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Screening of plants used in southern Africa for epilepsy and convulsions in the $GABA_A$-benzodiazepine receptor assay

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Abstract

A number of plants are traditionally used to treat mental diseases in South Africa. Aqueous and ethanol extracts of 43 plants that are traditionally used to treat against epilepsy and convulsions, were tested in the $GABA_A$-benzodiazepine receptor binding assay, where the binding of ^{3}H-Ro 15-1788 (flumazenil) to the benzodiazepine site is measured. The $GABA_A$-benzodiazepine receptor complex is involved in epilepsy and convulsions. Out of the 118 extracts tested, one aqueous and 18 ethanol extracts showed activity. The most active extracts were the ethanolic leaf extracts of *Rhus tridentata*, *Rhus rehmanniana* and *Hoslundia opposita* and the ethanolic corm extract of *Hypoxis colchicifolia*, which all showed good dose-dependent activity.

Keywords: Convulsions; Epilepsy; $GABA_A$-benzodiazepine receptor assay; South Africa; Traditional medicine

1. Introduction

It is estimated that about 70–80% of the African population is using traditional medicine totally or partly, this is often the only treatment available (Obijiofor, 2002). In South Africa many of plants are traditionally used to treat mental diseases (Sobiecki, 2002). The following screening is based on plants that are traditionally used against epilepsy and convulsions. Epilepsy is a symptom complex consisting of repeated unprovoked seizures. The seizures are classified according to the area of the brain in which the seizure originates; partial seizures and generalized seizures (Dhillon and Sander, 1999). During seizures some of the patients will experience convulsions. Some common anti-convulsing agents are the benzodiazepines, which bind to the $GABA_A$-benzodiazepine receptor complex where they enhance the affinity for the inhibitory neurotransmitter γ-aminobutyric acid (GABA). A GABA stimulus on the $GABA_A$-receptor causes an influx of chloride ions into the cell. This influx causes hyperpolarization of the membrane, making it more difficult to generate action potential (Ashton, 1999). As a result the cell is inhibited and an anticonvulsant activity is achieved. For this reason it is of interest to find plants that enhance GABA's affinity to the $GABA_A$-receptor.

2. Material and methods

2.1. Plant materials

Plant species traditionally used to treat epilepsy and convulsions, were selected based on information in a database on plants used to treat mental diseases, constructed at the Research Centre for Plant Growth and Development, University of Natal. The information in the database mainly originates from published literature. Plants were collected in KwaZulu-Natal. Voucher specimens are deposited in the University of Natal Herbarium. All plant materials were dried at 50 °C.

2.2. Preparation of extracts

One gram dried, powdered plant material was extracted with 10 ml water or ethanol for 1 h using an ultra-sound

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bath. The extracts were filtered and the solvent was removed under vacuum. The residues were dissolved in water and ethanol respectively at 10 mg ml^{-1}.

2.3. Tissue preparation

The preparation was performed at 0–4 °C. Cerebral cortex from four rats was homogenised for 5 s in 20 ml of Tris–citrate (50 mM, pH 7.1) using an Ultra-Turrax. The suspension was centrifuged at 27 000 × *g* for 15 min, and the pellet was washed three times with buffer. The washed pellet was homogenised in 20 ml of buffer and the suspension was incubated in a water bath (37 °C) for 30 min to remove endogenous GABA. Then the suspension was centrifuged for 10 min at 27 000 × *g*. The final pellet was resuspended in 30 ml buffer and stored in aliquots at −20 °C.

