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Screening of medicinal plants used in South African traditional medicine for genotoxic effects

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Abstract

Dichloromethane and 90% methanol extracts from 51 South African medicinal plants were evaluated for potential genotoxic effects using the bacterial Ames and VITOTOX® tests with and without metabolic activation. Dichloromethane extracts from bulbs of *Crinum macowanii* showed mutagenicity in strain TA98 with and without metabolic activation, whereas extracts from leaves of *Chaetacme aristata* and foliage of *Plumbago auriculata* showed mutagenicity and/or toxicity. Extracts from the leaves of *Catharanthus roseus* and twigs of *Combretum mkhzense* were mutagenic with metabolic activation only. The only 90% methanol extracts that were mutagenic in strain TA98 were from the leaves of *C. roseus* and *Ziziphus mucronata* in the presence of metabolic activation. No genotoxic effects were found in strain TA100 or in the VITOTOX® test.

Keywords: Plant extracts; Medicinal plants; Genotoxicity; Ames test; VITOTOX® test

1. Introduction

Fossils date human use of plants as medicine to approximately 60 000 years ago (Fabricant and Farnsworth, 2001). Today, almost 65% of the world's population relies on plants as an integral part of their primary health care. In South Africa up to 60% of the population consults one of an estimated 200 000 traditional healers, in preference to, or in addition to Western medical doctors, especially in the rural areas (Van Wyk et al., 1997).

There have been many validations of traditional remedies through scientific research (McGaw et al., 2000; Sparg et al., 2000; Rabe and van Staden, 1998). In addition, the use of ethnomedical information has contributed to health care world

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wide through the isolation of bioactive compounds for direct use in medicine (Fabricant and Farnsworth, 2001).

The adverse effects of widely used plants are not well documented in the literature. Based on their long-term use by humans one might expect plants used in traditional medicine to have low toxicity. However, recent investigations have revealed that many plants used as food or in traditional medicine have mutagenic effects in in vitro assays (Higashimoto et al., 1993; Schimmer et al., 1988, 1994; Kassie et al., 1996). This raises concern about the potential mutagenic hazards resulting from the long-term use of such plants. The purpose of this study was to investigate the potential mutagenic effects of plants used in South African traditional medicine using the in vitro bacterial Ames and VITOTOX® tests.

The VITOTOX® test is based on *Salmonella typhimurium* strain TA104 *rec*N2-4) that contain the lux operon of *Vibrio fisheri* under transcriptional control of the recN gene, which is part of the SOS system. Incubation of the bacteria in the presence of a genotoxic compound results in the derepression of the *rec*N promoter, and hence in expression of the *lux* operon. This expression finally results in light production as a function of genotoxicity (van der Lelie et al., 1997). Addition of another strain (TA104 *pr*1) that contains the *lux* operon under control of the strong promoter *pr*1 that is not part of the SOS-system allows detection of false positive responses (if light is also induced here) or of a toxic response (if background light production significantly decreases) (Verschaeve et al., 1999).

2. Materials and methods

South African plants were selected on the basis of their reported ethnobotanical use in South African traditional medicine (Table 1) and on availability (Hutchings et al., 1996; Van Wyk et al., 1997). Ten grams of dried and powdered plant material were extracted using a sonication bath (40 °C). Sequential extractions with 100 ml of CH_2Cl_2 and 90% CH_3OH were performed for 30 min each. Plant material was dried overnight between extractions. Crude extracts were filtered and the filtrate dried under vacuum at 45 °C. Aliquots were prepared from dried crude extracts and dissolved in 10% DMSO to give the initial concentrations of 5.0, 0.5 and 0.05 mg/ml for the Ames test. Concentrations of dried crude extracts between 0 and 2.0 mg/ml (sometimes 5.0 mg/ml) were used in the VITOTOX®test.

The Ames assay was performed with *S. typhimurium* strain TA98, and in some instances also strain TA 100. The well-known plate incorporation procedure described by Maron and Ames (1983) was used.

One hundred microliters of bacterial stock were incubated in 20 ml of Oxoid Nutrient broth for 16 h at 37 °C on a rotative shaker. Of this overnight culture 0.1 ml were added to 2.0 ml of top agar (containing traces of biotin and histidine) together with 0.1 ml test solution (plant extract, solvent control or positive control) and 0.5 ml phosphate buffer (for exposure without metabolic activation) or 0.5 ml metabolic activation mixture containing an adequate amount post-mitochondrial fraction (S9mix). The top agar mixture was poured over the surface of a minimal agar plate and incubated for 48 h at 37 °C. After incubation the number of revertant colonies (mutants) was counted. All cultures were made in triplicate (except the solvent control where five replicas were made). Absence of toxicity was examined by observing the background bacterial growth, which should be normally present. The positive controls used were 4-nitroquinoline 1-oxide (4-NQO) at a concentration of 2 μg/ml (for TA98 and TA100 without S9) and benzo[a] pyrene (B-[a]-P) at a concentration of 20 μg/ml (for TA98 and TA100 with S9).

