illnesses related to depression. Extracts from all four plants showed antidepressant-like *in vitro* and *in vivo* effects. This may explain their wide use in traditional South African medicine against depression.

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