Phytochemical and elemental studies of two indigenous medicinal plants of South Africa, *Bridelia micrantha* and *Sideroxylon inerme*

By

Bongisiwe Gladys Shelembe

A thesis is submitted in fulfilment of the academic requirements for the degree of Master of Science in the school of Chemistry and Physics, University of KwaZulu-Natal, Durban

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DECLARATION 1 – PLAGIARISM

I hereby declare that this dissertation is my own work, except where specifically acknowledged in the text. Neither the present dissertation nor any part thereof has been submitted by any other University for a degree.

Author: _____

Miss Bongisiwe G. Shelembe

Supervisor: _____

Prof. Sreekanth.B. Jonnalagadda

Co-supervisor: _____

Dr Roshila Moodley

DECLARATION 2 - PUBLICATIONS

Publication 1

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Manuscript in preparation.

In preparation of the above manuscripts, I performed all the experiments and interpreted the data. The co-authors contributed in editing, and verifying the scientific content as well as editing the manuscript.

Signed: .....

DEDICATION

This work is dedicated to my loving and caring brother Njabulo Sihle Emmanuel Shelembe and my parents Mr Sipho Elias Shelembe and Mrs Nombuso Busaphi Shelembe.

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ABSTRACT

Bridelia micrantha (Euphorbaceae) and *Sideroxylon inerme* (Sapoacaea) are two indigenous medicinal plants found in KwaZulu-Natal and are both known as 'uMhlalamagwababa' in isiZulu. These two plants species have similar physical properties, they both contain black, berry-like edible fruits and they both produce milk sap. This similarity has led to them being confused for one species so they are both used by traditional healers to treat a variety of the same illnesses. Different parts of *B. micrantha* and *S. inerme* are used traditionally to treat a variety of ailments; the bark is used to treat burns, wounds, venereal diseases, tapeworm and toothache. They are also used as anti-diarrhoeal and anti-bacterial agents.

The main aim of this study was to isolate, characterise and identify the phytocompounds in the leaves, stem bark, roots and fruits of the two plant species, *B. micrantha* and *S. inerme*, to evaluate if they can be used interchangeably in traditional medicine. The isolated compounds were also assessed for their antioxidant activity to determine the plants potential as natural antioxidants. The impact of soil quality parameters on elemental uptake of the wild fruits of *B. micrantha* from eight different geographic locations in KZN as well as the nutritional value of the edible fruits was also determined to evaluate the plants potential as a nutraceutical.

The phytochemical investigation of *S. inerme* revealed the presence of three pentacyclic triterpenes (friedelin, stigmasterol and a mixture of α and β –amyrin), one carotenoid (lutein) and one megastigmane (apocynol B). Two flavonoids (quercetin and quercetin-3-*O*-glucoside) and two triterpenes (oleanolic acid and acacic acid lactone) were isolated from *B. micrantha*. The free radical scavenging activity of the extracts and selected phytocompounds was determined by

the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The antioxidant activity for both plants was good but that of *B. micrantha* was better than that of *S. inerme*.

The concentrations of elements in the fruits were found to be in decreasing order of Mg > Ca > Mn > Zn ≈ Fe > Cu > Co > Ni > Cr > Pb > As ≈ Cd. It was also found that 100 g of the fruit contained about 90.1% moisture, 3.2% ash, 4.1% proteins, 0.9% oil and 1.7% carbohydrates. This study shows that the fruits of *B. micrantha* are a good source of essential elements and have low levels of the toxic metals investigated. The classes of compounds isolated from *B. micrantha* and *S. inerme* validate the ethnomedicinal use of these plant species in traditional medicine however, since the isolated compounds from each species were different it can be concluded that they cannot be used interchangeably for their medicinal benefit. The results obtained from this study provide baseline information on the plants' potential as a source of medicine and also revealed the nutritional benefits of consuming the edible wild fruits of *B. micrantha*.

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ABBREVIATIONS

^{13}C-NMR	^{13}C- nuclear magnetic resonance spectroscopy
^{1}H-NMR	Proton nuclear magnetic resonance spectroscopy
BAF	Bioaccumulation factor
CEC	Cation exchange capacity
COSY	Correlation spectroscopy
CRM	Certified reference material
d	Doublet
dd	Double doublet
DEPT	Distortionless enhancement by polarization transfer
DM	Dry mass
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRI	Dietary reference intake
EDTA	Ethylenediaminetetraacetic acid
Ex	Exchangeable
GC-MS	Gas chromatography-mass spectrometry
HMBC	Heteronuclear multiple bond coherence
HSQC	Heteronuclear single quantum coherence
Hz -	Hertz
ICP-OES	Inductively coupled plasma-optical emission spectrometry
IR	Infrared
NOESY	Nuclear overhauser effect spectroscopy
r	Correlation coefficient

RDA	Recommended dietary allowance
SD	Standard deviation
SOM	Soil organic matter
T	Total
TLC	Thin-layer chromatography
UL	Tolerable upper intake level
UV	Ultraviolet

CHAPTER 1

1.1 Introduction

Traditional medicine, including herbal remedies and spiritual therapies, has been used for years to treat chronic and acute illnesses, worldwide. More than one third of the world's population in developing countries lack access to conventional medicine, as a result traditional medicine is used to treat health-related issues (Srinivasan, 2005; Moodley *et al.,* 2012; WHO, 2008). Traditional medicine can be used either directly or to synthesise pharmaceutical drugs. In developed countries, pharmaceutical drugs are used to treat various diseases, even though these drugs create problems due to side effects such as carcinogenicity due to their synthetic characteristics. Apart from the availability of traditional medicine, its affordability makes it more attractive than pharmaceutical drugs. In countries like Ghana where malaria is endemic, antimalarial drugs are extremely expensive and unaffordable to most affected people therefore they turn to traditional healers or herbalists who sell concoctions purported to cure such ailments, at an affordable rate (Chen and Lopez, 2013 and Holliday, 2003).

Traditional medicine uses medicinal plants for the preparation of infusions, decoctions, concoctions, and ointments that are used to treat or cure illnesses or diseases. Different plant parts are used for this purpose which includes the stem bark, leaves and roots. Plants have contributed a lot to modern medicine with the origin of numerous drugs in use today being derived from medicinal plants (Table 1). The medicinal value of plants comes from the compounds that they produce or phytochemicals (secondary metabolites) that are produced as a defence against predators and are not involved in the growth and development of the plant (Bourgaud *et al.,* 2001). Important natural products include alkaloids, terpenoids and phenolic

compounds. Research on medicinal plants has shown promising constituents for effective treatment and management of various illnesses. Extensive research in the area of isolation and characterisation of the active principles of these plants is required so that better, safer and cost effective drugs for treating various illnesses can be developed.

Table 1: Drugs isolated from plants and their medicinal uses.

Drugs	**Plant species**	**Medicinal use**	**References**
Morphine	*Papaver somniferum*	Pain relief	Furuya *et al.*, 1972
Quinine	*Cinchona succirubra*	Anti-malaria	Andrade-Neto *et al.*, 2003
Taxol	*Taxus brevifolia*	Anti-cancer	Strobel *et al.*, 1996

Inappropriate use of traditional medicine can lead to negative effects which might result in death. For instance, Ephedra a traditional herb used to treat respiratory congestion in China was also used in the United States as a dietary supplement but when taken in excess, it can cause side effects that may lead to heart attacks, stroke and even death. Therefore, the provision of safe and effective traditional therapies is important to increase access to healthcare. The World Health Organisation (WHO) has shown its commitment in the designation of strategies to overcome such issues. One of them includes documentation of traditional medicines and their remedies and they are developing a policy of evaluation and regulation of traditional medicine and practice.

Apart from the medicinal value of plants they are also used as a source of vitamins and minerals to promote good health and growth (Bin Daud, 2006). People, especially those from rural areas,

utilise wild fruits as a source of nutrition. Plants need correct ratios and levels of nutrients for their normal growth and development. Plant nutrition is divided into macronutrients (nutrients required in large quantities) such as N, K, S, Mg, Ca and P and micronutrients (nutrients required in small quantities) such as Cr, Co, Mn, Ni, Fe, Se, Zn, Mo and Cu. Inappropriate supply of nutrients to the plant may result in metabolic disruptions which can result in poor growth and development. The availability of nutrients to the plant is affected by soil chemical or physical factors such as pH (acidity and alkalinity of the soil), soil organic matter (SOM) and cation exchange capacity (CEC).

Plants act as intermediate reservoirs through which metals from the soil are transferred to humans. Toxic metals are taken up by plants from the soil and in this way brought into the food chain. These toxic metals can threaten human health depending on factors such as mobility, bioaccumulation and geographic index (Moodley *et al.*, 2012). Since plants are collected from the wild, whether for medicinal use or as food, they need to be analysed for their metal content and other anthropogenic pollutants.

Bridelia micrantha (Euphorbiaceae) and *Sideroxylon inerme* (Sapotaceae) are two medicinal plants that are collected from the wild for both medicinal and nutritional purposes. These two medicinal plants are indigenous to KwaZulu-Natal (KZN); they both contain edible berry-like fruits that turn black when ripe and ripen in summer; they both produce milk sap. These morphological similarities have resulted in them being confused for one species (known as 'uMhlalamagwababa' in isiZulu) and as such, are used interchangeably by traditional healers to treat a variety of the same illnesses such as gastro-intestinal infections, paralysis, diabetes, painful joints, prehepatic jaundice, tape worms, abnormal pains, headaches, bloody diarrhoea,

coughs and wound infections (Lin e*t al.,* 2002; Green *et al.,* 2011; Ngueyen *et al.,* 2008; Atindehou *et al.,* 2004).

Despite the natural distribution of *S. inerme* in South Africa and the importance of the plants to the local people, no phytochemical or pharmacological studies have been done or documented on this plant. The isolation and identification of the phytocompounds is essential to support and understand indigenous knowledge. A number of studies have been conducted on *B. micrantha*. These include the antimycobacterial activity of the hexane extract (Green *et al.,* 2011) as well as the study conducted by Samie and co-workers (2005) which showed the *in vitro* activity of the stem bark of the methanolic extract of *B. micrantha* against Gram negative and Gram positive bacteria (Lin *et al.,* 2002; Samie *et al.,* 2005).

1.2 Problem Statement

WHO estimates that 80% of the people in developing countries rely on traditional medicine for their primary health care needs and about 85% of traditional medicine involve the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as a source of drugs. About 21 drugs prescribed in the USA today come from natural sources; 90 of which come either directly or indirectly from plant sources (Benowitz, 1996). More than 47% of the anti-cancer drugs in the market today are derived from natural products (Newman *et al.*, 2007). Therefore, there is a need to provide a scientific basis for the ethno-medicinal use of plants and also to isolate and characterise the secondary metabolites in these plants that impart medicinal value to them, to add to the potential list of drugs. (Falodun *et al.,* 2008). A bioassay-guided study is also necessary to test for different biological activities, which is the basis of some ethnomedicinal claims. Regulation of traditional medicine is a complicated and challenging issue as it is highly dependent on experience with use of these products. Evaluation of quality, safety and efficacy based on research is needed to improve approaches to assessment of traditional medicine.

It is also important to take note of the inorganic constituents when taking medicinal plants, since plants also contain heavy metals. High concentrations of certain metals result in adverse health effects and mental disorders. Heavy metals generally are absorbed by plants as a source of nutrients and toxic metals are absorbed in lieu of essential nutrients. Both essential and toxic metals are detrimental to human health if ingested at high concentrations. Factors such as bioaccumulation, geoaccumulation and exchangeability are considered when assessing the mobility and uptake of metals from soil to plants.

1.3 Aim

The aim of the study was to phytochemically and analytically investigate two medicinal plant species that are indigenous to KwaZulu-Natal namely *S. inerme* and *B. micrantha*, which produce edible fruits, to determine their potential as nutraceuticals. The phytochemical investigation was done to determine if these morphologically similar plant species that are used interchangeably by traditional healers have similar secondary metabolites or, at least, phytocompounds from the same classes, to validate this use. The analytical investigation was done to determine the nutritional value of the fruits and to evaluate the impact of soil quality parameters on elemental uptake.

1.4 Objectives

The research objectives were:

- To extract and isolate the secondary metabolites from various morphological parts of the plants.
- To identify and characterise isolated compounds using various spectroscopic techniques such as 1D (one Dimensional) and 2D NMR (two Dimensional Nuclear Magnetic Resonance) UV-Vis (Ultraviolet–visible spectroscopy), IR (infrared spectroscopy) and GC-MS (Gas Chromatography-Mass Spectrometry).
- To identify suitable bioassays, based on classification of compounds isolated, and to test the isolated compounds for biological activity therefore promoting further use of the plants or validating their ethno-medicinal use.

- To determine the elemental concentrations in *B. micrantha* fruits as a function of geographic location and soil quality parameters and to assess the soil for metal contamination and the fruit for metal toxicities.
- To evaluate the nutritional value of fruits by comparing their elemental concentrations to the recommended dietary allowances (RDAs).

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CHAPTER 2

LITERATURE REVIEW

2.1 Overview of traditional medicine

Despite the global economic growth in the last few decades, about 21% of the world's population remains poor, earning less than a dollar per day, (WHO, 2005). Poverty and health are somehow related, for instance poor people are unable to access modern methods of preventing and curing diseases, hence low labour productivity and high rate of mortality. A large proportion of the population in Africa is unable to afford modern medicine due to high costs and poor infrastructure; instead they rely on traditional medicine to treat diseases. Africans have also managed to cure a lot of diseases by using concoctions called 'Imbiza' made by mixing different plants species, and it has been passed from one generation to the other (Mahlangeni, 2012).

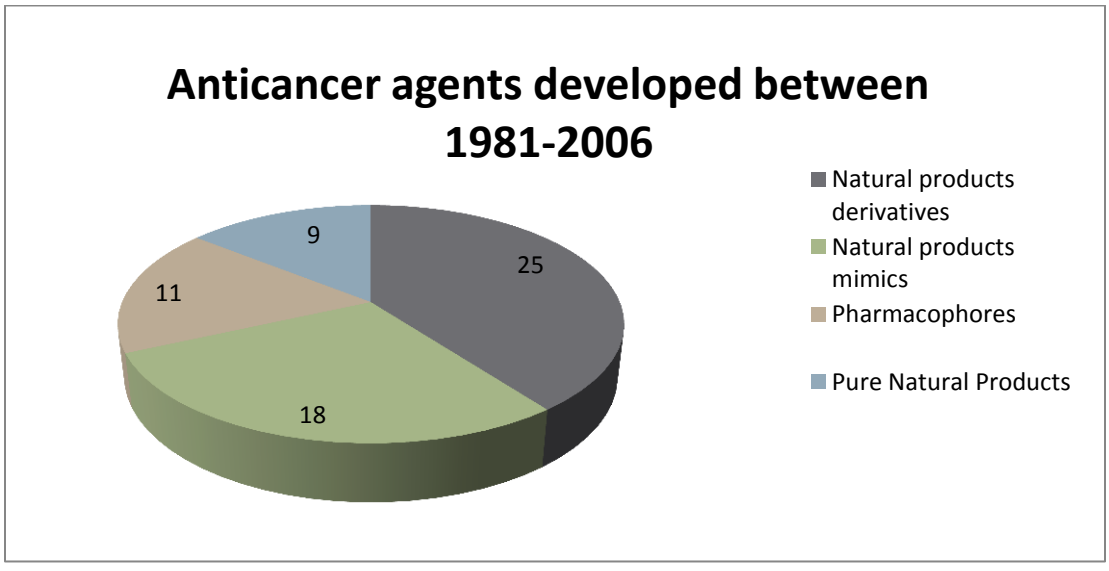


Figure 1: Uses of natural products as a source of drugs.

Natural products have been and continue to be used as baseline information for the discovery of new drugs. Cancer is one of the most dreaded diseases in the world today; about 25% of the anti-cancer drugs in the market today are derived from natural products or their derivatives (Figure 1). About 18 % of the cancer drugs are mimics of compounds isolated from natural products.

According to WHO, traditional medicine is defined as the sum of knowledge , skills and practices based on the theories , believes and experiences indigenous to different cultures, whether explicable or not (WHO, 2005). Traditional medicine is generally used to maintain health, preventing diagnosis and improve or treat physical and mental illnesses. According to (WHO), about 80% of the world's population still rely on traditional medicine for the treatment of many diseases/illnesses that have global economic importance such as HIV/AIDS, tuberculosis, cancers and malaria (WHO, 2008). The Chinese people today represent the largest users of traditional medicine, with over 5000 plants and plant products in their pharmacopeia (Bensky and Gamble, 1993). Traditional medicine incorporates medicinal plants which are used to cure diseases**.** Plants have been used as a basis of medicine and this is still in practised today. Synthetic drugs also make use of plant-derived compounds as the base.

Traditional medicines, including herbal medicine, have been, and continue to be used in every country around the world in some capacity. In much of the developing world, 70–95% of the population rely on traditional medicine for their primary care (WHO, 2008) (Figure 2). The Ethiopian population is the highest users of traditional medicine (up to 90 %).

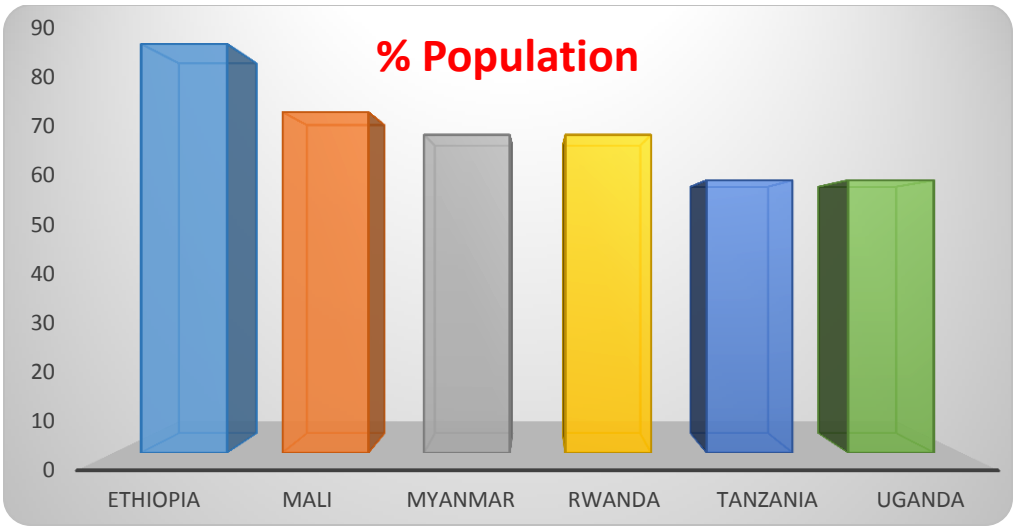


Figure 2: Population using traditional medicine for their primary health care (WHO, 2008).

Traditional medicine is also used in developed countries. Figure 3 shows the use of traditional medicine in selected developed countries and use of complementary and alternative medicine in selected developed countries.

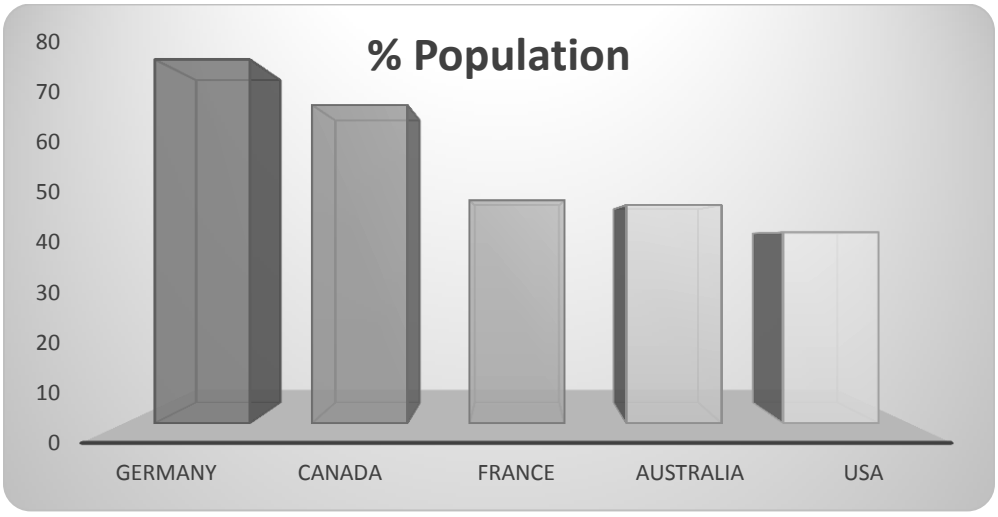


Figure 3: Population in developed countries who have used complementary and alternative medicine at least once (WHO, 2005).

2.2 Medicinal plants in South Africa

A large proportion of the South African population uses traditional medicine for their psychological needs. This has led to the sale of plants part in an open market in Durban, KZN, called the Durban Muthi Market.

2.3 Traditional medicine from medicinal plants

Many medicinal plants have recently gained popularity as ingredients in cosmetic formulations based on their ethnomedicinal values and many cosmetic products sold in stores are of natural origin (Namrita and Navneet, 2014). Medicinal plants are plants which are used in herbalism and thought to have certain extractable compounds in their leaves, stem, flowers and fruits which can be used as a source of medicine. Plants such as *Aloe ferox* are being used to cure or treat a variety of diseases including cancer, bacterial infections and aloe rust. There are several ways in which these plants can be used as a source of medicine. They can either be used as a tea or any other extracted form to obtain their natural constituents and they can also be used as agents in drug synthesis.

A plant synthesises substances that are useful to the maintenance of health in humans and animals called natural products. These are the organic substances found in plants; they are divided into primary and secondary metabolites. Primary metabolites are produced during the growth and development phase as a result of primary metabolism. Examples includes amino acids, nucleic acids as well as ethanol. Secondary metabolites are important for the plants survival and are produced through modification of primary metabolites. They include alkaloids, flavonoids and terpenoids**.**

The medicinal value of the plant depends on chemical substances or groups of compounds that produces a definite physiological action in the human body. About 12000 secondary metabolites have been isolated; these substances (particularly the alkaloids) serve as plant defence mechanisms against predation by microorganisms, insects, and herbivores.

Apart from plants, natural products are derived from various sources of materials including terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates (Rout *et al.,* 2009). It has been estimated that about 75 000 plant species have been used for traditional medicine, where known by scientists and accepted for commercial purposes (Kartal, 2007). They are considered important due to their biological activities which include their antioxidant properties (Shahwarl and Razal, 2012).

