JPP
Journal of
Pharmacy and Pharmacology

JPP 2010, 62: 368–373

Received July 6, 2009
Accepted November 30, 2009
DOI 10.1211/jpp/62.03.0012
ISSN 0022-3573

Research Paper

Evaluation of the effects of *Olea europaea* L. subsp. africana (Mill.) P.S. Green (Oleaceae) leaf methanol extract against castor oil-induced diarrhoea in mice

George J. Amabeoku and Kapinga Bamuamba

School of Pharmacy, University of the Western Cape, South Africa

Abstract

Objectives *Olea europaea* L. subsp. *africana* (Mill.) P.S. Green is widely used in South Africa by traditional medicine practitioners to treat diarrhoea. However, little is known scientifically about this South African species in the treatment of diarrhoea. The main aim of the study therefore was to investigate the antidiarrhoeal effect of the leaf methanol extract of the plant species in mice.

Methods The antidiarrhoeal activity of the leaf methanol extract of *O. europaea* subsp. *africana* was studied using a castor oil-induced diarrhoeal test. The antipropulsive activity of the plant extract was also investigated using the charcoal meal transit test. Standard methods were used to investigate the acute toxicity and effect of *O. europaea* subsp. *africana* on castor oil-induced intraluminal fluid accumulation.

Results Leaf methanol extract of *O. europaea* subsp. *africana* and loperamide, a standard antidiarrhoeal drug, significantly reduced the number of diarrhoeal episodes induced by castor oil, significantly decreased the stool mass, significantly delayed the onset of the diarrhoea and protected the animals against castor oil-induced diarrhoea. Both *O. europaea* subsp. a*fricana* and loperamide significantly decreased the gastrointestinal transit of charcoal meal and castor oil-induced intraluminal fluid accumulation in mice. The LD50 value was found to be 3475 mg/kg (p.o.).

Conclusions The results obtained suggest that the leaf methanol extract of *O. europaea* subsp. *africana* has an antidiarrhoeal property and that, given orally, it may be non-toxic and/or safe in mice.

Keywords acute toxicity; antidiarrhoeal activity; leaf methanol extract; Oleaceae; *Olea europaea* subsp. *africana*

Introduction

Numerous plant species with medicinal properties have been used extensively by traditional medicine practitioners in South Africa for the treatment of various ailments, including diarrhoea. These include the South African species of *Olea europaea*, known as *Olea europaea* L. subsp. *africana* (Mill.) P.S. Green.

The plant belongs to the family, Oleaceae. It is widely distributed in South Africa and known locally as 'olienhout' in Afrikaans, 'motholoari' in Sotho and 'umquma' in Zulu and Xhosa. It is called wild olive in English.[1] The early Cape settlers in South Africa used the fruit for the treatment of diarrhoea.[2]

The plant is widely used in the treatment of hypertension.[1,2] Infusions or decoctions of the leaves and young stems are used in Montagu District, Western Cape, South Africa for throat and kidney problems as well as backache (Mayeng, oral communication). Infusions of the leaves are used to treat eye infections and decoctions are used as diuretics and tonics. They are also used to treat diarrhoea and gargled to treat sore throat.[1]

According to isolation studies carried out by Bruneton *et al.*[3] and Hansen *et al.*,[4] the leaves of *O. europaea* subsp. *africana* contain two main active principles of secoiridoids, known as oleuropein and oleacein, triterpenoids-4 and flavonoids. Cortesi *et al.*, through HPLC studies on the leaf extracts of *O. europaea*, showed that tetra- and pentacyclic triterpenes, sterols, erythroidal, uvaol and oleanolic acid are also present.[5] Some of these compounds have been shown to contribute to the pharmacological activities of the plant

Correspondence: George J. Amabeoku,
Discipline of Pharmacology,
School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa.
E-mail: gamabeoku@uwc.ac.za

species. Petkov and Manolov have shown oleuropein to have hypotensive, coronary dilating and antiarrhythmic actions.[6]

