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Full Length Research Paper

In vitro antioxidant and cytotoxic activity of *Gunnera perpensa* L. (Gunneraceae) from South Africa

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***Gunnera perpensa* L. (Gunneraceae) is a medicinal plant used by Zulu traditional healers to induce labor, expel the placenta after birth and to relief menstrual pains. Phytochemical screening of the rhizomes revealed the presence of alkaloids, flavonoids, steroids, saponins, tannins and glycosides. Methanol extracts of *G. perpensa* exhibited strong scavenging of 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulfonate (ABTS), but showed poor (< 50%) radical scavenging of nitric oxide, superoxide and hydroxyl radicals. At a concentration of 5 mg/100 ml, the extract was able to inhibit lipid peroxidation of the whole rat brain homogenate (71.13%) and lipoxygenase (30%) activity. The plant extract also contained reduced form of nicotinamide adenine dinucleotide (NADH, 3.8 ρm/g), total phenol (248.45 mg/g) and traces of sulfhydryl groups (SH). The total antioxidative capacity was 36% relative to ascorbic acid (AA) and 64% relative to butylated hydroxyl toluene (BHT). The cytotoxicity of the extract (LC_{50}) to brine shrimp larvae was 137.62 mg/ml. It is apparent that the antioxidant activity of *G. perpensa* contributes to its effectiveness in folk medicine.**

Key words: *Gunnera perpensa*, gunneraceae, antioxidant activity, cytotoxicity.

INTRODUCTION

The genus *Gunnera* (Gunneraceae) is the only member of the family with about 45 - 50 species, that occurs naturally in Central and Southern Africa, New Zealand, Indonesia, Philippines, Hawaii, Mexico, Central and South America. In Africa, it is represented only by *Gunnera perpensa* L. (Mendes, 1978; Bergman et al., 1992). In the flora of South Africa, *G. perpensa* L., also known as *Ugobho* (Zulu) and *Iphuzilomlambo* (Xhosa) is a wetland perennial herb that grows (up to 1 m) in shallow water around the edge of pools in marshy areas or along streams. It is endemic to KwaZulu-Natal and Western Cape Provinces of the country (van Wyk and Gericke, 2000). *G. perpensa* has long been used in traditional medicine by the Zulus and Southern Sotho as a remedy to initiate labour, facilitate the expulsion of placenta and and clearing of the womb after birth (Veale et al., 1992; Hutchings et al., 1996; Kaido et al., 1997; van Wyk and Gericke, 2000; Ngwenya et al., 2003). Decoctions of the root or rhizome have been used for rheumatic pains, cold, wound-dressing, stomach aliments, menstrual pains, and to treat female infertility and male impotence (Iwalewa et al., 2007). Infusions of the root have also been used topically and taken orally for psoriasis, while, tinctures have been used for urinary stones (van Wyk et al., 1997, 2000).

Little is known about the chemical constituents of *G. perpensa*, although, an early study reported the presence of a bitter principle, celastrin (van Wyk and Gericke, 2000). However, pyrogallol, succinic acid, lactic acid, trimethyl ether of ellagic acid glucoside and venusol have been isolated from the aqueous extract of the dry rhizomes of this specie (Khan et al., 2004). In addition, 1,4-benzoquinones derivatives and *trans*-phyt-2-enol have also been secluded from the dichloromethane and methanol extracts of the leaves and stems and aerial parts of this plant, respectively (Drewes et al., 2005). Biological and pharmacological studies of various extracts and

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isolated compounds from the leaves, stems and roots of G. *perpensa* confirmed the activities such as antimicrobial, uterotonic, cytotoxicity and wound healing activities (Kaido et al., 1997; Grierson and Afolayan, 1999; McGaw et al., 2000; Khan et al., 2004; Steenkamp et al., 2004; Drewes et al., 2005; McGaw et al., 2005; Buwa and van Staden, 2006; Brookes and Dutton, 2007; Ndhlala et al., 2009; Nkomo and Kambizi, 2009).

Free radicals are known to cause various diseases in living tissues. Oxidative stress and more specifically, lipid peroxidation is known to play an important role in the pathophysiology of non-communicable diseases (Manuel et al., 2001) and fatigue syndrome (Vecchiet et al., 2003). Even though pregnancy is not a disease, it is often accompanied by a high-energy demand of many bodily functions and an increased oxygen requirement (Lachilli et al., 2001; Upadhyaya, et al., 2005). This triggered aerobic environment should primarily be responsible for raised oxidative stress in pregnancy. Oxidative damage might be prevented or limited by anti-oxidants. Considering the extensive utilization of *G. perp-ensa* in Zulu traditional medicine, the present study was undertaken to investigate the *in vitro* antioxidative activity and cytotoxicity of *G. perpensa* growing wild in KwaDlangezwa area of KwaZulu-Natal Province, South Africa.

