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RADICAL SCAVENGING ACTIVITY OF SELECTED MEDICINAL PLANTS FROM LIMPOPO PROVINCE OF SOUTH AFRICA

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Abstract

Plants collected from Limpopo province of South Africa were investigated for their antioxidative potential using the DPPH radical scavenging assay. Acetone extracts of *Flueggea virosa* had the highest antioxidant activity with an IC_{50} value of 30 µg/ml, closely matching the ascorbic acid with an IC_{50} value of 25 µg/ml. The lowest antioxidant readings were observed with extracts of *Rhynchosia venulosa* (root extract) and *Ficus ingens* (leaf extract). Acetone extract of *Bridelia virosa* leaves had the highest phenolic content (156 mg GAE/g extract), while the lowest content was recorded for *R. venulosa* root extract and leaf extract of *F. ingens* (8.3 and 17.7 mg GAE/g extract, respectively). There was a linear correlation between antioxidant activity and total phenolic content. Extracts with high phenolic content had low IC_{50} values, while extracts with low phenolic concentrations had high IC_{50} values.

Keywords: antioxidant activity; medicinal plants; DPPH; phenolics

Abbreviations: GAE, gallic acid equivalent; EC_{50}, effective concentration 50; DPPH, diphenyl-picrylhydrazyl

Introduction

Man is continually exposed to situations that increase the level of risk of exposure to oxidative stress, a phenomenon strongly linked with the onset and progression of several diseases (Sun et al., 2002). Oxidative stress is implicated in the development of diseases such as malaria, acquired immuno-deficiency syndrome (AIDS), heart diseases and diabetes mellitus (Hertog et al., 1993; Alho and Leinonen, 1999). These diseases may develop as a result of direct damage to molecules such as membrane lipids, DNA or proteins (Tippani et al., 2010). In many rural areas of South Africa, plants form the core of primary health care and dietary supply. The plants consumed as fruits, teas, wines, vegetables and medicines may contain a large quantity of antioxidant compounds which scavenge free radicals. Consumption of these plant-derived materials as well as synthetic antioxidant is alleged to reduce the risks of heart diseases, diabetes and cancer (Ames et al., 1993; Knekt et al., 1997; Willett, 2002; Halliwell, 1978), possibly due to presence of antioxidants in these materials (Kalt and Kushad, 2000).
Extracts of several medicinal plants possess antioxidant activity (Hinneburg et al., 2006; Shai et al., 2010; Cui et al., 2005). Many of the plants that have a high concentration of phenolics have good antioxidant activity (Rice-Evans et al., 1996; Zheng and Wang, 2001). However, little attention has been directed at the determination of antioxidant potential of plants (Tilak et al., 2004). The main aim of the study was to determine the antioxidant activity in some edible wild fruit-bearing plants that are also used as components of traditional medicine preparations.

Materials and Methods

Extraction

Plant material (leaves or roots) were collected in April 2011 at Mashishimale village in Phalaborwa, South Africa. *Ficus ingens* was collected from the Lowveld National Botanical Garden in Nelspruit, Mpumalanga, where plant species are identified by a name tag on the stem. Plant species used in the study are listed in Table 1. The dried plant material was ground to fine powder using a pestle and mortar. Five grams of each plant material was extracted overnight in 50 ml acetone. The extract was filtered using a Whatman no.1 filter paper. The filtrate was dried under a stream of air in pre-weighed beakers. The extracts were dissolved in dimethylsulphoxide (DMSO) to a final concentration of 10 mg/ml. All the plants tested, with the exception of *Rhynchosia venulosa* produce edible fruits. .

Antioxidant activity

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Antioxidant activity was determined in 96-well microtiter plates by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method. This method is used for quick and reliable measurement of *in vitro* antioxidant activities of plant extracts and pure compounds (Navarro et al., 1993; Thabrew et al., 1998). Plant extracts were two-fold serially diluted in microtiter plates using sterile distilled water. The total volume of the dilutions was 100 µl. One hundred µl of 0.025% DPPH was added, followed by incubation at room temperature for 5 min. The absorbance at 540 nm was determined. In control wells, 100 µl distilled water replaced plant extracts. Colour controls, which contained dilutions of plant extracts, with 100 µl distilled replacing DPPH, were used to account for the absorbance of extracts (background). The corrected absorbance was calculated by subtracting background absorbance from corresponding test reading. Antioxidant activity was calculated as follows:

$$\% \text{ antioxidant activity} = 100 - \left(\frac{\text{At x 100}}{\text{Ac}}\right)$$

where At is absorbance of test sample and Ac is absorbance of control sample.
The IC_{50} values (mg/ml) were estimated from concentration vs. antioxidant activity graphs.

