DISTRIBUTION OF SELECTED ESSENTIAL NUTRIENT ELEMENTS AND SECONDARY METABOLITES IN *MONSONIA BURKEANA*

BY

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Plant protection) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

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ABSTRACT

Monsonia burkeana is widely used as a decoction for plant protection in South Africa. However, the accumulative capabilities (ACs) for essential nutrient elements and phenolic-antioxidant relationship in its organs are not documented. A study was conducted to determine whether: (1) the ACs for nutrient elements in fruit, leaf, stem and root of *M. Burkeana* were similar, (2) total phenolic and antioxidant contents in fruit, leaf, stem and root of *M. burkeana* were distributed equally, and (3) phenolic levels have an effect on accumulation of antioxidants in the four organs. Ten plants per plot, with three replicates, were harvested whole, oven-dried and separated into the four organs and then quantified for the above enlisted variables. The ACs for essential nutrient elements differed among the four organs. Generally, reproductive organs and leaves had high ACs for macro-nutrients, whereas roots had high ACs for micro-nutrients. Similarly, reproductive organs and leaves were good sources of phenolic and antioxidant compounds. Saturation factors in various organs of *M. burkeana* suggested that more than 90% of antioxidants were derivatives of the phenolic compounds. Optimum levels of antioxidant activities were attained at 5.39, 5.49, 4.36 and 4.13 mg/ 100 g of phenolic content in fruit, leaf, stem and root, respectively. Consequently, *M. burkeana* organs have the potential to provide fertiliser effect on crops, and both phenolics and antioxidants required as active ingredients for sprays used in plant protection.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Generally, active ingredients with useful properties are concentrated in specific plant organs, which can be leaves, roots, stems or fruit (Cunningham, 1993). Due to limited knowledge of the site of concentration of active ingredients in indigenous plants, in most cases, the entire plant is harvested for use in plant protection. In the production of a commercial product, it would not be recommended to process the entire plant unless the active ingredient is equally distributed in the whole plant. Similarly, the use of the entire plant is ecologically unsustainable.

In wild cucumber (*Cucumis myriocarpus*) the active ingredients, namely, cucumin and leptodermin, collectively called cucurbitacin A, are concentrated in roots and fruit, with the highest concentration being in seeds (Rimington, 1998). However, in wild watermelon (*C. africanus)*, similar active ingredients were equally distributed in the whole plant (Rehm *et al*., 1957). Generally, users of *C. myriocarpus* use mainly roots and fruit, whereas those of *C*. *africanus* use the entire plant (Rehm *et al*., 1957). In South Africa, various communities use *Monsonia burkeana* Planch. ex Harv. (Family Geraniaceae) (Figure 1.1), native to Southern Africa (Venter, 1979), which is viewed as a special herb since it is used in plant and animal health.

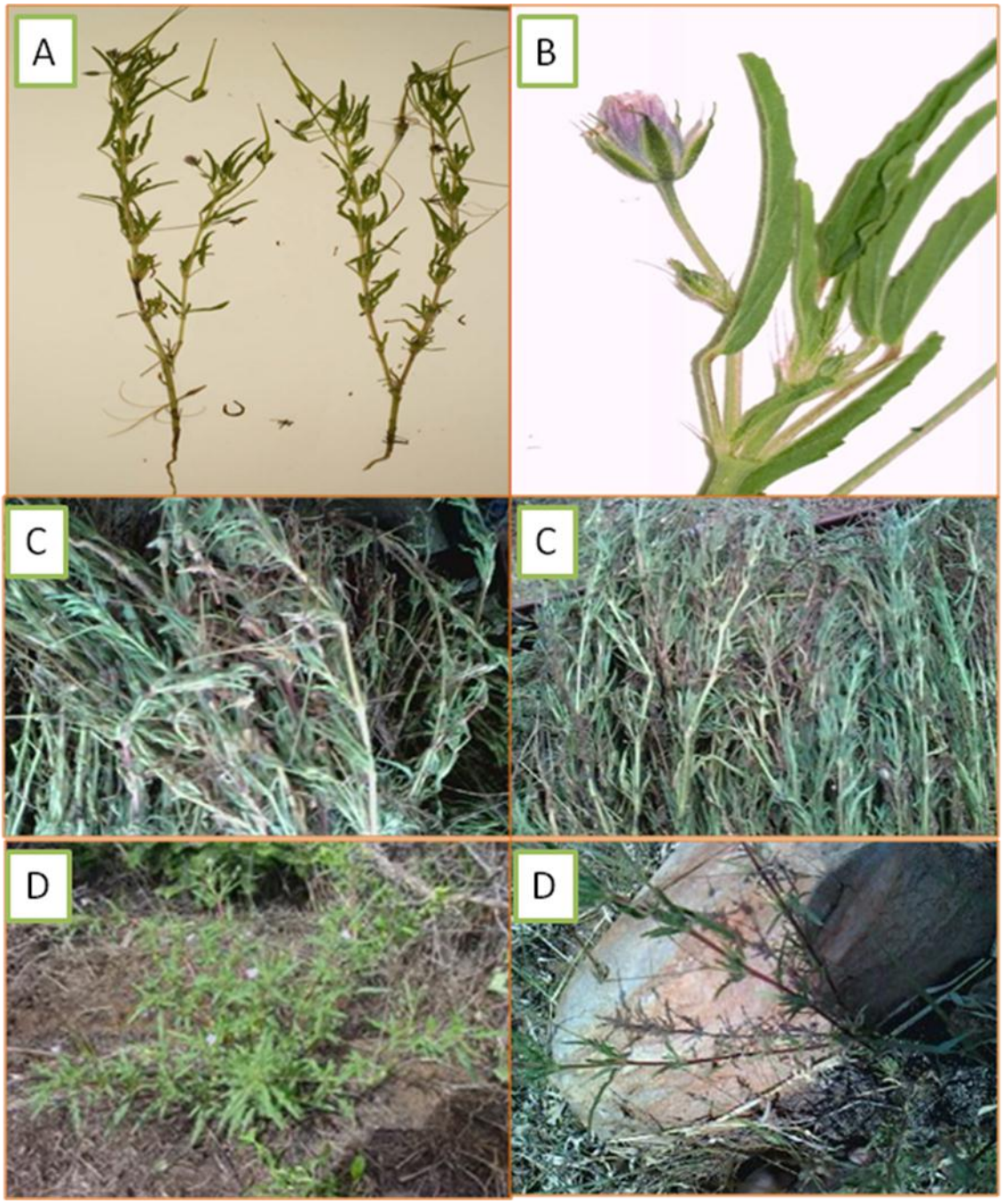

Figure 1.1 A. Photograph of *Monsonia burkeana* plant collected from Chuenespoort. B. Photograph of *Monsonia burkeana* showing flower and leaves. C. Photographs of harvested *Monsonia burkeana* plant material. D. Photographs of pre-harvested *Monsonia burkeana.*

In Africa, remedies made from plants by traditional healers play an important role in health care of millions of people, their animals and crops (Rukagira, 2001). The potential of certain plant materials in the maintenance of health and protection has raised interest in the ethno-healthcare sector as consumers move towards functional foods with specific health effects and less residues from synthetic pesticides. Using plant materials, in the absence of information pertaining to chemical content, incidentally leads to over dosages that result into fatalities and high residues on produce (Langille and MacLean, 1976).

