Screening of traditional South African leafy vegetables for selected anti-nutrient factors before and after processing

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REFERENCE DECLARATION

I, Ms. Humaira Essack- 20801815 and Dr. JJ Mellem do hereby declare that in respect of the following dissertation – Title: **Screening of traditional South African leafy vegetables for selected anti-nutrient factors before and after processing**

1. As far as we ascertain:

a) no other similar dissertation exists;

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2. All references as detailed in the dissertation are complete in terms of all personal communication engaged in and published works consulted.

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AUTHORS DECLARATION

This study presents original work by the author. It has not been submitted in any form to another academic institution. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was carried out in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, South Africa, under the supervision of **Dr. JJ Mellem** and **Prof Bharti Odhav**.

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Student's signature

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TABLE OF CONTENTS

ABSTRACT

The present study investigated the effect of processing on anti-nutritional factors of thirteen traditional leafy vegetables collected in KwaZulu-Natal, South Africa. The purpose of this study was to determine whether processing reduced anti-nutrient levels of the species. Three boiling parameters were used with a ratio of 1:4 vegetable to water for a time period of 0, 5 and 15 min. The vegetables studied were: *Amaranthus dubius, Amaranthus hybridus, Asystasia gangetica, Bidens pilosa, Ceratotheca triloba, Chenopodium album, Emex australis, Galinsoga parviflora, Guilleminea densa, Momordica balsamina, Oxygonum sinuatum, Physalis viscosa* and *Solanum nigrum*. From this study, it was determined that non processed samples contained anti-nutrients such as tannins (0.01–0.14 mg/ml), phytic acid (0.002–0.059 mg/ml), alkaloids (3.6–11%), oxalic acid (85.2–1079.3 mg/ml) and cyanogenic glycoside (17–33 mg/100g). *Solanum nigrum* was the highest in tannin content (0.14 mg/ml).*Ceratotheca triloba* was the highest in phytic acid content (0.06 mg/ml). *Momordica balsamina* (11.1%) and *Physalis viscosa* (10.3%) ranked the highest overall in alkaloid content. *Ceratotheca triloba* (1079.3 mg/ml), *Amaranthus hybridus* (796 mg/ml) and *Oxygonum sinuatum* (673.9 mg/ml) were the highest in oxalic acid. *Asystasia gangetica* (33.3 mg/g), *Ceratotheca triloba* (32.6 mg/g), *Momordica balsamina* (32.5 mg/g), *Physalis viscosa* (32.3 mg/g) and *Solanum nigrum* (32.2 mg/g) were the highest in cyanogenic glycoside. All anti-nutrients were reduced significantly through boiling in all the species. The results of this study provide evidence that the local traditional vegetables upon which the population is so reliant upon, are important contributors in micronutrient malnutrition in developing countries and can be eliminated through common boiling methods for a minimum of 5 and maximum of 15 minutes.

1. INTRODUCTION

Nutrition is of great importance especially when a plant or vegetable is being utilized as a source of food. It is imperative to note that endogenous toxic factors within the plant or vegetable can influence the nutrients present. These toxic factors are known as anti-nutrients. Anti-nutrients can be defined as chemicals which are present within the plant to protect the plant as a defence mechanism and aid in other biological functions. They reduce the ability of nutrients such as minerals, vitamins and even proteins within the plant material. This in turn affects the nutritional value of these nutrients. Anti-nutrients are comprised of amino acids to proteins, simple amines to alkaloids, glycosides and phenolic compounds. When a plant food is consumed as a nutritional source, along with this, anti-nutrients are consumed and pose a health risk to the consumer. Processing techniques are said to inactivate anti-nutrients (Ugwu, 2006).

In sub-Saharan Africa most of the population live in rural regions. Their nutrition, due to inaccessibility of resources, is sourced from local cereals and plant foods such as traditional leafy vegetables. However, many rural communities suffer from nutrition deficiency as well as diseases like malnutrition and micro-nutrient malnutrition. The World Health Organization (WHO) has stated that chronic nutrition deficiency affects 200 million of sub-Saharan population which is equivalent to 42% of the population. Traditional leafy vegetables grow wildly, do not require cultivation, are not expensive and can provide high nutrition for poor communities where malnutrition is rife (WHO, 1982). Anti-nutritional factors may affect health as well as the body's digesting ability. All things are toxic at high concentrations and it has been stated that 90% of all toxins occur naturally. A toxic substance is a substance that causes an unfavorable biological effect or disease and bodily damage if present at high doses. Anti-nutritional substances can be derived from natural components within food due to genetic predisposition of organisms, from substances that enter the food by mistake or they can be derived from foreign substances for example, that are added intentionally to improve the food's quality (Velisek, 2014).

Tannins are identified as plant polyphenols that are capable of forming complexes with metal ions and macro-molecules like proteins and polysaccharides thereby interfering with their bioavailability.

Tannins are capable of leaving behind available protein by antagonistic competition and can therefore elicit a protein deficiency syndrome known in Sub-Saharan Africa as 'Kwashiorkor'. Tannins are responsible for a decrease in growth rate as well as a non-palatable taste. Phytic acid is the major phosphorous storage compound in traditional leafy vegetables. Phytic acid has been recorded to inhibit the absorption of minerals and reduce the bioavailability of metal ions like zinc and iron as well as affect protein and starch digestion. A phytic acid intake of 4–9 mg/100g decreases iron absorption in humans by 4–5 fold. Alkaloids are basic nitrogenous compounds produced as metabolites that cause biological effects based on consumption. They occur quite often as a mixture of compounds of related structure. They are found in about 15–20% of vascular plants, especially in seeds, leaves, roots, bark etc. They are classified into three groups. True alkaloids (e.g. Pyridine), are heterocyclic nitrogenous bases derived from amino acids toxic to humans and animals, e.g. tobacco and nicotine. Pseudo alkaloids (e.g. Purine alkaloids), are heterocyclic nitrogenous bases and their precursors are not amino acids. They are less toxic than true alkaloids, e.g. caffeine in coffee. Protoalkaloids are basic amines derived from amino acids but the nitrogen is not heterocyclic, e.g. capsaicin in hot peppers. They are responsible for causing bitterness in some plants. Oxalic acid exists in many leafy vegetables and plant foods. Depending on species, oxalic acid can occur as soluble salts of potassium and sodium or as insoluble salts of calcium, magnesium or iron or it can occur as a combination of soluble and insoluble salts. This forms strong chelates with dietary calcium inhibiting its absorption. A high intake of this soluble oxalic acid can form kidney stones (Akwaowo et al., 2000).

There are many traditional leafy vegetables which are known to have a rich source in micro and macro nutrients, however, their anti-nutritional factors are unknown. Due to the high levels of macro and micro-nutrient malnutrition amongst developing countries the need for anti-nutrient testing and ways to reduce them is in great demand. The proposed research initiative for this project is to conduct a preliminary assessment of the anti-nutritional factors from an array of selected indigenous and underutilized South African traditional leafy vegetables and the effective processing method available to the population in order to minimize these anti-nutritional factors with a view to promote their utilization and contribute to the socio-economic development of the people living in areas where these plants are found and to play a major role in aiding the problem of food insecurity such as micro-nutrient malnutrition problems faced in sub-Saharan Africa.

Traditional leafy vegetables benefit sub-Saharan Africa by improving its nutritional status in vulnerable groups and therefore calls for the need of more in depth anti-nutrient studies to ensure maximum nutritional benefit of these leafy vegetables. Due to a lack of information on anti-nutrient levels and processing required to minimize their effects the aim of this study is to determine the effect of processing on selected anti-nutrient factors of 13 traditional South African leafy vegetables. This was achieved by carrying out the following objectives: (i) To examine the effect of processing on tannin content using spectrometric techniques; (ii) To determine the effect of processing on alkaloid content via gravimetric analysis; (iii) To test the effect of processing on phytic acid content using spectrometric methods; (iv) To analyze the effect of processing on oxalic acid using high performance liquid chromatography; (v) To evaluate the effect of processing on cyanogenic glycoside content using spectrometric techniques.

2. LITERATURE REVIEW

2.1. Food Insecurity

Sub-Saharan Africa is a region that is 24.3 million square kilometers and is made up of 48 countries. The population is estimated at 788 million, this covers just over 10% of the entire world's population (UNAIDS, 2007). South Africa is known for having one of the highest cases of under nutrition in the world. Statistics show that one in every three people are chronically hungry (FAO, 2008).

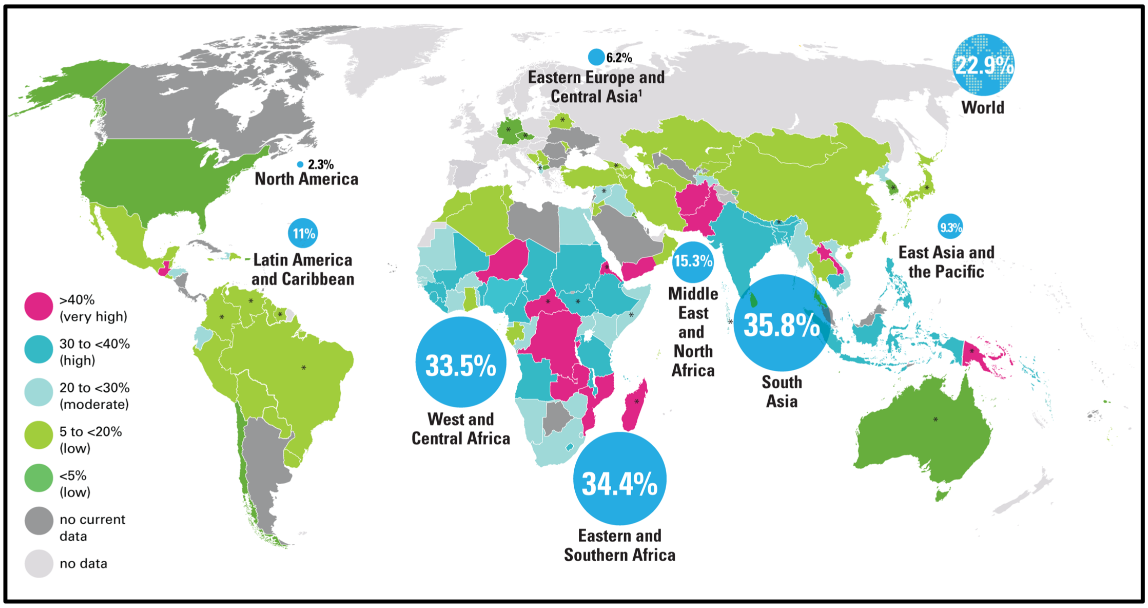

Figure 1: Undernourished people in the world (UNICEF/WHO/WorldBankGroup, 2017)

It has been reported that 45% of all deaths for children under the age of 5 years old are linked to malnutrition being the primary cause. In 2015, globally about 7.7% of children were wasted, 24.5% were stunted and 15% were underweight. The African region as well as South-East Asia have the highest incidents reported of malnutrition, with the African region accounting for about 39.4% of the stunted, 24.9% of the underweight and 10.3% of the wasted children (Figure 1). According to the 2015 millennium development goal (MDG) report, sub-Saharan Africa (SSA) is responsible for one third of all the undernourished children worldwide, emphasizing that malnutrition still remains to be a major health concern for children under 5 years of age in the sub-region, thereby reinforcing the need for urgent intervention. In the post-2015 development era, estimates of child malnutrition will help decide if the world is on the road to achieve the sustainable development goals (SDG) set out, particularly, to "end hunger, achieve food security and improved nutrition, and promote sustainable agriculture".

Between the years 2000 and 2016, the number of stunted children under the age of 5 years old worldwide decreased from 198 million to 155 million. At the same time, the numbers have increased at a disturbing rate in west and central Africa from 22.9 million to 28.1 million (Akombi et al., 2017). Throughout the world, it has been estimated that 44.4% of children younger than the age of five years old, (predominantly in Africa) are at a risk of vitamin A nutrition deficiency (WHO, 2009). A national food consumption survey conducted in South Africa proved that children between the ages 1–9 years old consumed a nutrient deficient diet (Uusiku et al., 2010). Although the staple food is cereal based which is poor in nutrients, the bulk of micronutrients are consumed from traditional leafy vegetables. Studies have indicated that a high intake of plant based food has beneficial effects and reduces the risk of chronic health conditions. Part of the plant based food's beneficial effects are due to the biological activities of their phytochemicals which possess antioxidant, anti-inflammatory, anti-cancer and antimicrobial effects. These phytochemicals also affect dietary intake and there is a lack of investigation into this amongst developing countries (Moyo et al., 2013).

The consumption of traditional leafy vegetables as a food source of essential micronutrients is a necessity for the poor population of sub-Saharan Africa which suffers from one of the highest rates of malnutrition in the world (Uusiku et al., 2010). These high rates are linked to famine and natural disasters prevalent within the country. It is estimated that in many of the countries in sub-Saharan Africa, 20% or more of its inhabitants suffer from food insecurity. Despite the efforts to reduce micronutrient malnutrition, it still exists as a great problem. Hunger and malnutrition both threaten the vast majority of the people in sub-Saharan Africa (Jansen van Rensburg et al., 2004).

The rural population in sub-Saharan Africa consumes traditional leafy vegetables because of their poverty status, the degree of urbanization, season and due to the long distance to travel to fresh produce markets. Therefore, traditional leafy vegetables are consumed by the poor more than the wealthy (Hutchings et al., 1996). Most of the population reside in rural and under developed areas. The hunger stems from poverty which can be attributed to the high rate of unemployment in the country as well as the growing HIV/AIDS pandemic and natural or human disasters (FAO, 2006).

