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Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport protein

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Abstract

Seventy five extracts from 34 indigenous plant species used in South African traditional medicine or taxonomically related to these were investigated for their affinity to the serotonin reuptake transport protein, making use of an in vitro serotonin reuptake transport protein binding assay. Aqueous and 70% ethanolic extracts of various plant parts were screened and 45 extracts derived from 15 plant species showed affinity. The affinity of 12 extracts from four plants was characterized as high (more than 50% inhibition at 5, 1, and 0.5 mg/ml). Plant species with high affinity to the serotonin reuptake transport protein included *Agapanthus campanulatus*, *Boophane disticha*, *Datura ferox* and *Xysmalobium undulatum*. *Agapanthus campanulatus* yielded high activity in aqueous extracts from leaves and flowers. *Boophane disticha* showed high activity both in aqueous and ethanolic extracts of leaves and bulbs. *Datura ferox* showed high activity in aqueous extracts from the seeds and *Xysmalobium undulatum* showed high activity in the ethanolic extract of the whole plant.

Keywords: Antidepressive plants; Depression; Citalopram; Indigenous plants; Radioligand assay; Serotonin reuptake transport protein; Traditional medicine

1. Introduction

In South Africa there are an estimated 200 000 indigenous healers and there are about 3500 species of higher plants used as traditional medicines (Gericke, 2002). It is possible that many of these plants contain chemical substances which have interesting pharmacological effects. Plants used in traditional South African medicine to treat mental disorders like schizophrenia or depression, so called psychotropic plants, are interesting plants from a pharmacological perspective. Investigation of chemical compounds from these plants for effects on receptors involved in mental disorders might improve our understanding of the use of these plants. Furthermore the discovery of active compounds from these plants may lead to the discovery of newer and better therapeutic agents with fewer side-effects in the treatment of mental disorders. There are examples of plants being used for depression with proven efficiency. *Hypericum perforatum* is used to treat mild depressions in many countries and represent an accepted alternative to synthetic antidepressants. In vitro experiments have shown that extracts from the plant has a clear inhibitory effect on neuronal uptake of serotonin, noradrenalin, dopamine, GABA and L-glutamate (Müller, 2003). Many of these transmitters are, according to the monoamine hypothesis of depression, associated with depression (Rang et al., 1999). Another example is *Sceletium tortuosum*, which has been used by pastoralists and hunter-gatherers in southern Africa as a mood altering substance from prehistoric times. Traditionally prepared dried plant material is chewed, smoked, or powdered and inhaled as a snuff. The plant is said to elevate mood and decrease anxiety, stress and tension. The major alkaloid mesembrine present in the plant is a potent serotonin reuptake inhibitor (Gericke, 2002).

Several antidepressants on the market exert their effect by selective inhibition of serotonin reuptake. These drugs are known as selective serotonin reuptake inhibitors (SSRI) and are currently amongst the most frequently prescribed therapeutic agents. The mechanism of the SSRI is to bind to a

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specific site on the neuronal serotonin transporter (SSRI site) with resulting inhibition of the transportation of serotonin from the synaptic gab back to the neuron (Stahl, 1998).

Screening medicinal plants for affinity to the serotonin transporter is a way to investigate these plants for possible antidepressant effects and a means to find new lead structures in the development of new antidepressant therapeutic agents.

2. Materials and methods

2.1. Plant material

All plant material was selected and collected on the basis of a database on psychotropic plants constructed at the University of Natal. They were collected from January to March 2003 in the province of KwaZulu-Natal, South Africa (Table 1). Freshly collected plant parts were washed thoroughly with water and dried in an oven at 50 °C for 48–72 h. The dried material was ground to a fine powder and stored in plastic bottles in the dark until use.

2.2. Preparation of plant extracts

Two grams of dried, grounded plant material were extracted in either 20 ml demineralized water or 70% ethanol for 60 min in an ultra sonic bath. The extracts were filtered through Whatman No. 1 filter paper and then evaporated to dryness.

2.3. Preparation of tissue suspension

All procedures were carried out at 0–4 °C. Whole rat brains except for the cerebellum from male Wistar rats (±200 g each) were homogenised with an Ultra Turax homogenizer in 1:10 w/v buffer (5 mM TRIS base, 150 mM NaCl and 20 mM EDTA, pH 7.5). The homogenate was centrifuged at 16000 × *g* for 10 min and the homogenized tissue pellet washed with 120 ml of the same buffer. The supernatant was discarded, the pellet was resuspended in buffer (5 mM TRIS base and 5 mM EDTA, pH 7.5), left to react for 20 min and centrifuged at 16000 × *g* for 10 min. The supernatant was discarded and the pellet was resuspended in 120 ml buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) and centrifuged at 16000 × *g* for 10 min. The supernatant was discarded and the protein pellet finally resuspended in 120 ml buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5). The tissue homogenate was kept at −70 °C until use.