Oleacein has been reported to inhibit angiotensin converting enzyme[4] and also to have antioxidant activity.[3] Bruneton has also reported that oleuropein has antispasmodic effect.[3] The use of the plant in the treatment of diarrhoea, especially in the rural areas of the Western Cape, is widespread (Mayeng, oral communication). This study attempts to better understand aspects of the scientific rationale for the wide use of *Olea europaea* subsp. *africana* by traditional medicine practitioners in the treatment of diarrhoea, by investigating the effect of the leaf methanol extract of the plant species on castor oil-elicited diarrhoea in mice. The effects of the leaf methanol extract of the plant species on the gastrointestinal transit of charcoal meal and castor oil-induced intraluminal fluid accumulation in mice and the acute toxicity of the plant species were also investigated.

Materials and Methods

Collection and identification of plant material

The plant was collected from Karoo Botanical Garden (NBI), Worcester 3319 CB, South Africa. The taxonomic identity of the plant was verified by Mr Franz Weitz, a taxonomist in the Department of Biodiversity and Conservative Biology, University of the Western Cape, and the voucher specimen (TRAD 201) was deposited in the Herbarium of the University.

Preparation of methanol extract of *O. europaea* subsp. *africana*

Leaves of *O. europaea* subsp. *africana* were dried in a ventilated oven for 72 h at 30°C. The dried plant materials were then ground into fine powders (852 g) using a Moulinex coffee grinder and passed through an 850-μm sieve. The dried powder (70 g) was extracted in a soxhlet extractor with methanol (750 ml) for 24 h. The methanol filtrate was evaporated to dryness using a Buchi RE11 evaporator and Buchi 461 water bath. A yield of 10.45 g crude methanol extract was obtained and stored in a desiccator for further use. Fresh solutions of the methanol extract were prepared on each day of the experiment by dissolving weighed quantities of the methanol extract in 0.85 ml of dimethylsulfoxide (DMSO) and made up to the appropriate volume with distilled water. The solutions were administered orally (p.o.) to mice in a volume of 1 ml/100 g of animal by means of a bulbed steel needle. The concentrations of the plant extract tested were 2.5–7.5 mg/ml.

Drugs and chemicals

Castor oil (GR Pharmaceuticals (Pty) Ltd, Atlantis, South Africa) was kept warm in a water bath at 30–35°C before and during the experiment, and administered orally in a constant volume to mice by means of a bulbed steel needle. Loperamide (4-[*p*-chlorophenyl]-4-hydroxy-*N*,*N*-dimethyl-diphenyl-1-piperidine-butyramine) hydrochloride (Sigma Chemical Co.) was dissolved in a minimum amount of 10% ethanol (Merck (Pty) Ltd) and made up to the appropriate volume with distilled water. Activated charcoal (Sigma Chemical Co.), an aqueous suspension of 5% charcoal and 5% acacia were prepared. Both the loperamide solution and the charcoal meal were administered orally in a volume of 1 ml/100 g of animals using a bulbed steel needle.

Animals

Male albino mice bred in the Animal House of the Discipline of Pharmacology, University of the Western Cape, Bellville, South Africa and weighing 18–25 g were used. The animals had free access to food and water before the commencement of the experiment. They were used in groups of eight per dose of drug or plant extract. Each animal was used for one experiment only.

Assessment of antidiarrhoeal activity

The method summarised in Williamson *et al.*[7] was modified and used to assess the antidiarrhoeal activity of the plant extract. Castor oil (0.7 ml, p.o.), known to cause frequent stooling within 4 h, was used to induce diarrhoea. A group of eight mice was used, each mouse pretreated with 0.25 ml of physiological saline for 15 min prior to the oral administration of 0.7 ml of castor oil (control). The castor oil treated animals were each placed in a large beaker (5000 ml) containing a weighed white tissue paper at the bottom for observation. The onset of diarrhoea and the number of diarrhoeal episodes were noted at 1 h intervals for a period of 5 h. The white tissue paper in each beaker was also changed at the same time and weighed to obtain the stool mass. The number of animals exhibiting diarrhoea at various time intervals over the 5 h period was also obtained. Experiments were repeated with animals pretreated for 15 min with leaf methanol extract of *O. europaea* subsp. *africana* (25–75 mg/kg), the standard antidiarrhoeal drug loperamide (25 mg/kg) or 0.25 ml of control vehicle (0.85 ml of DMSO dissolved in an appropriate volume of distilled water) prior to the administration of 0.7 ml (p.o.) of castor oil. The ability of plant extract to reduce the number of animals exhibiting diarrhoea and/or the number of diarrhoeal episodes is taken as an antidiarrhoeal activity.[3] The doses and pretreatment times used were obtained from preliminary studies in our laboratory. All experiments were carried out between 0800 h and 1700 h in a quiet laboratory with an ambient temperature of 22 ± 2°C.