Phytochemicals are biologically active, natural occurring compounds that exist in plants. They protect the plant from disease and damage as well as maintain the flavour, aroma and colour of plants. They are not required by humans but recent studies are looking into their effects in combatting and fighting common diseases in humans. There are more than 4000 phytochemicals recorded of which 150 have been studied in detail. They have been classified according to their function (protection of plant) as well as physical and chemical characteristics. Phytochemicals can be found in many fruit, vegetables, nuts, seeds, legumes, herbs and spices. They can be located in many parts of the plant such as the root, stem, leaves, fruit, flowers or even seeds. They have many biological properties including but not limited to antimicrobial effect, antioxidant activity and even anticancer effects. Phytochemicals cannot be classified due to the large variety, therefore they are classified into primary and secondary constituents based on the role they play in metabolism. Primary constituents consist of common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary constituents consists of remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignin, plant steroids, curcumins, saponins, phenolics and glucosides (Saxena et al., 2013).

A common approach in the prevention of micronutrient malnutrition lies in commercial food fortification. Commercial food fortification has been proven successful because of its long-term practicality and feasibility. Many programs have been implemented over the years to promote food fortification in developing countries. However, it has been discovered that whilst food fortification is a great way forward, it should be combined with locally available nutrient rich foods such as traditional leafy vegetables to improve the fight against food insecurity in sub-Saharan Africa (Kruger et al., 2015).

2.2. Anti-nutritional factors

In order for plants to protect themselves from specific growth conditions, insects, pathogens or herbivores, they synthesize a range of secondary metabolites called anti-nutritional factors or anti-nutrients. Once the plant is consumed, these factors then pose adverse physiological effects upon the consumer, having potential detrimental effects such as impaired growth rate, preventing the uptake of nutrients or even just discomfort to humans or animals (Bora, 2014). Anti-nutritional factors are found in almost all foods. The large fraction of human diets that come from consuming foods with anti-nutrients raise concern about the possible effects that anti-nutrients have on human health (Ileke, 2014).

Plants, like traditional leafy vegetables are made up of phytochemicals that form their characteristic properties (Soetan, 2009). Plant leaves contain nutrients that are required nutritionally for growth and development in humans and animals. The leaves are also sources of proteins, carbohydrates, pigments and minerals in foods. Anti-nutritional factors limit the effective use of the nutrient by binding to digestive enzymes and some dietary proteins (Oluwasola and Dairo, 2016). An anti-nutrient can be defined as a defense metabolite that has certain biological effects that depend upon the structure of the compound (Bora, 2014). Anti-nutritional compounds can be classified into primary and secondary metabolites. The primary metabolites consist of metabolic carbohydrates, proteins/amino acids, lipids, vitamins and minerals. Some secondary metabolites include alkaloids, cyanogenic glycosides and tannins (Soetan, 2009). They can be further categorized into specific groups based on their physical and chemical properties such as non-protein amino acids, quinolizidine, alkaloids, cyanogenic glycosides, pyrimidine glycosides, isoflavones, tannins, oligosaccharides, saponins, phytates, lectins or protease inhibitors (Bora, 2014). All vegetables contain minerals, vitamins and proteins, which are nutritious for humans and animals. The most common anti-nutritional factors in leafy vegetables are oxalic acid, tannins, phytic acid and hydrocyanic acid. Certain leafy vegetables are known to contain resins, alkaloids and saponins which have been known to be deadly to rats (Akwaowo et al., 2000).

2.2.1. Tannins

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the ability to form complexes usually with proteins, polysaccharides like cellulose, pectin, alkaloids, nucleic acids and minerals. These complexes can be either reversible or irreversible. Tannins are found mainly in fruits such as grapes, tea, corn, chocolate, blueberry and sorghum. Structurally they are divided into four groups. Gallotannins, ellagitannins, complex tannins and condensed tannins. Gallotannins are tannins in which galloyl units or their *meta*-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units. Ellagitannins are those tannins in which a minimum of two galloyl units are C-C coupled to each other, and do not contain a glycosidically linked catechin unit. Complex tannins are tannins where a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit. Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin (Figure 2).

In the medicinal industry, tannins are used in natural healing against diarrhoea as well as an anti-inflammatory and antiseptic. In the food industry, tannins are used to clarify wine, beer and juice. Tannins are also used in the textile and dyestuff industries (Saxena et al., 2013).

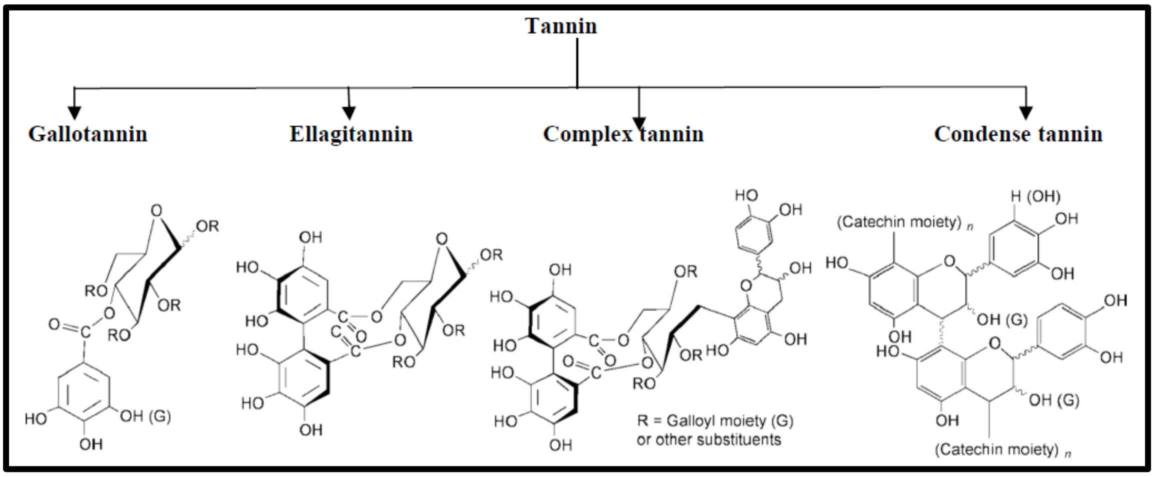

Figure 2: Classification of Tannins (Saxena et al., 2013).

Tannins have been known to adversely affect protein digestibility. By doing this, tannins are responsible for the reduction of nutritional value in food. Tannins usually form insoluble complexes with proteins, in so doing interfering with their bioavailability. Poor palatability is normally credited to diets that are high in tannins. Tannins are capable of leaving available protein by antagonistic competition and can therefore elicit a protein deficiency syndrome known in Sub-Saharan Africa as 'Kwashiorkor' (Bolanle et al., 2014). Tannins are responsible for a decrease in growth rate, a non-palatable taste and have also been known to affect digestive enzymes (Soetan, 2009). They are known to reduce the availability of iron and protein absorption (Sood et al., 2012). Tannins are found in vast amounts in seeds of leguminous plants. The complexes and reactions of tannins with proteins make it resistant to enzymatic hydrolysis which leads to low digestibility and reduced weight gain in animals. Extreme consumption of tannins can play a role in decreased mineral absorption which can in turn lead to damage to the intestinal mucosa (Velisek, 2014). The recommended allowed dosage for man is 0–0.3 mg/kg body weight (FAO/WHO, 1970).

2.2.2. Phytic acid

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6 hexakis-dihydrogen phosphate), also known as inositolhexakisphosphate (IP6) or phytate when in salt form is the major phosphorous storage compound in plant tissues of traditional leafy vegetables. It cannot be digested by humans or animals because of the lack of phytase (digestive enzyme) in the digestive tract which is needed to remove the phosphate from the inositol in the phytate molecule. Ruminants, on the other hand can digest the phytate because of the phytase produced by microorganisms in their rumen (Uusiku et al., 2010; Ileke, 2014). Phytic acid has been recorded to inhibit the absorption of minerals and reduce the bioavailability of metal ions like zinc and iron as well as affect protein and starch digestion (Uusiku et al., 2010). Phytic acid intake of 4–9 mg/100g decreases iron absorption in humans by 4–5 fold (Akwaowo et al., 2000). Phytic acid is a part of a complex class of anti-nutritional factors that occur naturally in plants that are consumed as food. In green leafy vegetables, phytic acid is responsible for the storage of phosphorus. Phytic acid affects the absorption of minerals as well as digestion in the human system (Sood et al., 2012). Phytic acid stores phosphorous that is used during germination of seeds, of cereals, pulses and oilseeds. Phytic acid in seeds occur as a mixture of calcium and magnesium salt (phytin) (Velisek, 2014). Phytic acid or myo-inositol hexakisphosphate is a naturally occurring compound in seeds and cereal grains where its role is as a storage molecule for phosphorus. In grains, phytic acid often occurs as phytate, which are mixed mineral salts of phytic acid. The synthesis of phytic acid starts after flowering of the plants and it accumulates in the seeds during development (Raes, 2014).

2.2.3. Oxalic acid

Oxalic acid exists in many traditional leafy vegetables and plant foods. Depending on species, oxalic acid/oxalate can occur as soluble salts of potassium and sodium or as insoluble salts of calcium, magnesium or iron or it can occur as a combination of soluble and insoluble salts. Insoluble salts are excreted through faeces but soluble salts are absorbed by the body. This forms strong chelates with dietary calcium inhibiting its absorption. A high intake of this soluble oxalate can form kidney stones. Therefore, diets high in oxalic acid need supplementation of minerals to avoid deficiency (Uusiku et al., 2010). Oxalates are a di-carboxylic acid. It is found as a soluble salt of potassium and sodium or an insoluble salt of magnesium, iron and calcium. Insoluble oxalate is excreted in the form of faeces through the human system. However, soluble oxalate is known to form chelates with calcium which in turn affects the absorption of calcium (Sood et al., 2012).

Oxalic acid is common in vegetable and plant foods. It yields insoluble calcium oxalate with calcium ions that can affect the metabolism of calcium. Kidney stones in humans are made up mostly of calcium oxalate. Boiling of vegetables has been recorded to decreases dietary soluble oxalate as opposed to steaming of vegetables (Velisek, 2014).

2.2.4. Alkaloids

Alkaloids are a class of naturally containing organic nitrogen containing bases. Some common alkaloids include morphine and nicotine (Sood et al., 2012). Alkaloids can be naturally synthesized by plants and animals, bacteria and fungi. Almost all alkaloids are known to impart a bitter taste. Alkaloids are a heterogeneous group of substances that consist of greater than ten thousand compounds that are differentiated by structure. They are basic nitrogenous compounds produced as metabolites that are responsible for biological effects based on consumption. They occur quite often as a mixture of compounds of related structure. They are found in about 15–20% of vascular plants, in seeds, leaves, roots and bark. They are classified into three groups. True alkaloids (e.g. pyridine), are heterocyclic nitrogenous bases derived from amino acids toxic to humans and animals, e.g. tobacco and nicotine. Pseudo alkaloids (e.g. purine alkaloids), are heterocyclic nitrogenous bases and their precursors are not amino acids. They are less toxic than true alkaloids, e.g. caffeine in coffee. Protoalkaloids (e.g. capsaicinoids), are basic amines derived from amino acids but the nitrogen is not heterocyclic, e.g. capsaicin in hot peppers (Velisek, 2014). Alkaloids contain organic nitrogen bases. Some common alkaloids are nicotine, morphine and quinine. They are responsible for causing bitterness in some plants (Sood et al., 2012). The fatal dose of alkaloids in humans have been reported as 50 mg/kg (Van Wyk et al., 2002).

Alkaloids are grouped depending on the type of heterocyclic ring system present in the molecule. There are different classes of alkaloids. Pyrrolidine alkaloids contain a pyrrolidine (tetrahydropyrrole) ring system e.g. hygrine found in *Erythroxylum coca* leaves. Pyridine alkaloids have a piperidine (hexahydropyridine) ring system e.g. coniine, piperine and isopelletierine. Pyrrolidine-pyridine alkaloids have a heterocyclic ring system present in their alkaloids which is pyrrolidinepyridine e.g. myosmine, nicotine alkaloid found in tobacco (*Nicotiana tabacum*). Pyridine-piperidine alkaloids contains a pyridine ring system joined to a piperidine ring system.

The simplest member is anabasine alkaloid isolated from poisonous Asiatic plant *Anabasis aphyllan*. Quinoline alkaloids have the basic heterocyclic ring system quinoline e.g. quinine occurs in the bark of cinchona tree. It has been used for centuries for treatment of malaria. Synthetic drugs such as primaquinine have largely replaced quinine as an anti-malarial. Isoquinoline alkaloids contain heterocyclic ring system isoquinoline e.g. opium alkaloids like narcotine, papaverine, morphine, codeine, and heroine (Figure 3).

