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Full Length Research Paper

Antibacterial, antifungal and antioxidant activity of the roots and leaves of *Pelargonium reniforme* Curtis (Geraniaceae)

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Pathogens such as bacteria and fungi and oxidative stress induce the progression of alcoholic liver disease. The antimicrobial and antioxidant activity of extracts from the roots and leaves of *Pelargonium reniforme* Curtis (Geraniaceae) were assessed in an effort to validate the traditional use of the plant in the treatment of liver diseases. Methanol, acetone and water extracts of the plant were investigated for antimicrobial activity against ten bacterial and five fungal species using the dilution method on solid agar medium. With the exception of *Streptococcus faecalis*, the extracts showed significant activity against all the gram-positive and three of the gram-negative bacteria tested in this study. Although not completely fungicidal, these extracts showed significant growth inhibition against all the fungi tested. The concentrations of the different classes of phenolic compounds were higher in the methanol extracts when compared with the water extracts. Antioxidant activities of the methanol and water extracts as assessed by three established *in vitro* methods, namely, 2,2´-azino*bis*-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric ion reducing power showed that the extract possessed strong scavenging activity and moderate reducing power. Results obtained in this study show that *P. reniforme* has good antimicrobial and antioxidant activity and this validates its traditional use in the treatment of liver diseases. Also, a comparative study of the antioxidant and antimicrobial activities of both the leaves and root showed that both parts had similar activity. The choice of the root by the traditional healers over the leaves may be arbitrary or due to easy collection. The leaves of *P. reniforme* may, as well, substitute for its roots in medicinal formulations.

Key words: *Pelargonium reniforme*, antimicrobial activity, antioxidant activity, phenolic compounds, liver diseases.

INTRODUCTION

Pathogens such as bacteria and fungi and oxidative stress are the causative agents of many diseases in man. They also induce the progression of many diseases including alcoholic liver disease. Despite the widespread use of broad spectrum antibiotics, bacterial infection is responsible for up to a quarter of the deaths of patients with liver disease (Wyke, 1987). Some of the bacteria implicated in liver diseases are *Staphylococci* sp., *Bacillus* sp., *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Jones et al., 1967; Wyke et al., 1982). One central component in the complex network of processes leading to the development of alcoholic liver disease is the activation of kupffer cells residing in the liver by several substance groups called endotoxins, which are released by bacteria living in the intestine (Wheeler, 2003). Alcohol consumption can lead to increased endotoxin levels in the blood and liver. When activated, kupffer cells

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produce signaling molecules (cytokines) that promote inflammatory reactions as well as reactive oxygen species (ROS), which can damage liver cells. Endotoxin activates kupffer cells by interacting with a complex of protein molecules that are located on the outside of the kupffer cells or which extend into the cell. Binding of endotoxin alters the activities of the proteins in the complex and triggers a cascade of biochemical signals in the kupffer cell, resulting in cytokine and ROS production and ultimately, liver damage (Wheeler, 2003).

Fungal infection has been identified as an important cause of morbidity and mortality in patients with acute liver failure. Fungal colonisation of superficial mucosal sites usually precedes invasive infection (Fisher et al., 1998). Like bacteria, fungi produce mycotoxins that cause mycotoxicoses. The most widespread and dangerous of these are the aflatoxins produced by the mould *Aspergillus flavus*. Aflatoxins pose a serious threat to humans because the mould grows on poorly stored grains. When eaten, the toxin is stored in the liver where it can eventually cause hepatitis and liver cancer (Charlotte, 2005).

Oxidative stress, induced by oxygen radicals, is believed to be a primary factor in various degenerative diseases as well as in the normal process of aging (Halliwell et al., 1992). It is also implicated in alcohol-induced liver disease. Alcohol abuse has the ability to disturb the balance between the pro- and antioxidant systems of the organism, therefore, leading to oxidative stress. This process can eventually cause increased membrane permeability and cell death. To counteract these oxidants, cells have several enzymatic antioxidants, but their levels are altered in alcoholics (Saravanan and Nalini, 2007).