2.4 Medicinal plants as antioxidants

There are numerous biochemical and physiological processes in the human body that produces reactive oxygen species (ROS) as by-products. The species includes superoxides ions (O_2^-), hydroxyl radical (OH radical) as well as non-radical species such as hydrogen peroxide (H_2O_2). Production of such molecules cause oxidative damage to biomolecules such as lipids & protein DNA, leading to diseases such as cancer, coronary heart disease, Alzheimer's disease, neuro degenerative disorders, atherosclerosis, cataracts and diabetes**.** Antioxidants (Figure 4) are molecules that inhibit oxidation, which is a chemical reaction that transfers an electron or proton from a substance to an oxidising agent (Diplock *et al.,* 1998). Free radicals are produced which are highly reactive chemicals that have the potential to harm cells, forming a chain reaction within the cell that damages or even kills the cell. Antioxidants, also known as free radical scavengers terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (Valko *et al.,* 2007; Hamid *et al.,* 2010). Antioxidant free radicals do not initiate the formation of free radicals due to the stabilization or delocalisation of radical electrons. Hydrogen donation to free radicals thereby forming stable radicals is shown in the reactions below (Figure 5).

$$R\bullet + AH \longrightarrow RH + A\bullet$$

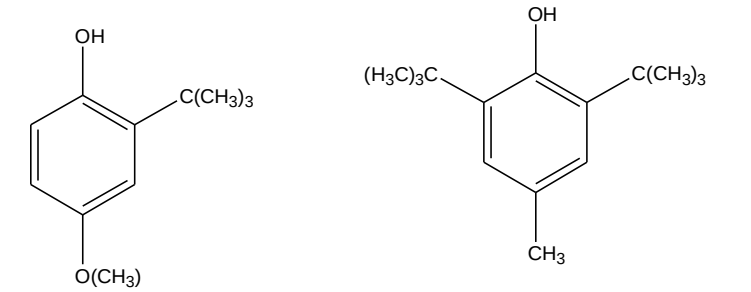
$$RO\bullet + AH \longrightarrow ROH + A\bullet$$

$$ROO\bullet + AH \longrightarrow ROOH + A\bullet$$

As a result, stable complexes are formed in between lipid radicals and antioxidant radicals.

$$R\bullet + A\bullet \longrightarrow RA$$

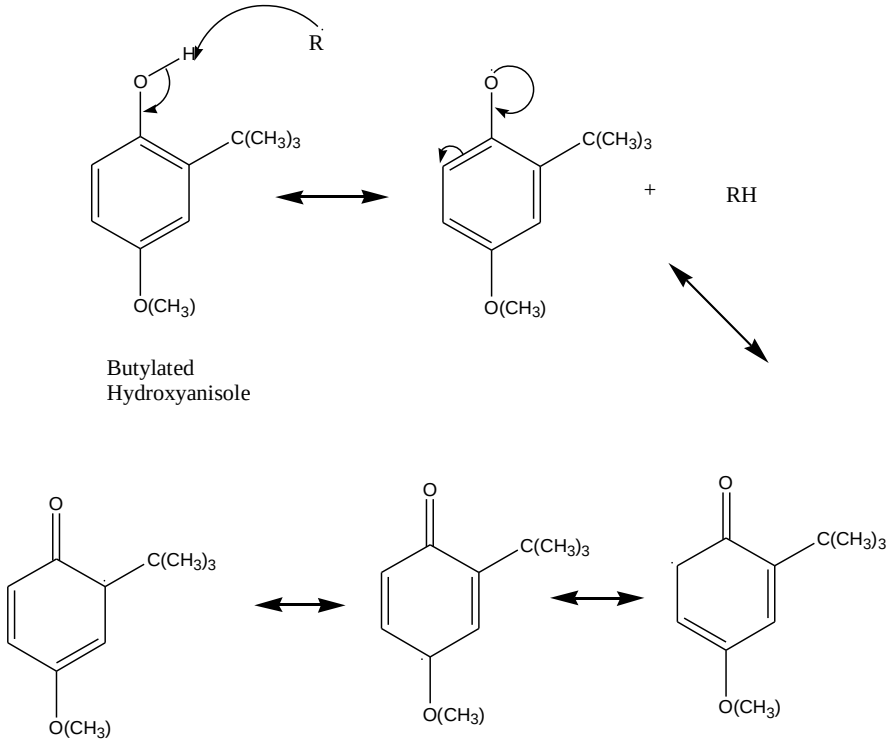
$$RO\bullet + A\bullet \longrightarrow ROA$$



Butylated hydroxyanisole (BHA)

Butylated hydroxytoluene (BHT)

Figure 4: Examples of antioxidant include BHA, ascorbic acid and BHT.



Butylated Hydroxyanisole

Figure 5: Mechanism of butylated hydroxanisole as antioxidant & radical stabilization.

Plants contain a variety of secondary metabolites that act as free radical scavengers such as phenolic compounds, terpenoids, flavonoids and nitrogen containing compounds. A variety of assays have been developed to measure the concentration of specific antioxidant including reducing power, chelating activity on Fe^{2+}, free radical-scavenging, total antioxidant, superoxide

radical scavenging, hydrogen peroxide scavenging and hydroxyl radical scavenging activities (Hussein, 2011). The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay is the most widely used assay due to its stability, sensitivity as well as simplicity (Prabhune *et al.*, 2013; Mandal *et al.*, 2009). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. The method is based on reduction of DPPH; a stable free radical, in the presence of hydrogen donating antioxidants to form non radical DPPH-H. DPPH•, gives a purple colour that changes to yellow when it is in the non-radical form. This change can be followed, spectrophotometrically at 517 nm.

2.5 Application of secondary metabolites in drug synthesis

Today, approximately two-thirds to three-quarters of the world's population are estimated to rely on medicinal plants as their primary source of medicines (Green *et al.*, 2011). As a result there has been an increasing interest in studying the biological properties of plants and their derivatives for discovering biologically active compounds (Green *et al.*, 2011). These compounds are divided into primary and secondary metabolites. Primary metabolites formed during the growth phase as a results of metabolism, they are essential for plant growth. Example includes ethanol, nucleic acid, and amino acids. While secondary metabolites are typically organic compounds produced through modification of primary metabolite phase. Phytochemicals may be classified as carotenoids, flavonoids, alkaloids and terpenoids.

2.6 Natural products from medicinal plants

Plants contain two classes of secondary metabolite, those that contain nitrogen atoms such as alkaloids, amides and glucosinolates and those that do not contain nitrogen atom such as terpenoids (monoterpenes, sesquiterpenes, triterpenes, sterols and saponins), phenolic compounds as well as flavonoids.

2.6.1. Flavonoids

Flavonoids are polyphenolic compounds with 15 carbon atoms (two benzene rings joined by a linear three carbon chain). They constitute some of the most characteristic classes of compounds in higher plants. Most flavonoids occur in flowering plants, but they are not restricted to flowers and are found in other parts of the plant as well (Rice-Evans *et al.,* 1996). Flavonoids possess a basic flavan moiety with two aromatic rings (A- and B-rings) interconnected by a three-carbon-heterocyclic ring (C-ring) (Park *et al.,* 2008). The most widespread flavonoids contain a double bond between C-2 and C-3 and a keto function at C-4 of the C-ring as shown in Figure 6.

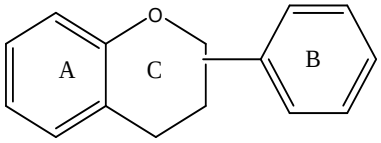


Figure 6: Basic skeleton of flavonoids

Recent studies have shown that flavonoids and related polyphenols contribute to antioxidant activity (Einbond *et al*., 2004). Fruits and vegetables are rich in flavonoids therefore humans should consume at least one hundred milligram of flavonoids per day (Rahman, 2011).

Flavonoids are classified according to the substitution in ring C yielding major groups such as chalcones, flavones, flavonol, flavanones, anthocyanins and isoflavonoids as shown in Figure 7.

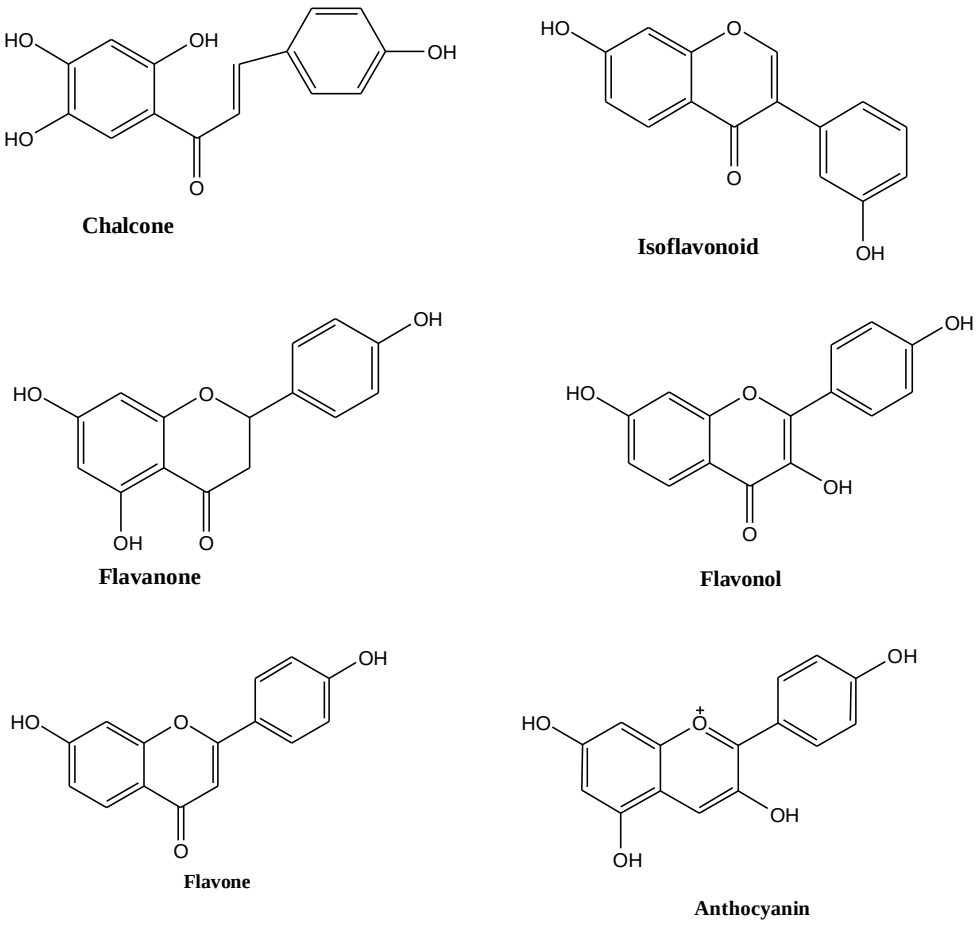
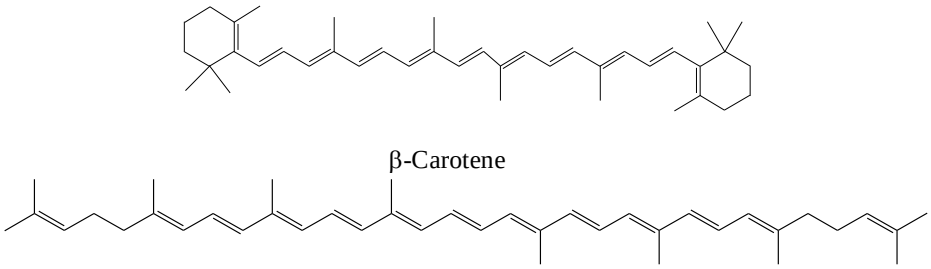


Figure 7: Constituents of flavonoids.

Knekt and co-workers (2002) suggested that dietary flavonoids are beneficial to health as they have a wide range of biological activities such as antibacterial, anti-inflammatory, anti-carcinogenic, antithrombotic and antioxidant activities.

2.6.2 Carotenoids

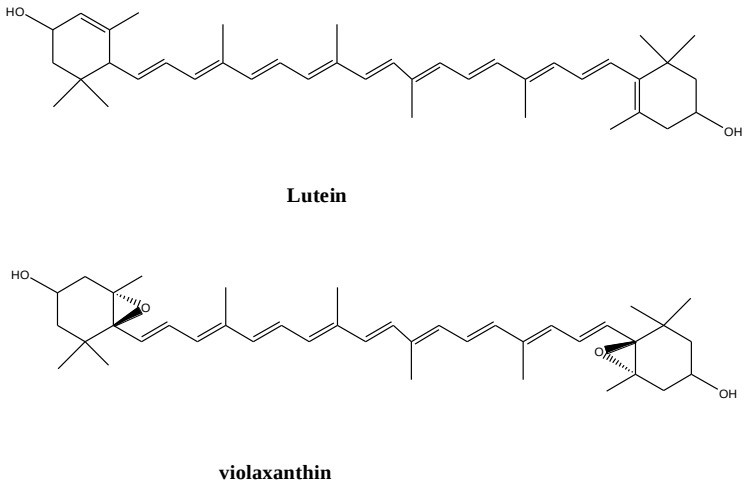
Carotenoids are pigments present in plants responsible for colours in fruits and vegetables. Carotenoids are classified as carotenes and oxocarotenes or xanthophyll. Carotene contains only hydrogen and carbon atoms, e.g β -carotene, γ-carotene and lycopene (Figure 8).



β-Carotene

Lycopene

Figure 8: Examples of carotenes found in plants.



Lutein

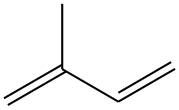
violaxanthin

Figure 9: Structure of Violaxanhin, a xanthophylls or oxocarotene

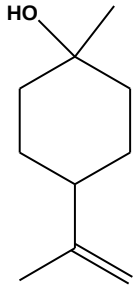
Oxocarotenes or xanthophylls are yellow pigments with molecular structures that are almost similar to those of carotenes except that they contain oxygen atoms. That makes them more polar than carotenes. They are found in leaves of many plants e.g. lutein and violaxanthin (Figure 9).

2.6.3 Terpenoids

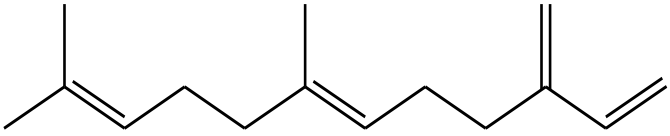
Terpernoids are volatile compounds mostly found in higher plants; they are used for perfumery, cooking, fuel transportation as well as medicines. Terpenoid is a plant constituent whose carbon skeleton is composed of isoprene units containing five carbon atoms. Most terpenes are hydrocarbons having molecular formulae $(C_5H_8)_n$ in a cyclic or acyclic, saturated or unsaturated structure. The classes of terpenoids are hemiterpenes (5C), monoterpenes (10C), sesquiterpenes (15C), diterpene (20C), triterpenes (30C), and tetraterpenes (40C) as shown in Figure 10.



Isoprene
(Hemiterpene)



β-terpineol
(Monoterpene)



Farnesene
(sesquiterpenes**)**

Figure 10: Classes of terpenoids and their examples.

2.6.4 Pentacyclic triterpenes

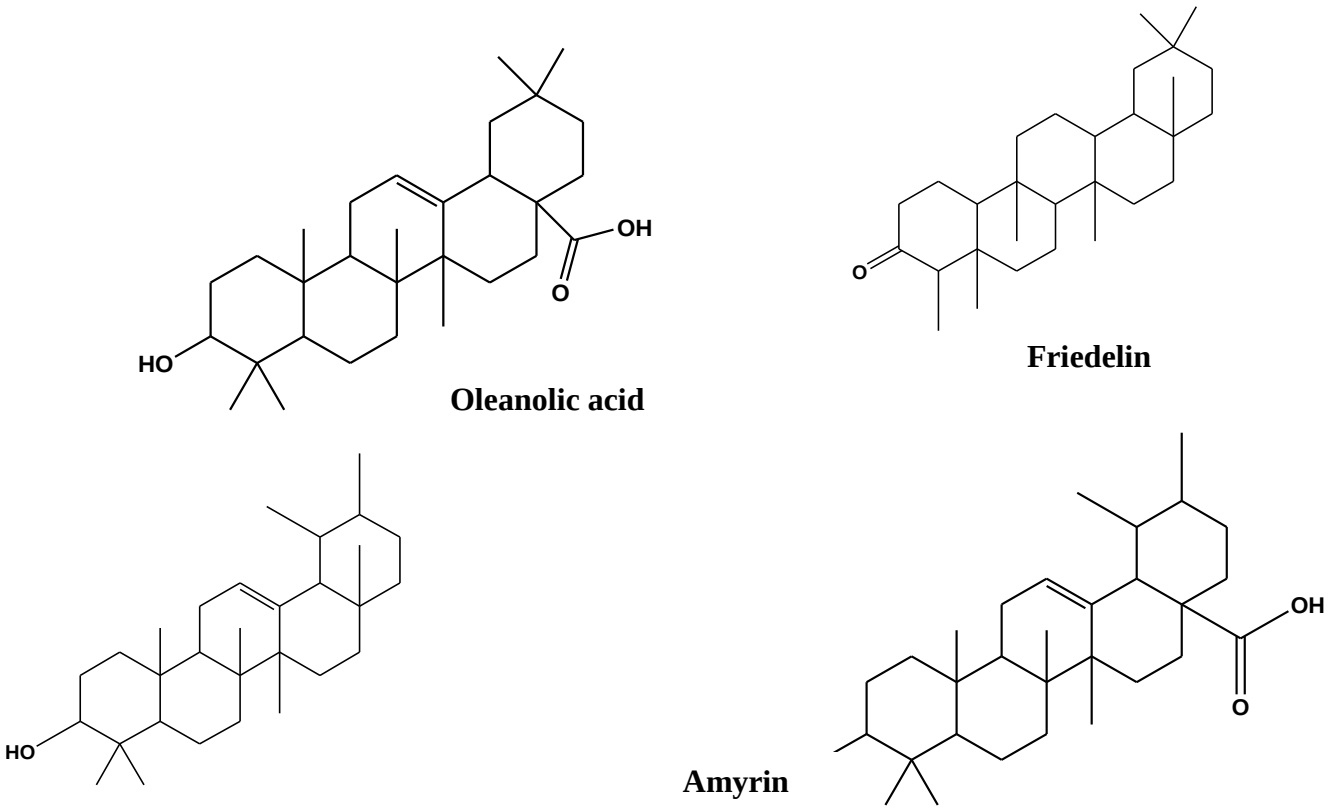


Figure 11: Structures of pentacyclic triterpenes; friedelin, amyrin, ursolic acid and oleanolic acid.

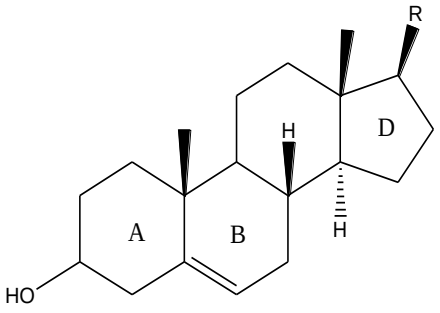
Pentacyclic triterpenes are secondary metabolites distributed in different parts of the plants worldwide. They are found in fruits, leaves and roots of some plants (Palaez *et al.,* 2013). Pentacyclic triterpenoids can be classified into several groups based on substitution in position C-3 and C-12 (see Figure 11) as well as oxidation to hydroxymethyl, aldehyde or carboxylic group. The class of compounds includes friedelin, amyrin, ursolic acid and oleanolic acid and its derivatives (Figure 11) have been proven to cure a wide range of diseases ranging from cancer to cardiovascular diseases (Sheng and Sun, 2011).

2.6.4.1 Biosynthesis of pentacyclic triterpenoids

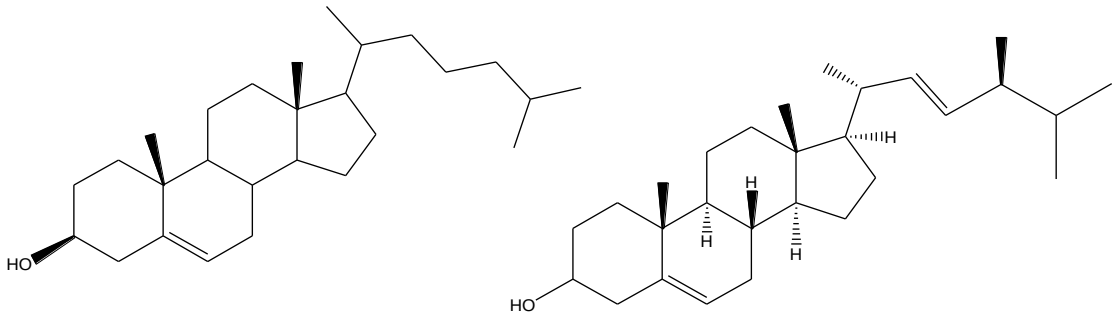
Pentacyclic triterpenoids basically are isopentanoids with 6 isoprene units (30C). The two most important types of triterpenes are derived by orientation of the precursor squalene epoxide in two distinct arrangements followed by condensation. One type leads to pentacyclic triterpenes and the other leads to steroids. They are derived from squaline via opening of squaline-2,3-epoxide and by cyclisation. Cyclisation yields dammareyl cation further undergoes 1,2-methyl and hydride shifts resulting in proton elimination.

2.6.5 Steroids

Steroids are essential components of the cell membrane. They are a class of organic compounds with a basic skeleton of 17C. Steroids include lanosterol, cholesterol, β-sitosterol, stigmasterol and campesterol (Figure 12).



Basic skeleton of steroid



Cholesterol **Stigmasterol**

Figure 12: Sterols commonly found in plants and animals.

2.6.6 Polyphenolic compounds

Polyphenolic compounds are naturally occurring compounds containing phenolic functionalities (Handque and Baruah, 2002). They are found in higher plants. Polyphenols are known to have a variety of biological and pharmaceutical activities. They are used as drugs to treat diseases such as AIDS, ulcers, bacterial infections and neural disorders (Tuckmentes *et al.*, 1999). Examples of hydroxycinnamic acid type of polyphenols include caffeic acid and *p*-coumaric acid, coumarin types of polyphenols include scopoletin and stilbenes type is resveratrol (Munin and Edwards-Le'vy, 2011).

2.7 *Sideroxylon inerme*

2.7.1 Botanical overview of the family Sapotaceae

Sapotaceae is a tropical family of forest trees, flowering plants and shrubs with much economic importance. The family has about 37-75 genera with over 1000 species (Yang, 1998, Alves-

Araújo and Marccus, 2013). *Sideroxylon* of the Sapotaceae was first reported in Taiwan by Forbes and Hemsley in 1889; thereafter several other scientists reported each species from the family (Forbes and Hemsley, 1889; Hayata, 1911; Matsumuru, and Hayata, 1906). In Brazil, the Sapotaceae are represented by 11 genera and 231 species, 86 of which have been registered in the region of Northeastern Brazil (Alves-Araújo and Marcus, 2013**).** Species of the family Sapotaceae are characterised by a huge diversity of secondary metabolites such as triterpenes, steroids, tannins, alkaloids and cyanogenic compounds (Beltrao *et al.*, 2008). Table 2 shows the different species, medicinal uses and specific compounds isolated from the family Sapotaceae.

Table 2: Different species, medicinal use and specific compounds isolated from the family Sapotaceae.

Species	Plant part used	Medicinal use	Compounds isolated	References
Manilkara hexandra	Stem bark	-fever -stomach disorder -flatulence	Saponins, Terpenoids, Cardiac glycoside, anthraquinone	Gopalkrishnam *et al.,* 2014
Mimusops elengi	Leaves	-HIV	flavanol glycoside, mearnsitrin.	Suedee *et al.,* 2014
Tridesmostemon omphalocarpoids Engl.	wood and stem bark	-gastroenteritis -skinlesion	Omphalocarpoidone, β-amyrin acetate, taraxerol , spinasterol, lichexanthone, epi-catechin, spinasterol-3-O-β-D-glucopyranoside, tormentic acid	Fru *et al.*, 2013
Chrysophyllum pruniforme	Stem bark	-coughs, -microbial infections -haemostatic agent	Phenols, flavonoids, tannins, saponins.	Angone *et al.,* 2013

Common phytocompounds previously isolated from the family include (1) α-amyrin, (2) gallic acid, (3) quercitrin, (4) myricitrin, (5) epigallocatechin, (6) epigallocatechin-3-O-gallate (Figure 13)

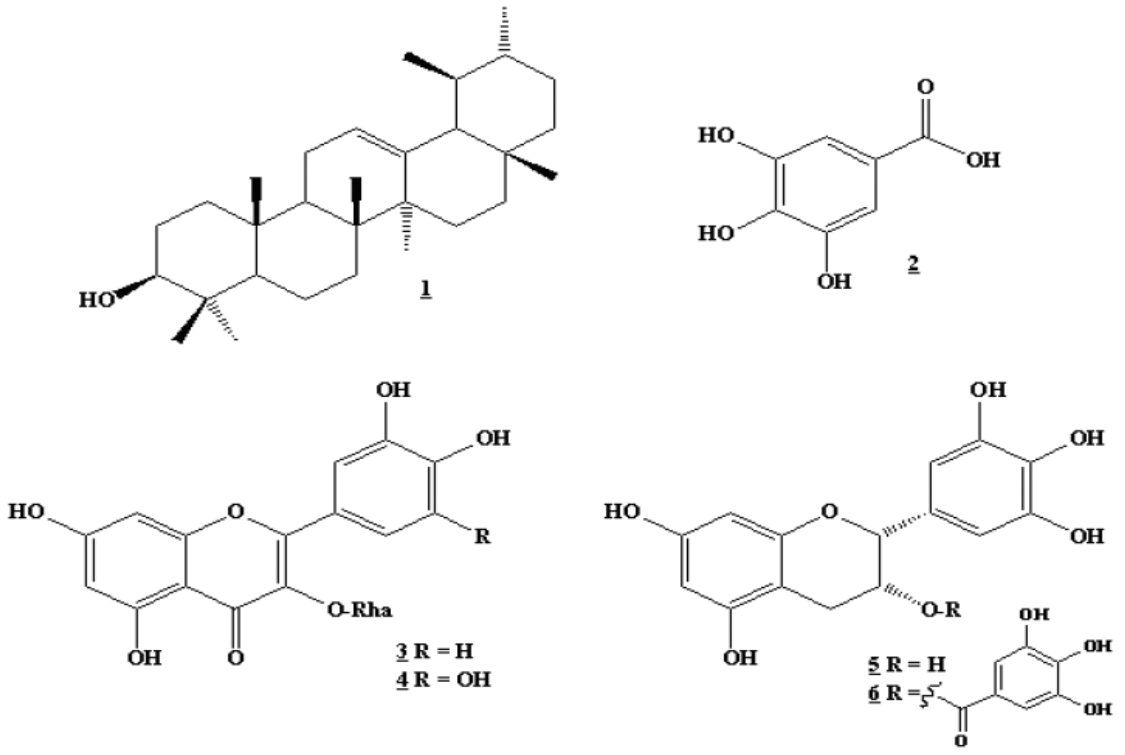


Figure 13: Compounds isolated from the plant family Sapotaceae: (1) α-amyrin, (2) gallic acid, (3) quercitrin, (4) myricitrin, (5) epigallocatechin, (6) epigallocatechin-3-O-gallate.

