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Evaluation of the mutagenic and antimutagenic effects of South African plants

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Abstract

Dichloromethane and 90% methanol extracts of 42 South African plants were screened for mutagenicity and antimutagenicity using the Salmonella/microsome mutagenicity assay (Ames) against *Salmonella typhimurium* TA98 and TA100 bacterial strains in the presence and absence of metabolic activator S9. The methanol extracts from whole plants of *Helichrysum simillimum*, *Helichrysum herbaceum* and *Helichrysum rugulosum* indicated mutagenicity. These are the first reported tests on the mutagenicity of *Helichrysum* species. Six species indicated antimutagenic properties, all in the presence of S9: methanol leaf extract of *Bauhinia galpinii*, and dichloromethane leaf extracts of *Bauhinia galpinii*, *Clerodendrum myricoides*, *Datura stramonium*, *Buddleja saligna*, *Millettia sutherlandii* and *Sutherlandia frutescens*.
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1. Introduction

Previously the possible mutagenic effects of 51 South African species used frequently in traditional medicine was reported (Elgorashi et al., 2003). In this study a further 42 South African plant species was screened for mutagenic and antimutagenic properties. Southern Africa has over 30 000 species of higher plants, mostly endemic, of which about 3000 are used in traditional medicine (Van Wyk and Gericke, 2000). Recently there has been considerable interest in mutagenicity (Schimmer et al., 1994; Kassie et al., 1996; Elgorashi et al., 2002, 2003; Marques et al., 2003) and antimutagenicity (Kusamran et al., 1998; Ferrer et al., 2001; Negi et al., 2003; Verschaeve et al., 2004) of medicinal plants used by traditional herbalists.

Frequently used plants in traditional medicine are assumed safe, due to their long-term use (Elgorashi et al., 2002) and are considered to have no side effects because they are 'natural' (Popat et al., 2001). This concept is largely circumstantial and it is important to determine toxicology of plant extracts, especially those that are used frequently over long periods.

The Ames test (Maron and Ames, 1983) was used in this study as it is widely used in the determination of possible gene mutations caused by extracts. 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 objective of this study was to evaluate the mutagenic and antimutagenic potential of plant extracts by studying their effects on two histidine requiring strains of *Salmonella typhimurium* in the absence and presence of a liver metabolizing system. The assay acts by detecting back mutations in the His^- operon ($\rightarrow His^+$) when growing *Salmonella typhimurium* bacteria in a His-poor medium.

2. Materials and methods

South African plant material was collected in the Province of KwaZulu-Natal during the late summer months. Deciding fac-

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tors for plant selection were ethnobotanical use and availability. Voucher specimens were made and deposited in the University of KwaZulu-Natal Herbarium, Pietermaritzburg (NU). The material was dried at 50 °C for 72 h, and then finely ground. Extraction was performed sequentially. Ten grams dried, ground plant material was extracted in 100 mL of dichloromethane (DCM) in a sonication bath (25 °C) for 30 min. The material was filtered and then rinsed with 50 mL, and finally with 20 mL of DCM. The plant material was dried overnight. It was then further extracted with 90% methanol in a sonication bath (25 °C) for 30 min, filtered and rinsed with 50 and 20 mL 90% CH_3OH. Crude DCM and 90% methanol extracts were filtered (Whatman filter paper no. 1) and air dried under a fume hood at 23 °C. Dried extracts were stored in glass vials at 4 °C.

Aliquots were prepared of the dried crude extracts and dissolved in 10% DMSO to give an initial concentration of 5.0, 0.5 and 0.05 mg/mL for mutagenic screening using the Ames test described by Maron and Ames (1983). One hundred microlitres *Salmonella typhimurium* bacterial stock (TA98 and TA100) were incubated in 20 mL Oxoid Nutrient Broth No. 2 for 16 h at 37 °C on a shaker. One hundred microlitres of overnight culture were added to 100 μL test solution (plant extract, solvent control, positive control) and 0.5 mL phosphate buffer (without metabolic activation) or 0.5 mL metabolic activation mixture containing 4% post-mitochondrial fraction (S9 mix). This mixture was added to 2 mL top agar containing histidine–biotin. The mixture was poured over the surface of a minimal agar plate and incubated (inverted in the dark) for 48 h at 37 °C. After incubation, revertant (mutant) colonies were counted and compared to the number of colonies formed in unexposed cultures. An extract was considered mutagenic when the mean number of revertants was at least double that found in the solvent control culture. Positive controls were 2-aminoanthracene (with S9) for TA98 and TA100, and 4NQO (4-nitroquinoline-1-oxide) (without S9) for TA98, sodium azide (without S9) for TA100. The negative control/blank was water and the solvent control 10% DMSO.