P. reniforme is an attractive erect shrublet, with pink flowers. It develops from a tuberous rootstock. It grows widely in the Eastern Cape province of South Africa and it has a variety of medicinal uses. It is used in the treatment of dysentery; its roots are used to prevent purging in horses (Smith, 1895). It is also used in the Eastern Cape to treat liver complaints in calves and sheep and as a remedy for diarhoeas, colic and fever (Watt and Breyer-Brandwijik, 1962). Europeans use the plant to treat asthma (Batten and Bokelman, 1966). It is also extensively used in Germany for bronchitis, antibacterial and antifungal infections (Mativandlela et al., 2006). The medicinally active ingredients are found in the bitter tasting roots of the plants (Helmstadter, 1996) and these results in the collection of only the roots of the species for local uses and export trade. This is a destructive method of plant harvesting. It reduces the opportunity for natural rejuvenation and could negatively affect plant demography. A number of strategies to solve the problem of over harvesting have been suggested among which is the use of leaves and stems as alternatives to tubers and roots for medicinal purposes (Lewu et al., 2006). In this paper, the antioxidant, antibacterial and antifungal properties of the methanol, acetone and water extracts of the roots and leaves of *P. reniforme* are studied to provide a rationale for the use of the plant in the treatment of liver diseases. The comparison of the activity of the leaves and roots was aimed at determining the differences and similarities in the pharmacological actions of the aerial and underground parts of the plant. This may encourage the use of an alternative (aerial) part of the herb rather than the roots.

MATERIALS AND METHODS

Plant material

The plant material was collected in December, 2008 from the vicinity of Grahamstown in the Eastern Cape Province in South Africa. The plant was identified by Prof. D.S. Grierson at the Department of Botany, University of Fort Hare and a voucher specimen (GER 3928) was deposited at the Griffen Herbarium.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium ferricyanide, catechin, butylated hydroxytoluene (BHT), ascorbic acid, tannic acid, quercetin and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA), vanillin from BDH chemicals Ltd. (Poole, England), Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical supplies (Damstadt, Germany). All other chemicals used including solvents, were of analytical grade.

Extract preparation

The leaves and roots were air-dried separately at room temperature. Dried plant materials (100 g each) were shaken separately in methanol, acetone and water for 48 h at room temperature on an orbital shaker (Stuart Scientific Orbital Shaker, UK). Extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper. The methanol and acetone extracts were evaporated to dryness under reduced pressure at 40^0C using a rotary evaporator (Laborota 4000-efficient, Heildolph, Germany). The water extracts were freeze-dried (Savant Refrigerated Vapor Trap, RVT4104, USA). Each extract was resuspended in its respective solvent to make a 50 mg/ml stock solution. This was diluted to the required concentrations (0.1, 0.5, 1 and 2 mg/ml) for the different assays.

Antibacterial and antifungal assay

The bacterial cultures used in this study were obtained as laboratory isolates from the Department of Biochemistry and Microbiology, University of Fort Hare. They consisted of five gram-positive (*Bacillus cereus, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus kristinae* and *Streptococcus faecalis*) and five Gram-negative strains (*Escherichia coli, Salmonella pooni, Serratia marcescens, Pseudomonas aeruginosa* and *Klebsiella pneumonia*). Each organism was maintained on

nutrient agar plates and was recovered for testing by growth in nutrient broth for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan and Meyer, 1997). Using the agar dilution method of Meyer and Afolayan (1995), test organism were streaked in radial patterns on sterile nutrient agar plates containing filtered extracts at final concentrations of 0.1, 0.5, 1.0 and 2.0 mg/ml. Plates containing only nutrient agar and another set containing nutrient agar and the respective solvents served as controls. Chloramphenicol and streptomycin were used as standard controls in the experiment at concentrations of 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 µg/ml. After inoculation, the plates were incubated at 37^0C for 24 to 48 h. Each treatment was performed in triplicates and complete inhibition of bacterial growth was required for an extract to be declared bioactive. This concentration was regarded as the minimum inhibitory concentration (MIC).

Three species of fungi (*Aspergillus flavus, A. niger* and *Penicillum notatum*) were used for the antimycotic investigation. The cultures were maintained on potato dextrose agar (PDA) and were recovered for testing by subculturing on fresh PDA for 3 days. PDA plates were prepared in the usual fashion by autoclaving before the addition of the filtered extracts. Each extract was mixed with the molten agar (at 45^0C) to final concentrations of 0.1, 0.5, 1.0 and 2.0 mg/ml, poured into petri dishes and left overnight for the solvent to evapo-rate (Koduru et al., 2006). Control plates containing only PDA or PDA with the respective solvents served as controls. The prepared plates containing the extracts were inoculated with plugs (5 mm in diameter) obtained from the actively growing margins of the recovered fungal cultures and were incubated at 25^0C for 5 days. The diameter of fungi growth was measured and expressed as percentage growth inhibition of three replicates (Afolayan and Meyer, 1997; Barreto et al., 1997; Quiroga et al., 2001). Significant differences within the means of the treatments and the controls were calculated using the LSD statistical test (Koduru et al., 2006). LC_{50} (the concentration at which there was 50% inhibition of the growth of the test fungi) was calculated by extrapolation.