2.4. Serotonin reuptake transport protein binding assay

The method described by Plenge et al. (1990) was used. Two hundred microlitre dilution of the extract (5, 1, 0.1, 0.01 and 0.001 mg/ml) in buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) making a final concentration in the assay of 3.3, 0.7, 0.07, 0.007, and 0.0007 mg/ml were mixed with 50 μl 4 nM [^{3}H]citalopram and 50 μl tissue suspension in the listed order. For determination of unspecific binding, 200 μl 1.5 μM paroxetine were mixed with 50 μl 4 nM [^{3}H]citalopram and 50 μl tissue suspension. The total binding of [^{3}H]citalopram was determined by mixing 200 μl buffer (50 mM TRIS base, 120 mM NaCl and 5 mM KCl, pH 7.5) with 50 μl 4 nM [^{3}H]citalopram and 50 μl tissue suspension. All samples were incubated for 2 h at 23–26 °C and filtered through Advantec GC-50/25 glass fibre filters under vacuum. After 24 h the radioactivity of the filters containing protein bounded [^{3}H]citalopram was measured by liquid scintillation, using 5 ml Beckman Ready Value II^{TM} as scintillation fluid.

3. Results and discussion

Four categories were set up to classify the investigated plant extracts. Extracts with high affinity had to exhibit concentration-dependent inhibition with less than 50% [^{3}H]citalopram binding with the three strongest concentrations (5, 1, and 0.1 mg/ml); medium affinity less than 50% [^{3}H]citalopram binding in the two strongest concentrations (5 and 1 mg/ml); low affinity less than 50% [^{3}H]citalopram binding in the strongest concentration (5 mg/ml); extracts with no affinity did not exhibit concentration-dependent inhibition and the obtained [^{3}H]citalopram binding had to be within 70–130%.

Seventy five extracts from 34 plant species were selected for investigation in this study. Thirty seven of these extracts, derived from 15 plants showed some degree of affinity to the serotonin reuptake transport protein (Table 2). Seven extracts from four plants had affinity, characterized as high, nine extracts from eight plants had medium affinity whereas 21 extracts from 11 plants showed low affinity. Thirty eight extracts from 26 plants did not show any affinity (Table 2).

Extracts from *Agapanthus campanulatus*, *Boophane disticha*, *Datura ferox* and *Xysmalobium undulatum* showed high affinity to the serotonin reuptake transport protein in the displacement assay utilized in this study. Aqueous extracts of leaves and flowers from *Agapanthus campanulatus* resulted in displacement of more than 60% transport protein bound [^{3}H]citalopram at the three strongest concentrations. Aqueous and ethanolic extracts of the leaves and the aqueous extract of the bulbs from *Boophane disticha* resulted in displacement of more than 50% of the transport protein bound [^{3}H]citalopram at the three strongest concentrations. *Boophane disticha* is used in traditional medicine for numerous purposes, e.g. hysteria in young women. It is known as a toxic plant and alkaloids from the bulb are known to possess hallucinogenic properties (De Smet, 1996; Du Plooy et al., 2001).

An aqueous extract from the seeds of *Datura ferox* almost displaced 80% transport protein bound [^{3}H]citalopram at the three strongest concentrations. *Datura* species are known to

Table 1
Plant species, family, plants parts and voucher numbers of material collected for screening

