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Antimicrobial, anti-inflammatory and mutagenic investigation of the South African tree aloe (*Aloe barberae*)

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ABSTRACT

Ethnopharmacology relevance: In recent times, many products ranging from aloe drinks to aloe gels, powders, capsules, and creams have appeared on the commercial market prepared from different aloe species including *Aloe barberae*. These products are used in ethnomedicine to treat various conditions including gastrointestinal disorders, insect bites, skin burns and other skin injuries by traditional communities.
Aim of the study: This study was aimed at evaluating the antibacterial, antifungal and anti-inflammatory activities as well as genotoxic effects of different extracts of *Aloe barberae*.
Materials and methods: Organic and water extracts of the upper stem, young bark, mature bark, leaves and roots of the South African tree aloe (*Aloe barberae*) were evaluated for their antimicrobial [Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria as well as the fungus *Candida albicans*], anti-inflammatory (COX-1 and COX-2) and mutagenic properties (Ames test). Thin layer chromatography (TLC) was used to compare the phytochemical profiles of different extracts of *Aloe barberae*.
Results: The petroleum ether (PE) and dichloromethane (DCM) extracts of the mature bark, leaves and roots exhibited good activity against all the bacteria and fungus *Candida albicans* with minimum inhibitory concentrations (MIC) ranging from 0.195 to 1.56 mg/ml. All the PE extracts evaluated showed a high activity (>70%) in both COX-1 and COX-2 assays. Apart from the organic extracts of the root with consistently good activity (>70%), all the remaining extracts showed moderate activity (40–69%) in COX-1 assay. The PE extracts also showed a dose dependent increase in activity. Ultraviolet (UV) spectrum of the leaves and root EtOH extracts indicated the presence of compounds that could absorb UV light (wavelength: 190–820 nm). None of the extracts had a mutagenic effect in the *Salmonella*/microsome assay against a tester strain, TA98.
Conclusion: Activity observed in the bark, leaves and roots of *Aloe barberae* validates its use in commercial herbal products, ethnobotany and ethnoveterinary medicine by South African communities and small scale farmers to treat various conditions.

1. Introduction

The succulent genus *Aloe* (Family: Asphodelaceae) is native to Africa, ranging from the Cape Province of South Africa to Madagascar, the Arabian peninsula and islands off the coast of Africa. They are adapted to harsh climates, thriving in many different types of habitats such as mountains, grasslands, deserts and beaches (Palgrave, 1997).

Aloe plants have been used in folk medicine for the treatment of various conditions including gastrointestinal disorders, insect bites, skin burns and other skin injuries (ElSohly et al., 2004). There are many products ranging from aloe drinks to aloe gels, powders, capsules and creams that have recently appeared on the commercial market prepared from different aloe species (ElSohly et al., 2004). Products such as 'Aloe 4 U' commercially sold in South Africa consists of many different aloe species mixed together to reportedly give a stronger effect.

Aloe barberae Dyer (*boomaalwyn* in Afrikaans, *inKalane enkulu* in Zulu) is South Africa's largest succulent tree aloe, growing up to 18 m tall. The bark of the tree-trunk is grey in colour. The leaves are long, narrow, deeply channelled and curved with a dark green surface and a toothed margin. The flowers are pink and appear

Abbreviations: 4NQO, 4-nitroquinoline-N-oxide; ATCC, American Type Culture Collection; ATM, African Traditional Medicine; COX, cyclooxygenase assays; DCM, dichloromethane; EtOH, 80% ethanol; INT, *p*-iodonitrotetrazolium chloride; MFC, minimum fungicidal concentration; MH, Mueller–Hinton; MIC, minimum inhibitory concentration; NFκB, nuclear factor κB; PE, petroleum ether; TLC, thin layer chromatography; UV, ultraviolet; VIS, visible; YM, yeast malt.

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mostly during June and July (Palmer and Pitman, 1972). The gel made from the leaf sap is applied to sores in traditional medicine. However, this species' utilisation in African Traditional Medicine (ATM) is less well documented (Arnold et al., 2002; Grace et al., 2008). This study was aimed at evaluating the antibacterial, antifungal and anti-inflammatory activities of different parts of *Aloe barberae*. The possible risk involved with the use of this plant in traditional medicine was also evaluated.