2.7.2 Genus: *Sideroxylon*

Sideroxylon is a genus of flowering plants, characterised by 70 species. It is distributed throughout Africa, Madagascar and Mascarene (Berry, 1916). Some species including *S. lanuginosum, S. tenax* and *S. lycloides* are found in the subtropic of North America. *S. inerme* is the only species found in South Africa with three subspecies. Species from the genus contain flavonoids and triterpenoid saponins (Erosa-Rejon *et al.,* 2010) (Table 3).

Table 3: Different species, medicinal use and specific compounds isolated from the genus *Sideroxylon.*

Species	Plant part used	Medicinal use	Compounds isolated	References
S. obtusifolium	Stem bark	-Pain -Anti-inflammatory	-	Beltrao *et al.,* 2008
S. foetodissimum	Leaf	-DNA interacting activity	Kaempferol-3-rutinoside, α-amyrin, β-amyrin, taraxasteril, stigmasterol	Erosa-Rejon *et al.,* 2010
S. inerme	Roots	-Stomach ache -Anti-inflammatory	Epigallocatechin gallate, procyanidin.	Van Wyk *et al.,* 1997

2.7.3 *Sideroxylon inerme*

S. inerme is a large evergreen tree that grows along the cost also known as White milkwood (Amasethole-amhlophe). It is used by traditional healers as a source of medicine (Van Wyk *et al.,* 1997). Its leaves are mostly alternate (Figure 14). It is characterised by the occurrence of milk sap and it contains blue-black grapelike fruits which ripens between November-April. *S. inerme* is divided into three sub-species; *S. inerme inerme* – found in Mozambique and South Africa, *S. inerme diospyroides* – found in Somalia, Kenya, Tanzania and Mozambique, and *S. inerme cryptophlebium* – found in Aldabra island.

Figure 14: Leaves and fruits of *S. Inerme.*

2.7.3.1 Medicinal uses of *S. inerme*

S. inerme is considered important due to their biological activity which includes antioxidant activity through various mechanisms of action including the scavenging of free radicals during the metabolism of normal cells. In South Africa, the Zulu and Xhosa people use the bark for different medicinal purposes; the stem bark and roots are used to treat fevers and broken bones (Watt and Breyer-Brandwijk, 1962).

2.7.3.2 Phytochemical studies of *S. inerme*

Despite the natural distribution of *S.inerme* in Africa and the importance of the plant to the local people, no in depth phytochemical or pharmacological studies have been done on this plant, except for preliminary screening which have shown the presence of two compounds epigallocatechin gallate(**1**) and procyanidin B1(**2**) (Figure 15)**.**

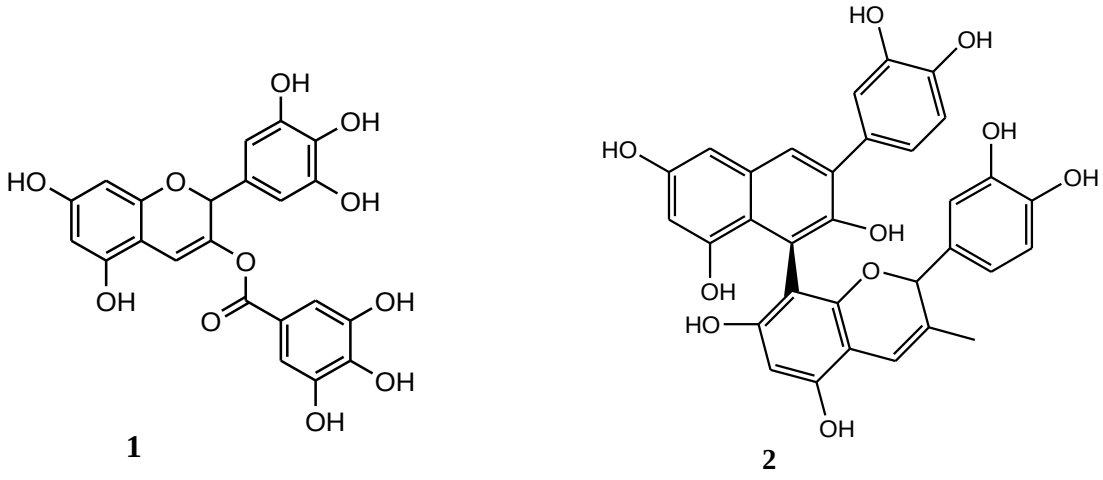


Figure 15: Compounds isolated from *S. inerme*; epigallocatechin gallate (1), procyanidin B1 (2).

2.8 Botanical overview of the plant family Euphorbiaceae

The family Euphorbiaceae is one of the biggest families of trees and shrubs with 280 genera and 8000 species. Generally, Euphorbiaceae is found in tropical regions. Species of this family are characterised by occurrence of milk saps which are believed to be toxic to cold blooded animals (Hecker, 1968).

2.8.1 Genus *Bridelia*

Table 4: Phytochemical constituents of *Bridelia* and their traditional uses.

Species	Isolated compounds	Traditional use	Part of plants used	References
B. monoica	Stigmasterol , sitosterol, friedelan-3-β-ol	Lack of appetite	Roots, stem and leaves	Watt and Breyer-Brandwijik, 196
B. grandis	Friedelin	Oral cavity affection purify breast	Bark and leaves	Ngueyem *et al.*, 2009
B. micrantha	Taraxerol, friedelin, gallic and ellagic acid	Painful joints, diabetes mellitus, retained placenta, headache,	Bark, root, leaves	Watt and Breyer-Brandwijik, 196
B. balansae	Bridelone, Balansenate, bridelonine	Bronchitis	leaves	Ngueyem *et al.*, 2009
B. ferruginea Benth		Diabetes mellitus, skin diseases and eruptions	Leaves and roots	Ngueyem *et al.*, 2009

Approximately 60 species *Bridelia* are found throughout tropical and subtropical regions of the world, dominating in Africa and Asia. Several *Bridelia* species are used in medicine as anti-amoebic, antiemetic, antibacterial, anti-convulsant, anti-diabetic, anti-diarrhoeal, anti-helminthic, anti-inflammatory, antimalarial, gynaecological and sexual diseases (Ngueyem *et al.*, 2009). *Bridelia* species generally occur along the sea; about 50 species are distributed in Tropical Africa, Madagascar, Yemen and Asia. Currently about 119 subspecies of *Bridelia* are recognised from the genus. Some of the species with their traditional uses are represented in Table 3.

2.8.2 *Bridelia micrantha*

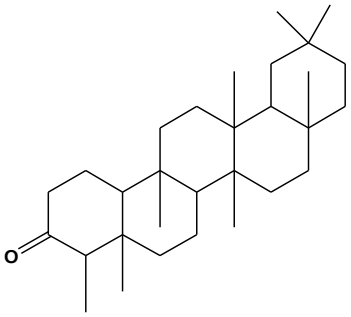
Bridelia micrantha (Coastal Gold Leaf) is a fast growing, beautiful shade tree for large gardens. It is found in coastal, riverine and swamp forests, usually in moist places in the Eastern Cape, KwaZulu-Natal and Limpopo Province (Murgananthan, 2012). *B. micrantha* belongs to the family Euphorbiaceae which is a large family of herbs and trees characterised by different features such as leafless branches and glands at the leaf base. The tree can grow up to 20 m tall, with a dense rounded crown; leaves are generally large, simple and alternate (Figure 16) (Nwaehujor *et al.*, 2011). Different plant parts have been employed for the treatment of different sicknesses.

Figure 16: *B. micrantha* growing along the coast and their leaves.

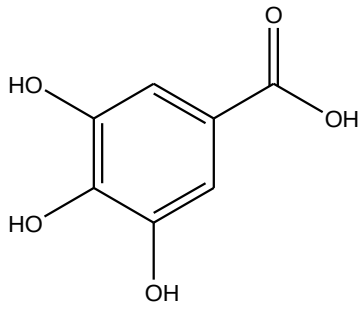
2.8.2.1 Medicinal uses of *B. micrantha*

Different plant parts of *B.micrantha* are used traditionally to treat a variety of ailments; the bark is used to treat burns, wounds, venereal diseases, tapeworm, and toothache. Zulu traditional healers also use *B. Micrantha* as an anti-diarrhoeal agent and against different pathogenic microorganisms that can cause diarrhoea (Lin, 2002). The leaf sap is mainly used to treat sore eyes. The roots are used to treat stomach pains such as gastric ulcers. The roots can also be powdered and mixed with fat or oil and rubbed into the head to cure headaches (Murgananthan, 2012). Herbalists of Western Nigeria use a stem bark decoction to prolonged pregnancy to full term (Ngueyem, 2009). They also use the bark for stomach and intestinal complaints. Biological tests have been conducted on the plant. According to Mainen and Moshi (2012), the methanol extract of the stem bark proved to inhibit *Helicobacter pylorin (*Mainen *et al.*, 2012).

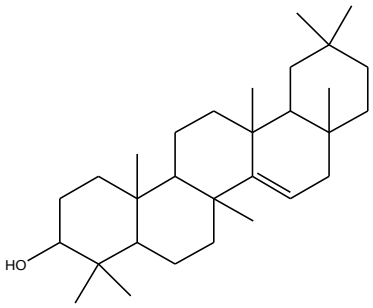
2.8.2.2 Phytochemical studies of *B. micrantha*



Friedelin



Gallic acid



Taraxerol

Figure 17: Compounds previously isolated from *B. micrantha*

Phytochemical and characterisation of the plant shows the presence of different secondary metabolites such as alkaloids, flavonoids, tanins, saponins, terpenes, terpenoids, sterols specifically taraxerol, friedelin, gallic and allagic acid (Pegel & Rogers, 1968)**.** Two major constituents were isolated from the stem bark; taraxerol and friedelin (Figure 17). Gallic acid and ellegic acid were isolated from the ether and acetone extract.

2.9 Soil analysis

Soil being the top layer of the earth's surface is defined as a heterogeneous mixture of air, water, inorganic solids, organic solids and microorganisms. The interactions of these mixtures influence plant growth. The breakdown of dead organic matter or of inorganic solids by the reaction of carbon dioxide and water, results in the release of nutrients (Donald, 2003). This promotes roots growth as a result increases nutrient uptake by plants. Soil is influenced and modified by living organisms. Plants and animals enhance the modification of soil through addition of organic matter which is decomposed by bacteria and fungi (in the presence of oxygen and water) into a semi-soluble biochemical substance known as humus. Humus plays a vital role in plant growth such as:

- It enhances the soils ability to hold and store moisture
- It reduces the level of soluble nutrients from the soil profile
- It acts as a primary source of carbon and nitrogen
- It improves soil structure which is important for plant growth

Soil consists of 4 layers (referred to as soil horizons) (Figure 18) they differ in terms of their physical properties such as structure, density, porosity, texture, colour and conductivity. The upper layer consists of undecomposed material, the layer below that is formed by most decomposed matter. Soil quality can be affected by a variety of factors such as low rainfall, high clay content, steep slope, cold temperatures, erosion, humidity, bedrock and deposition.

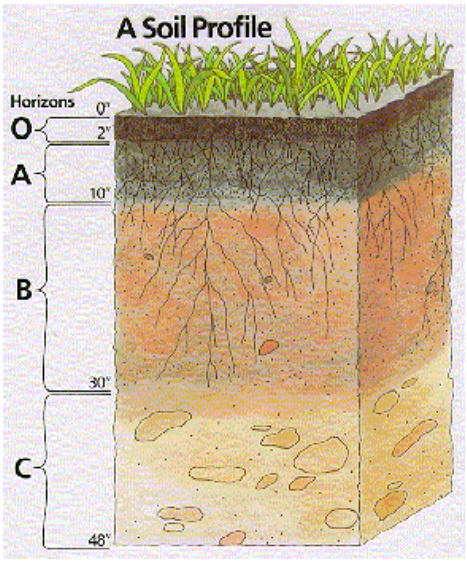


Figure 18: Soil profile.

(www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/office/ssr7/tr/?cid=nrcs142p2_047970)

2.9.1 Soil colour

Soil colour is generally derived from iron oxides and organic matter that coat the surface of the soil particle (Table 5)

Table 5: Implications of different colours on the soil attribution.

Soil colour	Soil attribution
Brown to black	Accumulation of organic matter
Black	Accumulation of Mn
Yellow to red	Fe^{3+} well aerated soil
Grey to blue	Fe^{2+} poor drained soil
White to grey	Accumulation of salt

2.9.2 Soil Organic matter (SOM)

Soil organic matter (SOM) is defined as the soil component consisting of plant and animal residue at different stages of decomposition; this involves cells and tissues of soil organisms, and well-decomposed substances (Moodley *et al.,* 2012). Organic matter acts as a reservoir of nutrients and water in soil, it increases compaction and surface crushing thus increases water infiltration into the soil. Organic matter can be influenced by variety of factors such as climate (temperature and rainfall), vegetation, soil texture, rate of decomposition, parent material and landscape. Temperature changes influence the amount and type of vegetation and the rate of decomposition hence amount of organic matter. Organic matter increases with increasing decomposition.

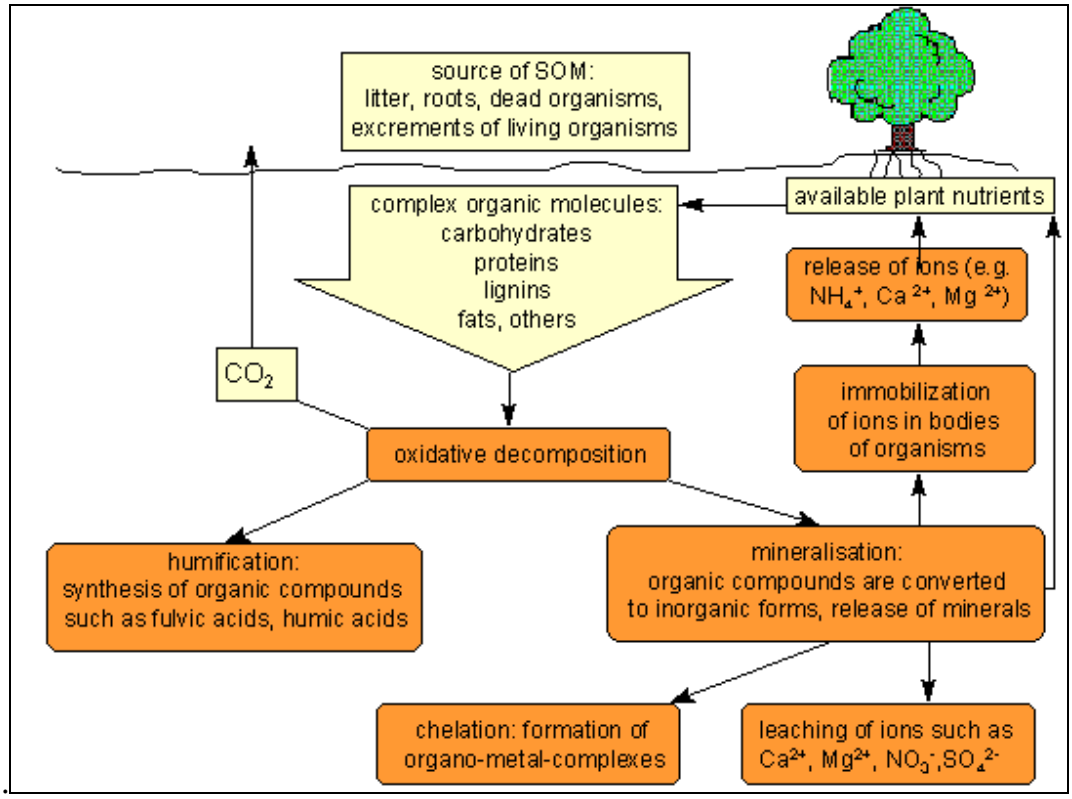


Figure 19: Basic nutrient cycle of formation of organic matter in the soil.

(http://soils.ifas.ufl.edu/faculty/grunwald/teaching/eSoilScience/organic.shtml)

Figure 19 shows how dead or living organisms are decomposed by microorganisms into minerals which can either leach as Ca^{2+}, Mg^{2+}, or chelate to form organic complexes or stick to the body of organisms and release them as ions to the surface of the soil, making them available to the plant. There are many methods for determination of SOM in soil; the Walker-Black method is one of the easiest and most reliable methods.

2.9.3 Determination of SOM by Walkey-Black method

This is one of the best methods used for determination of SOM. The method involves oxidation of organic matter in soil using dichromate ions which are added in excess therefore a known amount of reagent reacts with the organic matter in soil (Walkey & Black, 1934). The unreacted reagent is then obtained by back titration of a known amount of ferrous solution, as shown in the reactions below:

a. Dichromate ion reacts with carbon in soil as follows:

$$2Cr_2O_7^{2-} + 3C^0 + 16H^+ \rightarrow 4Cr^{2+} + 3CO_2 + 8H_2O$$

b. Ferrous ion reacts with dichromate as follows:

$$6Fe^{2+} + Cr_2O_7^{2-} + 14\ H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$$

2.9.4 Soil pH

The pH measures the concentration of hydrogen ions (H^+) in soil solution. The pH ranges are 1-14 of which a pH below 7 is considered acidic, pH of 7 neutral and pH above 7 basic/ alkaline. Soils with a relatively large concentration of hydrogen ions tend to be acidic. Alkaline soils have

a relatively low concentration of hydrogen ions. Soil pH plays an important role in determining the amount and strength of adsorption of nutrients on the soil surface. It also plays a role in the dissolution and precipitation of certain elements making them available for plant uptake, thus controlling the plant growth by controlling availability of essential nutrients and the concentrations of toxic elements. At low pH values (less than 5.5), many nutrients become very soluble and are leached from the soil profile. Elements such as Al and Mn maybe present in toxic concentrations while essential elements such as Ca, Mg, P, Mo and Si maybe deficient (Clark, 1997; Foy *et al.*, 1992). In moderately alkaline soil Mo and the availability of macronutrients increases. At high pH, nutrients become insoluble and plants cannot readily absorb them. Soil fertility is directly influenced by pH through the solubility of many nutrients. Maximum fertility occurs in the range of 6.0-7.2 (Figure 20).

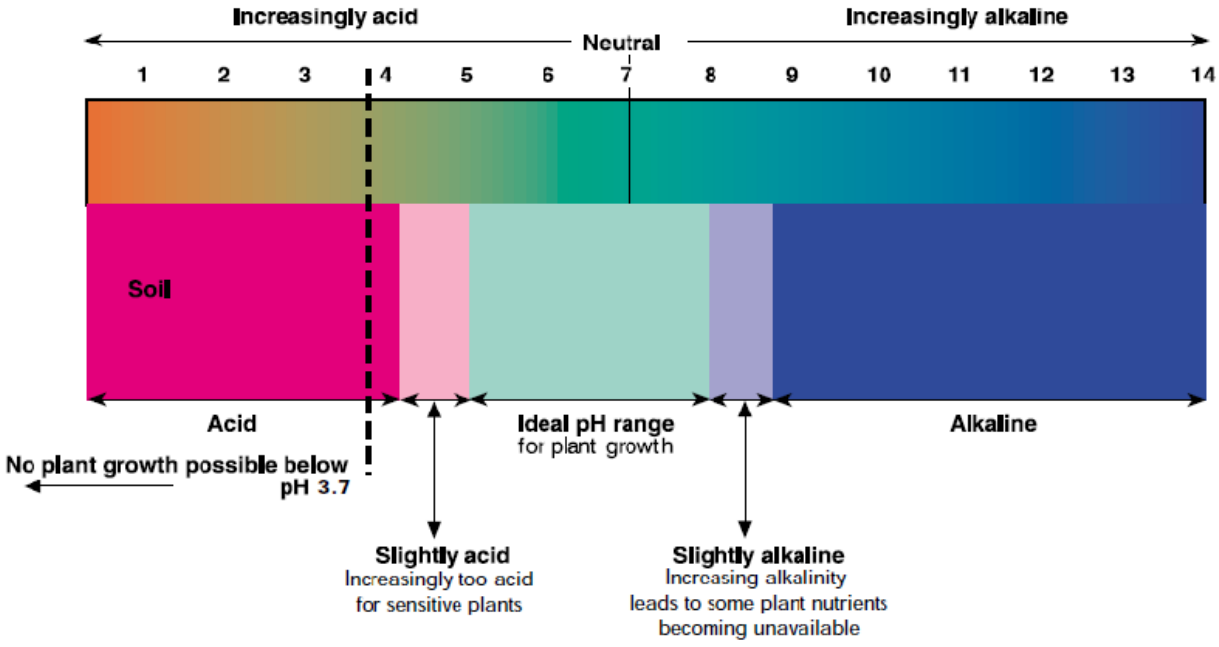


Figure 20: Plant growth and pH scale.

2.9.5 Salinity

Salinity is the measure of salt content in the soil. The salt can either come from irrigation or fertilizers. The ions responsible for salination include Na^{+}, Ca^{2+}, K^{+}, Mg^{2+} and Cl^{-}. Salinity is generally caused by irrigated soil due to high rates of evaporation and low rainfall, leaving salt to accumulate.

2.9.6 Cation Exchange Capacity (CEC)

The cation exchange capacity (CEC) is a measure of the quantity of negatively charged sites on soil surface that can retain positively charged ions such as Ca^{2+}, Mg^{2+} and K^{+} (Chapman, 1965). CEC is mainly influenced by soil pH; as pH increases, the number of negatively charged sites increase hence increasing the exchange capacity. Other factors include SOM and concentration of Fe, Al and Mn oxides. Exchangeable metals include Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and Al^{3+}.

2.9.6.1 Determination of CEC by Chapman method

CEC reflects soil ability to retain cations available for plant uptake, soil with high CEC may not necessary be more fertile because a soil's CEC can also be occupied by acid cations such as hydrogen (H^{+}) and aluminium (Al^{3+}). The method is not applicable to soils containing high amount of vermiculite clays, kaoli and halloysites. The method involves saturation of the cation exchange sites with ammonium acetate and removal of the excess ammonium ions with ethanol, replacement and leaching of exchangeable ammonium with protons from HCl acid (Horneck *et al.*, 1989).

2.9.7 Total and bioavailable or exchangeable metals in soil

Soils consist of different elements among which are trace and heavy metals. Some of these metals are the results of weathering of the parental rock which is generally made of elements such as calcite ($CaCO_3$), magnesite ($MgCO_3$) and dolomite ($CaMg(CO_3)_2$). Trace elements are those that occur at low concentrations, while heavy metals are those that have densities greater than 5 and molecular weights of 23 and above. These elements in soil are affected by substances they are binding to, hence affecting their bioavailability to the biota.

The total metal concentration is defined as the total amount of metals in soil after digesting with a strong acid while the bioavailable or exchangeable metals are those that are available to be absorbed by the organism (Moodley *et al.,* 2012). According to research conducted by Bettinelli and co-workers (2000), microwave-assisted acid solubilisation has proven to be the most suitable method for the digestion of complex matrices such as soils and sediments containing oxides, clay, silicates and organic substances. Determination of total metals in soil assists in collecting information on the genesis of the soil and on the level of contamination. At present, there are various official methods which employ different acid mixtures and microwaves for digestion of soils. Amongst the important methods of determination of total metals in soil is the use of closed vessel microwave digestion using nitric acid. The procedure allows for shorter digestion periods, using smaller quantities of acid, reduces the risk of contamination, gives good recoveries of all volatile elements and achieves complete digestion.

2.9.8 Heavy metals in soil

It is known that some elements from the soil are essential for plant growth and development such as Zn, Fe, Mg etc.; however plants accumulate heavy metals in soil that are not essential for their growth e.g. Cd, Ni, Cr and Pb. These metals are released in the environment by both natural (chemical and physical weathering) and anthropogenic (mining and industrial activities) sources. Concentrations of heavy metals in the soil play a vital role in controlling the availability of ions to plants. Heavy metals are those metals with an atomic weight greater than 5 amu with the properties of a metallic substance at room temperature. Some of these metals (Cu, Fe, Mn, Ca, Mo, Zn and Co) are required by living organisms. These metals may be detrimental to human health if at higher concentrations than required. Accumulation of toxic metals from the soil over time cause serious illness.

Table 6: Chemical form of metals in solids (Gunn *et al*., 1988; Salomons, 1995).