Assessment of gastrointestinal propulsion of charcoal meal

The methods described by Williamson *et al.*[7] and Kitano *et al.*[8] were used to assess the effect of the plant extract on the gastrointestinal transit of charcoal meal. Animals were used in groups of eight per dose of plant extract or the standard drug and fasted for 16 h, but had free access to water. The control group was pretreated for 15 min with 0.25 ml of physiological saline given orally with a bulbed steel needle, and then given 0.4 ml of charcoal meal (an aqueous suspension of 5% charcoal and 5% gum acacia) orally. Twenty minutes after the charcoal meal, the animals were killed by ether inhalation and the intestine was removed from the cardia to the rectal end. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. Experiments were repeated with other groups of animals pretreated for 15 min with either the leaf methanol extract of the plant

(25–75 mg/kg), the standard drug (25 mg/kg) or 0.25 ml of control vehicle (0.85 ml of DMSO dissolved in an appropriate volume of distilled water) prior to the administration of 0.4 ml of charcoal meal. All experiments were carried out between 0800 h and 1700 h in a quiet laboratory with an ambient temperature of 22 ± 2°C.

Assessment of castor oil-induced intestinal fluid accumulation

Modified methods of Robert *et al.*[9] and DiCarlo *et al.*[10] were used to assess the effect of the plant extract on castor oil-induced intestinal fluid accumulation. A group of eight mice were used, each pretreated with 0.25 ml of physiological saline for 15 min prior to the oral administration of 1.5 ml of castor oil (control). Thirty minutes after the administration of castor oil, the animals were killed by ether inhalation and the small intestine was removed from the pylorus to the caecum, its contents expelled into a Petri dish and the volume measured using a measuring cylinder. Experiments were repeated with other groups of animals pretreated for 15 min with the leaf methanol extract of the plant (25–75 mg/kg), the standard drug (25 mg/kg) or 0.25 ml of control vehicle (0.85 ml of DMSO dissolved in an appropriate volume of distilled water), prior to the administration of castor oil (1.5 ml, p.o.). All experiments were carried out between 0800 h and 1700 h in a quiet laboratory with an ambient temperature of 22 ± 2°C.

Acute toxicity testing

The modified method of Lorke[11] was used to assess the acute toxicity of *O. europaea* subsp. *africana* leaf methanol extract. Mice were used for the experiment. The animals were fasted for 16 h in groups of eight per dose of plant extract. *O. europaea* subsp. *africana* was administered orally by means of a bulbed steel needle to mice in graded doses (400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg). Another group of eight mice used as control received 0.25 ml of distilled water orally. Both the test and control animals were then allowed access to food and water, and observed over a period of 5 days for any deaths or acute toxicity symptoms. The log dose/response (% death) curve was then plotted, from which the median lethal dose (LD50) of the plant extract was obtained.

HPLC

Chromatographic system: Beckman HPLC system consisting of double pump programmable solvent module model 126; diode array detector module model 168; Samsung computer 386 with management System Gold (Gold V601) software supplied by Beckman; column, C18 Bondapak 5 μm and dimensions (250 × 4.6 mm). Chromatographic conditions: mobile phase: solvent A: 1% acetic acid; solvent B: methanol; mode: gradient; flow rate, 1 min/min; injection volume, 10 μl; detector, UV at 360 nm; reference standard, rutin (2.5 g dissolved in 100 ml of methanol). The HPLC operating conditions were programmed to give the following: 0 min, solvent B: 20%; 5 min, solvent B: 40%; 15 min, solvent B: 60%; 20 min, solvent B: 80% and 27 min, solvent B: 20%. The run rate was 30 min.

