

Full Length Research Paper

Phenolic content and phospholipids peroxidation inhibition by methanolic extracts of two medicinal plants: *Elionurus muticus* and *Hypoxis hemerocallidea*

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Two commonly known medicinal plants from Zimbabwe were analyzed for total phenolic content, superoxide radical scavenging activity and the ability to inhibit lipid peroxidation in rat brain extracts. The plant samples used in this study were *Elionurus muticus* and *Hypoxis hemerocallidea*. The Folin Ciocalteu method was used to determine the total phenolic content. The total phenolic content was high in *H. hemerocallidea* which had 0.268 ± 0.02 mg/100mg dry mass of plant extract and lowest in *E. muticus* root extract had 0.041 ± 0.03 mg/100mg dry mass of plant extract. The scavenging activities on the superoxide ion of the plant extracts was dose dependent with maximum scavenging activity being obtained from *E. muticus* whole plant ($69.12\% \pm 0.12$ at 80 mg/ μ l) and the least activity were detected in *H. hemerocallidea* ($52.92\% \pm 0.95$ at 80 mg/ μ l). The plant extracts also gave a dose dependent protection against lipid peroxidation in rat brain homogenate with the highest protection detected from *E. muticus* whole plant and the least from *H. hemerocallidea*.

Key words: Phenolic compounds, medicinal plants, phospholipid peroxidation.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Numerous investigations have proved that medicinal plants contain diverse classes of bioactive compounds such as polyphenols, tocopherols and alkaloids. Among them flavonoids and phenolic acids are particularly attractive as they are known to exhibit various pharmacological properties such as vasoprotection, anticarcinogenic, antimicrobial, anti-inflammatory as well as antiallergic and antiproliferative activity on tumour cells (Gordona *et al.*, 2004). Some of these potential health benefits of polyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers,

quenchers of singlet and triplet oxygen and inhibitors of peroxidation (Li-Chen *et al.*, 2005).

As a group, phenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases (Kyung-Hee *et al.*, 2005). Phenolic compounds are plant substances which possess in common an aromatic ring bearing one hydroxyl substituent. They are water soluble. They may occur combined with sugar, as glycosides and they are usually located in the vacuole of the plant cells. They are different classes of phenolic compounds and these include phenols, phenolic acids, phenylpropanols, flavonoids, minor flavonoids, tannins and quinines (Harborne, 1998).

Lipid peroxidation may be initiated by any primary free radical of sufficient reactivity to abstract an allylic hydrogen atom from a reactive methylene group of polyunsaturated fatty acid side chains. The formation of the initiating factors is accompanied by bond rearrangement that re-

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sults in stabilization by diene conjugate formation. The lipid radical then takes up oxygen to form the peroxy repeated many times.

Accumulation of hydroperoxides and their subsequent decomposition to alkoxyl and peroxy radicals can accelerate the chain reaction of polyunsaturated fatty acids peroxidation leading to oxidative damage to cells, membranes and proteins. Haem proteins through redox cycling properties catalyse decomposition of hydroperoxides generating alkoxyl and peroxy radical species. Lipid peroxy radicals oxidize cholesterol and react with proteins impairing functions of critical enzyme and receptor systems (Evans et al., 1995; Gordana et al., 2004; Govindarajan et al., 2005).

Recently, identifying phytochemicals with the capacity to interfere carcinogenesis has received considerable attention. Carcinogenesis involves initiation, promotion and progression processes. It is well established that oxidative insults to DNA can lead to mutations in crucial genes, which is involved in the initiation process of carcinogenesis. Furthermore, oxidation and inflammation are well recognized to be closely linked to the process of promotion (Tzung-Hsu et al., 2005). Consequently, determination of antioxidation functions has been proposed to be a good indicator for screening or evaluating plants for medicinal properties. Owing to their many uses in traditional medicines in Southern Africa and other potential health benefits, *Elionurus muticus* and *Hypoxis hemerocallidea* were selected for this study.

E. muticus commonly known, as lemon grass is a tufted grass found in open grassland and high rainfall areas. It grows wild or may be cultivated and is common in Southern Africa especially in South Africa. The roots are chewed to treat toothache, colic and to make young man strong and true. The aerial parts give an aromatic essential oil with possible use in the cosmetic industry. The essential oil has been reported to possess potential analgesic and antibacterial action (Tredgold, 1986; Boose; Holt, 1999).