	FRACTIONS	**MOBILITY**
TOTAL	Dissolved in pore water	High
	Exchangeable-weakly adsorbed	High
	Associated with carbonate	High
	Associated with Fe, Mn oxide	Medium
	Complexed by organics	Medium
	Associated with sulphide	Low
	In the mineral lattice	Low

Heavy metals are introduced into soil either from soil parental material (lithogenic source), natural sources (volcanic eruptions) and anthropogenic sources such a metal smelting. Metals in soil are divided as total or available (mobile). Total concentrations are the total concentration of metal that are present in the soil such as ions bound in minerals such as clays, oxides and carbonates, those bound in organic matter and free ions and soluble organic and inorganic complexes in soil solution. Bioavailable or mobile metals are those that are available to be taken up by plants. Metals such as those dissolved in water, those that are weakly adsorbed (Table 6) and those that are associated with carbonates are readily available to be taken up by plants.

2.10 Geoaccumulation index (*Igeo*)

Soil contains heavy metals which are introduced either due to anthropogenic or natural causes. Heavy metals have a great ecological significance attributing to their toxicity and accumulation behaviour. Water sediments and biota are good reservoirs of heavy metals (Rabee *et al.,* 2011). The presence of metals in the soil is not indicating the contamination but relates to the geology of the parental material from which the soil was formed (Reddy *et al.,* 2014). The level or degree of contamination can be determined in many ways; the common ones are geoaccumulaion index and enrichment factor. Geoaccumulation index has been widely utilised as a measure of pollution in sediment by comparing current metal contents with pre-indusrtial levels (Muller, 1986). The geoaccumulation index can be determined by the following equation (Muller, 1969).

$$Igeo = \frac{Cn}{1.5 \times Bn}$$

Where:

Cn – Concentration of heavy metal

Bn - Geoaccumulation background value

1.5 - Possible variation of the background data due to lithological variation.

The degree of pollution is divided into 7 levels (Table 7), level zero indicates less contamination in the soil and level 6 indicates extreme contamination.

The Igeo is used to determine the degree of anthropogenic influence on heavy metal concentration in sediments or soil.

Table 7: The degree of metal pollution in the seven enrichment classes (Stoffers *et al.*, 1986).

I geo value	**Igeo Class**	**Soil quality**
0	0	uncontaminated
0-1	1	Uncontaminated to Moderately contaminated
1-2	2	Moderately contaminated
2-3	3	Moderately contaminated to strong contaminated
3-4	4	Strongly contaminated
4-5	5	Strongly to extremely contaminated
>5	6	Extremely contaminated

2.11 Elemental uptake from the soil to the plant

Nutrients from the soil are taken up by plants in three pathways: mass flow, diffusion and root interception. There are numerous factors that affect elemental uptake by plants, factor such as concentration of elements, soil parameters (SOM, CEC, Temperature and pH) (Pongrac *et al.*, 2007). The elemental uptake depends on the mobility and transport capacity of nutrients. Plants have the ability to increase metal concentrations with reference to the soil (those plants are referred to as bioaccumulators). Other plants have the ability of altering the elemental uptake to meet their nutritional demands (those plants are referred to as extruders).

2.12 Plant nutrition

Plants need certain elements for them to grow; there are 15 essential elements that plants must have in order to grow properly. Three basic elements H, O and C are obtained from the atmosphere through photosynthesis and N, P, K, S, Mg, Ca, Fe, B, Mn, Zn, Mo and Cu are obtained from the soil. Nutrients are defined as essential elements or chemicals that are required in the body of a living organism for metabolism to take place (Moodley *et al.*, 2012). They are required at specific amounts. Those that are required in large amount (usually 50 mg Kg^{-1}) are called macronutrients, and those that are required in extremely small amounts (less than 50 mg Kg^{-1}) are referred to as micronutrients.

Macronutrients are further classified into primary (N, P and K) and secondary macronutrients (Ca, S and Mg); they are major nutrients usually obtained from the soil because plants use large amount for their growth and survival. Secondary macronutrients such as sulfur are obtained from decomposition of SOM and Ca and Mg are obtained from liming acidic soil. For normal growth

and development plants need correct ratios and levels of nutrients. Inappropriate nutrient supply results in metabolic disturbances.

2.13 Bioaccumulation and Bioaccumulation factor (BAF)

Accumulation refers to the tendency of pollutants to concentrate as they move from one trophic level to the next, increase in concentration of a pollutant from the environment to the first organism in a food chain. Lead is one of the most toxic metals to human health and it has been released into the environment by anthropogenic causes and accumulates in terrestrial and aquatic environments (Rezvani and Zaefarian, 2011). Cadmium is known to cause a wide variety of toxic effects when taken up by plants. It inhibits plant physiological processes including oxidative reactions and nitrogen metabolism (Rezvani and Zaefarian, 2011).

2.14 Essential elements to humans

Essential elements, also known as dietary minerals, are elements required by living organisms. They are required in either larger (macronutrient) or small (micronutrient) quantities. Macronutrients include N, C, K, S, Na, Ca and Mg. Micronutrient elements play a very important role in keeping the body functioning effectively. Metals like, Fe, Cu, Zn and Mn are essential metals since they play an important role in biological systems, whereas Pb and Cd are nonessential metals as they are toxic even in trace amount.

Fe in the body is found in haemoglobin which is responsible for transportation of oxygen from the lungs to the rest of the body (Davidson *et al.*, 1995). Copper along with amino acids and vitamins are required for normal metabolic processes. The body cannot synthesise Cu, humans

rely on the diet for the supply of Cu. At higher concentrations even essential nutrients can lead to poisoning.

Chromium generally occurs in two forms or oxidation states, trivalent or hexavalent. Inhaling the hexavalent form can irritate the skin and is carcinogenic. Long term exposure can cause cancer and liver damage (Frossard *et al.*, 2000).

Magnesium is essential for nerve impulses and muscle contraction. Calcium contributes to about 2% of the body weight and is used mainly in strengthening bones and teeth.

Zinc is essential for the normal growth of genital organs, healing wounds and it plays a role in managing the hormone insulin which treats diabetes (Davidsson *et al.,* 1995). Table 8 and Table 9 show the nutritional levels for most individuals.

Table 8: The upper intake levels (ULs) of elements for most individuals (Food and Nutrition Board, Institute of Medicine, National Academies, 2011).

Age	As **(µg d^{-1})**	Ca **(mg d^{-1})**	Cu **(µg d^{-1})**	Fe **(mg d^{-1})**	Mg (**mg d^{-1})**	Mn **(mg d^{-1})**	Ni **(mg d^{-1})**	Se **(µg d^{-1})**	Zn **(mg d^{-1})**	Cr **(µg d^{-1})**
15-50	ND	3000	8000	45	350	10	1	400	35	ND
50	ND	2500	10000	45	350	11	1	400	40	ND

ND – Not determinable

Table 9: Dietary Reference Intake (DRIs): Recommended intakes for intake for individuals (Food and nutrition Board, Institute of Medicine, National Academies, 2011).

Life stage group	**Ca (mg d^{-1})**	**Cr (µg d^{-1})**	**Cu (µg d^{-1})**	**Fe (mg d^{-1})**	**Mg (mg d^{-1})**	**Mn (mg d^{-1})**	**Se (µg d^{-1})**	**Zn (mg d^{-1})**
Children	800	15	440	10	130	1.5	ND	5.0
Adults	1000	35	900	8	420	1.9-2.2	55	11

2.15 Determination of protein by Kjeldahl method

This is a standard method for quantitative determination of nitrogen in a substance. This method was developed by John Kjeldahl in 1883 (Bremner, 1960). The method involves three basic steps; 1) Heating the sample with sulphuric acid to break down organic substances and oxidises nitrogen to ammonium sulphate; 2) the solution is then distilled with sodium hydroxide from ammonium sulphate to ammonia into a trapping solution, 3) quantitative determination of ammonia by back titration with a standard solution (Douglas *et al.*, 2000). Chemical reactions are shown below:

Degradation:

$$\text{Sample} + H_2SO_4 \rightarrow (NH_4)_2SO_4\,(aq) + CO_2\,(g) + SO_2\,(g) + H_2O\,(g)$$

Liberation of ammonia:

$$(NH_4)_2SO_4\,(aq) + 2NaOH \rightarrow Na_2SO_4\,(aq) + 2H_2O_{\,(l)} + 2NH_3\,(g)$$

Capture of ammonia:

$$B(OH)_3 + H_2O + NH_3 \rightarrow NH_4^+ + B(OH)_4^-$$

Back-titration:

$$B(OH)_3 + H_2O + Na_2CO_3 \rightarrow NaHCO_3(aq) + NaB(OH)_4(aq) + CO_2(g) + H_2O$$

2.16 Extraction method

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. They involve the separation of medicinally active portions of plants or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

2.17 Chromatography techniques

Chromatography is an analytical technique used for separation, identification and determination of chemical components in complex mixtures. Chromatography has been applied to a variety of systems and techniques, and all the methods have in common the use of a stationary and mobile phase in common. Components of a mixture pass through a stationary phase by the flow of either a gaseous or liquid mobile phase; separation is based on the interaction of the components with the stationary phase, the component with strong interactions will be slower than those with weaker interactions.

Examples of the application of chromatographic methods are:

- Purification of mixtures in synthetic reactions
- Checking the progress of a reaction
- Separation of secondary metabolites from plant extracts
- Purification of bio-molecules such as proteins
- Analysis of environmental samples

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2.17.1 Column chromatography

Column chromatography (CC) separates mixture of compounds based on their polarity. The technique uses silica gel (SiO_2) or alumina (Al_2O_3) as the stationary phase that is placed in a vertical glass column. The mixture to be analysed is placed on the packed column.

Figure 21: Column chromatography packed with silica gel.

The solvent forming the mobile phase is poured from the top of the column and flows down the column (Figure 21). As the solvent flows, it elutes the sample through the column, and the components of the mixture begin to flow at their respective rates down the column. This technique needs large volumes of solvent and any careless additions can cause disturbances to the stationary phase, leading to poor separations.

2.17.2 Thin layer chromatography

This is one of the most inexpensive and simple techniques that involve the use of glass coated with silica. The technique is used to determine the purity of isolated or synthesised compounds; it is also used to test the progress of a chemical reaction. The silica-coated plate (Figure 22) acts as a stationary phase and is placed in a chamber containing organic solvents of different polarities. The solvent moves up and carries a molecule of the solution to be detected at different rates. The ratio of the distance molecule travelled to the distance that the solvent travelled is called the retardation factor (R_f) which is calculated as follows:

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the mobile phase}}$$

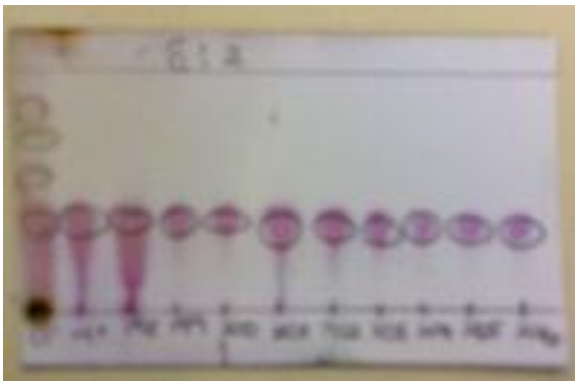

Figure 22: TLC plate.

Compounds with equal R_f values are considered the same and a single spot means that the fraction that was purified is clean.

2.17.3 Gas chromatography

Gas chromatography (GC) is a qualitative and quantitative method of separating components of a solution (Skoog *et al.*, 2004). The method separates components based on their boiling points and relative affinity for the stationary phase. Gas chromatography differs from other chromatographic techniques in that the mobile phase is a gas; components are separated as a vapour. The higher the component's affinity for the stationary phase, the slower it comes off the column. The components are detected and represented as peaks on the chromatogram. GC is made up of three important components, injector port, column and detector (Figure 23).

GC deals only with gaseous substances, if the sample to be analysed is non-volatile, they undergo the process of derivatisation which is a modification of a compound to produce a new compound which has properties that are suitable for analysis using a GC. Derivatisation increases the stability of compounds that undergoes thermal decomposition and it enhances sensitivity for electron capture detection. It also helps in separating sample peaks from solvent peaks. Types of derivatisation include silylation, alkylation and acylation.

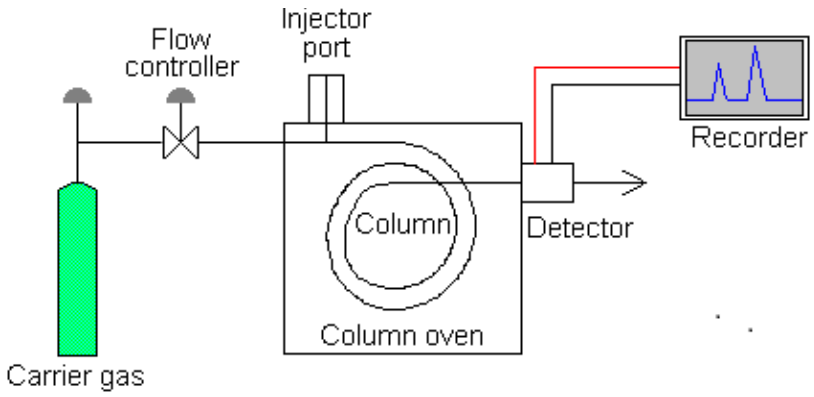

Figure 23: Components of gas chromatography.

The sample is injected in the injector port which is 50^0C higher than the boiling point of the least volatile component of the sample. Therefore the aerosols are transported to the column via inert gas. The column is located inside the oven to maintain a constant temperature. The components are thereafter sent to the detector. There are a variety of detectors that are used to identify the compounds isolated. Mass spectrometry (MS) is the most reliable detector. MS is used to identify various components from their mass spectra. Each compound has a unique or near unique mass spectrum that can be compared with mass spectral databases and thus the compound can be identified.

2.17.3.1 Limitations of the instrument

- Sample must either be volatile or capable of derivatisation.
- If the sample is not volatile (as in headspace, pyrolysis, or direct probe), then the analysed material must be volatile.

2.18 Microwave digestion

Microwave ovens (Figure 24) began to find widespread use in chemical laboratories in the late 1980's. The use of laboratory microwave units has become increasingly popular because of the improvement in chemical reaction rates that are possible using microwave radiation. A typical microwave acid digestion can be completed in a matter of minutes, whereas the same conventional hot plate digestion can take hours. Microwave digestion usually involves placing a sample in an acid solution and heating to high temperatures and pressures.

Figure 24: Microwave (Mars 6) Digester at the University of KwaZulu-Natal.

Microwave digestion systems have several advantages over open system such as:

- Simultaneous heating of more than 6 samples in less than an hour
- Consumption of less acid than open systems
- Microwave can withstand higher temperatures up to 300 °C to make sure complete digestion is achieved.
- There is no loss of elements by volatilisation
- There is no contamination from external sources.

Microwave digestion systems have one disadvantage which is the explosion and cracking of the vessels due to pressure build up along with increasing temperatures. However, microwave systems have built-in temperature and pressure sensors.

2.19 Inductively coupled plasma-optical emission spectrometry (ICP-OES)

Inductively Coupled Plasma-Optical Emission Spectrometry **(**ICP-OES) is one of the most popular analytical techniques for qualitative and quantitative determination of trace elements in samples and involves the use of an ICP-Optical Emission Spectrometry (Figure 25). The technique is normally based on emission of photons from atoms and ions that have been excited. Liquid samples are injected directly into the instrument while solid samples are to be extracted or acid digested so that the analyte is present in solution (Skoog *et al.,* 2004)

The sample is converted to an aerosol by high velocity gas and directed into the plasma where a gaseous mixture that is highly energetic excites atoms into ions (cation or anions). A plasma sustains a temperature of 10 000 K to remove the solvent from the aerosol leaving the sample as microscopic salt particles (Boss and Fredeen, 1997). After removing water the aerosol is vaporised. Analyte elements are liberated as free atoms in the gaseous state. Energy is often available to convert the atoms to ions (ionisation) and subsequently promote the ions to an excited state (excitation). In order for an atom or ion to emit its characteristic radiation, one of its electrons must be promoted to a higher energy level through an excitation process.

The excited atoms and ions emit their characteristic radiation which is collected by a device that sorts the radiation by wavelength. Thus the wavelength of the photons can be used to identify the elements from which they originated. The total number of photons is directly proportional to the concentration of the originating element in the sample.

Figure 25: ICP-OES at the University of KwaZulu-Natal**.**

2.19.1 Advantages of ICP-OES

- Multiple elements analyses
- Low detection limits
- Limited physical and chemical interferences eliminated by high operating temperatures of the argon plasma which breaks chemical bonds
- good stability
- low detection limit
- low matrix effects

2.19.2 Disadvantage of ICP-OES

- Uses only liquid samples

2.19.3 Interferences

The ICP-OES technique is a widely used analytical tool in the analysis of a wide range of elements simultaneously, it can analyse more than 60 elements per sample and it can also analyse over 100 samples per day. ICP-OES also suffers from interferences like all other instrument techniques, some interferences include:

- Spectral interference
- Matrix interferences

Interferences by definition are enhancers or suppressors of the analyte which result in incorrect results. They are caused by background emissions from other elements other than the analyte, causing the overlap of the spectral line with that of the analyte. They are also caused by the changes in viscosity or generation of compounds that have low atomisation efficiency (Morishige and Kimura, 2008). Spectral interferences can be avoided by changing the emission line or subtracting background emission for most analytical emission lines

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CHAPTER 3

PHYTOCHEMICAL INVESTIGATION OF TWO INDIGENOUS MEDICINAL PLANTS SPECIES *BRIDELIA MICRANTHA* AND *SIDEROXYLON INERME* AND THEIR ANTIOXIDANT ACTIVITIES

ABSTRACT

A phytochemical investigation was done on two indigenous medicinal plant species with morphological similarities namely *Bridelia macrantha* and *Sideroxylon inerme* to determine if they have a chemotaxonomic link. They are both known in KwaZulu-Natal as 'uMhlalamagwababa' by the Zulu people and are used interchangeably by traditional healers in South Africa. Three pentacyclic triterpenes (friedelin, stigmasterol and a mixture of α and β – amyrin), one carotenoid (lutein) and one megastigmane (apocynol B) were isolated from *S. inerme*. Two flavonoids (quercetin and quercetin-3-*O*-glucoside) and two triterpenes (oleanolic acid and acacic acid lactone) were isolated from *B. micrantha*. These compounds were not previously isolated from these plants. The free radical scavenging activity of the extracts and selected phytocompounds at varied concentrations was determined by the DPPH assay. The antioxidant activity for both plants was good but that of *B. micrantha* was better than that of *S. inerme*. The classes of compounds isolated from these two plant species and their antioxidant activity corroborates the use of *S. inerme* and *B. micrantha* by traditional healers but does not support their use interchangeably.

Keywords: flavonoids, triterpenes, DPPH assay, traditional medicine

3.1 INTRODUCTION

Plants have been used throughout history in traditional medicine for the treatment of a wide range of illnesses and diseases. Different parts of the plant contain secondary metabolites that function as a defence against various environmental threats (Gosh *et al.,* 2010) and some of these secondary metabolites are known to have medicinal benefits. The consumption of plants by humans, particularly fruits and vegetables, for their antioxidant value is common practise (Shwahwar and Raza, 2012). Natural antioxidants counter the effects of free radicals that promote oxidative damage which is involved in the pathogenesis of many diseases such as cancer, Alzheimer's and diabetes (Murthy *et al.,* 2012; Mandal *et al.,* 2009). In developing countries, high levels of infectious diseases and high mortality rate during pregnancy and childbirth, in addition to cancer and chronic respiratory diseases, accounts for most deaths (Fidele *et al.,* 2013). Most people in these countries usually turn to natural products for treatment and cure when faced with health-related problems due to accessibility and cost effectiveness.

Bridelia micrantha (known as 'uMhlalamagwababa' in isiZulu) is a fast growing shade tree from the plant family Euphorbiaceae. *B. micrantha* is generally found along the swamp forests in the Eastern Cape, KwaZulu-Natal and Limpopo provinces in South Africa. *Sideroxylon inerme* (known as 'uMhlalamagwababa' and 'Amasethole-amhlophe' in isiZulu) is a slow growing, evergreen tree that belongs to the plant family Sapotaceae that comprises 1100 species and 53 genera (Yang, 1998). *S. inerme* is found along the coast of South Africa from the Cape Peninsula to Northern Zululand. Both these plant species have similar looking berry-like fruits that ripen in summer, turn black when ripe and produce milk sap. Many people confuse these two plant species with each other due to morphological similarities, even traditional healers who use these species interchangeably.

Different parts of *B. micrantha* and *S. inerme* are used traditionally to treat a variety of ailments; the bark is used to treat burns, wounds, venereal diseases, tapeworms and toothache. Traditional healers in KwaZulu-Natal use the bark as anti-diarrhoeal and anti-bacterial agents (Lin *et al*., 2002). The leaf sap is used to treat sore eyes; roots are used to treat stomach pains, gastric ulcers and headaches. The herbalists of Western Nigeria use the stem bark to prepare decoctions that are used to prolong pregnancy to full term (Ngueyen *et al*., 2009).

Phytochemical studies have not been done on *S. inerme* despite its role in traditional medicine. However, preliminary screening of crude extracts indicated the presence of two phytocompounds, epigallocatechin gallate and procyanidin B (Momtaz *et al*., 2008). Studies on *S. inerme* species indicated the presence of triterpenoids and sterols which are used as chemotaxonomic markers (Da Silva *et al.*, 2006). The main aim of this study was to isolate, characterise and identify the phytocompounds in the leaves, stem bark, roots and fruits of the two plant species *B. micrantha* and *S. inerme* to evaluate if they can be used interchangeably in traditional medicine. The isolated compounds were also assessed for their antioxidant activity to determine the plants potential as natural antioxidants.

3.2 MATERIALS AND METHODS

3.2.1 General experimental procedure

3.2.2 Sample collection

The stem bark, leaves, roots and fruits of both plants (*S. inerme* and *B micrantha*) were collected from various sites in KwaZulu-Natal, South Africa. The plants were identified by a taxonomist, Prof A. Nicolas from the School of Life Sciences, University of KwaZulu-Natal (UKZN),

Westville campus and voucher specimens (Shelembe B1 and Shelembe B2) were deposited in the ward herbarium.

Plant materials were dried at room temperature for 3 weeks. The ground samples were sequentially extracted exhaustively with hexane, dichloromethane (DCM) and methanol (MeOH). The aqueous MeOH extract was further partitioned with equal volumes of DCM followed by ethyl acetate. All extracts were concentrated by rotatory evaporation. The crude extracts were dried and subjected to column chromatography (Merck Kieselgel 60, 0.063-0.200 mm, 70-230 mesh ASTM) to obtain fractions whose purity was monitored by thin layer chromatography (TLC) (Merck silica gel 60, 20 x 20 cm F254 aluminium sheets). Spots from the plates were visualised using 10% H_2SO_4 in MeOH and anisaldehyde spray reagent (12.5 mL anisaldehyde and 25 mL H_2SO_4 in 1 L of distilled water).

3.2.3 Characterisation and quantification methods

NMR spectra were recorded using a Bruker AVANCE III 400 MHz or 600 MHz spectrometer in deuterated chloroform ($CDCl_3$) or deuterated methanol (MeOD) with tetramethylsilane (TMS) as internal standard. UV-Vis spectra were obtained on a UV-Vis-NIR Shimadzu UV-3600 Spectrophotometer. IR spectra were recorded using a Perkin Elmer Universal ATR spectrometer. GC-MS data were recorded on an Agilent GC-MSD apparatus equipped with a DB-5SIL MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) fused silica capillary column. He (2 mL min^{-1}) was used as a carrier gas and MeOH or DCM was used to dissolve the samples. The injector was kept at 250 ^oC whilst the transfer line was at 280 ^oC. The column temperature was held at 50 ^oC for 2 min, and then ramped to 280 ^oC at 20 ^oC min^{-1} where it was held for 15 min.

3.2.4 Isolation and purification of compounds from *S. inerme*

The hexane extract (10.66 g) and DCM extract (7.58 g) of leaves were combined due to similar TLC profiles. The combined extract was subjected to column chromatography and separated using a hexane: ethyl acetate step gradient starting with 100% hexane and stepped by 10% to 100% ethyl acetate. Ten 10 mL fractions were collected for each solvent system and the purity of each fraction was examined by TLC. Fractions with similar retention factors (R_f) were combined and concentrated using a rotatory evaporator. Two compounds were isolated, compound **A1** (10.23 mg) in fractions 10-17 and compound **A2** (10.66 mg) in fractions 55-57.