The S9 mix was prepared by adding 2 ml of S9 (prepared from the liver of Aroclor-induced rats; Moltox™, Boone, North Carolina) to 18 ml of water in which one mutagenicity-test tablet (from Roche; Mannheim, Germany) was dissolved. The so-obtained ready-to-use working solution (S9mix) contains 4 mM NADP–Na_2, 5 mM glucose-6-phosphate–Na_2, 8 mM Mg–aspartate, 33 mM KCl, 0.1 M phosphate buffer (pH 7.4) and 10% v/v S9-fraction.

The VITOTOX® test was carried out as described by Verschaeve et al. (1999) using the test

Table 1
Medicinal plants, usage and summary of the results obtained with the bacterial Ames assay

Plant name	Plant family	Medicinal use	Plant part used	AMES test							
				Dichloromethane extracts				90% Methanol extracts			
				TA98		TA100		TA98		TA100	
				−S9	+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9
Tulbaghia violacea	Alliaceae	Leaves, bulb: asthma, tuberculosis, rheumatisms, paralysis, stomach problems, enemas	Leaf	−	−	−	−	−	−	−	−
Tulbaghia violacea			Bark	Toxic	−	−	−	−	−	−	−
Boophane disticha	Amaryllidaceae	Bulb: dressing for cuts, boils, septic wounds, headaches, abdominal pain, weakness, eye conditions, sedative	Bulb	−	−	−	−	−	−	−	−
C. macowanii		Bulb: scrofula, rheumatic fever, kidney and bladder diseases, fever, sores, glandular swellings	Bark	+	+	−	−	−	−	−	−
Harpephyllum caffrum	Anacardiaceae	Stem bark: emetics, blood purifiers, skin washes, sprains, fractures	Leaf	−	−	Nt	Nt	−	−	−	−
Rhus gueinzii		Root: gastro-intestinal infections	Root	−	−	−	−	−	−	−	−
Sclerocarya birrea		Leaf, bark, root: diarrhoea, dysentery, stomach problems, fever, malaria, tonic, diabetes	Bark	−	−	Nt	Nt	−	−	−	−
Sclerocarya birrea			Root	−	−	−	−	−	−	−	−
Sclerocarya birrea			Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Heteromorpha trifoliata	Apiaceae	Leaves, bark: scrofula, enemas, abdominal disorders, mental/nervous disorders, intestinal worms, headaches, antiscabies	Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Heteromorpha trifoliata			Twigs/ bark	−	−	Nt	Nt	−	−	Nt	Nt
Acokanthera oblongifolia	Apocynaceae	Leaves, root: emetics for snakebite, severe gastro-intestinal irritation, digitalis-like cardiac effects, irritant, convulsions	Twigs/ bark	−	−	Nt	Nt	−	−	Nt	Nt
Acokanthera oblongifolia			Leaf	−	−	Nt	Nt	−	−	Nt	Nt
C. roseus		Leaves: diabetes, rhematism	Leaf	−	+	−	−	−	+	−	−
Artemisia afra	Asteraceae	Leaves: coughs, colds, influenza, fever, loss of appetite, colic, headache, earache, malaria, intestinal worms	Leaf	−	−	−	−	−	−	−	−
Senecio serratuloides		Leaves: cuts, swellings, burns, sores, blood purifiers, headaches	Leaf	−	−	−	−	−	−	−	−
Vernonia colorata		Leaves, twigs: abdominal pain, colic, rheumatism, dysentery, diabetes, ulcerative colitis (roots)	Leaf	−	−	−	−	−	−	−	−
Vernonia colorata			Root	−	−	−	−	−	−	−	−
Balanites maughamii	Balanitaceae	Roots, bark: mulluscicidal properties	Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Balanites maughamii			Twigs	−	−	Nt	Nt	−	−	Nt	Nt
Kigelia africana	Begoniaceae	Fruit: ulcers, sores, syphilis, rheumatism, enema	Flower	−	−	−	−	−	−	−	−

Table 1 (*Continued*)