2. Materials and methods

2.1. Plant material

Plant material was collected in October 2007 from the University of KwaZulu-Natal Life Sciences Campus, Pietermaritzburg, South Africa and separated into upper stem, young bark, mature bark, leaves and roots. A voucher specimen [AR Ndhlala 03 NU] was deposited in the University of KwaZulu-Natal Herbarium, Pietermaritzburg. Plant parts were dried at 50 °C, ground and stored in airtight containers at 10 °C in the dark.

2.2. Preparation of plant extracts

Dried, ground plant parts were extracted sequentially with 20 ml/g of petroleum ether (PE), dichloromethane (DCM), 80% ethanol (EtOH) and water using a sonication bath containing ice for 1 h each. The extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum using a rotary evaporator. The concentrated sample was dried at room temperature and kept at 10 °C until required.

2.3. Antibacterial activity

Antibacterial activity of the extracts was evaluated using the micro-dilution bioassay in 96-well micro-plates for minimum inhibitory concentration (MIC) determination (Eloff, 1998). Overnight cultures (incubated at 37 °C in a water bath with shaking) of four bacterial strains: two Gram-positive (*Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* ATCC 12600) and two Gram-negative (*Escherichia coli* ATCC 11775 and *Klebsiella pneumoniae* ATCC 13883) bacteria were diluted with sterile Mueller–Hinton (MH) broth (1 ml bacteria/50 ml MH). The organic and water extracts were redissolved in 80% ethanol and water, respectively. One hundred microlitres of each redissolved extract were twofold serially diluted with sterile distilled water down a 96-well microplate for each of the four bacteria in duplicate. A similar twofold serial dilution of neomycin (Sigma) (0.1 mg/ml) was used as a positive control while bacteria-free broth, distilled water and 80% ethanol were included as negative controls (solvent controls) against each bacterium. One hundred microlitres of each bacterial culture were added to each well. The plates were covered with parafilm and incubated overnight at 37 °C. Bacterial growth was tested by adding 50 μl of 0.2 mg/ml *p*-iodonitrotetrazolium chloride (INT) to each well and the plates incubated at 37 °C for 1 h. Bacterial growth in the wells was indicated by a reddish-pink colour, whereas clear wells indicated growth inhibition by the tested sample. MIC values were recorded as the lowest concentrations of extracts showing clear wells. The assay was repeated twice.

2.4. Antifungal activity

The antifungal activity of the extracts was evaluated against *Candida albicans* (ATCC 10231) using the micro-dilution assay (Eloff, 1998) modified for fungi (Masoko et al., 2007). An overnight fungal culture was prepared in yeast malt (YM) broth. Four millilitres of sterile saline were added to 400 μl of 24-h-old *Candida* culture. The assay was repeated twice with three replicates per extract. MIC and minimum fungicidal concentration (MFC) values were recorded as lowest concentrations of extracts showing clear wells.

2.5. Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the enzyme-based cyclooxygenase assays (COX-1 and COX-2) (Eldeen and Van Staden, 2008). There were four controls in the assay. Two were backgrounds in which the enzyme was inactivated with HCl before adding [^{14}C] arachidonic acid and which were kept on ice, and two were solvent blanks. Indomethacin was used as a positive control at a concentration of 5 μM for COX-1 and 200 μM for COX-2. All the extracts were initially tested at 250 μg/ml for organic extracts and 2 mg/ml for water extracts. The PE extracts were subsequently further evaluated at 31.25, 62.5 and 125.0 mg/ml. Percentage inhibition by the extracts was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank. Results are the mean of two experiments (each experiment in duplicate).

2.6. Thin layer chromatography

The crude EtOH and PE extracts were applied to Silica gel 60 F_{254} on polyester plates, 10 cm × 20 cm, (Merck, Germany) and developed with ethyl acetate:methanol:water (100:16.5:13.5) in a pre-saturated chromatographic chamber (Shen et al., 2001). The developed plates were dried in a stream of warm air and visualised under visible (VIS) and ultraviolet (UV) light (254 and 366 nm). A pencil was used to carefully mark any florescence observed. The plates were then sprayed with Folin–Ciocalteu reagent (Folin C.: distilled water, 1:1, v/v).