Statistical analysis

The data obtained from the antidiarrhoeal and antipropulsive activity and intestinal fluid accumulation experiments were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison (GraphPad Prism, version 5.0, GraphPad Software, Inc., San Diego CA 92130, USA) and presented as mean ± standard error of mean (SEM). However, the data on the number of animals exhibiting diarrhoea were analysed using the chi-squared test. In the above cases, P values of less than 5% ($P < 0.05$), were considered significant.

Ethics clearance

The Ethics Committee of the University of the Western Cape approved the experimental protocol used in the present study and this conforms to the *Guide to the care and use of animals in research and teaching* of the university.

Results

Effect of leaf methanol extract of *O. europaea* subsp. *africana* on castor oil-induced diarrhoea

Castor oil (0.7 ml, p.o.) in the presence of physiological saline induced diarrhoea in under 30 min of observation in all the animals used. *O. europaea* subsp. *africana* (25–75 mg/kg, p.o.) significantly reduced the number of diarrhoeal episodes, significantly and dose-dependently decreased the stool mass and delayed the onset of castor oil-induced diarrhoea. *O. europaea* subsp. *africana* (50–75 mg/kg, p.o.) significantly decreased the incidence of the diarrhoea induced by castor oil by significantly reducing the number of animals suffering from diarrhoea. However, 25 mg/kg (p.o.) of the plant extract did not significantly affect the incidence of castor oil-induced diarrhoea since only 50% of mice were protected against the diarrhoea. Loperamide (25 mg/kg, p.o.) completely protected all the mice used against castor oil-induced diarrhoea. The control vehicle (0.25 ml) did not alter castor oil-induced diarrhoea to any significant extent (Table 1).

Effect of leaf methanol extract of *O. europaea* subsp. *africana* on the gastrointestinal transit of charcoal meal and castor oil-induced intestinal fluid accumulation

The mean length of mouse intestine travelled by the charcoal meal in the presence of physiological saline was 84.30 ± 1.48%. Leaf methanol extract of *O. europaea* subsp. *africana* (25–75 mg/kg, p.o.) significantly and dose dependently reduced the mean length of intestine travelled by the charcoal meal, by 21.3–56%. Loperamide (25 mg/kg, p.o.) significantly reduced the mean length of intestine travelled by the charcoal meal by 81.1%.

The mean intraluminal accumulation of fluid volume induced by castor oil in mice was 1.93 ± 0.09 ml. Leaf methanol extract of *O. europaea* subsp. *africana* (25–75 mg/kg, p.o.) in a dose-dependent manner

Table 1 Effect of leaf methanol extract of *O. europaea* subsp. *africana* on castor oil-induced diarrhoea in mice

Treatment groups	Mass of stool (g)		Faecal output	Onset of diarrhoea (min)		No. of animals exhibiting diarrhoea	No. of diarrhoeal episodes		Percentage episode inhibition
	Mean ± SEM			Mean ± SEM			Mean ± SEM		
DW									
0.25 ml	0.71	0.18	100	25.63	1.10	8/8	4.88	0.48	
O. europaea subsp. *africana*									
25 mg/kg	0.33**	0.03	46.5	40.50*	0.94	4/8	2.00*	0.29	59.0
50 mg/kg	0.29**	0.01	40.8	54.58**	1.75	2/8+	1.50**	0.25	69.3
75 mg/kg	0.26**	0.03	36.6	83.00**	3.00	2/8+	1.50**	0.25	69.3
Loperamide									
25 mg/kg	0			0		0/8++	0		100
DMSO									
0.25 ml	0.70	0.18	99	25.50	1.23	8/8	5.13	0.55	