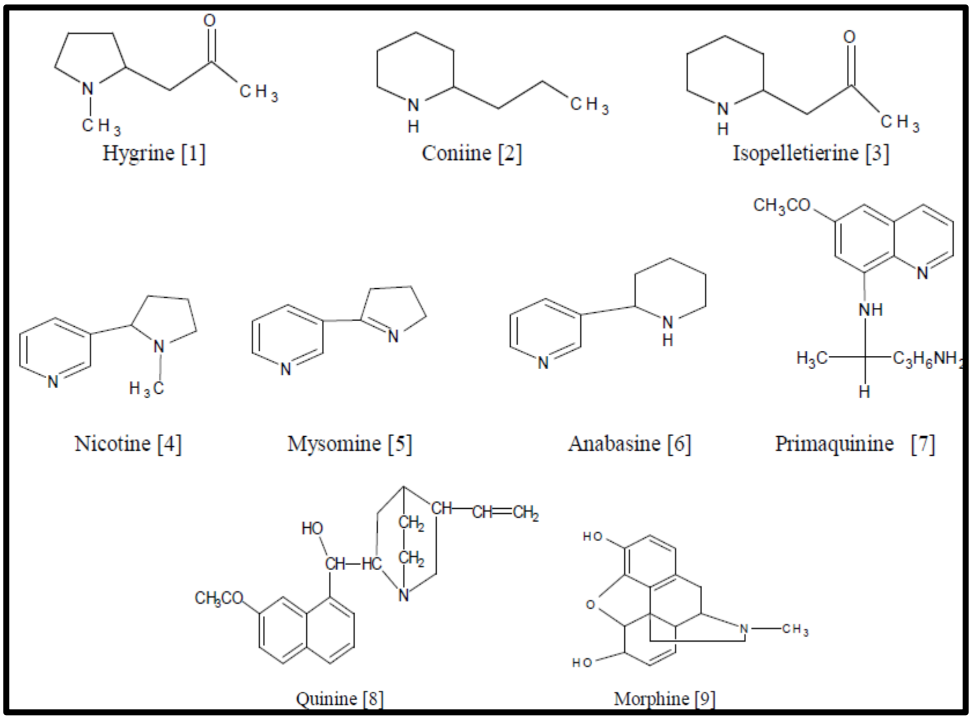

Figure 3: Classification of alkaloids (Saxena et al., 2013).

Alkaloids are known for protection and survival of plants due to their capacity to survive microorganisms, insects and herbivores. Plants that contain alkaloids within them have been used for many years as drugs, dyes and even poisons. A few examples of alkaloids in the pharmaceutical industry are that many indole alkaloids are used for antihypertensive effects and quinine is used for anti-malarial activity. In the food industry alkaloids are used as stimulants such as in caffeine and nicotine (Saxena et al., 2013). Alkaloids are responsible for the bitterness in many traditional leafy vegetables. There are two groups of alkaloids, namely pyrrolizidine and quinolizidine found in leafy vegetables. Pyrrolizidine is usually found in members of the Asteraceae family (*Bidens pilosa* and *Galinsoga parviflora*) which render these plants as toxic. Quinolizidines are more often found in *Amaranthus* species. Pyrrolizidines are not harmful on their own but become highly toxic when they are transformed by cytochrome P450 monooxygenases in the human liver (Uusiku et al., 2010).

2.2.5. Cyanogenic Glycosides

Cyanogens, when hydrolyzed, produce toxic products such as hydrogen cyanide. Cyanide is a deadly poison with a lethal dose of 0.5–3 mg/kg body weight. These metals are functional groups of many enzymes which in turn inhibit processes such as the reduction of oxygen in the cytochrome respiratory chain, electron chain transport in photosynthesis as well as the acting of enzymes like catalase and oxidase (Francisco and Pinotti, 2000). Cyanogenisis can be defined as the ability of a plant to synthesize cyanogenic glycosides, which usually releases cyanohydric acid. Plants use cyanogenisis as a mechanism to protect the plant against predators or herbivores. When the plant is intact, the enzyme (hydroxynitrile lyase) and the cyanogenic glycoside are separated but if the plant tissue becomes disrupted they make contact and cyanohydric acid is released. Cyanohydric acid is very toxic because of its ability to link with metals (Fe^{2+},Mn^{2+},Cu^{2+}) (Francisco and Pinotti, 2000).

2.3. Effects of anti-nutrients on humans

Food is a complicated substance comprising of many chemical compounds which are needed by the body for nourishment. These compounds are nutrients which consist of water, lipids, proteins, vitamins, minerals and carbohydrates. Many plant foods also contain naturally occurring anti-nutrients that aid the plant as a defence mechanism from animals and microorganisms. Anti-nutrients have the potential to be harmful as they render nutrients inactive and can affect human health by preventing digestion as well the absorption of vitamins and minerals. They also impair the nutritional value of a plant food by causing a deficiency of a nutrient or thwarting digestion upon consumption by humans or animals (Prathibha et al., 1995). Table 1 is a summary of the effect of anti-nutrients on humans and animals according to (Akwaowo et al., 2000; Mudzwiri, 2007).

Table 1: Common anti-nutrients found in traditional leafy vegetables and their effects on human and animals (Akwaowo et al., 2000).

Anti-nutrient	Plant source	Effects on humans and animals
Tannins	Fruits, Legumes & nuts	Damage to intestinal mucosa, carcinogenic effects, poor protein utilization, liver and kidney toxicity.
Phytic acid	Legumes & cereals	Prevents digestion, Inhibits the uptake of calcium and can cause problems with metabolic bone disease, renal failure, bladder stones, decreases iron absorption.
Alkaloids	Potato, tomato, tobacco	Kidney inflammation, carcinogen, birth defects, reduced iron uptake
Oxalic acid	Spinach, rhubarb, tomato	Result in kidney stones and reduces solubility of calcium, zinc and iron
Cyanogenic glycoside	Sweet potato, lima beans, cassava, sorghum	Gastro-intestinal inflammation and Inhibition of cellular respiration

2.4. Processing

Different processing techniques are often utilized in order to reduce anti-nutritional factors. Some processing techniques are performed on household level or domestically and others are performed on a larger scale in industry (Raes, 2014). Leafy vegetables are hardly consumed in the raw form, they are usually cooked in stews or soups to name a few. By this cooking, inhibitors are activated, low molecular weight compounds are leached into the cooking medium and discarded.

This simple technique has been employed for many years without man being aware of the technology in order to make these leafy vegetables more palatable and easier to digest (Bora, 2014). According to many studies in literature, soaking, cooking, and boiling have generally achieved significant reduction of anti-nutrients. Therefore foods high in anti-nutrients should be processed adequately in order to make them wholesome for consumption (Ileke, 2014). Different forms of processing are used based on the different cultures in sub-Saharan Africa and their resources. These include boiling, cooking, blanching and sun drying. All through the African region, the leaves, shoots and even flowers of traditional leafy vegetables are boiled and consumed as a potherb or as a relish, stew or side accompaniment to a dish.

The leaves, after harvest, are usually washed and thereafter cooked with water. The cooking time is dependent upon the species of the plant as well as the culture and can vary for up to two hours long. Whilst it is recorded that reduction of anti-nutrients can be achieved through post-harvest processing, in order for complete removal, physical or chemical methods must be employed. These can include soaking, cooking, boiling, germination, fermentation, selective extraction, irradiation and enzymatic treatment. Often, a combination of these methods are employed. Some of these methods, although effective, have a great financial cost factor and are not easily as accessible to the sub-Saharan population. Therefore common cooking methods such as boiling and drying techniques have been employed to mimic the resources that would be available to the rural population (Bora, 2014).

2.5. Different types of processing and their effects on anti-nutrients

2.5.1. Mechanical Treatment

There are numerous types of mechanical treatments, however, dehulling stands out. The technique, often used in cereal grains involves the removal of the bran (outer layer) from the grain and seeds. This is commonly used as a pre-treatment before other techniques. It has been recorded that anti-nutrients such as phenolic compounds, especially tannins, are located in these outer layers. Anti-nutrients, such as phytates (phytic acid) are located in both the germ and the bran of grains and seeds. Dehulling has been recorded to remove up to 50% of tannins and between 2–50% of phytate. This is dependent on the cereal or legume. It is important to note that whilst anti-nutritional factors may be removed, this is at the cost of important minerals which leads to a lower nutritional value in the grain or legume. It has been recorded that the loss of iron and zinc has been considerable due to this process. Milling is often used as a better pre-treatment without allowing the loss of anti-nutrients or minerals. However, it upsets the location of the anti-nutrients and minerals due to cell degradation and in turn affects mineral bioaccessability (Raes, 2014).

2.5.2. Soaking

In order to reduce cooking time and keeping the nutritional value of products, soaking is used. Soaking can be defined as a process in which a biological material is submerged into a water solution (if possible acidified) at a specific temperature of 4–80°C. This process is often used for legumes, grains and seeds.

Upon soaking, water is absorbed by the cells and the pH changes. This results in the activation of endogenous enzymes, the cell walls softening and solubilization of starch and water-soluble components leaching into the soaking medium. This is dependent upon time, temperature and pH. Upon soaking, cell wall degrading enzymes are activated, this causes the degradation of pectin. Pectin is responsible for a cell wall's rigidity. Once the cell wall loses its rigidity, it allows soluble components like minerals, phytate and phenolic compounds to be leached out into the soaking medium. Work done on phytate content has shown a reduction of 60% due to soaking after preheating of rice. Although soaking has had great results in phytate reduction, it also allows for minerals such as zinc and iron to be lost as an unwanted side effect (Raes, 2014).

2.5.3. Germination

Germination is a process in which nutritional value and palatability is increased. Dry seeds are usually soaked in water which begins the process of a series of biochemical reactions. Other types of germination are malting and sprouting. Malting involves the drying of germinated kernels in order to stop enzymatic reactions. Sprouting is a process in which soaked and drained seeds are left to germinate and sprout. Enzymes such as phytases are activated during germination which release nutrients from the seeds and thereby reduce anti-nutrients. Germination needs a warm temperature between 20–30°C to be effective. During germination, phytase activation leads to phytate degradation. Due to this process, phytate has been degraded by 4–50% in sorghum and millet. A decrease in tannin content has been observed due to germination in sorghum varieties. In addition, germination of some grains (e.g. millet) and legumes (e.g. lupine) resulted in an increase in total phenols, total flavonoid content and total tannin content. Although the increase in phenolic compounds is low compared to other process treatments, biochemical changes during germination leads to the production of phenolic compounds as secondary metabolites in germinated seeds (Raes, 2014).

2.5.4. Fermentation

Fermentation is a process whereby microbial enzymes are synthesized and the pH altered. These are the two processes that allow the increase of shelf-life for the altering of sensory properties of the material to be fermented. The pH is either increased or decreased. Both situations affect endogenous and microbial enzyme activities which in turn affect the complexation of anti-nutrients with minerals.

The most commonly used microorganisms for fermentation are lactic acid bacteria as well as yeasts and fungi. Phytate degradation during fermentation has been recorded between 0 and 90%. This is due to the endogenous phytase activity of the plant matrix (Raes, 2014).

2.5.5. Heat processing

Thermal processing is a domestic technique that is used to reduce anti-nutritional factors in green leafy vegetables, however, it is temperature, pH and species dependent. Heat processing, specifically moist heat, such as boiling, autoclaving, extrusion, cooking and microwaving have been known to decrease vast levels of hydrogen cyanide as well as tannins and phytate by up to 40% (Inyang et al., 2013). Roasting, however has been recorded to decrease phytate and tannin levels more significantly. Blanching has been recorded to reduce phytate and tannin levels in green leafy vegetables by 5–15% (Inyang et al., 2013). Partial cooking (blanching) refers to the vegetables being cooked partially without the addition of other ingredients, this is done in the summer months when there is an abundance of vegetables and stored for later use. Leafy vegetables do not have a long shelf life and are therefore dried if not cooked in order to extend their shelf life. They are also dried to ensure their availability during the winter months. They are dried on flat rocks and exposed to the sun for 2–3 days. The dried leafy vegetables are then stored in huge clay pots or sacks. The dried and stored leafy vegetables are retained for up to 3 years (Jansen van Rensburg et al., 2004).

The most common domestic processing method include cooking (Embaby, 2010). Processing treatments include heating which can be made up of cooking, sterilization, steaming and pasteurization as well as roasting or microwaving. All these heat treatments differ with regards to the maximum temperature that is reached during the heating process, as well as the use of wet or dry heating technology. As soon as the optimal temperature is reached, it is then maintained for a specific time period. This is what influences the anti-nutritional factors and the bioaccessability of minerals. Depending on the chosen heat treatment, endogenous enzymes like phytases, cellulases and pectinases are usually inactivated (Raes, 2014). Cooking is a common form of processing in plants that are consumed as a food source. Cooking causes changes in the phytochemistry of the traditional leafy vegetable affecting its bioaccessability and health benefit properties. The degree of these changes depends largely on the cooking methods as well as the traditional leafy vegetable (Odhav et al., 2007).

Studies show that cooking has been recorded to reduce the oxalic acid content more significantly than drying, in spinach and amaranth leaves. Open pan and pressure cooking achieved similar results. Blanching caused cell wall rupture and the leaching of oxalic acid into the cooking medium. These studies also showed that cooking did not significantly change the phytic acid content of leafy vegetables due to endogenous phytases being inactivated by the heat. Therefore, a higher temperature was required to degrade phytate. However, blanching reduced the phytic acid level in leafy vegetables; the longer the time period of blanching the greater the effect in reducing the phytic acid levels. Blanching causes the cell walls to rupture and in turn allows soluble phytic acid to leach into the blanching water (Yadav and Sehgal, 2003). Thermal processing (heating) leads to enzyme inactivation, texture changes of vegetables, as well as unavoidable leaching of water-soluble compounds. This in turn can alter the phytochemical profile and content of vegetables. Phytochemicals do not exist as individual compounds; they are usually bound to other compounds or to cell structures. The heat causes disruption and damage to the cell membranes. The once bound phytochemicals are then released into the medium and can be extracted. It has been reported that heating increases the chemical extractability of phytochemical compounds due to the release of phytochemicals from chromoplasts which leads to an increase in concentration. Heating similarly boosts the diffusion of cellular fluids containing phytochemicals from the plant cell to the water medium (Howard et al., 1999).