2.7. Ultraviolet spectrum

Leaf and root EtOH extracts were eluted with ethanol through celite and the absorbance was measured by scanning through wavelengths (λ) 190–820 nm using a UV–vis spectrophotometer (Varian, Cary 50 Conc).

Table 1
Antibacterial activity (MIC) of different extracts (mg/ml) of South African tree aloe (*Aloe barberae*).

Extract	Bacterium	Upper stem	Young bark	Mature bark	Leaf	Root
PE	*Bacillus subtilis*	3.125	1.56	**0.78**[a]	**0.78**	1.56
	Escherichia coli	1.56	1.56	**0.78**	1.56	**0.78**
	Klebsiella pneumoniae	1.56	3.125	1.56	**0.78**	**0.78**
	Staphylococcus aureus	3.125	1.56	**0.78**	1.56	**0.78**
DCM	*Bacillus subtilis*	1.56	1.56	**0.39**	**0.39**	**0.78**
	Escherichia coli	1.56	**0.78**	**0.78**	**0.39**	**0.195**
	Klebsiella pneumoniae	1.56	**0.78**	**0.78**	**0.78**	**0.39**
	Staphylococcus aureus	1.56	1.56	**0.39**	**0.78**	**0.39**
EtOH	*Bacillus subtilis*	3.125	1.56	1.56	**0.78**	1.56
	Escherichia coli	3.125	1.56	**0.78**	1.56	**0.78**
	Klebsiella pneumoniae	3.125	1.56	1.56	1.56	**0.78**
	Staphylococcus aureus	3.125	1.56	3.125	**0.78**	1.56
Water	*Bacillus subtilis*	6.25	3.125	3.125	1.56	1.56
	Escherichia coli	6.25	3.125	3.125	1.56	3.125
	Klebsiella pneumoniae	6.25	3.125	3.125	6.25	3.125
	Staphylococcus aureus	6.25	3.125	3.125	6.25	6.25

The MIC values (mg/ml) for neomycin (positive control) were *Bacillus subtilis* = 1.6×10^{-3}; *Escherichia coli* = 0.8×10^{-3}; *Staphylococcus aureus* = 0.8×10^{-3}; *Klebsiella pneumoniae* = 1.6×10^{-3}.

[a] Values boldly written are considered very active (<1 mg/ml).

Table 2
Antifungal activity (MIC and MFC) of different extracts (mg/ml) of South African tree aloe (*Aloe barberae*) against *Candida albicans*.

Extract	MIC/MFC (mg/ml)	Upper stem	Young bark	Mature bark	Leaf	Root
PE	MIC	3.125	3.125	1.56	**0.78**[a]	**0.78**
	MFC	6.25	6.25	6.25	6.25	1.56
DCM	MIC	3.125	3.125	**0.78**	**0.39**	**0.39**
	MFC	6.25	6.25	6.25	6.25	1.56
EtOH	MIC	6.25	3.125	1.56	**0.78**	**0.78**
	MFC	6.25	12.5	6.25	6.25	3.125
Water	MIC	12.5	6.25	6.25	1.56	1.56
	MFC	12.5	6.25	6.25	6.25	6.25

The MIC and MFC values (μg/ml) for amphotericin B (positive control) were 9.77×10^{-3} and 7.81×10^{-2}, respectively.
[a] Values boldly written are considered very active (<1 mg/ml).

2.8. Mutagenicity test

Mutagenicity was tested using the *Salmonella* microsome assay as performed by Maron and Ames (1983) based on the plate-incorporation procedure with *Salmonella typhimurium* tester strain TA98. Stock (100 μl) bacteria in 20 ml Oxoid nutrient No. 2 broth were incubated for 16 h at 37 °C. The bacterial cultures (100 μl) were added to 100 μl of plant extract in 500 μl phosphate buffer and 2 ml of agar containing biotin–histidine (0.5 mM). The mixture was poured on a minimal agar plate and incubated at 37 °C for 48 h. Samples were tested in triplicate. Three dilutions (50, 500, 5000 μg/ml) were used per sample. 4-Nitroquinoline-N-oxide (4NQO) was used as a positive control. Bacterial colonies were counted at the end of 48 h. Results are expressed as means of three independent experiments.

