

Full Length Research Paper

Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*

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Monsonia burkeana, widely used as 'special tea', is harvested unsustainably due to insufficient information on the accumulative abilities of its organs on the secondary metabolites. Using phenolics and antioxidants as focus chemical compounds, an investigation was carried out to (1) determine the accumulative abilities of organs of *M. burkeana* on phenolic and antioxidant compounds, and (2) determine whether phenolic acids and antioxidants in *M. burkeana* had density-dependent relationship patterns. Ten plants per plot, with three replicates, were harvested whole, oven-dried, separated into the four organs and quantified for phenolics and antioxidant components using the Folin Ciocalteu method and the Trolox Equivalent Antioxidant Capacity (TEAC) assay, respectively. Generally, reproductive and vegetative organs had high levels of phenolic and antioxidant compounds when compared to roots. The saturation factor suggested that more than 90% antioxidants were derivatives of the phenolic compounds. Optimum levels of antioxidant activity were attained at 5.39, 5.49, 4.36 and 4.13 mg/ 100 g of phenolics in fruit, leaf, stem and root, respectively. In conclusion, vegetative and reproductive organs are good sources of phenolic and antioxidant compounds in *M. burkeana*.

Key words: Phenolic, antioxidant, saturation factor, optimum.

INTRODUCTION

Interest has increased considerably in finding naturally occurring antioxidants for use in foods, medicinal materials or plant protection to replace synthetic compounds which are being restricted due to their carcinogenicity and environment-unfriendliness (Velioglu et al., 1998). Herbal teas are receiving much attention as functional medicinal beverages, because of their natural nutritive values that encompass essential nutrient elements such as N, P, K, Ca, Zn, Fe, vitamins, phenolics and antioxidant compounds. In most medicinal plants, for a particular organ to be considered as a harvestable organ, it must have a high accumulative ability for the focus chemical compound. Generally, when in doubt of which organ contains the highest concentration of the desired chemical compound; the entire plant is harvested - which is not sustainable. The accumulative abilities of organs on essential nutrient elements and secondary

metabolites follow the density-dependent pattern, which is expounded by the saturation factor model (Salisbury and Ross, 1992). In this model, as the independent factor increases the dependent factor also increases to reach a threshold above which it begins to have an effect. Thereafter, the response increases sigmoidally, until the system becomes saturated, and as the stimulus continues to increase, the response remains constant and then begins to decrease if the stimulus at its high levels becomes inhibitory.

Monsonia burkeana Planch. ex Harv. (Family Geraniaceae), is native to Southern Africa (Venter, 1979). Decoctions from this herb, referred to as 'special tea', are widely used in marginal communities that comprised former homeland areas of the Republic of South Africa. Most users of this herb believe that it has chemical properties that range from blood cleansing, amelioration of erectile dysfunction and improvement of libido (IKS). The organ in which medicinal properties in *M. burkeana* is concentrated is not documented, resulting in locals harvesting and using the whole plant in preparation of decoctions. The objective of this study was to determine

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the accumulative abilities of *M. burkeana* organs with respect to phenolics and antioxidants, along with the relationship of the two chemical compounds.

MATERIALS AND METHODS

Study location and experimental design

Fresh plant materials were sampled in 2008 and 2009 summer seasons from Chuenespoort, Limpopo Province, South Africa (24°21'4" S; 29°48'4" E) during fruiting. Plots of 10 × 10 m were arranged in a randomised complete block design with three replicates, where blocking was done for gradient. Ten plants within each plot were randomly sampled by collecting the entire plant and transported in cooler boxes to the Horticultural Skills Centre of the University of Limpopo, Turfloop Campus (23°53'10" S, 29°44'15" E).