H. hemerocallidea commonly known as African potato (*Hodzori*; *Shona*) is a plant in the tuber family. It is high in sterols and sterolins and natural immune substances that have yielded a breakthrough in the fight against infection. The compound hypoxoside has been isolated to be a major phenolic constituent of the plant. The compound has been shown to have anti-tumour properties, suggesting it as a promising oral drug for cancer.

The plant has immunity boosting properties (Tredgold, 1986). In Zimbabwe the main use of *H. hemerocallidea* is in HIV patients as a powder and of late capsules, which contains the powder has been produced (Tredgold, 1986; Pooley, 1998).

The aim of the study was to determine the amount of phenolic compounds, superoxide radical scavenging activity and inhibition of phospholipid peroxidation by methanolic extracts of the medicinal plants *H. hemerocallidea* and *E. muticus*.

MATERIALS AND METHODS

Plant material

The whole plant of *E. muticus* (roots and aerial parts) was collected from the University of Zimbabwe farm near Harare. Tubers of *H. Hemocallidea* were bought at Mbare market in Harare and authenticated at the National Herbarium of Zimbabwe. The roots of *E. muticus* were separated from the aerial parts and dried separately in the air.

Reagents

All the reagents used were of analytical grade. Folin-Ciocalteu, gallic acid, nitroblue tetrazolium salt (NBT), phenazine methosulphate (PMS) and ascorbic acid were obtained from Sigma – Aldrich Chemie (Steinheim, Germany). Reduced nicotinamide adenine dinucleotide (NADH) was obtained from Boehringer, Mannheim, Germany.

Extraction of phenolic compounds

Total phenolic compounds were extracted from the ground material as described by Makkar (1999). The sample (2 g) was extracted twice with cold 50% aqueous methanol (10 ml). The two volumes were combined, made up to 20 ml, centrifuged at 3000 rpm for 10 min and transferred into small sample bottles for analysis.

Folin C. assay for total phenolic compounds

Total phenolic compounds were determined following the method by Makkar (1999). To a sample (50 µl), distilled water (950 µl) was added to make up to 1 ml followed by 1N Folin C. reagent (500 µl) and sodium carbonate (2.5 ml). After 40 min at room temperature absorbance at 725 nm was read on a Spectronic 20® Genesys™ spectrophotometer against a blank that contained methanol instead of sample. Total phenolic compounds were in terms of gallic acid equivalent.

Superoxide anion radical scavenging activity

Anion radical scavenging activity was determined following the method by Kuda et al. (2005). The sample up to 80 µl was mixed with phosphate buffer (0.1 mL, 0.1 M, pH 7.2), NADH (mM, 0.025 mL) and NBT (0.5 mM, 0.025 mL). An aliquot (0.025 mL) was transferred into a microtitre plate and after incubation with PMS (0.03 mM, 0.025 mL) for 3 min, absorbance at 550 nm was read on a Spectra MAX 340 (USA, Sunnyvale, California) microtitre plate spectrophotometer. Ascorbic acid was used as a positive control. The anion scavenging activity was calculated as:

$$\text{Anion scavenging activity (\%)} = (\text{Abs sample} / \text{Abs control}) \times 100$$

Inhibition of phospholipid peroxidation

Female Sprague Dawley rats (*Rattus norvegicus*) were obtained from the Animal House, University of Zimbabwe and dissected in the Physiology Department to obtain rat brain. The rat brains were stored at -85°C until used. Homogenization of rat brain (2 g) was done in a chloroform:methanol mixture (2:1, v/v) followed by centrifugation at 3000xg for 5 min. The supernatant obtained was used as the source of phospholipids. The blank contained the phospholipid solution (50µl) mixed with distilled water instead of the sample (0.5 ml) and 50% methanol (0.2 ml). The test run contained the

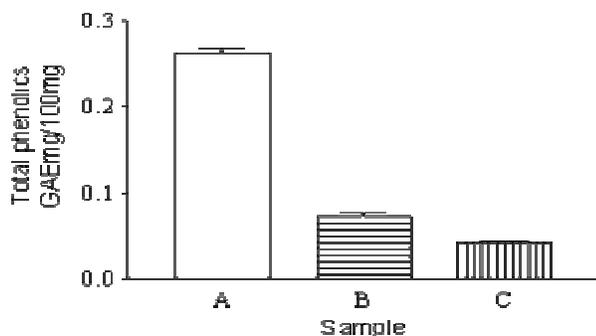


Figure 1. Total phenolic acid content of *Hypoxis hemerocallidea* (A), *Elionurus muticus*- whole plant (B) and *Elionurus muticus*- root (C).