Total phenolic content

Total phenolic content was determined according to the method of Singleton et al. (1999). Briefly, 25 µl of plant extract was oxidised with 250 µl Folin-Ciocalteau's phenol reagent for 5 minutes. The reaction was stopped by the addition of 750 µl of 20% anhydrous sodium carbonate. The volume was made up to 5 ml with distilled water. The mixture was incubated for 2 hr. at room temperature. Absorbance was read at 760 nm. Phenolic content was determined by extrapolation from a gallic acid calibration curve (0-500 µg gallic acid). Phenolic content was expressed as mg GAE/g extract. A correlation graph of antioxidant activity (at 0.08 mg/ml extract) versus total phenolic content was plotted.

Results and Discussion

Four plant species used in Limpopo either as medicines to treat different diseases were screened for their potential as antioxidants, using the DPPH radical scavenging activity method. *Ficus ingens* was collected for the study, though its medicinal use in Phalaborwa was not established.. DPPH may accept an electron or hydrogen radical, from phytochemicals, to form a stable diamagnetic molecule (Gulcin et al., 2003). *Flueggea virosa* leaf extract had the highest antioxidant activity, with an IC_{50} value of 30 µg/ml. *Flueggea virosa* had similar antioxidant activity as ascorbic acid, with an estimated IC_{50} value of 25 µg/ml. Extracts of *R. venulosa* roots and *F. ingens* leaves had the least antioxidant activity (IC_{50} values above 2500 µg/ml, the highest concentration of extracts used in the experiment) (Table 2 and Fig. 1). Antioxidant activity of all extracts tested was generally dose-dependent, with highest concentrations of the extracts showing the highest antioxidant activity. *Flueggea virosa* contains high amounts of bergenin (Nyasse et al., 2004), a compound with moderate antioxidant activity (IC_{50} value of 921 µM) (Takahashi et al., 2003). In view of this, some of the antioxidant activity of the extracts may be attributed to bergenin activity.

Total phenolic content, as mg GAE/g extract, was determined in all the extracts. Concentration of total phenolics in plant material is directly related to the antioxidant activity of the plant material (Velioglou et al., 1998). *Rhynchosia venulosa* leaf and root extracts, as well as *F. ingens* leaf extracts had the lowest phenolic content (8.32 and 17.68 mg GAE/g extract, respectively). *Bridelia mollis* had phenolic content of 39 mg GAE/g extract (Table 2). Extracts of these three had low antioxidant activity. Ndhlala et al. (2006) reported good antioxidant activity of *Bridelia mollis* fruit extract.
Leaf extract of *Flueggea virosa* had the highest phenolic content (156 mg GAE/g extract) (Table 2). All extracts with low total phenolic content also had low antioxidant activity, as displayed by the linear relationship between total phenolic content and antioxidant activity (% antioxidant activity at 0.08 mg/ml extract) on Fig, 2. In this study, extracts with a high phenolic content, as also reported elsewhere (Veliglou et al., 1998) had a high antioxidant activity. All extracts with low total phenolic content had a corresponding low antioxidant activity, suggesting that the bulk of the antioxidant activity in these extracts may be attributed to phenolic compounds. Phenolic compounds are potent antioxidants (Rice-Evans et al., 1995; Lu and Foo, 2001). Further work on the characterization of active compounds is underway.

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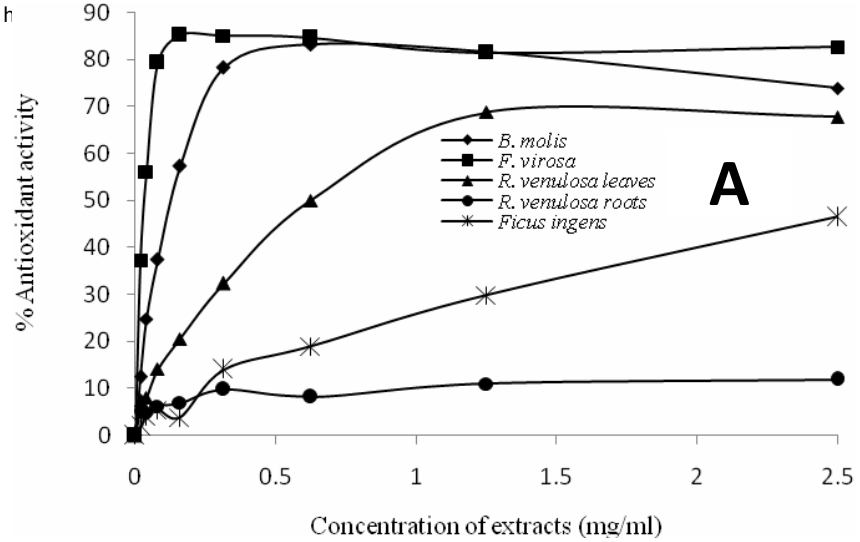