2.6. Traditional Leafy Vegetables

Traditional leafy vegetables are common in the diets of many rural as well as urban homes throughout Africa. Traditional leafy vegetables can be defined according to the fact that they were initially cultivated in Africa throughout the last few centuries. Introduced vegetables are vegetables that have been chosen by breeders and have been improved to impart a better taste. Traditional leafy vegetables however, have not been researched to that extent. Traditional leafy vegetables are known to contain great amounts of glycosides, oxalic acid, alkaloids and hydrocyanic acid. The cultivation of traditional leafy vegetables lies within the knowledge of the African people. The number of people in sub-Saharan Africa that still consume traditional leafy vegetables till this day is substantial.

In the year 1996, the yearly sales of traditional leafy vegetables in the urban markets of Cameroon were approximately 22 million US dollars.

If one had to take into consideration the value of rural consumption, the approximate total market value would have been more than 56 million US dollars (Gockowski et al., 2003). Leafy vegetables grow wild in soils of low fertility, are relatively drought tolerant, are more resistant to pathogens and pests, provide good ground cover and can be harvested within a short period of time. Most importantly, they are available freely to poor households and communities. They are mainly grown in small patches in homes in which exotic vegetables are not usually grown due to lack of infrastructure. Due to this, leafy vegetables compete well with exotic vegetables because exotic vegetables require high inputs and are not always affordable to farmers or the poor consumers (Jansen van Rensburg et al., 2004; van Jaarsveld et al., 2014; Kruger et al., 2015).

The sub-Saharan Africa has a prevalence of disease. This could be attributed to many factors such as diet changes or environmental factors. Traditional leafy vegetables are plentiful in South Africa and the presence of anti-nutrients indicates that the evaluation of anti-nutritional factors is therefore necessary (Hutchings et al., 1996). The staple food in sub-Saharan Africa is based on a diet containing cereals which are low in micronutrients therefore the daily requirements for these are obtained through traditional leafy vegetable consumption.

Traditional leafy vegetables are inexpensive, more accessible and provide protein and essential amino acids (Omotoso, 2006). Traditional leafy vegetables are also easy to cook, provide roughage and are rich in vitamins and minerals (Jau-Tien et al., 2011). A summary of the traditional leafy vegetables used in the study is depicted in Table 2 (Hutchings et al., 1996). These traditional leafy vegetables are high in mineral content and have been tested by (Odhav et al., 2007).

Table 2: Summary of Traditional leafy vegetables and their common uses (Hutchings et al., 1996).

Scientific Name	Family Name	Common name	Traditional Name	Common Uses	Source
Amaranthus dubius	Amaranthaceae	Wild Spinach	Terere	Potherb	Reservoir Hills
Amaranthus hybridus	Amaranthaceae	Cockscomb	Imbuya	Relish mixed with mealie meal	Reservoir Hills
Asystasia gangetica	Acanthaceae	Hunter's spinach	Isihobo	Treat asthma	Reservoir Hills
Bidens pilosa	Asteraceae	Black jack	Amalenjane	Used in tea, salads, stews	Reservoir Hills
Chenopodium album	Chenopodiaceae	Fat hen	Imbikilicane	Eaten as porridge	Reservoir Hills
Ceratotheca triloba	Pedaliaceae	Wild foxglove	Udonga	Cooked as spinach	Reservoir Hills
Emex australis	Polygonaceae	Devil's thorn	Inkunzane	Cooked as spinach	Reservoir Hills
Galinsoga parviflora	Asteraceae	Gallant soldier	Ushukeyana	Potherb,soup flavouring	Reservoir Hills
Guilleminea densa	Amaranthaceae	Small Matweed	*	Decoction	Reservoir Hills
Momordica balsamina	Cucurbitaceae	Balsam apple	inkaka	Cooked as spinach	National Botanical Institute, Durban
Oxygonum sinuatum	Polygonaceae	Stars Talk	Untabane	Boiled as a vegetable	Park Rynie
Physalis viscosa	Solanaceae	Grape ground-cherry	Uqadol	Fruit and berries edible	Park Rynie
Solanum nigrum	Solanaceae	Black common/garden/ woody nightshade	isihlalakuhle, udoye, ugqumgqumu, ugwabha, umaguqa	Cooked as a vegetable	Reservoir Hills

***Not recorded**

2.6.1. *Amaranthus dubius*

Amaranthus dubius (Figure 4) is found in tropical and warm climates. The members of the Amaranthaceae family often accumulate free oxalates, potassium nitrate and saponins and produce betalains but not anthocyanins. They are not tanniferous and lack both proanthocyanins and ellagic acid. Crystals of calcium oxalate are present in some cells of the parenchymatous tissues often as clustered crystals or crystal sand (Hutchings et al., 1996). *Amaranthus dubius* is often referred to as *umfino imbuya* in Zulu and *groot meerjarige* in Afrikaans. In English, it is commonly referred to as African spinach, Indian spinach, and green leaf, bush green and Chinese spinach. It is an annual plant that grows up to 1 meter in height. It has a lot of branches and is commonly found in rural and peri-rural areas of Africa. The stems are glossy green with maroon streaks. The leaves vary in length between 25 to 85 mm and are green with dark blotches.

Figure 4: *Amaranthus dubius*.

The species grows rapidly in hot conditions all year round and are pleasant in taste which makes it popular as a primary food source in developing countries. *A. dubius* is a popular nutritious leafy vegetable crop that is rich in protein, vitamins and minerals (Mellem, 2008). According to work done by (Odhav et al., 2007), *Amaranthus dubius* has a high manganese (82 mg/100g) and magnesium (806 mg/100g) content. The leaves and shoots are eaten as a vegetable. It is commonly used with other, more bitter vegetables in order to avoid discarding the boiling water used in the boiling of the vegetable. It therefore serves to improve the taste of many traditional leafy vegetables. (Tredgold, 1990). Amaranth leaves have medicinal properties for young children, lactating mothers and for patients with fever, hemorrhage, anemia, constipation or kidney complaints. In Tanzania the whole plant is used as a medicine against stomach ache. In Uganda, *Amaranthus dubius* plants are used for the preparation of potash (Grubben, 2004).

2.6.2. *Amaranthus hybridus*

Amaranthus hybridus (Figure 5) is an erect annual plant with a stem. It usually grows 30–200 cm tall and is found in tropical and warm climates. The members of the Amaranthaceae family often accumulate free oxalates, potassium nitrate and saponins and produce betalains but not anthocyanins. They are not tanniferous and lack both proanthocyanins and ellagic acid. Crystals of calcium oxalate are present in some cells of the parenchymatous tissues often as clustered crystals or crystal sand (Hutchings et al., 1996). It is very common because of its nutritional value as it contains high levels of carbohydrates and protein. According to work done by (Odhav et al., 2007)

Figure 5: *Amaranthus hybridus*.

Amaranthus hybridus has a high protein content (6 g/10 g) as well as calcium (2363 mg/100 g) and magnesium content (1317 mg/100 g). It is often said to taste like spinach, however, it has significantly higher calcium, phosphorous and iron levels than spinach. (Odjegba and Sadiq, 2002). *Amaranthus hybridus* is commonly known as "pigweed" or "amaranth" and is an annual, herbaceous plant that grows up to 2 meters in height. Its leaves are a dull green colour and are rough and hairy. It has small green-red flowers. In Nigeria it is used to prepare soups and in West Africa it is eaten as a salad. In the Congo, the leaves are consumed as spinach or green vegetables. In West Africa, the leaves are boiled and thereafter mixed with a groundnut sauce to be eaten as a salad (Akubugwo et al., 2007). The leaves are cooked as spinach. Once washed, they are slightly boiled with tomatoes and onions. Maize meal is often used as a thickening agent to form a thin porridge (Maundu et al., 1999).

2.6.3. *Asystasia gangetica*

Asystasia gangetica (Figure 6) is commonly known as "hunter's spinach" in English and "isihobo" in Zulu. It is found widely in Africa in disturbed lands and typically consumed during famine (Odhav et al., 2007). It is also found in largely tropical areas including open country and deserts extending to the Mediterranean. Quinazoline or quinolone alkaloids and diterpenoid bitter substances are frequently present within the plant. Members of the Acanthaceae family are rarely cyanogenic, saponiferous or tanniferous. Various forms of calcium oxalate crystals are often present in some cells of the parenchymatous tissues (Hutchings et al., 1996). *Asystasia gangetica* is an attractive, fast growing, spreading and herbaceous ground cover. It grows from 300 to 600 mm in height. It has a green, oval shaped leaf. The flower is whitish and cream coloured and the fruit contains purple markings. The fruit is a club shaped capsule that splits from the tip to the base. In East Africa, *Asystasia gangetica* has been used as a traditional medicine to manage asthma. It is also consumed as a food source as the leaves have been documented to contain protein, carbohydrates, lipids, minerals, amino acids and fibre (Ezike et al., 2008). According to work done by (Odhav et al., 2007) it has a high content of calcium (2566 mg/100 g), phosphorus (814 mg/100 g) and magnesium (961 mg/100 g). It is eaten as a vegetable mainly due to its short cooking time (Tredgold, 1990).

Figure 6: ***Asystasia gangetica.***

2.6.4. *Bidens pilosa*

Bidens pilosa (Figure 7) is an annual herb with an erect habit to 1.5 m in height. It is easily recognized by the elongated fruits that bear hooked bristles (burrs) that embed themselves in people's clothing as they brush past the stems. It is alternatively known as *B.leucantha*, and is more commonly known as black jack. The Zulu names are *amalenjane* and *uqadolo*. It is a plant originally from South America but is now widespread in South Africa. Its chemical constituents include polyacetylene phenylheptatriyene and chalcone okanin. A number of polyacetylenes have been known to be phototoxic to bacteria, fungi and human fibroblast cells in the presence of certain lights. These leaves have been known to attain a bitter astringent taste and contain a strong odour. The plant is commonly consumed as a pot herb especially in the Transkei regions and this has led to high rates of esophageal cancer in this area. The hot leaf and root infusions have been used as a Zulu medicinal treatment for enemas due to stomach complaints. The young shoots are chewed for rheumatism. In Transkei, powder from this plant is diluted in water for sickness such as syphilis. In East Africa the leaves are used for conjunctivitis and constipation in babies (Hutchings et al., 1996). It has been recorded to be high in protein (5 g/100 g) and fiber (3 g/100 g) and has a high copper (10 mg/100 g) and magnesium content (658 mg/ 100 g) (Odhav et al., 2007).

Figure 7: *Bidens pilosa.*

2.6.5. *Ceratotheca triloba*

Ceratotheca triloba (Figure 8), is an erect herb found in tropical and warm climates especially in the coast and arid areas. It is part of a genus in which iridoid compounds are produced. It is a tall annual plant that grows to 1.3 meters in height and has distinct white to dark pink flowers. Members of the Pedaliaceae family are not cyanogenic, saponiferous or tanniferous. However, sometimes small crystals of calcium oxalate are produced. *Ceratotheca triloba* is also known as C. *lamiifolia* and its common names are vingerhoedblom and wild foxglove. In Zulu, it is referred to as either *udoncalwabathwa, udongalwezithutha* or *udonqabathwa*. The fresh leaves give off a rather unpleasant odour and are sometimes used as insect repellents. The root infusions have been known to be used for sore ears. In Zimbabwe, the plant infusions are used to induce abortion. Amongst the Zulu medicinal usage, the roots have been used as traditional medicine and the leaf infusions for menstrual cramps (Hutchings et al., 1996). The new shoots along with the leaves are cooked as spinach. The unpleasant odour of the leaves are removed with boiling. It has a sweet taste and is easily digestible and therefore used as a relish for children to consume. The plant contains vitamins A and C, as well as calcium and iron, and is thus used to relive gastric disorders (Tredgold, 1990). The plant has a high energy content (62 kcal/100 g) and fat content (2 g/100 g). It is also high in magnesium (428 mg/ 100 g) (Odhav et al., 2007).

Figure 8: *Ceratotheca triloba.*

2.6.6. *Chenopodium album*

This plant (Figure 9) is a fast growing weed plant found in cosmopolitan areas and deserts as well as semi-deserts. It is an annual plant growing to 0.9 m by 0.2 m in height. Originally it was from Europe and Asia but is now found in many Southern African regions. Members of the Chenopodiaceae family accumulate organic acids and also oxalates or free nitrates. They often contain alkaloids as well as saponins and crystals of calcium oxalate. These plants are not tanniferous. Crystals of calcium oxalate, clustered or in the form of sand, are commonly found in some cells of the parenchymatous tissue. *Chenopodium album* is an annual or perennial herb or shrub that is commonly known as fat hen, hondepisbossie, misbredie, seepbossie or varkbossie. In Zulu, it is referred to as *mbikilicane* or *isijabane*. It has been reported to contain hydrocyanic acid, ascorbic acid and 7.22% potassium oxalate. The seeds contain albumen and fat and the plants contain calcium, iron and vitamin A. The plant contains much nutrients but this is depleted as the plant ages. Large amounts of the plant ingested by cattle has been reported to be poisonous and result in the cattle entering a coma. The leaves are finely powdered for Zulu medicinal usage to treat genital irritations in children. The Xhosa people use the plant as a blood purifier to treat malnutrition. The Tswana people have traditionally eaten this cooked plant on a weekly basis to purify blood and keep the stomach "working well". The plant is eaten as a cooked vegetable in Transkei and Lesotho (Hutchings et al., 1996). The plant has a high zinc content (109 mg/100 g) and a high protein content (5 g/100 g) (Odhav et al., 2007).