Plant name	Plant family	Medicinal use	Plant part used	AMES test							
				Dichloromethane extracts				90% Methanol extracts			
				TA98		TA100		TA98		TA100	
				−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Afzelia quanzensis	Caesalpinaceae	Bark: uterine pain, schistosomiasis (bladder), eye complaints, roots: aphrodisiac, snakebite	Root	–	–	–	–	–	–	–	–
Warburgia satutaris	Canellaceae	Bark: coughs, colds, chest complaints, influenza, rheumatism, malaria, venereal diseases, headache, toothache, gastric ulcers	Leaf	–	–	–	–	–	–	Nt	Nt
C. mkhzense	Combretaceae	Roots: stomach disorders, enemas (Genus)	Bark	–	+	–	–	–	–	–	–
Diospyros whyteana	Ebenaceae	Roots: dysmenorrhoea, imitating rashes, antibacterial?	Twigs	–	–	Nt	Nt	–	–	Nt	Nt
Diospyros whyteana			Leaf	–	+	Nt	Nt	–	–	Nt	Nt
Euclea divinorum		Roots: purgative, for headache, toothache, constipation, antihelmintics, tonics, chest pain, pneumonia, stomach pain	Root	Toxic	–	–	–	Nt	Nt	Nt	Nt
Euclea natalensis		Bark: heart disease, headache, anti-inflammatory, purgative, analgesic	Bark	Toxic	–	–	–	Nt	Nt	Nt	Nt
Antidesma venosum	Euphorbiaceae	Leaves, twigs: abdominal pain, enema	Leaf	–	–	Nt	Nt	–	–	Nt	Nt
Antidesma venosum			Root	–	–	–	–	–	–	–	–
Antidesma venosum			Twigs	–	–	Nt	Nt	–	–	Nt	Nt
Croton sylvaticus		Bark, roots: abdominal, internal inflammations, uterine disorders, tonic, febrile conditions, purgative, pleurisy, indigestion, TB, rheumatism	Leaf	–	–	Nt	Nt	–	–	Nt	Nt
Croton sylvaticus			Twigs/bark	–	–	Nt	Nt	–	–	Nt	Nt
Ricinus communis		Seed oil/fruits/leaves: purgatives, enemas, stomach ache, root and leaf applied to wounds, sores, boils	Root	–	–	–	–	–	–	–	–
Spirostachys africana		Wood: stomach ulcers, acute gastritis, eye washes, headaches, rashes, boils, emetic, renal ailment, purgative, blood purifiers, diarrhoea, dysentery	Leaf	–	–	Nt	Nt	–	–	Nt	Nt
Spirostachys africana			Twigs/bark	–	–	–	–	–	–	–	–
Ornithogalum longibracteatum	Hyacinthaceae	Bulb: charm, irritant	B	–	–	Nt	Nt	–	–	Nt	Nt
Ornithogalum longibracteatum			L/F	–	–	Nt	Nt	–	–	Nt	Nt
Hypoxis colchicifolia	Hypoxidaceae	Bulb: tonic, anti-HIV, anti-inflammatory	Bulb	–	–	Nt	Nt	Nt	Nt	Nt	Nt
Hypoxis hemerocallidea		Bulb: dizziness, bladder and urinary disease, tonic, burns	Bark	–	–	–	–	–	–	–	–
Tetradenia riparia	Lamiaceae	Leaves: coughs, sore throats, malaria, dengue fever, dropsy, fever, diarrhoea, haemoptysis, boils, mumps, induce drowsiness	Leaf	–	–	Nt	Nt	–	–	Nt	Nt

Table 1 (*Continued*)

Plant name	Plant family	Medicinal use	Plant part used	AMES test							
				Dichloromethane extracts				90% Methanol extracts			
				TA98		TA100		TA98		TA100	
				−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Ocotea bullata	Lauraceae	Bark: snuff, headaches, urinary disorders, stomach problems, infantile diarrhoea	Bark	−	−	−	−	−	−	−	−
Acacia sieberiana	Leguminosae	Bark: enemas, antiseptic, fever, stomach ache, tapeworm, astringent, haemostatic, diarrhoea, opthalmia	Bark	−	−	−	−	−	−	−	−
Erythrina caffra		Bark: sores, wounds, arthritis, sprains, aches	Bark	−	−	−	−	−	−	Nt	Nt
Merwilla natalensis	Liliacae	Bulb: enema, sprains, fractures, purgative, boils, sores, infertility	Bulb	−	−	−	−	−	−	−	−
Ekebergia capensis	Meliaceae	Bark: emetic for dysentery and heartbum, abscesses, boils	Bark	−	−	−	−	Nt	Nt	Nt	Nt
Trichilia emetica		Bark: stomach and intestinal complaints, dysentery, kidney problems, indigestion, parasites, fever, purgative, bruises, rheumatism	Bark	−	−	−	−	−	−	−	−
Turraea floribunda		Bark, root: emetic, rheumatism, dropsy, heart disease, swollen and painful joints	Twigs	−	−	Nt	Nt	−	−	Nt	Nt
Turraea floribunda			Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Bersama lucens	Melianthaceae	Bark: barreness and impotence, menstral pain, leprosy	Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Syzygium cordatum	Myrtaceae	Leaves, bark: respiratory disorders, TB, stomach complaints, emetics, diarrhoea, colds, fever, headaches, amenorrhoea, wounds	Leaf	-	−	Nt	Nt	−	−	Nt	Nt
Ochna serrulata	Ochnaceae	Root: enema, gangrenous rectitis, bone diseases (children)	Leaf	−	−	−	−	−	−	−	−
Oenothera biennis	Onagraceae	Leaves: antiasthmatic, anti-viral, arthritis, antithrombic, antioxidant, fungistatic	Leaf	−	−	−	−	−	−	−	−
P. auriculata	Plumbaginaceae	Roots, leaves: headaches, emetics, warts, fractures, scrofula, oedema, malaria, skin lesions	Twigs	−	−	Nt	Nt	−	−	Nt	Nt
P. auriculata			Foliage	+	+	Nt	Nt	−	−	Nt	Nt
Polygata virgata	Polygalacaea	Leaves: emetic, blood purifier, antiviral (vs. rhinovirus, polio, herpes simplex), TB	Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Rhamnus prinoides	Rhamnaceae	Leaves, root: sprains, blood purifiers, pneumonia, emetics, purgative, colic, stimulants	Twigs/Bark	−	−	Nt	Nt	−	−	Nt	Nt
Rhamnus prinoides			Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Z. mucronata		Leaves, bark, roots: boils, sores, glandular swellings, diarrhoea, dysentery, expectorant, emetic for coughs chest problems, boils, sores, glandular swellings	Leaf	−	−	−	−	−	+	−	−
Ziziphus mucronata			Root	−	−	−	−	−	−	−	−
Z. mucronata			Twigs	−	−	Nt	Nt	−	−	Nt	Nt
Prunus africana	Rosaceae	Fruit/bark: intercostal pain, prostate hypertrophy, hair tonics	Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Prunus africana			Twigs	−	−	Nt	Nt	−	−	Nt	Nt