MATERIALS AND METHODS

Chemicals

Unless otherwise stated, all the chemicals used including the solvents, were of analytical grade from Sigma-Aldrich Co., Ltd (Steinheim, Germany).

Plant materials

Fresh plant materials of *G. perpensa* were collected from KwaDlangezwa area in the city of Umhlathuze, KwaZulu-Natal Province, South Africa, at the flowering stage in March, 2008. The plant was identified by Mrs. N.R.Ntuli, Department of Botany, University of Zululand, KwaDlangezwa. A voucher specimen was deposited at the University Herbarium [Simelane, MBC/01(ZULU)].

Preparation of the extract

The air-dried and pulverized rhizomes of *G. perpensa* were extracted (1:5 w/v) exhaustively with methanol. The resultant extract was concentrated to dryness under reduced pressure in a rotary evaporator (40°C) to yield dried methanolic extract, which was 17.15% of the starting material. Dried extract was re-dissolved in methanol for further experiments.

Phytochemical screening

The procedures prescribed by Edeoga et al. (2005), Harborne (1984), Sofowora (1993) were used to screen the crude extract.

Total phenolic, sulfhydryl and NADH contents

The total phenolic content (measured as gallic acid equivalent) was determined using Folin-Ciocalteu reagent (Chandler and Dodds, 1983). The sulfhydryl content was measured fluorimetrically using the o-pthaladehyde condensation method of Cohn and Lyle (1966). The colorimetric method of Stern et al. (2002) was used to determine the reduced form of nicotinamide adenine dinucleotide (NADH) content.

In vitro antioxidant activity

Free radical scavenging assay

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl: The DPPH radical scavenging activity of *G. perpensa* extract was determined by modifying the methods of Brand-Williams et al. (1995). 2 mL of various concentrations (0 - 50 mg/L) of the extract in methanol was added to 2 mL of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken and allowed to stand for 30 - 60 min; the absorbance of the resulting solution was measured at 517 nm.

$ABTS^+$ radical scavenging activity: The $ABTS^+$ radical scavenging activity of *G. perpensa* extract was determined according to the improved assay of Re et al. (1999) with some modifications. $ABTS^+$ was dissolved in PBS (0.01 M, pH 7.4) to a 7 mM concentration. ABTS radical was produced by mixing the solution with 2.45 mM potassium persulfate and allowing the mixtures to stand in the dark at room temperature for 16 h. The $ABTS^+$ solution was diluted with PBS (0.01 M, pH 7.4) to an absorbance of (0.70 ± 0.02) at 734 nm and equilibrated at 30°C for 30 min. A methanolic solution (0.2 mL) of *G. perpensa* extract at various concentrations (0 - 50 mg/L) was mixed with 2.0 mL of diluted $ABTS^+$ solution. After 20 min reaction at room temperature, the absorbance at 734 nm was measured.

Superoxide anion (O_2^-) radical scavenging: The ability of the extract to inhibit photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin–light–NBT system was assessed according to the modified method of Nagai et al. (2001). The reaction mixture containing 0.02 mL each of (50.0 mM, pH 10.5) sodium carbonate buffer, 0.15% bovine serum albumin, 3 mM xanthine, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.75 mM NBT and 0.02 mL of different concentrations (0 - 50 mg/L) of *G. perpensa* extract was incubated for 20 min at 25°C. Then, 0.02 mL of xanthine oxidase (XOD) was added to initiate the reaction. The production of blue formazan was monitored for 20 min at 25°C, after adding 0.02 mL of 6 mM copper (II) chloride. The absorbance of the mixture was measured at 560 nm.

Hydroxyl radical ($^{\cdot}OH$) scavenging: The assay was based on benzoic acid hydroxylation method, as described by Chung et al. (1997). In a screw-capped tube, 0.2 mL of $FeSO_4.7H_2O$ (10 mM), EDTA (10 mM), different concentrations (0 - 50 mg/L) of *G. perpensa* extract, DNA (10 mM) and phosphate buffer (0.1 M, pH 7.4) were added. Then, 200 µL of H_2O_2 solution (10 mM) were added to the mixture. The reaction mixture was incubated at 37°C for 2 h. Thereafter, 1.0 mL of TCA (2. 8%) and 1.0 mL of TBA (1%) were added and the mixture was boiled for 10 min and allowed to cool on ice. The absorbance of the resulting mixture was measured at 520 nm against the corresponding blank solution.