For antimutagenic screening a variation of the Ames test was used. Here, 50 μL test solution were added to 0.5 mL phosphate buffer or 0.5 mL S9 mixture, 50 μL of mutagen were added to this mixture and then preincubated for 3 min before the addition of 100 μL of overnight culture. After incubation revertant (mutant) colonies were counted and the percentage inhibition calculated: $[1 - T/M] \times 100$ (Negi et al., 2003), where T is the revertants per plate in the presence of mutagen and plant extract and M is the revertants per plate in the control (10% DMSO, no plant extract). A non-antimutagenic effect was considered to give a value smaller than 25%, a moderate effect a value between 25% and 40% and strong antimutagenicity a value greater than 40% (Negi et al., 2003). Mutagens were 2-aminoanthracene, 4NQO and sodium azide. The control was water and the solvent control 10% DMSO. All tests for mutagenicity and antimutagenicity were done in triplicate and extracts indicating activity were repeated.

3. Results

Table 1 lists the plant species investigated and indicates the antimutagenic activity exhibited by each plant. Positive results (+) indicate where plant extracts stimulated revertant colonies to twice or more that of the positive control. No DCM extracts exhibited mutagenic activity (Table 2). The 90% methanol extracts of three plants, *Helichrysum simillimum*, *Helichrysum herbaceum* and *Helichrysum rugulosum*, exhibited mutagenic activity (Table 2). Most concentrations of *Helichrysum simillimum* tested, exhibited a positive response of mutagenicity against TA98 (9.4, 2.1 for 5 and 0.5 mg/mL, respectively) and TA100 (4.9, 4.0, 2.6 for 5, 0.5 and 0.05 mg/mL, respectively) bacterial strains of *Salmonella typhimurium*, in the presence of S9. Mutagenicity was also exhibited by *Helichrysum simillimum* in the absence of S9 against TA98 at a concentration of 5 mg/mL (3.8) and 0.5 mg/mL (2.2). Both *Helichrysum herbaceum* and *Helichrysum rugulosum* exhibited mutagenicity against the TA98 strain in the presence of S9 at the highest concentration of 5 mg/mL (2.9 and 1.7, respectively). For these three plants extracts were made from the shoots (leaves, stems and flowers) (Table 1). *Helichrysum herbaceum*, *Helichrysum simillimum* and *Helichrysum rugulosum* are extensively used in traditional medicine in the treatment of coughs, colds, fever, infections, headache and menstrual pain (Van Wyk et al., 1997).

For antimutagenic screening, positive responses, where the percentage inhibition by the plant extract against either the TA98 or TA100 bacterial strain of *Salmonella typhimurium* was of a value greater than 40%, were indicated by six of the 42 species screened (Table 3), all in the presence of S9. The 90% methanol leaf extract of *Bauhinia galpinii* indicated antimutagenic activity against TA98 at 5.0 mg/mL (48%) and TA100 at 5.0 mg/mL (78%). The dichloromethane leaf extract of *Bauhinia galpinii* inhibited TA98 at 5.0 mg/mL (49%), the leaf extract of *Buddleja saligna*, inhibited TA98 at 5.0 mg/mL (69%) and TA100 at 5.0 mg/mL (56%), the leaf extract of *Clerodendrum myricoides* indicated antimutagenicity against both TA98 at 5.0 and 0.5 mg/mL (91% and 40%, respectively) and TA100 at 5.0, 0.5 and 0.05 mg/mL (73%, 66% and 42%, respectively). DCM extracts of the seed pod and leaf extracts of *Datura stramonium* each exhibited a degree of antimutagenicity. The seed pod extract inhibited TA98 at 5.0 mg/mL (82%) and TA100 at 5.0 mg/mL (71%) and the leaf material TA98 at 5.0 mg/mL (65%). *Millettia sutherlandii* leaf extract (62% and 55%) and the extract from *Sutherlandia frutescens* (55% and 58%), comprising both leaf and stem material, inhibited TA98 and TA100 at 5.0 mg/mL, respectively.

