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Mutagenicity, antimutagenicity and cytotoxicity evaluation of South African *Podocarpus* species

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ABSTRACT

Ethnopharmacological relevance: Four species of *Podocarpus* are used in traditional medicine both in human and animal healthcare in South Africa. *In vitro* pharmacological screening of leaf and stem extracts of these species exhibited potent antimicrobial, anti-inflammatory, anti-tyrosinase, anthelmintic, acetylcholinesterase inhibitory and antioxidant activities.
Aim of the study: To investigate the mutagenicity, antimutagenicity and cytotoxicity effects of leaf and stem extract of South African *Podocarpus* species.
Material and methods: The mutagenicity and cytotoxic effects of extracts from four species of *Podocarpus* were tested using the *Salmonella*/microsome assay with and without metabolic activation, based on the plate-incorporation method and neutral red uptake (NRU) assay respectively. Five *Salmonella typhimurium* tester strains; TA98, TA100, TA102, TA1535 and TA1537 were used for mutagenicity testing. The relative cytotoxicity of the extracts was assessed by determining their NI_{50} values (50% inhibition of NRU).
Results: The extracts did not show any mutagenic effects against all the tester strains with or without metabolic activation. All extracts demonstrated a strong antimutagenic effect on the mutations induced by 4NQO, decreasing its mutagenic effect in a dose-dependent manner. Strong cytotoxic effects were exhibited by petroleum ether extracts as compared to 80% ethanol extracts. When HepG2 cells were in contact with plant extracts in an increasing concentration, slopes of NRU decreased (highest–lowest %) following a concentration-dependent pattern. For 80% ethanol extracts, the most toxic extract in terms of percentage viability was leaves of *Podocarpus falcatus* whereby at 0.2 mg/ml, the viability of the cells was 38.9%. Stem extract of *Podocarpus latifolius* was the most toxic among PE extracts, giving a percentage viability of 46.4 at 0.1 mg/ml.
Conclusion: Absence of mutagenicity does not indicate lack of toxicity, as was observed from these extracts. These findings will help in assessing the safety measures to be considered in the use of these species and also the need to determine the cytotoxic potential of these species against various forms of human cancer cells.

1. Introduction

The identification of substances capable of inducing mutations has become an important procedure in safety assessment. Chemicals that can induce mutations can potentially damage the germ line leading to fertility problems and to mutations in future generations. Mutagenic chemicals are also capable of inducing cancer. This concern has driven most of the mutagenicity testing programs (Mortelmans and Zeiger, 2000). Many plants are known to contain mutagenic compounds such as furocoumarins (Varanda et al., 2002), tannins, anthraquinones (Ferreira and Vargas, 1999) and flavonoids (Rietjens et al., 2005). The enzymes responsible for the activation of the promutagens are present in different cells of mammals, and such activation happens frequently (Goldstein and Faletto, 1993) and in many cases, even a very low exposure to the mutagenic agent may be enough to induce a genotoxic effect. The accumulation of mutations relates to the development of most cancers and various degenerative disorders, as well as aging and genetic defects in offspring (Migliore and Coppedè, 2002).

The search for antimutagenic agents is essential, since mutagenic and carcinogenic factors are present in the human environment and elimination of all of them seems to be impossible (De Flora and Ramel, 1988). It is important to assess for both mutagenic and antimutagenic responses for the same plant extracts, since many edible plants or plant products are known to contain a variety of antimutagenic substances as well as the

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enzymatic machinery to activate environmental mutagens/carcinogens (Cortés-Eslava et al., 2004). A sample indicating both of these activities is referred to as 'Janus' carcinogens and mutagens (Zeiger, 2003). There is also the need of enhancing the human exposure to antimutagenic agents, especially those naturally occurring in plants as secondary metabolites (Ikuma et al., 2006; Jeong et al., 2006). In the last two decades, a wide range of evidence from epidemiological and laboratory studies has demonstrated that some plants eaten whole, or their active ingredients have protective effects against human carcinogenesis and mutagenesis (Surh and Ferguson, 2003). Antimutagenicity determination of plant extracts is important for the discovery of new effective natural anticarcinogenic treatments. Although an antimutagenic effect found in a plant extract does not necessarily mean that it is an anticarcinogen, it is however an indication of the possibility of acting as one. Medicinal plants are candidates for chemoprevention of cancer, because they may possess chemopreventive agents with inhibitory effects on the initiation, promotion and progression of carcinogenesis (Surh and Ferguson, 2003).

Four species of *Podocarpus*; *Podocarpus elongatus* (Ait.) L' Herit. ex Pers., *Podocarpus falcatus* (Thunb.) R. Br. ex Mirb., *Podocarpus henkelii* Stapf. ex Dallim. & Jacks. and *Podocarpus latifolius* (Thunb.) R. Br. ex Mirb. occur in South Africa. These species are used in traditional medicine both in human and animal healthcare (Beentje, 1994; Hutchings et al., 1996; Dold and Cocks, 2001; Masika and Afolayan, 2003; Abdillahi et al., 2010a). The sap from these species is used to treat chest complaints (Watt and Breyer-Brandwijk, 1962). The bark of *Podocarpus latifolius* and *Podocarpus falcatus* is used to treat distemper in dogs and gallsickness in cattle (Dold and Cocks, 2001; Masika and Afolayan, 2003). A bark decoction of *Podocarpus latifolius* is used as a remedy for stomach ache and the powder for curing headaches (Beentje, 1994; Pankhurst, 2000). Oil from *Podocarpus falcatus* is said to have medicinal properties in curing gonorrhoea (Pankhurst, 2000). *In vitro* pharmacological screening of leaf and stem extracts of the four *Podocarpus* species elucidated various biological activities. The extracts exhibited broad-spectrum antibacterial and antifungal activity (Abdillahi et al., 2008); pronounced antioxidant, anti-inflammatory and tyrosinase inhibitory activities (Abdillahi et al., 2010b). In addition to these pharmacological activities, anthelmintic and acetylcholinesterase inhibitory activities were observed in the leaf and stem extracts of these species (Abdillahi, 2011). Based on these findings, *Podocarpus* species have promising biological activities and in order for them to be recommended for further research such as *in vivo* testing and bioprospecting, it is important to determine their safety. This was done by assessing the mutagenicity and genotoxicity of extracts of these species. The antimutagenic effects were also investigated because these extracts may contain antimutagenic substances as well as the enzymatic machinery to activate environmental mutagens/carcinogens. In addition to this, the cytotoxicity activity of the extracts was investigated. With respect to ethnopharmacology, the lack of toxicity and genotoxicity is important for the whole population.

2. Material and methods

2.1. Plant material collection and extraction

Leaves and young stems of *Podocarpus henkelii* (HA 001NU) were obtained from the University of KwaZulu-Natal Botanic Garden, Pietermaritzburg, while leaves and stems of *Podocarpus falcatus* (HA 002NU), *Podocarpus latifolius* (HA 003 NU) and *Podocarpus elongatus* (HA 004NU) were collected from the National Botanic Garden, Pietermaritzburg. Voucher specimens were deposited at the University of KwaZulu-Natal Herbarium (NU). The plant materials were dried at 37 °C and then ground into fine powders using an electric blender. The powders were sealed in airtight containers and stored in the dark at room temperature (25–28 °C), until use. Four solvents; petroleum ether (PE), dichloromethane (DCM), 80% ethanol and water in order of increasing polarity were used for sequential extraction. A 100 ml aliquot of PE was added to 5 g of dried material, then sonicated for 1 h in an ultra-sound bath, kept overnight and then filtered under vacuum using Whatman No. 1 filter paper. This process was then repeated with DCM, ethanol and finally water. The clear filtrates were either dried under vacuum, using a rotary evaporator or freeze dried.