Determination of total phenolics

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extracts was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VIS spectrophotometer. Samples of extract were evaluated at a final concentration of 1 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x was the absorbance and y was the tannic acid equivalent (mg/g).

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran (2007). To 2 ml of sample (standard), 2 ml of 2% $AlCl_3$ ethanol and 3 ml (50 g/l) sodium acetate solutions were added. The absorbance at 440 nm was read after 2.5 h at 20^0C. Extract samples were evaluated at a final concentration of 1 mg/ml. Total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and y was the quercetin equivalent (mg/g).

Determination of total proanthocyanidins

The procedure reported by Sun et al. (1998) was used to determine the total proanthocyanidin. A volume of 0.5 ml of 0.1 mg/ml extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x was the absorbance and y is the catechin equivalent (mg/g).

Determination of total flavonoids

Total flavonoid contents were determined using the method of Ordonez et al. (2006). A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After 1 h at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g), using the following equation based on the calibration curve: $y = 0.025x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

Antioxidant assays

ABTS radical scavenging assay

The method of Re et al. (1999) was adopted for the ABTS radical scavenging assay. The stock solution which was allowed to stand in the dark for 16 h at room temperature contained equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate. The resultant $ABTS^{*+}$ solution was diluted with methanol until an absorbance of about 0.70 ± 0.01 at 734 nm was reached. Varying concentrations of the plant extracts (1 ml) was reacted with 1 ml of the $ABTS^{*+}$ solution and the absorbance taken at 734 nm between 3 - 7 min using the spectrophotometer. The $ABTS^{*+}$ scavenging capacity of the extract was compared with that of BHT and rutin and the percentage inhibition calculated as:

ABTS radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control}) \times 100$

where $Abs_{control}$ was the absorbance of ABTS radical + methanol; Abs_{sample} was the absorbance of ABTS radical + sample extract /standard.

DPPH radical scavenging assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). A solution of DPPH (0.135 mM) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of varying concentrations of the methanolic extract. The reaction mixture was vortex thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using rutin and BHT as references. The ability to scavenge DPPH radical was calculated as:

Table 1. Antibacterial activity of the methanol, acetone and water extracts of the leaves and roots of *P. reniforme*.

Bacteria	Gram (+/-)	MIC* (mg/ml)							
		MeOH extract		Acetone extract		Water extract		Chloramphenicol (µg/ml)	Streptomycin (µg/ml)
		Leaf	Root	Leaf	Root	Leaf	Root		
Bacillus cereus	+	1.0	1.0	na	1.0	na	0.5	<2	<2
Staphylococcus epidermidis	+	0.1	0.1	0.5	0.5	0.1	0.1	<2	<2
Staphylococcus aureus	+	1.0	1.0	na	1.0	na	na	<2	<2
Micrococcus kristinae	+	2.0	0.1	1.0	1.0	0.1	0.1	<0.5	<2
Streptococcus faecalis	+	na	na	na	na	na	na	<2	<4
Escherichia coli	-	na	na	na	na	na	na	<2	<2
Salmonella pooni	-	na	2.0	na[b]	na	na	na	<10	<4
Serratia marcescens	-	na	na	na	na	na	na	<2	<2
Pseudomonas aeruginosa	-	na	2.0	na	2.0	na	na	<2	<2
Klebsiella pneumoniae	-	na	2.0	na	2.0	na	na	<2	<2

*Minimum inhibitory concentration, [b]na = not active.

(%) DPPH radical scavenging activity = $[(Abs_{control} - Abs_{sample})]/(Abs_{control}) \times 100$

where $Abs_{control}$ was the absorbance of DPPH radical + methanol; Abs_{sample} was the absorbance of DPPH radical + sample extract /standard.