Determination of total phenolic content

Whole plants were dried in air-forced ovens at 52°C for 48 h (Makkar, 1999). Fruit, leaves, stems and roots were individually ground in a Wiley mill to pass through a 1 mm sieve and stored in air-tight plastic containers at 5°C prior to analysis. Extractions were carried out using the solid to solvent ratio and solvent mixture (Justesen, 2000). Methanol was used as an extraction solvent for the determination of the Total Phenolic Content (TPC). Approximately 2 g of *M. burkeana* ground material of each organ were extracted using 40 mL of solvent. Methanol (20 mL) was added to 2 g sample in centrifuge tubes and the samples were vortex mixed every 10 min for 2 h to improve extraction efficiency. Samples were then centrifuged at 3500 rpm for 10 min (25°C), with the supernatant decanted. Sample residues were rinsed once with 20 mL solvent, vortex mixed for 5 min, centrifuged and then decanted.

The Folin Ciocalteu method (Singleton and Rossi, 1965), modified by Waterman and Mole (1994), was used to determine TPC content in *M. burkeana* extracts. Methanol extract (0.5 mL) was added to a 50 mL volumetric flask containing distilled water and mixed. Folin Ciocalteu phenol reagent (2.5 mL) was added and mixed, followed by 7.5 mL sodium carbonate solution (20 g/100 mL) within 1 to 8 min after addition of the Folin Ciocalteu phenol reagent. The contents were mixed and the flask made up to volume with distilled water, stopper and thoroughly mixed. Tannic acid was used as standard to prepare a standard curve and results were expressed as mg equivalents/100 mg of samples dry weight basis. Absorbance of the reactants was read after 2 h at 760 nm using an Ultraviolet (UV-visible) Genesys 20 Spectrophotometer.

Determination of total antioxidant activity

Total Antioxidant Activity (TAA) of the extracts was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) assay, as described by Miller and Rice-Evans (1996). This is a spectrophotometric technique that measures the relative ability of hydrogen-donating antioxidants to scavenge the ABTS⁺ radical cation chromogen in relation to that of Trolox (the water soluble vitamin E analogue which is used as an antioxidant standard). The ABTS⁺ mother solution was prepared by mixing equal volumes of 8 mM ABTS⁺ with 3 mM potassium persulfates prepared in distilled water and allowed to react in the dark for at least 12 h at room temperature before use. The ABTS⁺ solution was diluted with a phosphate buffer solution (pH 7.4) prepared by mixing 0.2 M of NaH₂PO₄, 0.2 M NaHPO₄ and 150 mM NaCl in 1 L of distilled water, with pH adjusted using NaOH when necessary. The solution was

freshly made for each analysis. The ABTS⁺ solution (2900 µL) was added to the methanol extracts (100 µL) of Trolox in a test tube and mixed. Absorbance values (734 nm) were taken at 30 min for the samples and at 15 min for the standard after the initial mixing. Results were expressed as µM Trolox equivalents /g of sample on a dry weight basis.

Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistix software in Linear Model procedure. The Tukey's Honestly Significant Difference (HSD) test was used to identify differences among the means at the probability level of 5%. TAA (y-axis) and TPC (x-axis) were subjected to the lines of the best fit using Statistical Package for the Social Sciences (SPSS). The responses of TAA to increasing TPC level were modelled by the regression curve estimations resulting to a quadratic equation:

$$Y = b_2 x^2 + b_1 x + a$$

Where Y = TAA levels; x = TPC with $-b_1/2b_2 = x$ value for the saturation point for each organ.

RESULTS

The TPC in the four organs of *M. burkeana* differed significantly ($P \leq 0.05$). Fruit had the highest TPC, with the leaf and stem having intermediate values, whereas roots had the lowest content (Table 1). The TAA among the four organs also differed. Fruit and leaf had higher antioxidant activity than the stem, whereas the root exhibited the lowest TAA. The TAA and TPC had quadratic relationships in all four organs. The treatment TPC contributed 97% TTV in TAA of fruits (Figure 1), 92% in leaves (Figure 2), 92% in stems (Figure 3) and 96% in roots (Figure 4). The TAA in fruit, leaf, stem and root was optimised at different levels of phenolic content, viz. 5.39, 5.49, 4.36 and 4.13 mg/ 100 g, respectively (Table 2). Amongst the four organs, the stem had the highest TAA saturation point at lower level of TPC as compared to the fruit and the leaf (Table 2).