2.2. Mutagenicity test

Mutagenicity was tested using the *Salmonella*/microsome assay with and without metabolic activation, based on the plate-incorporation method (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). Five *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) tester strains with different mutation mechanisms were used. PE and DCM extracts were dissolved in 10% DMSO while 80% ethanol and water extracts were dissolved in water. For each extract, three different concentrations (50, 500 and 5000 μg/ml) were used to test for mutagenicity. The five bacterial strains were incubated in Oxoid Nutrient Broth Number 2 for 16 h at 37 °C in a water bath on an orbital shaker to obtain a density of 2×10^9 colony forming units (CFU/mL). For the experiment without the S9 metabolic activation, 100 μl of the test solutions (plant extracts, negative and positive controls) were placed in a test tube and 500 μl of phosphate buffer (0.1 mM, pH 7.4) was added. This was preincubated for 3 min before the addition of 100 μl overnight bacterial cultures (2×10^8 cell/ml). Two millilitres of top agar containing 0.5 mM histidine-biotin were added to this mixture. The mixture was then poured over the surface of a labelled minimal agar plate and incubated (inverted in the dark) at 37 °C for 48 h. After incubation, his^+ revertant colonies were counted with the help of a binocular microscope and compared to the number of revertant colonies in the controls. For the experiment with S9 metabolic activation, the S9 mixture was freshly prepared before the assay and kept on ice until needed. The S9 mixture consisted of 5% (v/v) S9 fraction (Sigma–Aldrich, Co., St. Louis) pooled from Sprague–Dawley male rats in mixed enzymic cofactors containing NADP. The experiment was repeated as above and the only difference was that 500 μl of S9 was added in place of the phosphate buffer. Two microgram per plate of nitroquinoline-N-oxide (4NQO) and 2-aminoanthracene-(2-AA) were used as positive controls for the assay without and with S9 metabolic activation respectively. Ten percent DMSO and 80% ethanol were used as negative controls. An extract was considered mutagenic when the mean number of revertants was double or greater than two times that of the negative control and/or if a dose dependant increase in the number of revertants was observed. Each concentration per sample was tested in triplicate; in two different experiments.

2.3. Antimutagenicity testing

A variation of the Ames test was used to screen for antimutagenic activity of extracts from the four *Podocarpus* species. For this activity, only two strains; TA98 and TA100 without S9 metabolic activation were used due to cost implications. It is also known that these two strains (TA98 and TA100) are capable of identifying up to 90% of the mutagens (Mortelmans and Zeiger, 2000). Here, 500 μl of phosphate buffer was added to 50 μl of test sample in a test-tube. Fifty microlitres of the 4NQO was added to the mixture and then pre-incubated for 3 min before the addition of 100 μl of overnight TA98 and TA100 strains of bacterial culture. After incubation for 48 h at 37 °C, revertant colonies were counted and percentage

Table 1
The range in which the neutral red uptake test was determined.

Podocarpus species	Plant part	Extract	
		80% ethanol	Petroleum ether
Podocarpus elongatus	Leaves	0–1.5 mg/ml	0–1.5 mg/ml
	Stem	0–1.5 mg/ml	0–1.5 mg/ml
Podocarpus falcatus	Leaves	0–0.6 mg/ml	0–1.5 mg/ml
	Stem	0–0.6 mg/ml	0–1.5 mg/ml
Podocarpus henkelii	Leaves	NT	0–0.6 mg/ml
	Stem	NT	0–1.5 mg/ml
Podocarpus latifolius	Leaves	0–1.5 mg/ml	0–1.5 mg/ml
	Stem	0–0.6 mg/ml	0–0.3 mg/ml

NT, not tested.

inhibition calculated using the formula shown below. All extracts were tested in triplicate and extracts with strong activity were repeated twice. The antimutagenicity was classified negative, weak, moderate or strong on the basis of the percentage inhibition. The percentage inhibition was calculated using the formula below (Ong et al., 1986):

$$\text{Percent inhibition}\,(\%) = \left[1 - \frac{T}{M}\right] \times 100$$

where T is the number of revertants per plate in the presence of the mutagen 4NQO, and M is the number of revertants per plate in the positive control (mutagen).

2.4. Cytotoxicity testing

2.4.1. Extract preparation

In this assay only extracts of two solvents (PE and 80% ethanol) were tested. Initially the PE extracts were dissolved in DMSO to a concentration of 300 mg/ml stock solution. The stock solution was further diluted to 3 mg/ml in 1% DMSO. This was taken as the highest concentration for determining the 50% inhibition of NRU (NI_{50}). The 80% ethanol extracts did not dissolve in ethanol and most of the extracts partially dissolved in 1% DMSO to make a concentration of 300 mg/ml after prolonged heating and vortexing. PE leaf extracts of *Podocarpus henkelii* and *Podocarpus latifolius* did not dissolve in 1% DMSO, hence they were dissolved in a double volume of DMSO to a concentration of 150 mg/ml (stock solution) in DMSO. This was further diluted in 2% DMSO to a working solution of 3 mg/ml. Due to poor solubility in DMSO and the strong toxic quality of the extracts, as well as the slight toxicity of 2% DMSO, the extracts were finally tested at 1.5 mg/ml instead of 3 mg/ml in 1% DMSO. However, some of the extracts had to be further diluted to lower concentrations and 1.5 mg/ml was taken as the maximum test range. The different test range for each extract is indicated in Table 1. 80% ethanol leaf and stem extracts of *Podocarpus henkelii* did not dissolve at all in ethanol or DMSO and hence the NI_{50} was not determined.

2.4.2. Neutral red uptake assay

The cytotoxic effects of PE and 80% ethanol leaf and stem extracts of the four *Podocarpus* species were tested with the NRU assay (Borenfreund and Puerner, 1985) using the human hepatocellular liver carcinoma cell line 2 (HepG2). Cell suspensions of HepG2 in Dulbecco's modified Eagle's culture medium (DMEM) supplemented with 10% foetal calf serum was seeded into each well of a 96-well microtitre plate (1×10^4 cells/well). The plates were incubated overnight at 37 °C, 5% CO_2 and humidity was maintained using a water bath (milli-q water) inside an incubator. After the 24 h incubation period, the cells were tested with suitable dilutions of the extract preparations depending on the preliminary toxicity results. They were further incubated for 24 h. After the 24 h incubation, the medium was removed and cells were washed with 0.2 ml of a phosphate buffered saline (PBS) solution. To each well, 200 μl of medium containing 0.05 mg/ml neutral red dye were added. The plates were then incubated for another 3 h. The medium was removed and the cells were again rapidly washed with 0.2 ml of PBS solution. The dye was extracted from the cells using a 0.2 ml acetic acid-ethanol mixture. The positive control sodium dodecyl sulphate (SDS) was tested on a separate plate at varying concentrations and the NI_{50} determined as described above. The NI_{50} was kept within limits obtained from 10 independent experiments from which the average NI_{50} and standard deviations were calculated.

Using a microtitre plate shaker, the plates were agitated for at least 1 h or until a homogenously stained medium was obtained. Absorbance was measured against a blank reference at 540 nm using a micro plate spectrophotometer. The optical density (OD) values were calculated by subtracting the measured value of the extracts from the blank control value. Results were expressed as percentage of the OD obtained from the average of the blank control culture read at 540 nm and set at 100%. The NI_{50} was determined from the dose response curve of the mean OD values of the tested concentration range.

3. Results

3.1. Mutagenicity test

The results were based on the number of induced revertant colonies detected. The mean number of revertant colonies per plate in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 are shown in Tables 2–5. None of the extracts gave double or more than double the number of revertant colonies as the negative control. In addition to this, an increase in the number of revertant colonies for each extract tested against all the bacterial strains were not dose dependent. The numbers of revertant colonies shown by both the positive and negative controls were within the normal range found in the laboratory and in accordance with the literature (Mortelmans and Zeiger, 2000). All extracts of the four *Podocarpus* species were not mutagenic in the range of concentrations tested. Increase in the concentration of extracts did not influence the viability and mutation frequencies for each indicator bacterium.

3.2. Antimutagenicity test

A non-antimutagenic effect was considered to give a value smaller that 25% inhibition of the mutagen activity, a moderate effect, a value between 25% and 40% and strong antimutagenecity a value greater than 40% (Negi et al., 2003). At the highest concentrations, all extracts demonstrated a strong antimutagenicity effect on the mutations induced by 4NQO, decreasing its mutagenic effect in a dose-dependent manner (Figs. 1–4). These extracts reduced the number of mutant colonies in the TA98 and TA100 strains protecting against the mutagenicity by the 4NQO-induced frameshift mutation and base pair substitution. DCM extracts exhibited the highest percentage of antimutagenic activity using both TA98 and TA100 when compared to 80% ethanol, PE and water extracts (Figs. 1 and 2).

For TA98, at the highest concentration (5000 μg/ml) and lowest concentration (50 μg/ml), DCM extracts gave a percentage inhibition of mutations induced by 4NQO ranging from 87.4 to 102.2; and 76.4 to 87.7 respectively (Fig. 1). This was followed by 80% ethanol extracts with percentage antimutagenic activity ranging between 75.7 and 95.1 and 64.8 and 88.9 for the highest and lowest concentrations respectively (Fig. 1). PE extracts gave a percentage antimutagenic activity ranging from 77.3 to 93.8 for the highest concentration and 57.7 to 87.2 for the lowest concentration (Fig. 1). Water extracts exhibited the lowest antimutagenic activity when

Table 2
Number of his$^+$ revertants in *Salmonella typhimurium* strains produced by dichloromethane extracts of four *Podocarpus* species used in traditional medicine in South Africa.