Traditionally, herbalists identified herbal plant species and use them without further establishing the exact active ingredient properties. As a result, there is no guarantee of the authenticity and quantity of plant material used in preparations (Rukangira, 2001). Generally, in plant health it is worse since measurements of the material are hardly taken.

In South Africa, the bulk of phytopharmaceutical trade, situated in informal street markets, involves the sale of unprocessed or semi-processed plant products. Raw plant materials undergo very little processing before they are used in plant protection (Mander and Le Breton, 2006; Mashela, 2002). Most traditional health practitioners believe that isolated compounds have weaker efficacy than whole plant crude extracts (Rodriguez-Fragoso *et al*., 2008). Since crude extracts are complex mixtures containing several active ingredients, possibilities of interaction to form toxins are high (Ernst, 2000).

Seeing that millions of South Africans rely on traditional methods for both their primary healthcare needs and plant protection, it is becoming imperative that phytochemical constituents be determined. Investigations on chemical distribution in *M*. *burkeana* organs would improve the sustainable use of this plant.

1.2 Problem statement

The organ(s) which should be harvested in *M*. *burkeana* for use in plant protection has not been determined, although the plant is widely used in poor resource crop production systems of Limpopo Province, South Africa. The researcher intends to establish the distribution of chemical compounds in fruit, leaf, stem and root of *M*. *burkeana* plants in order to identify the plant organ(s) that should be used in the preparation of concoctions in pesticide sprays.

1.3 Motivation of the study

Result of this study will provide information on the distribution of selected chemicals throughout *M*. *burkeana*, which will then provide essential information on organ(s) to be harvested for commercial packaging.

1.4 Aim and objectives

1.4.1 Aim

The aim of this study is to determine the distribution of selected chemical elements and chemical compounds in organs of *M*. *burkeana.*

1.4.2 Objectives

Objective 1: To determine whether the distribution of selected essential nutrient elements in fruit, leaves, stems and roots of *M*. *burkeana* differ.

Objective 2: To determine whether the distribution of selected secondary metabolites in fruit, leaves, stems and roots of *M*. *burkeana* differ.

Objective 3: To determine whether phenolic compounds have an effect on accumulation of antioxidants in fruit, leaf, stem and root of *M. burkeana*.

1.5 Hypotheses

Hypothesis 1: Distribution of selected essential nutrient elements in fruit, leaves, stems and roots of *M. burkeana* do not differ.

Hypothesis 2: Distribution of selected secondary metabolites in fruit, leaves, stems and roots of *M*. *burkeana* do not differ.

Hypothesis 3: Phenolic compounds do not have an effect on accumulation of antioxidants in fruit, leaf, stem and root of *M. burkeana*.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Plant organs contain more than 700 chemicals, among which are compounds that are closely related to plant health such as flavonoids, amino acids, vitamins, caffeine and polysaccharides (Mondal, 2007). Also, there are those chemicals that can be described as being highly toxic to the kingdom animalia. This literature review will be limited to concepts in chemical composition and chemical distribution in plants.

2.2 Chemical composition in plants

Plants consist of a complex mixture of a wide variety of components, with biologically active and inactive properties. Generally, most inactive chemical compounds are secondary metabolites, whose precise biological functions in plants are unknown. Biologically active substances in plants, which have beneficial effects on both plant and human health, had been isolated (Colegate and Molyneux, 1993). Evidence is growing that certain phytochemicals may reduce the risk of diseases and pest in plants (Grayer and Harborne, 1994). Generally, plants exposed to environmental stresses (hash extrinsic factors) require additional supplies of primary and secondary metabolites, particularly N, P, K, Mg, Ca, Zn and phenolic compounds, to minimise the adverse effect of stress (Agrios, 2005; Cakmak, 2005).

Possible mechanisms for the beneficial health effects of phytochemicals include the antioxidant capacity, modification of hormonal profile, anti-inflammatory effects and modification of lipid profile (Craig, 1999). Further knowledge on the distribution and toxicity of these phytochemicals is essential in order to fully understand their potential benefits in both animal, human or plant healthcare (Houghton and Raman, 1998).

2.2.1 Secondary metabolites

Numerous secondary metabolites have been classified under various chemical groups (Macheix *et al*., 1990). Among the groups, phenolics embrace a range of substances which possess aromatic rings with one or more hydroxyl substituents (Macheix *et al*., 1990). Generally, phenolics are present in all plant tissues (Macheix *et al*., 1990). Polyphenols, which have been isolated in plants, can be grouped into several classes, based on the number of constitutive carbon atoms and the light of the structure of the basic skeleton (Macheix *et al*., 1990). In addition to simple soluble forms found mainly in vacuoles, there are also polymerised forms of varying solubility such as tannins or completely insoluble lignins (Macheix *et al*., 1990). Lignin, a complex polymer of phenylpropane units, is quantitatively the most important phenolic compound in plants (Daniel *et al*., 1999).

Distribution of the constituents of the different classes in plant organs is not homogenous (Macheix *et al*., 1990). Some compounds, such as hydroxycinnamic acids and flavonoids are widely distributed. Amounts of total

phenols broadly reflect the presence of the major compounds that occur in fruit (Macheix *et al*., 1990). After the plant has wilted, phenolics may persist for months and affect decomposing organisms and the decomposition processes (Horner *et al*., 1988). Their effects are not restricted to single plants but may extend to the functioning of whole ecosystems (Daniel *et al*., 1999). Some of these secondary metabolites are essential in neutralising the toxic free radicals in animals, thus, acting as antioxidants, whereas others are antimicrobial and anti-helmenthic.

2.2.2 Essential nutrient elements

Plants of different species have different absorptive capabilities of the essential nutrient elements. Consequently, different plants have different nutrient element contents. However, little is reported on essential nutrient element content of indigenous plants.