Determination of ferric reducing power

The ferric reducing potential of the extract was assayed as described by Duh et al. (1999). The different concentrations of the extract and the standards, rutin and BHT (0.025 - 0.50 mg/ml; 1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1 % w/v). The mixture was incubated at 50°C for 20 min. 2.5 ml of TCA (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml 0.1% w/v $FeCl_3$. The absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

RESULTS AND DISCUSSION

Antibacterial activity and antifungal activity

The results of the antibacterial activity of methanol, acetone and water extracts are shown in Table 1. With the exception of *S. faecalis*, all the extracts demonstrated activity against the gram-positive bacteria tested in this study. The MIC ranged from 0.1 to 2 mg/ml. All the extracts showed more activity against the gram-positive bacteria than the Gram-negative strains. Two gram-negative bacteria, *E. coli* and *Serratia mercescens*, were not inhibited by any of the extracts at the highest concentration (2 mg/ml) tested.

P. reniforme has been traditionally used for the treatment of various diseases caused by bacterial infections including liver diseases. *Staphylococci* sp., *Bacillus* sp., *P. aeruginosa* and *K. pneumonia* are bacterial pathogens implicated in liver diseases. These bacteria release high endotoxin levels in the liver especially after alcohol abuse. High endotoxin levels in the liver promote inflammatory reactions as well as release of ROS, which in turn leads to liver damage (Wheeler, 2003). All the extracts showed significant activity against *S. epidermidis, B. cereus, S. aureus, P. aeroginosa* and *K. pneumonia*. The inhibitory property of extracts from *P. reniforme* against these pathogens may justify its use in the treatment of liver diseases by the people of the Eastern Cape, South Africa.

The acetone, methanol and water extracts showed significant inhibition against most of the fungi tested in this study (Table 2). Extracts from the leaves were more inhibitory to the growth of the tested fungi. With the exception of the acetone root extracts against *A. flavus, A. niger* and *P. notatum* and the methanol root extracts against *P. notatum*, all the extracts showed significant inhibition of the fungus with activity ranging from 50.56% on *A. niger* (acetone leaf extract) to 100% on *A. niger, A. flavus* and *P. notatum* (methanol and water extract of leaf) at 2 mg/ml which is the highest concentration tested in the study. The susceptibility of *A. flavus and A. niger* to the extracts of *P. reniforme* is noteworthy, as *Aspergillus* sp. is implicated in liver diseases. They produce aflatoxins which pose a serious threat to humans because the mould grows on poorly stored grain. When eaten, the toxin is stored in the liver where it can eventually cause hepatitis and liver cancer (Charlotte, 2005). The inhibitory effect of *P. reniforme* on the growth of the fungi may also justify its use in the treatment of liver diseases by the people of the Eastern Cape, South Africa.

Table 2. Antifungal activity of the methanol, acetone and water extracts of the leaves and roots of *P. reniforme.*

Concentrations (mg/ml)	Growth inhibition (%)					
	Leaf			Root		
	A. flavus	*A. niger*	*P. notatum*	*A. flavus*	*A. niger*	*P. notatum*
Acetone extracts						
2.0	50.00^{d}	50.56^{d}	50.00^{c}	48.33^{c}	45.00^{b}	40.00^{b}
1.0	38.33^{c}	36.67^{c}	31.11^{b}	33.33^{b}	0.00^{a}	0.00^{a}
0.5	20.83^{b}	19.72^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
0.1	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	2.00	1.96	2.00	0.00	0.00	0.00
Methanol extracts						
2.0	81.94^{e}	100.00^{e}	56.11^{c}	66.67^{d}	56.67^{d}	45.00^{b}
1.0	74.72^{d}	70.83^{d}	23.06^{b}	41.67^{c}	40.83^{c}	0.00^{a}
0.5	68.61^{c}	56.94^{c}	0.00^{a}	24.17^{b}	20.00^{b}	0.00^{a}
0.1	56.94^{b}	41.67^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.09	0.32	1.82	1.33	1.58	0.00
Water extracts						
2.0	100.00^{b}	83.33^{e}	100.00^{e}	83.33^{e}	83.33^{e}	55.28^{b}
1.0	100.00^{b}	75.00^{d}	91.67^{d}	75.00^{d}	75.00^{d}	0.00^{a}
0.5	100.00^{b}	62.50^{c}	83.33^{c}	66.67^{c}	66.67^{c}	0.00^{a}
0.1	100.00^{b}	45.83^{b}	75.00^{b}	50.00^{b}	50.00^{b}	0.00^{a}
Control	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
LC_{50}	0.05	0.20	0.07	0.10	0.10	1.90

Values are means of percentage growth inhibition of three replicates: values within a column followed by the same superscript of the same species are not significantly different at $p < 0.05$ according to the LSD test.
LC_{50} values in mg/ml.