2.4. ^{3}H-*Ro 15-1788 (flumazenil) binding assay*

The assay was carried out according to Kahnberg et al. (2002). The membrane preparation was thawed and washed with 20 ml Tris–citrate (50 mM, pH 7.1, 0–4 °C). The suspension was centrifuged at 0–4 °C for 10 min at 27 000 × *g*. The pellet was resuspended in Tris–citrate (50 mM, pH 7.1, 2 mg original tissue per ml buffer), and then used for the binding assay. Membrane suspension (500 μl) was added to 25 μl test solution (plant extract/standard/blank) and 25 μl of flumazenil (Ro 15-1788, purchased from Perkin-Elmer Life Sciences) (0,5 nM, final concentration in assay), mixed and incubated for 40 min in an ice-bath (0–4 °C). Non-specific binding was determined using clonazepam (1 μM, final concentration in assay) added to separate samples. After incubation 5 ml of ice-cold buffer were added to the samples and the mixture poured directly onto Adventic glass fibre filters (GC-50) under suction, and immediately washed with 5 ml of ice-cold buffer. The amount of radioactivity was determined by conventional liquid scintillation counting. Specific binding was calculated as total binding minus non-specific binding. All experiments were done in triplicate.

3. Results and discussion

Forty three plants were selected for investigation as potential sources of antiepileptic compounds. A total of 118 extracts were tested in the flumazenil binding assay. The binding of ^{3}H-flumazenil obtained with five concentrations of all plant extracts are shown in Table 1. Activity was found almost exclusively in ethanol extracts, only the aqueous extract of *Oncoba spinosa* had some activity at the highest concentration tested. This indicates that the active compounds are of a more apolar nature. Several ethanol extracts had activity at the highest concentration tested, but not at lower concentrations. This could be due to low activity of the extracts or an unspecific interaction with other components of the plant extract.

The most active extracts were the ethanolic leaf extracts of *Rhus tridentata*, *Rhus rehmanniana* and *Hoslundia opposita* and the ethanolic corm extract of *Hypoxis colchicifolia*, which all showed good dose-dependent activity.

The *Rhus* species and *Hypoxis colchicifolia* have not been tested for CNS activity in animal studies, neither have compounds with known CNS activity been isolated from these plants. A chloroform extracts of *Hoslundia opposita* roots has been evaluated for effects on the central nervous system in mice. This extract exhibited anticonvulsant activity, with 60% of animals being protected against leptazol-induced convulsions (Olajide et al., 1999). Further, the *Hoslundia opposita* extract potentiated the phenobarbitone sleeping time. It was suggested that the *Hoslundia opposita* extract acted in a similar fashion to tranquilizers such as benzodiazepines (Olajide et al., 1999).

The ethanol extract of *Clausena anisata* bark had a weaker, but dose-dependent activity. An aqueous extract from the roots of *Clausena anisata* are reported to depress the central nervous system and demonstrated anticonvulsant action in mice when administrated intraperitoneally (Makanju, 1983). Chemical studies of the root of *Clausena anisata* have led to the identification of heliettin and imperatorin. Heliettin showed anticonvulsant activity in mice; the same effect has not been reported for imperatorin (Adesina and Ette, 1982).

The ethanol extracts of the three species of *Leonotis* had weak activity at the highest concentration tested; the aqueous extracts were not active. An aqueous extract of *Leonotis leonurus* was tested for anticonvulsant activity against seizures produced in mice by pentylenetetrazole, picrotoxin, bicuculline and *N*-methyl-DL-aspartic acid (Bienvenu et al., 2002). The *Leonotis leonurus* extract protected against or delayed seizures induced by pentylenetetrazole, picrotoxin and *N*-methyl-DL-aspartic acid, but did not protect against bicuculline-induced seizures. The data suggest that the extract of *Leonotis leonurus* has anticonvulsant activity and may probably act through non-specific mechanisms (Bienvenu et al., 2002). The results from this study, where the aqueous extract of *Leonotis leonurus* did not exhibit activity, suggest that the anticonvulsant mechanism is not via $GABA_A$-benzodiazepine receptor activity.

The few studies on CNS activity in animals of plants active in the flumazenil assay indicate that the active compounds in the tested extracts might be able to pass the blood brain barrier. In the search for new antiepileptic and anticonvulsant compounds with effect on the $GABA_A$-receptor, it is important that the compounds are able to pass the blood brain barrier. Therefore it is important to confirm this activity in an in vivo animal model.