Podocarpus species	Plant part	Concentration (μg/ml)	Bacterial strains									
			TA98		TA100		TA102		TA1535		TA1537	
			−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Podocarpus elongatus	Leaves	50	28.0 ± 2.0	35.0 ± 1.5	156.3 ± 3.1	176.7 ± 10.6	361.3 ± 2.4	329.3 ± 7.4	19.3 ± 0.3	22.0 ± 1.1	9.3 ± 1.7	18.3 ± 1.2
		500	27.3 ± 0.6	34.7 ± 0.8	161.7 ± 9.1	184.0 ± 8.0	325.3 ± 12.8	334.3 ± 15.5	18.7 ± 0.3	23.7 ± 0.6	9.0 ± 0.5	19.7 ± 2.0
		5000	27.3 ± 0.6	33.0 ± 1.7	158.3 ± 15.9	201.0 ± 2.0	332.7 ± 19.8	343.3 ± 8.1	18.0 ± 1.1	20.7 ± 0.6	8.3 ± 0.8	17.0 ± 1.1
	Stem	50	27.0 ± 1.0	34.7 ± 0.6	154.0 ± 12.0	222.0 ± 23.5	325.3 ± 16.7	395.3 ± 7.3	20.0 ± 1.5	24.0 ± 1.5	10.0 ± 1.3	17.3 ± 1.4
		500	27.7 ± 0.8	35.3 ± 1.2	169.3 ± 3.9	182.7 ± 7.7	381.0 ± 1.5	369.3 ± 23.7	19.0 ± 1.5	26.0 ± 0.5	12.0 ± 1.1	15.0 ± 1.5
		5000	26.7 ± 1.3	34.0 ± 1.0	148.3 ± 5.5	180.0 ± 15.2	348.7 ± 8.8	336.0 ± 6.9	18.0 ± 1.0	23.7 ± 2.3	8.7 ± 0.8	17.0 ± 1.1
Podocarpus falcatus	Leaves	50	26.0 ± 1.5	33.7 ± 1.4	163.0 ± 2.5	201.0 ± 2.0	378.0 ± 28.3	362.7 ± 12.7	20.7 ± 0.6	24.7 ± 0.3	8.7 ± 0.3	17.7 ± 1.2
		500	22.0 ± 1.1	29.7 ± 1.4	148.3 ± 9.3	184.0 ± 7.2	386.0 ± 8.0	426.3 ± 3.4	21.0 ± 3.6	26.7 ± 1.8	7.7 ± 0.3	16.7 ± 1.7
		5000	27.7 ± 2.4	35.3 ± 3.7	148.3 ± 7.3	200.0 ± 1.7	320.7 ± 5.2	419.0 ± 18.7	19.0 ± 1.5	21.0 ± 1.0	7.0 ± 0.5	14.7 ± 0.8
	Stem	50	25.7 ± 0.8	33.3 ± 0.6	159.3 ± 4.0	171.0 ± 2.3	358.7 ± 8.7	398.7 ± 24.2	21.7 ± 0.8	25.0 ± 1.5	8.0 ± 0.5	24.7 ± 0.8
		500	24.3 ± 1.7	32.3 ± 1.7	142.0 ± 2.0	182.0 ± 2.0	354.3 ± 18.7	458.7 ± 9.3	21.7 ± 1.2	25.7 ± 0.8	8.0 ± 0.5	24.0 ± 0.5
		5000	24.3 ± 1.8	32.3 ± 1.8	151.3 ± 0.8	202.7 ± 3.7	334.0 ± 18.5	449.3 ± 3.5	19.0 ± 1.0	22.7 ± 1.4	7.0 ± 0.5	22.3 ± 1.2
Podocarpus henkelii	Leaves	50	26.0 ± 2.0	33.7 ± 2.9	158.3 ± 20.6	186.0 ± 13.7	330.7 ± 20.8	446.3 ± 23.2	19.7 ± 0.3	27.0 ± 1.5	10.7 ± 0.7	20.7 ± 2.9
		500	25.3 ± 0.8	33.0 ± 1.0	161.0 ± 17.1	194.0 ± 4.3	374.0 ± 30.2	446.3 ± 23.2	18.3 ± 2.1	28.7 ± 2.4	10.3 ± 0.7	19.3 ± 1.8
		5000	26.0 ± 2.0	33.7 ± 2.1	153.0 ± 10.5	197.7 ± 1.2	357.3 ± 18.4	428.0 ± 17.4	18.7 ± 2.3	24.7 ± 0.3	7.3 ± 0.8	20.0 ± 1.7
	Stem	50	25.0 ± 0.5	32.7 ± 0.3	155.3 ± 8.8	189.7 ± 13.7	381.3 ± 3.5	441.3 ± 12.7	23.3 ± 1.4	26.3 ± 1.8	8.0 ± 0.1	24.0 ± 1.1
		500	23.3 ± 1.4	31.0 ± 1.1	176.3 ± 4.7	192.3 ± 10.4	375.0 ± 1.7	478.2 ± 9.6	23.3 ± 1.7	28.0 ± 0.5	7.7 ± 0.8	23.3 ± 0.8
		5000	25.0 ± 1.1	32.7 ± 1.2	145.3 ± 19.8	198.7 ± 5.2	362.0 ± 19.0	454.7 ± 3.5	18.3 ± 0.6	25.7 ± 0.6	7.0 ± 0.5	21.7 ± 1.6
Podocarpus latifolius	Leaves	50	25.7 ± 2.4	36.3 ± 2.3	171.0 ± 14.0	184.3 ± 13.8	374.0 ± 8.0	431.3 ± 22.1	19.7 ± 2.0	25.3 ± 0.8	8.3 ± 1.2	17.7 ± 2.1
		500	26.7 ± 4.8	33.7 ± 4.2	170.7 ± 8.7	183.3 ± 8.5	348.7 ± 18.7	451.7 ± 7.3	21.0 ± 1.5	26.7 ± 0.8	7.7 ± 0.8	18.3 ± 0.8
		5000	26.3 ± 1.2	33.3 ± 0.6	176.3 ± 7.8	212.0 ± 1.5	372.0 ± 24.0	378.7 ± 9.6	19.0 ± 0.5	23.3 ± 0.8	8.0 ± 0.5	16.3 ± 0.6
	Stem	50	27.3 ± 2.5	35.0 ± 2.0	147.0 ± 11.9	193.7 ± 4.4	357.3 ± 10.7	441.3 ± 14.1	23.7 ± 1.4	27.0 ± 1.5	8.7 ± 0.3	19.0 ± 2.5
		500	28.7 ± 1.6	38.0 ± 1.5	166.0 ± 8.7	184.3 ± 6.8	357.3 ± 16.1	437.7 ± 7.2	25.3 ± 1.2	24.7 ± 2.0	8.0 ± 0.0	19.0 ± 1.5
		5000	29.0 ± 1.5	36.3 ± 1.8	185.7 ± 2.9	208.0 ± 4.0	345.0 ± 17.3	408.0 ± 2.3	19.3 ± 1.7	29.0 ± 2.0	7.3 ± 0.3	18.0 ± 1.0
Positive control			167.3 ± 0.8	196.0 ± 3.6	1463.0 ± 36.8	2047.3 ± 42.6	1313.3 ± 80.1	1507.0 ± 10.4	847.0 ± 6.5	260.0 ± 8.3	47.3 ± 1.7	120.7 ± 10.4
Negative control			22.0 ± 1.1	31.0 ± 2.1	137.0 ± 1.7	196.0 ± 4.73	209.3 ± 19.3	254.0 ± 8.5	15.0 ± 0.5	18.0 ± 0.5	6.3 ± 0.8	17.0 ± 1.5

Table 3
Number of his$^+$ revertants in *Salmonella typhimurium* strains produced by 80% ethanol extracts of four *Podocarpus* species used in traditional medicine in South Africa.