Nitric oxide radical ($NO^{\cdot}$) scavenging: Nitric oxide, produced from the spontaneous reaction of sodium nitroprusside in aqueous solution at physiological pH, reacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction (Garrat, 1964). Scavengers of nitric oxide compete with oxygen and reduce the production of nitric oxide (Badami et al.,

2005). The 3 ml reaction mixture containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 mL of different concentrations (0 - 50 mg/L) of *G. perpensa* extract was incubated at 25°C for 150 min. Thereafter, 0.5 mL of the reaction mixture (containing nitrite) was pipetted and mixed with 1.0 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotisation. Then, 1.0 mL of naphthylethylenediamine dihydrochloride (0.1%) was added and allowed to stand for 30 min in diffused light. The absorbance was then measured at 540 nm.

Reducing power

The reducing power of *G. perpensa* extract was evaluated according to the method described by Oyaizu (1986). To 2.5 mL of different concentrations (0 - 50 mg/L) of *G. perpensa* extract in methanol was added 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide $K_3Fe(CN)_6$. The mixture was incubated at 50°C for 20 min, 2.5 mL of 10% TCA was added to the mixture and centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured spectrophotometrically at 700 nm. The higher the absorbance value the stronger the reducing power.

Metal chelating activity

The Fe^{2+} chelating effect of the extract was measured according to the method of Decker and Welch (1990). To 0.5 mL of various concentrations (0-50 mg/L) of *G. perpensa* extract in methanol, 1.6 mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) were added. After 30 s, the reaction was initiated by the addition of 5 mM ferrozine (0.1 mL). Then, the mixture was shaken and left at room temperature for 10 min. Absorbance of the mixture was measured spectrophotometrically at 562 nm. Citric acid (CA) and EDTA were used as standards.

Lipid peroxidation

Effect of extract on the Fe^{2+}/Ascorbate system: The antioxidant activity of the plant extract on the inhibition of oxidation induced by ascorbic acid (AA)/$FeCl_3$ was evaluated. Liver obtained from Sprague-Dawley rats (180 – 200 g) was perfused and homogenized (1:10 w/v) with 120 mM KCl, 50 mM phosphate buffer (pH 7.40). After centrifugation (700 xg, 10 min, 4°C) the supernatant was kept at -20°C until use. Protein content was quantified by the Lowry et al. (1951) method and the protein concentration of the supernatant was adjusted to 10 mg/mL. The reaction mixture containing 0.1 mL of the diluted homogenate, 0.1 mL Tris-HCl buffer (20 mM, pH 7), 0.1 mL KCl (30 mM), and 0.1 mL of various concentration (0 - 50 mg/L) of *G. perpensa* extract, was incubated for 5 min at 37°C. The AA/Fe^{2+} (0.1 mL; 0.1 mM) was added to the mixture and then incubated for 30 min at 37°C. The products of lipid peroxidation were detected by the thiobarbituric acid method measuring the absorbance at 532 nm (Ohkowa et al., 1979).

Effect of extract on lipid peroxidation in rat brain

Whole rat brain homogenate (0.1 mL of 10% w/v homogenized in phosphate buffer saline) and 0.1 mL of various concentration (0 – 50 mg/L) of *G. perpensa* extract were mixed and incubated in a water bath at 35°C for 5 min; 0.15 mL H_2O_2 (10 mM) was added to induce lipid peroxidation and the mixture reincubated at 37°C for 15 min. Then, thiobarbituric acid reactive species (TBARS) were measured as indication of the lipid peroxide formed (Ohkowa et al., 1979).

Effect of extract on lipoxygenase activity

The method reported by Mathisen et al. (2002) was used to determine the ability of the extract to inhibit lipoxygenase activity. *G. perpensa* extract (0.5 mL, 0 - 50 mg/L) was mixed with 0.95 mL borate buffer (0.2 M; pH 9.0), 2.0 mL substrate solution (linoleic acid) and enzyme solution (lipoxygenase). The absorbance was immediately measured at 234 nm.

Calculation of percentage inhibitory effect of *G. perpensa* extract

Unless otherwise stated, ascorbic acid, BHA and BHT were used as standards. All assays were repeated three times and the mean ± S.E reported. The inhibitory effect of *G. perpensa* extract of each parameter was calculated as: % Inhibition = $\{(A_0 – A_1)/A_0 \times 100\}$ where, A_0 is the absorbance value of the fully oxidized control and A_1 is the absorbance of the extract. The inhibitory concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against *G. perpensa* extract concentrations.

Determination of total antioxidant capacity

The assay was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH, according to the method of Prieto et al. (1999). *G. perpensa* extract (0.3 ml) was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and BHT.