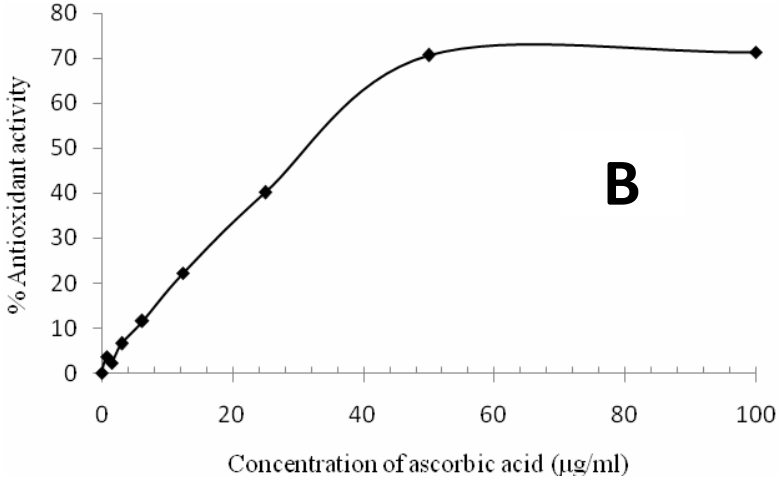

Figure 1. DPPH radical scavenging activity of extracts of different plant species (A) and ascorbic acid, the positive control (B). Correlation of antioxidant activity and total phenolic content is displayed in C.

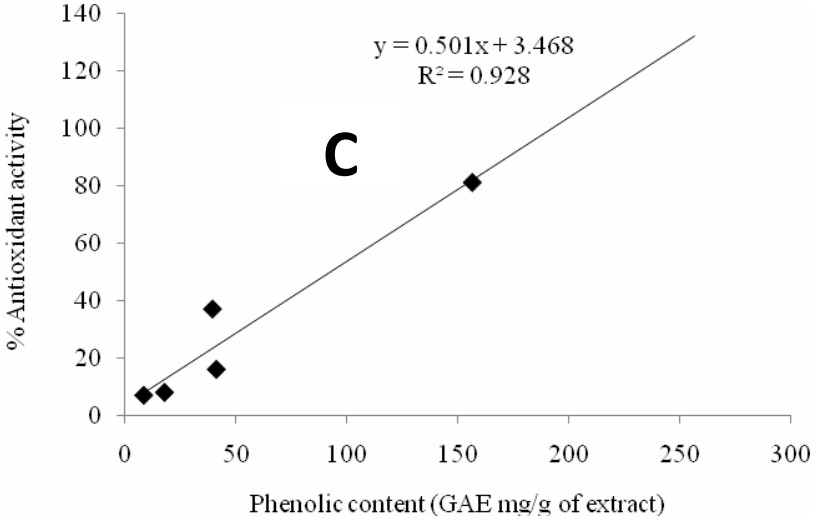

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Table 1: Plant species used in this study. All plant material, except *Ficus ingens*, were collected from Phalaborwa district of Limpopo Province, South Africa.

Plant species	Family	Voucher No.	Plant part used in study	Medicinal uses
Fluggea virosa (Roxb. Ex Wild) Voigt	Phyllanthaceae	PRU115081	Leaves	Malaria, liver disorders, sexual asthenia, gastric ulcers, pregnancy and coughs (Nadembega et al., 2011)
Bridelia mollis Hutch	Phyllanthaceae	PRU 117187	Leaves	Anti-emetic, wounds, piles, dysentery, itching (Mabogo, 1990).
Rhynchosia venulosa (Hiern) K.Schum.	Fabaceae	PRU 117189	Leaves and roots	Beverage component (Munkoyo) (Simwamba and Elahi, 1986)
Ficus ingens	Moraceae	Plants are identified by a name tag at The Lowveld National Botanical Garden	Leaves	*F. exasperata* used for heamostative opthalmia, coughs and heamorrhoid (Odunbaku et al., 2008).

Table 2: Antioxidant activity, expressed as EC_{50} values (mg/ml), of acetone extracts of various plant species.

Plant species	IC_{50} (µg/ml)	Phenolic content (mg GAE/g extract
B. mollis	130	39.31
F. virosa	30	156.43
R. venulosa leaves	600	41.03
R. venulosa roots	>2500	8.32
F. ingens	>2500	17.68
Ascorbic acid	25	-

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