Podocarpus species	Plant part	Concentration (μg/ml)	Bacterial strains									
			TA98		TA100		TA102		TA1535		TA1537	
			−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Podocarpus elongatus	Leaves	50	25.3 ± 0.8	33.0 ± 3.0	192.7 ± 3.1	250.0 ± 7.9	277.0 ± 3.6	329.3 ± 7.4	20.7 ± 1.2	23.7 ± 1.4	16.0 ± 0.5	24.0 ± 2.5
		500	24.7 ± 1.2	32.3 ± 2.0	192.7 ± 4.6	237.7 ± 19.3	266.0 ± 5.0	334.3 ± 15.4	24.0 ± 2.6	29.0 ± 1.7	17.7 ± 0.8	21.3 ± 0.8
		5000	26.0 ± 1.7	33.7 ± 0.8	187.0 ± 5.6	213.3 ± 7.2	252.0 ± 2.0	326.7 ± 0.6	20.0 ± 1.1	25.0 ± 0.5	14.3 ± 1.2	24.0 ± 0.8
	Stem	50	25.0 ± 2.6	32.7 ± 1.4	179.3 ± 10.9	237.0 ± 3.6	271.3 ± 12.3	362.0 ± 26.0	24.0 ± 1.5	28.0 ± 0.5	18.0 ± 0.5	21.7 ± 1.6
		500	26.7 ± 3.7	34.3 ± 2.6	162.3 ± 13.7	247.7 ± 12.9	277.3 ± 4.6	336.0 ± 22.7	22.3 ± 0.8	25.0 ± 1.1	14.3 ± 0.8	14.7 ± 1.2
		5000	25.3 ± 1.7	33.0 ± 2.8	160.3 ± 4.3	196.7 ± 10.3	232.0 ± 6.1	336.0 ± 6.9	23.0 ± 1.7	26.3 ± 2.0	9.7 ± 1.2	16.7 ± 0.8
Podocarpus falcatus	Leaves	50	26.0 ± 1.1	33.7 ± 2.3	192.0 ± 3.0	228.7 ± 4.9	286.7 ± 10.9	362.7 ± 9.3	21.7 ± 2.0	24.0 ± 1.1	12.7 ± 0.6	23.0 ± 1.5
		500	24.0 ± 1.1	31.0 ± 1.5	165.3 ± 7.8	212.0 ± 7.5	263.0 ± 12.7	326.3 ± 3.4	21.0 ± 2.5	23.3 ± 1.7	12.0 ± 0.5	19.7 ± 1.4
		5000	28.7 ± 1.2	36.3 ± 2.0	182.0 ± 7.9	203.7 ± 10.3	213.3 ± 4.8	352.3 ± 17.1	19.7 ± 0.8	23.7 ± 2.0	11.3 ± 0.8	17.0 ± 1.5
	Stem	50	27.7 ± 1.6	34.7 ± 4.1	196.0 ± 2.0	250.7 ± 4.9	242.3 ± 6.3	365.3 ± 17.9	28.3 ± 0.5	29.0 ± 1.5	14.0 ± 0.5	19.0 ± 0.5
		500	24.7 ± 1.4	32.7 ± 1.2	180.7 ± 12.5	207.3 ± 15.5	230.7 ± 8.7	358.7 ± 9.3	24.3 ± 1.4	26.7 ± 1.3	12.7 ± 0.6	17.3 ± 1.4
		5000	25.3 ± 0.8	34.7 ± 1.7	174.0 ± 2.0	199.0 ± 6.6	238.0 ± 11.1	349.3 ± 3.5	28.3 ± 1.4	30.7 ± 0.6	11.7 ± 0.8	16.0 ± 0.5
Podocarpus henkelii	Leaves	50	27.3 ± 0.8	35.0 ± 3.0	183.0 ± 1.7	225.7 ± 13.9	237.7 ± 1.2	396.0 ± 24.1	22.3 ± 2.3	26.3 ± 1.8	13.0 ± 1.5	17.3 ± 2.7
		500	25.0 ± 1.1	32.7 ± 1.2	149.0 ± 16.2	211.3 ± 8.4	281.3 ± 1.3	379.7 ± 10.2	27.3 ± 0.3	28.7 ± 0.8	11.0 ± 1.0	15.3 ± 2.0
		5000	27.0 ± 0.5	34.7 ± 1.7	170.0 ± 4.6	209.0 ± 4.7	245.3 ± 6.7	361.3 ± 21.9	21.3 ± 1.6	23.7 ± 1.8	10.3 ± 0.3	14.7 ± 1.4
	Stem	50	27.0 ± 1.1	35.0 ± 1.1	184.7 ± 8.4	224.0 ± 27.0	284.7 ± 2.3	374.7 ± 20.7	20.7 ± 1.4	25.0 ± 1.1	12.0 ± 2.0	16.3 ± 2.9
		500	25.7 ± 0.8	33.3 ± 2.6	167.3 ± 4.8	199.7 ± 6.1	246.3 ± 17.2	378.7 ± 9.6	25.0 ± 1.0	28.7 ± 0.8	13.0 ± 1.1	17.3 ± 1.8
		5000	25.3 ± 1.8	33.3 ± 1.6	170.3 ± 2.8	205.7 ± 5.6	255.0 ± 11.5	354.7 ± 3.5	24.3 ± 2.5	27.3 ± 1.4	11.7 ± 1.2	16.0 ± 0.0
Podocarpus latifolius	Leaves	50	26.7 ± 1.7	34.3 ± 3.8	169.3 ± 6.6	230.0 ± 22.2	290.0 ± 1.1	398.0 ± 13.3	18.7 ± 1.3	26.3 ± 1.7	14.3 ± 1.3	18.0 ± 2.5
		500	23.3 ± 0.8	31.0 ± 1.5	161.7 ± 7.8	204.0 ± 4.4	260.7 ± 11.1	351.7 ± 7.3	18.3 ± 2.7	23.3 ± 2.1	15.0 ± 0.5	19.0 ± 1.5
		5000	26.3 ± 0.8	32.7 ± 1.4	161.3 ± 8.4	200.7 ± 6.9	244.3 ± 8.8	378.7 ± 9.6	19.0 ± 0.5	21.7 ± 0.8	12.3 ± 1.2	17.0 ± 1.1
	Stem	50	28.3 ± 0.3	36.0 ± 2.0	176.7 ± 0.8	240.3 ± 22.0	256.7 ± 7.5	374.7 ± 24.4	27.0 ± 2.0	29.3 ± 1.4	15.7 ± 1.8	19.7 ± 2.9
		500	24.7 ± 0.6	31.7 ± 2.1	162.3 ± 1.4	211.0 ± 23.1	224.7 ± 2.4	337.7 ± 7.2	28.3 ± 0.6	30.7 ± 0.6	13.3 ± 0.8	17.7 ± 2.0
		5000	24.3 ± 1.2	32.0 ± 3.2	173.7 ± 3.8	204.7 ± 4.1	231.3 ± 8.1	408.0 ± 2.3	29.7 ± 1.7	29.3 ± 1.7	12.0 ± 1.1	16.3 ± 0.8
Positive control			175.7 ± 2.0	216.3 ± ± 2.0	1950.0 ± 18.1	2350.7 ± 19.2	1321.3 ± 14.1	1373.7 ± 25.5	852.0 ± 6.5	262.0 ± 2.5	70.3 ± 1.4	97.3 ± 1.7
Negative control			23.7 ± 0.6	31.3 ± 1.8	131.7 ± 10.4	200.3 ± 6.6	182.0 ± 3.0	213.3 ± 2.4	18.0 ± 0.5	19.0 ± 1.5	12.0 ± 0.5	16.3 ± 0.8

Table 4
Number of his$^+$ revertants in *Salmonella typhimurium* strains produced by petroleum ether extracts of four *Podocarpus* species used in traditional medicine in South Africa.