4. Discussion

South African plant material was screened for mutagenicity and antimutagenicity. It is necessary to look at possible mutagenic and antimutagenic responses by the same plant extracts. A sample indicating antimutagenicity as well as mutagenicity is referred to as ‘Janus carcinogens and mutagens’ (Zeiger, 2003).

The absence of a mutagenic response by plant extracts against *Salmonella typhimurium* bacterial strains in the Ames assay is a

Table 1
Antimutagenic results obtained from the bacterial Ames assay on 46 South African medicinal plants, their usage and the plant part used

Plant species	Family	Medicinal use	Plant part screened	Dichloromethane				90% Methanol			
				TA98		TA100		TA98		TA100	
				−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Agapanthus praecox	Alliaceae (Liliaceae)	Womens' health[a], aphrodisiac[a,b]	L	–	–	–	–	–	–	–	–
Bauhinia galpinii	Fabaceae	Tuber: pneumonia, veneral disease, diarrhoea[a]	L	–	+	–	–	–	+	–	+
Berkheya mantana	Asteraceae (Compositae)		L	–	–	–	–	–	–	–	–
Brunsvigia grandifolia	Amaryllidaceae	Bulb: coughs, colds, renal and liver complaints, wounds[b]	B (inner)	–	–	–	–	–	–	–	–
Buddleja saligna	Loganiaceae	Coughs, colds, purgatives[b]	L	–	+	–	+	–	–	–	–
Clerodendrum myricoides	Verbenaceae	Dysmenorrhoea, sterility, impotence, coughs, furunulosis, body swellings Leaf: analgesic, antipyretic bark: snakebites[b]	L	–	+	–	+	–	–	–	–
Clerodendrum myricoides	Verbenaceae	Root bark: fever and diarrhoea in cattle. Root: treat enlargement of the spleen, headaches, convulsions[b]	R	–	–	–	–	–	–	–	–
Cussonia sp 1	Araliaceae		L	–	–	–	–	–	–	–	–
Cussonia sp 2	Araliaceae		L	–	–	–	–	–	–	–	–
Cussonia sp 3	Araliaceae		L	–	–	–	–	–	–	–	–
Datura stramonium	Solanaceae	Relieve asthma, reduce pain, hypnotic, aphrodisiac[c]	Se	–	–	–	–	–	–	–	–
Datura stramonium	Solanaceae	Fruit: toothache, sore throat, tonsillitis[c]	SP	–	+	–	+	–	–	–	–
Datura stramonium	Solanaceae	Leaf: rheumatism, gout, boils, abcesses, wounds, bronchitis[c]	L	–	+	–	–	–	–	–	–
Dioscorea dregeana	Dioscoreaceae	Tuber: wound treatment, paralyses and kills small livestock, narcosis[d]	L	–	–	–	–	–	–	–	–
Ekebergia capensis	Meliaceae	Leaves: vermifuge. Root: coughs, dysentry, gastritis, headache, scabies. Bark: dysentry, heartburn, abcesses, boils, acne[a]	L	–	–	–	–	–	–	–	–
Helichrysum herbaceum	Asteraceae (Compositae)	Coughs, colds, fever, infections, headache and menstrual pain[c]. Leaves and stems burnt to invoke goodwill of ancestors[b]	WP	–	–	–	–	–	–	–	–
Helichrysum nudifolium	Asteraceae (Compositae)	Root: coughs, colds. Leaves: smoke inhaled for headaches, dress wounds. Plant: poultice for swellings[b,d]	L	–	–	–	–	–	–	–	–
Helichrysum ruderate	Asteraceae (Compositae)	Coughs, colds, fever, infections, headache and menstrual pain[c]	WP	–	–	–	–	–	–	–	–
Helichrysum rugulosum	Asteraceae (Compositae)	Coughs, colds, fever, infections, headache and menstrual pain[c]	WP	–	–	–	–	–	–	–	–
Helichrysum simillimum	Asteraceae (Compositae)	Coughs, colds, fever, infections, headache and menstrual pain[c]	WP	–	–	–	–	–	–	–	–
Helichrysum umbraculigerum	Asteraceae (Compositae)	Coughs, colds, fever, infections, headache and menstrual pain[c]	WP	–	–	–	–	–	–	–	–
Hermannia gerrandii	Sterculiaceae		L	–	–	–	–	–	–	–	–
Hoslundia opposita	Lamiaceae	Antimalarial	L	–	–	–	–	–	–	–	–
Hyptis suaveolens	Lamiaceae	Mosquito repellant	L	–	–	–	–	–	–	–	–
Leonotis dubia	Lamiaceae		L	–	–	–	–	–	–	–	–
Maytenus senegalensis	Celastraceae	Root: coughs, bronchitis, pneumonia, tuberculosis, menstration, uterine cramps, aphrodisiac, headache, haemoptysis, snakebites[a,b]	RB	–	–	–	–	–	–	–	–
Millettia sutherlandii	Papilionaceae		L	–	+	–	+	–	–	–	–
Milletia grandis	Papilionaceae	Seed: anthelmintics. Root: roasted residue induces sleep, arrow poison[b]	L	–	–	–	–	–	–	–	–