Plant species	Voucher specimen	Plant parts investigated
Acokanthera oblongifolia (Hochst.) Codd (Apocynaceae)	*Stafford 14 NU*	Leaves
Agapanthus campanulatus Leighton (Alliaceae)	*Stafford 59 NU*	Leaves, flowers and roots
Alepidea natalensis Wood & Evans (Apiaceae)	*Stafford 61 NU*	Leaves and roots
Artemisia afra Jacq. ex Willd. (Asteraceae)	*Stafford 63 NU*	Leaves
Artemesia dracunculoides Pursh. (Asteraceae)	*Stafford 64 NU*	Leaves
Asclepias fruticosa L. (Asclepiaceae)	*Stafford 65 NU*	Leaves
Boophane disticha (L.f.) Herb (Amaryllidaceae)	*Stafford 53 NU*	Leaves, roots and bulbs
Brunsvigia grandiflora Lindl. (Amaryllidaceae)	*Stafford 10 NU*	Leaves and bulbs
Bulbine frutescens (L.) Willd. (Asphodelaceae)	*Stafford 17 NU*	Roots
Cinnamomum camphora (L.) Presl. (Lauraceae)	*Stafford 69 NU*	Leaves
Clausena anisata (Willd.) Hook.f. (Rutaceae)	*Stafford 47 NU*	Leaves and bark
Conostomium natalense (Hochst.) Bremek. (Rubiaceae)	*Stafford 70 NU*	Leaves and roots
Cotyledon orbiculata L. (Crassulaceae)	*Stafford 71 NU*	Leaves
Datura ferox L. (Solanaceae)	*Stafford 72 NU*	Leaves, flowers and seeds
Datura stramonium L. (Solanaceae)	*Stafford 73 NU*	Leaves, seeds and seedpods
Diclis reptans Benth (Scrophulariaceae)	*Stafford 74 NU*	Leaves
Gasteria croucheri (Liliaceae)	*Stafford 18 NU*	Leaves
Gethyllis ciliaris L.f. (Amaryllidaceae)	*Stafford 76 NU*	Roots
Hemezyga obermeyern (Lamiaceae)	*Stafford 78 NU*	Leaves
Hypericum lanandii Choisy (Clusiaceae)	*Stafford 79 NU*	Leaves
Hypericum revolutum Vahl (Clusiaceae)	*Stafford 80 NU*	Leaves
Indigofera tristis E.Mey. (Fabaceae)	*Stafford 27 NU*	Leaves
Indigofera woodii Bolus (Fabaceae)	*Stafford 28 NU*	Leaves
Leonotis leonurus R.Br. (Lamiaceae)	*Stafford 38 NU*	Leaves
Loebelia alata Labill. (Campanulaceae)	*Stafford 83 NU*	Leaves
Malva parviflora L. (Malvaceae)	*Stafford 57 NU*	Leaves
Mentha aquatica L. (Lamiaceae)	*Stafford 84 NU*	Leaves
Mondia whitei (Hook.f.) Skeels (Periplocaceae)	*Stafford 43 NU*	Leaves and flowers
Olea africana (Mill) P.S. Green (Oleaceae)	*Stafford 87 NU*	Bark
Phytolacca L. (Phytolaccaceae)	*Stafford 88 NU*	Aerial parts
Piper capense L. (Piperaceae)	*Stafford 89 NU*	Leaves and roots
Rubus ludwigii Eckl. et Zeyh. (Rosaceae)	*Stafford 44 NU*	Roots
Stropharanthus speciosus (Ward et Harv.) Reber (Apocynaceae)	*Stafford 94 NU*	Leaves
Xysmalobium undulatum (L.) Aiton.f. (Asclepiadaceae)	*Stafford 95 NU*	Aerial parts and roots
Zanthoxylum ssp. (Rutaceae)	*Stafford 96 NU*	Leaves

contain tropane alkaloids such as hyoscyamine and hyoscine (Evans, 1996), the latter being a muscarinic receptor antagonist and a CNS depressant (Rang et al., 1999).

Xysmalobium undulatum was capable of displacing more than 50% transport protein bound [^{3}H]citalopram at the three strongest concentrations. *Xysmalobium undulatum* is sold as a herbal remedy under the name 'Uzara' in the West, where it is used as an intestinal smooth muscle relaxant. It is known that the $5HT_3$-receptor in the CNS is involved in contraction of intestinal smooth muscle (Stahl, 1998; Rang et al., 1999), so the observed clinical effects could be due to serotonin receptor affinity of compounds in the plant extract.

Different levels of activity were obtained with extracts from different plant parts. Extracts of other plant parts than those that yielded extracts with high affinity, exhibited medium inhibition of [^{3}H]citalopram binding at the serotonin reuptake protein SSRI binding site (Table 2). For example the aqueous extract of *Agapanthus campanulatus* roots and the ethanolic extract of *Boophane disticha* roots. The reason for this could be that the same active compounds are present in various parts of the plants in differing amounts.

The findings in this study are the first step in evaluating the use of these plants as traditional medicines. On the basis of the presented results any general conclusions on the antidepressive effect of the investigated plants cannot be made, as the plants might have other mechanisms of action. Depression includes various mechanisms and only a combination of in vitro and in vivo studies and a pharmacokinetic profile of isolated compounds can allow an in depth evaluation. Some of the plants screened showed interesting activities that warrants further investigation.