The results of the antibacterial activity showed that both the roots and leaves had similar activity on gram-positive bacteria. However, the roots showed more activity against gram-negative bacteria than the leaves. In addition, both plant parts were observed to be effective against the fungal strains tested, though the leaves had better activity. The results suggest that compounds identified in the roots of the plant may be similarly present in the leaves but with different concentrations of active compounds. The choice of the root by the traditional healers over the leaves may be arbitrary. The leaves of *P. reniforme* may, as well substitute for its roots in medicinal formulations especially in the treatment of liver diseases.

Antioxidant activity

Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties (Zheng and Wang, 2001), which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Afolayan et al., 2007). The methanol and water extracts of the plant extract were chosen for the antioxidant activity based on the results obtained from the antimicrobial assay. The methanol extracts showed the best activity and the water extract is the conventional form in which the plant is administered in traditional medicine. Results obtained revealed that the methanol extracts have higher levels of phenolic compounds than the water extracts (Figure 1). The presence of significant amount of phenolic compounds in the leaves and roots of *P. reniforme* may account for its high antioxidant activity (Sofidiya et al., 2008).

Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm that decreases with the scavenging of the proton radicals (Afolayan et al., 2007). *P. reniforme* extracts were fast and effective scavengers of the $ABTS^{*+}$ radical (Figure 2). It was also observed that while the scavenging activity of BHT and

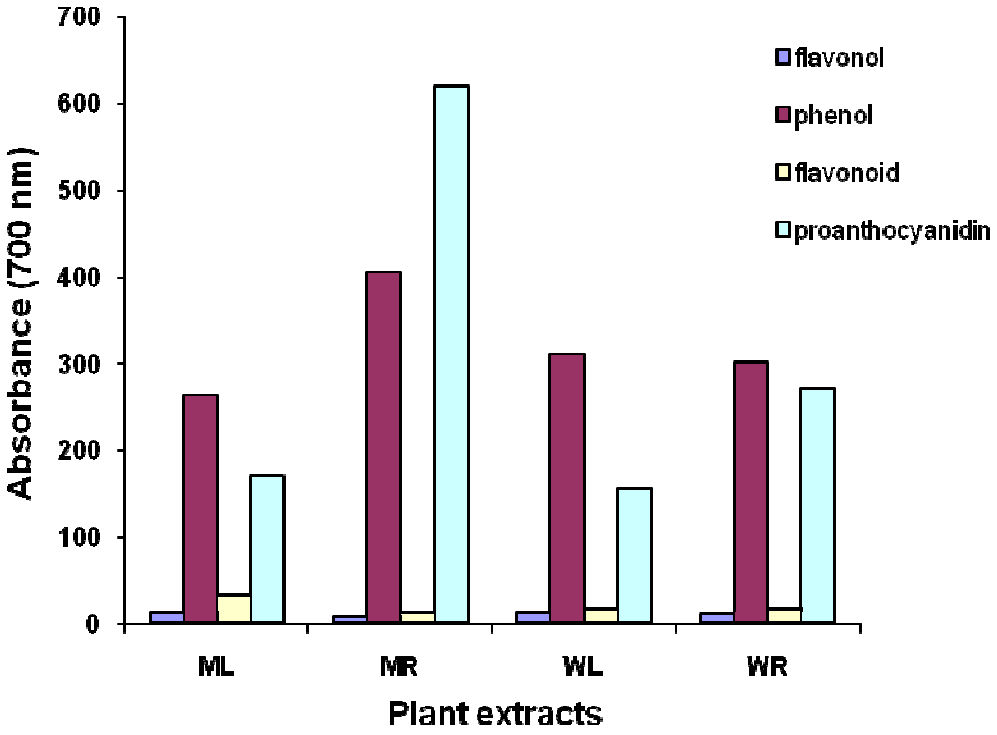

Figure 1. Polyphenolic contents of *P. reniforme.* ML = Methanolic leaf extract, MR = methanolic root extract, WL = water extract of leaf, WR = water extract of roots.