The present results appear to justify the use of some of the tested plants in traditional South African medicine. The fact that some plants did not show any activity in this assay

Table 1
Plant extracts screened for activity in the $GABA_A$-benzodiazepine receptor assay

Family	Species	Voucher specimen	Plant part analysed	Extraction solvent	Binding (%)				
					10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
Amaryllidaceae	*Brunsvigia grandiflora* Lindl.	*Stafford 10* NU	Leaves	Water	92 ± 6	102 ± 1	101 ± 4	95 ± 5	89 ± 5
				Ethanol	38 ± 9	84 ± 5	105 ± 1	108 ± 2	106 ± 3
Anacardiaceae	*Rhus chirindensis* Bak.f.	*Stafford 11* NU	Leaves	Water	55 ± 6	106 ± 2	117 ± 1	103 ± 7	95 ± 6
				Ethanol	38 ± 1	107 ± 8	116 ± 9	110 ± 10	120 ± 13
			Roots	Water	63 ± 2	106 ± 5	101 ± 6	89 ± 4	102 ± 4
				Ethanol	30 ± 2	92 ± 5	122 ± 15	85 ± 6	112 ± 12
	Rhus rehmanniana Engl.	*Stafford 12* NU	Leaves	Water	53 ± 2	101 ± 6	96 ± 1	99 ± 10	94 ± 2
				Ethanol	6 ± 1	4 ± 1	9 ± 1	57 ± 2	82 ± 3
	Rhus tridentata (L.F.) Wild & Drum.	*Stafford 13* NU	Leaves	Water	50 ± 1	88 ± 2	96 ± 4	107 ± 6	110 ± 8
				Ethanol	8 ± 2	6 ± 0	26 ± 1	86 ± 5	94 ± 2
Apocynaceae	*Acokanthera oblongifolia* (Hochst.) Codd	*Stafford 14* NU	Leaves	Water	61 ± 6	71 ± 4	67 ± 10	102 ± 5	110 ± 2
				Ethanol	81 ± 4	101 ± 3	104 ± 2	106 ± 3	101 ± 4
Araliaceae	*Cussonia* sp.1	*Stafford 15* NU	Leaves	Water	112 ± 4	109 ± 6	106 ± 3	110 ± 3	114 ± 5
				Ethanol	62 ± 2	106 ± 8	105 ± 2	99 ± 4	92 ± 2
	Cussonia spicata Thunb.	*Stafford 16* NU	Roots	Water	97 ± 6	110 ± 15	109 ± 4	112 ± 16	116 ± 5
				Ethanol	97 ± 14	99 ± 19	95 ± 11	246 ± 22	121 ± 5
Asphodelaceae	*Bulbine frutescens* Willd.	*Stafford 17* NU	Stem/root	Water	113 ± 5	114 ± 3	101 ± 7	98 ± 5	105 ± 5
				Ethanol	67 ± 4	122 ± 1	122 ± 7	119 ± 7	125 ± 9
	Gasteria croucheh Baker	*Stafford 18* NU	Leaves	Water	99 ± 3	94 ± 6	91 ± 4	96 ± 9	92 ± 4
				Ethanol	55 ± 4	85 ± 2	91 ± 3	107 ± 2	95 ± 5
			Root	Water	124 ± 5	122 ± 1	122 ± 7	119 ± 7	125 ± 9
				Ethanol	66 ± 0	101 ± 2	111 ± 6	107 ± 2	109 ± 2
Combretaceae	*Combreturn bracteosum* Brandis ex Eng.	*Stafford 19* NU	Leaves	Water	62 ± 1	88 ± 1	85 ± 1	96 ± 2	93 ± 2
				Ethanol	56 ± 4	105 ± 3	105 ± 5	106 ± 3	104 ± 7
			Root	Water	53 ± 3	81 ± 3	103 ± 7	105 ± 5	107 ± 4
				Ethanol	38 ± 3	85 ± 1	98 ± 4	100 ± 4	106 ± 2
	Combretum imberbe Wawra.	*Stafford 20* NU	Leaves	Water	53 ± 3	80 ± 1	98 ± 6	93 ± 2	95 ± 1
				Ethanol	13 ± 0	86 ± 2	99 ± 2	105 ± 4	101 ± 7
			Root	Water	41 ± 1	74 ± 2	104 ± 3	110 ± 3	106 ± 2
				Ethanol	41 ± 2	82 ± 1	95 ± 4	104 ± 4	107 ± 2
Euphorbiaceae	*Antidesma venosum* E. Mey. Ex Tul.	*Stafford 21* NU	Roots	Water	63 ± 3	97 ± 9	107 ± 15	112 ± 24	96 ± 2

