ANTIOXIDANT ACTIVITIES, PHYTOCHEMICAL, AND MICRO-NUTRIENTS ANALYSIS OF AFRICAN MORINGA (*MORINGA OVALIFOLIA*)

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NATALIA KWADJONYOFI ANANIAS

(200539744)

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Main Supervisor: Dr. Martha Kandawa-Schulz

Co- Supervisors: Prof. Luke Chimuka (University of Witwatersrand, South Africa)

Dr. Marius Hedimbi

ABSTRACT

Moringa ovalifolia (African Moringa) is endemic to southern Africa, and it is distributed from southern to central Namibia to south western Angola. At the moment, little is documented on the phytochemical content, miro-nutrients and antioxidant activities of *M. ovalifolia*. Hence, this study was aimed at evaluating the phytochemical, micro- nutritients and antioxidant activities of *M. ovalifolia*. Fresh Moringa leaves, bark, flowers and seeds (ponds) were collected from five different sites. Soxhlet extraction method was used for extraction, and thereafter different analyses were performed using UV-Vis spectrophotometer. Antioxidant properties were investigated using three indicators: reducing activity (700 nm), DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activities (515 nm) and total phenolic content (740 nm). High perfomance liquid chromatography (HPLC) with UV detector (254 nm) was used in the identification and quantification of flavonols. Elemental composition of the leaves was determined using the Inductively coupled plasma optical emission spectroscopy (ICP-OES). The total phenolic content of *M. ovalifolia* remained almost the same in samples from different sites, but varies in different plant parts. *M. ovalifolia* leaves, flowers reduced DPPH by nearly 20%, while the seed and bark reduced the DPPH by 12%. The following flavonols were present: Kaempferol, Quercetin and Myrietin. High amounts of quercetin were recorded, with the highest amount of about 16 844.47 ± 194.336 mg/Kg in leaves from site 4. Micro-nutrients analysis revealed the presence of: Ba (2.1±0.13 mg/Kg), Cd (6.2±0.29 mg/Kg), Co (3.4±0.17 mg/Kg), Li (10.9±0.05 mg/Kg), Mn (17.7±0.17 mg/Kg), Si (12.6±0.14 mg/Kg), Al (28.4±2.63 mg/Kg), Zn (59.1±0.88 mg/Kg), Cu

(29.8±0.42 mg/Kg), Ni (27.4±0.99 mg/Kg), Fe (84.7±2.21 mg/Kg), Na (190.5±7.15 mg/Kg), Ca (2427.90±187.62 mg/Kg), K (6399.92±387.04 mg/Kg) and Mg (3974.94±262.69 mg/Kg) from site 1. The leaves have a high conentration of these elements: Ca, K and Mg compared to other elements present. The concentration of different elements varies from site to site, and sample to sample. These variations could have been influenced by geographical location, soil conditions, climatic conditions and other factors. Hence results of this study indicates that *M. ovalifolia* can play a role in improving the health and nutrition, particularly in malnourished populations.

Keywords: *Moringa ovalifolia*, Macronutrients, Micronutrients, Metal content, Elemental analysis, Flavonoids, Total phenolics, ICP-OES, HPLC, Soxhlet extraction, DPPH radical scavenging activies, Reducing power, UV-Vis spectrophotometer

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DEDICATION

To my Parents, My late mom (Kristofina Ndahambelela Hakandonga) and my dad (Abraham Nghipundukavali Ananias) who have always been there for me through life struggles. If not for them, I would not be where I am today. I am forever indebted to you. Thank you Lord!

PUBLICATIONS AND CONFERENCE PRESENTATIONS

1. N. K. Ananias, M. Kandawa-Schulz, M. Hedimbi, H. K. Kwaambwa, H. Tutu, C. Makita, & L. Chimuka. (2015). Comparison of metal content in seeds of *Moringa ovalifolia* and *Moringa oleifera*. Journal of Food Science (Wiley Online) (manuscript submitted).
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DECLARATIONS

I, Natalia Kwadjonyofi Ananias, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree at any other institution of higher education.

[Signature]

Date……..

Natalia Kwadjonyofi Ananias

List of abbreviations and symbols used

$C_6H_8O_7$	Anhydrous Citric Acid
$C_6N_6FeK_3$	Potassium Ferricyanide
$C_7H_6O_5$	Gallic Acid
CH_2O_2	Formic Acid
DPPH	2,2-diphenyl-1-picryl-hydrazyl
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric acid
HNO_3	Nitric Acid
HPLC	High Performance Liquid Chromatography
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
Na_2CO_3	Sodium Carbonate
Na_2HPO_4	Sodium hydrogen phosphate
O_2HClO_2	Trichloroacetic acid
RP	Reducing power
TPC	Total phenolic contents
UV-vis	Ultraviolet-visible

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CHAPTER 1: INTRODUCTION

1.1. Orientation of the proposed study

Moringa is the sole genus in the flowering plant family Moringaeceae. It is a small medium sized tree that grows ranging between 10 and 15 m in height. It is widely cultivated in East Asia, Polynesia and the West Indies (Chumark, Khunawat, Sanvarinda, Phornchirasilp, Morales, Phivthonng, et al, 2008). The tree is widely distributed in India, Egypt, Phillipines, Ceylon, Thailand, Malaysia, Burma, Pakistan, Singapore, West Indies, Cuba, Jamaica and Nigeria (Ramachandran, Peter & Gopalakrishman, 1980). It is also cultivated in African countries such as Ghana, Kenya, Malawi, South Africa and Namibia. It is known by the names of drumstick plant (Khalafalla, 2010), Kelor tree and horse radish tree (Sreelatha & Padma, 2010; Farooq, 2012).

Moringa shows great promise as a dietary supplement in areas with minimal access to healthcare, due to both high vitamin content and documented antibacterial and anti-carcinogenic properties (Milugo, 2013). All parts of Moringa are edible and can be consumed by humans (Jed & Fahey, (2005). Flowers and young leaves are eaten as vegetables ((Siddhuraju & Becker, 2003); (Farooq, Rai, Tiwari, Khan & Farooq, 2012). The tender pods are cooked or pickled and used in culinary preparations (Anwar & Bhanger, 2003). Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (Sreelatha & Padma, 2010; Jed & Fahey, 2005). The seeds can be consumed fresh or pounded, roasted, or pressed into sweet, non-desiccating oil,

commercially known as Ben oil, of high quality (Anwar, Latif, Ashraf & Gilani 2007).

Moringa has various uses and special features, including medicinal, nutritional and industrial (cosmetics and in animal feed), (Pakade, Cukrowska & Chimuka, 2013a). The tree has been used to combat malnutrition, especially among infants and baby nursing mothers (Jed, 2005). It has been shown that the leaves, flowers and fruits of Moringa are more important for nutritional usage, whereas the roots, stems and barks are known for their medicinal value (Pakade et al, 2013a). Jed (2005) reported that since much of the tree is edible, it is enhanced by the fact that moringa products are loaded with nutritional value.

1.1.1 *Moringa oleifera*

The mostly cultivated species is *Moringa oleifer*a; which is cultivated throughout the tropics as a multipurpose tree and hence referred to as the miracle tree (Ramachandran, Peter & Gopalakrishnan, 1980). *M. oleifera* can grow well in the humid tropics or hot dry lands; it can survive destitute soils, and is hardly affected by drought (Morton, 1991). *M. oleifera* can be grown even in the hardest and driest of soils, where barely anything else will grow. In fact, one of the nicknames of Moringa is “never die” due to its incredible ability to survive harsh weather and even drought conditions. In Namibia, *M. oleifera* is found in the Zambezi and Kavango regions, where it is grown domestically.

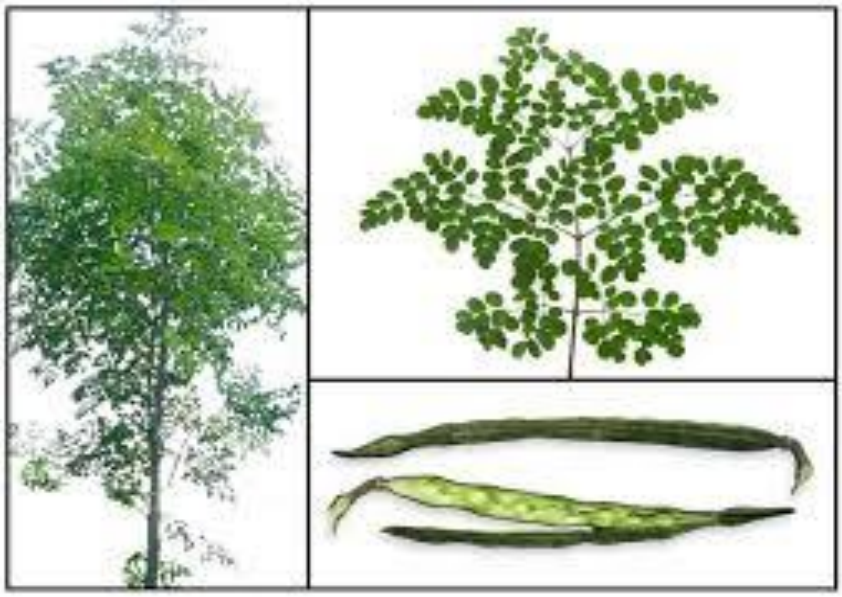

Figure 1: *Moringa oleifera*

1.1.2 *Moringa ovalifolia*

The only endemic Southern Africa Moringa species is *Moringa ovalifolia* (African Moringa/Ghost tree) which is distributed from central to southern Namibia and south western Angola (Dyer, 1975). *M. ovalifolia* is a deciduous tree with a thick and succulent trunk (Curtis & Mannheimer, 2009). It has leaves clustered near end of branches, small white flowers and grey brown fruits (pods). Figure 2 shows the picture of *M. ovalifolia*. *M. ovalifolia* occurs mainly in desert or arid savannah vegetation and in rocky hillsides. It starts flowering in December to May (Curtis & Mannheimer, 2009). Fruits appear in October to May, with occasional records in other months according to Curtis & Mannheimer (2009). Very little is documented and hence little is known about this species. In Namibia, it is protected under the

Nature Conservation Ordinance 4 of 1975, and under the Preservation of Tree and Forest Ordinance 1952 (Curtis & Mannheimer, 2005).