Podocarpus species	Plant part	Concentration (μg/ml)	Bacterial strains									
			TA98		TA100		TA102		TA1535		TA1537	
			−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Podocarpus elongatus	Leaves	50	27.0 ± 3.6	29.0 ± 1.0	225.7 ± 5.3	261.7 ± 31.0	362.7 ± 6.6	371.0 ± 8.1	21.0 ± 1.5	24.3 ± 1.7	10.3 ± 0.3	15.7 ± 0.8
		500	26.0 ± 5.0	25.0 ± 4.0	262.3 ± 14.7	268.7 ± 10.9	383.3 ± 5.2	385.3 ± 4.6	19.7 ± 2.6	24.3 ± 0.3	9.7 ± 0.3	15.0 ± 1.5
		5000	28.7 ± 1.3	24.0 ± 1.5	230.7 ± 5.3	231.7 ± .26.7	328.0 ± 12.0	331.3 ± 11.3	17.7 ± 1.2	22.7 ± 0.8	10.0 ± 0.5	14.3 ± 1.2
	Stem	50	29.0 ± 1.0	34.0 ± 1.5	252.3 ± 20.7	258.7 ± 14.3	372.7 ± 12.3	382.0 ± 6.1	18.0 ± 0.5	28.7 ± 3.1	10.7 ± 0.3	15.7 ± 0.3
		500	24.0 ± 1.5	30.3 ± 31	219.7 ± 6.3	251.3 ± 8.6	354.7 ± 6.3	377.7 ± 5.7	19.0 ± 0.5	28.3 ± 2.0	9.3 ± 0.3	14.3 ± 0.3
		5000	27.0 ± 2.0	31.3 ± 2.1	222.7 ± 11.3	249.4 ± 7.8	357.3 ± 4.	365.3 ± 7.8	18.0 ± 3.1	24.3 ± 3.2	10.0 ± 1.5	14.0 ± 1.1
Podocarpus falcatus	Leaves	50	23.0 ± 1.5	32.3 ± 1.2	255.7 ± 3.9	276.7 ± 4.0	356.0 ± 20.5	370.7 ± 7.4	22.4 ± 5.3	26.7 ± 7.4	11.0 ± 0.5	18.7 ± 0.8
		500	22.0 ± 1.5	31.0 ± 2.0	270.3 ± 12.6	282.0 ± 14.4	330.7 ± 30.7	355.0 ± 17.2	18.0 ± 1.5	25.3 ± 2.0	10.0 ± 0.5	18.0 ± 0.5
		5000	28.3 ± 1.7	31.0 ± 0.5	262.2 ± 1.6	278.0 ± 4.6	349.3 ± 20.8	364.7 ± 12.7	17.0 ± 2.5	21.7 ± 4.1	9.0 ± 1.1	14.3 ± 1.2
	Stem	50	24.7 ± 2.1	30.3 ± 2.3	278.0 ± 1.78	251.7 ± 25.8	357.3 ± 12.7	364.7 ± 12.9	22.3 ± 3.1	26.3 ± 1.8	10.3 ± 0.3	17.3 ± 0.3
		500	22.7 ± 0.8	31.0 ± 1.5	205.3 ± 2.0	255.0 ± 6.43	328.0 ± 24.0	341.3 ± 14.4	24.7 ± 0.8	28.7 ± 2.9	9.7 ± 1.2	16.7 ± 1.2
		5000	25.3 ± 1.4	31.7 ± 2.4	229.7 ± 3.3	242.7 ± 1.4	373.3 ± 9.33	378.0 ± 7.2	20.0 ± 2.6	24.0 ± 4.5	10.7 ± 0.8	17.7 ± 0.8
Podocarpus henkelii	Leaves	50	22.3 ± 2.0	30.7 ± 4.0	236.3 ± 22.6	266.0 ± 18.3	366.0 ± 23.3	382.7 ± 5.4	24.0 ± 3.7	28.0 ± 5.5	10.7 ± 0.5	17.0 ± 1.0
		500	22.3 ± 1.7	32.7 ± 0.8	244.0 ± 2.0	257.0 ± 5.1	330.0 ± 15.0	349.0 ± 10.6	22.7 ± 2.4	26.0 ± 4.1	10.0 ± 1.0	18.7 ± 1.4
		5000	21.7 ± 2.3	34.0 ± 1.1	259.7 ± 8.9	267.7 ± 10.7	344.0 ± 21.1	361.3 ± 9.4	27.0 ± 2.3	31.3 ± 4.6	8.7 ± 0.8	15.3 ± 0.8
	Stem	50	24.3 ± 1.2	31.3 ± 3.3	256.0 ± 27.6	263.3 ± 20.9	354.7 ± 20.7	385.7 ± 3.8	22.0 ± 2.6	26.3 ± 4.2	10.0 ± 0.5	17.0 ± 0.5
		500	22.0 ± 1.7	32.7 ± 1.7	244.0 ± 3.0	257.0 ± 5.1	364.7 ± 23.7	360.3 ± 6.3	18.0 ± 2.0	22.0 ± 4.3	8.33 ± 0.8	14.7 ± 0.8
		5000	21.3 ± 2.3	32.7 ± 0.8	262.0 ± 8.1	267.3 ± 6.9	350.7 ± 19.3	365.0 ± 2.8	19.3 ± 0.6	23.3 ± 2.4	8.7 ± 0.6	13.7 ± 0.6
Podocarpus latifoli±us	Leaves	50	27.0 ± 1.0	30.3 ± 2.0	265.3 ± 7.9	270.7 ± 4.7	340.7 ± 6.5	368.3 ± 0.8	18.0 ± 2.0	22.0 ± 0.5	10.3 ± 1.1	17.3 ± 1.7
		500	24.0 ± 1.1	29.0 ± 1.7	230.7 ± 9.2	251.0 ± 5.5	328.0 ± 8.0	359.0 ± 9.0	18.0 ± 2.6	22.0 ± 4.5	9.7 ± 0.8	16.3 ± 1.3
		5000	27.7 ± 0.8	29.0 ± 2.6	236.0 ± 3.5	256.7 ± 5.2	373.3 ± 10.6	378.3 ± 11.1	17.7 ± 2.4	23.7 ± 2.9	9.7 ± 0.3	16.7 ± 1.4
	Stem	50	28.0 ± 0.5	32.3 ± 2.0	260.7 ± 4.0	280.3 ± 2.6	358.7 ± 13.3	376.3 ± 5.3	20.8 ± 1.7	24.3 ± 3.7	11.0 ± 1.1	17.3 ± 1.3
		500	25.3 ± 2.7	31.0 ± 2.6	258.3 ± 6.4	274.7 ± 2.4	370.0 ± 13.5	337.3 ± 26.1	16.3 ± 3.7	20.3 ± 5.3	9.7 ± 0.3	16.7 ± 1.2
		5000	28.3 ± 1.20	33.0 ± 0.5	292.3 ± 1.86	294.7 ± 2.9	320.0 ± 10.5	380.0 ± 10.0	16.7 ± 3.7	20.7 ± 2.7	8.0 ± 0.5	15.0 ± 1.5
Positive control			165.0 ± 2.0	191.3 ± 7.6	1668.3 ± 20.8	1701.3 ± 11.2	1930.3 ± 10.3	1997.0 ± 51.7	864.7 ± 51.6	263.7 ± 4.91	54.0 ± 2.3	82.3 ± 1.2
Negative control			20.3 ± 0.8	25.3 ± 0.8	178 ± 6.7	193.0 ± 1.7	248.0 ± 18.5	247.3 ± 18.5	14.3 ± 1.4	18.3 ± 0.8	6.3 ± 0.3	13.3 ± 0.8

Table 5
Number of his$^+$ revertants in *Salmonella typhimurium* strains produced by water extracts of four *Podocarpus* species used in traditional medicine in South Africa.