DW, distilled water; DMSO, dimethylsulfoxide. *$P < 0.05$, **$P < 0.001$ vs castor oil (0.7 ml, p.o.) control, ANOVA ($n = 8$); +$P < 0.01$, ++$P < 0.001$ vs castor oil (0.7 ml, p.o.) control, chi-squared test ($n = 8$).

significantly reduced the mean intraluminal accumulation of fluid volume induced by castor oil by 45.6–69.9%. Loperamide (25 mg/kg, p.o.) significantly reduced the mean intraluminal accumulation of fluid volume induced by castor oil by 86.5%. The control vehicle (0.25 ml) did not significantly affect the gastrointestinal transit of charcoal meal or castor oil-induced intestinal accumulation of fluid volume (Table 2).

Acute toxicity studies

Leaf methanol extract of *O. europaea* subsp. *africana* (400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600, 4000 mg/kg) given orally caused one death at a dose of 2800 mg/kg, three deaths at 3200 mg/kg, five deaths at 3600 mg/kg and eight deaths at 4000 mg/kg. However, from the doses of 2000 to 4000 mg/kg (p.o.), all the animals showed hypoactivity. The LD50 value for the plant species was found to be 3475 mg/kg.

HPLC analysis

The HPLC fingerprint of the leaf methanol extract of *O. europaea* subsp. *africana* showed major peaks at the following retention times (min): 22.35, 22.68, 22.84, 24.52 and 24.71 (Figure 1).

Discussion and Conclusion

The present study investigated the antidiarrhoeal activity of *O. europaea* subsp. *africana* in mice. Castor oil, used to induce diarrhoea, is an irritant or contact laxative, which is hydrolysed in the upper small intestine to ricinoleic acid,[12]

Table 2 Effect of leaf methanol extract of *O. europaea* subsp. *africana* on the gastrointestinal transit of charcoal meal and castor oil-induced intestinal fluid accumulation in mice

Treatment groups	Length of intestine travelled		Percentage inhibition	Intestinal fluid volume (ml)		Percentage inhibition
	Mean ± SEM			Mean ± SEM		
DW						
0.25 ml	84.30	1.48		1.93	0.09	
O. europaea subsp. *africana*						
25 mg/kg	66.33*	2.56	21.32	1.05**	0.07	45.60
50 mg/kg	45.08**	1.48	46.52	0.89**	0.08	53.89
75 mg/kg	37.05**	2.37	56.05	0.58**	0.06	69.95
Loperamide						
25 mg/kg	11.69	0.70	86.13	0.30	0.05	84.46
DMSO						
0.25 ml	83.18	2.07	1.33	1.91	0.13	1.04

DW, distilled water; DMSO, dimethylsulfoxide. *$P < 0.05$, **$P < 0.001$ vs castor oil (0.7 ml, p.o.) control, ANOVA ($n = 8$).