Figure 2: *Moringa ovalifolia*

1.2. Statement of the problem

In Namibia, lots of different plants have been and are still being used to improve nutrition. While a lot has been reported about nutritional and health benefits of *M. oleifera*, there are few reports on the possible health and nutritional benefits of *M. ovalifolia*. Lack of information on the benefits of *M. ovalifolia* resulted in this species being less cultivated and its products not commercialized. Hence, this study seeks to evaluate the phytochemical, micronutritional and antioxidant activities of *M. ovalifolia.*

1.3. Objectives of the research

1. To measure the total phenolic content present in *M. ovalifolia.*
2. To measure the ferric reducing activities (reducing power) of *M. ovalifolia*.
3. To measure the DPPH radical scavenging activities of *M. ovalifolia.*
4. To extract and determine total flavonoids found in *M. ovalifolia*
5. To determine the elements in *M. ovalifolia*

1.4. Significance of the study

Moringa tree is generally being advocated for wide scale propagation by communities as a food supplement and for poverty alleviation in developing world. There is also a commercial interest to come up with various Moringa products in Namibia. However, all the current products in the market are mainly from *M. oleifera*. It is therefore important to know the amount of essential compounds in *M. ovalifolia* in order to adequately advice communities wishing to grow it on a large scale. Therefore, the findings of this study can be used as a justification for any future programs to commercialise or grow *M. ovalifolia* in the country.

1.5. Limitation of the study

Currently, the University of Namibia doesn't have all the instruments needed to carry out all the analyses outlined in this study. Hence, most of the analyses were done at the University of Witwatersrand, South Africa. Another limitation is that moringa is a protected plant by the Namibian conservation law; therefore the amount of samples to be taken at each site is limited to a few grams. Due to insufficient funding, samples cannot be collected from each and every site in the country.

CHAPTER 2: LITERATURE REVIEW

2.1 Moringa species

There are thirteen species that are found in Moringaceae. These are *Moringa oleifera, Moringa arborea, Moringa borziana, Moringa concanensis, Moringa drouhardii, Moringa hildebrandtii, Moringa longituba, Moringa ovalifolia, Moringa peregrina, Moringa pygmaea, Moringa rivae, Moringa ruspoliana* and *Moringa stenopetala.* (Mugal & Haq, (2010). *Moringa oleifera* is grown in many parts of the world including Namibia, and is the most studied species. *Moringa ovalifolia* was studied in this project. *Moringa ovalifolia* is found naturally in Namibia and very little is documented about it.

2.2 Moringa Nutritional value

The most incredible fact about Moringa is the amount of nutritional and medicinal chemicals and compounds found in this plant (Simopoulos & Gopalan, 2003). **Table 1** gives a quick overview of some of the notable nutrients contained in Moringa.

Table 1: Comparison of nutrient levels in 100 g of dried *Moringa oleifera* leaves with other food (Fahey, 2005).

Type of Nutrient in different food types	**Amount of nutrient in dried *Moringa oleifera* leaves (100 g)**
The Vitamin A of carrots	10 times
The Vitamin C of Oranges	1/2 times
The Calcium of Milk	17 times
The Potassium of Bananas	15 times
The Iron of Spinach	25 times
The Protein of Yogurt	9 times

As seen in **table 1**, not only does Moringa contain vitamin A, vitamin C, Calcium, Potassium, Iron, and Protein, but it contains them in high amounts. **Table 1** highlights some of the commonly known nutrients needed by the human body. **Table 2** and **table 3** provide more detailed view of the vitamins, minerals and amino acids (proteins), contained in *Moringa oleifera* fresh leaves and dried leaf powder.

Table 2: Vitamin and Mineral content of *Moringa oleifera* leaves (fresh and dried leaves) (All values are per 100 grams of edible portions) (Simopoulos & Gopalan, 2003).

Vitamin and Mineral	**Fresh leaves**	**Dried leaves**
Carotene (vit. A)	6.78 mg	18.9 mg
Thiamin (B1)	0.06 mg	2.64 mg
Riboflavin (B2)	0.05 mg	20.5 mg
Niacin (B3)	0.8 mg	8.2 mg
Vitamin C	220 mg	17.3 mg
Calcium	440 mg	20003 mg
Calories	92 cal	205 cal
Carbohydrates	12.5 g	38.2 g
Copper	0.07 mg	0.57 mg
Fat	1.70 g	2.3 g
Fiber	0.90 g	19.2 g
Iron	0.85 mg	28.2 g
Magnesium	42 mg	368 mg
Phosphorus	70 mg	204 mg
Potassium	259 mg	1324 mg
Protein	6.70 g	27.1 g
Zinc	0.16 mg	3.29 mg

Table 3: Amino Acid Content of Moringa (all values are per 100 grams of edible portion) (Simopoulos & Gopalan, 2003).

Different amino acids	**Fresh leaves (mg)**	**Dried leaves (mg)**
Arginine	406.6	1325
Histidine	149.8	613
Isoleucine	299.6	825
Leucine	492.2	1950
Lysine	342.4	1325
Methionine	117.7	350
Phenylalinine	310.3	1388
Threonine	117.7	1188
Tryptophan	107	425
Valine	374.5	1063

Moringa products are known to alleviate protein deficiency in developing countries as the leaves and other parts of the tree contain high amount of crude proteins and amino acids, comparably to soy bean (Joy, Thomas, Mathew & Skaria, 1998). Moringa leaves contain more Vitamin A than carrots, more Calcium than milk, more Iron than spinach, more Vitamin C than oranges, and more Potassium than Bananas, and the protein quality of moringa leaves rivals that of milk and eggs (Fahey, 2005). Moringa can provide the calcium needed by the body in a safe way (Stampfer, Hennekens, Manson, Colditz. Rosner & Willett, 1993). Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers (Fahey,

2005). Successful treatment of malnourished children has been well-documented. Some pharmacies are now selling Moringa leaf powder to mothers with malnourished children (Kiple, 2000).

Moreover, leaves of Moringa are rich in iron. In human body, iron is present in all cells and has several vital functions, as a carrier of oxygen to the tissues from the lungs in the form of haemoglobin, as a transport medium for electrons within the cells in the form of cytochromes, and as an integral part of enzyme reactions in various tissues (Hercberg, Preziosi & Galan, 2001). Iron is also highly recommended for women who are anaemic due to their menstrual cycles (Farooq et al, 2012).

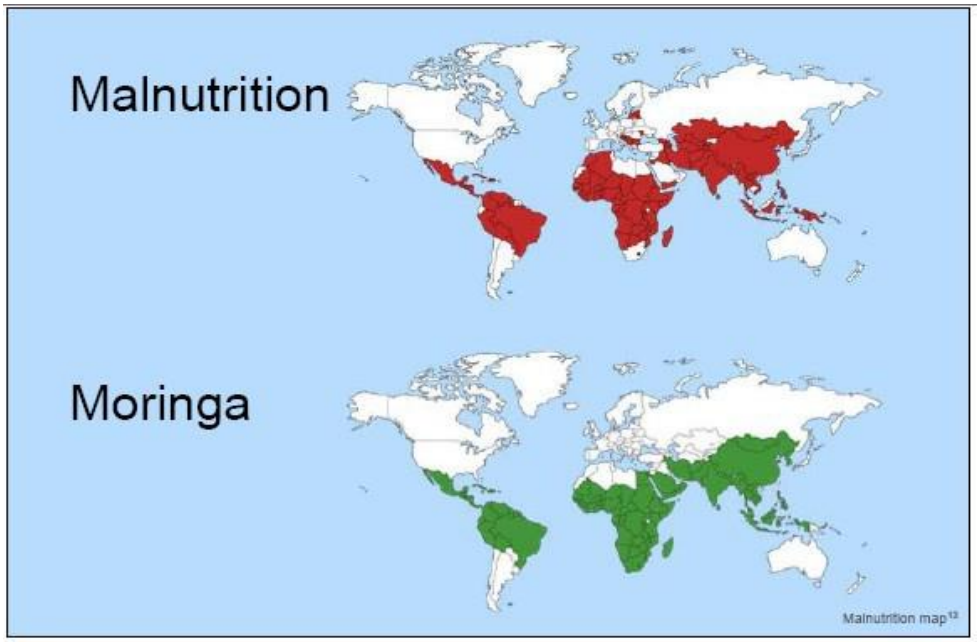

Figure 3: The map of the places where malnutrition is a major issue is the same as the map of where Moringa grows wild (Hiawatha, 2010).

2.3 Moringa Medicinal Value

In Namibia and other developing countries with lots of cultural diversities and traditional practices, different plants have been used and are still being used to improve nutrition and meet health care needs. Among these plants, *Moringa oleifera* has its great contribution from ancient time with exceptional medicinal and nutritional properties which can resolve the health care needs in several situations (Farooq et al, 2012). For centuries, people all over the world, including traditional healers have utilized different parts of moringa tree as traditional medicine (Padayachee & Baijnath, 2012). Different parts of *M. oleifera* such as the roots, leaves, flowers, fruits and seeds are also known to be good sources of phytochemicals compounds. It is reported that *M. oleifera* contain alkaloids, carotenoids, tannins, anthraquinones, anthocyanins and proanthocyanidns (Padayachee, & Baijnath, 2012).