Figure 9: ***Chenopodium album.***

2.6.7. *Emex australis*

Emex australis is a herbaceous plant and is a member of the Polygonaceae family (Figure 10). It is known to produce anthocyanins and often accumulate anthraquinone glycosides as well as oxalic acid. They are commonly tanniferous. Calcium oxalate crystals are found in the parenchymatous tissue. The species are found in northern temperatures of Africa. *Emex australis,* is also known as *E.spinosa*. It is commonly referred to as cape spinach or devils thorn. In Zulu, it is called *inkunzama*. It is a monoecious herb reported to have diuretic effects. The Zulu people employ it medicinally to treat colic in babies. It is also used as a strong enema. The leaves are boiled by the Xhosa people and eaten to treat biliousness and relieve dyspepsia as well as to increase appetite (Hutchings et al., 1996). It is high in protein (5 g/100 g) and magnesium (1018 mg/100 g) (Odhav et al., 2007).

Figure 10: *Emex australis*.

2.6.8. *Galinsoga parviflora*

Galinsoga parviflora (Figure 11) is a part of the Asteraceae family and is commonly known as "gallant soldier" in English. It is a slender annual plant, growing from 20-70cm tall. In Zulu, it is referred to as "ushukeyana". It is cultivated and consumed regularly throughout Africa (Odhav et al., 2007). Members of this family are characteristic of toxic pyrrolizidine alkaloids as well as pyridine, quinolone and diterpenoid alkaloids. The plants are occasionally cyanogenic. They are not usually tanniferous. Crystals of calcium oxalate are seldom present in some cells of the parenchymatous tissues (Hutchings et al., 1996). The leaves are cooked as spinach and are also used as a relish (Tredgold, 1990). It has a high magnesium content (681 mg/100 g) (Odhav et al., 2007).

Figure 11: ***Galinsoga parviflora.***

2.6.9. *Guilleminea densa*

Guilleminea densa (Figure 12) belongs to the Amaranthaceae family and is found in tropical and warm climates. The members of this family often accumulate free oxalates, potassium nitrate and saponins and produce betalains but not anthocyanins. They are not tanniferous and lack both proanthocyanins and ellagic acid. Crystals of calcium oxalate are present in some cells of the parenchymatous tissues often as clustered crystals or crystal sand (Hutchings et al., 1996). It is also referred to as *Brayulinea densa*. It is a woody perennial herb with a fibrous fleshy root. It has a basal rosette of leaves with white hairs that are crowded on the stems. It is low growing and grows up to 5 cm tall and 40 cm wide. The flowers are yellowish-cream to off-white. The flowers are up to 6mm. It is found in Africa on the roadsides, grasslands and open woodlands (Henrickson, 1987). It is used as a remedy for diarrhoea. The whole plant, with the addition of hot water is used to prepare this remedy. The dose is given thrice daily depending on the age of the patient and this can range from three teaspoons to a cup. The decoction is also mixed with maize meal to form a soft porridge to be fed to patients (Mathabe et al., 2006).

Figure 12: ***Guilleminea densa.***

2.6.10. *Momordica balsamina*

Momordica balsamina (Figure 13) is a tendril-bearing annual vine. It can grow up to 4.5 meters tall. It is from the genus *Momordica* L. and is commonly known as aloentjie, bursting beauty and laloentjie. In Zulu, it is named *inkaka, intshugu* or *intshungwana yehlathi*. It is found in tropical areas. It contains the bitter principle momordocin. The compounds isolated from the plant's seed oil include two conjugated octadecatrienoic fatty acids, punicic acid, α-eleostearic acid, campesterol and β-sitosterol. The leaf and plant extracts have shown to have depressant effects as well as protein synthesis inhibitory activity. It has been used for cold infusion of runners and the roots to soothe squeamish stomachs as a Zulu medicinal usage. In West Africa the plant is used to treat fevers. In other medicinal usage the fruit has been used to treat colic and the seed oil to treat burns. The roots are also used as an aphrodisiac. However, some fruit have been known to cause fatality in dogs as a result of aggressive vomiting (Hutchings et al., 1996). According to (Odhav et al., 2007), it is high in protein (5 g/100 g), calcium (2688 mg/100 g) and magnesium (613 mg/100 g).

Figure 13: *Momordica balsamina*.

2.6.11. *Oxygonum sinuatum*

Oxygonum sinuatum (Figure 14) is part of the Polygonaceae family. It is also known as "stars talk" and in Zulu it is referred to as "untabane". It is a roadside weed and is consumed as a famine food (Odhav et al., 2007). The leaves are eaten raw for their acidic taste and are also boiled as a vegetable (Maundu et al., 1999). It is found in cosmopolitan areas and northern temperatures. Members of this family produce anthocyanins and commonly accumulate oxalic acid. They are commonly tanniferous. Crystals of calcium oxalate are present in some cells of the parenchymatous tissues often as clustered crystals or solitary crystals. The roots are used in Zimbabwe as medication for abdominal pain, whooping cough, to avoid abortion and convulsions. The leaves are also applied to snake bites. The fruit is burnt and inhaled for nose bleeds (Hutchings et al., 1996). It has been recorded by (Odhav et al., 2007) to be high in sodium (1460 mg/100 g) and magnesium (521 mg/100 g).

Figure 14: *Oxygonum sinuatum.*

2.6.12. *Physalis viscosa*

Physalis viscosa (Figure 15) belongs to the Solanaceae family and is found in sub cosmopolitan areas especially Southern America. Various kinds of alkaloids are present within this family of plants. They are usually not tanniferous and seldom cyanogenic. Solitary or clustered crystals of calcium oxalate of various forms are commonly present in some of the parenchymatous tissue cells. It is an erect, perennial evergreen herb. The genus *Physais-L.* has been reported to contain hygrine alkaloids found in the roots of some species and flavonoid glycosides in other plant parts. The unripe fruit has been known to cause poisoning and symptoms such as fever. Leaf infusions are administered by the Zulus as enemas for children. In East Africa, the sap from the roots are used to treat gastric ulcers. Decoctions are used for labor pains, gonorrhea, skin rashes, infant colds and general ill-health (Hutchings et al., 1996). It has been recorded by (Odhav et al., 2007) to be high in energy (69 kcal/100 g), protein (6 g/100 g) and magnesium (535 mg/100 g).

Figure 15: *Physalis viscosa.*

2.6.13. *Solanum nigrum*

Solanum nigrum (Figure 16) belongs to the Solanaceae family and is found in sub cosmopolitan areas especially Southern America. Various kinds of alkaloids are present within this family of plants. They are usually not tanniferous and seldom cyanogenic. Solitary or clustered crystals of calcium oxalate of various forms are commonly present in some of the parenchymatous tissue cells. *Solanum* nigrum is commonly known as black/common/garden/woody nightshade and is referred to as isihlalakuhle, udoye, ugqumgqumu, ugwabha, umaguqa and even umsoba in Zulu. The species in the genus are perennial evergreen shrubs or herbs that can reach a height of 30 to 120 cm.

The fruit are known to commonly contain steroidal glycoalkaloids. The alkaloid (solanine) has been reported to have caused human toxicity symptoms such as vomiting, dizziness and irritation to the throat. Solanine has been found in the unripe fruit of *Solanum nigrum*. However, the leaves and seeds have been reported to contain ascorbic acid and carotene. The plants are widely cooked as a vegetable in places like Transkei. The leaves are also used as an anti-neuralgic. It has been employed amongst the Zulus for medicinal purposes by administering the leaf infusions to infants with upset stomachs. The paste from the leaves is also used to treat wounds (Hutchings et al., 1996). The leaves are usually eaten as a vegetable and typically cooked alongside the Amaranth species. The leaves are only picked and boiled and never fried. Salt is usually added to offset the leaves bitter taste. The fruit, when orange, is edible (Maundu et al., 1999). In the dry seasons, the leaves are boiled twice. Potash is added to soften the cooked leaves (Tredgold, 1990). The un-ripened fruit is applied to sore teeth or used as a teething relief. The leaves are used to aid stomach ache. The powdered leaves combined with the fruit are used to treat tonsillitis. The roots are also boiled in milk and given to children as a tonic (Maundu et al., 1999). The berries are crushed to form a paste to treat ringworm. A paste from the soaked leaves is used to treat ulcers, black-water fever and dysentery. An infusion of the plant is a common enema for children. The powdered burnt root is rubbed into an incision in the back to treat lumbago (Tredgold, 1990). It has been recorded by (Odhav et al., 2007), to be high in calcium (2067 mg/100 g), magnesium (277 mg/100 g) and iron (85 mg/10 g).

Figure 16: ***Solanum nigrum*.**

3. MATERIALS AND METHODS

3.1. Method overview

This section describes the methods that were used to investigate whether traditional leafy vegetables contained specific anti-nutrients and the levels of these anti-nutrients as well as whether these levels were decreased with thermal processing. Figure 17 shows a brief over view of the methodology used to conduct anti-nutritional testing as well as the processing parameters applied. The experiments involved testing the effect of processing on the anti-nutritional levels of thirteen traditional leafy vegetables. Statistical analysis was used to correlate the relationship between the anti-nutritional levels before processing and after two processing parameters. The plants used in the study were selected based on the information gathered from reviewing literature and were sourced in Durban, Kwa-Zulu Natal, South Africa. A number of these plants are mainstays in the diets of rural and urban households across most of sub-Saharan Africa, including South Africa and are also used in some traditional medicines (Hutchings et al., 1996). All the reagents used in this study were of analytical grade and were purchased from Sigma (Germany) and Merck (Germany).

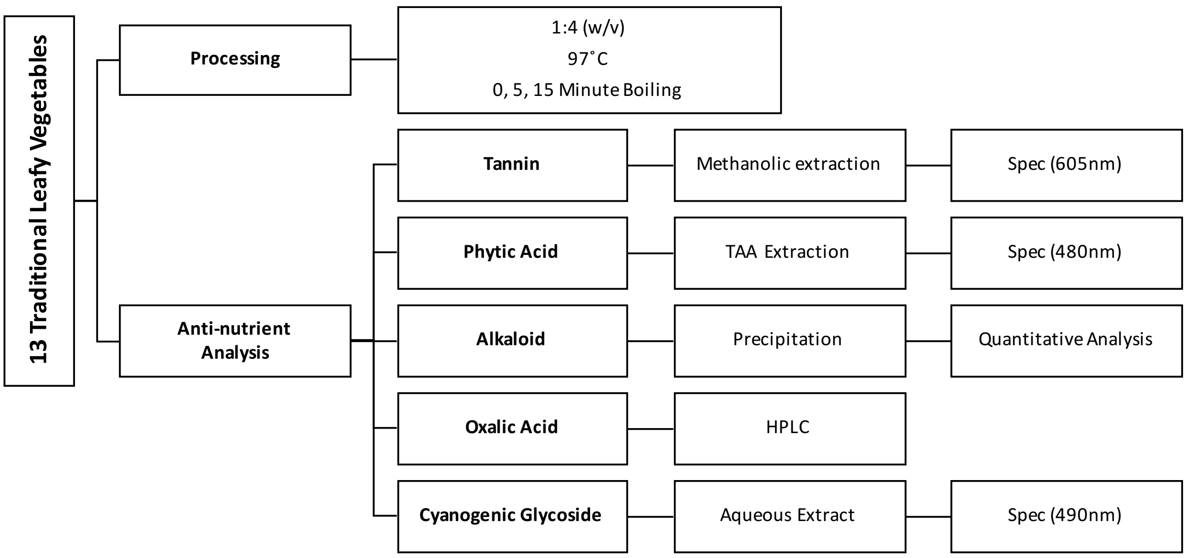

Figure 17: Brief overview of the methodology for the anti-nutritional analysis of 13 traditional leafy vegetables before and after processing.

3.2. Sample collection and Preparation

Anti-nutritional analyses (tannins, phytic acid, oxalic acid, alkaloids and cyanogenic glycoside) were conducted using the raw leaves of thirteen traditional leafy vegetables. Table 2 provides a brief overview of the traditional leafy vegetables used in the study. The plants were identified and sourced from general farm land during the months of January to March in Durban, Kwa-Zulu Natal, South Africa and voucher specimens were housed in Ward Herbarium, University of Kwa-Zulu Natal. Biodata on the plants are listed in Table 2 (Hutchings et al., 1996). The leaves were carefully inspected and damaged or infected leaves were discarded, as the collection period for the samples were during a period of rain. Appropriate leaves were cleaned and dried in a custom-built convection oven for drying plant material at 60°C for a time period of 48 h in order to mimic sun drying. The dried leaves were then processed and ground to a fine powder in a blender and stored until further use. All tests were done in triplicate.

3.3. Phytochemical analyses

3.3.1. Determination of Tannins

The tannin content was determined by Van-Buren and Robinson method. 50 ml distilled water was added to 500 mg sample and subjected to a mechanical shaker for 1 hour. The sample was then filtered into a 50 ml volumetric flask and made up in distilled water. 5 ml of the filtered sample was removed and mixed with 0.1M $FeCl_3$ in 0.1N HCl and 0.008M Potassium Ferrocyanide (Sigma-Aldrich P9387). A standard curve was prepared using tannic acid (Appendix 1). The absorbance was read on a Spectrophotometer (Varian Cary 100 UV-Vis Spectrophotometer, USA) at 605nm (Van-Buren and Robinson, 1969).

3.3.2. Determination of Phytic Acid

The phytic acid content was established using a modified method by (Omotoso, 2006) and (Wheeler, 1971). Phytic acid reacts with a coloured complex for example Fe(III)-sulphosalicylate to form a colourless Fe(III)-phytate complex. The method measured the Fe (II) content which links to the phosphorus content (4:6) and the phosphorus content correlates to the phytic acid content (1:1). A standard curve was prepared using Fe $(NO_3)_3$ (Sigma-Aldrich F3002) in the range 0.025–2 mg/ml (Appendix 2).