Brine shrimp lethality screening

Brine shrimp (*Artemia salina*) lethality test was carried out using the procedure described by Meyer et al. (1982) and McLaughlin (1992). Methanol extract of *G. perpensa* (reconstituted in 1% DMSO in artificial seawater) was added to brine shrimp suspension to give final concentrations of 9.2, 18.4, 29.9, 39.1 and 48.3 mg/mL (three replicates each, gallic acid and DMSO as positive and negative controls, respectively). The plates were maintained under illumination and the survivors were recorded after 24 h. The percentage mortality at each concentration and control were calculated using the Abbots formula (Abbott, 1925). LC_{50} and 95% confidence intervals values were determined using the computer model (Probit Program Version 1.5) prepared by the US Environmental Protection Agency.

Statistical analyses

The mean and standard error mean of three experiments were

determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated using Microsoft Excel Program, 2003 and Origin 6.0 for IC_{50}. Data were subjected to one way analysis of variance (ANOVA). P values ≤ 0.05 were regarded as significant and P values ≤ 0.01 as very significant.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of methanolic extract of *G. perpensa* rhizomes revealed the presence of alkaloids, flavonoids, steroids, saponins, tannins and glycosides.

Free radical scavenging assay

The ability of *G. perpensa* extract to scavenge DPPH, $ABTS^{+}$, O_2^{-}, $^{\cdot}OH$ and NO was evaluated and the results are shown in Table 1. The results show that the *G. perpensa* extract significantly and dose dependently reduced DPPH radicals. At a concentration of 50 mg/L, *G. perpensa* extract scavenged over 78% of DPPH radicals with IC_{50} value of 16 mg/L which was 2 times greater than the synthetic antioxidant, BHA (36.1mg/L) and BHT (35.9 mg/L). The extract was also found to be very effective scavenger of $ABTS^{-+}$ and the activity increased in a concentration dependent manner (Table 1). At 50 mg/L, the extract exerted the highest $ABTS^{-+}$ scavenging activities of 78.45% (IC_{50} of 9 mg/L) whereas BHA and BHT exhibited lower activities (IC_{50} of 36.7 and 31.8 mg/L, respectively). It is apparent that *G perpensa* is a good free radical scavenger, and it could effectively act as a primary antioxidant against free radicals and be considered a good source of natural antioxidant in preventing lipid peroxidation and protection from oxidative damage (Moure et al., 2001).

Superoxide anions and hydroxyl radicals have damaging effects on bio-macromolecules leading to various pathogenic conditions with severe consequences (Cotelle et al., 1992: Radi et al., 1991). Hydroxyl radicals are, among the ROS, the most reactive and predominant radicals, generated endogenously during aerobic metabolism (Waling, 1975). Owing to their high reactivity, they have a very short half-life and can only be effectively scavenged at high concentrations (Rathee et al., 2007). In addition, nitric oxides an essential bioregulatory required for several physiological processes like immune response, smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and control of blood pressure, have been implicated in several pathological conditions, including cancer (Palmer et al., 1987). Accordingly, the ability of *G. perpensa* to scavenge these radicals has been evaluated. The results of the scavenging ability of various concentrations of *G. perpensa* (0 – 50 mg/L) and standard antioxidants (ascorbic acid, BHA and BHT) against superoxide radical anions, hydroxyl radicals and nitric oxide radical are given in Table 1. The extent of scavenging activities of the extract and antioxidants were low (IC_{50} values ranged between 36.7 - 50.0 mg/L). How-ever, *G. perpensa* was found to be significantly better in hydroxyl radical scavenger than BHA, under the condition of investigation. The percentage of hydroxyl radical scavenging values was 46.24 and 36.36% for *G. perpensa* and BHA respectively. Comparing the overall free radical scavenging activities of *G. perpensa* extract and standard antioxidants against DPPH, ABTS, O_2.-, $^{\cdot}OH$ and NO (Table 1) it is noted that the extract showed the highest antioxidant activity.

Reducing power

The reducing capacity of many plant extracts has been correlated with their antioxidant activity (Pin-Der-Duh, 1998). In addition, reducing properties have been associated with the presence of reductants, which exert antioxidant action by breaking the free radical chains through hydrogen atom donation (Gordon, 1990; Pin-Der-Duh, 1998). Furthermore, reductones have also been reported to prevent peroxide formation by reacting with certain precursors of peroxide (Rathee et al., 2007). Figure 1 shows the reducing potential of *G. perpensa* extract in comparison with ascorbic acid and BHA. The results show the reducing power of the extract to be dose dependent and better reducing than ascorbic acid and BHA. At the concentration of 20 mg/L, the reducing power of *G. perpensa* extract (0.483 ± 0.023) was higher than those of ascorbic acid (0.453 ± 0.007) and BHA (0.463 ± 0.028), respectively. It is thus likely that certain compounds present in *G. perpensa* may act as primary and secondary reductants thereby inhibiting lipid peroxidation.