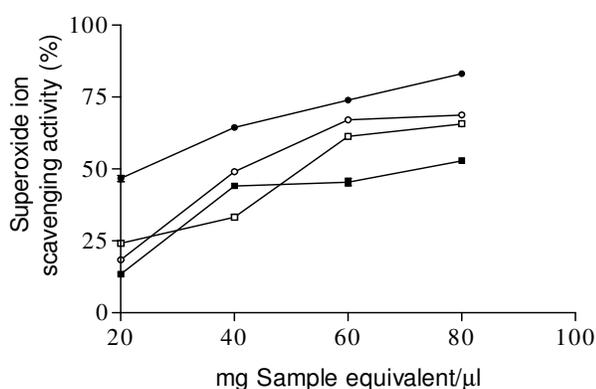


Figure 2. Scavenging activity of *Hypoxis hemerocallidea* (○), *Elionurus muticus*- whole plant (■), *Elionurus muticus*- root (□) and ascorbic acid (●) on the superoxide ion. (The results above are the ones discussed below).

phospholipids solution (50μl), the plant extract (0.5 ml), 50% methanol (0.2 ml), FeSO₄ (0.5 ml), thiobarbituric acid (0.5 ml) and trichloroacetic acid (4 ml). Ascorbic acid (5%) was used as the positive control. Incubation at 37°C was followed by the addition of TBA and TCA and the solution was then heated in a boiling water bath for 15 min. After cooling the sample on ice, absorbance was read at 532 nm on a Spectronic 20®Genesys™ Spectrophotometer.

Statistical analysis

Samples were analysed in duplicate and results are given as averages ± standard deviations. Oneway ANOVA and the Student's t-test, both packaged in the Statistical Package for Social Sciences (SPSS) for Windows Standard Version 8.0.0 were used for the statistical evaluation with P < 0.05 considered statistical significant.

RESULTS

Total phenolic acid content

Total phenolic content was determined by the Folin Ciocalteu method and expressed as Gallic acid equivalents

equivalents mg per 100 mg (GAE mg/100mg). The results are shown in Figure 1, *H. hemerocallidea* had 0.268 ± 0.02 mg/100 mg dry mass of plant extract, *E. muticus* whole plant had 0.068 ± 0.001 mg/100mg dry mass of plant extract and *E. muticus* root extract had 0.041 ± 0.03 mg/100mg dry mass of plant extract total phenolic compounds.

Scavenging activity on the superoxide ion

The percentage superoxide scavenging activity of the methanolic plant extracts are shown in Figure 2. The maximum scavenging activity was obtained from the positive control ascorbic acid (82.76% ± 0.27 at 80 mg/μl), followed by *E. muticus* whole plant (69.12% ± 0.12 at 80 mg/μl), followed by *E. muticus* root (65.82% ± 0.52 at 80 mg/μl) and the least activity was detected in *H. Hemerocallidea* (52.92% ± 0.95 at 80 mg/μl). In all cases there was a steady increase in the anion scavenging effect with increase in concentration of extract with activity levelling off at high concentration except for *H. hemerocallidea* which continued to increase between 60 and 80 mg sample equivalent/μl.

Statistical analysis of the results gave R² values close to one, showing correlation between the concentration of the plant extract and the capacity to scavenge the superoxide ions. P-values were less than 0.05 except for *E. muticus* (whole plant) where p = 0.064 showing no dependency of the scavenging activity and concentration variation.

Inhibition of lipid peroxidation in rat brain homogenate

The capacity to inhibit lipid peroxidation in the rat brain by the plant extracts are shown in Table 1. The plant extracts gave a dose dependent protection against lipid peroxidation in rat brain homogenate. The maximum inhibition values are: *H. hemerocallidea* (69.84% ± 0.22), *E. Muticus* whole plant (80.15% ± 0.14), *E. muticus* root (74.43% ± 0.04) and ascorbic acid (97.85% ± 0.4).

Statistical analysis of the results gave R² values close to one, showing a significant correlation between the concentration of the plant extract and the lipid peroxidation inhibition capacity. P- values were less than dependency of the inhibition activity on the concentration. Ascorbic acid showed no significant dependency of activity on the concentration (p = 0.051).