Table 1 (*Continued*)

Plant name	Plant family	Medicinal use	Plant part used	AMES test							
				Dichloromethane extracts				90% Methanol extracts			
				TA98		TA100		TA98		TA100	
				−S9	+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9
Catunaregam spinosa	Rubiaceae	Root/fruit: emetic, fever, aphrodisiac, gonorrhoea, headaches, nausea, respiratory and febrile compliants, gynaecological ailments, epilepsy, arthritis	Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Gardenia volkensii		Fruit/root: emetic, sore eyes, headaches, asthma, dysmenorrhoea, infertility, epilepsy, convulsions, earache	Twigs/ bark	−	−	−	−	−	−	Nt	Nt
Gardenia volkensii			Leaf	−	−	Nt	Nt	−	−	Nt	Nt
Dombeya rotundifolia	Sterculiaceae	Bark, roots, wood: internal ulcers, haemorrhoids, diarrhoae, stomach problems, nausea, chest complaints	Bulb	−	−	−	−	−	−	−	−
Dombeya rotundifolia			Leaf	−	−	−	−	−	−	−	−
Celtis africana	Ulmaceae	Wood: charm, protective	Root	−	−	−	−	−	−	−	−
C. aristata		Bark, roots: haemorrhoids	Leaf	+	+	Nt	Nt	−	−	Nt	Nt
C. aristata			Twigs	−	−	Nt	Nt	−	−	Nt	Nt
Pouzolzia mixta	Urticaeae	Bark: fever, wounds, dysentery (roots)	Leaf	−	−	−	−	−	−	Nt	Nt
Pouzolzia mixta			Root	−	−	−	−	Nt	Nt	Nt	Nt
Siphonochitus aethiopicus	Zingiberaceae	Rhizome: colds, coughs, influenza, hysteria, asthma, dysmennorhoea	Root	−	−	−	−	−	−	−	−

−, Negative; +, positive; Nt, not tested.

strains TA104 *Rec*N2-4 and TA104 *pr*1 of *S. typhimurium* in the presence and absence of S9 mix. The two bacterial strains (20 μl) were incubated overnight (approximately for 15 h) on a rotative shaker (180 rpm) at 37 °C in 5 ml of 869 medium (Mergeay et al., 1985) which is a normal well-known bacterial growth medium. The next morning the bacterial suspension was diluted ten times in the same growth medium and incubated for a further 1 h at 37 °C on a rotative shaker (180 rpm) to obtain log phase growth.

In the meantime the S9 mix was prepared. Here no Ames test tablets were used because much smaller amounts of S9 are necessary compared with the Ames test. The final S9mix is, however, the same as used in the Ames test. This mixture (400 μl) was added to 860 μl culture medium and 140 μl of the bacteria (*rec*N2-4 or *pr*1). From this 90 μl were added to 10 μl of the test compound in 96-well plates. The 96-well microtiter plate was then placed in a Microlumat LB96B luminometer (EG|G Berthold) or in a Luminoscan Ascent (Labsystems) and measuring was performed according to the following parameters: 1 s/well; cycle time = 5 min; 60 cycles; incubation temperature 30 °C. The signal to noise ratio (S/N) being the light production of exposed cells divided by the light production of non-exposed (control) cells, is automatically calculated for each measurement. An extract is considered genotoxic when the max S/N (recN2-4)/max S/N (pr1) > 1.5 and when the signal is not generated in the first 20 min of the measurements. However, the extract is considered toxic if S/N (for *rec*N2-4 and/or *pr*1) rapidly decreases below 0.8. Positive controls were 4-NQO (without S9) and B-[a]-P (with 39) at a concentration of 0.04 and 8 μg/ml, respectively.

3. Results

Table 1 lists the plant species and parts used, together with their main use in traditional medicine. It also contains the results on the mutagenic effects of CH_2Cl_2 and 90% CH_3OH extracts examined with the Ames test. Negative results (−) mean that the extracts were not able to enhance the number of $His^- \rightarrow His^+$ revertants by a factor of two or more above the spontaneous background level. This background level as well as positive control values, were in all cases within the normal limits found in the laboratory (Table 2) and in accordance with literature data (Mortelmans and Zeiger, 2000). Detailed results are represented for plant species where a mutagenic response was observed (Table 2).