The same procedure was followed for the combined hexane and DCM extracts of the stem bark (11.50 g) and roots (9.5 g). After elution, fractions 35-37 from the stem bark yielded compound **A2** (16.23 mg), fractions 42-43 yielded compound **A3** (12.70 mg) and fractions 56-60 yielded compound **A4** (20.50 mg). After elution, fractions 22-24 from the roots yielded compound **A2** (5.33 mg) and fractions 31-32 yielded compound **A3** (4.99 mg).

Approximately 100 mL of the aqueous methanol extract of the stem bark was partitioned with an equal volume of DCM, in triplicate, to afford the DCM fraction (10.34 g). This fraction was subjected to column chromatography using a hexane: ethyl acetate step gradient, starting with hexane (80%): ethyl acetate (20%) and stepped by 20% to 100% ethyl acetate. Fifty fractions of 20 mL each were collected and fractions 1-26 were combined to yield compound **A5** (23.01 mg).

Compound A1: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.62 (2H, d, J=16 Hz, H-11, 11’), 5.52 (1H, brs, H-4’), 6.36 (1H, s, H-12’), 6.32 (1H, s, H-14, 14’), 6.23 (1H, s, H-7), 6.10 (3H, m, H-8, 8’, 10, 10’), 5.52 (1H,brs, H-4’), 5.41 (1H, m, H-7’), 5.09 (2H, brs, H-7), 4.23 (1H, m, H-3’),

3.97 (1H, m, H-3), 2.38 (2H, m, H- 6', 2', 2, 4), 2.03 (3H, s, H-16), 1.89 (3h, s, H-16'), 1.71 (3H, s, H-17), 1.66 (3H, s, H-20), 1.59 (3H, s, H-20'), 1.44-1.33 (2H, d, m, H-2 and 2'), 0.97 (3H, s, H-18'), 1.05 (6H, s, H-18,19), 0.84 (3H, s, H-19').

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 138.5 (C-8), 137.9 (C-6), 137.7 (12 and 12'), 136.5 (C-13), 136.4 (13'), 135.7 (C-9'), 135.1 (C-9), 132.6 (C-14), 131.3 (C-7'), 130.8 (C-8), 130.1 (C-8'), 128.7 (C-7'), 126.2 (C-5'), 125.6 (C-5'), 125.6 (C-10'), 124.9 (C-11'), 124.8 (C-4'), 124.5 (C-11), 65.9 (C-3'), 65.1(C-3), 54.9 (C-6'), 48.4 (C-2), 44.6 (C-4'), 42.6 (C-4), 34.1 (C-1'), 37.1 (C-1), 30.3 (C-17), 29.5 (C-17'), 28.7 (C-16), 24.3 (C-16'), 22.9 (C-18'), 21.6 (C-18), 13.1 (C-19'), 12.8 (C-18 and 20), 12.8 (C-20').

Compound A2: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.36 (2H, m, H-4), 2.23 (1H, s, H-4), 1.54 (1H, t, H-10), 1.46 (2H, m, H-7), 1.37 (1H, t, H-8), 1.32 (2H, t, H-6), 1.15 (3H, s, H-28), 1.09 (3H, s, H-27) 0.98 (6H, d, *J* = 6.87 Hz, H-30,26), 0.93 (3H, s, H-29), 0.86 (3H, s, H-25) , 0.70 (3H, s, H-24), 0.84 (3H, s, H-23).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 22.3 (C-1), 41.5 (C-2), 210.3 (C-3), 58.2 (C-4), 42.1 (C-5), 41.2 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 60.2 (C-10), 35.6 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.4 (C-15), 36.2 (C-16), 30.0 (C-17), 42.7 (C-18), 35.3 (C-19), 28.1 (C-20), 32.7 (C-21), 39.2 (C-22), 12.8 (C-23), 14.6 (C-24), 17.9 (C-25), 20.2 (C-26), 18.6 (C-27), 32.1 (C-28), 35.2 (C-29), 31.7 (C-30).

Compound A3: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.33 (1H, m, H-6), 5.10 (1H, dd, *J* = 15.3, 8.0 Hz, H-22), 5.02 (1H, dd, *J* = 15.3, 8.0 Hz, H-23), 3.47-3.52 (1H, m, H-3), 0.90 (3H, d, *J*

= 6.5 Hz, Me-21), 0.83 (3H, d, *J* = 6.6 Hz, M-26), 0.84 (3H, t, *J* = 7.0 Hz, Me- 29), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.80 (3H, s, Me-19), 0.65 (3H, s, Me-18).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.8 (C-17), 56.0 (C-24), 50.1 (C-9), 45.8 (C-13), 42.3 (C-4), 42.3 (C-20), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 39.1 (C-8), 34.0 (C-25), 31.6 (C-7), 29.8 (C-2), 29.1 (C-16), 28.2 (C-28), 26.1 (C-15), 24.3 (C-27), 23.1 (C-21), 21.0 (C-11), 19.0 (C-19), 18.9 (C-26), 12.0 (C-18), 11.9 (C-29).

Compound A4: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.10 (1H, t, *J* = 3.6, 7 Hz, H-12), 3.20 (1H, m, H-3), 1.13 (3H, *J* = 1.0 Hz, H-27), 0.997 (1H, s, H-23), 0.968 (3H, s, H-26), 0.938 (3H, s, H-25), 0.87 (6H, s, H-29, H-30), 0.832 (3H, s, H-28), 0.792 (3H, s, H-24), 0.742 (1H, d, *J* = 1.9 Hz, H-5).

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 145.09 (C-13 β), 139.48 (C-13 α), 124.32 (C-12 α), 121.62 (C-12 β), 78.74 (C-3), 58.99 (C-5, α), 55.08 (C-5, β), 47.61 (C-9), 47.53 (C-9), 47.13 (C-18), 47.54 (C-18), 46.70 (C-19), 41.43 (C-14), 41.98 (C-14), 39.91 (C-4), 39.69 (C-4), 39.56 (C-8), 39.51 (C-8), 38.69 (C-1), 38.67 (C-10), 38.49 (C-22), 36.96 (C-21), 33.65 (C-29), 33.24 (C-29), 32.84 (C-7), 32.39 (C17), 31.16 (C-20), 30.98 (C-20), 29.61 (C-23), 28.65 (C-23), 28.30 (C-28), 28.03 (C-23), 27.17 (C-2), 27.13 (C-16), 26.52 (C-15), 26.05 (C-27), 23.59 (C-30), 23.43 (C-11), 18.26 (C-6), 17.37 (C-26), 16.77 (C-25), 16.71 (C-24).

Compound A5: ^{1}H NMR (600 MHz, Temperature 25 ^{0}C, $CDCl_3$) δ (ppm): 0.89 (3H, s, Me-13), 1.07 (3H, s, Me-12), 1.27 (3H, d, *J* = 6.2 Hz, Me-10), 2.24 (1H, d, *J* = 17 Hz, H-2β), 2.40 (1H,

d, *J* = 17 Hz, H-2α), 4.21 (2H, d, *J* = 15 Hz, H-11), 4.38 (1H, m, H-9), 5.80 (1H, d, *J*=12 Hz, H-7), 5.81 (1H, dd, *J* = 4.68, 7.4 Hz, H-8), 6.06 (1H, s, H-4).

^{13}C- NMR, DEPT90 and DEPT135 NMR (600 MHz, Temperature 25 ^{0}C, $CDCl_3$) δ (ppm): 162.4 (C-5, -C-), 135.7 (C-7, CH), 129.6 (C-8, CH), 124.6 (C-4, CH), 78.2 (C-6, -C-), 68.0 (C-9, CH), 62.7 (C-11, CH_2), 49.9 (C-2, CH_2), 41.5 (C-1, -C-), 23.7 (C-10, CH_3), 23.5 (C-12, CH_3), 22.9 (C-13, CH_3).

3.2.5 Isolation and purification of compounds from *B. micrantha*

The MeOH extract of the leaves and stem bark were combined due to similar R_f values on the TLC plate. The combined extract (124 g) was subjected to column chromatography using silica gel as the stationary phase and a hexane: ethyl acetate solvent system starting with hexane (10%): ethyl acetate (90%) and stepped by 10% to 100% ethyl acetate. Fifty fractions, 5 × 20 mL for each solvent system, were collected and fractions 34-44 were combined and further purified using Sephedex as stationary phase to produce two compounds (**B1** (5.12 mg) and **B2** (10.20 mg)).

The hexane and DCM extract from the roots (10.66 g) were combined due to similar R_f values on the TLC plate and was subjected to column chromatography. The compounds were eluted with a hexane: ethyl acetate solvent system starting with 100% hexane that was stepped by 20% to 100% ethyl acetate. Sixty fractions, 10 × 20 mL for each solvent system, were collected and profiled using TLC. Fractions with similar profiles were combined and concentrated using a rotatory evaporator. Fractions 9-10 produced compound **B3** (7.55 mg) and fractions 54-55 produced compound **B4** (8.04 mg)**.**

Compound B1: ^{1}H NMR spectral data (600 MHz, Temperature 30°C, MeOD) δ (ppm): 7.76 (1H, d, *J* = 1.50 Hz, H-2'), 7.66 (1H, dd, *J* =1.5, 8.5 Hz , H-6'), 6.91 (1H, d, *J*=8.5 Hz, H-5'), 6.41 (1H, d, *J* = 1.4 Hz, H-8), 6.21 (1H, d, *J* = 1.4 Hz , H-6).

^{13}C NMR spectral data (600 MHz, Temperature 30°C, MeOD) δ (ppm): 175.95 (C-4), 164.22 (C-7), 160.08 (C-5), 156.86 (C-9), 146.38 (C-3'), 146.67 (C-4'), 144.8 (C-2), 135.8 (C-3), 122.76 (C-6'), 120.32 (C-1'**)**, 114.87 (C-5'), 114.64 (C-2'), 103.13 (C-10), 97.91 (C-6), 93.08 (C-8).

Compound B2: ^{1}H-NMR spectral data (600 MHz, Temperature 30°C, MeOD) δ (ppm): 7.76 (1H, d, *J* = 1.5 Hz, H-2'), 7.66 (1H, dd, *J* = 1.5, 8.5 Hz , H-6'), 6.91 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, d, *J* = 1.4 Hz, H-8), 6.21 (1H, d, *J*=1.4 Hz , H-6), 4.99 (1H, d, *J* = 7.7 Hz, H-1''), 3.5 (1H, m, H-2''), 3.4 (1H, m, H-3''), 3.4 (1H, m, H-4''), 3.2 (1H, m, H-5''), 3.7 (2H, d, *J* = 5.2 Hz, H-6'').

^{13}C NMR and DEPT spectral data (600 MHz, Temperature 30°C, MeOD) δ (ppm): 178.12 (C-4, -C-), 164.67 (C-7, -C-), 160.00 (C-5, -C-), 158.22 (C-2, -C-), 157.10 (C-9, -C-), 148.50 (C-3', -C-), 144.30 (C-4', -C-), 134.70 (C-3, -C-), 121.88 (C-1', -C-), 121.72 (C-6', -CH-), 116,71 (C-5', -CH-), 114.88 (C-2', -CH-), 104.46 (C-10, -C-), 104.26 (C-1''-CH-), 99.09 (C-6, -CH-), 93.94 (C-8, -CH-), 104.25 (C-1'', -CH-), 74.10 (C-2'', -CH), 76.55 (C-3'', -CH-), 70.54 (C-4'', -CH-), 76.63 (C-5'', -CH-), 61.17 (C-6'', -CH-).

Compound B3: ^{1}H NMR spectral data (400 MHz, $CDCl_3$) δ (ppm): 5.23 (1H, t, H-12), 3.18 (1H, dd, *J* = 5, 10 Hz, H-3), 2.21 (1H, d, *J* = 11.1 Hz , H-18), 1.93 (2H, m, H-22, 2, 11, 16, 19), 2.21

(2H, t, H-15), 1.67 (1H, t, H-9), 0.99 (1H, s, H-23), 0.97 (3H, s, H-26), 0.94 (3H, s, H-25), 0.88 (6H, s, H-29, H-30), 0.79 (3H, s, H-24), 0.77 (3H, s, H-27).

^{13}C NMR spectral data (400 MHz, $CDCl_3$) δ (ppm): 181.8 (C-28), 139.6 (C-13), 126.9 (C-12), 79.8 (C-3), 56.8 (C-5), 54.3 (C-17), 48.0 (C-9), 43.3 (C-19), 40.8 (C-14), 40.5 (C-18), 40.4 (C-8), 40.1 (C-4), 39.9 (C-1), 38.2 (C-10), 38.1 (C-21), 34.4 (C-7), 31.9 (C-22), 29.3 (C-15), 29.0 (C-20), 27.9 (C-23 and C-16), 25.4 (C-30), 25.4 (C-29), 24.5 (C-27), 24.5 (C-2), 21.9 (C-11), 19.6 (C-6), 18.0 (C-26), 17.9 (C-24), 16.3 (C-25)

Compound B4: ^{1}H NMR spectral data (600 MHz, Temperature 30°C, MeOD) δ (ppm): 5.31 (1H, brs, H-12), 4.47 (1H, s, H-21), 3.94 (1H, s, H-5), 3.19 (1H, dd, J = 5 and 11 Hz, H-3), 3.03 (1H, dd, J = 4.0 and 14.4 Hz, H-18), 2.30 (2H, t, H-22), 1.91 (2H, m, H-13), 1.93 (2H, m, H-, 2, 11, 16, 19), 2.21 (2H, t, H-15), 1.67 (1H, t, H-9), 0.997 (1H, s, H-23), 0.968 (3H, s, H-26), 0.938 (3H, s, H-25), 0.88 (6H, s, H-29, H-30), 0.792 (3H, s, H-24), 0.77 (3H, s, H-27).

^{13}C NMR spectral data (600 MHz, Temperature 30°C, MeOD) δ (ppm): 181.2 (C-28), 145.1 (C-13), 123.5 (C-12), 79.8 (C-3), 75.3 (C-21), 56.9 (C-5), 48.1 (C-17), 47.7 (19), 42.6 (C-9), 42.1 (C-14), 40.6 (C-18), 39.9 (C-1), 39.9 (C-8), 38.2 (C-4), 36.6 (C-10), 36.2 (C-22), 34.3 (C-2), 33.4 (20), 32.7 (C-7), 31.4 (C-11), 28.8 (C-29), 27.9 (C-30), 27.3 (C-16 and C-23), 24.9 (C-27), 24.5 (C-15), 19.5 (C-6), 17.8 (C-24), 16.4 (C-25), 16.0 (C-26)

3.2.6 Antioxidant activity

The scavenging ability of the crude extracts of *S. inerme* and *B. micrantha* and compounds **A5**, **B1** and **B2** was measured using the DPPH stable free radical method outlined by Murthy (2012) with modifications. A volume of 300 µL of methanolic solution of crude extract at different

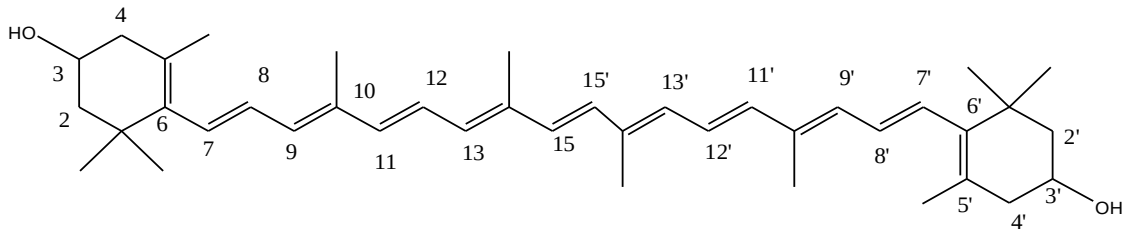
concentrations ranging from 1000 µg mL^{-1} to 15 µg mL^{-1} was mixed with 900 µL of a methanolic solution of DPPH (0.10 mM) and kept in the dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. Each sample was evaluated in triplicate. The percentage scavenging activity of the radical was determined by the following formula:

$$\%\, Scavenging \;= \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

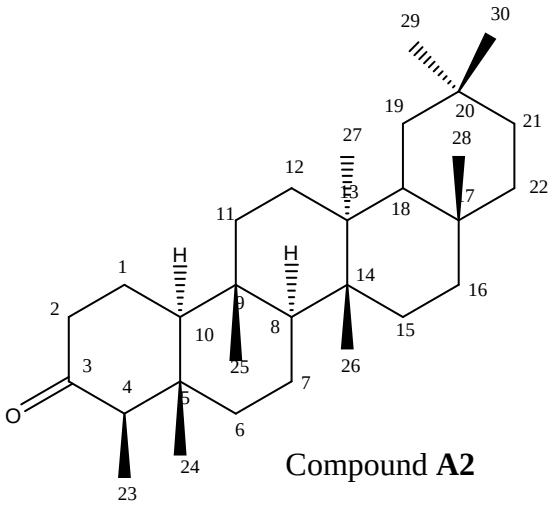
3.3 RESULTS AND DISCUSION

3.3.1 Structure elucidation of compounds from *S. inerme*

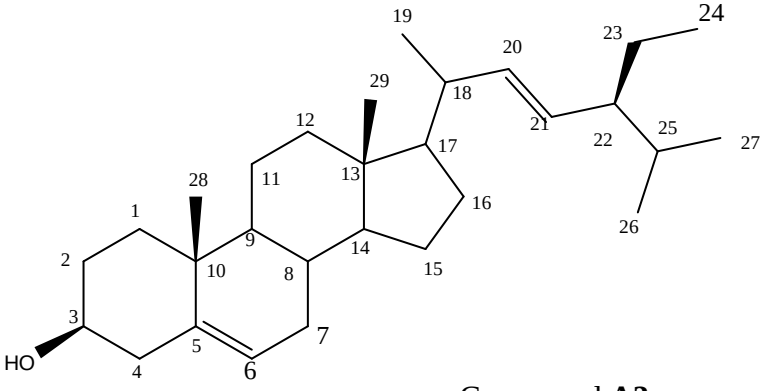
Compound **A1** was isolated as a dark yellow oily solid with a mass of 10.23 mg. The IR spectrum showed a broad absorption band at 3401 cm^{-1} (O-H stretch) and 1712 cm^{-1} (C=C). GC-MS data showed molecular ion peak [M^+] at m/z 568.9 which is in agreement with the molecular formula $C_{40}H_{56}O_2$. This data together with ^{1}H-NMR and ^{13}C-NMR data that corresponded with that in literature (Ragasa *et al*., 2012; Sohn *et al.*, 2008; Shotipruk *et al*., 2012) confirmed compound **A1** to be lutein. Lutein is one of the major carotenoids known for its antioxidant activity. Previous studies have shown that lutein prevents age-related macular degeneration (Serrano *et al.,* 2005; Seddom *et al.,* 1994 and Ribaya-Mercado *et al.,* 2004). Lutein has previously been isolated from many plant species including *S. spinosum* (Khallouki *et al.,* 2005).



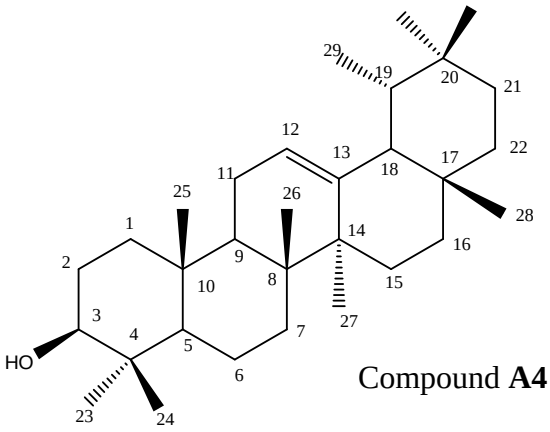
Compound **A1**



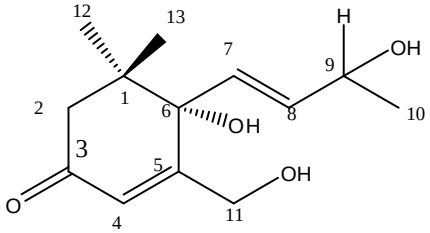
Compound **A2**



Compound **A3**



Compound **A4**



Compound **A5**

Figure 26: Compounds isolated from the crude extract of *S. inerme*; compound **A1** (lutein), compound **A2** (friedelin), compound **A3** (stigmasterol), compound **A4** (α- and β-amyrin) and compound **A5** (apocynol B).

Compound **A2** (Figure 26) was isolated as a white crystalline solid with a mass of 32.22 mg. The ^{1}H-NMR spectrum showed characteristic resonances for a pentacyclic triterpene with seven methyl resonances between δ_H 0.7-1.20 but no double bond or carbinylic proton resonances. The ^{13}C-NMR spectrum showed a carbonyl resonance at δ_C 213.14 which was assigned to C-3 due to HMBC correlations. The carbonyl group was further confirmed by the IR spectrum which showed an absorption band at 1714 cm^{-1} (C=O). The UV spectrum of compound **A2** gave maximum wavelength (λ_{max}) at 275 nm. GC-MS data showed molecular ion peak [M^{+}] at m/z 426 which is in agreement with the molecular formula $C_{29}H_{48}O$. This data together with NMR data that corresponded with that in literature (Mohato and Kundu, 1994, Sousa *et al.,* 2012) confirmed compound **A2** to be friedelin. This compound has previously been isolated from *Maytenus ilicifolia (*Queiroga *et al*., 2000) and *Quercus suber (*Ghosh *et al.*, 2010*).*

Compound **A3** (Figure 26) was isolated as a white powder with a mass of 17.69 mg. The ^{1}H-NMR spectrum showed characteristic resonances for sterols at δ_H 5.33 (H-6) and a multiplet at δ_H 3.50 (H-3). The double doublets at δ_H 4.98 (H-21) and δ_H 5.12 (H-20) indicated the sterol to be stigmasterol. This was confirmed by the ^{13}C-NMR spectrum that resolved 29 carbon resonances comprising six methyl, nine methylene, eleven methine and three quaternary carbon resonances identified using DEPT90 and 135 experiments. The UV spectrum of compound **A3** gave maximum wavelength (λ_{max}) at 256 nm. The IR spectra showed a broad absorption band at 3414 cm^{-1} (OH group) and 1645 cm^{-1} (C=C). GC-MS data showed molecular ion peak [M^{+}] at m/z 426 which is in agreement with the molecular formula $C_{29}H_{48}O$. This data together with NMR data that corresponded with that in literature (Mohato and Kundu, 1994; Vasquez *et al*., 2012) confirmed compound **A3** to be stigmasterol. Stigmasterol is one of the most ubiquitous phytosterols and has been isolated from numerous plant species (Kaur *et al.,* 2011).

Compound **A4** (Figure 26) was isolated as a white powder with a mass of 20.50 mg. The ^{1}H-NMR spectrum showed characteristic resonances for the oleanane-type pentacyclic triterpene with a vinylic proton resonance at δ_H 5.10 (H-12), a carbinylic proton resonance at δ_H 3.20 (H-3), a double doublet at δ_H 2.81 (H-18) and eight methyl resonances. Resonances at δ_C 145.1 and δ_C 121.6, δ_C 139.5 and δ_C 124.3 in the ^{13}C-NMR spectrum were assigned to C-13 and C-12, respectively. This corroborated the presence of a Δ^{12}-double bond of the olean-12-ene-type and indicated the presence of a mixture of α and β-amyrin in a ratio of 80:20%. The UV spectrum of compound **A4** gave maximum wavelength (λ_{max}) at 229 nm. The IR spectrum showed a broad absorption band at 3337 cm^{-1} (OH group) and a band at 1555 cm^{-1} (C=C). GC-MS data showed molecular ion peak [M^{+}] at m/z 426 which is in agreement with the molecular formula $C_{30}H_{50}O$. This data together with NMR data that corresponded with that in literature (Mohato and Kundu, 1994; Vasquez *et al*., 2012) confirmed compound **A4** to be a mixture of α and β-amyrin.