Metal chelating activity

Transition metals can arouse lipid peroxidation by generating hydroxyl radicals through Fenton reaction and hasten lipid peroxidation by lipid hydroperoxides into peroxyl and alkoxyl radicals (Zhao et al., 2006). Most effective pro-oxidants in food and drugs are ferrous ions (Yamaguchi, 1988). Chelating agents which form σ-bond with metal (Gordon, 1990) are effective as secondary antioxidants because they decrease redox potential, thereby stabilizing the oxidized form of the metal ion. The chelating activity for ferrous ion of the extract was assayed by inhibition of the formation of red-colored ferrozine and ferrous complex. Figure 2 shows the results

Table 1. Percentage scavenging of DPPH, ABTS, superoxide, hydroxyl radical and nitric oxide by *G. perpensa* rhizome extract[a] (mg/mL).

	DPPH	ABTS	($O_2^{\cdot}$-)	($^{\cdot}OH$)	($NO^{\cdot}$)
Control	100.0 ± 0.02	100.0 ± 0.02	100.0 ± 0.09	100.0 ± 0.02	100.0 ± 0.0.01
G. perpensa					
1	41.49 ± 0.00	52.54 ± 0.02	10.74 ± 0.06	16.13 ± 0.02	5.76 ± 0.00
2	56.02 ± 0.02	63.61 ± 0.08	19.88 ± 0.11	32.26 ± 0.00	7.89 ± 0.00
3	68.00 ± 0.06	68.69 ± 0.08	20.51 ± 0.08	33.33 ± 0.00	9.15 ± 0.01
4	72.27 ± 0.01	71.68 ± 0.01	22.77 ± 0.04	40.86 ± 0.00	12.54 ± 0.02
5	78.77 ± 0.04	78.17 ± 0.01	25.91 ± 0.13	46.24 ± 0.00	15.25 ± 0.01
IC_{50}[b]	1.6	0.9	>5	> 5	> 5
AA (IC_{50})	-	-	4.49	-	-
BHA (IC_{50})	3.61	4.01	-	> 5	3.67
BHT (IC_{50})	3.59	3.59	-	-	-

[a](n = 3, X ± SEM),[b]IC_{50} -inhibitory concentration, (O_2-) - Superoxide anion radical scavenging; ($^{\cdot}OH$) - Hydroxyl radical scavenging; ($NO^{\cdot}$)- Nitric oxide radical scavenging.

Table 2. Polyphenol contents, antioxidative and cytotoxic activities of *G. perpensa* rhizome extract and standard controls (mg/Ml)[a].

	G. perpensa	BHA	BHT	AA	Gallic acid
Polyphenol contents					
Total phenol[b]	248.45 + 1.28	-	-	-	-
TAC (%)[c]	-	-	64	36	-
Antioxidative activity (%)					
Lipid peroxidation					
(i) Fe^{2+} / Ascorbate	48.01	22.69	-	-	-
(ii) TBARS	71.13	63.56	-	-	-
Lipoxygenase	30.00	28.27	-	-	-
NADH[d]	3.8	-	-	-	-
Cytotoxicity [e]LC_{50} (95% CI)	137.62 (72.63 - 2151.07)	-	-	-	11.45 (6.63 - 38.15)

[a](n = 3, X ± SEM), [b]Expressed as mg gallic acid/g of dry plant material, [c]TAC - Total antioxidant capacity relative to ascorbic acid and BHT, [d]NADH - ρm/g,[e]LC_{50} (95% CI) - Lethal concentrations with 50 % larvae mortality rate and 95% confidence intervals (95% CI).

obtained, indicating weak chelating activity. At the concentration of 5 mg/L, the extract and citric acid chelated 7.51 and 7.42% of ferrous ions respectively, while, EDTA showed moderate ferrous ion chelating activity of 14.81%.

Lipid peroxidation and lipoxygenase activity

Plant constituents that have been reported to be free radical scavengers and anti-lipoperoxidative include polyphenol (Maisuthisakul et al., 2007; Adedapo et al., 2009), reduced glutathione (Bhatia and Jain, 2004) and NADH (Stern et al., 2002). NADH therapy is known to have beneficial effects in patience with chronic fatigue syndrome (Santaella et al., 2004). Polyphenols have antioxidant and iron-chelating properties and can combat oxidative stress. *G. perpensa* extract had a relatively high total polyphenol and NADH contents (Table 2) indicating a potential for anti-lipoperoxidative activity. The extracts significantly inhibited hydrogen peroxide induced lipid peroxidation in the rat brain, and also inhibited lipoxygenase activity, and the AA/Fe^{2+} system. Apparently, *G. perpensa* is a good free radical (especially the peroxy type) scavenger.