Five grams of ground sample was extracted in 50 ml of 3% trichloroacetic acid (Gupta et al. 2005) (Sigma-Aldrich T4885). The samples were placed in a shaking incubator (Labcon,USA) for 30 minutes at a constant speed of 156 rpm. The suspensions were thereafter centrifuged (Eppendorf 5810R, Germany) at 10 000 rpm for 15 minutes and the supernatants (2.5 ml each) were transferred to 15 ml centrifuge tubes. Two millilitres of $FeCl_3$ solution (2 mg/ml) was added to each sample. The sample was heated for 45 minutes in a water bath at a temperature of 90°C. The solutions were centrifuged again (10 000 rpm for 15 min) and the supernatants poured off. The pellets were washed by adding 10 ml 3% TAA solution, heated for 5 minutes and centrifuged (10 000 rpm for 15 min). The resultant pellet was washed once with distilled water and re-suspended in 1 ml distilled water and 1.5 ml of 1.5N NaOH (Sigma-Aldrich S5881) solution and stirred. The volume was brought up to 15 ml with distilled water, heated in boiling water for 30 minutes and centrifuged (10 000 rpm for 15 min). The solution was filtered while hot (Whatman No. 2 filter paper). The precipitate was washed with 40 ml of hot distilled water and the filtrate discarded. The precipitate left in the paper was dissolved with 20 ml 3.2N solution of HNO_3 (Sigma-Aldrich) transferring it to a 50 ml volumetric flask. The sample was then cooled at room temperature and calibrated with distilled water. A 2.5 ml sample was transferred to a volumetric flask and diluted to 35 ml with dH2O. Thereafter 10 ml of 1.5 M potassium thiocyanate (KSCN) solution was added and the solution calibrated to 50 ml with distilled water. The absorbance of the samples was read within 1 min at an absorbance of 480 nm using a spectrophotometer (Varian Cary 100 UV-Vis Spectrophotometer, USA).

3.3.3. Alkaloid precipitation

The presence of alkaloids was established using a precipitation method by (Harborne, 1973) and (Edeoga et al., 2005) with slight modifications. Ammonium hydroxide was added to plant extracts in order to precipitate alkaloids. The dried sample was treated with 200 ml of 10% acetic acid in ethanol (v/v) for 4 hours at room temperature. The extract was thereafter filtered and concentrated to 50 ml on a rotary evaporator at a temperature of 60°C. 1 ml of concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The solution was left to stand in order for the precipitate to settle. The precipitate was collected and washed with a ratio of distilled water and ammonium hydroxide (5 ml: 5 ml) (v/v) and thereafter filtered. The remaining residue was dried at room temperature and weighed. The results were recorded in grams per 5 g dried leaves and converted to percentage.

3.3.4. Quantification of Oxalic acid

The oxalic acid content was established using high performance liquid chromatography (HPLC) analysis modified method by (Miller, 2004). A standard curve was used to establish the concentration of the unknown oxalic acid in the plant extracts. Oxalic acid standards were prepared in the range 1- 20 mg/ml (Appendix 3) and run chromatographed on an HPLC system (D7000 Lichrom Merck-Hitachi, Germany). The parameters included were a C18 column (250 x 4 mm id, particle size 5 µm Luna 5µ C-18 (Phenomenex, USA) at room temperature, injection volume of 5 µl, mobile phase (80:20 HPLC grade methanol: 0.4% acetic acid v/v), flow rate of 1 ml/min, run time of 5 min and UV detection at 290 nm. The retention time of oxalic acid under the above conditions should be approximately 1.4 min. The mean absorbance units obtained with the standards were used to plot a standard curve. Oxalic acid was extracted from 0.5 g of dried leafy material using 4 ml of 0.025 M HCL. The extract was centrifuged at 10 000 rpm for 20 minutes at a temperature of 25°C. The supernatant was collected in 1 ml centrifuge tubes and passed through the Phenomenex C18 solid-phase extraction cartridge (Phenomenex, USA). The concentrations of oxalic acid in plant extracts were then calculated from the standard curve using the formula $y = mx + c$.

3.3.5. Quantification of Cyanogenic Glycoside

Cyanogenic glycoside was determined using the alkaline picrate method of (Onwuka, 2005) with minor modifications. 2.5 grams of sample was dissolved in 25 cm^3 distilled water. The cyanide extraction was left to stand overnight and then filtered (Inuwa et al., 2011). In order to prepare the cyanide standard curve, various concentrations of KCN solution containing 0.1–1 mg/ml cyanide were prepared (Appendix 4). 4 ml of alkaline picrate solution (1 g of picrate and 5 g of Na_2CO_3 in 200 cm^3 distilled water) was added to 1 ml of the sample filtrate and standard cyanide solution in test tubes and incubated in a water bath for 15 minutes. After colour was developed, the absorbance was read at 490 nm on a spectrophotometer with a blank consisting of 1 ml distilled water and 4 cm^3 alkaline picrate solution.

The cyanide content was extrapolated from the cyanide standard curve (Appendix 4). Calculation:

$$\text{Cyanogenic glycoside (mg/100 g)} = \frac{\text{C (mg)} \times 10}{\text{Weight of sample}}$$

C (mg) = concentration of cyanide content read off the graph.

3.4. Processing of selected traditional South African leafy vegetables

Ground plant material was boiled according to the cooking methods employed by (Shimelis and Rakshit, 2007) with slight modifications by (Mosha and Gaga, 1999) , using a seed-to-distilled water ratio of 1:4 (w/v) at 97°C for 0, 5 and 15 minutes. The cooking water was drained off and left to air dry for 24 hours. All cooking parameters were done in triplicate.

3.5. Statistical Analysis

All determinations were carried out in triplicate. Differences were evaluated by two-way analysis of variance, ANOVA (Graph Pad Prism), followed by Tukey test for multiple comparisons. Values are expressed as a mean±standard deviation (n=3). Significance was accepted as $P<0.05$.

4. RESULTS

4.1. Tannins

The tannin concentration was determined spectrophotometrically in thirteen species as shown in Figure 18. These values were obtained from 500 mg dried leafy material. All 13 species contained tannins in this study with *S. nigrum* having the highest tannin content (0.14 mg/ml). Tannin content at 0 minute processing ranged from 0.01–0.14 mg/ml. The species lowest in tannin content were *A. hybridus*, *O. sinuatum*, *C. album*, *E. australis*, *G. densa* and *G. parviflora* which all contained concentrations of less than 0.08 mg/ml. The tannin content after 5 minute processing ranged from 0.01–0.09 mg/ml. The tannin content was highest in *P. viscosa* (0.09 mg/ml) and lowest in *G. densa* (0.01 mg/ml). Tannin content after 15 minute processing ranged from 0.01–0.07 mg/ml. The tannin content was highest in *S. nigrum* (0.07 mg/ml). There was a significant difference in tannin content between the 0 and 5 minute processing as observed with all the leafy vegetables except *P. viscosa, A. hybridus, O. sinuatum* and *C. album* (Table 3). There was no significant change in the tannin content between 5 and 15 minute processing in all 13 species. The tannin content was changed significantly between 0 and 15 minute boiling in all 13 species except *A. hybridus, O. sinuatum* and *C. album*. *A. hybridus, O. sinuatum* and *C. album* had no significant effect in the reduction of tannin content between both boiling parameters. Therefore, a longer time period would be required in order to reduce the tannin content of the above species. *P. viscosa* had a significant effect in the decrease of tannin content only between 0 and 15 minute boiling which indicates that a minimum of 15 minute boiling was required in order to reduce the tannin content in *P. viscosa*. *C. triloba* had no significant effect in tannin content decrease between 5 and 15 minute boiling meaning that a minimum of 5 minute boiling was adequate to reduce the tannin content in *C. triloba* as can be seen Table 3.

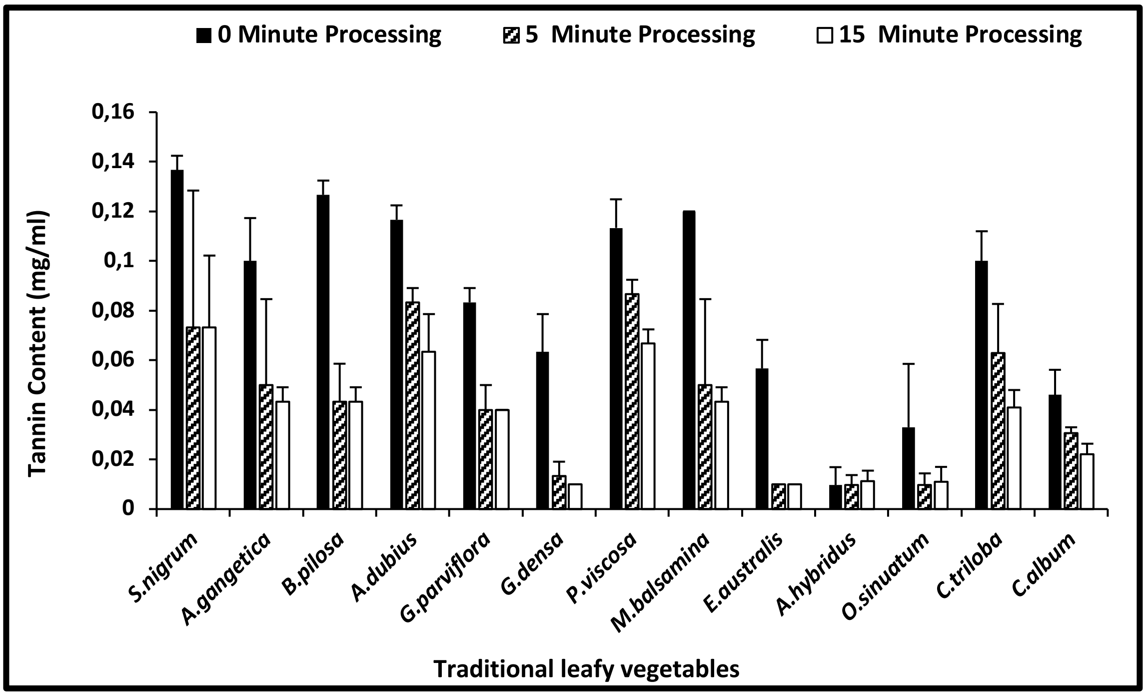

Figure 18: Effect of boiling on Tannin concentration in 13 traditional leafy vegetables at 0, 5 and 15 minute processing [Bars denote mean ± standard deviation (n=3)].

Table 3: Statistical analysis of boiling parameters on Tannin content in 13 traditional leafy vegetables.

Tukey's Multiple Comparison Test			
Plant Species	**0 and 5**	**5 and 15**	**0 and 15**
Solanum nigrum	****	ns	****
Asystasia gangetica	***	ns	****
Bidens pilosa	****	ns	****
Amaranthus dubius	*	ns	***
Galinsoga parviflora	**	ns	**
Guilleminea densa	***	ns	***
Physalis viscosa	ns	ns	**
Momordica balsamina	****	ns	****
Emex australis	**	ns	**
Amaranthus hybridus	ns	ns	ns
Oxygonum sinuatum	ns	ns	ns
Ceratotheca triloba	*	ns	****
Chenopodium album	ns	ns	ns

Ns=not significant, * $p<0.05$, ** p 0.0031, **** $p<0.0001$

4.2. Phytic acid

The concentration of phytic acid was determined spectrophotometrically in 13 species as shown in Figure 19. The concentration of phytic acid was obtained from 5 grams of dried plant material at 0 minute, 5 and 15 minute boiling. All 13 species contained low detections of phytic acid in this study. The phytic acid content at 0 minute processing varied between 0 mg/ml to 0, 06 mg/ml. Out of the 13 traditional leafy vegetables, phytic acid content was highest in C. *triloba* (0.06 mg/ml) and lowest in *A. dubius* (0.002 mg/ml). The phytic acid content after 5 minute processing ranged from 0–0.02 mg/ml. Phytic acid content was highest in O. *sinuatum* (0.02 mg/ml) and completely removed in six of the traditional leafy vegetables. The phytic acid content after 15 minute processing ranged from 0–0.02 mg/ml. C. *triloba and A. hybridus* both contained the highest concentrations of phytic acid after 15 minute boiling at 0.01 mg/ml. A total time of five minute boiling was adequate to eliminate the phytic acid content in *S. nigrum, M. balsamina, G. densa, G. parviflora, E. australis* and *A. dubius* whereas *P. viscosa* and *A. gangetica* required a total of fifteen minutes boiling to completely eliminate the phytic acid content. There was no significant difference in the phytic acid between 5 and 15 minute boiling in all the leafy vegetables except for *A. hybrid*us. Due to the initial phytic acid concentration being minimal, there was no significant effect in the decrease of phytic acid between 0–5.5 and 15 or 0 and 15 minute boiling in 12 species. Only *A. hybridus* attained a significant effect in the decrease of phytic acid content after 15 minute boiling as can be seen in Table 4.