DISCUSSION

In this study, *M. burkeana* leaves and fruits had the highest amount of TPC and TAA contents, with the stems having intermediate levels and the roots the lowest. Results of this study agree with those in creosote bush (*Larrea tridentata*), cup plant (*Silphium perfoliatum*), St. Johnswort (*Hypericum perforatum*), spotted St. Johnswort (*Hypericum maculatum*) and sweet-amber (*Hypericum androsaemum*) studies (Hyder et al., 2002; Kowalski and Wolski, 2006; Radusiene et al., 2004; Valentao et al., 2003). Generally, differences in the accumulation of secondary metabolites by various organs occur in plants with medicinal attributes (Ayan et al., 2004), with concentrations of the secondary metabolites varying from plant to plant species and even in different

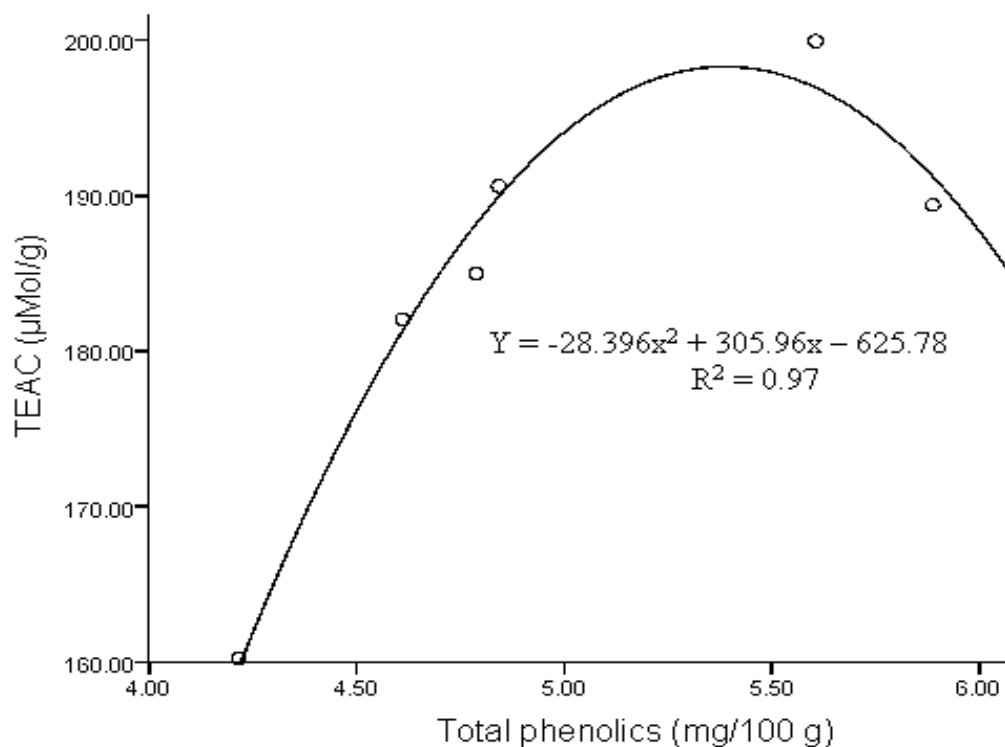
Table 1. Quantities of phenolic content and antioxidants in fruit, leaf, stem and root of *M. burkeana*.

Organ	Mean total phenolic content (mg/100 g)	Mean total antioxidant levels ($\mu\text{mol/g}$)
Fruit	4.9087a	172.16a
Leaf	4.6003ab	170.72a
Stem	3.3466ab	142.81ab
Root	2.9739b	90.836b

Column means with the same letter were not different ($P \leq 0.05$) according to the Tukey' honest significant difference test.