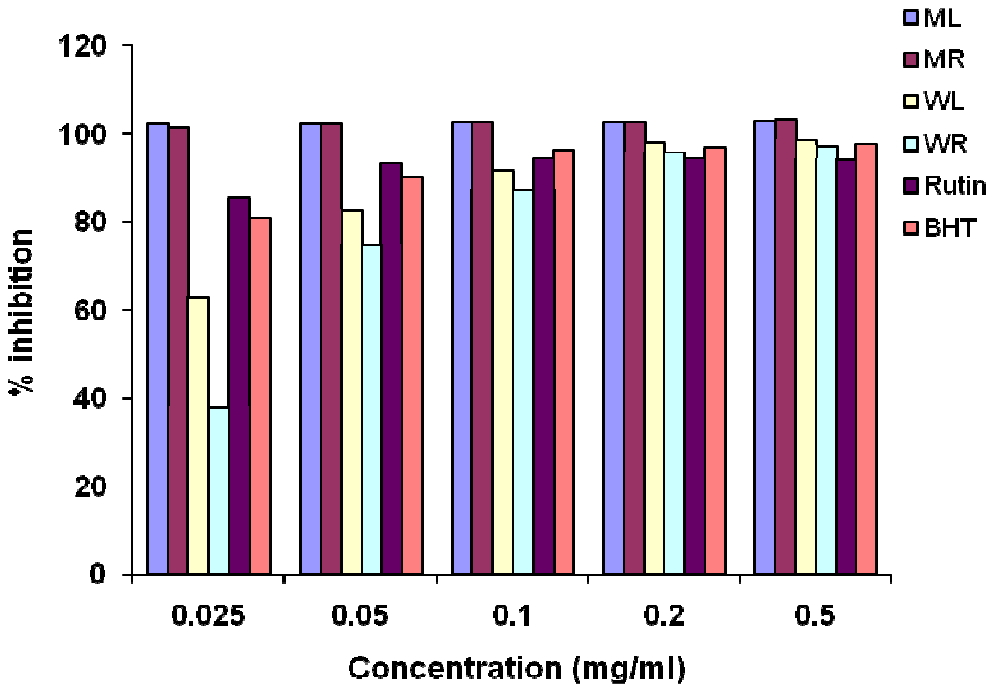

Figure 2. ABTS radical scavenging activities of the different extracts of *P. reniforme.* ML = Methanolic leaf extract, MR = methanolic root extract, WL = water extract of leaf, WR = water extract of roots.

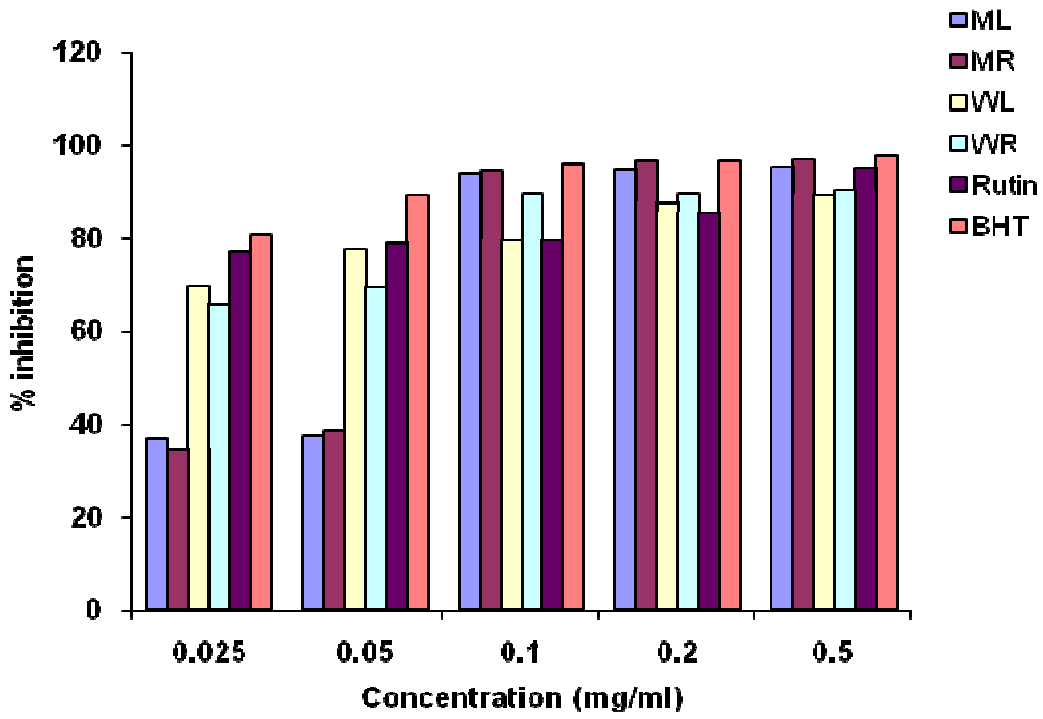

Figure 3. DPPH* radical scavenging activities of the different extracts of *P. reniforme*. ML = Methanolic leaf extract, MR = methanolic root extract, WL = water extract of leaf, WR = water extract of roots.