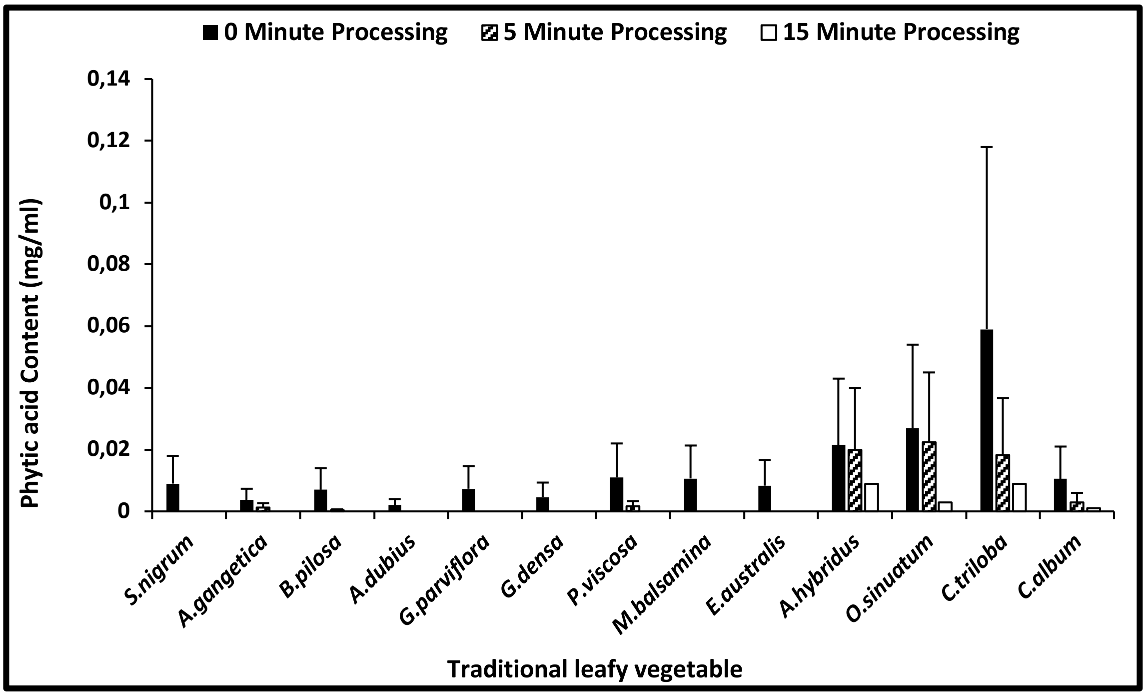

Figure 19: Effect of boiling on phytic acid concentration in 13 traditional leafy vegetables at 0, 5 and 15 minute processing. [bars denote mean ± standard deviation (n=3)].

Table 4: Statistical analysis of boiling parameters on phytic acid content in 13 traditional leafy vegetables

Tukey's Multiple Comparison test			
Plant Species	**0 and 5**	**5 and 15**	**0 and 15**
Solanum nigrum	ns	ns	ns
Asystasia gangetica	ns	ns	ns
Bidens pilosa	ns	ns	ns
Amaranthus dubius	ns	ns	ns
Galinsoga parviflora	ns	ns	ns
Guilleminea densa	ns	ns	ns
Physalis viscosa	ns	ns	ns
Momordica balsamina	ns	ns	ns
Emex australis	ns	ns	ns
Amaranthus hybridus	ns	*	ns
Oxygonum sinuatum	ns	ns	ns
Ceratotheca triloba	ns	ns	ns
Chenopodium album	ns	ns	ns

ns = not significant, * p0.0003, ****=p<0.0001**

4.3. Alkaloid

Alkaloid precipitates were obtained using 5 grams of dried plant material at 0 minute, 5 and 15 minute boiling. Alkaloid content measured in the 13 species are shown Figure 20. All 13 species contained alkaloids. At 0 minute processing, the alkaloid content in the 13 species ranged from 4 to 11%. Out of the 13 species, alkaloid content was highest in *M. balsamina* (11%) and lowest in *O. sinuatum* (3.62%). The alkaloid content after 5 minute processing ranged from 2, 5% to 10%. The highest alkaloid content was in *M. balsamina* (10%) and lowest in *Emex australis* (2, 5 %). The alkaloid content after 15 minute processing ranged from 1, 7 to 5, 6%. The highest alkaloid content was *C. triloba* (5.6%) and the lowest *B. pilosa* (1.7%). There was no significant difference between 0 and 5 minute boiling as well as 5 and 15 minute boiling for the following plants; *A.dubius, G. densa, A. hybridus, C. triloba and C. album,* indicating a time period of longer than 15 minute boiling in order to reduce their alkaloid contents as well. There was no significant difference between 0 and 15 minute boiling in *B. pilosa* indicating that *B. pilosa* would require a time period of longer than 15 minute boiling in order to reduce its alkaloid content. The following plants had no significant difference between 5 and 15 minute boiling namely *S. nigrum* and *A. gangetica,* therefore 5 minute boiling was adequate to reduce the alkaloid content in the above mentioned species. There was no significant difference between all boiling parameters for *O. sinuatum*, indicating a time period of longer than 15 minute boiling in order to significantly reduce its alkaloid content. *G. parviflora* had a significant effect in reduction of alkaloid content with all boiling parameters and therefore responded the best to the boiling parameters as can be seen in Table 5.

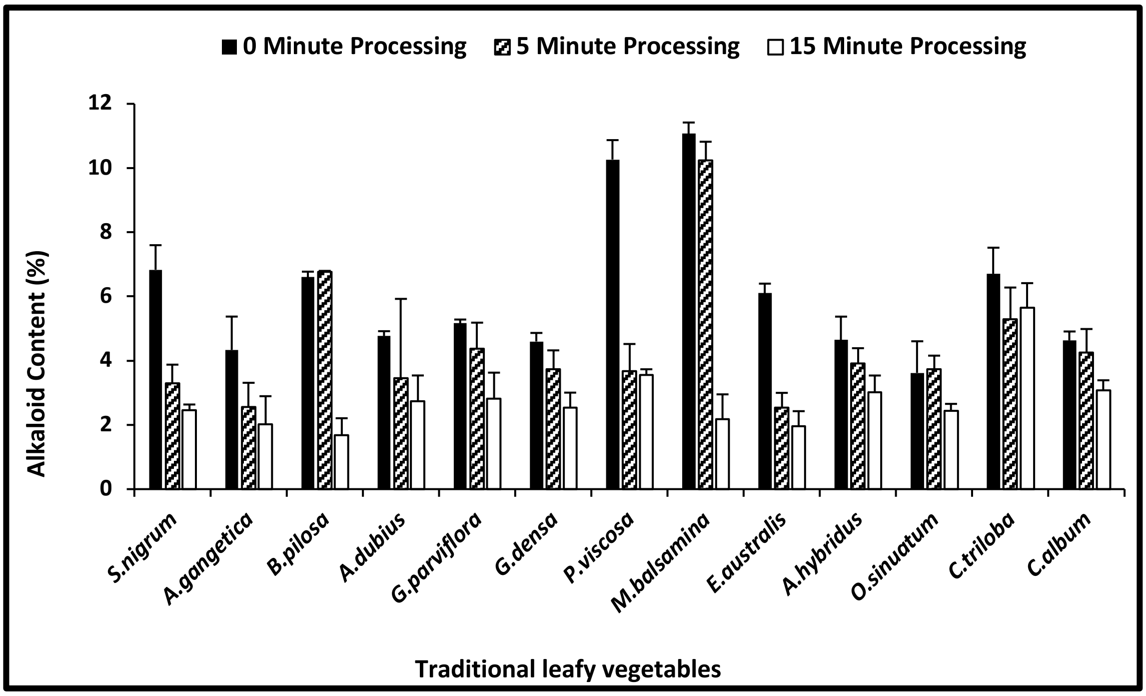

Figure 20: Effect of boiling on alkaloid percentage in 13 traditional leafy vegetables at 0, 5 and 15 minute processing. [bars denote mean ± standard deviation (n=3)].

Table 5: Statistical analysis of boiling parameters on alkaloid content in 13 traditional leafy vegetables

Tukey's Multiple Comparison test			
Plant Species	**0 and 5**	**5 and 15**	**0 and 15**
Solanum nigrum	****	ns	****
Asystasia gangetica	**	ns	***
Bidens pilosa	ns	****	****
Amaranthus dubius	ns	ns	*
Galinsoga parviflora	*	*	****
Guilleminea densa	ns	ns	**
Physalis viscosa	****	ns	****
Momordica balsamina	ns	****	****
Emex australis	****	ns	****
Amaranthus hybridus	ns	ns	*
Oxygonum sinuatum	ns	ns	Ns
Ceratotheca triloba	ns	ns	*
Chenopodium album	ns	ns	*

Ns = not significant, ****=p<0.0001

4.4. Oxalic acid

Oxalic acid was quantified using High Pressure Liquid Chromatography (HPLC). The concentrations of oxalic acid were obtained from 0.5 grams dried plant material at 0 minute, 5 and 15 minute boiling. The oxalic acid contents of the leaves boiled at 0 minutes, 5 and 15 minutes are shown in Figure 21. The oxalic acid content at 0 minute boiling ranged from 85–1079 mg/ml. All 13 species contained oxalic acid with the highest oxalic acid content in *C. triloba* (1079 mg/ml) and the lowest *G. parviflora* (85 mg/ml). The oxalic acid content after 5 minute processing ranged from 33 mg/ml to 651 mg/ml. *C. triloba* contained the highest concentration oxalic acid content after 5 minute boiling (651 mg/ml) and the lowest was *G. densa* (33 mg/ml). The decrease in oxalic acid content after 15 minutes processing ranged from 38 mg/ml to 487 mg/ml. *A. hybridus* contained the highest concentration of oxalic acid after 15 minutes boiling (487 mg/ml) and the lowest concentration was *G. densa* (38 mg/ml). The following species were high in oxalic acid content and had no significant effect in the decrease of oxalic acid content with all boiling parameters namely *A. gangetica, B. pilosa, G. parviflora, G. densa, P. viscosa, M. balsamina* and *C. album*. *S. nigrum, E. australis* and *A. hybridus* all required only 5 minute boiling to reduce their oxalic acid content significantly. *O. sinuatum* and *C. triloba* had a significant effect in the decrease of oxalic acid content in all boiling parameters and responded the best to the processing parameters as can be seen in

Table 6. *C. triloba*, *O. sinuatum*, *S. nigrum* and *E. australis* had a significant difference in the decrease of oxalic acid content after a mere 5 minutes of boiling ($p<0.0001$). However both Amaranthus species required a total of 15 minute boiling to attain a significant difference in the reduction of oxalic acid content. The oxalic acid contents were significantly lower in boiled leaves than fresh leaves. The decrease was highest in the leaves boiled at fifteen minutes.

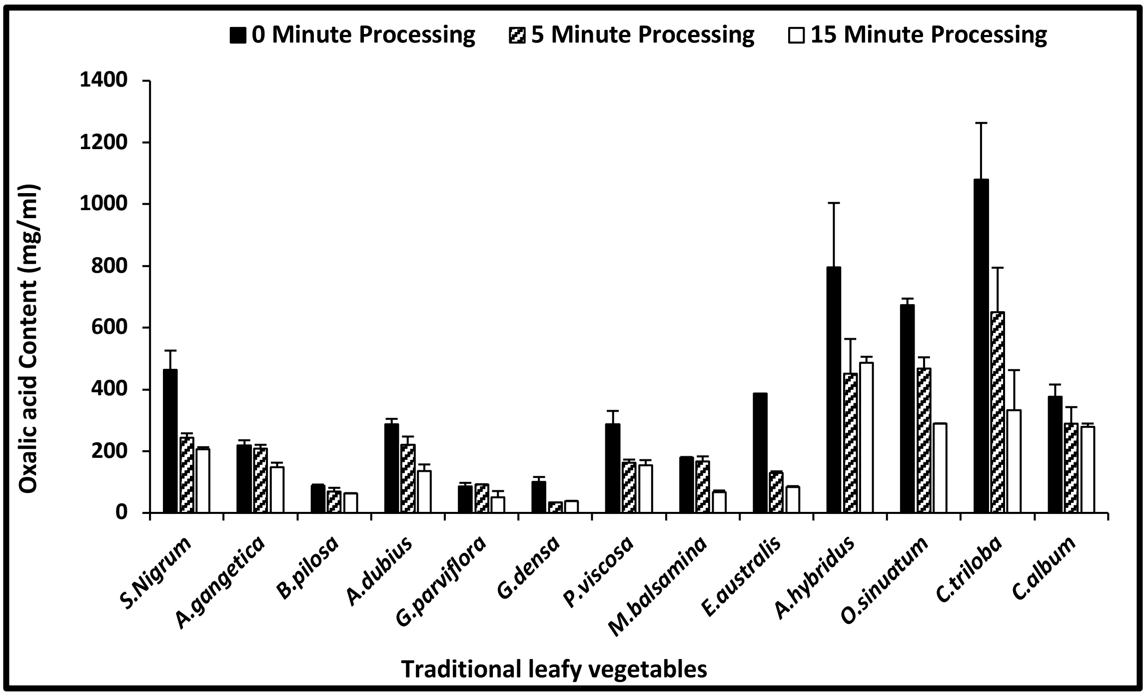

Figure 21: Effect of boiling on oxalic acid concentration in 13 traditional leafy vegetables at 0, 5 and 15 minute processing. [bars denote mean ± standard deviation (n=3)].