Moringa tree has great medicinal uses both for disease prevention and treatment (Ramachandran et al, 1980). The essential nutrients present in the leaves prevent diseases. For instance the leaves contain Vitamin A, which acts as a shield against eye disease, skin disease, and heart ailments, diarhoea (Jed, 2005). Vitamin C is also present in Moringa leaves, which fights a host of illnesses including cold and flu. Furthermore, calcium is also present, which builds strong bones and teeth, and helps prevent asteoporosis. Elements like potassium are also found in the leaves, and is essential for the functioning of the brain and nerves. Proteins, the basic building blocks of all body cells, are also present in the leaves (Jed, 2005).

M. oleifera is considered as one of the world's most useful trees, as almost all parts of this plant have been used for various treatments of ascites, rheumatism as well as cardiac and circulatory stimulants (Jongrungruangchok, Bunrathep & Songsak, 2010). Leaves of Moringa are known to have various biological activities, including hypolipidaemic, antitheroslerotic (**Table 4**), prevention of cardiovascular diseases and antioxidant (Matshediso, Cukrowska & Chimuka, 2015). The seed kernels of Moringa showed promising effect in the treatment of bronchial asthma (Farooq et al, 2012). **Table 5** summarizes some of the nutrients present in Moringa and how the lack of these nutrients often leads to various diseases and maladies.

Table 4: Great medicinal values of Moringa being suggested by traditional medicine from different countries (Jed, 2005).

Different countries	**Types of different diseases that can be cured by moringa (conditions/effects)**
Guatemala	Skin infections, sores
India	Anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera, conjunctivitis, cough, diarrhoea, eye and ear, blood pressure, hysteria, pain in joints, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis
Malaysia	Intestinal worms
Nicaragua	Headache, skin infections, sores
Philippines	Anaemia, glandular swelling, lactation
Puerto Rico	Intestinal worms
Senegal	Diabetes, pregnancy, skin infections, sores
Venezuela	Intestinal worms, colitis, diarrhea, dropsy, dysentery, gonorrhoea, jaundice
Other countries	Malaria, stomach ulcers, tumor, urinary disorders, wounds

Table 5: Different types of nutrients and different diseases and maladies that occur in their absence (Hiawatha, 2010)

Different types of nutrients present in Moringa	**The disease and maladies that occur in the absence of the nutrients**
Vitamin A	Blindness, Maternal mortality, Pregnancy and lactation, Weak immunity and inability.
Vitamin C	Scurvy, high blood pressure (Hypertension), weakness lassitude, swollen gums, nosebleeds
Iron	Anaemia, fatigue, irritability, weakness, shortness of breath, dizziness, pale skin, sore tongue, brittle nails, decreased appetite, headache
Calcium	Anaemia, Osteoporosis, muscle damage, nerve damage, abnormal heartbeat and functioning
Protein (Amimo Acids)	Weight loss, thinning or brittle hair, hair loss, skin becomes very light, reduced pigmentation in the hair on scalp and body, skin rashes, dryness, flakiness, general weakness and lethargy, muscle soreness and weakness, cramps, difficulty sleeping, headache, nausea and stomach pain, fainting, crankiness, moodiness, severe depression, anxiety, lack of energy.

Potassium	Hypokalemia, fatigue, acme problem, temporary memory loss, ringing/noise in ear, digestive system will be affected, skin related problems, hyponatremia, heart related problems

2.4 Some health benefits of Moringa

Moringa contains an anti-aging compound called Zeatin, (Dhakar et al, 2011). Zeatin is a plant hormone, derived from the purine adenine (Sondheimer, & Tzou, 1971). According to Sondheimer & Tzou, (1971), zeatin helps promote small cell size, a key component to more youthful skin. It also influences the structural and functional integrity of the cell, and prevents accumulation of macromolecular damage in the cell. Their study reported that zeatin increases the activity of some antioxidant enzymes, counteracting the free radical-induced oxidative damaged incurred during cell aging (Dhakar, Maurya, Pooniya, Bairwa & Gupta, 2011).

Several studies have shown Moringa's health benefits. It is a strong antioxidant effective against prostate and skin cancers, and anti-tumor. As a detoxifying agent, it is effective against snake and scorpion bites. It is effective against nervous disorders including headaches, migraines, hysteria, and epilepsy (Moyo, Masika, Hugo & Muchenje, 2013).

2.5 Flavonoids

The flavonoids are polyphenolic compounds that are ubiquitous in nature, possessing 15 carbon atoms, two benzene rings joined by a linear three carbon chain (Agrawal, 2013). The flavonoids subgroups mainly consist of chalcones, flavonols, flavones, catechins, anthocyanins and Isoflavonpoids (Miranda, Stevens, Ivanov, McCall, Frei, Deinzer et al, 2000). Flavonoids constitute one of the most characteristic classes of compounds in higher plants. These flavonoids occur as secondary metabolites in most angiosperm class plants which attribute to giving flowers and fruits the vibrant colours and protecting the plants from microbe and insect attack. However, their occurrence is not limited to flowers and fruits but, extends throughout all the other parts of the plant. Over 4000 flavonoids have been identified, many of which occur in ordinary fruits and vegetables and some wild animals (Dangles, Fargeix & Dufour, 1999).

Flavonoids have led to considerable interest in a broad spectrum of research fields due to their prospective beneficial effects to human health. These compounds have been reported to have anti-viral, anti-allergic, anti-inflammatory, anti-tumour and anti-oxidant activities (Saleem, 1995; Eklund, Langvik, Warna, Salmi, Willfor & Sjoholm, 2005). Antioxidants are molecules that are capable of inhibiting oxidation processes. Antioxidants supply the free atoms needed by the human body and mitigate the effect of free radicals (Hiawatha, 2010). The contribution of flavonoids enhancement of the human health is mostly antioxidative related, which makes the antioxidant property of the flavonoids the most prominent property of this class of

compounds (Eklund et al, (2005). In this project three flavonols (Quercetin, Myricetin and Kaempferol) were studied and their basic structures are shown in **figure 4**.

Flavonols act as antioxidants boosting the effects of vitamins, regulating nitric oxide and scavenging free radicals while regulating the blood flow and keeping the heart healthy (Hollman & Katan, 1999). As antioxidants, some flavonols such as quercetin, protect LDL **(**low density lipoprotein) cholesterol from oxidative damage and are found in a wide range of food, for instance flavones are found in citrus, isoflavanones in soy products (Ho, Chen, Leung, Chan, Huang & Chen, 2002) and flavans in apples (Guyot, Le Bourvellec, Marnet, & Drilleau, 2002).

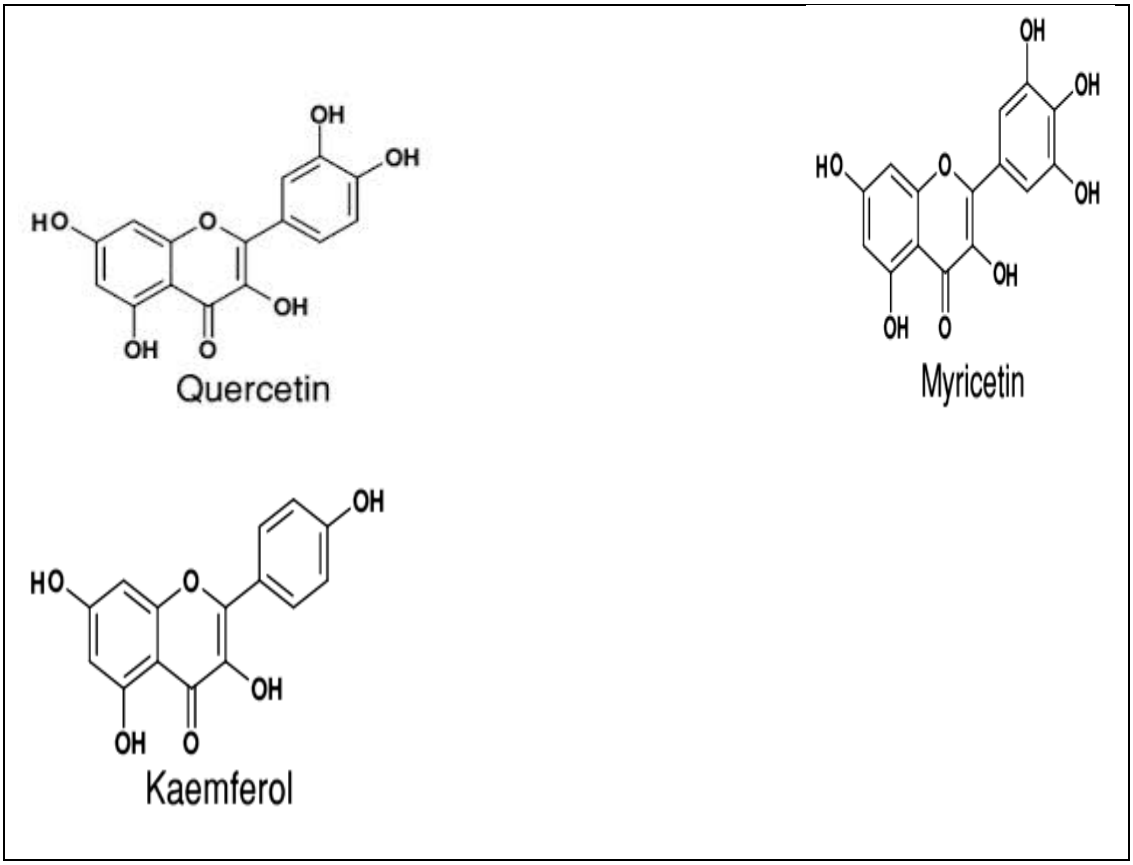

Figure 4: Schematic diagram of the molecular structures of flavonols (Quercetin, Myricetin and Kaempferol).

2.6 Phenolic compounds

Phenolic compounds are important due to their ability to serve as antioxidants which are widely found in secondary products of medicinal plants (Li, Xu, Wang & Huang, 2005, Ignat, Volf & Popa, 2011). Phenolics are chemicals characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups (Sakihama, Cohem, Grace & Yamasaki, 2002). Phenolic acids and other polyphenolic compounds that is flavonoids, present in fruits, vegetables and other parts of the plants are known to

possess antioxidant activity due to presence of hydroxyl groups in their structures (Dimitrios, 2006).