Table 1 (*Continued*)

Plant species	Family	Medicinal use	Plant part screened	Dichloromethane				90% Methanol			
				TA98		TA100		TA98		TA100	
				−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Nauclea latifolia	Rubiaceae	Dysentry, dyspepsia, fever, gastritis, gonorrhea, malaria, leprosy, measles, piles, toothache[e]	R	–	–	–	–	–	–	–	–
Olea africana	Oleaceae	Root, bark, leaves: urinary and bladder infections, colic, headache. Leaves: eye lotions, sorethroats, diphtheria[b]	T	–	–	–	–	–	–	–	–
Oncoba spinosa	Flacourtiaceae	Root: dysentry, bladder problems, urethral discharge. Leaf and fruit: colds, fever[b]	R	–	–	–	–	–	–	–	–
Phyllanthus amarus	Euphorbiaceae	Digestive, diuretic, diabetes, malaria[e]	R	–	–	–	–	–	–	–	–
Phytolacca octandra	Phytolaccaceae	Root: lung sickness, snakebites, sores, kill lice. Leaves: septic wounds. Shoots: stimulant[b]	L	–	–	–	–	–	–	–	–
Pleurostylia capensis	Celastraceae	Charms[b]	L	–	–	–	–	–	–	–	–
Rhus chirindensis	Anacardiaceae	Heart complaints. Root: schistosomiasis. Leaves: biliousness[b]	L	–	–	–	–	–	–	–	–
Rhus rehrianmana	Anacardiaceae		L	–	–	–	–	–	–	–	–
Strophanthus speciosus	Apocynaceae	Roots: snuff, snakebites. Fruit: used in TM. Seeds and foliage/poisons for arrows and homicides[b]	L	–	–	–	–	–	–	–	–
Uvaria chamae	Annonaceae	Catarrh, dysentry, fever, hematemesis, inflammation, jaundice, wounds, yellow fever[e]	R	–	–	–	–	–	–	–	–
Xysmalobium undulatum	Asclepiadaceae	Stem: poisoning. Root: indigestion, malaria, colic, stomach aches, headaches. Leaf: supplemet for spinach[e]	L	–	–	–	–	–	–	–	–
Hypoxis hemerocallidea	Hypoxidaceae	Infusions used as emetics to treat dizziness, bladder disorders and insanity. Juice is applied to burns[c]	B	–	–	–	–	–	–	–	–
Hypoxis hemerocallidea	Hypoxidaceae	Prostrate problems, testicular tumors, prostate hypertrophy and urinary infections[c]	L	–	–	–	–	–	–	–	–
Sutherlandia fructescens	Fabaceae	Stomach problems, internal cancers, colds, influenza, chicken-px, diabetes, varicose veins, piles, inflammation, liver problems[c]	L& S	–	+	–	+	–	–	–	–
Catharanthus roseus	Apocynaceae	Diabetes, rheumatism, breast and uterine cancer, Hodgkin's and non-Hodgkin's lymphoma[c]	L	–	–	–	–	–	–	–	–
Tulbaghia violaceae	Alliaceae	Leaves: cancer of oesophagus. Fever, colds, asthma and tuberculosis[c]	L	–	–	–	–	–	–	–	–
Tulbaghia violaceae	Alliaceae	Fever, colds, asthma and tuberculosis[c]	B	–	–	–	–	–	–	–	–
Coleonema album	Rutaceae	Used as 'Buchu', a dusting powder[e]	S, L, Sh	+	–	–	–	–	–	–	–

AP: aerial parts; B: bulbs; Ba: bark; F: fruit; L: leaves; R: roots; RB: root bark; Rh: rhizome; S: stem; SB: stem bark; Se: seeds; Sh: shoots; SP: seed pod; T: twigs; WP: whole plant.