Podocarpus species	Plant part	Concentration (μg/ml)	Bacterial strains									
			TA98		TA100		TA102		TA1535		TA1537	
			−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Podocarpus elongatus	Leaves	50	26.0 ± 1.0	30.7 ± 1.2	153.7 ± 13.5	187.3 ± 2.1	263.7 ± 1.5	272.7 ± 2.3	24.3 ± 1.5	25.0 ± 1.0	16.0 ± 1.0	25.7 ± 0.6
		500	27.0 ± 1.0	25.0 ± 1.0	154.0 ± 6.1	163.0 ± 2.6	242.7 ± 3.1	226.7 ± 1.2	19.3 ± 1.2	27.7 ± 1.5	13.7 ± 1.5	21.3 ± 1.5
		5000	24.3 ± 1.5	29.7 ± 0.6	137.3 ± 17.1	159.0 ± 2.6	249.3 ± 3.1	251.0 ± 11.5	22.3 ± 3.1	23.0 ± 1.0	15.7 ± 0.6	24.0 ± 1.0
	Stem	50	28.0 ± 2.0	33.3 ± 15	167.0 ± 9.9	237.0 ± 6.2	222.0 ± 3.5	236.0 ± 12.0	25.0 ± 1.0	29.0 ± 1.0	17.3 ± 0.6	20.7 ± 1.2
		500	25.3 ± 3.1	32.0 ± 2.0	150.0 ± 6.0	170.7 ± 2.1	254.7 ± 4.0	288.7 ± 1.5	21.3 ± 0.6	23.0 ± 3.0	11.3 ± 1.2	13.7 ± 0.6
		5000	26.7 ± 1.5	28.7 ± 1.2	155.3 ± 6.0	186.7 ± 3.5	244.0 ± 3.5	252.7 ± 2.3	21.7 ± 1.5	26.0 ± 1.0	13.0 ± 1.0	16.0 ± 1.0
Podocarpus falcatus	Leaves	50	28.0 ± 1.0	32.3 ± 2.1	159.0 ± 12.5	225.3 ± 22.0	276.7 ± 2.6	253.0 ± 3.6	21.7 ± 3.2	24.7 ± 1.2	11.7 ± 1.2	15.3 ± 0.6
		500	26.0 ± 0.1	29.7 ± 0.6	136.0 ± 10.6	187.0 ± 2.6	213.3 ± 8.3	239.0 ± 12.3	23.3 ± 1.5	24.7 ± 1.2	13.0 ± 1.0	23.6 ± 1.5
		5000	27.0 ± 0.2	27.7 ± 2.1	139.3 ± 13.1	200.3 ± 1.5	243.0 ± 2.6	279.3 ± 3.6	21.7 ± 1.0	24.0 ± 1.0	12.3 ± 0.6	20.3 ± 1.5
	Stem	50	27.3 ± 2.3	31.3 ± 1.5	146.0 ± 4.0	224.0 ± 4.6	224.7 ± 5.0	263.7 ± 3.2	22.7 ± 2.5	28.0 ± 1.0	15.0 ± 0.5	19.7 ± 1.5
		500	27.0 ± 1.0	32.3 ± 1.0	133.7 ± 3.5	184.0 ± 3.6	265.6 ± 0.6	249.3 ± 6.1	23.7 ± 1.5	26.0 ± 0.6	12.0 ± 1.0	15.7 ± 0.6
		5000	25.0 ± 1.0	31.0 ± 1.0	137.7 ± 12.7	197.0 ± 7.9	244.0 ± 4.0	252.0 ± 6.9	23.0 ± 1.0	27.7 ± 1.0	13.0 ± 1.5	16.7 ± 1.5
Podocarpus henkelii	Leaves	50	29.0 ± 0.6	31.7 ± 0.6	123.0 ± 4.2	169.7 ± 3.2	241.3 ± 2.3	239.7 ± 8.7	26.0 ± 1.0	25.0 ± 1.0	12.0 ± 1.0	16.3 ± 1.2
		500	25.0 ± 1.0	33.3 ± 1.5	153.3 ± 4.2	219.0 ± 16.5	232.0 ± 0	214.7 ± 12.7	22.7 ± 0.6	28.7 ± 1.5	14.7 ± 0.6	20.0 ± 1.0
		5000	26.0 ± 1.0	30.0 ± 2.0	132.0 ± 2.0	201.3 ± 3.2	264.3 ± 3.9	276.0 ± 2.0	25.0 ± 1.0	25.0 ± 1.0	13.3 ± 0.6	17.7 ± 1.5
	Stem	50	27.3 ± 1.5	34.0 ± 0.7	138.0 ± 11.4	207.3 ± 18.1	263.0 ± 3.6	262.0 ± 2.0	22.0 ± 1.0	26.0 ± 0.7	12.7 ± 1.2	16.3 ± 0.6
		500	27.0 ± 1.0	32.3 ± 3.1	138.3 ± 11.6	173.0 ± 3.6	235.0 ± 1.7	254.7 ± 6.1	26.3 ± 0.6	28.0 ± 1.6	15.0 ± 1.0	23.0 ± 1.7
		5000	24.0 ± 1.0	30.0 ± 1.5	113.3 ± 3.5	196.0 ± 5.3	281.0 ± 1.0	271.3 ± 5.0	22.7 ± 1.2	25.3 ± 1.5	14.0 ± 1.0	19.0 ± 2.6
Podocarpus latifolius	Leaves	50	27.0 ± 2.6	32.7 ± 2.1	149.3 ± 6.0	187.3 ± 3.8	244.0 ± 4.0	251.7 ± 12.7	21.3 ± 1.2	28.0 ± 0.6	16.0 ± 2.6	21.7 ± 1.5
		500	24.3 ± 2.1	31.3 ± 2.3	126.3 ± 1.5	226.7 ± 4.0	273.3 ± 4.2	271.0 ± 2.3	23.7 ± 1.5	21.7 ± 1.5	13.7 ± 0.6	23.0 ± 5.6
		5000	25.0 ± 1.0	31.0 ± 1.7	142.7 ± 4.6	201.7 ± 1.5	224.0 ± 3.9	262.0 ± 2.0	20.7 ± 1.2	24.7 ± 1.5	15.3 ± 0.6	17.7 ± 1.2
	Stem	50	28.3 ± 0.6	32.7 ± 1.2	122.7 ± 2.3	201.0 ± 4.4	244.7 ± 4.2	244.7 ± 3.1	25.0 ± 1.0	29.7 ± 2.1	17.7 ± 0.5	23.0 ± 1.7
		500	24.7 ± 1.2	28.3 ± 2.1	153.0 ± 9.8	230.3 ± 26.3	24.7 ± 1.2	237.7 ± 12.5	29.3 ± 0.6	22.7 ± 3.1	13.7 ± 1.2	17.0 ± 1.0
		5000	25.0 ± 1.0	28.7 ± 2.1	128.3 ± 8.6	201.3 ± 1.5	273.3 ± 3.1	264.7 ± 4.2	22.7 ± 3.1	29.3 ± 1.2	14.7 ± 1.5	19.7 ± 1.5
Positive control			175.7 ± 4.0	216.3 ± 3.5	1886.3 ± 22.9	2331.3 ± 8.1	1930.3 ± 17.9	1997.0 ± 4.0	894.7 ± 4.5	263.7 ± 8.5	70.3 ± 2.5	97.3 ± 3.1
Negative control			23.7 ± .2	31.3 ± 3.2	113.7 ± 1.5	123.7 ± 4.7	248.0 ± 32.2	214.0 ± 4.0	15.0 ± 1.7	19.0 ± 1.7	12.0 ± 1.0	16.3 ± 1.5

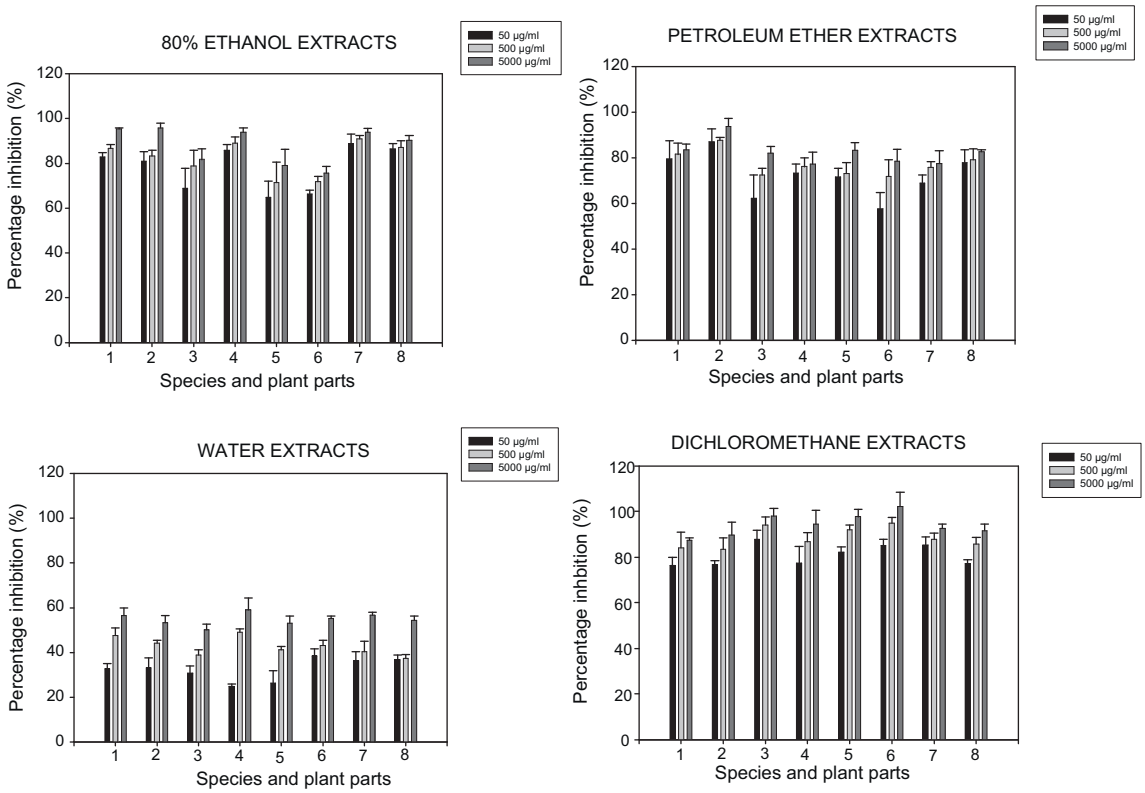

Fig. 1. Antimutagenic activity of leaf and stem extracts of four *Podocarpus* species using *Salmonella typhimurium* TA98. [1]*Podocarpus elongatus* leaves; [2]*Podocarpus elongatus* stem; [3]*Podocarpus falcatus* leaves; [4]*Podocarpus falcatus* stem; [5]*Podocarpus henkelii* leaves; [6]*Podocarpus henkelii* stem; [7]*Podocarpus latifolius* leaves; [8]*Podocarpus latifolius* stem.