Mashela (2002) demonstrated that fruit crude extracts of *C. myriocarpus* had fertilizer effect on tomato plants, with the content of nutrient elements used to provide some explanation to the observed increased dry matter of treated plants. Also, it is well-documented that Fe, K, Ca, Cu, Zn, Mg, Mn and Na play a vital role for general well being and the cure of diseases in plants (O'Dell and Sunde, 1997; Prasad, 1993).

2.3 Chemical distribution in plants

Even within a plant species, individual plants may vary chemically and also pharmacologically due to genetic differences, environmental differences and

plant age (Tyler *et al.,* 1976). In general, individuals from the same population may be more chemically similar, thus allowing for greater replicability (Malone, 1981).

Studies on alkaloids, demonstrated that the active ingredients in medicinal plant are manufactured, translocated and stored in various plant organs (Waller and Nowacki, 1978). Rutin content in plant parts of three species was higher in the order of flower > leaf > seed > stem > root (Park *et al*., 2004). Therefore, collecting the correct plant organ at the right stage of development or time of year is necessary for maximum concentration of active compounds.

2.4 Findings within *Monsonia* species

Extracts obtained from *M*. *angustifolia,* using a methanol/dichloromethane mixture as solvent, may treat erectile dysfunction and enhance libido in males (Wo, 2007). In the same study, the plant materials used comprised roots, stems, leaves and purple flowers. Extracts obtained from *M*. *angustifolia* included five compounds, namely (i) 5-methoxyjusticidin A, (ii) justicidin A, (iii) chinensinaphthol, (iv) suchilactone and (v) retrochin (Wo, 2007). Also, *M*. *angustifolia* contains medicinal properties such as vitamin C, nutrient elements, protein and fats (Lyimo *et al*., 2003). High values of up to 42.0, 124.0 and 3.0 mg/100 g of vitamin C, Ca and Fe, respectively, were recorded. Protein content was 3.2; crude fiber 2.2 and fat content 1.0 % (Lyimo *et al*., 2003). Work done on *M. burkeana* is limited to botanical classification and its widespread use by

marginal rural communities as “special tea” and its use as sprays against pests in plant health.

2.5 Focus of current research

The study intended to determine chemical composition of selected essential nutrient elements, secondary metabolites and the secondary metabolites relationship in fruit, leaf, stem and root of *M*. *burkeana.*

CHAPTER 3
ACCUMULATIVE CAPABILITIES OF SELECTED ESSENTIAL NUTRIENT ELEMENTS IN ORGANS OF *MONSONIA BURKEANA*

3.1 Introduction

Most crude extracts used in plant protection have fertiliser effect. Crude extracts of wild Cucumber fruit have high concentrations of essential nutrient elements, with significant fertiliser effect when the material is used in the Ground Leaching Technology (GLT) system (Mashela, 2002). Despite the widespread use of *Monsonia burkeana* by most of the marginal communities in South Africa and its economic potential as herbal tea, information on its nutrient composition is scant.

The content of essential nutrient elements in a given organ depends on the organ's accumulative capabilities (ACs) and the interactions of the nutrient elements in that organ (Salisbury and Ross, 1992). Information on the ACs of organs is important since in addition to providing the nutrition value of an organ, it also provides information which assist in selective harvesting of organs instead of harvesting the whole plant. The objective of this study was to determine the ACs of fruits, leaves, stems and roots using selected essential nutrient elements in order to establish whether the plant should be harvested in whole or in part.

3.2 Materials and methods

Fresh plant materials were sampled during fruiting in 2008 and 2009 from Chuenespoort, Limpopo Province, South Africa (24°21'4" S; 29°48'4" E). Plots of 10 m × 10 m were arranged in a randomised complete block design with three replicates, where blocking was done for slope. 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).

Essential nutrient element determination: Whole plants were dried in air-forced ovens at 52°C for 48 hours (Makkar, 1999). Fruit, leaves, stems and roots were individually ground (Figure 3.1) in a Wiley mill to pass through a 1-mm sieve and stored in air-tight plastic containers at room temperature prior to analysis.

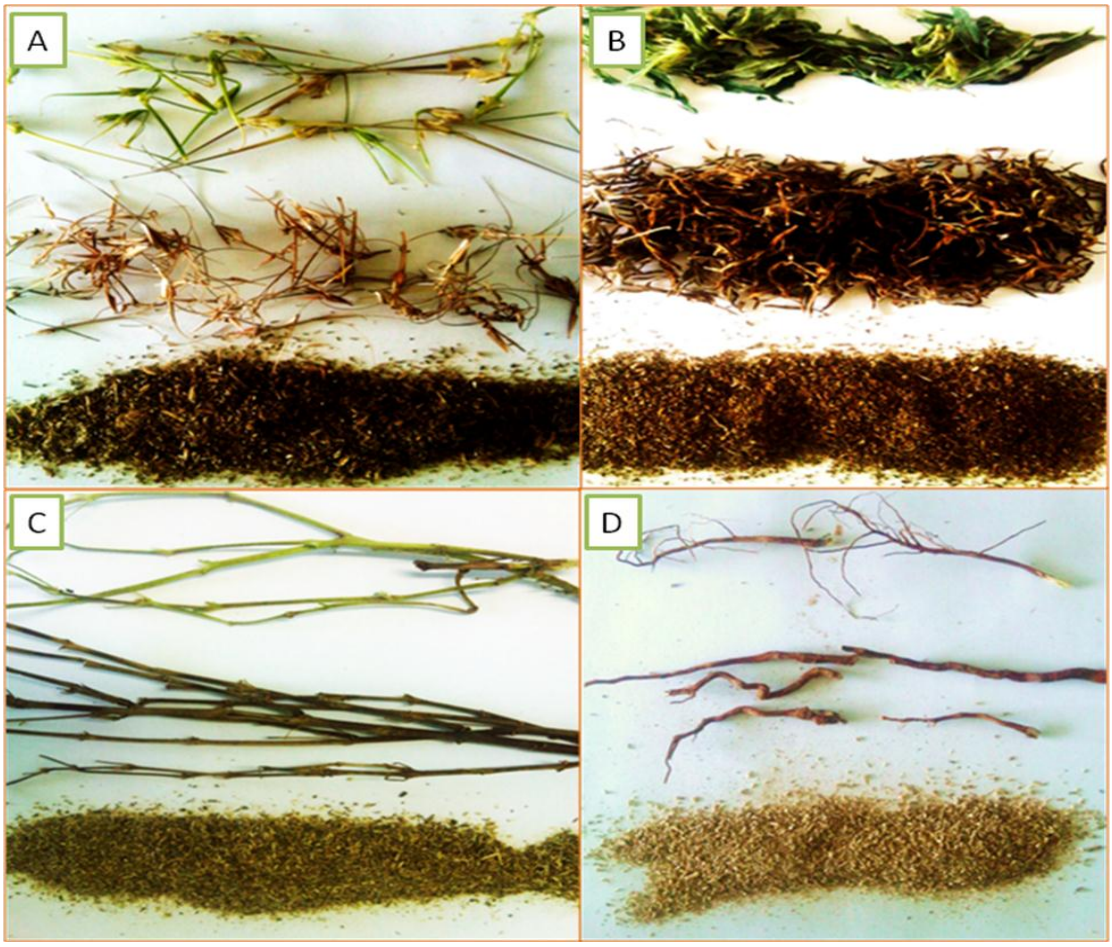

Figure 3.1 A. Photograph of *Monsonia burkeana* fresh, dry and ground fruit sample. B. Photograph of *Monsonia burkeana* fresh, dry and ground leaf sample. C. Photograph of *Monsonia burkeana* fresh, dry and ground stem sample. D. Photograph of *Monsonia burkeana* fresh, dry and ground root sample.