[a] See Van Wyk and Gericke (2000).

[b] See Hutchings et al. (1996).

[c] See Van Wyk et al. (1997)

[d] See Watt and Breyer-Brandwijk (1962).

[e] See Johnson (1999).

Table 2
Mutagenicity indicated by 90% methanol extracts obtained from *Helichrysum* species

Plant species	Extract concentration tested (mg/mL)	+S9		−S9	
		TA98	TA100	TA98	TA100
Helichrysum herbaceum Sweet	5	2.9	1.4	1.3	1
	0.5	1	1.1	1.1	1.1
	0.05	1.3	0.9	1	0.9
Helichrysum rugulosum Less.	5	1.7	1.3	0.6	0.9
	0.5	1.2	1.2	0.7	0.9
	0.05	1.3	1	0.9	0.9
Helichrysum simillimum DC.	5	9.4	4.9	3.8	1.4
	0.5	2.1	4	2.2	1.3
	0.05	1.1	2.6	0.9	0.9

Expressed as a ratio of revertants of *Salmonella typhimurium* strains TA98 and TA100 to the solvent control.

positive step forward in determining the safe use of those plants utilized in traditional medicine. However, plant extracts exhibiting a positive response and hence a mutagenic effect need to be extensively investigated to determine their possible genotoxicity to humans as their safe use in traditional medicine is questionable. Screening is required to identify and eradicate the use of all mutagenic plants as numerous studies have shown that the proportion of carcinogens identified as mutagens by the Ames test ranges from about 50% to 90% (Zeiger, 2001). Most mutagenicity was exhibited in the presence of metabolic activation in this study. The active extracts (*Helichrysum simillimum*, *Helichrysum herbaceum* and *Helichrysum rugulosum*) are all administered orally for the treatment of a number of ailments and the smoke from leaves is often inhaled (imphepho) and may therefore be subjected to metabolic activation within the human body. The methanol extracts exhibited mutagenicity suggesting that the mutagens are more hydrophilic than hydrophobic. There is no previous mention in the literature of mutagenicity screening of Southern African *Helichrysum* species. The genotoxic effect of these *Helichrysum* species is not automatically noticeable in human health but the long-term effects could be substantial.

The occurrence rate of cancer is increasing worldwide and the determination of chemoprevention or chemoprophylaxis compounds is important in the effort to reduce the risk of cancer (Kundu et al., 2004). Antimutagenicity determination of plant extracts is important in the discovery of new effective anti-

Table 3
Antimutagenicity of dichloromethane and 90% methanol plant extracts

Plant species	Concentration tested (mg/mL)	Dichloromethane extracts		90% Methanol extracts	
		TA98	TA100	TA98	TA100
Bauhinia galpinii N.E.Br	5	49	19	48	78
	0.5	4	16	1	20
	0.05	10	3	2	−10
Buddleja saligna	5	69	56	−2	15
	0.5	−43	20	−11	3
	0.05	−15	18	2	17
Clerodendrum myricoides R. Fern.	5	91	73	27	7
	0.5	40	66	−22	−1
	0.05	14	42	−24	−16
Datura stramonium (SP) L.	5	82	71	−16	−50
	0.5	21	−5	−3	−29
	0.05	6	−1	−2	−59
Datura stramonium (L) L.	5	65	13	−11	−61
	0.5	11	−5	7	−40
	0.05	3	−34	−3	−8
Millettia sutherlandii Harv.	5	62	55	13	−9
	0.5	29	17	7	19
	0.05	14	−9	9	11
Sutherlandia frutescens (L.)R.Br. ex W.T.Aiton	5	55	58	−3	5
	0.5	13	16	0	1
	0.05	13	17	−28	12

Expressed as percentage inhibition on the mutagen, 2-aminoanthracene, in the presence of S9.

carcinogenic treatments. A plant extract indicating antimutagenicity is not necessarily an anticarcinogen, however, it is an indication of a possible anticarcinogen. Six species indicated antimutagenicity in this study, mostly from DCM extracts, none of which had previously been screened for antimutagenicity: *Bauhinia galpinii*, *Buddleja saligna*, *Clerodendrum myricoides*, *Millettia sutherlandii* and *Sutherlandia frutescens*. All plants indicating antimutagenicity in this study have the potential to act as anticarcinogens or chemopreventative agents.