The antioxidant capacity of phenolic compounds is determined by their structure, in particular the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to a free radical and ability of an aromatic compound to support an unpaired electron as the result of delocalization around M-electron system (Ross & Kasum, 2002). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Kähkönen, Hopia, Vuorela, Rauha, Pihlaja, Kujala, et al, 1999). Extraction of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method employed, sample particle size, storage time and conditions, as well as presence of interfering substances (Naczk & Shahidi, 2004).

Moreover, like vitamins, phenolic acids also help with many ailments such as coronary heart disease, and blood clots which can lead to strokes and cancers (Prakash, Suri, Upadhyay & Singh, 2007). *M. oleifera* has been reported to contain phenolic acid, chlorogenic acid, ferulic acid, caffeic acid and ellagic acid (Prakash et al, 2007; Siddhuraju & Becker, 2003; Bajpai, Pande, Tewari & Prakash, 2005).

2.7 Formation and degradation of radicals

Heavy exercises, sunshine, air pollution and smoking are known to substantially increase the amount of free radicals being produced. These free radicals oxidize biologically essential molecules such as lipids, proteins, sugars, and nucleic acids, which results in the loss of their physiological functions and induction of deleterious effects (Takashima, Horie, Shichiri, Hagihara, Yoshida & Niki, 2012). Our bodies have mechanisms of antioxidants to neutralize free radicals before they can cause any damage. The body has the capacity to produce its own antioxidants from nutrients obtained from food or the nutrients can be used directly as antioxidants. Vitamin C and E are known to supply the body with direct antioxidants (Halliwell, 1996).

2.8 DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging activity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) is one of a few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at 515 nm (Huang, Ou & Prior, 2005). The DPPH antioxidant assay is based on the ability of DPPH radical, to decolourize upon the addition of antioxidants. When DPPH accepts an electron donated by an antioxidant compound, it is decolourized which can be quantitatively measured from the changes in absorbance (Kumarasamy, Byres, Cox, Jaspers, Nahar & Sarker, 2007). Antioxidant efficiency is measured at ambient temperature and thus eliminates the risk of thermal degradation of the molecules tested (Bondet, Brand-Williams & Berset, 1997). This allows fair projections of the efficiency of the plant extracts in the presence of radicals in the body as the

experiments are done at room temperature. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Kähkönen et al, 1999).

Epidemiological studies have shown that food rich in vitamins provide protection against degenerative diseases including cancer, coronary heart disease and even Alzheimers's disease (Kohen, Fanberstein & Torosh, 1997). Plants containing antioxidants like Vitamin C, Vitamin E, carotenes, polyphenols, and many other compounds reduce these disease risks. Most of the antioxidant compounds in a typical balanced diet are derived from plant sources with a wide variety of biological and chemical properties (Scalbert, Johnson & Saltmarsh, 2005).

Polyphenols scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and inhibit the oxidative mechanisms that can lead to degenerative diseases. There are a number of clinical studies confirming the powerful anti-cancerous and anti-heart disease properties of polyphenols (Siddhuraju & Becker, 2003; Bajpai et al, 2005). Alkaloids, responsible for bio-enhancing properties and the therapeutic effect of many plants, can help the body's central nervous system and absorption in the gastrointestinal tract (Sreevidya & Mehrotra, 2003).

2.9 Mineral content in plants

Minerals are inorganic elements needed by the body as structural components and regulators of body processes. They combine with other elements in the body, while retaining their chemical identity. In many developing countries, the supply of minerals is inadequate to meet the mineral requirements of farm animals and rapidly growing population (Anjorin, Ikokolah & Okolo, 2010). Minerals cannot be synthesized by animals and must be provided from plants or mineral-rich water (Anjorin et al, 2010). Minerals are essential in animal feed and human nutrient. Human bodies need more than 100 mg of major elements and less than 100 mg of minor minerals daily (O'Dell & Sunde, 1997).

The determination of minerals and trace elements is important to enhance production efficiency in plants (Rodriguez, Morales, Rodriquez & Romero, 2011). Some of the trace elements including, iron, manganese, zinc and copper are essential micronutrients with a variety of biochemical functions in all living organisms (Umran, Canan, Sermin, Ali & Serap, 2012). Different elements have many functions in plant growth and development. Metals ions, including iron, zinc and copper, are required for catalytic and structural properties of many proteins and are therefore essential for growth and development of all organisms. Essential elements also play a major role in nerve transmission, blood circulation, cellular integrity, energy production and muscle contraction (Belay & Kiros, 2014). However, excessive amounts of these metals, or of non-essential metals such as cadmium (Cd)

and lead (Pb), are toxic and inhibit plant growth (Guo, Meetam & Goldsbrough, 2008).

The macronutrients are distinguished between two sub groups, major ones and secondary ones. The nutrients like nitrogen (N), phosphorus (P) and potassium (K) are referred to as major macro-elements, and calcium (Ca), magnesium (Mg), and sulfur (S) are the secondary ones. The micronutrients, which are needed only in trace amounts, are iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), sodium (Na), nickel (Ni), silicon (Si), cobalt (Co) and selenium (Se) (Pakade, Cukrowska & Chimuka, 2013b). The micro-nutrients are important just like macro-elements. Plant performance is crucially dependent on adequate supply of all elements including those that are demanded in relatively small quantities (Belay, & Kiros, 2014). **Table 6** shows the average concentration of the nutrients needed for plant growth. Together, the macronutrients and micronutrients provide energy, structure, and regulation which are needed for growth, maintenance, repair, and reproduction (Raigón, Prohens, Munoz-Falcon & Nuez, 2008). Each nutrient provides one or more of these functions, but all nutrients together are needed to maintain human health.

Table 6: Average concentration of macro and micro nutrients in plants (Epstein, 1965)

Element	**Average concentration in tissue (mg/kg)**
Fe	100
Mn	50
Zn	20
B	20
Cu	6
Mo	0.1
Cl	100
Na	15 000
K	10 000
Ca	5 000
Mg	2 000
P	2 000
S	1 000
Zn	20

2.10 Moringa and Water Purification

Contamination of ground water remains a major challenge because of human activities such as mining, industrialization, and agricultural, that result in deposit of toxic substances in water streams. A billion people across Asia, Africa, and Latin

America are estimated to rely on untreated surface water sources for their daily water needs. Of these, some two million are thought to die from diseases caught from contaminated water every year, with the majority of these deaths occurring among children under five years of age (Mahmood, Mugal & Hag, 2010). Moringa seeds can be used to purify water. *M. oleifera* seeds have the potential to be used in the treatment of hard-water for domestic use in tropical and developing countries (Muyibi & Evison, 1995).

Figure 5: Moringa for purification of turbid water (before and after treatment) (Lea, 2010).

Powdered seed acts as a natural flocculent; able to clarify even the most turbid water seed powder can be used as a quick and simple method for cleaning dirty water (Mahmood et al, 2010). The powder joins with the solids at the bottom (**figure 5**). In Sudan, dry *M. oleifera* seeds are used in place of alum by rural women to treat highly

turbid Nile water (Jahn, Musnand & Burgstaller, 1986). Studies by Muyimbi and Evison (1995) identified the presence of an active antimicrobial agent in *M. oleifera* seeds. The active agent isolated was found to be 4a L-rhamnosyloxy-benzyl isothiocyanate, at present the only known glycosidic mustard oil. Madsen Schlundt & Omer, (1987) carried out coagulation and bacterial reduction studies on turbid Nile water in the Sudan using *M. oleifera* seeds and observed turbidity reduction of 80 - 99.9 % paralleled by a bacterial reduction of 1-4 log units (90 - 99%) within the first one to two hours of treatment, the bacteria being concentrated in the coagulated sediment (Jahn et al, 1986).

2.11 Moringa and Plant growth

Laboratory experiments have shown that Moringa spray had a wide range of beneficial effects on plant crops. Moringa contain plant hormones (including zeatin) that help plants and crops to produce greater yields (Hiawatha, 2010). Hiawatha (2010), reported that juice from fresh moringa leaves can be used to produce an effective (spray containing) plant growth hormone, increasing yields by 25-30 % for crops such as: onions, bell pepper, soya, maize, sorghum, coffee, tea, chilli, and melon.

CHAPETR 3: MATERIALS AND METHODS

3.1. Sample Collection

Fresh leaves, bark and seeds (pods) of *Moringa ovalifolia* were collected from Moringa farm **'Site 1'** (which is 40 km west of Okahandja, Otjozondjupa region), Okaukuejo **'Site 2'** and Halali **'Site 3'** (situated in Etosha national park, Kunene Region), near Tsumeb **'site 4'** (about 3 km south of Tsumeb along B1 main road, Oshikoto Region) and near Keetmanshoop (!Karas region) **'Site 5'** (**Figure 6**).