Table 1 (*Continued*)

Family	Species	Voucher specimen	Plant part analysed	Extraction solvent	Binding (%)				
					10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
				Ethanol	66 ± 1	134 ± 3	126 ± 15	117 ± 3	98 ± 10
	Jatropha panduaeifolia Andr.	*Stafford 22* NU	Leaves	Water	114 ± 1	116 ± 4	104 ± 2	97 ± 7	111 ± 3
				Ethanol	56 ± 7	101 ± 5	101 ± 2	100 ± 2	100 ± 2
			Bark/root	Water	92 ± 2	107 ± 1	112 ± 2	107 ± 5	105 ± 4
				Ethanol	28 ± 1	89 ± 6	102 ± 5	109 ± 6	102 ± 3
	Jatropha zeyheri Sond.	*Stafford 23* NU	Roots	Water	122 ± 4	103 ± 23	102 ± 4	88 ± 13	109 ± 8
				Ethanol	79 ± 3	120 ± 1	101 ± 9	105 ± 4	120 ± 5
Fabaceae	*Bauhinia galpinii* N.E. Br.	*Stafford 24* NU	Leaves	Water	72 ± 4	117 ± 10	136 ± 32	109 ± 23	127 ± 9
				Ethanol	27 ± 2	93 ± 16	104 ± 3	111 ± 8	96 ± 5
	Bauhinia tomentosa L.	*Stafford 25* NU	Leaves	Water	114 ± 7	113 ± 9	123 ± 5	123 ± 6	154 ± 53
				Ethanol	46 ± 3	115 ± 45	93 ± 7	95 ± 8	108 ± 2
	Dichrostachys cinera Miq.	*Stafford 26* NU	Leaves	Water	78 ± 12	120 ± 3	117 ± 1	108 ± 14	96 ± 8
				Ethanol	30 ± 6	66 ± 5	67 ± 6	52 ± 20	56 ± 12
	Indigofera tristis E. Mey.	*Stafford 27* NU	Leaves	Water	83 ± 6	105 ± 1	95 ± 3	107 ± 2	97 ± 4
				Ethanol	36 ± 2	98 ± 2	99 ± 6	105 ± 4	109 ± 9
	Indigofera woodii Bolus	*Stafford 28* NU	Leaves	Water	95 ± 1	96 ± 10	115 ± 12	118 ± 2	112 ± 9
				Ethanol	33 ± 0	106 ± 2	115 ± 1	114 ± 3	120 ± 1
	Senna didymobotrya (Fresenius) N.W. Irwin & R.C. Barneby	*Stafford 29* NU	Leaves	Water	90 ± 1	90 ± 3	91 ± 2	91 ± 4	94 ± 1
				Ethanol	28 ± 1	79 ± 2	91 ± 5	95 ± 4	
			Root	Water	101 ± 4	106 ± 4	102 ± 5	104 ± 3	104 ± 2
				Ethanol	33 ± 5	89 ± 3	102 ± 3	95 ± 3	98 ± 3
	Senna petersiana (Bolle) J.M. Lock	*Stafford 30* NU	Leaves	Water	75 ± 3	88 ± 3	96 ± 8	94 ± 2	88 ± 2
				Ethanol	24 ± 3	78 ± 2	92 ± 4	95 ± 5	95 ± 3
			Root	Water	63 ± 3	113 ± 8	110 ± 4	102 ± 7	97 ± 4
				Ethanol	47 ± 3	87 ± 7	113 ± 2	97 ± 5	110 ± 4
Flacoutiaceae	*Oncoba spinosa* Forssk.	*Stafford 31* NU	Leaves	Water	86 ± 6	80 ± 33	99 ± 9	102 ± 7	103 ± 5
				Ethanol	46 ± 1	89 ± 2	94 ± 7	96 ± 2	82 ± 2
			Roots	Water	27 ± 2	70 ± 2	91 ± 8	93 ± 5	94 ± 6
				Ethanol	16 ± 4	73 ± 13	94 ± 16	102 ± 5	110 ± 5
Hypoxidaceae	*Hypoxis angustifolia* Lam.	*Stafford 32* NU	Leaves	Water	101 ± 1	113 ± 2	96 ± 0	97 ± 2	98 ± 4
				Ethanol	36 ± 1	97 ± 2	102 ± 4	103 ± 8	105 ± 3
			Corm	Water	85 ± 1	90 ± 1	98 ± 3	103 ± 6	98 ± 4
				Ethanol	38 ± 4	79 ± 8	96 ± 1	104 ± 0	104 ± 3
	Hypoxis colchicifolia Bak.	*Stafford 33* NU	Corm	Water	95 ± 6	104 ± 9	107 ± 4	107 ± 5	104 ± 8
				Ethanol	23 ± 3	77 ± 1	83 ± 6	88 ± 3	91 ± 3
	Hypoxis hemerocallidea Fisch. & C.A. Mey.	*Stafford 34* NU	Leaves	Water	103 ± 4	105 ± 2	100 ± 2	99 ± 7	111 ± 5
				Ethanol	57 ± 5	100 ± 2	101 ± 3	110 ± 1	112 ± 2
			Corm	Water	68 ± 5	107 ± 3	111 ± 4	108 ± 9	98 ± 9