Compound **A5** (Figure 26) was isolated as a brown oily liquid with a mass of 23.01 mg. The ^{1}H-NMR spectrum showed 3 methyl singlets resonating at δ_H 0.82 (H-13), 1.09 (H-12) and 1.23 (H-10). Vinylic proton resonances were observed at δ_H 6.06 (s, H-4), 5.81 (d, H-7) and 5.80 (dd, H-8). Two doublets resonating at δ_H 2.26 and 2.42 integrated to one proton each. The HSQC spectrum correlated these 2 protons to the same carbon at δ_C 49.80 (C-2). The HSQC spectrum correlated protons resonating at δ_H 4.21, 4.38, 5.80, 5.81 and 6.06 to carbon resonances at δ_C 62.7 (C-11), 68.0 (C-9), 135.7 (C-7), 129.6 (C-8) and 124.6 (C-4), respectively. The ^{13}C-NMR and DEPT spectra showed 3 quaternary carbons resonances at δ_C 41 (C-1), δ_C 78 (C-6) and δ_C 162 (C-5); 4 olefinic carbon resonances between δ_C 124 and 163, of which 3 were methine resonances (C-4, C-7, C-8) and one was a quaternary carbon resonance (C-5); a methylene at δ_C 62.5 (C-11) bearing a hydroxyl group that correlated with the proton at δ_H 4.22 and a methine at δ_C 68.0 (C-9)

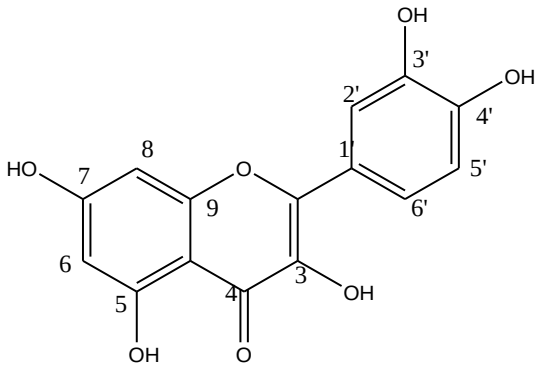
that correlated with the doublet at δ_H 4.37. GC-MS spectral data showed molecular ion peak [M^+] at *m/z* 239 and base peak at *m/z* 207 which was in agreement with molecular formula $C_{13}H_{20}O_4$. The fragmentation pattern as well as spectroscopic and physical data of compound **A5** was consistent with that reported in literature for Apocynol B (Murakami *et al.*, 2001) confirming compound **A5** to be Apocynol B.

Apocynol B has not previously been isolated from plant extracts, but was obtained from enzymatic hydrolysis of apocynoside II. Apocynoside II was isolated from the roasted leaves of *Apocynum venetum* L. by Murakami and co-workers (Murakami *et al.*, 2001). Biological testing has not been done on these compounds, but compounds from this class are known to possess anti-obesity activity. They are good inhibitors of pancreatic lipase and adiposity differentiation which hydrolyses and stores fats in the small intestines (Ahha *et al.*, 2013).

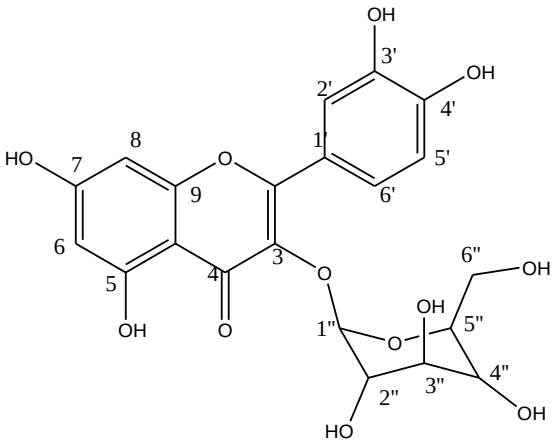
3.3.2 Structure elucidation of compounds from *B. micrantha*

Compound **B1** (Figure 27) was isolated as a yellow solid with a mass of 5.12 mg and compound **B2** (Figure 2) was isolated as a yellow powder with a mass of 10.20 mg. The ^{1}H-NMR spectra of compounds **B1** and **B2** exhibited characteristic resonances for a flavonol. The aromatic region exhibited the ABX system with protons resonating at δ_H 7.76 (H-2'), δ_C 7.66 (H-6') and δ_C 6.91 (H-5') due to disubstitution of ring B and a meta-coupled pattern with protons resonating at δ_H 6.41 (H-8) and δ_H 6.21 (H-6) due to disubstitution of ring A. The ^{13}C-NMR spectra indicated the presence of 15 and 21 carbon signals for compounds **B1** and **B2**, respectively which resolved 5 methylene and 10 quaternary carbon resonances in the DEPT90 and 135 experiments for compound **B1**. For compound **B2**, the resonance at δ_H 4.9 (H-1'') due to the anomeric proton

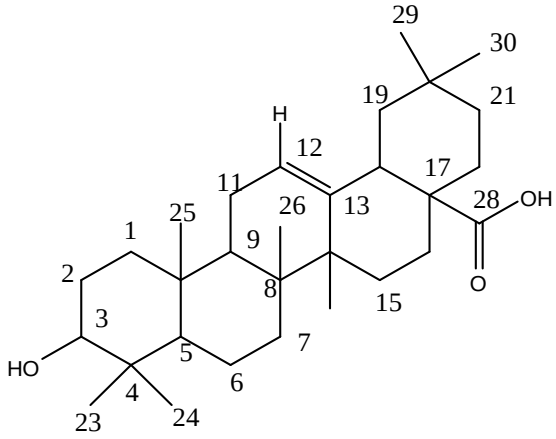
indicated the presence of a glycosidic linkage. This was confirmed by resonances between δ_H 3.2-3.7 (H-2''- H-6''). The carbon resonance at δ_C 62.5 (C-6'') was shown to be a methylene indicating that the sugar is a glucose attached at position 3 as confirmed by HSQC and HMBC correlations.



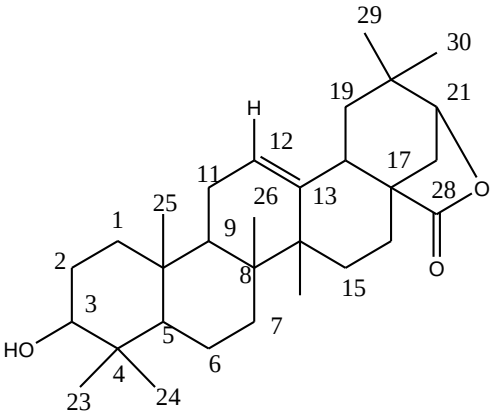
Compound **B1**



Compound **B2**



Compound **B3**



Compound **B4**

Figure 27: Compounds isolated from the crude extract of *B. micrantha*; compound **B1** (quercetin), compound **B2** (quercetin-3-O-glucoside), compound **B3** (oleanolic acid), compound **B4** (acacic acid lactone).

The $[M]^+$ ion at *m/z* 302 for compound **B1** is in agreement with the molecular formula $C_{15}H_{10}O_7$ for quercetin and the $[M]^+$ ion at *m/z* 465 for compound **B2** is in agreement with the molecular formula $C_{21}H_{20}O_{12}$ for quercetin-3-*O*-glucoside. The UV spectrum showed two prominent peaks at 250 nm and 350 nm originating from the A and B rings. The IR spectrum showed a broad absorption band at 3343 cm^{-1} (O-H group), absorption bands at 2943 and 2832 cm^{-1} (–CH stretch), an absorption peak at 1656 cm^{-1} (C=O) and a sharp band at 1594 cm^{-1} (aromatic rings). The physical and spectroscopic data for compounds **B1** and **B2** matched those published in literature (Guvenalp and Demirezer 2005; Kazuma *et al*., 2003) therefore these compounds were identified as the aglycone, quercetin and its glycoside, quercetin-3-*O*-glucoside, respectively.

Compound **B3** (Figure 27) was isolated as a white amorphous powder with a mass of 7.55 mg. The ^{1}H-NMR spectrum showed characteristic resonances for the oleanane-type pentacyclic triterpene with a vinylic proton resonance at δ_H 5.23 (H-12), a carbinylic proton resonance at δ_H 3.18 (H-3), a double doublet at δ_H 2.21 (H-18) and seven methyl resonances. The ^{13}C-NMR spectrum showed resonances at δ_C 126.9 (C-12), δ_C 139.6 (C-13) and δ_C 180.8 (C-28), which corroborated the presence of a Δ^{12}-double bond and carboxylic acid functionality. The UV spectrum of compound **B3** gave maximum wavelength (λ_{max}) at 229 nm. The IR spectrum showed a broad absorption band at 3462 cm^{-1} (OH group) and a band at 1703 cm^{-1} (C=O). GC-MS data showed molecular ion peak $[M^+]$ at m/z 456 which is in agreement with the molecular formula $C_{30}H_{48}O_3$. This data together with NMR data that corresponded with that in literature (Mohato and Kundu, 1994; Vasquez *et al*., 2012) confirmed compound **B3** to be oleanolic acid.

Compound **B4** (Figure 27) was isolated as a white amorphous powder with a mass of 8.04 mg. The ^{1}H-NMR spectrum showed characteristic resonances for the oleanane-type pentacyclic triterpene with the presence of a Δ^{12}-double bond. Spectral data of compound **B4** was similar to

that of compound **B3**, except for the signals corresponding to the lactone ring at C-21. The IR absorption band at 1766 cm^{-1} and the peak at δ_C 181.8 in the ^{13}C-NMR spectrum showed the presence of a γ-lactone ring. The formation of the lactone ring with the carboxyl group at C-17 was confirmed by the key HMBC correlation of δ_H 4.21 (H-21) with the carboxyl carbon at δ 181.8 (C-28). The UV spectrum of compound **B4** gave maximum wavelength (λ_{max}) at 229 nm. GC-MS data showed molecular ion peak [M^+] at m/z 454.34 which is in agreement with the molecular formula $C_{30}H_{46}O_3$. This data together with NMR data that corresponded with that in literature (Mohato and Kundu, 1994; Garai and Mahato, 1996) confirmed compound **B4** to be acacic acid lactone.

3.3.3 Antioxidant activity

The DPPH method is one of the quickest methods of evaluating antioxidant activity because of its widely used index and its stability (Shahwar and Razal, 2012). The DPPH free radical scavenging activity of the four crude MeOH extracts from leaves, bark, roots and fruits of *S. inerme* and *B. micrantha* were evaluated (Table 10 and Figure 28) and recorded as percentage inhibition. Higher percentage inhibition indicates better scavenging activity. Radical scavenging activity generally is measured in terms of the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% (IC_{50}) (Toit *et al.*, 2001). The IC_{50} values were also obtained as presented in Table 10. Ascorbic acid was used as a standard. The IC_{50} value for each sample was determined graphically by plotting the % inhibition as a function of sample concentration in µg mL^{-1} for the standard and samples.

Table 10: Percent inhibition of crude MeOH extracts of the roots (R), bark (B), leaves (L) and fruits (F) of *S. inerme* and Apocynol B isolated from the bark with IC_{50} (half maximum inhibitory concentration). Data represented as mean ± SD (n=3).

Concentration µg L^{-1}	**% Inhibition**					
	Ascorbic acid	**MeOH (R)**	**MeOH (B)**	**MeOH (L)**	**MeOH (F)**	**Apocynol B**
1000	95.8±0.00	83.5±0.34	95.4±0.02	75.8±1.01	57.2 ±3.60	71.7±1.05
500	88.8±0.06	74.8±0.51	75.1±0.93	65.3±0.23	38.6 ±2.08	32.9±0.17
250	91.4±0.127	52.8±0.30	65.9±0.29	44.6±0.23	20.6±3.10	25.3±0.22
125	96.0±0.12	52.9±0.063	58.1±0.14	37.2±0.02	18.8±3.10	24.2±0.24
62	59.3±0.52	16.7±0.36	55.1±0.069	35.9±0.04	17.2±0.42	19.2±0.17
31	45.3±1.07	1.9±2.10	48.9±0	33.8±0.02	10.5±0.12	15.3±0.06
15	15.8±0.23	0.8±0	42.3±0.080	30.6±0.72	7.2 ±0.23	6.9±0.06
IC_{50} µg mL^{-1}	41.0	191.1	41.1	178.3	815.4	71.4

The IC_{50} values for ascorbic acid (41 µg mL^{-1}) and the MeOH extract of the bark of *S. inerme* were similar (41.1 µg mL^{-1}) indicating the high antioxidant potential of the plant especially the bark. For both plants, *S. inerme* and *B. micrantha*, the scavenging effect of each extract increased with increasing concentrations and that of the standard was higher than extracts at 1000 µg mL^{-1}. However, all extracts exhibited appreciable scavenging activity at 1000 µg mL^{-1} with the scavenging ability of the MeOH extracts of the bark being 95.4% and 85.5% in *S. inerme* and *B. micrantha*, respectively.

The higher antioxidant activity of the *S. inerme* extract from the bark could be attributed to the stabilising effect of apocynol B in the extract that has four hydroxyl groups capable of donating protons thereby reducing the DPPH radical to DPPH-H. The higher antioxidant activity of the *B. micrantha* extract from the bark could be attributed to the two flavonoids present in this extract. Of the two flavonoids, the activity of the glycoside (96.7%) is much higher than the aglycone (70.9%) and even the standard, ascorbic acid. This indicates that the sugar moiety enhances the antioxidant activity of the compound. It would appear that the effect of the compounds in the extract is additive as seen by the decreased activity in the extract (85.5%) when compared to the individual activities especially that of quercetin-3-*O*-glucoside.

At low concentrations (15 μg mL^{-1}), the bark extract of *S. inerme* has higher antioxidant activity (42.3%) than ascorbic acid (15.8%). At low concentrations (50 μg mL^{-1}), quercetin-3-*O*-glucoside has higher antioxidant activity (85.5%) than ascorbic acid (59.2%). In *S. inerme*, the antioxidant activity of the leaves could be due to the presence of lutein which is a known antioxidant. Similarly, fruits of both plants had high free radical scavenging activity which is comparable to ascorbic acid especially in *B. micrantha*. Therefore, consuming fruits from these plants may be beneficial to human health as it can help minimise oxidative stress.

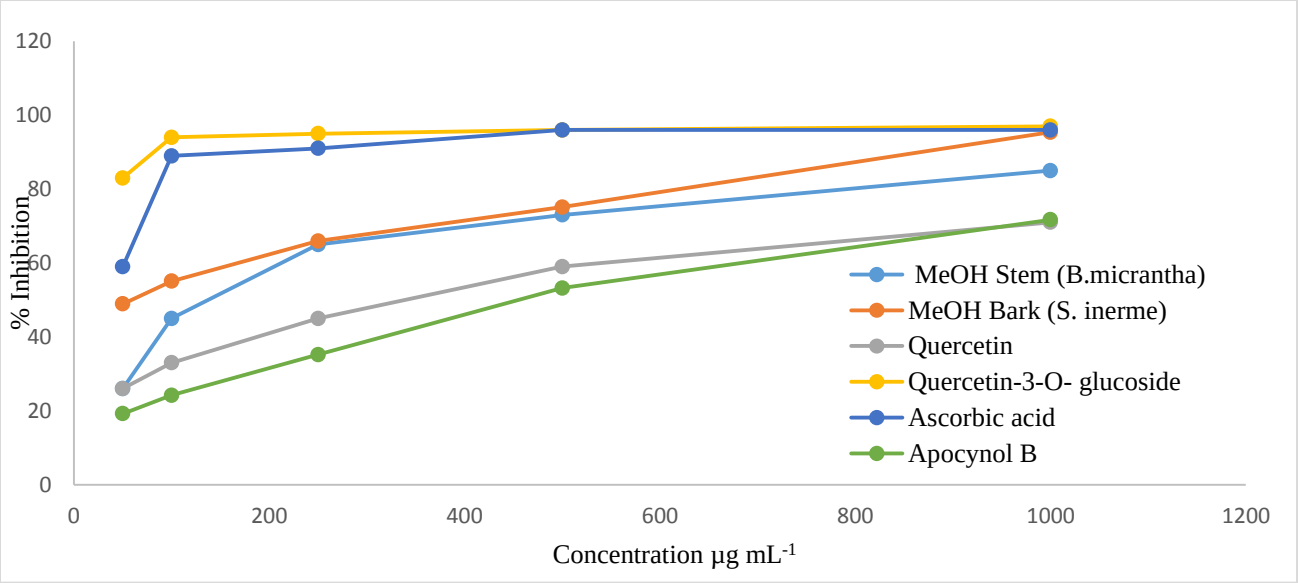
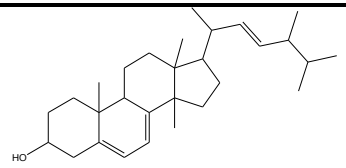
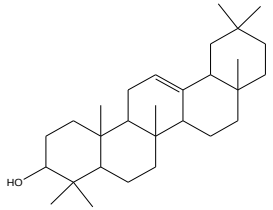
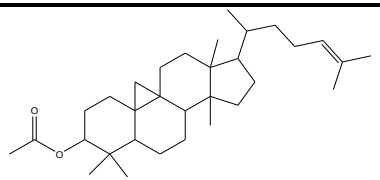
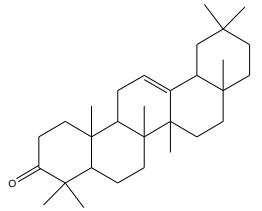
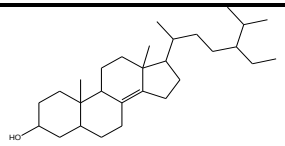
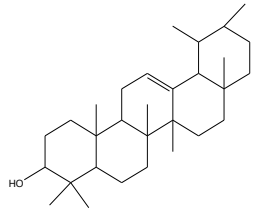


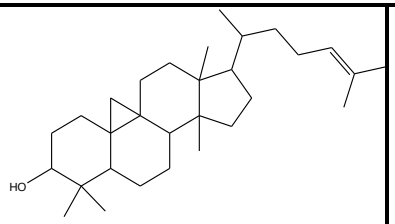
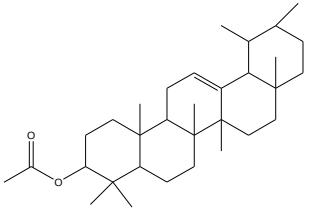
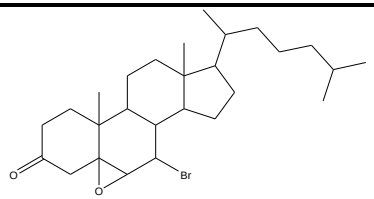
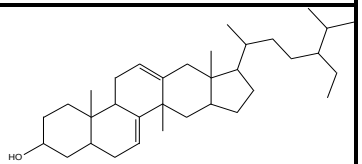
Figure 28: Percentage inhibition of the crude MeOH extract of *B. micrantha* and isolated compounds measured by DPPH method.

3.3.4 GC-MS profiling of fruit extracts from *S. inerme* and *B. micrantha*

GC-MS profiling led to the identification of 11 compounds from the fruit extracts of *B. micrantha* and *S. inerme* (Table 11). The structures and names for each compound were obtained from the National Institute of Standard and Technology data bank (NIST 05, 2005). The investigation of *B. micrantha* revealed the presence of mostly sterols including ergosterol and cycloartenol while *S. inerme* revealed the presence of mostly triterpenes including fridelin and α- and β-amyrin. Triterpenes and plant sterols have previously been reported by many researchers to possess pharmacological activities such as anti-inflammatory, antioxidant, antiviral, antibacterial, gastroprotective, cytotoxic and antiulcerogenic activities (Mohatu and Kundu, 1994, Fru *et al.*, 2013, Mann *et al.*, 2011 and Oliveira *et al*., 2005). GC-MS profiling of the fruit extracts show the two plants to possess different secondary metabolites.

Table 11: GC-MS comparison of the fruits extracted with methanol from *B. micrantha* and *S. inerme.*

B. micrantha			*S. inerme*		
Compound	Molecular ion peak	Structure	Compound	Molecular ion peak	Structure
Ergosterol	414		β -amyrin	426	
Cycloartenol acetate	468		Friedelin	424	
Stigmast-8(14)-en-3-ol	414		α -amyrin	426	

Cycloartenol	426		α -amyrin acetate	468	
5β,6β-Epoxy-7-bromocholestan-3-one	399		Stigmast-7-en-3-ol	414	

3.4 CONCLUSION

The phytochemical investigation lead to the isolation of three pentacyclic triterpenes, one carotenoid and one megastigmane from S. *inerme*, and two flavonoids and two triterpenes from *B. micrantha*. These compounds were not previously isolated from these plants. The MeOH extract of the bark of *S. inerme* appeared to have high antioxidant activity which was similar to that of ascorbic acid whilst the antioxidant activity of the glycoside isolated from *B. micrantha* bark had the highest antioxidant activity, even higher than ascorbic acid. Although the antioxidant activity of these two plant species were good, the antioxidant activity of *B. micrantha* was better than that of *S. inerme*. Also, the secondary metabolites isolated from the bark, leaves and roots or those identified in fruits using GC-MS analysis of these two plant species, were different. This shows that these two plant species, although morphologically similar, do not have chemotaxonomic similarities. This study corroborates the use of *S. inerme* and *B. micrantha* by traditional healers but does not support its use interchangeably.

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CHAPTER 4

ELEMENTAL COMPOSITION AND NUTRITIONAL VALUE OF THE EDIBLE FRUITS OF *BRIDELIA MICRANTHA* AND THE IMPACT OF SOIL QUALITY ON CHEMICAL PROPERTIES

ABSTRACT

Bridelia micrantha is a known medicinal plant indigenous to South Africa, particularly KwaZulu-Natal. It is used by traditional healers to treat a variety of illnesses. The plant bares berry-like edible fruits that are consumed by the local people. The distribution of essential and toxic elements in soil and fruits of *B. micrantha* from eight different sites in KwaZulu-Natal were investigated. The concentrations of elements in the fruits were found to be in decreasing order of Mg > Ca > Mn > Zn ≈ Fe > Cu > Co > Ni > Cr > Pb > As ≈ Cd. It was also found that 100 g of the fruit contained about 90.1% moisture, 3.2% ash, 4.1% protein, 0.9% oil and 1.7% carbohydrate. This study shows that the fruits of *B. micrantha* are a good source of essential elements and has low levels of toxic elements.

Keywords: Soil Organic Matter, elemental analysis, nutritional value and *Bridelia micrantha*

4.1 INTRODUCTION

Food security is related to the ongoing availability of food and it is an extremely important social determinant of health. Researchers, government and organisations working with food are concerned with the nutritional status of people in developing countries especially children, pregnant woman, lactating mothers and the sick. Maternal malnutrition during pregnancy can lead to growth retardation and reduced birth weight of the offspring especially if malnutrition is prevalent in the last trimester of pregnancy. Low birth weight in children increases their susceptibility to infectious diseases which reduces their chances of normal growth or survival (Andersen *et al.*, 2002).

In South Africa, a large number of communities living in rural areas rely on crops such as wheat and rice, wild fruits and vegetables for their nutritional needs because of inaccessibility to mainstream formal markets and affordability. Information on the nutritional and anti-nutritional properties of most wild fruits is not known even though they form a significant proportion of the global food basket. Studies on these food sources need to be undertaken to determine their nutritional quality and to promote and manage sustainable use of these wild foods towards poverty elimination.

Heavy metals that accumulate in agricultural soils influence the ecosystem as soils are the reservoirs through which these heavy metals are introduced into the food chain. Heavy metals, whether essential or non-essential, can have adverse effects on human health if at elevated levels. Toxic metals can enter the food chain through uptake by edible plants or may be leached into groundwater that contaminates drinking water. Toxicity has become a problem to human health, mainly because of exposure to heavy metals released into drinking water, edible fruits and vegetables, soils as well as house dusts (Boisa *et al.*, 2013). The Soils

Generic Assessment Criteria (SGAC) has been developed as a screening tool to assess the risks to human health from exposure to contaminated soils.

Bridelia micrantha is a fast growing shade tree found in the Eastern Cape, KwaZulu-Natal and Limpopo Province. It has edible berry-like fruit that ripen in summer and are enjoyed by the local people in KwaZulu-Natal. Extracts of different morphological parts of *B. micrantha* are used by traditional practitioners to treat a variety of ailments (Lin *et al*., 2002). The main objective of this study was to determine the elemental composition of the edible wild fruit of *B. micrantha* and to assess for nutritional value. The impact of soil quality on elemental uptake by the plant was also evaluated by investigating soils and plant material from eight different sites. The thirteen elements selectively investigated included As, Cd, Co, Cr, Cu, Ca, Fe, Mg, Mn, Ni, Pb, Se and Zn.

4.2 MATERIALS AND METHODS

4.2.1 Sample collection

B. micrantha fruit and soil samples were collected from eight different sites in the KwaZulu-Natal east coast region between the month of March and April 2014. The topography of sampling sites was flat and the soils were sandy (Sites B1, B2, B6, B7) or sandy loam (Sites B3, B4, B5, B8) in texture, see Table 12 and Figure 29. The average temperature was 27 °C with sunshine. A thin-walled stainless steel coring tube, 20 cm long and 3.8 cm wide, was driven into the soil along the drip line of the trees to a depth of approximately 15 cm to collect cores (six per tree). Extruded cores were placed into a plastic mixing bowl and were manually homogenised, reduced by method of coning and quartering then placed in separate

polyethylene bags and refrigerated at 4 ^{0}C. Samples of tree-ripened fruit were collected and stored in separate polyethylene bags and refrigerated.

4.2.2 Sampling sites

Figure 29 is a map showing the eight sampling sites and Table 4.1 provides the geographical coordinates for each site.