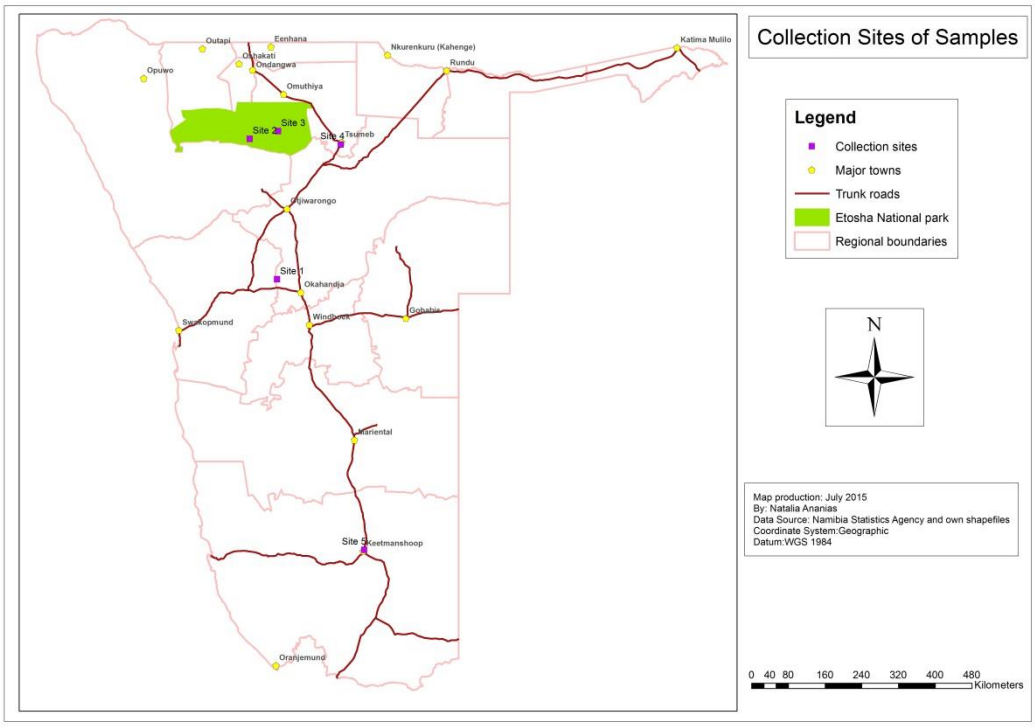

Figure 6: Namibian Map showing different sites of sample collection

3.2. Research Equipments, chemicals and reagents

A high performance liquid chromatography (HPLC)-UV, SRI 210D (Los Angeles, CA, USA) equipped with a UV/Vis detector (VUV-24) and a Phenomenex C_{18} column (150 x 4.6 mm, 5 µm) was used for the identification and quantification of

flavonols from methanolic extracts. Metal contents were analysed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Spectro Genesis, Spectro, Germany).

All reagents used were of analytical grade. Quercetin, myricetin, kaempferol, methanol (HPLC grade), formic acid (CH_2O_2), sodium hydrogen phosphate (Na_2HPO_4) anhydrous, citric acid ($C_6H_8O_7$), nitric acid (HNO_3), Folin – Ciocalteu reagent, potassium ferricyanide ($C_6N_6FeK_3$), ferric chloride ($FeCl_3$), gallic acid ($C_7H_6O_5$), sodium carbonate (Na_2CO_3), trichloroacetic acid ($C_2HCl_3O_2$) and 2,2-diphenyl-1-picryihydrazyl free radical (DPPH), were purchased from Sigma Aldrich (Johanessburg, South Africa). Ultra-pure water from a purification system, Milli-Q, Millipore (MA, USA) was used in all the experiments. All standards were prepared as stock solutions at 10mg/100 mL in methanol and stored in an opaque container at 4 °C.

3.3. Experimental Procedures

3.3.1.Sample preparations

All samples collected were left to air dry in shade at room temperature. The dry samples were grounded to powder and were stored in a cool dry place until use.

3.3.2. Extraction/hydrolysis of plant material

Extraction and hydrolysis of the samples was carried out using a modified method of Sultana Anwar & Ashraf, (2009). Acidified methanol (50 mL) containing 1% (v/v) HCl was added to 2.5 g grounded leaves powder contained in a 250 mL round-bottom flask. The flask was fitted with a reflux condenser. About 10 mL of 1.2 M HCl was added to this flask, and the mixture was heated at 90 °C for 4 hours. The extracts were cooled to room temperature and then sonicated for 15 min to remove air and filtered through Whatman filter paper. The extract solution was stored at -20 °C until further use.

3.3.3. Extraction of total phenolics

Total phenolic contents were determined by the Folin-Ciocalteau method. The extract samples were prepared using the method of Moraes-de-Souza (2008). 0.5 mL of extract in distilled water (1: 9) was mixed with 2.5 mL of Folin- Ciocalteau reagent diluted in distilled water (1: 9 v/v). The mixture was hand shaken and after 5 minutes, 2 mL of sodium carbonate 4% (v/v) were added. Samples were incubated for 2 hours in the dark and absorbance measured at 740 nm using UV/Vis spectrometry. The calibration curve was prepared by four data points ranging from 10 to 100 mg/L solutions of gallic acid in water. The total phenolic contents were estimated according to the Folin-Ciocalteau method using gallic acid (Pakade et al, 2013a).

3.3.4. Determination of total phenolic content

Total phenolic content (TPC) in the acetone extracts of Moringa samples were analysed using Folin–Ciocalteau reagent assay. About 200 μL of extract was added to a freshly prepared solution mixture of 750 μL Folin–Ciocalteu reagent (1:10) and 2mL of 7.5% sodium carbonate. The final mixture was diluted to 7 mL with deionized water. The reaction mixture was incubated at ambient conditions in the dark for 2 h to complete the reaction. Then the absorbance was measured at 765 nm using UV-visible spectrophotometer (Varian, Cary 50 Conc, Germany). All the experiments were conducted in triplicates using gallic acid as a calibration standard, and results were recorded as gallic acid equivalents (/100 g of extract).

3.3.5. Measurement of reducing power

The reducing power of the *Moringa ovalifolia* extracts was determined using the method described by Siddhuraj & Becker (2003). The extract (1 mL) was mixed with 0.2 M phosphate buffer (5 mL, PH 6.6), 1% potassium ferricyanide (5 mL) and then incubated at 50 °C for 20 minutes. Trichloroacetic acid 10% (5 mL) was added to the mixture to stop the reaction and centrifuged at 3000 rpm for 10 minutes. The supernatant (5 mL) was mixed with distilled water (5 mL) and 0.1% ferric chloride (1 mL) and then the absorbance was measured at 700 nm using UV- visible spectrophotometer.

3.3.6. Measurement of DPPH scavenging activities

The antioxidant activity of different extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH method

adapted from Siddhuraj & Becker (2003). 0.1 mL of extract at various concentrations was added to 3.9 ml (0.025 g L^{-1}) of DPPH solution. The decrease in absorbance at 515 nm was determined continuously at every minute with a spectrophotometer for 20 minutes. The remaining concentration of DPPH in the reaction was calculated from a calibration curve obtained with DPPH. The radical activity was calculated using: **$(A_{control} - A_{sample}) / A_{control} \times 100$ = antiradical activity**, where $A_{control}$ is the absorption of the DPPH at time = 0 minutes and A_{sample} is the absorption of the DPPH solution after the addition of the sample at a particular

3.3.7. Determination of total flavonoids

A modified method of Siddhuraju & Becker (2003) was used. To 1.0 mL of the extract, 1.0 mL of 60% 2.8 M HCl in methanol was added and incubated in a water bath at 90 °C for 2.5 hours. After hydrolysis, the extracts were allowed to cool to room temperature, filtered with a 0.45 µm filter paper prior to injection in HPLC. Samples were eluted under isocratic condition with methanol and 20 mM sodium dihydrogen phosphate buffer adjusted to pH 2.5 with citric acid (55: 45 v/v and 0.1 % formic acid) at flow rate of 1.0 mL/minute. The injection volume was 20 µL for both samples (extracts) and standards. Samples were prepared in triplicates. Quantification of myricetin, quercetin and kaempferol were done using a four-point calibration curve of standard solutions at concentrations between 1.0 and 30 µg/mL.

3.3.8. Analysis of elelment

Methods of Pakade et al, (2013b) were followed. A sample of 0.1 g was placed in a microwave digestion vessel. About 8 mL concentrated nitric acid was added to the digestion vessel and 2 mL hydrogen peroxide (H_2O_2) was added. Digestion was

carried out for about 30 minutes in the microwave. After digestion, the samples were transferred into a 25 mL volumetric flask and made up to volume with deionised water. Samples were then taken for elemental analysis on the ICP-OES (Inductively Coupled Plasma-Optical Emission).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Total Phenolic Content (TPC) studies

During the process of incubation time, the process of oxidation of phenol, Folin-Ciocalteau which is a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids is reduced to blue oxides of tungsten (W_8O_{23}) and molybdene (Mo_8O_{23}) (Banerjee & Bonde, 2011). The longer the incubation time; the more phenolics are released. In this study however, an incubation period of 2 hours was used. The extraction temperature also influenced the phenolics released. In general, extractions at higher temperature give increased mass transfer rates and higher extraction yields as a result of improved solute desorption from matrix active sites (Co, Zettersten, Nyholm, Sjoberg & Turner, 2012). In this experiment, the samples were incubated at 90 °C.

In **table 7** the TPC were expressed as mg/kg. Flower extract showed the highest phenolic content with 206221 mg/kg, followed by leaves with 143493 mg/kg when compared to other parts extract from site 1. Fakurazi, Sharifudin & Arulselvan (2012) reported the same results, the same trend of high phenolic contents on moringa flowers compared to other parts.

Table 7: Total phenolics content (TPC) and Reducing Power (RP) of different parts of *M. ovalifolia* (site 1).

Parts	**TPC** **Concentration (mg/kg)**	**RP** **Absorbance**
Leaves	143492.5 ± 336.7	0.362 ± 0.001
Flowers	206221.1 ± 806.9	0.305 ± 0.004
Seeds	111609.4 ± 79.8	0.280 ± 0.001
Bark	31054.36 ± 50.7	0.0039 ±0.001

The total phenolic content of the leaves, barks and seeds from different sites as shown in **table 8,** it indicates that leaves have more phenolic contents followed by seeds and then bark. However, the phenolic contents of different parts (leaves, barks and seeds) from different sites did not differ much. Leaves from site two, have the highest phenolic contents 168681.6 ± 946.2 mg/kg and the lowest phenolic contents were found on site three 117861.7 ± 557.4 mg/kg. Moreover, the phenolic contents in bark were high on site one 34071.8 ± 723.0 mg/kg and low in site four 29498.1 ± 395.9 mg/kg. While, seeds from site four have the highest phenolic contents 138924.7 ± 1019.3 mg/kg and seeds from site 1 88126.2 ± 773.0 mg/kg have the lowest phenolic contents. The difference in the phenolic contents of the leaves and barks of *M. ovalifolia* could only be due to geographical location of the trees. It is reasonable to expect the concentration of seed elements of plants from Etosha to be different from others as these have been exposed to drought conditions since there is a low rainfall in that region unlike other sites. Moreover, there is a possibility that moringa plants

from Tsumeb may have accumulated some heavy metals, as that region has copper mining.