Table 1 (*Continued*)

Family	Species	Voucher specimen	Plant part analysed	Extraction solvent	Binding (%)				
					10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
				Ethanol	36 ± 0	89 ± 3	111 ± 3	130 ± 2	109 ± 4
			Root	Water	71 ± 2	95 ± 1	106 ± 0	106 ± 1	108 ± 4
				Ethanol	57 ± 2	88 ± 3	99 ± 4	108 ± 3	109 ± 1
Lamiaceae	*Hoslundia opposita* Vahl	*Stafford 35* NU	Leaves	Water	102 ± 1	77 ± 38	95 ± 30	106 ± 9	115 ± 6
				Ethanol	10 ± 2	30 ± 2	46 ± 1	54 ± 6	62 ± 10
	Leonotis dubra E. Mey.	*Stafford 36* NU	Leaves	Water	128 ± 7	110 ± 7	111 ± 5	111 ± 8	109 ± 8
				Ethanol	38 ± 9	81 ± 8	104 ± 8	106 ± 3	112 ± 1
	Leonotis intermedia Lindl.	*Stafford 37* NU	Aerial parts	Water	98 ± 4	108 ± 4	104 ± 4	106 ± 3	98 ± 1
				Ethanol	36 ± 6	85 ± 7	95 ± 4	100 ± 7	97 ± 3
	Leonotis leonurus R. Br.	*Stafford 38* NU	Leaves	Water	110 ± 2	115 ± 1	111 ± 3	114 ± 1	113 ± 2
				Ethanol	47 ± 3	97 ± 2	110 ± 6	111 ± 4	114 ± 5
			Aerial parts	Water	66 ± 4	89 ± 3	115 ± 2	108 ± 6	107 ± 4
				Ethanol	35 ± 7	97 ± 6	88 ± 5	91 ± 3	102 ± 9
	Salvia chamelaeagn ea Berg.	*Stafford 39* NU	Leaves	Water	84 ± 3	95 ± 3		95 ± 18	116 ± 10
				Ethanol	28 ± 3	93 ± 11	139 ± 10	132 ± 11	155 ± 19
Loganiaceae	*Buddleja saligna* Willd.	*Stafford40* NU	Leaves	Water	151 ± 36	120 ± 5	126 ± 5	132 ± 10	149 ± 40
				Ethanol	55 ± 0	74 ± 16	100 ± 13	116 ± 19	131 ± 25
	Buddleja salviifolia Lam.	*Stafford 41* NU	Leaves	Water	111 ± 5	123 ± 1	111 ± 5	110 ± 7	116 ± 2
				Ethanol	74 ± 4	91 ± 2	94 ± 2	96 ± 3	90 ± 2
Meliaceae	*Ekebergia capensis* Sparrm.	*Stafford 42* NU	Leaves	Water	96 ± 3	115 ± 4	120 ± 7	121 ± 5	96 ± 8