Table 2
Screening of plant extracts for affinity to the serotonin transporter protein

Plant species	Plant part	Extract	Yield (mg)	[^{3}H]citalopram binding in percent, (mg/ml)				
				5 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
Acokanthera oblongifolia	Leaves	Aqueous	377	62	95	107	102	100
Agapanthus campanulatus	Leaves	Aqueous	268	29	13	36	55	64
		Ethanolic	367	86	91	91	56	63
	Flowers	Aqueous	143	7	10	24	43	62
		Ethanolic	193	19	69	84	73	85
	Roots	Aqueous	187	9	23	78	97	92
		Ethanolic	286	97	119	93	115	109
Alepidea natalensis	Leaves	Aqueous	262	84	94	105	91	99
	Roots	Aqueous	121	117	115	98	115	126
Artemisia afra	Leaves	Ethanolic	352	28	104	106	110	127
Artemisia dracunculoids	Leaves	Aqueous	308	69	96	106	116	121
		Ethanolic	244	22	85	107	110	103
Asclepias fruticosa	Leaves	Aqueous	337	66	87	92	102	97
		Ethanolic	294	45	99	103	101	101
Boophane disticha	Leaves	Aqueous	212	12	14	42	86	83
		Ethanolic	256	7	8	35	75	91
	Roots	Aqueous	91	79	69	127	126	133
		Ethanolic	101	40	46	64	93	84
	Bulbs	Aqueous	50	34	14	39	75	85
		Ethanolic	307	61	36	83	90	74
Brunsvigia grandiflora	Leaves	Aqueous	265	16	46	82	79	98
	Outer bulb	Aqueous	142	53	79	109	111	107
		Ethanolic	463	31	52	80	96	96
	Inner bulb	Aqueous	216	41	72	97	108	94
		Ethanolic	415	47	65	110	106	94
Bulbine frutescens	Roots	Aqueous	135	38	17	28	7	24
Cinnamomum camphora	Leaves	Aqueous	225	83	101	102	109	110
Clausena anisata	Leaves	Aqueous	156	62	101	125	125	140
		Ethanolic	132	63	73	103	130	129
	Bark	Aqueous	181	94	121	119	116	118
		Ethanolic	345	34	80	93	82	78
Conostonium natalense	Leaves	Aqueous	231	98	128	117	121	127
		Ethanolic	285	77	117	135	116	118
	Roots	Ethanolic	206	53	101	120	119	102
Cotyledon orbiculata	Leaves	Aqueous	732	84	86	74	75	70
Datura ferox	Leaves	Aqueous	148	24	18	80	71	99
	Flowers	Aqueous	151	20	15	75	74	79
	Seeds	Aqueous	126	20	21	13	80	56
Datura stramonium	Leaves	Aqueous	422	14	57	112	123	123
		Ethanolic	329	20	57	110	114	94
	Seeds	Aqueous	94	28	62	90	119	112
		Ethanolic	96	56	45	84	105	102
	Seed pods	Ethanolic	150	33	78	107	117	102
Diclis reptans	Leaves	Aqueous	300	100	107	111	109	108
		Ethanolic	461	70	99	98	108	101
Gasteria crouchii	Leaves	Aqueous	351	76	96	113	113	106
		Ethanolic	280	62	100	104	113	115
Gethyllis ciliaris	Roots	Aqueous	414	73	108	103	118	113
		Ethanolic	357	44	96	110	114	112

Table 2 (*Continued*)

Plant species	Plant part	Extract	Yield (mg)	[^{3}H]citalopram binding in percent, (mg/ml)				
				5 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
Hemezyga obermeyern	Leaves	Aqueous	362	55	105	87	88	99
Hypericum lanandii	Leaves	Aqueous	178	127	105	109	105	92
Hypericum revolutum	Leaves	Aqueous	305	139	93	110	101	114
Indigofera tristis	Leaves	Ethanolic	369	125	134	117	110	124
Leonotis leonurus	Leaves	Ethanolic	318	24	91	131	131	117
Lobelia alata	Leaves	Aqueous	235	75	50	23	22	11
Malva parviflora	Leaves	Aqueous	202	39	62	70	92	94
		Ethanolic	272	6	53	85	92	95
Mentha aquatica	Leaves	Ethanolic	---	67	57	124	134	114
Mondia whitei	Leaves	Aqueous	299	57	77	101	98	99
		Ethanolic	364	4	21	61	83	89
	Flowers	Aqueous	388	41	82	103	106	107
Olea Africana	Bark	Aqueous	186	44	65	89	84	75
		Ethanolic	308	11	80	90	108	104
Phytolacca octandra	Aerial parts	Aqueous	244	67	104	102	106	133
		Ethanolic	35	53	58	92	105	95
Piper capense	Leaves	Aqueous	155	21	40	89	80	73
		Ethanolic	168	73	79	98	96	98
	Roots	Ethanolic	448	68	87	108	110	110
Rubus ludwigii	Roots	Ethanolic	128	171	161	118	104	124
Strophanthus speciosus	Leaves	Aqueous	339	35	56	78	68	73
Xysmalobium undulatum	Aerial parts	Aqueous	332	40	61	73	88	52
		Ethanolic	332	20	31	47	73	70
	Roots	Aqueous	179	51	68	81	117	114
		Ethanolic	165	76	93	107	82	111
Zanthoxylum ssp.	Leaves	Aqueous	182	35	81	106	76	96
		Ethanolic	242	10	47	89	110	112

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