Table 8: Total phenolics content (TPC) in different parts of *M. ovalifolia* from different sites.

Different sites	**Concentration (mg/kg)**		
	Leaves	**Barks**	**Seeds**
1	122414 ± 946.2	34071.8 ± 723.9	88126.2 ± 773.0
2	168681.6 ± 1220.6	30242.5 ± 320.3	-
3	117861.7 ± 557.4	30405.1 ± 684.6	107777.3 ± 1251.5
4	151088.5 ± 3564.2	29498.1 ± 395.9	138924.7 ± 1019.3
5	157416.4 ± 3793.4	-	-

The reducing power of different parts of *M. ovalifolia* from different sites, have behaved in similar way as total phenolics. The leaves were highest followed by seeds and barks the lowest. Within different sites, there is no difference between the reducing power of leaves, seeds and barks (**Table 9**).

Table 9: Reducing power (RP) in different sites of different parts of *M. ovalifolia*.

Sites	Absorbance		
	Leaves	**Seeds**	**Bark**
1	0.37 ± 0.023	0.229 ± 0.004	0.781 ± 0.028
2	0.346 ± 0.022	-	0.91 ± 0.017
3	0.322 ± 0.020	0.297 ± 0.002	0.889 ± 0.005
4	0.380 ± 0.034	0.313 ± 0.010	1.008 ± 0.031
5	0.392 ± 0.020	-	-

4.2 The Ferric reducing activity and DPPH radical scavenging activity

Antioxidant property of *M. ovalifolia* was investigated by their reducing power and DPPH radical scavenging capacity. In the reducing power assay, the presence of reductants (antioxidants) in tested samples would reduce Fe^{3+} /ferricyanide complex to the ferrous form (Fe^{2+}) (Chung, Bloking & Chiang, 2002). At higher temperature, analytes of interest are released more from the active sites of the matrix and as such, more antioxidants are extracted. Plant extracts were found to have antioxidant properties as upon the addition of the extracts to the DPPH radical, there were colour changes from blue to yellow (Matshediso et al, 2015).

The DPPH assay, showed the bark with highest antioxidant capacity (20% - 50%), **figure 7**. A similar study by Basma, Zakaria, Latha & Sasidharan, (2011) on

Euphorbia hirta, showed that leaves had the highest DPPH scavenging activity of (72.96 ± 0.78%) followed by flowers, roots and stem.

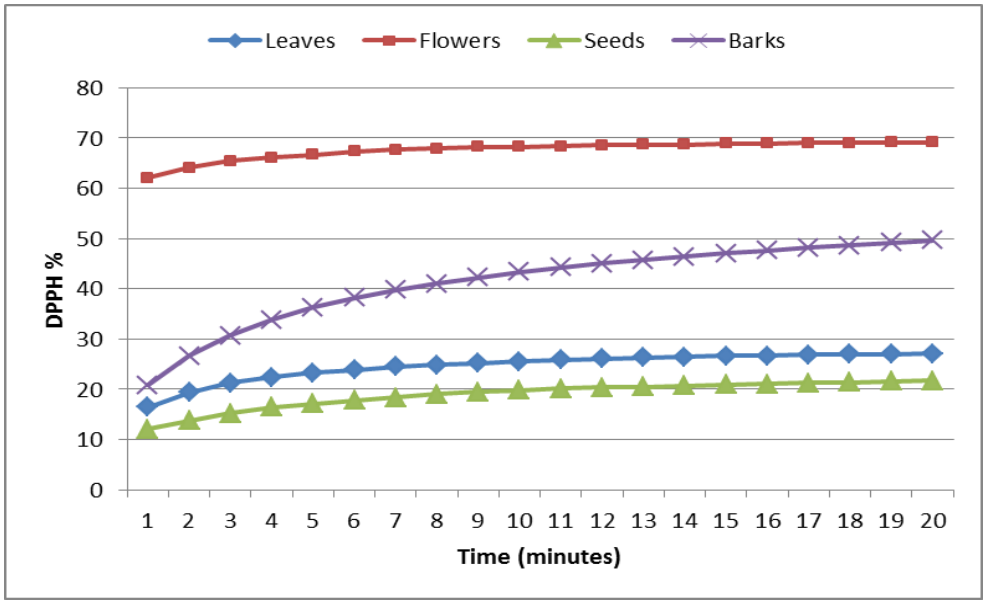

Figure 7: DPPH % (antiradical activity) of M. valifolia in different plant parts (site 1)

4.3 HPLC analysis of the flavonols

The flavonols were separated in a Gemini C-6 phenyl, 5 µm column. Myricetin was eluted first followed by Quercetin and lastly Kaempferol which is less polar hence has more affinity for the stationary phase (**Figure 8**).

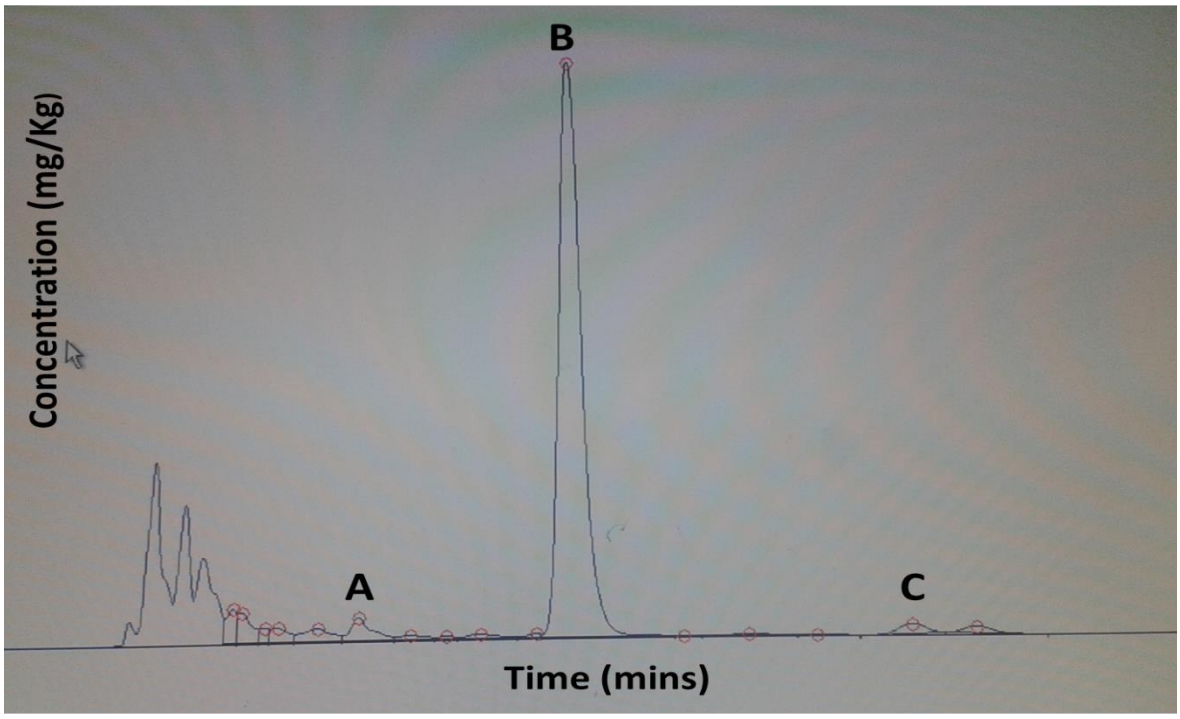

Figure 8: Chromatograms of A: Myrcetin, B: Quercetin and C: Kaempferol in the extract of M. ovalifolia leaves

The results in **figure 8** confirms the presence of the flavonols myricetin, quericetin and kaempferol. The two peaks for myricetin (peak A) and kaempferol (peak C) in the plant extract are clearly resolved with reasonable peak height while for quercetin (peak C) the peak height is too high. This is because quercetin can survive the harsh conditions of acid hydrolysis of which other flavonols does not really survive. Acid hydrolysis breaks the glycosidic bonds to release the flavonoids aglycones which can then be detected and quantified. This is because in nature, flavonoids can occur either in the free or conjugated forms, often being esterified to one or two sugar molecules, through at least one hydroxyl group (Fresco, Borges, Diniz & Marques, 2006).

Table 10: Flavonoids content in *M. ovalifolia* leaves.

Flavonls (concentration mg/kg)		
Myricetin	**Quercetin**	**Kaempferol**
581.62 ± 12.91	11909.31 ± 453.19	283.19 ± 58.55

The results of the flavonoids content showed that all the tested flavonols (Myricetin, Quercetin and Kaempferol) were present (**figure 8**). This corresponds with the report by Pakade et al (2013b), where they reported the presence of these three flavonols. Looking at the three different flavonols from the plant samples, Quercetin has the highest concentrations (11909.31 mg/kg), followed by Myricetin (581.62 mg/kg) and Kaempferol has the lowest concentration (283.19 mg/kg). However, the distribution of flavonols in the leaves differs from those reported in *M. oleifera*. Pakade et al, (2013b) reported that *M. oleifera* contained more Quercetin (1972.8 mg/kg), followed by Kaempferol (2145.2 mg/kg) and Myricetin (ranging from 1004 mg/kg – 1809 mg/kg). These differences are due to the genetic makeup or growth parameters of *M. oleifera* and *M. ovalifolia.* The flavonoids results showed that *M. ovalifolia* is better than *M. oleifera* in the content of flavonols.