Plant extracts inducing revertant colonies numbering at least twice the revertant control number were considered positive for mutagenic activity. Of the CH_2Cl_2 extracts tested, that from the bulbs of *Crinum macowanii* and *Ziziphus mucronata* were mutagenic for strain TA98 with and without metabolic activation. Dichloromethane extracts of the leaves of *Chaetacme aristata* and foliage of *Plumbago auriculata* were at first sight highly mutagenic at the lowest concentration tested whereas higher doses gave a very high and uncountable number of small colonies. However, the results presumably reflects bacterial toxicity rather than mutagenicity, especially since the normal background bacterial growth was missing, and also since no indication of genotoxicity was found in the micronucleus test and alkaline comet assay in human white blood cells (Taylor et al., 2003). Dichloromethane extracts of the leaves of *Catharanthus roseus*, *Diospyros whyteana* and twigs of *Combretum mkhzense* showed mutagenicity for TA98 with metabolic activation only. *D. whyteana* was mutagenic only in the lowest dose (0.05 mg/ml). At higher doses no background bacterial layer was present indicating a toxic response. The 90% CH_3OH leaf extracts of *C. roseus* and *Z. mucronata* showed a mutagenic effect for TA98 in the presence of S9 mix alone.

It was also noticeable that extracts from different organs of the same species gave different responses, e.g. CH_2Cl_2 extracts of the leaves and twigs of *C. aristata*, and foliage and twigs of *P. auriculata*.

Plant extracts tested for mutagenic effects using TA100, with and without metabolic activation, invariably gave a negative response.

In the VITOTOX®-test neither CH_2Cl_2 or 90% CH_3OH extracts induced a genotoxic response. However, results indicated that many of the plant extracts were toxic according to the above set of

Table 2
Ratio of His^+ revertants in *S. typhimurium* strain TA98 produced by crude plant extracts to that in the controls

Plant name	Plant part	Dichloromethane extracts						90% Methanol extracts					
		−S9			+S9			−S9			+S9		
		5.0[a]	0.50[a]	0.05[a]	5.0[a]	0.50[a]	0.05[a]	5.0[a]	0.50[a]	0.05[a]	5.0[a]	0.50[a]	0.05[a]
C. macowanii	Bulb	12.4	2.7	1.1	2.2	1.6	1.3	1.1	1.1	1.4	1.5	1.3	1.1
C. aristata	Leaf	NC[b]	NC[b]	80.0	NC[b]	NC[b]	NC[b]	1.6	1.4	1.6	1.1	1.1	1.4
P. auriculata	Foliage	NC[b]	NC[b]	NC[b]	NC[b]	NC[b]	120.4	0.7	0.8	0.8	1.2	1.2	1.2
C. roseus	Leaf	1.1	0.8	1.1	4.1	3.6	3.2	1.1	1.0	0.9	4.1	3.9	2.0
D. whyteana	Leaf	0.8	0.7	0.5	1.1	1.2	69.0	0.6	0.6	0.6	1.5	0.9	1.0
C. mkhzense	Twigs	1.1	1.1	1.2	2.2	1.9	1.5	0.8	1.2	0.9	1.5	1.7	1.7
Z. mucronata	Leaf	1.0	1.1	1.1	1.1	0.9	0.7	1.6	1.2	1.2	2.3	1.1	1.1

Ranges of control values (number of revertants) observed over the different experiments reported in Tables 1 and 2; 45 > solvent control values with and without S9 > 10; 412 > positive control values without S9 > 119; 447 > positive control values with S9 > 162.
[a] Concentration of dried plant extract used (mg/ml).
[b] Uncountable colonies (number of colonies was too high to be counted).

criteria, preventing a proper evaluation of genotoxicity. Extracts of plants such as *Acokanthera oblongifolia*, *D. whyteana*, *Antidesma venosum* and *Turraea floribunda* retained this apparently toxic response with the addition of S9. In others, toxicity was lost when S9 was added, e.g. *Acacia sieberiana*, *Merwilla natalensis* and *Celtis africana*. An example of VITOTOX®-test results is given in Figs. 1 and 2 where positive controls are presented (indicating clear genotoxicity without a sign of toxicity or a false positive response), and in Fig. 3 for *Senecio serratuloides* where a toxic response was found at the highest dose (2000 μg/ml) and lack of genotoxicity at the lower doses (16, 80 and 400 μg/ml). Figs. 1 and 2 show a wide range of doses in order to better demonstrate the dose–effect relationships. However, positive controls used in a typical VITOTOX®-test comprise only one dose as indicated before.

4. Discussion

The results of this study indicated that extracts from bulbs of *C. macowanii*, leaves of *C. roseus*, *D. whyteana*, *C. roseus*, *Z. mucronata* and twigs of *C. mkhzense* caused frame shift mutations in *S. typhimurium* TA98. Leaves of *Chaetacme aristata* and foliage of *P. auriculata* were most probably toxic at all doses. All extracts tested in Strain TA100 were negative and thus TA100 was not used for testing the remaining extracts. Earlier work in our laboratory also indicated that *S. typhimurium* TA100 added little to the information obtained from TA98, at least for the kind of samples used (unpublished).