3. Results and discussion

The minimum inhibitory concentrations of different extracts of *Aloe barberae* evaluated for antibacterial activity are presented in Table 1. All the extracts showed activity against the four bacterial strains used. The organic extracts with MIC ranging from 0.195 to 3.125 mg/ml generally showed a better activity compared to water extracts. Extracts obtained from mature bark, leaves and roots exhibited good activity (<1 mg/ml) in most cases compared to those from young bark and the upper stem. The DCM extract of roots showed the lowest MIC of 0.195 mg/ml against *Escherichia coli*.

In the antifungal assay, all the extracts evaluated showed activity against *Candida albicans* with MIC and MFC values ranging from 0.39 to 12.5 mg/ml (Table 2). The organic extracts were generally more active than the water extracts. The DCM extracts of the leaves and roots in particular showed the highest activity with a MIC value of 0.39 mg/ml.

Aloe species contain a number of pharmacologically active compounds including homonataloin, aloeresin, aloe emodin, aloin (the C-glucoside of aloe emodin) and chrysophanol, all of which have been reported to exhibit antimicrobial activity (Shen et al., 2001; Kambizi and Afolayan, 2008). The level of these compounds in *Aloe* plants is highly variable and appears to depend on the species and strain of *Aloe* as well as their growing conditions (Wamer et al., 2003). The antibacterial and antifungal activities recorded in the different parts of *Aloe barberae* could therefore be as a result of the

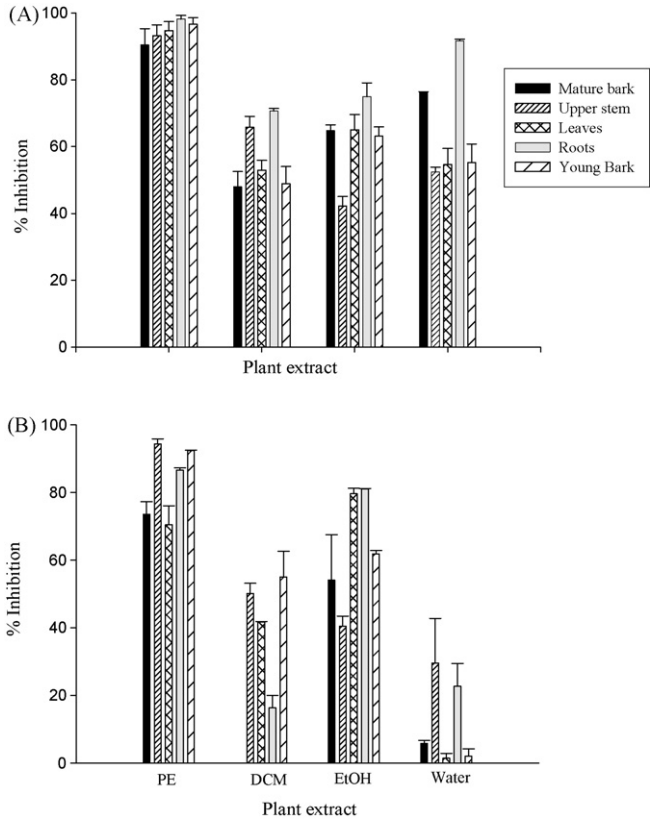

Fig. 1. Anti-inflammatory activity of *Aloe barberae* extracts. (A) COX-1 assay (B) COX-2 assay. Water extract evaluated at 2 mg/ml, other extracts at 250 μg/ml. Percentage inhibition by indomethacin in COX-1 was 64.18 ± 3.10; COX-2 was 68.50 ± 2.57.