				Ethanol	27 ± 3	55 ± 20	36 ± 7	41 ± 10	51 ± 4
			Bark	Water	69 ± 3	103 ± 3	110 ± 4	102 ± 5	109 ± 4
				Ethanol	29 ± 2	60 ± 3	63 ± 8	42 ± 3	76 ± 30
Periplocaceae	*Mondia whitei* (Hook.f.) Skeels	*Stafford 43* NU	Leaves	Water	106 ± 9		88 ± 4	89 ± 4	95 ± 3
				Ethanol	63 ± 4	101 ± 2	98 ± 1	97 ± 6	96 ± 10
Rosaceae	*Rubus ludwigii* Enkl. & Zeyh.	*Stafford 44* NU	Leaves/fruits	Water	71 ± 1	86 ± 7	108 ± 9	102 ± 6	100 ± 8
				Ethanol	50 ± 3	91 ± 2	98 ± 2	99 ± 4	102 ± 3
			Root	Water	79 ± 3	100 ± 6	105 ± 10	101 ± 2	102 ± 2
				Ethanol	65 ± 2	104 ± 2	106 ± 2	106 ± 2	103 ± 4
	Rubus phoeniculacius Maxim	*Stafford 45* NU	Leaves/stem	Water	74 ± 4	85 ± 9	95 ± 9	89 ± 9	88 ± 10
				Ethanol	58 ± 1	97 ± 6	94 ± 2	93 ± 3	99 ± 6
Rubiaceae	*Catunaregam spinosa* Thunb.	*Stafford 46* NU	Leaves	Water	108 ± 7	134 ± 14	147 ± 20	151 ± 28	104 ± 7
				Ethanol	54 ± 1	111 ± 2	87 ± 6	113 ± 10	115 ± 3
			Roots	Water	96 ± 8	111 ± 0	117 ± 8	144 ± 51	127 ± 28
				Ethanol	79 ± 3	97 ± 15	120 ± 1	114 ± 6	116 ± 1
Rutaceae	*Clausena anisata* (Willd.) Hook.f.	*Stafford 47* NU	Bark, aerial	Water	108 ± 7	111 ± 2	115 ± 2	107 ± 1	110 ± 9
				Ethanol	18 ± 1	54 ± 2	91 ± 3	91 ± 4	87 ± 1
			Bark, root	Water	82 ± 7	101 ± 2	111 ± 4	114 ± 4	113 ± 6
				Ethanol	46 ± 1	67 ± 2	93 ± 3	104 ± 1	103 ± 7
	Ruta graveolens L	*Stafford 48* NU	Leaves/stem	Water	87 ± 5	114 ± 10	113 ± 9	112 ± 6	124 ± 7
				Ethanol	34 ± 1	97 ± 2	111 ± 0	115 ± 5	113 ± 2
	Zanthoxylum capense (Thunb.) Harv.	*Stafford 49* NU	Leaves	Water	105 ± 2	113 ± 8	101 ± 2	97 ± 1	100 ± 7