Table 2. Total phenolic content (mg/100g) for optimal Trolox equivalent antioxidant activity ($\mu\text{mol/g}$) in *M. burkeana* organs.

Organ	Formula	R ²	TPC level (x)	TAA saturation level	P <
Fruit	$Y = -28.39x^2 + 305.96x - 625.78$	0.97	5.39	198.38	0.05
Leaf	$Y = -22.988x^2 + 256.78x - 516.57$	0.92	5.59	200.50	0.05
Stem	$Y = -30.969x^2 + 270.03x - 328.08$	0.92	4.36	260.54	0.05
Root	$Y = -7.8122x^2 + 64.49 - 10.245$	0.96	4.13	122.87	0.05

**Figure 1.** Relationship between antioxidant activity and total phenolic content in fruit samples of *M. burkeana*.

parts of the same plant species (Achakzai et al., 2009). For instance, in wild watermelon (*Cucumis africanus*) cucumin and leptodermin are concentrated in the whole plant, whereas in wild cucumber (*Cucumis myriocarpus*) they are exclusively concentrated in seeds and roots (Van Wyk et al., 2002).

Plants produce phenolic compounds in different organs in response to adverse environmental conditions (Pasqualini et al., 2003). Climatic changes like high temperatures promote production of phenolic compounds (Christie et al., 1994; Dixon and Paiva, 1995; Sivaci and Sökmen, 2004). Inderjit (1996) provided an extensive

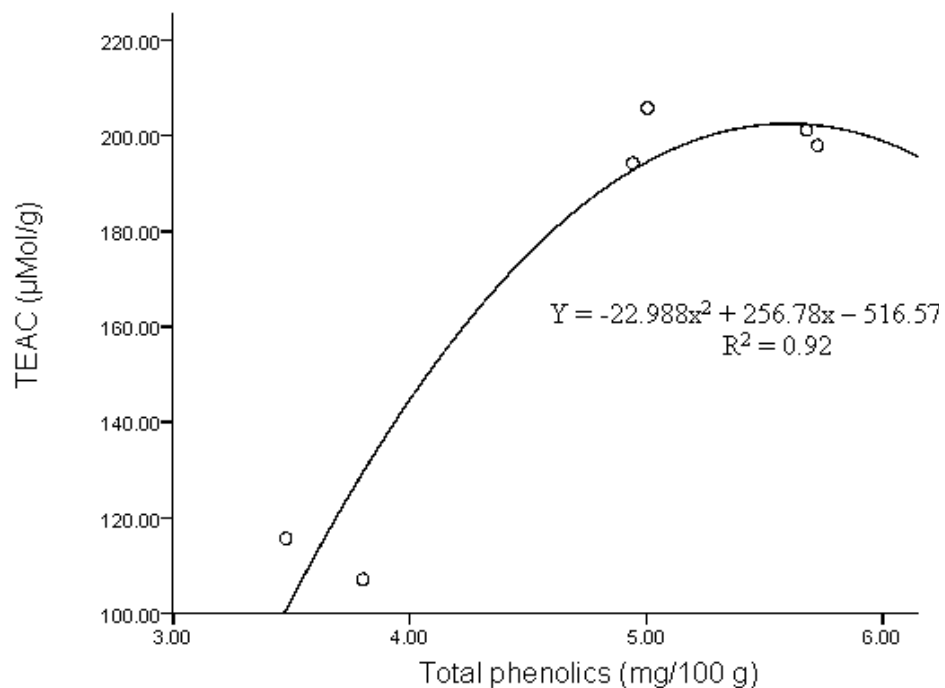


Figure 2. Relationship between antioxidant activity and total phenolic content in leaf samples of *M. burkeana*.