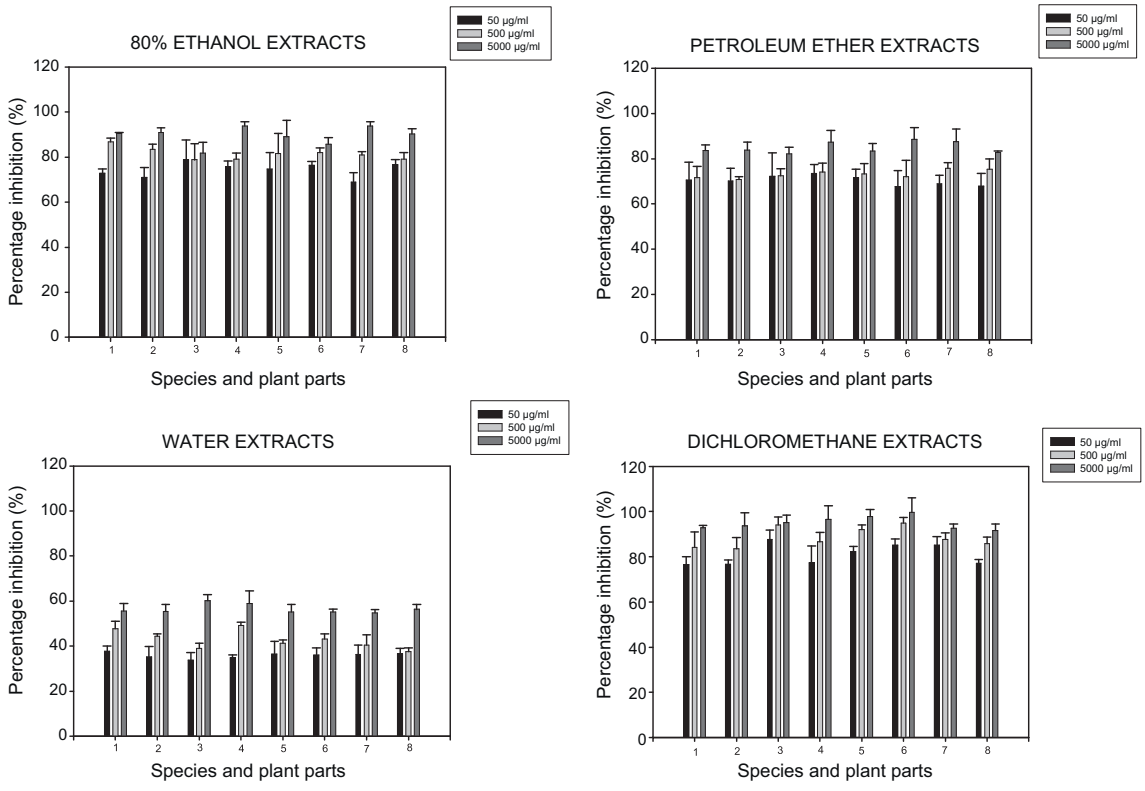

Fig. 2. Antimutagenic activity of leaf and stem extracts of four *Podocarpus* species using *Salmonella typhimurium* TA100. [1]*Podocarpus elongatus* leaves; [2]*Podocarpus elongatus* stem; [3]*Podocarpus falcatus* leaves; [4]*Podocarpus falcatus* stem; [5]*Podocarpus henkelii* leaves; [6]*Podocarpus henkelii* stem; [7]*Podocarpus latifolius* leaves; [8]*Podocarpus latifolius* stem.

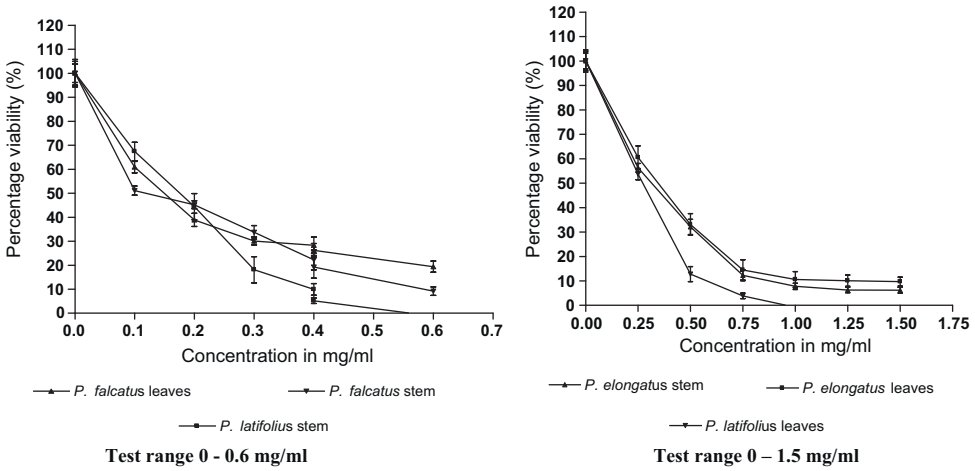

Fig. 3. Percentage viability of HepG2 cells exposed to 80% ethanol extracts of *Podocarpus* species for 48 h.

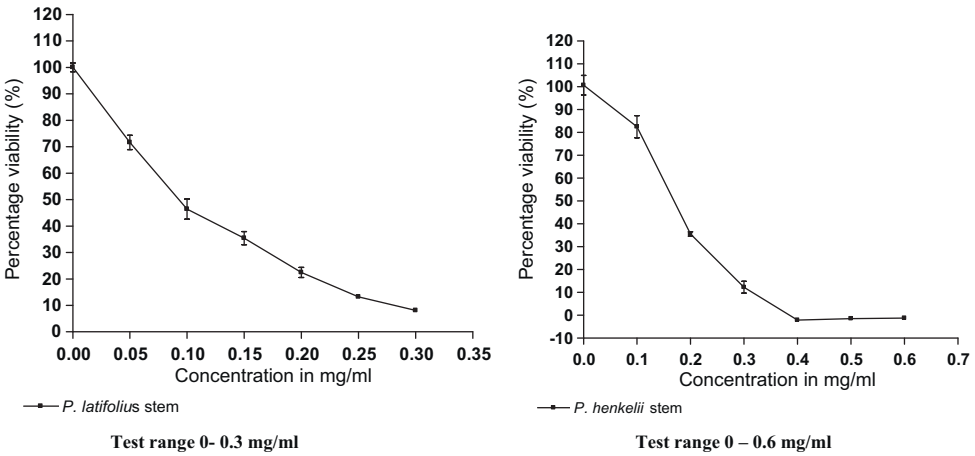

Fig. 4. Percentage viability of HepG2 cells exposed to petroleum ether stem extract of *Podocarpus latifolius* and *Podocarpus henkelii* for 48 h.

compared to the other three solvents; a strong effect (50.2–59%) was observed at the highest concentration and a moderate effect (24.8–36.7%) at the lowest concentration (Fig. 1).

A similar trend was also observed with TA100. DCM extracts gave the highest antimutagenic effect ranging between 91.6–99.7% and 76.4–87.7% for the highest and lowest concentration respectively (Fig. 2). 80% ethanol extracts exhibited antimutagenic activity that ranges between 81.7% and 93.9% for the highest concentration and 68.9% and 78.9% for the lowest concentration (Fig. 2). This was followed by PE extracts with antimutagenic activity of 82.1–88.6% and 67.1–73.4% for the highest and the lowest concentration respectively (Fig. 2). Water extracts showed a similar activity with TA100 as for TA98. At the highest concentration, water extracts gave a percentage antimutagenic activity of 54.7–60.2% and 33.9–57.7% for the lowest concentration (Fig. 2).

3.3. Cytotoxicity test

The viability test was performed in order to assess the toxicity of these four species and to determine the dose effective range. The cell growth and viability were determined by measuring the amount of neutral red taken up into the cells. When HepG2 cells were in contact with plant extracts in an increasing concentration, slopes of NRU decreased (highest–lowest %) following a concentration-dependent pattern (Figs. 3–5). Dose-dependent percentage cell viability was observed in these test ranges. The NRU

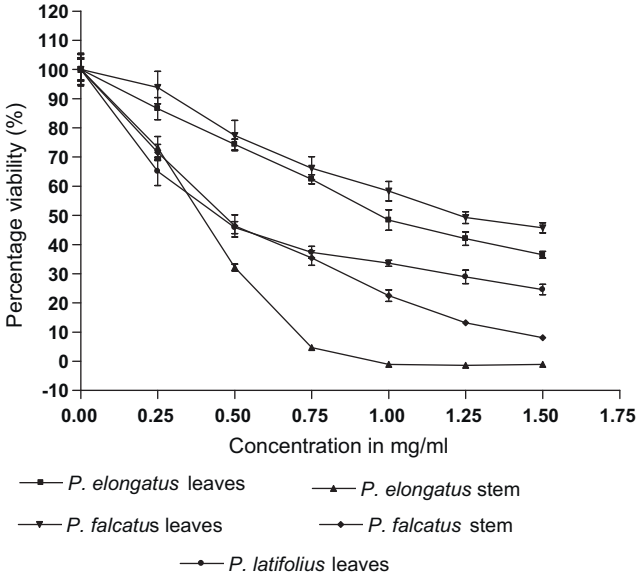

Fig. 5. Percentage viability of HepG2 cells exposed to petroleum ether extracts of *Podocarpus* species for 48 h at test range 0–1.5 mg/ml.