Ground samples were prepared for data collection using the modified method recommended by the Association of Official Analytical Chemists (1984). Briefly, the method entailed taking 2 g plant material per organ and ashing in porcelain crucibles at 550°C in a muffle furnace for 24 hours. The ash was then dissolved in 5 ml of HNO_3/HCl/H_2O (1:2:3 v/v/v) and heated gently on a hot plate until brown fumes disappeared. Five-ml de-ionised water was added to the remaining content in each crucible and the mixture was heated until a colourless solution was obtained. The solution in each crucible was transferred into a 100-ml volumetric flask, filled up to the mark with de-ionised water and filtered using Whatman no. 42. Then, Cu, Ca, K, Mg, Mn, Zn and Fe were quantified using Perkin Elmer atomic absorption spectrophotometer; whereas Auto Analyzer 3 (AA3) segmented flow was used to determine P (Association of Official Analytical Chemists, 1984). Boron was quantified through colorimetry using azomethine-H (Gaines and Mitchell, 1979) and Cl with chlorometer.

One gram of ash sample for N was shaken in 40 ml of a 10% trichloroacetic acid (TCA) solution at 20°C for 1 hour using a wrist-action shaker. In accordance with Association of Official Analytical Chemists (1984) the insoluble residue was removed by centrifugation at 5000 rpm for 10 min, with residues treated three times with 15 ml of a 10% (w/v) TCA solution. The supernatant was collected, its volume made up to 100 ml with distilled water; with the aliquot taken for the determination of soluble N using the Kjeldahl procedure.

Data analysis: Prior to analysis, data for micro nutrients were transformed using $\log_{10}(x+1)$ in order to homogenise the variance (Gomez and Gomez, 1984). However, untransformed data were reported. 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 separate differences among the means at the probability level of 5%. Unless otherwise stated, means for essential nutrient elements were different at the probability level of 5%.

3.3 Results

Macro-nutrients: Except for Mg alone, N, P, K, Ca showed statistical differences ($P \leq 0.05$) within organs of *M. burkeana* (Appendices 3.1 to 3.5). The four test organs had different ACs for macro-nutrient elements N, P, K and Ca (Table 3.1). The fruit and the leaf had the highest ACs for N, with those of the stem and the root being moderate and low, respectively. The stem had the highest ACs for P, whereas those in the other three organs were not different. The stem had the highest ACs for K, followed by the root and then the fruit and the leaf. The leaf had the highest ACs for Ca, with those of the fruit and the stem being moderate, whereas those in the root were the lowest. All four test organs had similar ACs for Mg. On average, the increasing order of macronutrients in the tested organs was as follows: fruit = Ca > K > N > P > Mg, leaf = Ca > N > K > P ≥ Mg, stem = K > Ca > N > P ≥ Mg and root = K > Ca > N > P ≥ Mg.

Table 3.1 Accumulative capabilities of nitrogen, phosphorus, potassium, calcium and magnesium in the fruit, leaf, stem and root of *Monsonia burkeana*.

	Macro nutrients (%)				
Organ	N	P	K	Ca	Mg
Fruit	1.35a	0.32b	1.41c	1.79b	0.27a
Leaf	1.40a	0.28b	1.25c	2.68a	0.27a
Stem	0.84b	0.41a	2.51a	1.75b	0.25a
Root	0.73c	0.26b	1.91b	0.74c	0.25a
SE	0.02	0.02	0.13	0.07	0.01

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

Micro-nutrients: Except for Cu and Zn alone, Fe, Mn, B and Cl where distributed unequally in different organs of *M. burkeana* (Appendices 3.6 to 3.11). The four test organs had different ACs for the six micro-nutrients measured (Table 3.2). The ACs for Cu in the leaf, the stem and the root were not different, whereas those in the fruit were lower than those in the leaf, but were not different to those in the stem and the root. The ACs for Fe was the highest in the leaf and the root and the lowest in the fruit and the stem. The leaf and the root had the highest ACs for Zn, with the fruit and the stem having the lowest. The root had the highest ACs for Mn followed by the leaf, whereas the fruit and the stem had the lowest. The leaf had the highest ACs for B, whereas the fruit and the stem had moderate ACs for B and the root had the lowest. The fruit and the stem had the highest ACs for Cl, with the leaf having moderate ACs for Cl, whereas the root had the lowest. On average, the increasing order of micronutrients was as

follows: fruit = Cl > Fe > Mn > Cu > Zn > B, leaf = Cl > Cu > Fe > Zn > Mn > B, stem = Cl > Cu > Fe > Mn > Zn > B and root = Fe > Cl > Cu > Mn > Zn > B.

Table 3.2 Accumulative capabilities of copper, iron, zinc, manganese, boron and chlorine in the fruit, leaf, stem and root of *Monsonia burkeana*.

	Micro-nutrients (ppm)					
Organ	Cu	Fe	Zn	Mn	B	Cl
Fruit	81.5b	137.00b	47.33c	93.67c	26.50b	2 600a
Leaf	795.0a	336.00a	231.00a	153.00b	28.33a	1 400c
Stem	148.3ab	117.33b	76.33bc	94.00c	22.33b	1 900ab
Root	373.3ab	415.33a	182.00ab	191.00a	20.00c	400d
SE	193.20	17.89	43.00	3.56	1.05	0.01

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

3.4 Discussion

The differences in the partitioning of the quantified macro- and micro-elements within various organs of *M. burkeana* may be attributed primarily to the ACs of individual organs. Factors such as the preferential absorbability of a particular organ for the corresponding element, the age of the plant, the mineral composition of the soil in which the plant grows and the ambient climatological conditions all have a role in accumulation of different quantities of essential nutrient elements in different plant organs (Serfor-Armah *et al.,* 2001). Different ACs for essential nutrient elements in different organs of *M. burkeana* confirmed those observed in barley *(Hordeum vulgare)*, flax (*Linum usitatissimum*), seagrass *(Posidonia australis*; *P. sinuosa)* and annual lupins (*Lupinus* species)

(Bowen, 1976; Epstein, 1972; Hocking and Pate, 1978; Hocking *et al*., 1980; Moraghan, 1993).