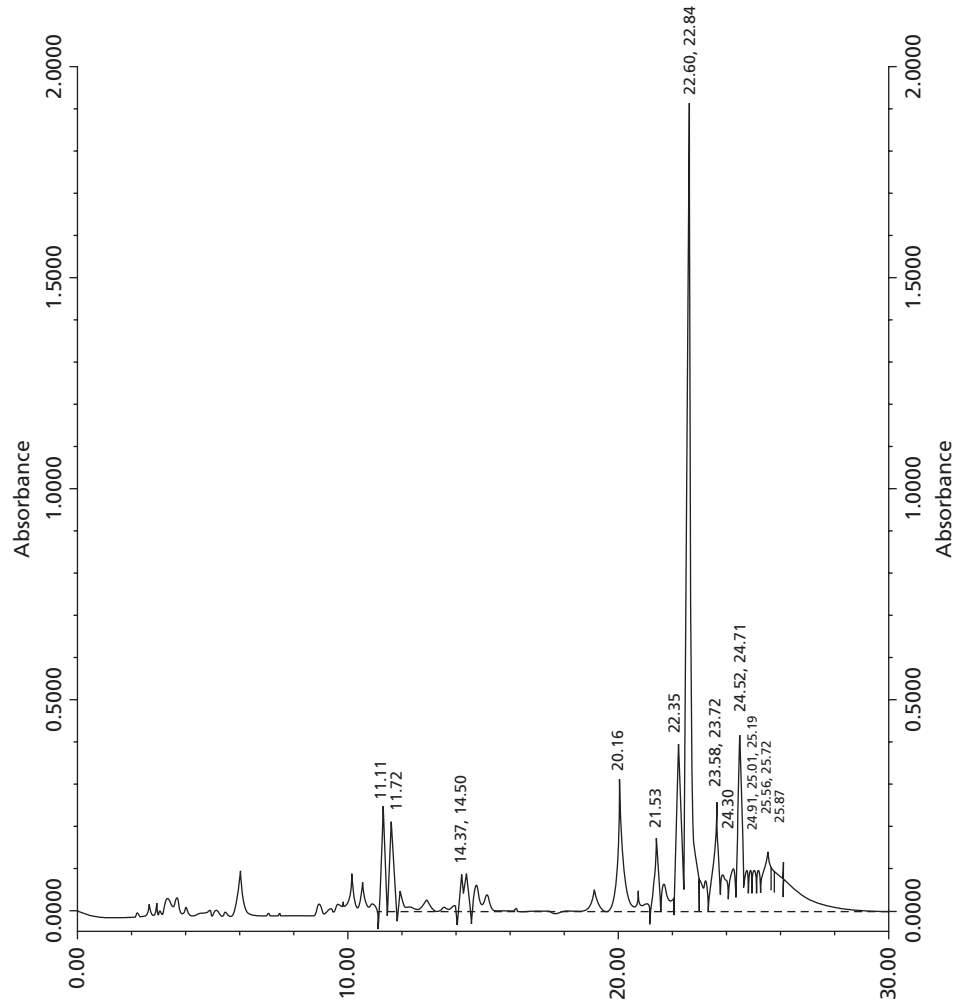

Figure 1 HPLC fingerprint of leaf methanol extract of *Olea europaea* subsp. *africana*

which is thought to exert its effect by irritating the mucosa of the gastrointestinal tract, resulting in an increase in intestinal motility. Ricinoleic acid is also known to diminish sodium ion and chloride ion permeability and is associated with stimulation of prostaglandin release.[13,14] The nitric acid mechanism has also been shown to be involved in castor oil-induced diarrhoea.[15,16]

The data obtained in the present study show that loperamide, a standard antidiarrhoeal agent, profoundly inhibited castor oil-induced diarrhoea in mice. Loperamide, an opioid derivative, decreases intestinal motility by binding to mu receptors on neurons in the submucosal neural plexus of the intestinal wall and by its antimuscarinic activity in the gastrointestinal tract.[12,17,18] It is not surprising therefore that loperamide protected mice against castor oil-induced diarrhoea.

In our study, the leaf methanol extract of *O. europaea* subsp. *africana* significantly antagonised castor oil-induced diarrhoea in mice. It is possible that the plant extract may be exerting its antidiarrhoeal effect by slowing intestinal motility. In the present study, both *O. europaea* subsp. *africana* and loperamide inhibited the intestinal propulsion of charcoal meal and also reduced castor oil-induced intraluminal accumulation of fluid volume. According to DiCarlo *et al.,*[10] agents that reduce intestinal motility and secretion may possess antidiarrhoeal activity. Furthermore, a report by Nwafor *et al.*[19] suggested that agents that suppress intestinal fluid accumulation may inhibit gastrointestinal function. All these support the above suggestion that *O. europaea* subsp. *africana* may be exerting its antidiarrhoeal effect by slowing intestinal motility. Phytochemical studies of the leaves of *O. europaea* carried out by Bruneton and Hansen *et al.* have shown the presence of oleuropein and oleacein.[3,4] Furthermore, Bruneton showed that oleuropein has antispasmodic effect.[3] Antispasmodic agents, which have a powerful inhibiting effect on gut motility, are particularly useful for treating diarrhoea.[7] It is possible that oleuropein may also be present in the leaves of the South African species *O. europaea* subsp. *africana* and may contribute to its antidiarrhoeal activity. In our study, the HPLC analysis of the plant extract showed the presence of distinct peaks that may characterise the plant.