The VITOTOX® test is very useful as it is very rapid (results are obtained within 4 h), requires only minimal amounts of test sample, usually detects much lower concentrations of a genotoxin than other short term tests (e.g. concentrations of the positive controls used in the VITOTOX® test were considerably lower than in the Ames test), and correlates well with other bacterial tests, as for example the Ames assay (Verschaeve et al., 1999; Van Gompel, 2001). This is the reason why we used this test in the present work as we also did for other studies, e.g. for analyses of complex mixtures as, for example, effluents and extracts from air samples. Results that we already obtained from such investigations do demonstrate, however, that the VITOTOX® test, indeed is particularly interesting for testing pure compounds or, compounds that do not require extraction or preconcentration steps (Verschaeve et al., 1999; Verschaeve, 2002; Corbisier et al., 2001), but when samples required extraction or preconcentration procedures other tests were found more appropriate. The results obtained in the present work seem to confirm these preliminary results and conclusions. Indeed, none

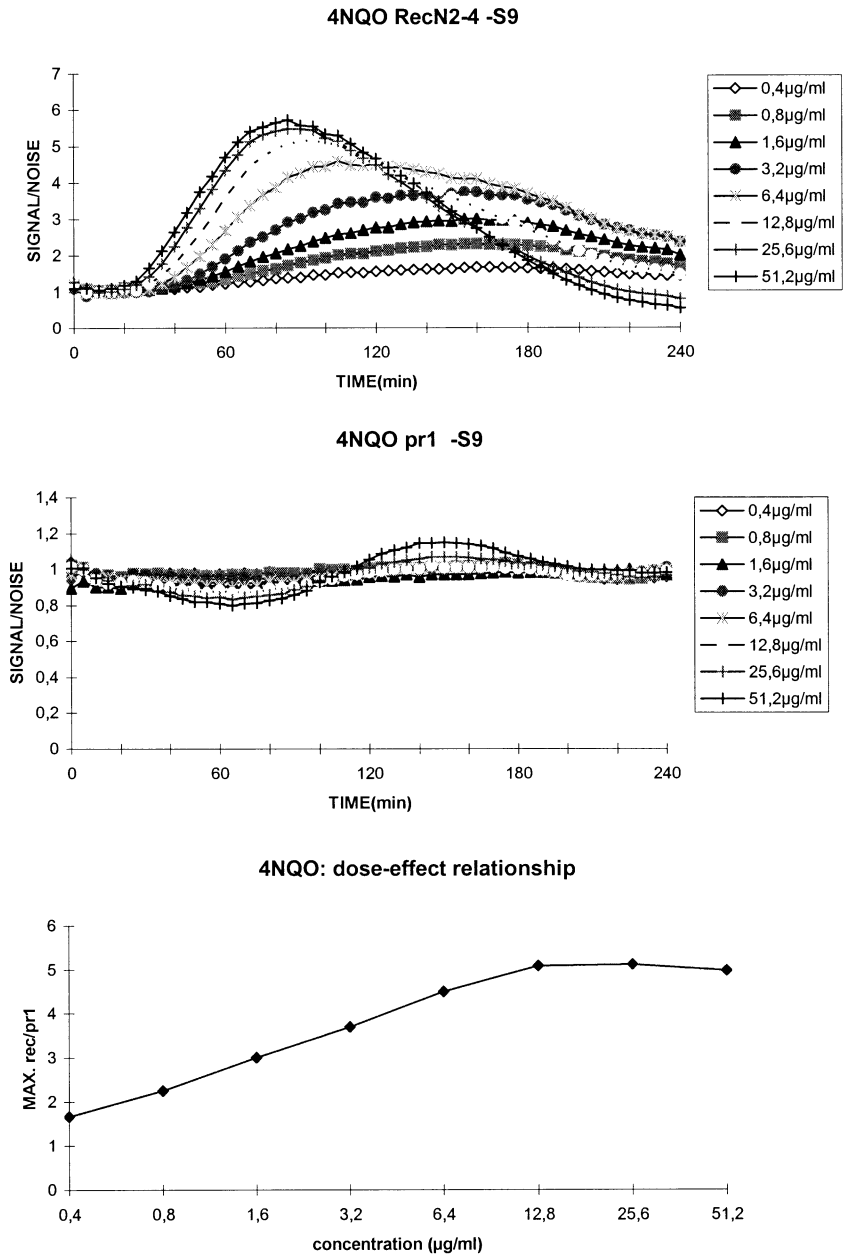

Fig. 1. Example of VITOTOX® test results for the positive control 4-NQO. A S/N ratio around 1 for the *pr* 1 strains indicates absence of toxicity or false positive responses. In the *rec* N2-4 strain there is a clear dose–response relationship well above "2" indicating genotoxicity. This can also be seen by the dose–effect relationship showing the $max_{s/n}$ recN2-4/$max_{s/n}$ pr1 in function of test sample concentration. A dose dependent increase reaching >1.5 levels indicates genotoxicity.

of the plant extracts tested caused genotoxicity as evidenced by the negative results obtained in the VITOTOX® test, but most of the plant extracts exhibited toxicity according to the above mentioned criteria. This toxicity could mask the genotoxic response (Schimmer et al., 1994).