In addition, when the analysis of flavonoids in the leaves from different sites was done, there were variable differences in all concentrations from site to site. The biggest difference in the concentration was between site three 349.20 mg/kg ± 21.1 (the lowest) and site two 855.17 mg/kg ± 44.8 (the highest). The value of Quercetin

was highest at all sites compared to other flavonoids, whereas that of Kaempferol was lowest (**Table 11**).

Table 11: Flavonoids content (Mean ± SD) of *M. ovalifolia* leaves in different sites.

Different sites	Concentration (mg/Kg)		
	Myricetine	**Quercetin**	**Kaempferol**
Site 1	618.48 ± 40.5	16844.5 ± 1194.3	590.53 ± 144.5
Site 2	855.17 ± 44.8	8501.10 ± 287.6	305.87 ± 56.9
Site 3	349.20 ± 21.1	11025.47 ± 323.8	222.77 ± 7.8
Site 4	503.72 ± 16.45	8682.22 ± 542.9	106.56 ± 2.6
Site 5	581.51 ± 20.78	14493.28 ± 1191.0	190.22 ± 22.3

4.4 Metal Content

Elements found in the *M. ovalifolia* were divided in two categories: major (Ca, K, Mg and Na) and minor (Ba, Cd, Co, Li, Mn, Si, Al, Zn, Cu, Ni and Fe) elements. The concentrations of minor and major elements in Moringa are shown in **Tables 12, 13** and **14**.

Furthermore, the results show that the presence of these elements varies from site to site. The variation observed in the concentration of *M. ovalifolia* seeds, leaves and flowers may have been due to either different genetic makeup of the plants or more probably due to the environmental effects. It is reasonable to expect the

concentration of elements of plants from Etosha to be different from others as these have been exposed to drought conditions since there is a low rainfall rate in that region unlike other sites where the Moringa grows on rocky hilltops.

4.4.1 Metal content in seeds

According to the results, barium (Ba) has the lowest concentration (1.5 mg/kg – 2.1mg/kg) in all three sites. There was no difference in the site concentration of Ba, Li and Si but there was a difference in Cd concentration in site one (6.2 mg/kg) compared to the site three (3.9 mg/kg) and four (3.9 mg/kg). There was a lower concentration of cobalt at site 4 (1.92mg/kg) compared to site one and three. However site four had a higher concentration of Mn compared to site one and three. This illustrates that the concentration of individual elements varies from site to site.

The concentration of K was the highest at all sites compared to other elements; this is because potassium is basic to plant and animal life. Except for N, the plants require more K than any other nutrient. Potassium is the third most abundant mineral in the human body, and it plays many vital roles in plant nutrition. Thus, its mobility in the plant allows it to influence almost all aspects of plant growth. Potassium increases crop yield and improves quality, it increases root growth and improves drought resistance (Römheld & Kirkby, 2010). Ajayi, (2008) also reported that the seeds are good sources of mineral elements. The results by Ajayi, (2008) revealed potassium to be the prevalent mineral elements with concentration of 2470 mg/kg and 1680 mg/kg in *Artocarpus heterophyllus* and *Treculia africana*, respectively. In this study, *M.*

ovalifolia seeds contain high potassium concentration, with the average of 6399.917 mg/kg.

Iron (Fe) concentration was relatively high (85 mg/kg), compared to other minor elements. Iron prevents anaemia, fatigue and weakness, if *M. ovalifolia* contains high concentration it can be used as a supplement. A study done by Pakade et al, (2013b) also showed presence of high iron content in *Moringa oleifera* leaves.

Table 12: Concentration (mg/kg) of minor and major elements in *Moringa ovalifolia* seeds from different sites (Mean ± SD of 3 replicates are presented).

Elememts	Site 1	Site 3	Site 4
Ba	2.1±0.13	1.5±0.00	1.5±0.00
Cd	6.2±0.29	4.0±0.90	4.0±0.14
Co	3.4±0.17	3.8±0.00	1.9±0.38
Li	10.9±0.05	10.8±0.05	10.8±0.05
Mn	17.7±0.17	18.8±0.10	26.5±1.01
Si	12.6±0.14	13.9±0.39	13.3±0.65
Al	28.4±2.63	25.8±1.54	23.2±0.84
Zn	59.1±0.88	63.9±0.43	65.5±0.27
Cu	29.8±0.42	29.0±0.19	12.5±0.27
Ni	27.4±0.99	11.1±1.41	19.2±1.15
Fe	84.7±2.21	79.1±0.18	62.7±0.71
Na	190.5±7.15	196.2±7.05	176.7±7.69
Ca	2427.90±187.62	2939.56±43.68	2244.56±13.02
K	6399.92±387.04	6501.58±99.61	7938.25±39.66
Mg	3974.94±262.69	3953.28±60.38	4104.94±20.28

Combining the results of elements present in *M. ovalifolia* from different sites as shown in **Table 12**, there was no significant difference in the site concentration of magnesium, lithium, barium, silicon, aluminium, sodium and zinc ($P > 0.05$). However, there was a significant difference in site concentration of calcium,

potassium, iron, nickel, copper, manganese, cobalt and cadmium ($P < 0.05$). Potassium shows the highest concentration compared to all other elements ($P < 0.05$), followed by Magnesium and Calcium.

4.4.2 Metal contents in leaves

The Moringa leaves samples contained relatively high amount of metals. As the data in **table 13** shows for the leaves samples, the metal with highest concentration in leaves is Potassium from all sites with a concentration range of 10998±485 mg/kg to 63223±583 mg/kg. When looking at the calcium level from different sites, the concentration was high on all sites compared to other elements. Nutritionally, these data are indicating that *M. ovalifolia* is a good source of Calcium.

Zn, Cu, Cd of the leave samples were compared to the normal levels of heavy metals concentration in plants. Zn concentration in *M. ovalifolia* leaves was well within the range 10 - 150 mg/kg (the standard range). Cu trace nutrients also fell within the range of 3 - 30 mg/kg. Moreover, the concentration of Cd from the leaves also fell within the range of 0.05 - 2 mg/kg.

4.4.3 Metal content in flowers

M. ovalifolia flowers were found to have both macro and micro elements which are essential in human body. Similarly, as in seeds and leaves, potassium was one of the elements with the highest concentration (35223.30 ±725.00 mg/Kg).

Table 13: Mean concentrations (mg/kg) of minor and major elements (Mean ±SD) in *M. ovalifolia* leaves from different sites.

Elements	**Different sites**				
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Al	85.98±1.04	96.65±2.12	42.40±2.18	32.88±2.19	90.15±2.54
Li	35.04±1.74	34.88±0.50	35.01±1.74	35.10±1.75	35.35±1.76
Ba	17.35±0.87	15.52±0.76	3.35±0.71	14.85±0.24	18.60±0.35
Mn	55.93±0.69	53.60±1.80	112.60±0.35	115.85±1.24	57.85±3.47
Co	4.00±0.00	4.17±0.29	3.50±0.71	4.25±0.35	3.75±0.35
Cd	5.30±0.00	5.30±0.00	4.80±0.00	4.55±0.35	4.80±0.00
Cr	11.20±0.71	12.45±1.00	13.20±0.71	23.87±0.29	11.47±2.12
Ni	52.28±5.07	50.53±4.93	47.28±4.22	36.10±0.88	51.03±4.46
Cu	29.03±3.09	18.84±2.74	17.40±2.48	18.64±0.42	28.80±1.65

Si	39.05±0.75	82.45±5.06	12.50±2.50	72.30±3.50	62.30±5.02
Fe	123.55±0	260.05±7.07	263.80±5.76	184.30±5.30	238.05±6.06
Zn	22.73±1.78	29.40±0.70	14.40±0.00	24.57±0.79	12.25±0.75
Na	451.20±3.36	620.3±3.56	531.2±19.98	492.95±14.1	634.2±2.65
Ca	16643.4±495	16568.4±666	22993±414	23864±787	13100±839
K	13773±654	15023±944	12315±460	63223±583	10998±485
Mg	8248.65±50	8023.7±124	8148.7±141	8051±147	12373±371

Table 14: Major and Minor elements (Mean ±SD) in *M. ovalifolia* flowers (site 1).

Elements	**Concentration (mg/Kg)**
Al	32.93 ±6.72
Ba	2.10 ±0.35
Zn	33.77 ±1.25
Cd	5.05 ±0.35
Co	4.25 ±1.06
Cr	22.20 ±1.00

Cu	17.55 ±1.41
Fe	172.30 ±24.40
Si	34.70 ±6.60
Li	35.10 ±6.75
Ni	33.85 ±0.71
Mn	27.27 ±1.36
Na	231.00 ±5.66
Ca	1543.40 ±545.67
K	35223.30 ±725.00
Mg	2348.65 ±883.88

Zn, Cu, Cd of the flower samples were compared to the normal levels of heavy metals concentration in plants. Zn concentration in *M. ovalifolia* flowers was well within the range 10 - 150 mg/kg (German standard range). Cu trace nutrients also fell within the range of 3 - 30 mg/kg. Lastly, the concentration of Cd from the flowers also fell within the range of 0.05 - 2 mg/kg.

4.4.4 Variations in element composition of *M. ovalifolia* Seeds, leaves, and flowers

The variations of metal content of different parts of *M. ovalifolia* were observed. It is very important to know how macro and micro elements differ in different parts of Moringa tree in order to estimate their role as sources of human diet. According to **figure 9** and **10**, Ca (18634.01 ± 355 mg/kg), Mg (8969.15 ± 50 mg/kg), Na (545.966 ± 3.36 mg/kg), Mn (85.166 ± 0.69 mg/kg) and Fe (213.95 ± 0 mg/kg) levels

were high in leaves compared to seeds and flowers. But, K (35223.3 ± 355 mg/kg) concentration was high in flowers compared to leaves and seeds, whereas Zn (62.83 ±0.88 mg/kg) and Cu (23.80 ± 0.42 mg/kg) were high in seeds compared to leaves and flowers. However, according to the study done by Fakankun, Babayemi & Utiaruk, (2013) on *M. oleifera*, Ca (9400 mg/kg), Mg (762 mg/kg) and Na (9050 mg/kg) levels were highest in root bark, K (11300 mg/kg) in the seed, Mn (86 mg/kg) and Fe (214 mg/kg) in the leaves, and Zn (18 mg/kg) in powder and stem wood.