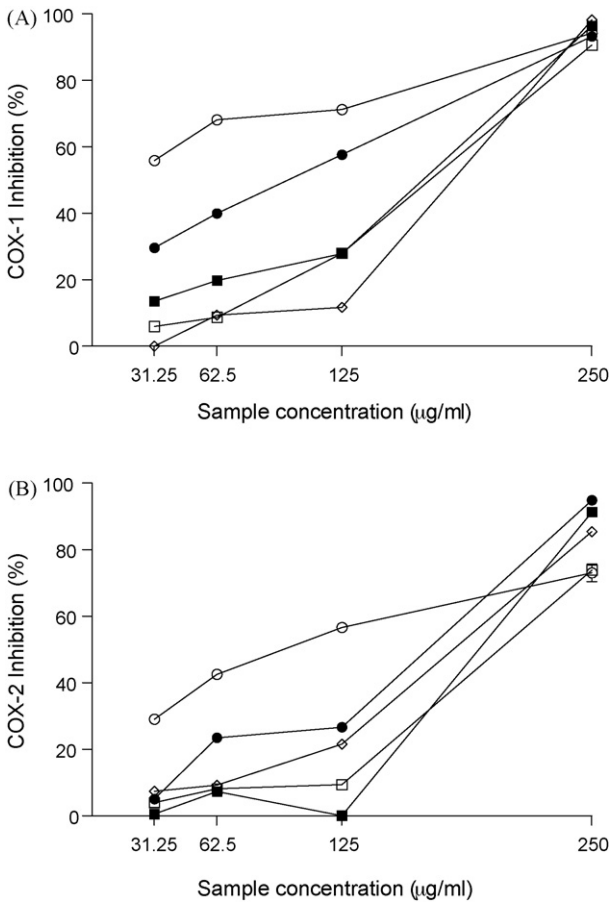

Fig. 2. Anti-inflammatory dose dependent curves of *Aloe barberae* PE extracts at different concentrations (31.25, 62.5, 125 and 250 μg/ml). (A) COX-1 and (B) COX-2. Leaves (○), upper stem (●), young bark (■), mature bark (□) and roots (◊).

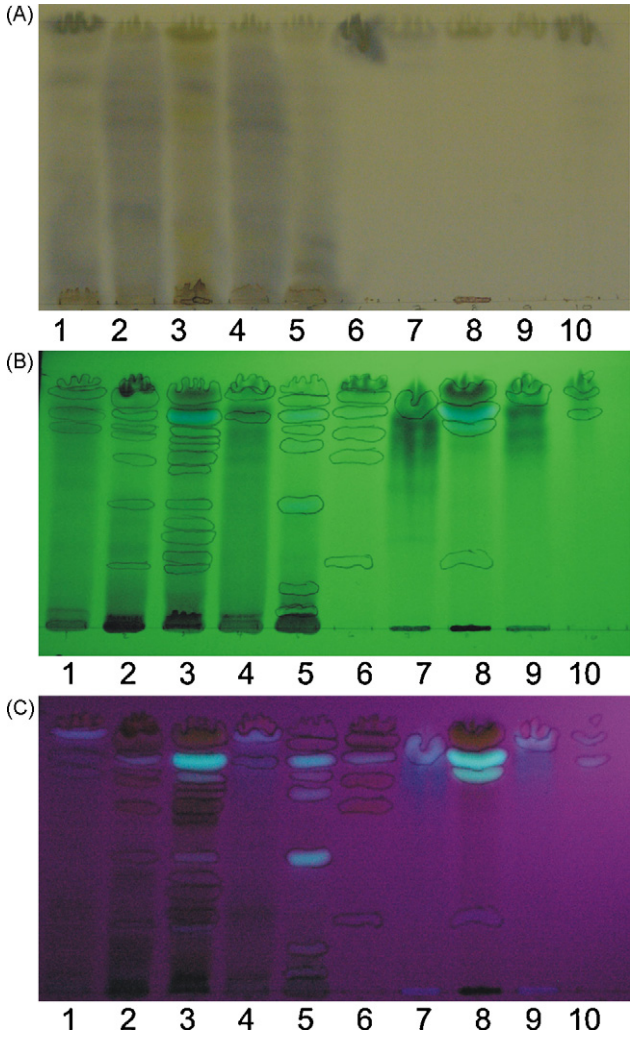

Fig. 3. TLC profiles of crude EtOH extracts of leaves, mature bark, roots, young bark, upper stem (1–5, respectively) and PE extracts of leaves, roots, mature bark, young bark, fresh bark (6–10, respectively). (A) Folin C. spray, (B) UV λ = 366 and (C) UV λ = 254.

growing conditions and/or other compounds. Crude extracts may contain dilute amounts of the active components thus resulting in high MIC values (MIC > 1.0 mg/ml) in some of the extracts evaluated in the assays (Rabe and Van Staden, 1997). Antimicrobial activity observed in the bark, leaves and roots of *Aloe barberae* validates its use in ethnobotany and ethnoveterinary medicine by South African communities and small scale farmers to treat various conditions.