In conclusion, the data obtained in this study suggest that *O. europaea* subsp. *africana* reduces intestinal motility,

justifying, in some cases, its use by traditional medicine practitioners in the treatment of diarrhoea. It is probable that the antidiarrhoeal effect of *O. europaea* subsp. *africana* may involve the inhibition of electrolyte permeability, inhibition of prostaglandin release and/or inhibition of nitric acid mechanism. The relatively high LD50 value of 3475 mg/kg (p.o.) obtained for the plant extract indicates that it is safe and/or non-toxic in mice.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was funded by the National Research Foundation, South Africa (grant number: 67983).

Acknowledgements

We are grateful to Messrs Lilburne Cyster, Vinesh Jeaven and Yusuf Alexander for their technical assistance.

References

1. Van Wyk B *et al. Medicinal Plants of South Africa*. Pretoria: Briza Publications, 1997.
2. Watt JM, Breyer-Brandwijk MG. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd edn. London: Livingstone, 1962.
3. Bruneton J. *Pharmacognosy, Phytochemistry, Medicinal Plants*. Hampshire: Intercept, 1995.
4. Hansen K *et al*. In vitro screening of traditional medicines for anti- hypertensive effect based on inhibition of the angiotensin converting enzyme (ACE). *J Ethnopharmacol* 1995; 48: 43–51.
5. Cortesi N *et al*. High performance liquid chromatography in the analysis of *O. europaea* leaf extracts. *Chem Abst* 1985; 102: 227.
6. Petkov V, Manolov P. Pharmacological analysis of the iridoid oleuropein. *Arzn-Forsch (Drug Res)* 1972; 22: 1476–1488.
7. Williamson EM *et al. Pharmacological Methods in Phytotherapy Research: Selection, Preparation and Pharmacological Evaluation of Plant Material*, vol. 1. Chichester: Wiley, 1996.
8. Kitano Y *et al*. General pharmacological profile of the new cognition enhancing agent nefiracetam. *Arzn-Forsch (Drug Res)* 1994; 44: 199–210.
9. Robert A *et al*. Enteropooling assay: a test for diarrhoea produced by prostaglandin. *Prostaglandins* 1976; 11: 809–814.
10. DiCarlo GD *et al*. Effects of quercetin on the gastrointestinal tract in rats and mice. *Phytother Res* 1994; 8: 42–45.
11. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol* 1983; 54: 275–287.
12. Altman DF. Drugs used in gastrointestinal diseases. In: Katzung BG, ed. *Basic and Clinical Pharmacology*, 8th edn. San Francisco: McGraw Hill, 2001: 1070–1071.
13. Zavala MA *et al*. Antidiarrhoeal activity of *Waltheria Americana*, *Commelins cuelestris* and *Alternanthra repens*. *J Ethnopharmacol* 1998; 61: 41–47.
14. Gaginella TS, Phillips SF. Ricinoleic acid: current view of an ancient oil. *Digest Dis Sci* 1975; 23: 1171–1177.
15. Capasso F *et al*. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of N^G-nitro-L-arginine methyl ester. *Br J Pharmacol* 1994; 113: 1127–1130.
16. Mascolo N *et al*. Nitric oxide and castor oil-induced diarrhoea. *J Pharmacol Exp Ther* 1994; 268: 291–295.
17. Waller DG *et al. Medical Pharmacology and Therapeutics*, 2nd edn. Edinburgh: Elsevier Saunders, 2005.
18. Camillen M *et al*. Consensus report: clinical mechanisms, diagnosis and management of irritable bowel syndrome. *Aliment Pharmacol Ther* 2002; 16: 1407.
19. Nwafor PA *et al*. Antidiarrhoeal and antiulcerogenic effects of methanol extract of *Asparagus* pubescent root in rats. *J Ethnopharmacol* 2000; 72: 421–427.