Species from the same genus as *Bauhinia galpinii* have been researched in cancer related experiments, especially *Bauhinia purpurea* (Codington et al., 1975) and *Bauhinia variegata* (Rajkapoor et al., 2003), indicating positive results. *Buddleja saligna* has no previous screening although ethnopharmacological work has been performed on a number of other *Buddleja* species (Houghton et al., 2003).

The toxicity of *Datura stramonium* is well documented and had been linked to deaths and poisonings for centuries (Friedman, 2004; Steenkamp et al., 2004). Surprisingly, the leaf and seed pod material indicated antimutagenicity. This species has many uses in traditional medicine (Van Wyk et al., 1997), but is administered at low concentrations. Antimicrobial activity has been identified in petroleum ether seed extracts (Uzun et al., 2004).

Millettia sutherlandii indicated antimutagenicity. Screening studies for anti-tumor promoters have been performed on 15 isoflavonoids isolated from plants of the genus *Millettia.* All compounds showed inhibitory activity, without showing any cytotoxicity, indicating that some of the isoflavonoids might be valuable anti-tumor promoters (Ito et al., 2000).

In the literature it is mentioned that four of the plants tested are used in cancer treatment: *Hypoxis hemerocallidea*, *Sutherlandia frutescens*, *Catharanthus roseus* and *Tulbaghia violaceae* (Hutchings et al., 1996; Van Wyk et al., 1997). Of the extracts screened, only the *Sutherlandia frutescens* extract indicated activity at the highest concentration screened. *Sutherlandia frutescens* is commonly known as the ‘kankerbos’ ‘cancer bush’, and antimutagenicity in the leaf and stem mixture was anticipated. A toxicity study has been performed using 16 Vervet monkeys, where consumption of leaf powder did not exhibit any toxic or side effects (Final report by the Medical Research Council, South Africa, 2002; Fernandes et al., 2004). Chemical studies have identified one compound, canavanine, a non-protein α-amino acid with anti-tumor properties (Bell et al., 1978; Tai et al., 2004). Its major metabolite, canaline is being developed as a new anticancer drug, for pancreatic cancer (Crooks and Rosenthal, 1994). Extracts of *Sutherlandia frutescens* have been shown to exhibit anti-inflammatory activity in the COX-2 assay (Kundu et al., 2004) with mention made that anti-inflammatory activity may be related to anticarcinogenic activity. Ethanol extracts have been shown to indicate a concentration dependent antiproliferative effect on several human tumor cell lines (MDA-MB 468 breast cancer cells and HL60 Leukemia cells) (Tai et al., 2004). *Sutherlandia frutescens* has received international attention as a cheap herbal medicine that can improve the health of AIDs patients (Harnett et al., 2005). There is little scientific documentation on mechanisms of *Sutherlandia frutescens* in the immune system of humans (Fernandes et al., 2004) and in AIDs patients (Harnett et al., 2005). Recent studies indicated *Sutherlandia frutescens* extracts to contain inhibitory compounds active against HIV target enzymes (Harnett et al., 2005). The mode of action is unknown.

The determination of toxicity of South African plants is particularly necessary as the use of some traditional medicines has resulted in several cases of acute toxicity, leading to increased morbidity and mortality. Popat et al. (2001) refers to three case-studies in South Africa indicating a high rate of acute toxicity resulting from the use of traditional medicines. The presence of the background layer in all agar plates, in the mutagenic and antimutagenic screening, is an indication that the extracts were not toxic.

The Ames test was used in this study as it is reliable, quick and easy. This test is used to screen for possible carcinogens, however, a positive result does not necessary indicate the substance as being a carcinogen. Also, if a substance screened does not suggest a mutagenic response it does not necessary confirm that it is not mutagenic or not carcinogenic. It confirms that the substance is not mutagenic to the particular bacterial strain used and for the genetic endpoint tested. To test for carcinogenicity a 2 year carcinogenicity test would have to be performed by testing the effect of the mutagenic sample in mice and rats (male and female) (Zeiger, 2001). The same can be said for antimutagenic screening.

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