Table 1 (*Continued*)

Family	Species	Voucher specimen	Plant part analysed	Extraction solvent	Binding (%)				
					10 mg/ml	1 mg/ml	0.1 mg/ml	0.01 mg/ml	0.001 mg/ml
				Ethanol	46 ± 2	92 ± 4	105 ± 7	107 ± 4	107 ± 3
Verbenaceae	*Clerodendru m myricoides* (Hochst.) Watke	*Stafford 50* NU	Leaves	Water	68 ± 7	75 ± 8	80 ± 9	80 ± 4	91 ± 8
				Ethanol	16 ± 3	74 ± 11	130 ± 8	145 ± 29	113 ± 8
Vitaceae	*Rhoicissus tomentosa* (Lam.) Wild & Drum.	*Stafford 51* NU	Leaves	Water	37 ± 10	43 ± 9	96 ± 2	88 ± 11	86 ± 7
				Ethanol	56 ± 2	98 ± 2	105 ± 2	107 ± 6	112 ± 3
	Rhoicissus tridentate (L.f.) Wild & Drum.	*Stafford 52* NU	Leaves	Water	91 ± 2	95 ± 3	99 ± 4	98 ± 4	102 ± 4
				Ethanol	61 ± 3	104 ± 1	94 ± 4	102 ± 5	103 ± 1

does not mean that they do not have any antiepileptic and anticonvulsant effect at all. These plants may have a total different mechanism of action, or a different way of administration.

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References

Adesina, S.K., Ette, E.I., 1982. The isolation and identification of anticonvulsant agents from *Clausena anisata* and *Afraegle paniculata*. Fitoterapia 53, 63–66.

Ashton, C.H., 1999. Insomnia and anxiety. In: Walker, R., Edwards, C. (Eds.), Clinical Pharmacy and Therapeutics, second ed. Churchill Livingstone, Edinburgh, pp. 393–408.

Bienvenu, E., Amabeoku, G.J., Eagles, P.K., Scott, G., Springfield, E.P., 2002. Anticonvulsant activity of aqueous extract of *Leonotis leonurus*. Phytomedicine 9, 217–233.

Dhillon, S., Sander, J.W.A.S., 1999. Epilepsy. In: Walker, R., Edwards, C. (Eds.), Clinical Pharmacy and Therapeutics, second ed. Churchill Livingstone, Edinburgh, pp. 435–451.

Kahnberg, P., Lager, E., Rosenberg, C., Schougaard, J., Camet, L., Sterner, O., Nielsen, E.O., Nielsen, M., Liljefors, T., 2002. Refinement and evaluation of a pharmacophore model for flavone derivatives binding to the benzodiazepine site of the $GABA_A$ receptor. Journal of Medicinal Chemistry 45, 4188–4201.

Makanju, O.O.A., 1983. Behavioral and anticonvulsant effects of an aqueous extract from the roots of *Clausena anisata* (*Rutaceae*). International Journal of Crude Drug Research 21, 29–32.

Obijiofor, C., 2002. Integrating African ethnomedicine into primary healthcare: a framework for South Eastern Nigeria. In: Iwu, Wootton (Eds.), Ethnomedicine and Drug Discovery. Elsevier Science, Amsterdam, pp. 71–79.

Olajide, O.A., Awe, O.S., Makinde, J.M., 1999. Central nervous system depressant effect of *Hoslundia opposita* Vahl. Phytotherapy Research 13, 425–426.

Sobiecki, J.F., 2002. A preliminary inventory of plants used for psychoactive purposes in southern African healing traditions. Transactions of the Royal Society of South Africa 57, 1–24.