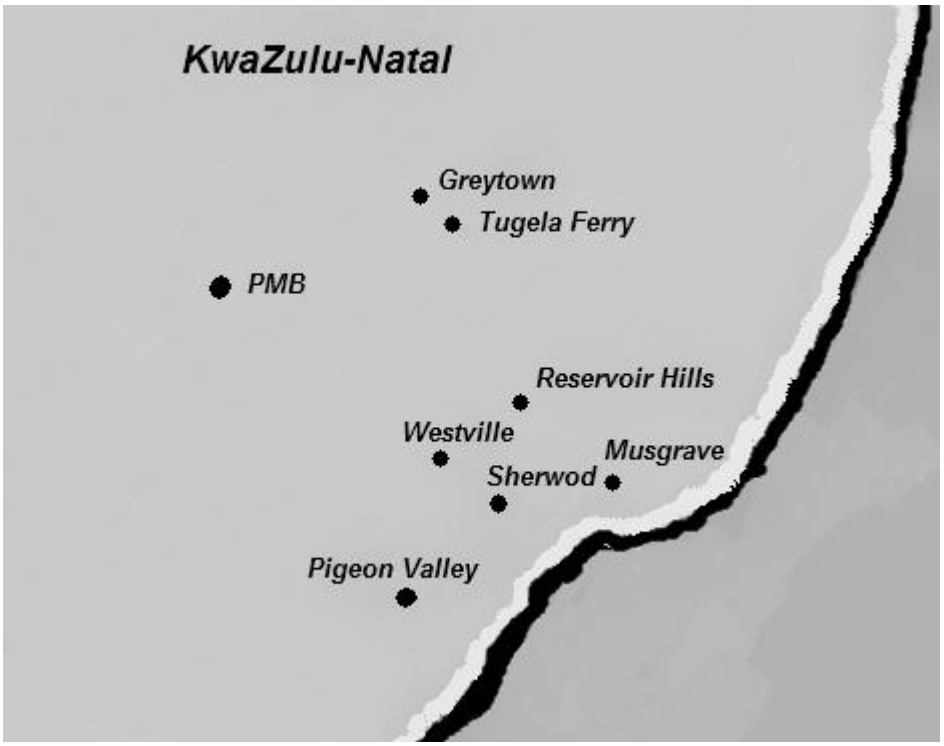

Figure 29: Sampling sites in KwaZulu-Natal, South Africa.

Table 12: Geographical coordinates of the eight sampling sites.

Site code	Location	Latitude	Longitude
B1	Scottsville	29° 37’ 43” S	30° 24’ 7” E
B2	Pigeon Valley	29° 51’ 52’’ S	30° 59’ 12’’ E
B3	Westville	29° 49’ 0” S	30° 56’ 43” E
B4	Greytown	29° 0.3’ 03’’ S	30° 36’ 10’’ E
B5	Reservoir Hills	29° 47’ 36’’ S	30° 56’ 08’’ E
B6	Tugela Ferry	28° 47’ 45” S	30° 29’ 26” E
B7	Sherwood, Jan Smuts 848	29° 50’ 10” S	30° 58’ 17” E
B8	Musgrave, Halfords Rd	29° 50’ 54” S	31° 0’ 22” E

4.2.3 Reagents and standards

All the analytical reagents that were used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade. Double distilled water was used throughout the experiments. To minimise the risk of contamination, 6.0 M HNO_3 was used to clean plastic bottles and glassware and rinsed with double distilled water prior to use. The standard solutions used were prepared from a 1000 ppm stock solution of each element.

4.2.4 Sample preparation

Fruits were oven dried at 50 °C for 24 hr to ensure complete removal of moisture. These were then ground into a fine powder using a food processor (Kenwood True Compac Blender). The powdered samples were stored in labelled polyethylene bottles in the refrigerator at 4 °C

until analysed. Soil samples were allowed to pass through a 2 mm mesh sieve to remove gravel and stones. Soil was air dried, crushed using a pestle and mortar to reduce the particle size then stored in polyethylene bottles and kept in a refrigerator at 4 °C until analysed.

4.2.5 Elemental analysis

4.2.5.1 Microwave digestion

The microwave-assisted closed vessel digestion technique was used for digestion of fruit and soil samples due to its superior digestion capability and sample throughput. Digestions were performed using the Mars 6 Microwave Sample Preparation System (1000 W) with eight-high-pressure Teflon (TFM) lined Xpress vessels (HF 50). To ensure improved precision, five sub-samples (both fruit and soil) were digested. Fruit (0.5 g) or soil samples (0.25 g) were weighed into ceramic easy prep vessels, to which, 10 mL of 70% HNO_3 was added and allowed to pre-digest for 1 hr before sealing. For fruit samples, the microwave power was ramped to 500 W for the first 15 min, where it remained for the next 15 min, then ramped to 650 W for 15 min during which complete digestion occurred. The microwave power was reduced and the bombs cooled by forced ventilation for 15 min. The digestion method for soil samples was harsher; the power was ramped from 100 W to 600 W for 10 min then ramped from 600 W to 900 W for the next 12 min. The microwave power was reduced and the bombs cooled by forced ventilation for 15 min. Fruit and soil digests were filtered through a 0.45 µm filters into 50 mL volumetric flasks, made up to the mark with double distilled water then transferred into polyethylene bottles for elemental analysis. All samples were analysed for As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

The analytical wavelengths were selected based on minimum spectral interferences and maximum analytical performance. Table 13 shows the wavelengths chosen for each of the elements analysed by ICP in this study. Initially the 3 most sensitive lines were chosen. From these lines, the lines with no interfering elements were chosen.

Table 13: Emission lines for the selected elements.

Element	**Emission line (nm)**	**DL*/ppb**
As	188.9	0.90
Ca	317.9	0.04
Cd	214.4	0.07
Co	228.6	0.07
Cr	267.7	0.25
Cu	327.4	0.25
Fe	239.4	0.20
Mg	279.6	0.04
Mn	259.1	0.03
Se	196	0.20
Ni	231	0.37
Pb	217	1.40
Zn	206.2	0.20

*DL- Instrument Detection Limit

4.2.6 Extraction of bioavailable metals

Bioavailable or exchangeable metals were determined by extracting 1.0 g of soil sample with 10 mL of the solution which was prepared by mixing ammonium acetate (1.0 M), ethylenediaminetetraacetic acid (EDTA, 0.05 M) and acetic acid (0.43 M). The soil was then shaken on an orbital shaker for 1 hr. The mixture was filtered through a 0.45 µm filter paper by gravity into a 50 mL volumetric flask, made up to the mark with double distilled water then transferred into plastic bottles and stored at 4 °C until analysed (Dean, 2005).

4.2.7 Soil pH

The pH of the soil was measured using a 1:1 ratio of soil: 0.01 M $CaCl_2$. A mass of 1.0 g of soil was transferred into a 50 mL plastic beaker and dissolved in 1 mL of 0.01 M calcium chloride; the slurry was allowed to stand for 30 min, thereafter the pH of the solution was measured using a pH meter which was previously calibrated with buffer solutions (pH 5 and pH 7).

4.2.8 Soil organic matter (SOM)

The SOM was estimated using the Walkley-Black Method (Schulte & Hoskin, 2009). Depending on the type of soil, a mass between 0.1 - 1.0 g of soil sample was weighed into a 500 mL conical flask to which 10 mL potassium dichromate and 20 mL conc. H_2SO_4 was added. The solution was swirled, allowed to stand for 30 min, diluted with 200 mL double distilled water then 10 mL of 85% phosphoric acid and 0.2 g sodium fluoride was added to it. The solution was titrated against ferrous ammonium sulphate using diphenylamine as indicator to a colour change from turbid blue to light green.

Calculations

$$\% \text{ C} = \frac{(\text{B} - \text{S}) \times \text{M of } \text{Fe}^{2+} \times 12 \times 100}{\text{Mass of soil (g)} \times 4000}$$

Where:

B = mL of Fe^{2+} solution used to titrate blank

S = mL of Fe^{2+} solution used to titrate sample

12/4000 = milliequivalent weight of C in g

Organic matter was then calculated using the following equation:

$$\% \text{ OM} = \frac{\% \text{ total C} \times 1.72}{0.58}$$

4.2.9 Cation exchange capacity (CEC)

The cation exchange capacity was determined using the Chapman method using ammonium acetate (NH_4OAc) at pH 7.0 with a few modifications (Chapman, 1965; Horneck et el., 1989). Approximately 5 g of soil and 25 mL of NH_4OAc was added to a 500 mL Erlenmeyer flask which was shaken thoroughly and allowed to stand overnight. The solution was filtered by suction until the filtrate was clear. The soil was then washed with eight separate additions of 95% ethanol (EtOH) to remove excess saturating solution. The leachate was then discarded. The adsorbed ammonium ions (NH_4^+) were then extracted by leaching with 25 mL portions of 1.0 M KCl, leaching slowly. The soil was then discarded and the leachate was transferred into a 250 mL volumetric flask then diluted with KCl to the mark. The concentration of NH_4-N in the KCl extract was determined by the Kjeldahl distillation method (Bremner, 1960). The amount of NH_4-N in the original KCl extracting solution (blank) was also determined.

4.2.10 Bioaccumulation factor (BAF)

BAF was obtained by calculating the ratio of metal concentration in the fruit to total or exchangeable concentration in the soil. A BAF value greater than 1 indicated that the plant accumulated that particular element while a BAF less 1 indicated that the plant excluded that element.

$$BAF = \frac{[\text{Metal}]_{\text{Fruit}}}{[\text{Metal}]_{\text{Soil}}}$$

A plot of BAF against the total / exchangeable concentration indicates essentiality and non-essentiality of the element based on the shape of the graph. Essential elements are indicated by a rectangular hyperbola while non-essential elements are indicated by a parallel line opposite the x-axis.

4.2.11 Geoaccumulation index (I_{geo})

The Geoaccumulation index (I_{geo}) has been widely utilised as a measure of pollution in sediment by comparing current metal contents with pre-indusrtial levels (Muller, 1986). The I_{geo} value is determined by the following equation according to Muller (1969).

$$Igeo = \frac{Cn}{1.5 \times Bn}$$

Where:

Cn – Concentration of heavy metal

Bn - Geoaccumulation background value

1.5 - Possible variation of the background data due to lithological variations.

The degree of pollution is divided into 7 levels (Stoffers *et al.*, 1986). Level 0 indicates no contamination and level 6 indicates extreme contamination. I_{geo} is used to determine the degree of anthropogenic influence on heavy metal concentration in the soil.

4.2.12 Statistical analysis

The significance of plant–soil relationships were established by calculating the Pearson's correlation coefficients for the soil parameters (SOM, CEC and pH), exchangeable concentration of elements in soil and the concentration of elements in the fruit of *B. micrantha*. The coefficients were obtained using Statistical Package for the Social Sciences (SPSS). The correlation ranges between + 1 and -1, where + 0.8 correlation indicates a strong positive relationship, while - 0.8 correlation indicates a negative relationship.

4.3 RESULTS AND DISCUSSION

4.3.1 Elemental Analysis

For the purpose of quality assurance, the analyses were carried out in triplicate and the accuracy of the method for elemental analysis was validated by comparison of the measured values obtained for the two different Certified Reference Materials (CRMs), BCR-402 (white clover powder) for the plant and D081-540 (Metals in soil) for the soil samples, to certified values and the accepted limits (Tables 14a and 14b). In Table 14b, the values for As, Co and Se are certified whilst those for Fe, Ni and Zn are suggestive therefore no uncertainties are provided.

Table 14a: Comparison of measured and certified values (mean ± SD, n = 3) in the CRM, D081-540 (Metals in soil) (ERA A Waters Company).

Elements	Certified mg kg^{-1}	Accepted limits	Measured µg g^{-1}
As	101 ± 5.92	61.0-116	83.2 ± 3.36
Ca	6200 ± 7.27	5620-9440	6240 ± 542
Cd	143 ± 5.60	104-182	122.4 ± 3.78
Co	232 ± 4.10	148-250	209.2 ± 4.27
Cr	320 ± 6.06	60.0-114	63.8 ± 2.50
Cu	268 ± 4.72	204-332	237 ± 23.7
Mg	2850 ± 5.51	1860-3840	2362 ± 52.4
Mn	425 ± 9.69	324-525	349 ± 17.7
Ni	236 ± 4.17	175-302	212 ± 21.7
Pb	97.9 ± 11.3	69.3-126	95.8 ± 4.52
Zn	130 ± 11.5	87-173	90.9 ± 5.29
Se	127 ± 4.47	84.6-170	88.9 ± 2.95

Table 14b: Comparison of measured and certified values in the CRM (white clover powder) (BCR-402)

Elements	**Certified mg kg^{-1} ****	**Measured µg g^{-1}****
As	0.093 ± 0.010	0.0677 ±0.058
Co	0.178 ± 0.08	0.167 ± 0.055
Fe	244	240 ±37.70
Ni	8.25	8.23 ± 0.46
Zn	25.2	25.43 ± 0.64
Se	6.70 ± 0.25	6.60 ± 0.265

Based on dry mass, **Mean ± S.D, at 95% confidence interval, n = 3.

Table 15: Elemental concentrations in μg g^{-1} (Mean (SD), n=5) of selected elements in *B. micrantha* fruits and corresponding growth soil (Total (T) and Exchangeable (Ex)).

	Sites	**$[Soil]_T$**	**$[Soil]_{Ex}$**	**[Fruit]**	**[Fruit] / $[Soil]_T$**	**[Fruit] / $[Soil]_E$**	**Ex%**
Ca	B1	11717 (1025)	10590 (485)	417 (43.3)	0.0356	0.0394	90.4
	B2	2841 (169.9)	2070 (932)	562 (28.4)	0.198	0.271	72.9
	B3	3000 (316)	1519 (168)	299 (2.78)	0.0997	0.197	198
	B4	4367 (450)	124 (2.29)	616 (98.0)	0.141	4.97	2.84
	B5	1774 (281)	53.4 (7.05)	418 (10.7)	0.236	7.83	3.01
	B6	2459 (2015)	16.8 (8.88)	352 (17.2)	0.14	20.9	0.68
	B7	3148 (2137.9)	83.0 (65.6)	1056 (57.7)	0.336	12.7	2.64
	B8	1473 (198.9)	157 (41.1)	624 (2.90)	0.424	3.97	10.7
Co	B1	3.05 (0.370)	1.81 (0.19)	0.20 (0.05)	0.066	0.05	59.3
	B2	1.02 (0.118)	ND	0.35 (0.06)	0.34	ND	ND
	B3	0.370 (0.0386)	ND	0.73 (0.08)	1.97	ND	ND
	B4	2.88 (0.590)	2.46 (0.60)	1.46 (0.14)	0.51	0.28	85.4

	B5	0.293 (0.072)	ND	0.30 (0.02)	1.02	ND	ND
	B6	0.254 (0.066)	ND	ND	ND	ND	ND
	B7	5.23 (0.066)	3.65 (0.47)	3.98 (0.78)	0.76	1.09	69.8
	B8	2.20 (0.384)	0.263 (0.01)	40.80 (3.95)	18.5	155	12.0
Cr	B1	4.06 (0.81)	0.18 (0.045)	1.50 (0.36)	0.37	8.33	4.43
	B2	2.23 (0.14)	0.02 (0.01)	1.58 (0.34)	0.71	79.0	0.90
	B3	0.588 (0.034)	0.04 (0.018)	0.87 (0.55)	1.48	21.8	6.80
	B4	5.24 (0.59)	0.12 (0.044)	7.27 (0.27)	1.39	60.6	2.30
	B5	2.14 (0.46)	1.02 (0.074)	1.86 (0.73)	0.87	1.83	47.7
	B6	1.29 (0.20)	1.01 (0.35)	3.50 (0.85)	2.71	0.499	78.3
	B7	5.30 (0.45)	0.53 (0.09)	3.50 (0.72)	0.66	6.60	10.0
	B8	8.31 (0.72)	0.15 (0.12)	2.59 (0.24)	0.31	17.27	1.81
Cu	B1	32.7 (2.09)	8.70 (1.43)	9.83 (1.94)	0.30	1.13	26.6
	B2	11.4 (2.21)	6.65 (3.58)	9.51 (1.97)	0.83	1.43	58.1
	B3	10.5 (1.94)	6.55 (0.83)	8.50 (2.17)	0.81	1.30	62.2
	B4	31.8 (2.83)	9.95 (1.80)	7.89 (1.22)	0.25	0.79	31.3

	B5	21.2 (1.77)	15.2 (1.25)	10.5 (0.33)	0.49	0.69	71.7
	B6	11.4 (0.3)	7.61 (0.35)	10.5 (0.78)	0.922	1.37	67.0
	B7	34.2 (4.37)	12.8 (1.32)	5.98 (1.04)	0.175	0.468	37.4
	B8	30.8 (7.97)	15.7 (0.85)	9.22 (0.85)	0.299	0.587	51.0
Fe	B1	20858 (2308)	391 (59.8)	27.95 (1.51)	0.0013	0.07	1.88
	B2	6058 (6675)	183 (96.4)	27.49 (1.54)	0.0041	0.15	3.02
	B3	14985 (77.8)	166 (16.30)	7.71 (0.77)	0.0012	0.05	2.567
	B4	6497 (838)	150 (14.02)	855.84 (25.88)	0.06	5.69	1.004
	B5	6507 (601)	404 (17.92)	38.71 (1.72)	0.01	0.10	6.20
	B6	4422 (1009)	64.4 (1.20)	8.88 (0.308)	0.002	0.138	1.46
	B7	15458 (829)	65.0 (5.92)	345.33 (11.29)	0.02234	5.31	0.420
	B8	4835 (699)	90.7 (5.22)	16.67 (2.92)	0.00345	0.184	1.88
Mg	B1	1789 (115)	68.3 (18.7)	834 (13.1)	0.47	12.2	3.82
	B2	359 (6.41)	45.8 (7.39)	838 (14.10)	2.33	18.3	12.8
	B3	757 (24.9)	25.9 (4.33)	1018 (8.71)	1.34	39.44	3.41
	B4	2368 (417)	15.5 (0.70)	956 (54.4)	0.40	61.6	0.655

	B5	609 (35.7)	71.4 (7.91)	984 (55.0)	1.61	13.8	11.7
	B6	346 (41.82	27.7 (1.21)	1221 (86.0)	3.53	44.1	8.01
	B7	2222 (128)	15.95 (1.38)	648 (67.0)	0.292	40.7	0.718
	B8	641 (115)	44.8 (2.33)	369 (69.5)	0.546	8.23	6.99
Mn	B1	941 (190.86)	484 (66.7)	287 (8.62)	0.30	0.59	51.40
	B2	389 (18.18)	315 (214.09)	283 (7.22)	0.89	0.74	81.05
	B3	122 (23.55)	85.1 (8.2)	321 (17.65)	2.63	3.77	69.63
	B4	402 (16.01)	383 (39.7)	712 (54.71)	1.77	1.86	95.32
	B5	106 (6.24)	57.8 (2.41)	555 (9.48)	5.23	9.62	54.4
	B6	213 (15.41)	45.8 (6.42)	450 (8.78)	2.11	9.83	21.4
	B7	3517(4.57	483 (59.1)	286 (10.33)	0.0815	0.593	13.7
	B8	56570 (3769.7)	2150 (75.5)	43900 (4601)	0.776	20.42	3.80
Ni	B1	56.1 (4.01)	ND	1.92 (0.26)	0.034	ND	ND
	B2	17.7 (2.44)	ND	2.02 (0.18)	0.11	ND	ND
	B3	6.62 (2.05)	ND	1.19 (0.34)	0.18	ND	ND
	B4	37.7 (4.19)	ND	6.26 (1.13)	0.17	ND	ND

	B5	8.08 (0.94)	ND	3.99 (0.34)	0.49	ND	ND
	B6	10.7 (18.3)	1.35 (0.11)	1.78 (0.19)	0.166	1.32	12.6
	B7	32.1 (3.31)	7.68 (0.67)	1.25 (0.77)	0.039	0.16	23.9
	B8	13.9 (1.56)	5.62 (0.72)	2.14 (1.22)	0.153	0.380	40.3
Pb	B1	56.0 (4.01)	3.01 (0.55)	0.98 (0.71)	0.017	0.326	5.37
	B2	17.6 (2.44)	5.02 (0.85)	2.00 (0.14)	0.11	0.40	28.44
	B3	1.92 (1.93)	0.92 (0.17)	1.59 (0.40)	1.42	0.83	47.92
	B4	31.6 (2.12)	ND	2.75 (0.43)	0.087	ND	ND
	B5	25.0 (1.86)	3.78 (0.26)	1.54 (0.32)	0.062	0.41	15.12
	B6	15.2 (11.72)	6.15 (0.45)	ND	ND	ND	40.25
	B7	29.3 (2.43)	1.46 (0.21)	ND	ND	ND	3.58
	B8	8.06 (1.14)	0.20 (0.02)	0.3 (0.1)	0.0372	1.5	2.48
Zn	B1	39.0 (9.13)	8.70 (3.73)	247 (21.2)	6.34	28.42	22.30
	B2	32.3 (1.56)	9.56 (8.13)	250 (24.3)	7.75	26.24	29.57
	B3	17.1 (5.86)	6.77 (3.61)	358 (48.4)	20.9	52.89	39.52
	B4	18.1 (2.59)	5.87 (0.28)	365.5 (24.6)	20.2	62.3	32.4

B5	48.7 (5.63)	7.22 (9.51)	341.8 (28.2)	7.10	47.3	14.8
B6	17.9 (5.73)	7.65 (7.28)	18.2 (1.41)	1.01	2.39	42.7
B7	14.7 (1.46)	11.9 (1.88)	16.20 (3.17)	1.10	1.42	77.4
B8	8.12 (3.07)	5.83 (1.52)	213. (20.2)	26.2	36.5	71.8

B1 = Scottsville, B2 = Pigeon Valley, B3 = Westville, B4= Greytown, B5 = Reservoir Hills, B6 = Tugela Ferry, B7 = Sherwood, B8= Musgrave

[F] = Fruit; T = Total; E = Exchangeable;

ND = not determinable;

$Ex\% = [Soil]_{Ex} / [Soil]_{T} \times 100$

The concentrations (exchangeable and total) of 13 selected metals present in the soil and the fruit samples of *B. micrantha* from 8 different sites in KwaZulu-Natal were analysed and represented in Table 15. The exchangeable percent (% Ex) was also calculated to measure the percent of elements from the soil that are available for plant uptake. Arsenic and Cd in soil and in fruit were below the detection limit of the instrument therefore they were excluded from the discussion.

On analysis of Table 15, it was observed that the total concentration of Fe in soil was higher than all other elements at all 8 sites. Total soil Fe at Site B1 (Scottsville) was highest (20858 μg g^{-1}) but was low in the fruit (27.95 μg g^{-1}) and total soil Fe at Site B4 (Greytown) was lowest (6497 μg g^{-1}) but was high in the fruit (855 μg g^{-1}). This indicated that the plant controlled uptake of Fe where, if Fe was not readily available then uptake increased but if Fe concentrations in soil were sufficient then uptake was reduced. On average, about 2.3% of total soil Fe was in exchangeable form.

Calcium, which contributes about 2% towards body weight, was found to be high in both soil and fruits. Calcium in the fruits is believed to delay ripening and it helps to reduce biological aging or degradation of the fruit (Valvi and Rathod, 2011). Calcium in soil ranged between 1473 and 11717 μg g^{-1} and between 299 and 1056 μg g^{-1} with highest concentrations in the fruit (11717 μg g^{-1}) being observed at site B1 (Scottsville). Most of the Ca at sites B1 (Scottsville), B2 (Pigeon Valley) and B3 (Westville) were in exchangeable form (> 70%). Although the available concentration of Ca was high, the plant controlled uptake to meet its physiological requirement levels.

Magnesium concentrations in both soil and fruits were high, but the exchangeable concentrations were low. This shows that the plant accumulated Mg with BAFs ranging from 0.29 to 3.53 and 8.23 to 61.6 for total and exchangeable soil concentrations, respectively.

High concentrations of Fe, Mg and Ca in soil are likely due to calcite ($CaCO_3$), magnesite ($MgCO_3$) and dolomite ($CaMg(CO_3)_2$) which are major carbonates found in soil from parental rock material.

Cobalt concentrations in both soil and fruit were relatively low with concentrations ranging from 0.254 to 5.23 μg g^{-1} in soil and 0.3 to 3.98 μg g^{-1} in fruit. Site B8 (Musgrave) was found to accumulate Co with the concentration in the fruit being higher than the other sites with BAFs of 18.5 and 155 for total and exchangeable soil concentrations, respectively.