All the PE and root EtOH extracts evaluated showed good anti-inflammatory activity (>70%) in both COX-1 and COX-2 assays (Fig. 1). Other EtOH extracts however, showed moderate activity (40–69%). Due to the high COX-1 and COX-2 activity observed in PE extracts, three other concentrations (31.25, 62.5 and 125 μg/ml) were included to investigate whether the activity was dose dependent. Fig. 2 represents the dose dependent curves for the PE extracts. Both COX-1 and COX-2 of the PE extracts increased as the concentration of sample increased.

Water extracts showed moderate activity in the COX-1 assay but poor activity (<40%) in the COX-2 assay. In traditional medicine, people seldom weigh the plant material when making decoctions or infusions and they tend to use more of the plant material which could result in extraction of more active components. The observed activity, though mainly moderate, validates the inclusion of this species in treating pain related conditions. The anti-inflammatory activity observed in the non-polar solvents at a low concentration (0.25 μg/μl) is interesting since lipophilic compounds soluble in these extracts have far better resorption through the cell membrane (Zschocke and Van Staden, 2000). Poor or no inhibition of COX enzymes in some extracts does not, however, rule out anti-inflammatory activity. Some plant compounds act in other pathways such as the inhibition of the nuclear factor κB (NFκB) mediated signalling pathways in immune cells that leads to the production of the inducible COX enzymes themselves and other pro-inflammatory cytokines (Polya, 2003).

The TLC profiles (Fig. 3) showed the presence of compounds which are phenolic in nature. Other compounds that need to be investigated if they are possibly present in the plant extracts include aloin and chrysophanol. Aloin and chrysophanol contains multiple hydroxyl groups (common in most phenolic compounds) which undergo a reduction reaction with Folin C. reagent upon spraying the TLC plates to form deep blue spots. Phenolic compounds, including tannins have been reported to exhibit antimicrobial, anti-inflammatory and antioxidant properties (Bourrel et al., 1993; Polya, 2003; Ndhlala et al., 2008). The extracts of the mature bark showed more bands on the TLC profiles compared to the young bark (Fig. 3). This suggests that the mature bark contains more compounds compared to the young bark. The likely presence of more compounds in the mature bark could also explain the better activity shown in the antimicrobial and anti-inflammatory assays.

Besides providing defense against microbial invasion and herbivores, phenolic compounds in *Aloe* plants also play a crucial role in protection against UV irradiation damage (Shen et al., 2001). This makes *Aloe* plants an important pharmaceutical ingredient for skin products and gels. Fig. 4 shows the UV spectrum of leaf and root EtOH extracts. High absorbance was observed within the UV region. This corresponds to the compounds homonataloin (342–344 nm), aloeresin-1 (298–300 nm) and aloeresin-2 and -3 (300–302 nm) reported by Shen et al. (2001). TLC profiles (Fig. 3B and C) also shows

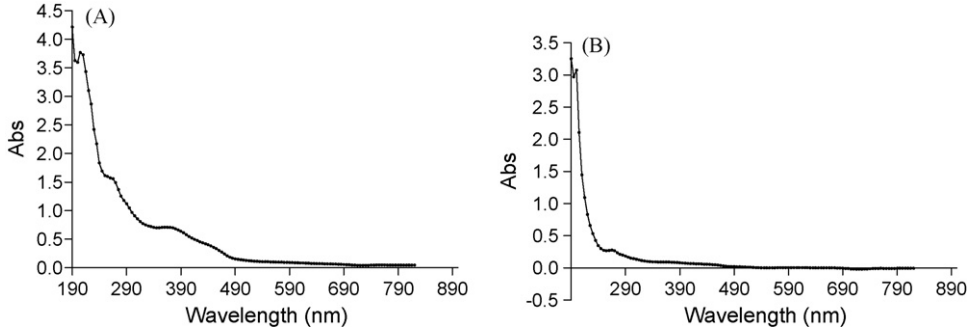

Fig. 4. UV absorption spectrum of phenolic compounds in the South African tree aloe (*Aloe barberae*). EtOH leaf extract (A) and root extract (B) at wavelength range from 190 to 820 nm.