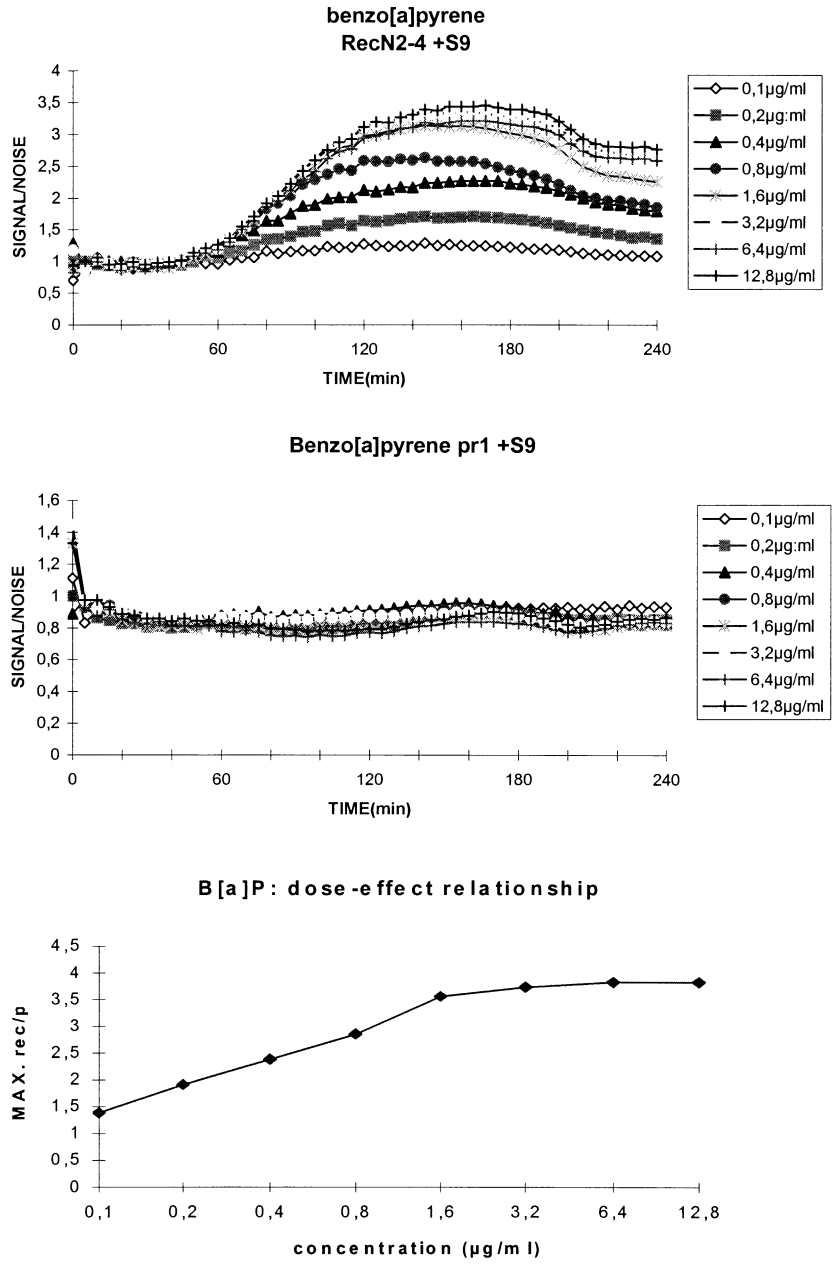

Fig. 2. Example of VITOTOX test results for the positive control B-[a]-P. A S/N ratio around 1 for the *pr* 1 strains indicates absence of toxicity or false positive responses. In the *rec* N2-4 strain there is a clear dose–response relationship well above "2" indicating genotoxicity. This can also be seen by the dose–effect relationship showing the $max_{s/n}$ recN2-4/$max_{s/n}$ pr1 in function of test sample concentration. A dose dependent increase reaching >1.5 levels indicates genotoxicity.

Crude extracts from *C. macowanii*, *Catharanthus rosus*, *S. serratuloides* were further fractionated into acidic, neutral and basic fractions to eliminate the toxic fractions. Further testing of these fractions in the VITOTOX® test showed even higher "toxicity". We, therefore, believe that