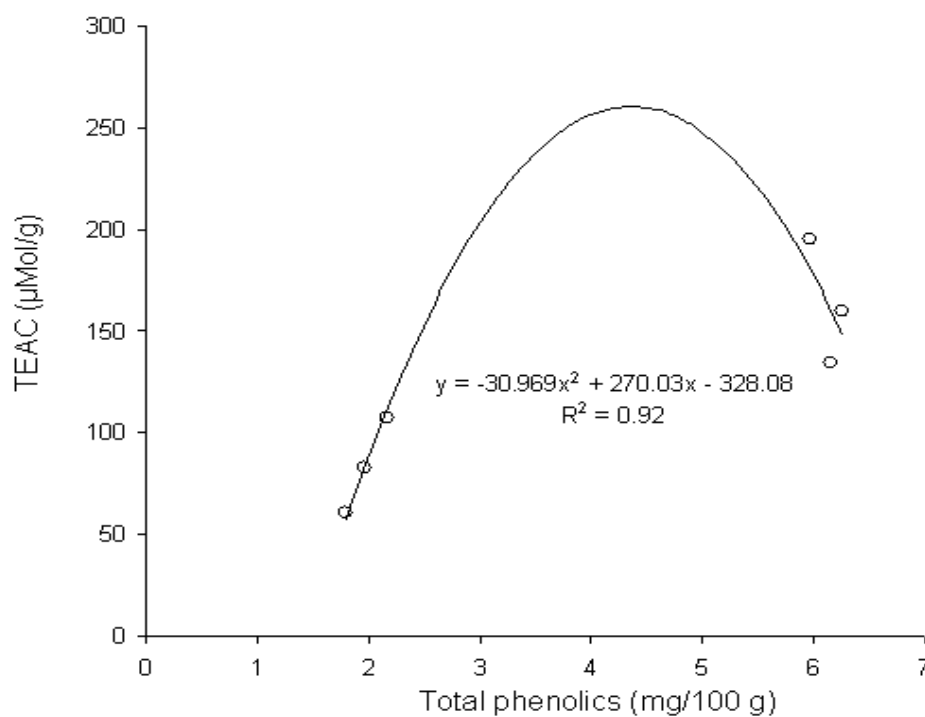


Figure 3. Relationship between antioxidant activity and total phenolic content in stem samples of *M. burkeana*.

review of the roles of phenolic compounds in allelopathy. The general role of phenolic compounds in plant

physiology and allelopathy had been reported for years (Heisey, 1990). A well reported aspect of phenolics in