Accumulation capability for macronutrient elements: Generally, *M. burkeana* conspicuously accumulated the highest concentration of macro-elements in reproductive and vegetative organs, whereas the roots accumulated appreciable concentrations of micro-elements. The lower concentrations of the macro-nutrient elements in the roots are indicative of high rates of their transportation to shoots (Baldantoni *et al.,* 2009), resulting in high ACs for macronutrients in reproductive and vegetative organs of *M. burkeana*. The high level of macronutrients in aerial organs, particularly in the leaves, agreed with the ACs of leaves in *Phragmites australis* (Baldantoni *et al.,* 2009).

The high ACs of the leaves for Ca could be explained in relation to the role of this element in plants. Calcium is the constituent of calcium pectate in the middle lamella, which binds adjacent cell walls together (Campbell, 1990). Apart from this structural function, Ca promotes ACs for K and also prevents K from leaching out of organs as senescence sets in (Epstein, 1961). Calcium ions also serve a protective function. For instance, it protects organs from the injurious effect of H^+ ions, high salinity ions and other toxic ions (Epstein, 1961). The addition of Ca into an organ substantially reduces protein loss and maintains active accumulation of ions required by plant organs (Bonner, 1976). Additionally, Ca enhances the ACs for B (Wildes and Neales, 1971), which may provide some explanation to the higher content of B in leaves than in roots.

In view of the listed facts, high levels of essential nutrient elements in shoots, particularly in the leaves, were due to the ability of Ca to enhance the ACs for other minerals such as B and K as well as its protective role. Low Ca in the roots of *M. burkeana* failed to enhance the ACs for other essential nutrient elements, and therefore, their low concentrations. The cell wall-binding capability of Ca translates to minimum leaching out of essential nutrient elements in above ground organs of *M. burkeana*.

Generally, concentrations of sucrose in an organ are inversely proportional to those of osmoticum ions (K, Na, Cl) as a measure of balancing the turgor pressure (Bonner, 1976; Mashela and Nthangeni, 2002). Translocation of sucrose is closely linked to the concentration of K (Hartt, 1970); with circulation of K around the sieve plates of the phloem being part of the mechanism that increases translocation of sucrose in sieve tubes (Spanner, 1958). Consequently, a decrease in K content incidentally reduces translocation of sucrose by depressing the electro-osmotic potential gradient across the sieve plates. Any factor that decreases transportation of K alters the electro-osmotic potential between sieve tubes, thereby, reducing sucrose translocation. Also, K is essential for the synthesis of starch (Campbell, 1990). In view of these facts, accumulation of leaf K in *M. burkeana* may translate to accumulation of proximate compounds such as carbohydrates, proteins, fats and vitamins. *Monsonia angustifolia* leaves have vitamin C, protein and fat ranging from 249.6

to 266 mg/100 g material, 0.6 to 5.0% and 0.1 to 1.0%, respectively (Lyimo *et al*., 2003).

Normal levels of macronutrient elements in plants are defined as 0.11% to 0.17% for P (Smidt, 1988), 0.35% to 0.66% for K (Zöttl and Hüttl, 1989), 0.23% to 0.50% for Ca and 0.05% to 0.13% for Mg (Smidt, 1988). Relative to the listed nutrient element levels, results from the current study suggested that *M. burkeana* has a much better ACs for essential nutrient elements, and therefore may serve as a good source for those elements.

Accumulation capability for micro-nutrients: Among the evaluated micro-elements, the concentration of Cl was consistently the highest in all organs, except in the roots where it was second highest. Boron was the lowest in all organs. Except under conditions where extrinsic stresses force more sucrose to be channelled towards roots, as an osmoticum ion, the Cl ion is mobilised to leaves (Mashela and Nthangeni, 2002), where toxic concentrations are avoided through leaf abscission.

Relative to other micronutrients, the roots exhibited the highest concentration of Fe. The chemical properties of Fe are responsible for its role in oxidation-reduction reactions and since Fe is a transitional metal, it is capable of existing in more than one oxidation state (Bonner, 1976). Iron forms stable chelates with molecules containing oxygen, sulphur or nitrogen and also accumulates in nuclei of root cells (Possingham and Brown, 1957). This may be the reason Fe was the

highest in roots. The same applies to Mn. The high levels of Fe and Mn in the roots might also have been a result of low rates of transportation to the shoots.

Generally, the ranges of micro-nutrients in crops are between 4 and 15 ppm for Cu and 15 to 200 ppm for Zn (Allaway, 1986; Bowen, 1966). Relative to the cited ranges, the 795 ppm for Cu and 231 ppm for Zn in the leaves of *M. burkeana* were remarkable.

In *C. myriocarpus*, most of the essential nutrient elements are accumulated in fruit(Mashela, 2002), which are the harvestable part for uses in plant protection.
In terms of nutrient elements, this study suggested that the leaves, the stems and the fruitof *M. burkeana* have the potential to serve as the harvestable material for commercial packaging of a plant protection product.

CHAPTER 4
ACCUMULATIVE CAPABILITIES OF SELECTED SECONDARY METABOLITES IN ORGANS OF *MONSONIA BURKEANA*

4.1 Introduction

Interest has increased considerably in finding naturally occurring pesticides for use in plant protection to replace synthetic compounds which are being restricted due to their environment-unfriendliness (Velioglu *et al.,* 1998). Bio-pesticides are receiving much attention due to their environment-friendliness. In most bio-pesticides, 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.

The accumulative ability of organs for phenolic-antioxidants in *M. burkeana* is not documented, resulting in locals harvesting and using the whole plant in

preparations of bio-pesticides. The objective of this study was to determine the accumulative abilities of *M. burkeana* organs with respect to phenolics and antioxidants, along with the relationship of the two chemical compounds.

4.2 Materials and methods

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 m × 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 hours (Makkar, 1999). Fruit, leaves, stems and roots were individually ground (Figure 3.1) 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 minutes for 2 hours to improve extraction efficiency. Samples were then centrifuged at 3500 rpm for 10 minutes (25°C), with the supernatant decanted.

Sample residues were rinsed once with 20 mL solvent, vortex mixed for 5 minutes, centrifuged and then decanted.