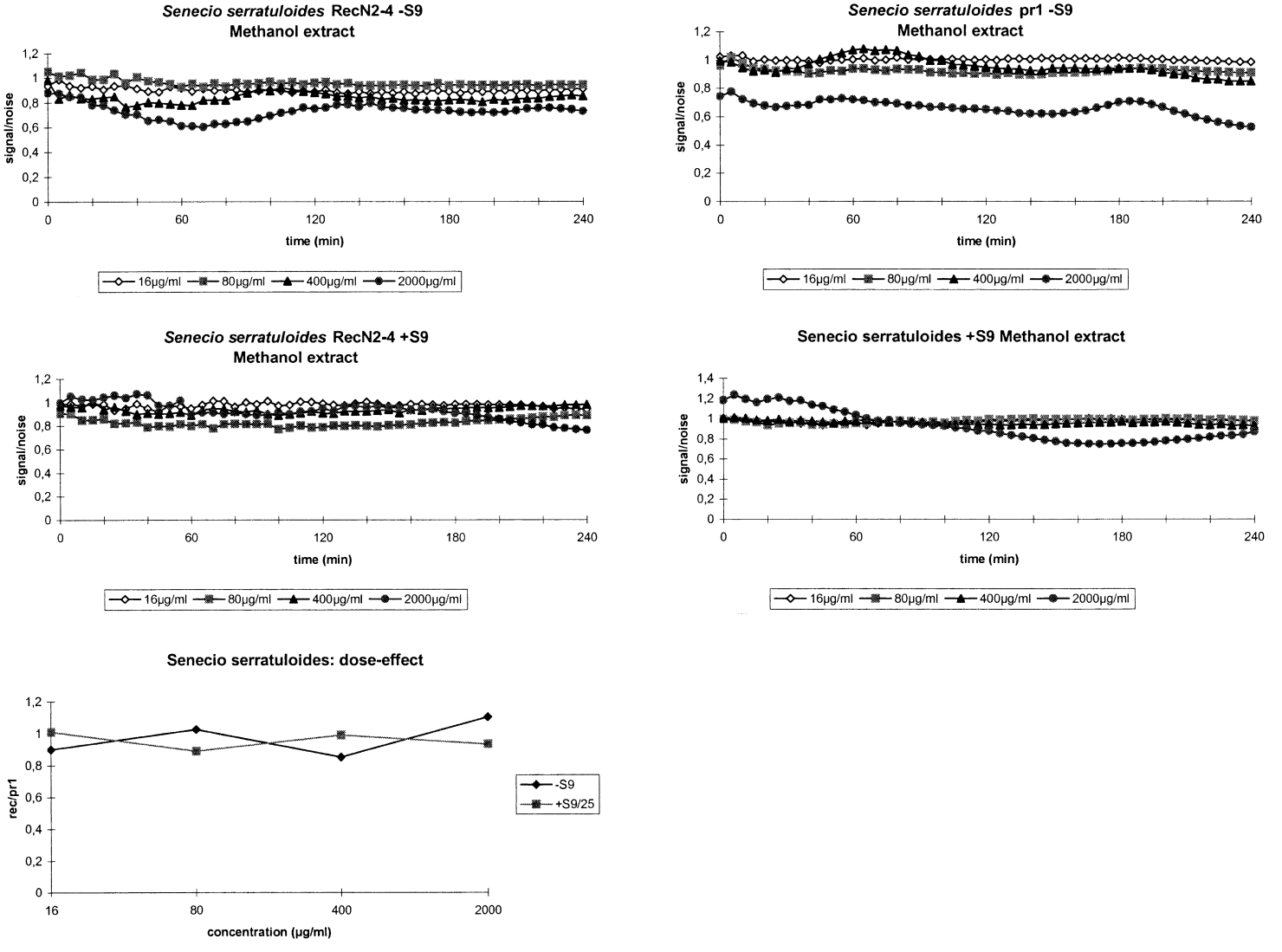

Fig. 3. S/N ratios in the *rec* N2-4 and *pr* 1 strains of *S. typhimurium* indicating absence of genotoxicity of *S. serratuloides* methanol extracts (no increased light production in *rec* N2-4) and signs of "toxicity" (see text) at the highest concentration (S/N ratio slightly below 0.8 in both the pr1 and *rec* N2-4 strains, especially in the absence of S9). There is no dose dependent increase reaching >1.5 levels indicating lack of genotoxicity.

the VITOTOX® test is, at least in its present form, not very suitable for the testing of plant, and eventually also other, extracts. It is possible that residues of extraction products are responsible for "toxicity" in the VITOTOX® test. However, as they are usually not toxic in the Ames test this is rather unlikely (both tests use *S. typhimurium*). A more plausible explanation is that the residues interact with the *lux* operon in TA104 *rec*N2-4 and TA104 *pr*1 and, therefore, disturb its "normal" functioning. This certainly needs further investigation.

For the present, it is only possible to state that results of the VITOTOX® test applied to plant extracts, should be treated with caution and that at least some of the South African medicinal preparations have a genotoxic potential in *S. typhimurium* TA98.

There are several reports on plant extracts exhibiting mutagenic and/or genotoxic effects (Morimoto et al., 1982; Xue-jun et al., 1991; Schimmer et al., 1994). Quercetin, furoquinoline alkaloids and isothiocyanates are considered to be among the possible mutagens of plant origin (Schimmer et al., 1988, 1994; Kassie et al., 1996). However, there are no reports on mutagenic compounds from plant extracts investigated in this study. The fact that plant extracts are complex mixtures of organic compounds makes it difficult to speculate on the compounds responsible for the mutagenic response detected with these extracts. These findings warrant the isolation of the compounds responsible for these mutagenic effects.

Many of the plants used in traditional medicine have solid scientific support with regard to their efficacy. However, the findings in this study raise questions about the safety of these plants and their continued extensive use in primary health care in the rural areas of South Africa. Further investigation of plant extracts in the micronucleus test (for detection of chromosome breakage and aneuploidy) and the alkaline comet assay (for DNA damage), both performed on human peripheral white blood cells, supported this finding. Extracts that showed genotoxic effects in bacterial tests (Table 2) also were genotoxic in at least one of the genotoxicity tests in human blood, for at least one of the extraction procedures (Taylor et al., 2003). Therefore, thorough screening for potential harmful genotoxic effects of plants used in traditional medicine is recommended before long-term usage is entered into.

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