Cytotoxic activity

The cytotoxic activity of *G. perpensa* extract was

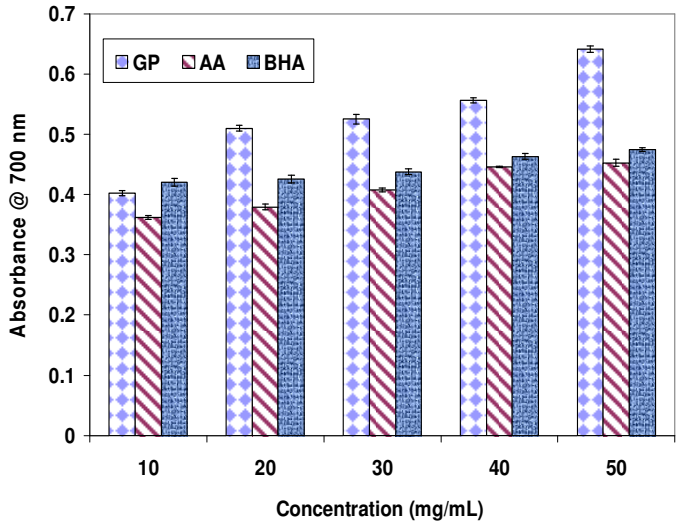

Figure 1. Reducing power of *G. perpensa* (GP) rhizome extract, ascorbic acid (AA) and butylated hydroxyl anisole (BHA). Each value is mean ± S.E (n = 3).

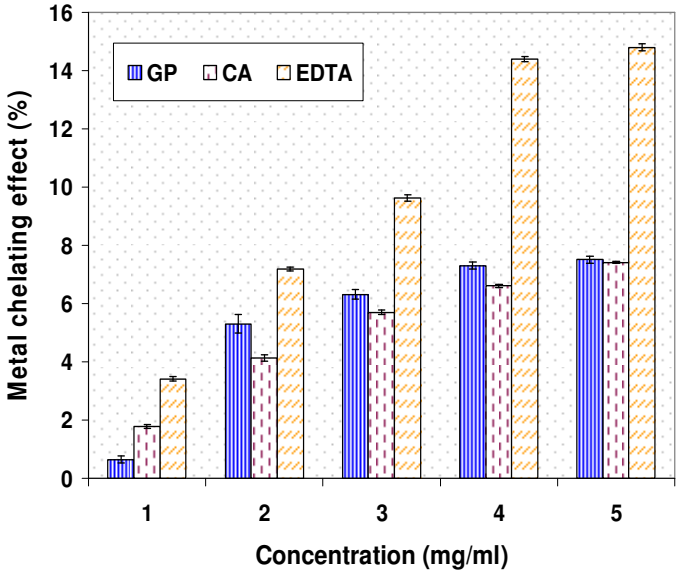

Figure 2. Metal chelating assay of *G. perpensa* (GP) rhizome extract, citric acid (CA) and ethylenediaminetetraacetic acid (EDTA). Each value is mean ± S.E (n = 3).

determined using the brine shrimp (*A. salina*) lethality assay. Table 2 shows the result of the cytotoxicity of *G. pe-rpensa* extract and the control (gallic acid) after 24 h exposure. The degree of the brine shrimp lethality was found to be directly proportional to the different concentrations of the extract, with lethal concentration (LC_{50}) of 137.62 mg/100 ml). Compared to the standard gallic acid (LC_{50} = 11.45 mg/100 ml) it is apparent that *G. perpensa* has a weak toxic activity. In toxicity evaluation of plant extracts by brine shrimp lethality bioassay, LC_{50} values lower than 1000 µg/mL are considered bioactive (Meyer et al., 1982).

Conclusions

The relationship between antioxidants and prevention of chronic degenerative diseases is the interest of many researchers and the restricted use of synthetic antioxidants, such as BHA and BHT, has led to the search for natural antioxidants from plant extracts. Growing evidence indicates that reactive oxygen species are responsible for exercise-induced protein oxidation and contribute highly to muscle fatigue (Powers et al., 2004). Thus, the treatments that reverse muscle fatigue may be acting through mechanisms that scavenge reactive oxygen species. The potential antioxidants capability and weak toxic effect of *G. perpensa* has been established in this study. It is apparent that *G. perpensa* contains compounds that might improve the action of natural dietary antioxidant. The relatively high total polyphenol and NADH contents and the significant total antioxidant capacity, expressed as the percentage of BHT, could possibly contribute to *G. perpensa* antioxidant properties. Even though, the detailed mechanisms of its effectiveness in inducing labor and facilitating the expulsion of placenta or relief of menstrual pains have not yet been known, it is however, apparent that the antioxidant properties and the relatively weak toxic activity of the plant may contribute to its use in folk medicine.