The Folin Ciocalteau 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 Ciocalteau 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 minutes after addition of the Folin Ciocalteau phenol reagent. The contents were mixed and the flask made up to volume with distilled water, stoppered 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 hours at 760 nm using a 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 hours 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 NaH_2PO_4, 0.2 M $NaHPO_4$ 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 minutes for the samples and at 15 minutes 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.

4.3 Results

The TPC in the four organs of *M. burkeana* differed at $P \leq 0.05$ (Appendix 4.1 and 4.2). Fruit had the highest TPC, with the leaf and stem having intermediate, whereas roots had the lowest content (Table 4.1). The TAA among the four organs differed. Fruit and leaf had higher antioxidant activity than the stem, whereas the root exhibited the lowest TAA (Table 4.1).

Table 4.1 Quantities of phenolic content and antioxidants in fruit, leaf, stem and root of *Monsonia burkeana.*

Organ	Total phenolic content (mg/100g)	Total antioxidant levels (µmol/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 ≤ 0.05) according to the Tukey' honest significant different test.

The TAA and TPC had quadratic relationships in all four organs. The treatment (TPC) contributed 97% TTV in TAA of fruit(Figure 4.1), 92% in leaves (Figure 4.2), 92% in stems (Figure 4.3) and 96% in roots (Figure 4.4). The TAA in fruit, leaf, stem and root was saturated at different levels of phenolic content, viz. 5.39, 5.49, 4.36 and 4.13 mg/ 100 g respectively (Table 4.2). 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 4.2).

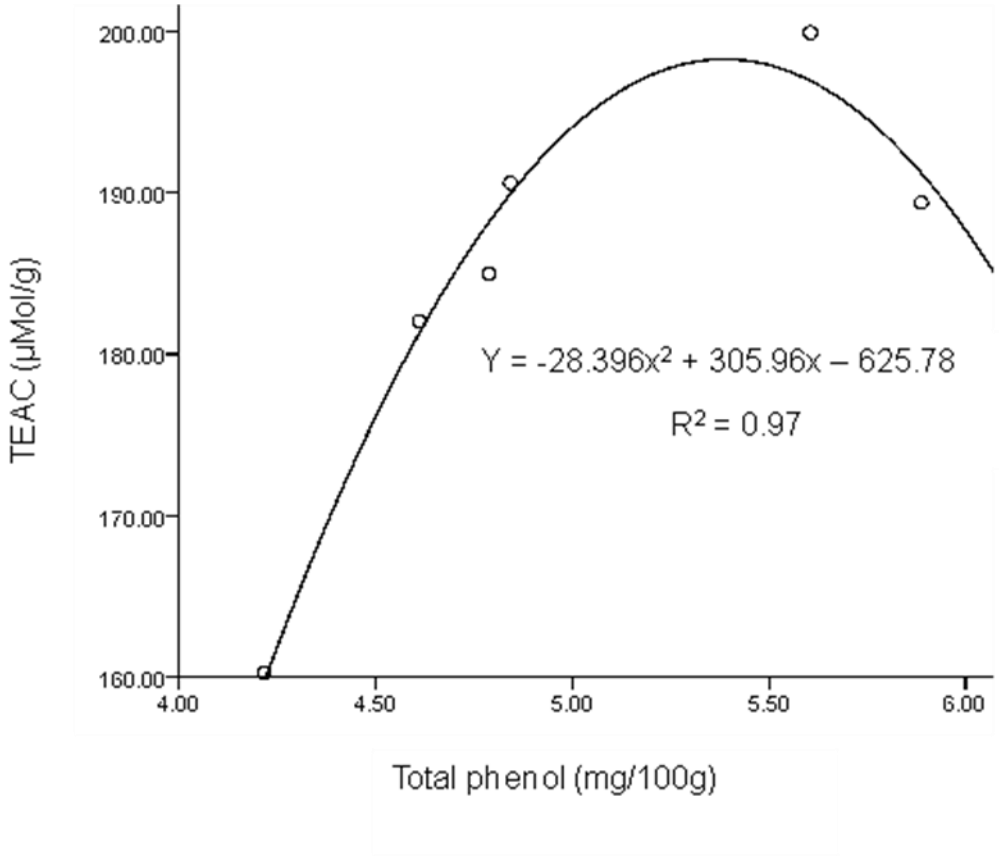

Figure 4.1 Relationship between antioxidant activity and total phenolic content in fruit samples of *Monsonia burkeana.*

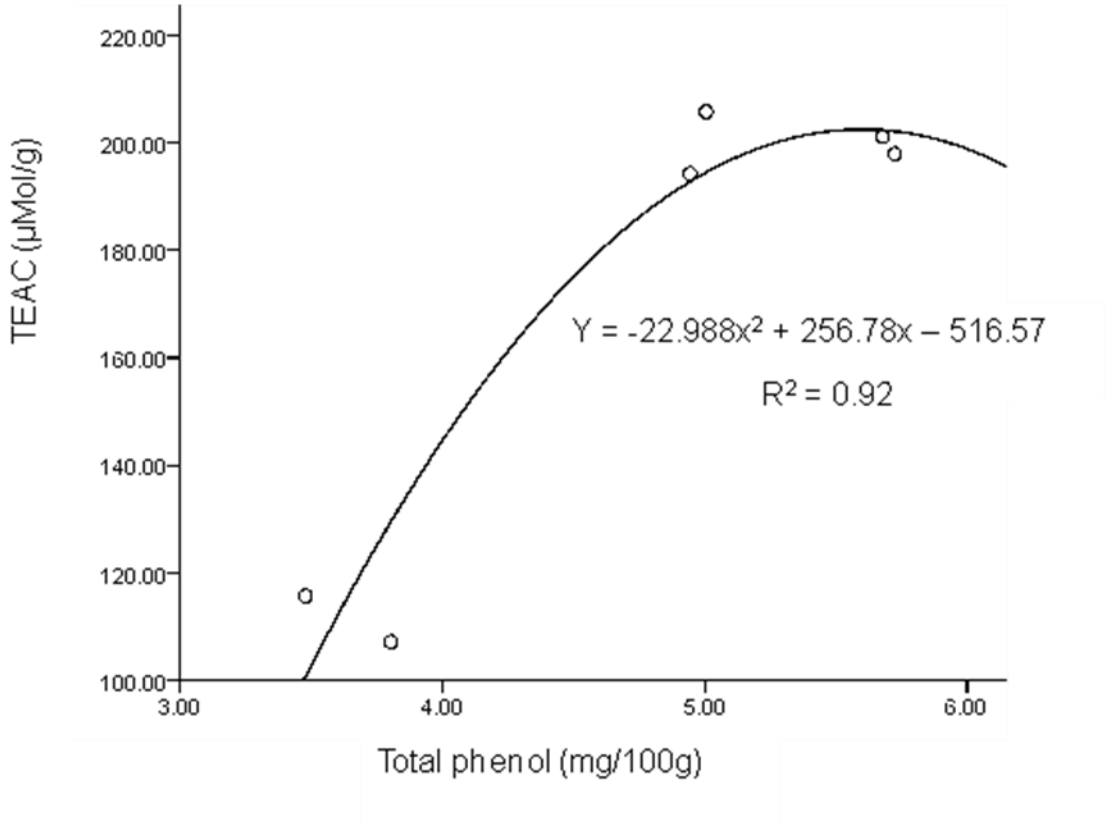

Figure 4.2 Relationship between antioxidant activity and total phenolic content in leaf samples of *Monsonia burkeana.*