Chromium exists in two oxidation states, trivalent and hexavalent form. Both soil and fruit concentrations of Cr were low with concentrations ranging from 1.29 to 8.31 μg g^{-1} in soil and from 1 to 3.5 μg g^{-1} in the fruit. The average exchangeable percent of Cr was found to be 28% with site B4 (Greytown) having the highest exchangeable percent of 85.4%.

Copper is one of the essential elements in both humans and plants, but poses toxic effects if at higher concentrations than recommended. Total soil Cu ranged from 10 to 32 μg g^{-1} and on average about 50.6% was in available form. The concentration of Cu in the fruit ranged from 5.9 to 10.47 μg g^{-1}.

Total soil Mn was high with concentrations ranging between 283 and 56570 μg g^{-1} with the highest concentration being observed at site B8 (Musgrave). Manganese in fruit ranged from 286 to 43900 μg g^{-1}. High concentrations of Mn in the fruit were observed at sites B3 (Westville), B4 (Greytown), B5 (Reservoir Hills) and B6 (Sherwood); these concentrations were higher than total soil concentrations thereby producing BAFs >1. The study showed the plants tendency to accumulate Mn. When Mn in soil was too high, then the plant concentration increased accordingly.

The exchangeable concentrations of Ni were below the instruments detection limit at most sites. Nickel concentrations in the fruits ranged between 1.19 to 6.26 μg g^{-1}, with site B4 (Greytown) having the highest concentration and site B3 (Westville) having the lowest.

Lead contamination occurs due to anthropogenic sources such as combustion and smelting of leaded gasoline. Lead is also toxic in plants as it impairs plant growth by damaging seed germination and root elongation (Pourrut *et al.,* 2011). Lead concentrations were low in the fruits and ranged between 0.3 and 2.8 μg g^{-1} with site B4 (Greytown) having the highest concentration and site B8 (Sherwood) having the lowest. According to the Department of health, the maximum level of Pb in fruits and berries in South Africa is set at 0.20 mg kg^{-1} (Department of Health, 2004). Five of the sites had concentrations of Pb above this limit.

Zinc concentration in fruits was higher than total soil concentrations with concentration ranging between 16.2 and 365.5 μg g^{-1} in fruit. The highest concentration of Zn was observed at site B4 (Greytown). BAFs ranging from 1.01 to 26.6 and 1.42 to 52.8 for total and exchangeable soil concentrations respectively indicated the plants tendency to accumulate Zn.

On analysis of the data, in particular the concentration of the elements in the fruit of *B. micrantha*, it was observed that concentrations of Ca, Fe, Mg, Mn, and Zn were higher than other metals in the fruit. This distribution of the major elements in fruits is illustrated in Figure 30. Of the major elements, the concentration of Mg was highest at most sites and that of Fe and Zn were lowest.

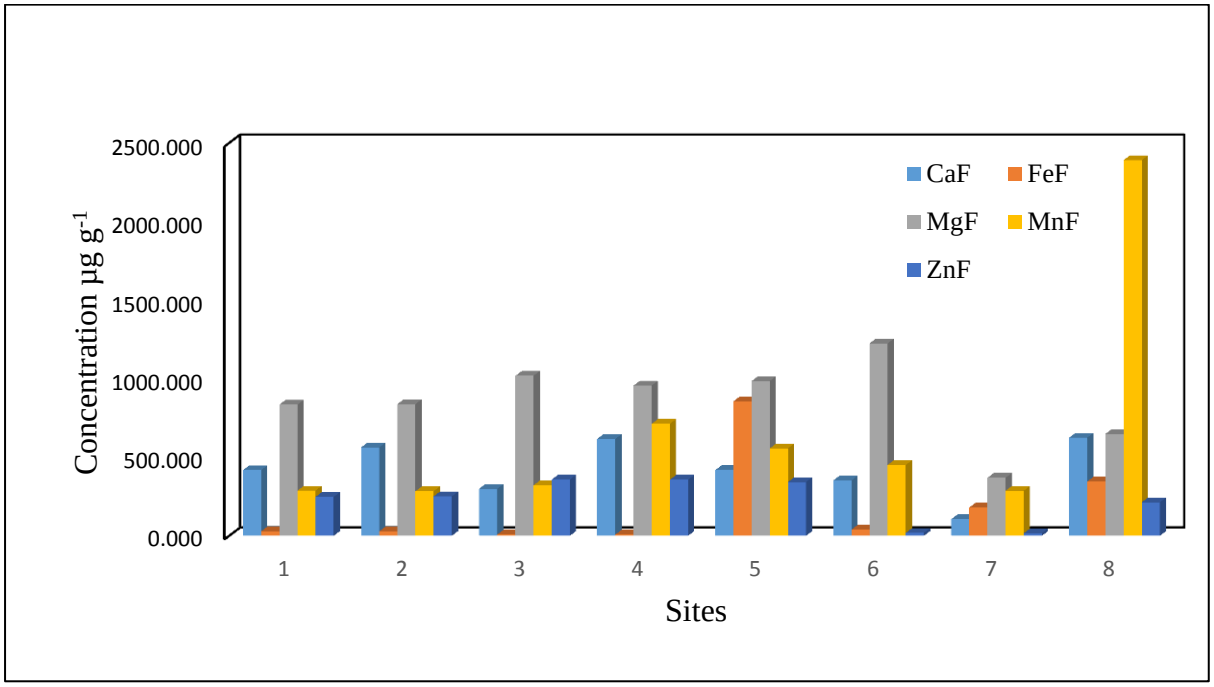

Figure 30: Major elements in the fruits (F) of *B. micrantha*

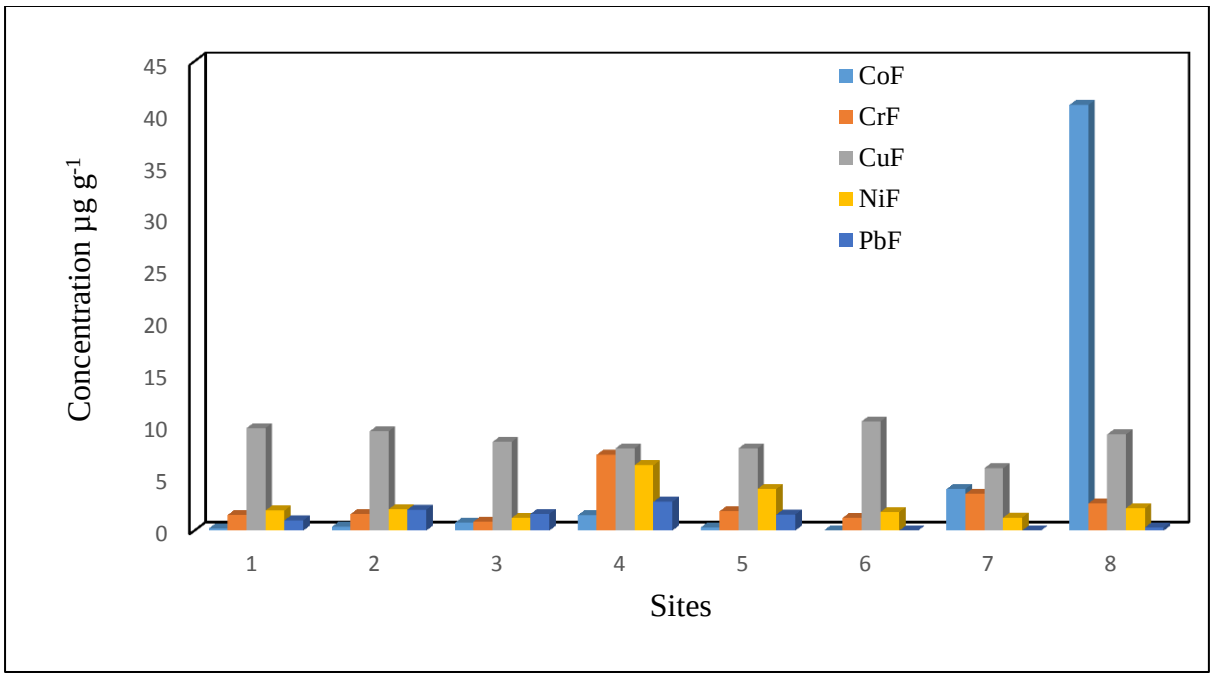

Figure 31: Minor elements of *B. micrantha* fruits (F)

This distribution of the minor elements in fruits is illustrated in Figure 31. These elements are Co, Cr, Cu, Ni and Pb with Cu exhibiting the highest concentrations at most sites. In general, the concentrations of the elements in the fruits of *B. micrantha* were found to be in decreasing order of Mg > Ca > Mn > Zn ≈ Fe > Cu > Co > Ni > Cr > Pb > As ≈ Cd.

Plants are not specific when taking heavy metals from the soil, either essential such as Cr, Mn, Fe, Co, Cu, Zn and Se or non-essential elements such as As, Cd and Pb. These metals have a tendency of accumulating in plants and vegetables. Some plants are able to eliminate or accumulate heavy metals to meet their metabolic demands. When the concentration (total and exchangeable) of the plant essential element in the soil is below the concentration required by the plant, the plant tends to accumulate that particular element to meet its physiological demand, as a result we observe higher concentrations in plant soil. Likewise, when the soil concentrations (total and exchangeable) exceed its physiological demand the plant tends to exclude the excess amount. The study showed that *B. micrantha* accumulated Mg, Mn and Zn as the concnetrations in the fruits were higher than that of soil (exchangeable and total) and tended to exclude the other elements to meet its physiological requirement levels.

4.3.2 Estimated contribution of *B. micrantha* fruits to the diet

A balanced diet is one of the important parameters in support of normal growth and development of the human body. A balanced diet means eating the right types and amounts of food to supply nutrition and energy for maintenance of body cells, tissues and organs. An imbalance in the diet can lead to malnutrition; eating low amounts of certain nutrients can lead to deficiencies while eating too much can lead to toxicities.

Table 16: Dietary Reference Intake (DRI)=Recommended Dietary allowance (RDA) and Tolerable Upper Intake Level (UL) of elements, compared to the average concentration of elements obtained from *B. micrantha* fruits (n=8).

Element	Average Concentration ($\mu g\ g^{-1}$)	Average Concentration ($mg\ 10g^{-1}$)	DRI ($mg\ day^{-1}$)		Estimated contribution to RDA (%)
			RDA	UL	
Ca	543	5.4	1000-1300	2500	0.4-0.5
Cr	3	0.03	0.024-0.035	ND	81-118
Cu	9	0.09	0.9	8.00	10
Fe	166	1.7	8-15	45.00	11-21
Mg	859	8.6	310-320	350	2.7-2.8
Mn	414	4.1	1.6- 3	9.00	180-258
Ni	3	0.03	ND	1.00	ND
Pb	1	0.01	ND	ND	ND
Zn	226	2.3	8 -11	34.0	21-28

ND: Not Determined

The elemental distribution in the fruit was compared to Dietary Reference Intakes (DRIs) (Table 16). If an average serving size of 10 g (dry mass) of the fruit of *B. micrantha* is consumed it would contribute significantly towards the daily intake of important nutrients.

Most of the nutrients did not exceed their Tolerable Upper Intake Levels (ULs). Manganese is one of the essential nutrients for nerve impulses and muscle contraction. About 10 g of *B. micrantha* fruits contributes about 180-258% towards the RDA for Mn. Although the percentage contribution towards the RDA for Mn was high, the UL for Mn (9 mg per day) was not exceeded. If approximately 10 g of *B. micrantha* fruit is consumed per day the contribution to the diet for the elements Cr and Fe would be >81% and >21% for these elements, respectively. For the toxic elements, As, Pb and Cd, concentrations in the fruit were at safe levels for human consumption. In South Africa, especially in rural communities, many households rely on natural resources for food and B. *micrantha* fruits are frequently consumed by the people in these communities. This study provides information on the nutritive value of *B. micrantha* fruits which indicates that it is good for health and does not have a tendency to accumulate toxic elements.

4.3.3 Geoaccumulation index

The background concentration of trace elements in soil conveys the information on the natural range of concentrations that can be expected before contamination (Herselman, 2007). Table 17 shows the status of heavy metals pollution in different sites around KwaZulu-Natal, which was evaluated by examining the geoaccumulation index (*Igeo*) and background concentrations. According to the level of contamination established by Muller (1986), *Igeo* values less than 0 (negative) would mean the soil is uncontaminated while positive values indicates enrichment of soil by heavy metals. In this study, the assessment of contamination at the eight sampling sites indicated non-contamination by the elements examined as the *Igeo* values were less than 0 (negative).

Table 17: Total baseline concentrations of metals in South African soil (µg g^{-1}) and geoaccumulation index (*Igeo*) for 8 sites

Elements	**TBT***	**Site B1**	**Site B2**	**Site B3**	**Site B4**	**Site B5**	**Site B6**	**Site B7**	**Site B8**
		Igeo							
Cd	2.7	-1.28	-2.80	-	-	-	-	-	-
Co	69	-4.23	-	-	-4.90	-	-	-4.08	-0.038
Cr	353	-4.47	-5.34	-7.25	-4.10	-5.39	-6.21	-2.36	0.0925
Cu	117	-2.42	-3.94	-4.06	-2.46	-3.05	-3.95	-1.75	0.176
Zn	115	-2.14	-2.41	-3.33	-3.25	-1.99	-2.70	-3.55	0.047
Pb	66	-0.82	-2.49	-6.47	-1.65	-1.82	-3.27	-4.83	0.0814

* Total Baseline Concentration (Herselman *et al.*, 2007)

4.3.4 Soil properties

In Table 18 the values for the soil properties pH, SOM and CEC from the eight sampling sites in KwaZulu-Natal are presented. Soil pH ranged from 5 to 7.7, indicating that the plant grows in a slightly acidic soil. The ideal soil pH for normal plant growth of *B. micrantha* is between 6-8 where cations such as Ca, Mg and Fe become more available. The CEC ranged between 4-15.5 meq $100g^{-1}$ and SOM (%) ranged between 6-16.7% with site B2 (Pigeon Valley) having the minimum CEC and SOM and site B4 (Greytown) having the highest values.

Table 18: pH, Soil Organic Matter (SOM) and Cation Exchange Capacity (CEC) of soil sample from 8 different sites of KZN.

Sites	pH	SOM	CEC
B1	6.56 ± 0.079	16.68 ± 2.19	4.00 ± 3.5
B2	5.40 ± 0.015	4.65 ± 2.30	4.97 ± 4.8
B3	5.85 ± 0.02	6.19 ± 1.36	4.88 ± 2.11
B4	7.60 ± 0.006	10.35 ± 2.50	15.54 ± 2.75
B5	5.37 ± 0.042	10.03 ± 0.85	8.08 ± 3.88
B6	5.21 ± 0.015	6.04 ± 1.75	14.87 ± 9.32
B7	7.62 ± 0.031	7.14 ± 1.38	9.70 ± 2.90
B8	7.52 ± 0.25	10.8 ± 1.20	8.23 ± 2.33

4.3.5 Chemical composition of *B. micrantha* fruit

B. micrantha fruit were found to contain about 90.1% moisture, 3.2% ash, 4.1% protein, 0.9% oil and 1.72% carbohydrate (Table 19). Most natural foods contain about 0-5% of protein while processed foods contains more than 10%. Water contributes up to 60% of the human body; we need to consume enough water daily for the normal functioning of the body. The moisture content in the fruit was 90.1%, therefore the fruit can also be hydrating and refreshing to eat.

Table 19: Nutritional composition of *B. micrantha* (per 100g dry sample)

Composition per 100g dry mass	
Moisture content	90.1 ± 0.60
Ash	3.2 ± 0.46
Protein	4.1 ± 0.13
Oil	0.9 ± 0.01
Carbohydrate **	1.7

**Carbohydrate obtained by subtracting the sum of oil, ash & protein from the total dry mass.

4.3.6 Statistical analysis

Concentrations of elements in the fruits of *B. micrantha* were, to varying degrees, correlated with total and exchangeable concentrations in the soil (Table 20). The metals in the soil interact with each other thereby influencing their exchangeability hence uptake by plants of the metals with which they interact. Metals in the soil can interact synergistically with each other where, an increase in the concentration of one element increases the concentration of the other or antagonistically where, an increase in the concentration of one element decreases the concentration of the other. The correlation matrix provides the correlation efficient (r) which indicates the extent to which interactions are related either synergistically (positive correlations) or antagonistically (negative correlations).

Synergistic relationships in soil were observed between Zn and Co (r = 0.7) as well as Mg and Fe (r = 0.8). This could be due to the similarity in charge and size of Zn and Co and the similarity in charge between Mg and Fe. SOM positively correlated with exchangeable Ca (r = 0.7) whilst pH negatively correlated with exchangeable Pb (r = -0.8). This indicates that as

the organic matter of the soil increases, so too does the available form of Ca and also that the availability of Pb is pH dependent.

Synergistic correlations in the plant were observed between Co and Mn (r = 1.0), Cr and Ni (r = 0.8), Pb and Zn (r = 0.8) and Pb and Ni (r = 0.7). This indicates that an increase in uptake of Ni and Zn increases uptake of the toxic metal Pb. For Co and Mn or Cr and Ni, the synergies observed could be due to the plant needing proportionate amounts of these metals where, if the concentration of one metal increases then requirement for the other metal increases thereby promoting its uptake.

Generally, an antagonistic relationship in the plant will occur when the plant takes up two elements by the same mechanism and the increase in uptake of one element decreases the uptake of the other (Kalavrouziotis *et al.,* 2008). Antagonistic correlations in the plant were observed between Ca and Cu (r = -0.7) as well as Ca and Mg (r = -1.0). The mechanism of uptake for these similarly charged metals could be similar thereby promoting the observed competition.

Strong positive correlations were observed between pH and Ca (r = 0.7) as well as Cr (r = 0.7) in the plant. Strong antagonistic correlations were observed between pH and Mg in the plant (r = -0.7). This indicates that at high pH the concentrations of Ca and Cr increases and that of Mg decreases.

Table 20: Correlation matrix for concentrations of elements in soil (Exchangeable (E)) and fruits (F) of *B. micrantha*.

	CaE	CaF	CoE	CoF	CrE	CrF	CuE	CuF	FeE	FeF	MgE	MgF	MnE	MnF	NiF	PbE	PbF	ZnE	ZnF	pH	SOM	CEC
CaF	-0.3																					
CoE	0.4	0.3																				
CoF	-0.2	0.2	-0.4																			
CrE	-0.3	-0.3	-0.3	-0.2																		
CrF	-0.3	0.5	-0.1	0.1	-0.3																	
CuE	-0.4	0.4	0.2	0.6	-0.2	0.2																
CuF	0.4	**-0.7**	-0.4	0.1	0.4	-0.5	-0.4															
FeE	0.6	-0.4	0.6	-0.3	-0.3	-0.2	0.1	0.1														
FeF	-0.3	0.0	0.4	0.2	-0.1	-0.1	**0.8**	-0.3	0.5													
MgE	0.5	-0.4	0.5	0.0	-0.2	-0.5	0.3	0.4	**0.8**	0.6												
MgF	0.0	**-1**	-0.4	-0.4	0.6	-0.2	-0.5	0.6	0.2	-0.1	0.1											
MnE	-0.1	0.3	-0.3	1.0	-0.3	0.1	0.6	0.1	-0.3	0.1	0.1	-0.5										
MnF	-0.2	0.1	-0.4	1.0	-0.2	0.0	0.6	0.2	-0.3	0.2	0.1	-0.3	1.0									
NiF	-0.3	0.0	-0.1	-0.1	-0.2	**0.8**	0.2	-0.2	0.2	0.2	0.0	0.3	-0.1	-0.1								
PbE	0.1	-0.4	0.2	-0.5	**0.7**	-0.6	-0.4	0.5	0.2	0.0	0.3	0.5	-0.5	-0.4	-0.3							
PbF	0.1	-0.3	-0.1	-0.4	-0.5	0.4	-0.3	-0.1	0.4	-0.1	0.0	0.3	-0.3	-0.3	0.7	-0.2						
ZnE	0.2	0.6	**0.7**	-0.4	0.0	-0.2	-0.2	-0.3	0.0	-0.1	0.0	-0.5	-0.3	-0.4	-0.5	0.4	-0.4					
ZnF	0.2	-0.5	-0.1	-0.1	-0.6	0.2	0.0	0.0	0.6	0.2	0.3	0.3	-0.1	0.0	0.5	-0.4	**0.8**	-0.6				
pH	-0.1	**0.7**	0.0	0.5	-0.5	**0.7**	0.4	-0.5	-0.3	-0.1	-0.4	**-0.7**	0.6	0.4	0.2	**-0.8**	-0.1	0.0	-0.1			
SOM	**0.7**	-0.1	0.4	0.2	-0.3	0.2	0.3	0.1	0.6	0.1	0.5	-0.1	0.3	0.2	0.3	-0.3	0.0	-0.2	0.3	0.4		
CEC	-0.6	0.2	-0.4	0.0	0.6	0.6	0.1	-0.1	-0.5	-0.1	-0.6	0.3	-0.1	0.0	0.5	0.0	0.0	-0.3	-0.3	0.2	-0.2	

$[X]_{Exchangeable}$ where X= various elements; $[X]_{Fruits}$ where X= various elements;

SOM = Soil Organic Matter; [d] CEC = Cation Exchange Capacity

4.4 CONCLUSION

The impact of soil quality parameters on elemental uptake of the wild fruits of *B. micrantha* from eight different geographic locations in KwaZulu-Natal was determined. This was done for 13 elements. The elemental concentrations in the fruits were also assessed for their nutritional value. The concentration of the elements in the fruits were found to be in decreasing order of Mg > Ca > Mn > Zn ≈ Fe > Cu > Co > Ni > Cr > Pb > As ≈ Cd. The elemental analysis in the fruits of *B. micrantha* showed that the plant controls the uptake of the elements to meet its physiological demands. The plant tended to accumulate Mg, Mn and Zn and tended to exclude the other elements studied. Statistical analysis revealed competition effects, both antagonistic and synergistic. *B. micrantha* fruits were found to be rich in essential elements and contained low concentrations of the toxic elements studied. The high concentration of Ca in the fruits supports the medicinal use of *B. micrantha* which is used to treat toothache.

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CHAPTER 5

5.1 Summary

S. inerme and *B. micrantha* are two medicinal plant species indigenous to KwaZulu-Natal. These plants have morphological similarities as a result they are used in traditional medicine to treat a variety of the same illnesses. The study aimed to investigate the impact of soil quality on the chemical composition of the fruit and to investigate if the two plants contain similar phytocompounds to validate its use interchangeably by traditional herbalists. The antioxidant activity of the four crude extracts and pure phytocompounds fom *S. inerme* and *B. micrantha* were also evaluated using the DPPH assay.

5.2 Findings from the phytochemical study *S. inerme*

Three pentacyclic triterpenes (friedelin, stigmasterol, and a mixture of α and β-amyrin), one carotenoid (lutein) and one megastigmane (apocynol B) were isolated and identified from different parts of *S. inerme.* These compounds were not isolated previously from this plant. Apocynol B has not been previously isolated from plant extracts, but was obtained from enzymatic hydrolysis of apocynoside II. Apocynoside II was isolated from the roasted leaves of *Apocynum venetum* L. The extract and isolated compounds showed good antioxidant activity.

5.3 Findings from the phytochemical study of *B. micrantha*

Two flavonoids (quercetin and quercetin glucoside) and two pentacyclic triterpenes (oleanolic acid and acacic acid lactone) were isolated and identified from *B. micrantha*. These compounds were not isolated previously from this plant. The extract and isolated compounds showed good antioxidant activity.

5.4 Findings from elemental analysis of *B. micrantha*

The impact of soil quality parameters on elemental uptake of the wild fruits of *B. micrantha* from eight different geographic locations in KwaZulu-Natal was determined. This was done for 13 elements. The elemental concentrations in the fruits were also assessed for their nutritional value. The concentration of the elements in the fruits were found to be in decreasing order of Mg > Ca > Mn > Zn ≈ Fe > Cu > Co > Ni > Cr > Pb > As ≈ Cd. The elemental analysis of the fruits of *B. micrantha* showed that the plant controls the uptake of the elements to meet its physiological demands and concentrations of essential elements in the fruit were within acceptable limits therefore can contribute positively to the diet without the threat of toxic effects.

5.5 Overall conclusion

The phytochemical analysis revealed the medicinal benefits of *S. inerme* and *B. micrantha*. However, the classes of compounds isolated from the two plant species were different therefore it can be concluded that these two plant species cannot be used interchangeably for their medicinal benefit. This was further confirmed by GC-MS profiling which showed different

compounds in the fruit of both plant species. The elemental analysis revealed the nutritional benefits of consuming the wild fruits of *B. micrantha.* The results obtained from this study reveal the potential of the fruits of these plant species as neutraceuticals.