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REFERENCES

Abbott WS (1925). A method of computing the effectiveness of insecticide. J. Econ. Entomol., 18: 265–267.

Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ (2009). Assessment of the medicinal potentials of the methanol extracts of the leaves and stems of *Buddleja saligna*. BMC Complement Altern. Med., 9: 21.

Badami S, Rai SR, Suresh B (2005). Antioxidant activity of *Aporosa lindleyana* root. J. Ethnopharmacol., 101: 180–184.

Bergman B, Johansson C, Soderback E (1992). The *Nostoc* - *Gunnera* symbiosis. New Phytol., 122: 379-400.

Bhatia AL, Jain M (2004). *Spence oleracea* L. protects against gamma radiations: A study on glutathione and lipid peroxidation in mouse liver. Phytomed., 11: 607-615.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol-LEB.

28: 25-30.
Brookes KB, Dutton MF (2007). Bioactive components of the uteroactive medicinal plant, *Gunnera perpensa* (*Ugobo*). S. Afr. J. Sci., 103: 187-189.
Buwa LV, van Staden J (2006). Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J. Ethnopharmacol., 103: 139-142.
Chandler SF, Dodds JH (1983). The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. Plant Cell Rep., 2: 105-110.
Chung SK, Osawa T, Kawakishi S (1997). Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). Biosci. Biotechnol. Biochem., 61: 118-123.
Cohen VH, Lyle J (1966). A fluorometric assay for glutathione. Anal. Biochem., 14: 434-440.
Cotelle N, Bernier JL, Henichart JP, Catteau JP, Gaydou E, Wallet JC (1992). Scavenger and antioxidant properties of ten synthetic flavones. Free Radical Biol. Med., 13: 211-219.
Decker EA, Welch B (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem., 38: 674-677.
Drewes SE, Fatima Khan F, van Vuuren S, Viljoen AM (2005). Simple 1,4-benzoquinones with antibacterial activity from stems and leaves of *Gunnera perpensa*. Phytochem., 66: 1812-1816.
Edeoga HO, Okwu DE, Mbaeble BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.
Garrat DC (1964). The qualitative analysis of drugs, 3. Japan; Chapman and Hall, pp. 456-458.
Gordon MH (1990). The mechanism of antioxidant action *in vitro*. In Food antioxidants. B. J. F. Hudson (Ed.), Elsevier. London: pp. 1-18.
Grierson DS, Afolayan AJ (1999). Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. J. Ethnopharmacol., 66: 103-106.
Harborne JB (1984). Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd edition, Chapman and Hall Ltd, London, UK. pp 49-150.
Hutchings A, Scott AH, Lewis G, Cunningham AB (1996). Zulu Medicinal Plants: An Inventory. University of Natal Press: Scottsville, South Africa. pp. 186-187.
Iwalewa EO, McGaw LJ, Radio V, Eloff JN (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. Afr. J. Biotechnol., 6: 2868-2885.
Kaido, TL, Veale DJH, Havlik I, Rama DBK (1997). Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. J. Ethnopharmacol., 55: 185-191.
Khan F, Peter XK, Mackenziea RM, Katsouli L, Gehring R, Munro OQ, van Heerden FR, Drewes SE (2004). Venusol from *Gunnera perpensa*: Structural and activity studies. Phytochem., 65:1117-1121.
Lachilli B, Hininger I, Faure H, Armaud J, Richard MJ, Favier A, Roussel AM (2001). Increased lipid per-oxidation in pregnant women after iron and vitamin C supplementation. Biol. Trace Elem. Res., 83(2): 103-110.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.
Maisuthisakul P, Suttajit M, Pongsawatmanit R (2007). Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem., 100: 1409-1418.
Manuel Y, Keenoy B, Moorkens G, Vertommen J, De Leeuw I (2001). Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. Life Sci., 68: 2037-2049.
Mathisen E, Diallo D, Andersen OM, Malterud KE (2002). Antioxidants from the bark of *Burkea Africana*, an African medicinal plant. Phytother. Res., 16: 148-153.
McGaw LJ, Jager AK, van Staden J (2000). Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J. Ethnopharmacol., 72: 247–263.
McGaw LJ, Gehring R, Katsoulis L, Eloff JN (2005). Is the use of *Gunnera perpensa* extracts in endometritis related to antibacterial activity. Onderstepoort, J. Vet. Res., 72: 129-134.