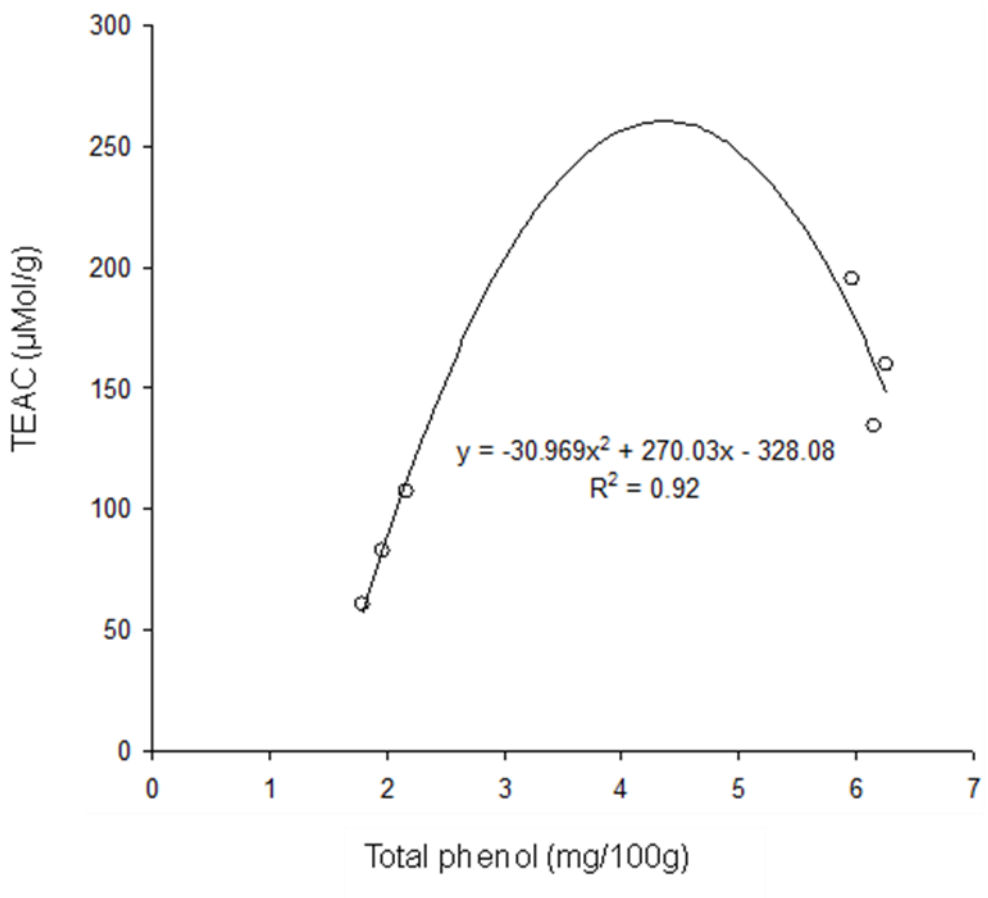

Figure 4.3 Relationship between antioxidant activity and total phenolic content in stem samples of *Monsonia burkeana.*

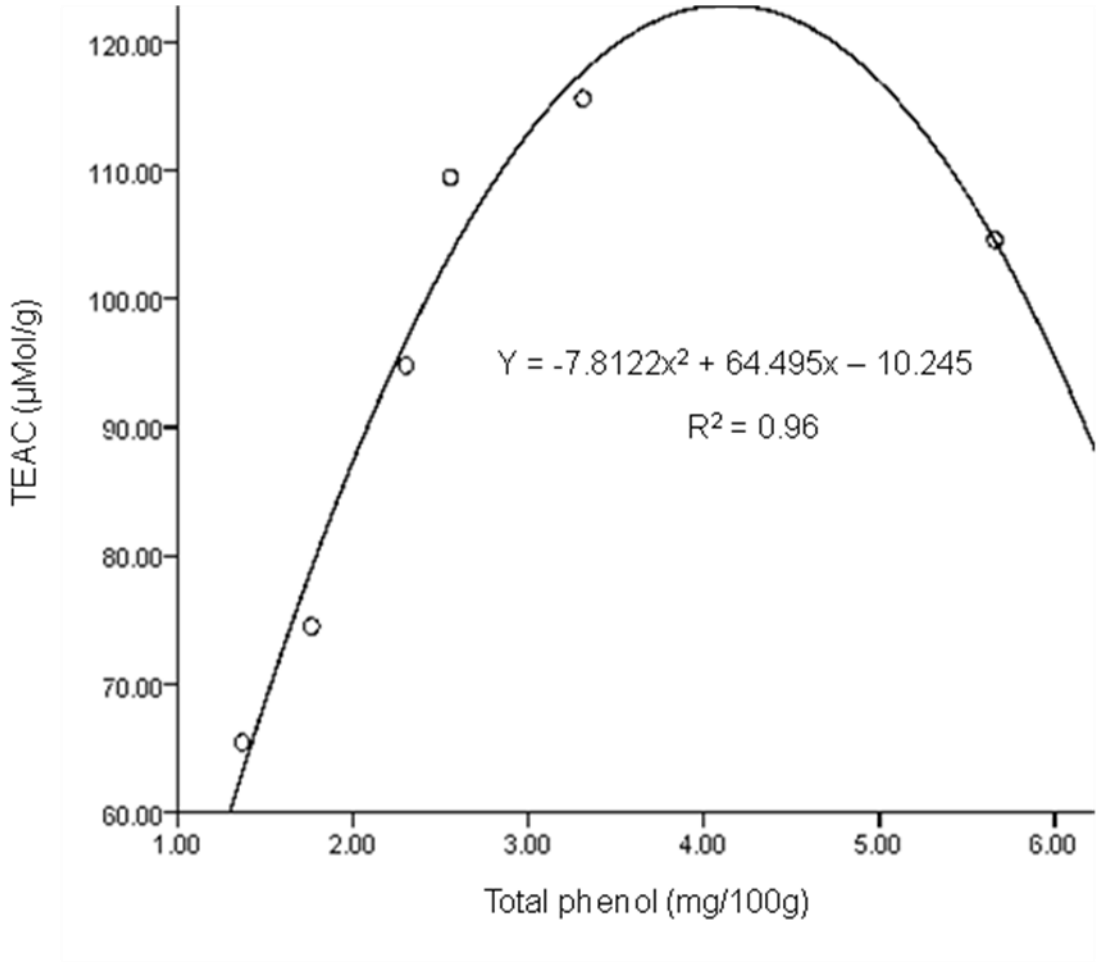

Figure 4.4 Relationship between antioxidant activity and total phenolic content in root samples of *Monsonia burkeana.*