The levels of Ca observed in the leaves have the concentration of 18634 mg/kg. It was compared to the concentration of calcium present in the leaves of *M. oleifera* from literature which was 30300 mg/kg. The levels of Ca concentration from the literature reports similar trends ranging from 15100 mg/kg to 30300 mg/kg (Fakankun et al (2013)). The concentrations of Mg ranged from 4011 to 8969 mg/kg. Magnesium and Calcium have similar properties when comparing this study to the findings reported by Fakankun et al (2013).

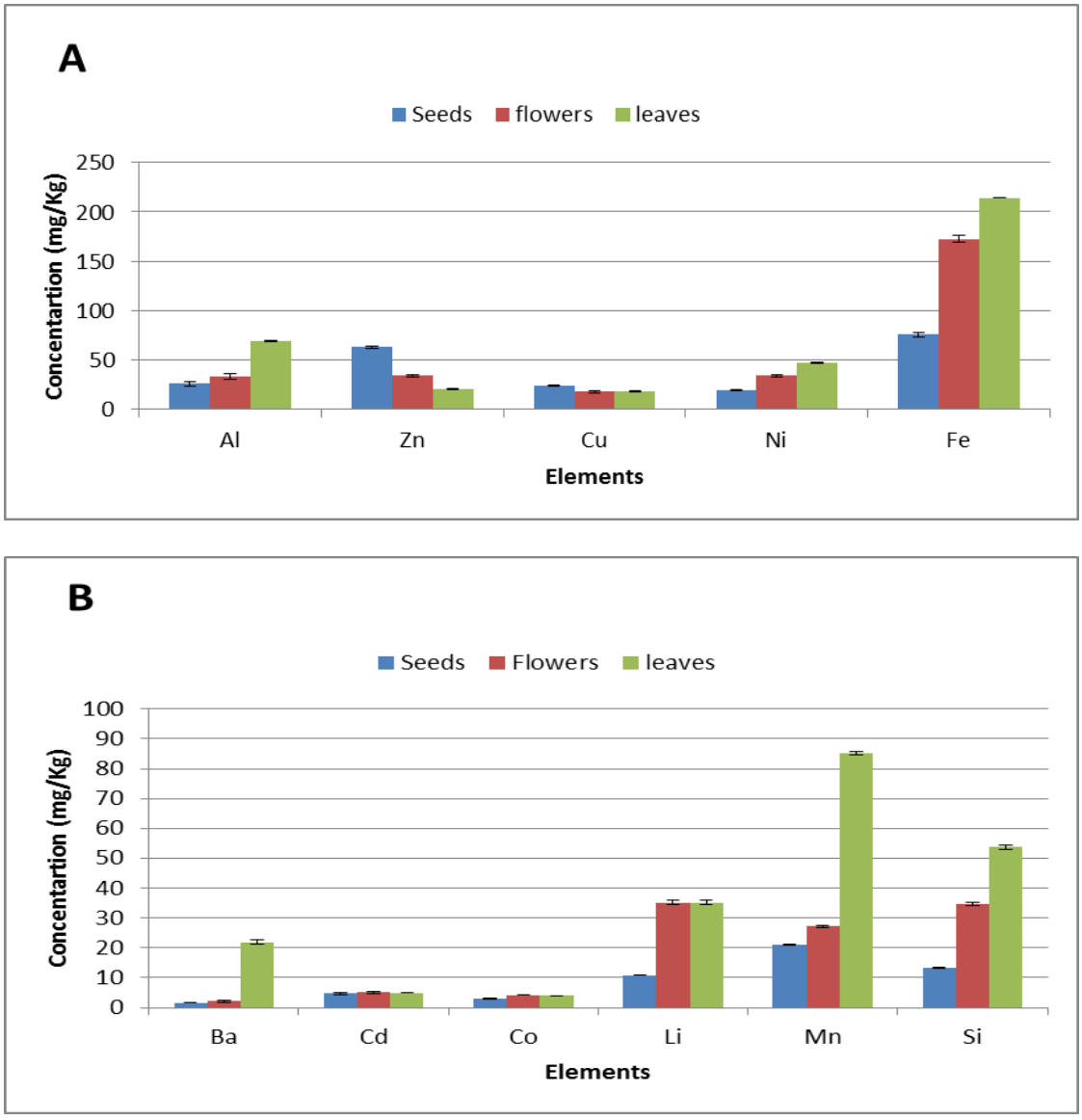

Figure 9: Comparison of minor metal contents in different parts of M. ovalifolia

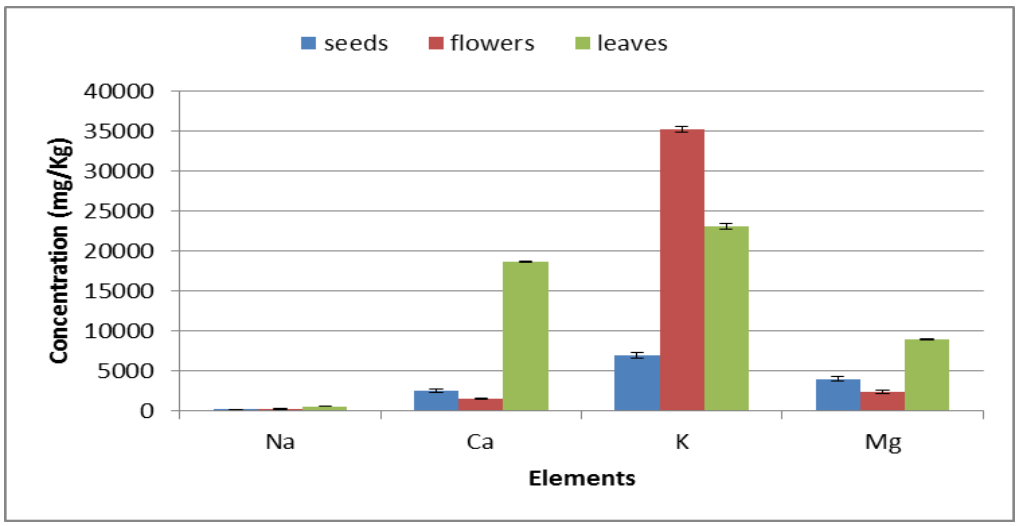

Figure 10: Comparison of major elements present in different parts of M. ovalifolia

Potassium levels ranged from 6946 - 35223 mg/kg with the highest levels in the flowers and lowest in the seeds. The flowers of *M. ovalifolia* can be recommended as a good source of potassium for supplement in diet. The study done by Fakankun et al (2013), showed that, the highest levels of K was found in seeds, but not in flowers as indicated by this study. Concentration of potassium in the leaves was 23066 mg/kg, about three times higher than the levels of potassium in Banana (Fahey, 2005).

The highest concentration of Na (545.9 mg/kg) was observed in the leaves. In all the parts studied, the levels ranged from 187.7 to 545.9 mg/kg. Maida, Farooq, Raziya, Umer, Kazi & Nadeem, (2005) reported the same levels of potassium. The concentration of Mn observed in this study ranged from 21 to 85 mg/kg. This range is comparable to those reported (32 to 86 mg/kg) by Foidl, Makkar & Becker, (2001). Mn helps in breaking down of fats, carbohydarates and proteins.

Iron levels were high in leaves (213.9 mg/kg) and lowest in seeds (75 mg/kg). Fakankun et al (2013) reported high levels (214 mg/kg) of Iron in leaves compared to other parts. The leaves and fruits of Moringa are the most consumed, hence potential sources of Fe in human diet. Fe functions in the formation of haemoglobin and myglobin which are carriers of oxygen in the blood and blood vessels. Concentration of Zn observed ranged from 20 to 62 mg/kg. Zn enhances the function of immune system and proper functioning of some enzymes.

CHAPTER 5: CONCLUSIONS AND RECOMENDATIONS

5.1. Conclusions

This study has shown that different parts of *M. ovalifolia* indeed have phytochemicals of known health benefits, for instance:

- The extracts of different parts of *M. ovalifolia* were found to be rich in phenolic compounds and flavonoids.
- The extracts of different parts of *M. ovalifolia* were found to have important minerals.

The antioxidant properties of *M. ovalifolia* were assessed. The findings showed that plant extracts had the ability to reduce Fe^{3+}, to donate hydrogen and scavenge for the DPPH radical. Three flavonoids namely kaempferol, myricetin and quercetin were determined. The flavonoids which have been identified can be used to justify the medicinal potential of *M. ovalifolia*. The plant extracts were also found to contain phenolic constituents. Phenolic constituents are likely to contribute to the overall antioxidant capacity of *M. ovalifolia.*

The metal content analysis of *M. ovalifolia* seeds, leaves and flowers shows that *M. ovalifolia* can provide the needed minerals in the human body. This study supports the use of Moringa as a source of minerals, particularly calcium, potassium, iron, magnesium and phosphorus for our diet. This plant needs to be explored further for use as a supplement and ready source of dietary minerals in animal and human food.

5.2. Recommendations

This study aimed at evaluating the phytochemical, micro- nutritients and antioxidant activities of *M. ovalifolia,* further studies on other classes of flavonoids need to be done to have detailed understanding of polyphenols present in *M. ovalifolia.* Different techniques need to be used in the analyses of phytochemicals and micro-nutrients. More studies need to be done on *M. ovalifolia* for potential as food (commercialization of moringa product) and for medicinal properties.

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