McLaughlin JL (1991). Crown gall tumors on potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. Method Plant Biochem., 6: 1-32.
Mendes EJ (1978). Haloragaceae. *Flora zambesiaca*, 4: 79-81.
Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta. Med., 45: 31-34.
Moure A, Cruz JM, Fraco D, Dominguez JM, Sineiro J, Dominguez H, Nunez MJ, Parajo JC (2001). Natural antioxidants from residual sources. Food Chem., 72: 145-171.
Nagai T, Sakai M, Inoue H, Suzuki N (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chem., 75: 237-240.
Ndhlala AR, Stafford GI, Finnie JF, van Staden J (2009). *In vitro* pharmacological effects of manufactured herbal concoctions used in KwaZulu-Natal South Africa. J. Ethnopharmacol., 122: 117-122.
Ngwenya MA, Koopman A, Williams R (2003). Zulu botanical knowledge: An introduction. National Botanical Institute, Durban. South Africa, p. 67.
Nkomo M, Kambizi L (2009). Antimicrobial activity of *Gunnera perpensa* and *Heteromorpha arborescens* var. *abyssinica*. J. Med. Plants Res., 3: 1051-1055.
Ohkowa M, Ohisi N, Yagi K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358.
Oyaizu M (1986). Studies on products of browning reactions: Antioxidant activities of products of browning reaction prepared from glucose amine. Jap. J. Nut., 44: 307-315.
Palmer RMJ, Ferrige AG, Moncada S (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature, 327: 524-526.
Pin-Der-Duh X (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): Its scavenging effect on free-radical and active oxygen. J. Am. Oil Chem. Soc., 75: 455-461.
Powers SK, DeRuisseau KC, Quindry J, Hamilton KL (2004). Dietary antioxidants and exercise. J. Sports Sci., 22: 1-94.
Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269: 337-341.
Radi R, Beckman JS, Bush KM, Freeman BA (1991). Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys., 288: 481-487.
Rathee JS, Hassarajani SA, Chattopadhyay S (2007). Antioxidant activity of *Nyctanthes arbor-tristis* leaf extract. Food Chem., 103: 1350-1357.
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med., 26: 1231-1237.
Santaella ML, Font I, Disdier OM (2004). Comparison of oral Nicotinamide Adenine Dinucleotide (NADH) versus conventional therapy for chronic fatigue syndrome. PR. Health Sci. J., 23: 89-93.
Sofowora A (1993). Medicinal Plants and Traditional Medicines in Africa. Spectrum Books Ltd. Ibadan, Nigeria, pp. 150-156.
Steenkamp V, Mathivha E, Gouws MC, van Rensburg CEJ (2004). Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. J. Ethnopharmacol., 95: 353-357.
Stern N, Hochman A, Zemach N, Weizman N, Hammel I, Shiloh Y, Rotman G, Barzilai A (2002). Accumulation of DNA damage and reduced levels of nicotine adenine dinucleotide in the brains of Atm-deficient mice. J. Biol. Chem., 277: 602-608.
Upadhyaya C, Mishra S, Singh PP, Sharma P (2005). Antioxidant status and peroxidative stress in mother and newborn—A pilot study. Indian J. Clin. Biochem., 20: 30-34.
van Wyk BE, van Oudtshoorn B, Gericke N (1997). Medicinal plants of

South Africa. Briza Publications, Pretoria. South Africa. pp. 160-161.
van Wyk BE, Gericke N (2000). People's plants: A guide to useful plants of southern Africa. Briza Publications, Pretoria. South Africa. pp. 186-187.
Veale DJH, Furman KI, Oliver DW (1992). South African traditional herbal medicines used during pregnancy and childbirth. J. Ethnopharmcol., 36: 185-191.
Vecchiet J, Cipolin F, Falasca K, Mezzetti A, Pizzigallo E, Bucciarelli T, De Laurentis S, Affaitati G, De Cesare D, Giamberardino MA (2003). Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome**.** Neurosci. Lett., 335: 151-154.
Waling C (1975). Fenton's reagent revisited. Acc. Chem. Res., 8: 125-131.
Yamaguchi R, Tatsumi MA, Kato K, Yoshimitsu V (1988). Effects of metal salts and fructose on the antioxidation of methyl lineate in emulsion. Afr. Chem., 52: 849-850.
Zhao G, Xiang Z, Ye T, Yuan Y, Guo Z (2006). Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem., 99: 767-774.