Table 3
Number of histidine$^+$ revertants in *Salmonella typhimurium* strain TA98 produced by different parts of South African tree aloe (*Aloe barberae*).

Plant part	Extract	Number of colonies		
		5000 μg/ml	500 μg/ml	50 μg/ml
Upper stem	PE	20.7 ± 0.1	22.7 ± 0.8	23.3 ± 0.4
	DCM	22.0 ± 1.1	20.0 ± 1.6	25.0 ± 0.2
	EtOH	19.3 ± 1.0	24.3 ± 0.5	22.6 ± 0.8
	Water	24.3 ± 0.2	21.3 ± 1.3	25.3 ± 0.3
Young bark	PE	22.0 ± 1.5	20.0 ± 0.1	24.0 ± 0.6
	DCM	27.3 ± 2.3	23.0 ± 1.0	19.0 ± 0.5
	EtOH	23.0 ± 0.7	22.0 ± 0.5	21.4 ± 0.7
	Water	23.3 ± 1.2	20.3 ± 0.2	18.3 ± 2.2
Mature bark	PE	20.3 ± 0.8	26.2 ± 1.5	19.3 ± 4.2
	DCM	22.3 ± 1.0	22.0 ± 1.1	18.0 ± 0.5
	EtOH	17.0 ± 2.6	20.0 ± 0.4	21.3 ± 1.7
	Water	24.3 ± 0.2	20.3 ± 1.2	26.3 ± 2.2
Leaf	PE	19.3 ± 0.9	20.7 ± 1.0	25.7 ± 1.9
	DCM	26.2 ± 0.1	24.0 ± 0.5	21.3 ± 0.9
	EtOH	21.8 ± 1.2	22.0 ± 1.3	23.0 ± 1.0
	Water	19.1 ± 0.6	22.7 ± 0.9	21.3 ± 0.2
Root	PE	21.0 ± 1.2	25.0 ± 0.3	20.7 ± 1.6
	DCM	20.3 ± 2.1	20.3 ± 1.9	19.7 ± 2.2
	EtOH	21.0 ± 1.0	26.0 ± 0.2	22.0 ± 0.2
	Water	24.2 ± 0.1	19.0 ± 2.0	21.0 ± 1.0
4NQO (positive control, 2 μg/ml)			60.3 ± 0.6	
Water (negative control)			21.0 ± 1.3	

several bands that had strong fluorescence under UV. However, the quality of the phenolic compounds may also affect the efficiency of UV absorption. The higher the concentration of the phenolic compounds in the extracts, the more efficient the extract in protection against UV.

Plants sometimes synthesize toxic substances, which in nature act as a defense against infections, insects and herbivores, but also may affect the organisms that feed on them. An assessment of cytotoxic and mutagenic potential of extracts used in traditional medicine is necessary to ensure their safe usage (Cavalcanti et al., 2006). The mutagenicity results shown in Table 3 revealed that all the extracts were non-mutagenic towards *Salmonella typhimurium* strain TA98. A compound is considered a mutagen if the results show a dose dependent increase in the number of revertants and if the number of revertants is equal to or greater than two times of the negative control (Bulmer et al., 2007). The average His$^+$ revertants observed ranged between 17.0 and 27.3 for all the *Aloe barberae* extracts at all concentrations (50, 500 and 5000 μg/ml) while it was 21.0 for the negative control (water) and 60.3 for the positive control (4NQO). Therefore, none of the extracts could be regarded as mutagenic.

4. Conclusions

High antimicrobial and anti-inflammatory activities were observed in the leaves, roots and mature bark of the South African tree aloe. The observed activity validates the plants use in traditional medicine. It is interesting from a conservation point of view to observe similar activities in the leaf compared with other parts of the plant since the harvesting of the leaf is sustainable. The absence of mutagenic effect suggests that this plant is probably safe for use in traditional medicine, though further safety testing may be appropriate. The results reported here might be considered sufficient for further studies aimed at isolating and identifying the active compounds.

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