Table 6
NI_{50} values (mg/ml) after 48 h treatment of HepG2 with leaf and stem extracts of four *Podocarpus* species.

Podocarpus species	Plant part	NI_{50} (mg/ml)	
		80% ethanol	Petroleum ether
Podocarpus elongatus	Leaves	0.344	0.971
	Stem	0.318	0.392
Podocarpus falcatus	Leaves	0.150	1.221
	Stem	**0.119**	0.318
Podocarpus henkelii	Leaves	NT	0.412
	Stem	NT	0.169
Podocarpus latifolius	Leaves	0.273	0.445
	Stem	0.176	**0.095**

Sodium dodecyl sulphate (SDS) NI_{50} = 0.252 mM; Values in **bold** indicates the most toxic extracts with the lowest NI_{50} value.
NT, not tested.

test is only an indicative assay and there is no standard rule as to how the results should be interpreted. However, many authors so far indicate a percentage viability of >70 to be non-toxic, 50–70% weak toxicity and <50% toxic. All extracts showed strong toxicity at varying concentration. At the highest tested concentrations for the three different test range (0–0.3; 0–0.6; and 0–1.5 mg/ml), all extracts were toxic giving a percentage viability of less than 50 (Figs. 3–5). For 80% ethanol extracts, the most toxic extract in terms of percentage viability was leaves of *Podocarpus falcatus* whereby at 0.2 mg/ml, the viability of the cells was 38.9% (Fig. 3). Stem extract of *Podocarpus latifolius* was the most toxic among PE extracts, giving a percentage viability of 46.4 at 0.1 mg/ml (Fig. 4). These two values were selected as an indication as to how toxic these extracts are to HepG2 cells. The toxicity levels of the other extracts in terms of concentration ranged between these two values.

Table 6 shows the NI_{50} values of the 80% ethanol and PE extracts of the four tested *Podocarpus* species. The uptake of neutral red into cells was markedly reduced by treating the cells with leaf and stem extracts of the four species of *Podocarpus*. A 50% reduction of the uptake was observed at 24 and 48 h after exposure to the extracts. From these NI_{50} values, the non-polar (PE) extracts showed higher cytotoxic activity against HepG2 cells as compared to the more polar (80% ethanol) extracts (Table 6). In terms of the NI_{50} values, for 80% ethanol extracts, instead of leaves of *Podocarpus falcatus* being most toxic, it was the stem with an NI_{50} value of 0.119 mg/ml (Table 6). For PE extracts, stems of *Podocarpus latifolius* that was the most toxic in terms of percentage viability was still the most toxic with an NI_{50} value of 0.095 mg/ml (Table 6). This shows that the NRU test is actually indicative, that there is no real significance in terms of direct human health, except of course that the more toxic the more hazardous the extract and the more cautious the user should be.

4. Discussion and conclusions

Assessment of mutagenicity is very important as an initial test for complex mixtures because there is a possibility that one or more components can be positive (Reid et al., 2006). The Ames test is recommended for initial screening of medicinal plants since studies have shown that the proportion of carcinogens identified as mutagens ranges from about 50% to 90% (Zeiger, 2001). A positive response in any single bacterial strain either with or without metabolic activation is sufficient to designate a substance as a mutagen (Zeiger, 2001). The mutagenicity test results revealed that the leaf and stem extracts of the four *Podocarpus* species have no mutagenic potential towards all five tested *Salmonella typhimurium* strains, since the number of revertant colonies observed in each extract was less than two times the number of revertant colonies of the negative control (spontaneous mutation) and no dose-dependent response was exhibited by the extracts. Bacterial toxicity was assessed by observing the background lawn of bacterial growth. Presence of a granular thin film layer on the background lawn confirmed absence of toxicity (Mortelmans and Zeiger, 2000). These was seen in both the negative control and plates with plant extracts in all concentration tested.

Substances with antimutagenic properties may be useful for combating the damage caused by environmental mutagenic agents, which we are exposed to in foods, drinks and medicines among others (Lakashmi et al., 2003). Mutagenic and antimutagenic effects of plants have been related to the presence of certain phytochemical substances (Ferreira and Vargas, 1999). A relationship between the structure and activity of flavonoids for both mutagenic activity (Beudot et al., 1998) and for the protection of the genetic material (Edenharder et al., 1993) has been reported. Antimutagenic activity of plants has also been attributed to tannins and their derivatives (Tanaka et al., 1998). When the effects of tannin components extracted from green and black tea were studied on mutagen-induced sister-chromatid exchange and chromosome aberrations, the tea tannins in the presence of S9 mix promoted DNA excision repair activity at low concentrations resulting in an antimutagenic effect (Imanishi et al., 1991). Since 4NQO induces DNA lesions that can be corrected by nucleotide excision repair, the fact that tannins possess this property and may have played a role in the observed antimutagenic activity cannot be overruled. Species of *Podocarpus* are known for their tannin contents and the four species studied were found to contain both condensed tannins and gallotannins (Abdillahi et al., 2010b).

Cytotoxicity effects against a number of human cancer-cell lines have previously been reported from several species of *Podocarpus*. Norditerpenes and totarols isolated from *Podocarpus* species possess cytotoxic and antiproliferative properties against several forms of cancer cells (Park et al., 2003, 2004). Nagilactone C isolated from *Podocarpus totara* and *Podocarpus neriifolius* has potent antiproliferative activity against human fibrosarcoma and murine colon carcinoma tumor cell lines, exhibiting ED_{50} values of 2.3 and 1.2 μg/ml (Shrestha et al., 2001). Taxol, a significant anticancer agent isolated from *Podocarpus gracilior*, inhibited the growth of HeLa cells (Stahlhut et al., 1998). Totarol, a diterpenoid that has been found in several species of *Podocarpus* and responsible for a number of biological activities, exhibited cytotoxic activity against three human proliferative cell lines (CH2988, HeLa and MG63) at concentration over 30 μmol/L (Evans et al., 1999). An antileukemic norditerpene dilactone known as podolide was found to be responsible for the tumor-inhibiting activity of ethanol extract of twigs and leaves of *Podocarpus gracilior*. This compound also occurs in *Podocarpus falcatus* (Kupchan et al., 1975). Rakanmakilactone A-F, sulphur-containing norditerpene dilactones isolated from leaves of *Podocarpus macrophyllus* var. *maki* exhibited a potent cytotoxic effects against P388 murine leukemia cells in a dose-response manner (Park et al., 2004). HepG2 cells contain enzymes that are responsible for the activation of a number of xenobiotics. Inhibition of HepG2 activity involves inhibiting the activation of xenobiotics. Plant polyphenols such as flavonoids and tannins have been found to inhibit various enzymatic activities responsible for xenobiotic metabolism (Wall et al., 1990). Flavonoids are also known to cause cytotoxic effects on malignant cells in culture (Saeki et al., 1999). The presence of methoxyl and hydroxyl groups in biflavonoids isolated from species of *Podocarpus* play an important role in mediating cytotoxic effects (Kuo et al., 2008).

Even though the inhibition of mutagenic effects is always complex, acting via multiple mechanisms, the significant antimutagenic activity of these extracts against a direct acting mutagen (4NQO), implies that these *Podocarpus* species may directly protect DNA damage from mutagens. More studies needs to be carried out to

assess the antimutagenic mechanisms of the phytochemical components of these four species of *Podocarpus*. In addition to this, the antimutagenic activity of these extracts should be tested against other mutagens such as 2AA, sodium azide, 2-nitrofluorene and 3-nitroflouranthene with other mutagenic mechanisms, in order to establish whether the antimutagenic effect was only specific to 4NQO or to other mutagens as well. It is not certain what is responsible for the observed non-mutagenic, antimutagenic and cytotoxic activities since, properties such as synergism and antagonism of chemical interactions of mutagenic and antimutagenic responses may be involved, resulting in an activity with a specific response pattern which is difficult to evaluate in interactions with DNA (Saxena, 1984). The strong cytotoxic activity of extracts of the four *Podocarpus* species against HepG2 cells could be an indication of the potentiality of these species to be further screened for antiproliferative or cytotoxic activities against a number of cancer cell lines. Extracts with low cytotoxicity could further be studied for inhibitory effects on the invasion of cancer cells at a non-lethal concentration.

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