rutin increased gradually with increase in concentration, that of *P. reniforme* was not significantly different at all concentrations, but high scavenging effects were observed even at a low concentration of the extract for the methanol extracts, while the activity of the water extracts increased with increasing concentration.

The effect of antioxidants on DPPH* is thought to be due to their hydrogen-donating ability (Baumann et al., 1979). Figure 3 shows the DPPH radical scavenging activity of the different extracts of the leaf and root of *P. reniforme* compared with BHT and rutin. The activity increased with increasing concentration and compared well with BHT and rutin. Radical scavenging activity is considered to be an authentic parameter for the measurement of antioxidant activity, which depends on the hydrogen donating ability of the sample (Iqbal et al., 2006). The radical scavenging power of this plant might be important in fighting diseases by conferring protection against free radical cellular DNA, lipids and proteins (Jimoh et al., 2007).

The reducing power of a compound is related to its electron transfer ability and may therefore serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). Figure 4 shows the reducing power of the methanol and water extracts of the leaves and roots of *P. reniforme* and the reference antioxidants. It was observed that the reducing power of the extracts increased with increasing concentrations.

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators (Miliaus-Kas et al., 2004). Synergism of polyhenolic compounds in the extract may contribute to the overall antioxidant activity (Shahidi et al., 1994) and its ability to scavenge ROS, which are implicated in liver diseases. This may also justify the use of the plant by the traditional people of the Eastern Cape, South Africa in the treatment of liver diseases.

Results obtained showed that both the leaves and roots showed antioxidant activity which was concentration dependent. This may also suggest that similar antioxidant compounds are found in both the leaves and the roots. These results also imply that *P. reniforme* leaves may substitute for its roots in medicinal formulations especially in the treatment of liver diseases.

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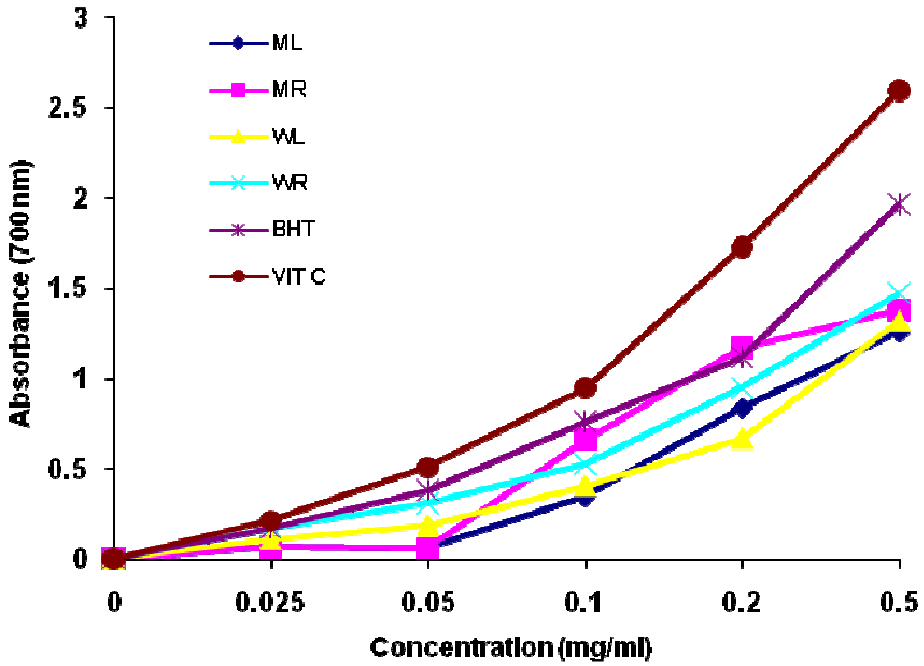

Figure 4. Reducing power of the different extracts of *P. reniforme.* ML = Methanolic leaf extract, MR = methanolic root extract, WL = water extract of leaf, WR = water extract of roots.

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