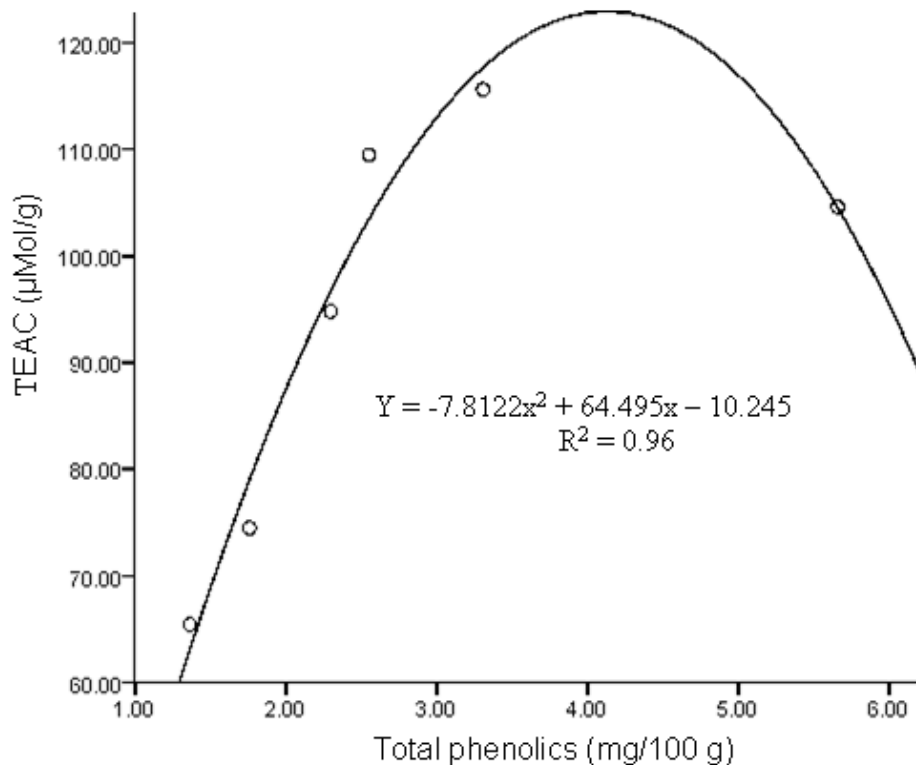


Figure 4. Relationship between antioxidant activity and total phenolic content in root samples of *M. burkeana*.

plant physiology is their activity in defense mechanism against various types of stresses caused by pathogens, pests or adverse environmental conditions during the course of plant ontogenesis (Agrios, 2005; Grace et al., 1998; Paliyath et al., 1997; Treutter, 2001).

In the analysed four organs of *M. burkeana*, the TAA increased, reached an optimum, and then started to decline with the increasing TPC levels. In *M. burkeana*, TPC optimised TAA at concentrations ranging from 4.13 to 5.59 mg/100 g by the saturation levels ranging from 122.87-260.54 µmol/g. The highest TAA in *M. burkeana* was evident in vegetative organs and fruit which are comparable to other studies (Hakulinen and Julkunen-Tiitto, 2000; McCune and Johns, 2007), whereas TAA in root was the lowest. Results of this study are in agreement with various phenolic-antioxidant relationships, which suggested that TPC was the primary source of TAA (Javanmardi et al., 2003; Katalinic et al., 2006). Using the linear regression relationships or correlations of TAA and TPC, various workers demonstrated that TAA and TPC had density-dependent relationships (Javanmardi et al., 2003; McCune and Johns, 2007; Katalinic et al., 2006).

Generally, the presented biological model is characterised by quadratic relationships (Salisbury and Ross, 1992). In studies where linear relationships were depicted (Javanmardi et al., 2003; McCune and Johns, 2007; Katalinic et al., 2006), the workers might have studied

phenolic-antioxidant relationships at the level below the saturation point, whereas in studies where there was no phenolic-antioxidant relationships (Anagnostopoulou et al., 2006; Ghasemi et al., 2009; Heinonen et al., 1998; Nickavar et al., 2007), the workers might have studied the relationship at the saturation point. Similarly, when there was a negative relationship, the concentrations of the phenolics (x-axis) were above the saturation point.

Briante et al. (2003) reported that phenolic compounds can be active as antioxidants by a number of potential pathways. The most important is likely to be by free radical scavenging in which the phenols can break the free radical chain reaction (Prakash et al., 2007). The presence of different substituents within the backbone structure of phenolic compounds modulates their antioxidant properties, in particular, their hydrogen-donating capacity (Prakash et al., 2007). Generally, antioxidant activities of phenolics are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Javanmardi et al., 2003). Biological functions of phenolic compounds and antioxidants include free radical scavenging, protection against allergies, cancer, platelets aggregation, blood cleansing, anti-viral and improving blood flow. In plants they serve as intrinsic defence structures against pathogens, pests and minimising the deleterious effect of unfavourable climatic conditions - (Agrios, 2005; Khan and Mukhtar, 2007). Also, phenolic

compounds are extrinsically used in crude extracts as sprays against pest in agricultural production systems (Hwang and Lindroth, 1993; Lindroth, 1993).

Conclusion

The TPC and TAA relationship in this study supported the density-dependent relationship patterns (Salisbury and Ross, 1992), where TPC serves as a sustainable source of TAA. The present investigation suggested that *M. burkeana* leaves and fruits may have great potential as a health beverage and/or a bio-pesticide ingredient, particularly as a source of phenolic-antioxidants. Consequently, when phenolic-antioxidants are a focus in 'special tea' only leaves and fruits should be harvested.

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