Table 4.2 Total phenolic content (mg/100g) for optimal Total antioxidant activity (µmol/g) in *M. burkeana* organs.

Organ	Formula	R^2	TP 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	$Y = -7.8122x^2 + 64.49x – 10.245$	0.96	4.13	122.87	0.05

4.4 Discussion

In this study, *M. burkeana* leaves and fruit 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 (*H. 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 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 (*C.*

myriocarpus) they are exclusively concentrated in seeds and roots (Van Wyk and Gericke, 2000).

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; Sıvacı and Sökmen, 2004). Inderjit (1996) provided an extensive 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 plant physiology is their activity in defence 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 the 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), 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).

In plants, phenolic compounds serve as intrinsic defence structures against pathogens and pests, and also minimise the deleterious effect of unfavourable climatic conditions (Agrios, 2005; Khan and Mukhtar, 2007). Phenolic compounds are extrinsically used in crude extracts as sprays against pests in agricultural production systems (Hwang and Lindroth, 1993; Lindroth, 1993).

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 fruit may have great potential as raw products for use in bio-pesticides as a source of phenolic-antioxidants. Consequently, when phenolic-antioxidants are a focus in plant protection, only leaves and fruit should be harvested.

CHAPTER 5
SUMMARY AND CONCLUSIONS

5.1 Summary of the study

The study determined accumulative capabilities and distribution of selected essential nutrient elements and phenolic-antioxidants in *Monsonia burkeana* organs. Results of accumulative capabilities and distribution provide information as to which organ should serve as a harvestable unit.

5.2 Essential nutrient element accumulative capabilities

Macro-essential nutrient elements differed in the four studied organs of *M. burkeana* with ACs highest in the leaves, stems and fruit. Similarly, micro-nutrient elements differed in the studied organs of *M. burkeana*. However, the leaves had distinctively high ACs than all other organs in terms of micro essential nutrient elements ACs.

5.3 Phenolic-antioxidant content distribution and density dependent relationship

Both TPC and TAA were highest in the leaves and fruit of *M. burkeana* whereas the stem and the roots had low accumulative abilities of the two compounds. Also, the TPC and TAA relationship in this study supported the density-dependent relationship patterns (Salisbury and Ross, 1992), where TPC served as a sustainable source of TAA.

5.4 Conclusion

The study confirmed that concentrations of chemical ingredients vary from organ to organ of the same species. The study further suggested that the variation of such ingredients is largely resulting from a particular organ due to its accumulative capability when compared to other organs. Results of this study suggested that for optimum utilisation of essential nutrient elements, phenolic and antioxidant compounds, leaves and fruit should serve as harvestable units.

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APPENDICES

Appendix 3.1 Analysis of variance for nitrogen (N) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	0.00209	0.2	0.99	0.41
ORGAN	4	1.07804	99	255.46	0.01
ERROR	8	0.00844	0.8		
TOTAL	14	1.08857			

Appendix 3.2 Analysis of variance for phosphorus (P) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	0.00228	5	1.80	0.23
ORGAN	4	0.03703	84	14.65	0.01
ERROR	8	0.00505	11		
TOTAL	14	0.04436			

Appendix 3.3 Analysis of variance for potassium (K) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	0.16912	5	2.10	0.19
ORGAN	4	3.04043	86	18.85	0.01
ERROR	8	0.32261	9		
TOTAL	14	3.53216			

Appendix 3.4 Analysis of variance for calcium (Ca) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	0.01764	0.3	0.64	0.55
ORGAN	4	5.74583	98	104.38	0.01
ERROR	8	0.11009	2		
TOTAL	14	5.87356			

Appendix 3.5 Analysis of variance for magnesium (Mg) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	0.00121	32	4.92	0.04
ORGAN	4	0.00153	41	3.11	0.08
ERROR	10	0.00099	27		
TOTAL	14	0.00373			

Appendix 3.6 Analysis of variance for copper (Cu) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	165710	7	0.69	0.53
ORGAN	4	1126035	50	2.36	0.14
ERROR	8	954102	43		
TOTAL	14	2245847			

Appendix 3.7 Analysis of variance for iron (Fe) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	2168	1	1.17	0.36
ORGAN	4	197596	95	53.14	0.01
ERROR	8	7436	4		
TOTAL	14	207200			

Appendix 3.8 Analysis of variance for zinc (Zn) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	10448	8	0.93	0.43
ORGAN	4	74263	57	3.30	0.07
ERROR	8	45031	35		
TOTAL	14	129743			

Appendix 3.9 Analysis of variance for manganese (Mn) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	192.53	1	4.09	0.06
ORGAN	4	20837	98	221.51	0.01
ERROR	8	188.13	1		
TOTAL	14	21217.73			

Appendix 3.10 Analysis of variance for boron (B) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	1.73333	1	0.22	0.81
ORGAN	4	134.93	80	8.63	0.05
ERROR	8	31.2667	19		
TOTAL	14	167.933			

Appendix 3.11 Analysis of variance for chlorine (Cl) content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P ≤
REP	2	0.00057	1	1.21	0.35
ORGAN	4	0.08231	97	86.94	0.01
ERROR	10	0.00189	2		
TOTAL	14	0.08477			

Appendix 4.1 Analysis of variance for total phenolic content in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P≤
REP	8	14.1230	15	1.06	0.42
ORGAN	4	28.0961	29	4.20	0.01
ERROR	32	53.4971	56		
TOTAL	44	95.7162			

Appendix 4.2 Analysis of variance for antioxidant activity in organs of *Monsonia burkeana*

SOURCE	DF	SS	%	F-value	P≤
REP	8	13829.9	12	0.92	0.51
ORGAN	4	39068.1	35	5.21	0.01
ERROR	32	59942.9	53		
TOTAL	52	112841			