DISCUSSION

H. hemerocallidea plant extract showed the highest phenolic compound content among the plants tested. Drewes and Khan (2004) detected high content in sterols and sterolins in *H. hemerocallidea*, the main one being hypoxoside, a phenolic compound. Despite its high total phenol-

Table 1. Percentage inhibition of lipid peroxidation in rat brain homogenate of the methanolic extracts of the samples and ascorbic acid in varying concentrations.

Plant extract	% Inhibition			
	20 mg equivalent/ μ l	40 mg equivalent/ μ l	60 mg equivalent/ μ l	80 mg equivalent/ μ l
<i>Hypoxis hemerocallidea</i>	56.42 \pm 0.83	58.56 \pm 0.31	65.56 \pm 1.36	69.84 \pm 0.22
<i>E.muticus</i> (whole plant)	76.84 \pm 0.04	77.62 \pm 0.03	78.59 \pm 0.97	80.15 \pm 0.14
<i>Elionurus muticus</i> (root)	75.29 \pm 0.14	75.87 \pm 0.03	76.65 \pm 0.74	77.43 \pm 0.04
Ascorbic acid	96.69 \pm 0.02	97.01 \pm 0.03	97.66 \pm 0.64	97.85 \pm 0.4

lic content *H. hemerocallidea* showed the lowest scavenging activity for the superoxide radical and the lowest inhibition of lipid peroxidation of rat brain homogenate. This can be explained by the fact that the phenolics present in the plant extract are not potent in scavenging for the superoxide radical and for inhibition of lipid peroxidation. Response of phenolic compounds in Folin-Ciocalteu assay depends on their chemical structure and the radical scavenging capacity cannot be predicted on the basis of total phenolic compound content (Makkar, 1999; Shahidi et al., 1994). According to Katalinic et al. (2005) various phenolic groups have different responses in this assay. The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups is oriented ortho or para (Frankel et al., 1995). There is a wide variation between different phenolic compounds and their effectiveness as antioxidants (Robards et al., 1999). Kähkönen et al. (1999) reported no significant correlations between the total phenolic content and antioxidant activity of 92 plants extracts they studied.

Evaluation of the free radical scavenging activity of the methanolic plant extracts in a biological system was done by determining their ability to inhibit FeSO₄-induced lipid peroxidation in rat brain. Lipid peroxidation was induced by ferrous sulphate through the formation of ferryl-perferryl complex or the OH[•] radical (Govindarajan et al., 2005). In biological systems, lipid peroxidation of polyunsaturated fatty acid generates a number of degradation products such as malondialdehyde (MDA) and is found to be an important cause of cell membrane damage. MDA is thus measured as an index of lipid peroxidation and as a marker for oxidative stress (Nandita and Rajini, 2003).

The root of *E. muticus* gave a percentage superoxide scavenging activity and lipid peroxide inhibition of rat brain homogenate so close to that of the whole plant, showing that the roots contribute much to the free radical scavenging activity and lipid peroxide inhibition of the whole plant. This can be due to the fact that the molecules that scavenge for free radicals and inhibit lipid peroxidation are more concentrated in the roots than in the aerial parts.

The results obtained showed that the roots of *E. Muticus* contribute more total phenolic compound content,

free radical scavenging activity and antioxidant activity than the aerial parts. The plants are therefore helpful in alleviating oxidative stress- induced or linked diseases such as cancer, diabetes, arteriosclerosis, AIDS symptoms and aging. Several epidemiological studies have shown that increased dietary intake of natural phenolic antioxidants correlates with a reduced incidence of diseases such as coronary heart disease, certain forms of cancer (Pena-Neira et al., 2000) and even AIDS, where it appears to work by reduction of oxidative stress which plays a major role in the progression of human immunodeficiency virus infection (Zhang et al., 1997).

Conclusion

In conclusion, we can say that our results further support the view that some medicinal plants are promising sources of natural antioxidants. Methanolic extracts of *H. hemerocallidea*, *E. muticus* (whole plant), and *E. muticus* (root) contain phenolic compounds with the highest in *H. hemerocallidea*. The extracts contain phenolic compounds which can inhibit lipid peroxidation in rat brain homogenate. Maximum inhibition was obtained in *E. Muticus* (whole plant). This represents an ability to scavenge for hydroxyl and hydroperoxides by the methanolic plant extracts. The plant extracts scavenge for the superoxide radical *in vitro*, with maximum scavenging activity shown by *E. muticus* (whole plant). The plants studied can be seen as potential source of useful drugs.

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