Table 6: Statistical analysis of boiling parameters on oxalic acid content in 13 traditional leafy vegetables

Tukey's Multiple Comparison Test			
Plant Species	**0 and 5**	**5 and 15**	**0 and 15**
Solanum nigrum	**	ns	***
Asystasia gangetica	ns	ns	ns
Bidens pilosa	ns	ns	ns
Amaranthus dubius	ns	ns	*
Galinsoga parviflora	ns	ns	ns
Guilleminea densa	ns	ns	ns
Physalis viscosa	ns	ns	ns
Momordica balsamina	ns	ns	ns
Emex australis	***	ns	****
Amaranthus hybridus	****	ns	****
Oxygonum sinuatum	*	****	**
Ceratotheca triloba	****	****	****
Chenopodium album	ns	ns	ns

Ns = not significant, ****=p<0.0001

4.5. Cyanogenic glycoside

Cyanogenic glycoside content was quantified spectrophotometrically in 13 species. The concentration of cyanogenic glycoside was obtained using 2.5 grams dried plant material at 0 minute, 5 and 15 minute boiling as shown in Figure 22. All 13 species contained cyanogenic glycoside at 0 minute processing in this study. Cyanogenic glycoside content at 0 minute processing ranged from 17–33 mg/100g with *A. gangetica* attaining the highest cyanogenic glycoside contents at 0 minute processing (33 mg/100g) and *E. australis* the lowest (17mg/100g). The decrease in cyanogenic glycoside after 5 minute processing ranged from 8–31 mg/100g. *M. balsamina* contained the highest concentration of cyanogenic glycoside after 5 minute boiling (31 mg/100g) and C. *album* the lowest (8 mg/100g). *A. hybridus* had the greatest decrease after 5 minute boiling with a concentration of 29.5–10.5 mg/100g. There was no significant difference in the change between 0 and 5 minute boiling in *P. viscosa*, M. *balsamina* and *E. australis* indicating that these three plant required a longer boiling period than the others. The decrease in cyanogenic glycoside content after 15 minute processing ranged from 30–3 mg/100g. C. *triloba* contained the highest concentrations of cyanogenic glycoside after 15 minute boiling (30 mg/100g) and *A. hybridus* the lowest (4 mg/100g). *M. balsamina* and *A. hybridus* were the only two plants that had a significant difference between 5 and 15 minute boiling. *P. viscosa* and *E. australis* had no significant effect in the decrease of cyanogenic glycoside content between both boiling parameters which indicates that a longer time period than 15 minute boiling was required in order to reduce the cyanogenic glycoside content (Table 7).

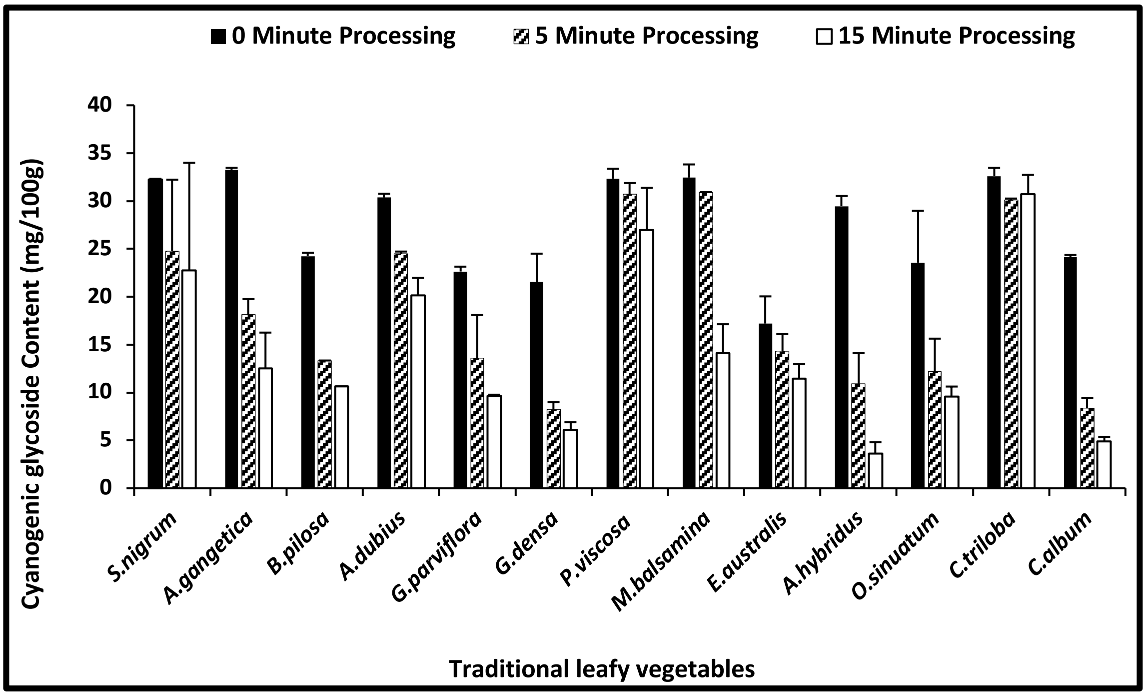

Figure 22: Effect of boiling on cyanogenic glycoside content in 13 traditional leafy vegetables at 0, 5 and 15 minute processing. [bars denote mean ± standard deviation (n=3)].

Table 7: Statistical analysis of boiling parameters on cyanogenic glycoside content in 13 traditional leafy vegetables

Tukey's Multiple Comparison test			
Plant Species	**0 and 5**	**5 and 15**	**0 and 15**
Solanum nigrum	*	ns	**
Asystasia gangetica	****	ns	****
Bidens pilosa	****	ns	**
Amaranthus dubius	**	ns	ns
Galinsoga parviflora	**	ns	***
Guilleminea densa	****	ns	****
Physalis viscosa	ns	ns	ns
Momordica balsamina	ns	****	****
Emex australis	ns	ns	ns
Amaranthus hybridus	****	*	****
Oxygonum sinuatum	***	ns	****
Ceratotheca triloba	ns	ns	ns
Chenopodium album	****	ns	****

ns = not significant, ****=p<0.0001

5. DISCUSSION

The main aim in consumption of famine foods such as traditional leafy vegetables are their great source of nutrition and excellent medicinal properties. One approach to maintaining their benefit is to maintain a low level of anti-nutrients through common forms of processing. Processing causes changes in the phytochemistry of the traditional leafy vegetable affecting its bioaccessability and health benefit properties. The degree of these changes depends largely on the processing methods as well as the traditional leafy vegetable (Hutchings et al., 1996).

The current study focused on the anti-nutritional content of 13 species to evaluate the effect of processing on them. To determine if the anti-nutritional content of the 13 species tested could effectively be decreased, they were subjected to three processing parameters. Thus far there has been little work regarding phytochemical processing of these specific species so that the nutritive values of foods could be efficiently maximized. However, previous studies have shown anti-nutrients are capable of being reduced in leafy vegetables after 5, 10 and 15 minute heat processing (Yadav and Sehgal, 2003).

Despite the long history of the consumption of traditional leaf vegetables by the sub-Saharan population, the potential of traditional leafy vegetables has not been fully investigated, therefore there is an urgent need for scientific investigation and documentation regarding the phytochemical values. Many workers such as (Odhav et al., 2007) have reported the nutritional value of some of these traditional leafy vegetables, however the anti-nutritional value remains unexplored. All thirteen species studied contained anti-nutrients namely tannins, phytic acid, alkaloids, oxalic acid and cyanogenic glycoside.

5.1. Tannins

Tannins are polyphenolic compounds that form complexes with proteins, starches and digestive enzymes consequently reducing the nutritional value of the food consumed. They also affect protein absorption and reduce the availability of iron (Saxena et al., 2013). The recommended dosage allowed for tannins in humans is 0–0.3 mg/kg body weight (FAO/WHO, 1970). This indicates that all the species except *A. hybridus* could be potential harm to the consumer if consumed prior to processing. *A. hybridus* had little change with the processing parameters but was detected as the lowest in tannin content. According to similar work done by (Akubugwo et al., 2007), steam processing with oven drying resulted in the greatest reduction of the tannin content of *A. hybridus*. High tannin content can lead to damage to intestinal mucosa, have carcinogenic effects as well as lead to liver and kidney toxicity (Akwaowo et al., 2000). The tannin content of the species in this study was highest in *S. nigrum* and varied widely amongst the other species. This corresponds to another study which had similar results on *Clerodendrum volubile* (Erukainure et al., 2011) as well as other work on *Ipomoea batatas* (sweet potato leaves) (Antia et al., 2006). However, other studies were much higher and found that high levels of tannin in raw and cooked leaves had a negative correlation on calcium bioavailability (Amalraj and Pius, 2015). The difference in tannin content could be attributed to different varieties or agro-climatic conditions as the previous work was done on plants in India. The differences could also be attributed to environmental conditions, soil, seasonal variations, state of maturity of the plant, different methods of sampling and analysis, preparation or cooking practices, or genetic factors (Somsub et al., 2008). The observations of a decrease in tannin content through processing in this study are in agreement with other works by (Singh et al., 2015) where the tannin content was decreased through boiling in *Eryngium foetidum* by 3.6% and *Enhydra flactuans* by 83.8%. This could be due to loss of hydrolysable tannin into the surrounding leachate as depicted by (Somsub et al., 2008). The reduction in tannin content through processing in this study are also in agreement with other works by (Zhang and Hamauzu, 2004) and by (Nworgu et al., 2007) where the reduction of tannin content of *Telfaria occidentalis* with soaking was reported. Tannin content between raw and cooked vegetables were found to have decreased after cooking according to work by (Somsub et al., 2008). This suggests that processing is an effective way to reduce tannins in traditional leafy vegetables. Furthermore, these results prove that tannin content may be decreased through processing treatments of 5 to 15 minutes.

5.2. Phytic acid

Phytic acid is a hexaphosphate of inositol and binds calcium and iron rendering these unavailable (Gupta et al., 2005). Phytic acid has been recorded to prevent digestion, inhibit the uptake of calcium and can cause problems such as metabolic bone disease, renal failure, bladder stones as well as decreases iron absorption (Akwaowo et al., 2000). A phytic acid intake of 4-9mg/100g is said to decrease iron absorption by 4–5 fold in humans (Hurrel et al., 1992).

According to the amount of plant food in the diet as well as the processing method, the daily intake of phytic acid is allowed to be 4500 mg (Reddy, 2002). The daily intake of phytic acid, however, for rural areas in developing countries is 150–4000 mg (Reddy et al., 1982). The results from the phytic acid content of the 13 species were all well within the daily intake and therefore posed no threat upon consumption. All the species were found to have low phytic acid concentrations. The phytic acid concentration was the highest in C. *triloba* at 0 minute processing. There is not much work in literature regarding the relationship between phytic acid and these specific species but the results were consistent with work done by (Gupta et al., 2005) on other leafy vegetables. Phytic acid was reported much lower than work done by (Akwaowo et al., 2000) on leafy vegetables. According to similar work done by (Yadav and Sehgal, 2003) cooking did not change the phytic acid content of leafy vegetables.

The reason being was that during cooking endogenous phytases were inactivated by the heat and therefore need to be broken down with higher temperatures (Amalraj and Pius, 2015). The interaction of the leaves with the hot water causes the cell wall to be ruptured and soluble phytic acid may leach into the medium which can account for phytic acid losses (Yadav and Sehgal, 2003). Phytic acid content between raw and cooked vegetables were found to have decreased after cooking according to work done by (Somsub et al., 2008). The overall reduction and subsequent elimination of phytic acid in most species with processing and a time period of a mere 5 minutes proves processing treatments along with their time are an adequate form of phytic acid removal. This has also been proved by other work in which a further reduction of phytic acid was observed when the germinated seeds of Lupin were boiled rather than other processing parameters (Mohammed et al., 2017). According to similar work done by (Akubugwo et al., 2007), steam processing with oven drying resulted in the greatest reduction of the phytic acid content of *A. hybridus*. According to the results obtained, it proved that longer processing time was effective in completely eliminating the phytic acid content of the species.

5.3. Alkaloid

Alkaloids are a class of naturally occurring organic nitrogen containing bases which are responsible for a bitterness in some plants (Sood et al., 2012). High levels of alkaloid content in food is a carcinogen and also leads to kidney inflammation, birth defects as well as reduced iron uptake (Akwaowo et al., 2000). The fatal dose of alkaloids in humans have been reported as 50 mg/kg (Van Wyk et al., 2002). Alkaloids cause gastrointestinal and neurological disorders. The glycoalkaloids, solanine and chaconine are recorded to be present in potato and the Solanum species and are toxic to humans (Soetan, 2009). Work done by (Uusiku et al., 2010) tested positive for alkaloid content in the Amaranthus species. This is in correlation with the present results which indicate that the Amaranthus species contain alkaloid content at 0 minute processing. The high alkaloid content in the Amaranthus species is in agreement with previous literature reports that state that plant leaves contain a substantial amount of alkaloids (Adeniyi et al., 2009). The percentage of alkaloid content in the 13 species at 0 minute processing was much higher than work done by (Erukainure et al., 2011) on *Clerodendrum volubile*, a tropical non-conventional vegetable with an alkaloid content of 0,79%.

The results from the alkaloid screening of the 13 species showed the highest alkaloid content to be that of *M. balsamina*. Amongst the highest alkaloid containing species, were *S. nigrum* and *P. viscosa*. This is consistent with work done by (Van Wyk et al., 2002) that states tropane alkaloids occur in the Solanaceae family. However, the results of the current work are slightly lower than work reported by (Adedapo et al., 2011), the discrepancy could have been due to different varietal and agro-climatic conditions of the species. These results are similar to work done by (Akubugwo et al., 2007) on *S. nigrum* which proved that processing treatments such as steaming with sun drying produced the greatest reductions in the amounts of alkaloids. The alkaloid levels were significantly decreased with processing and a time period of 5 minutes was adequate but the alkaloid content was further reduced after 15 minute processing. The results obtained proved that processing was effective in reducing alkaloid content of the species. It is important to note that the following species increased in alkaloid content after 5 minute processing but then decreased after 15 minutes, these were *B. pilosa* and *O. sinuatum*. The slight increase, although not significant, could be explained by work done by (Mohammed et al., 2017), which states that Lupin seeds increased in alkaloid content when soaked but then decreased with long boiling as these compounds are water soluble and their contact to the soaking and boiling water accelerate their removal from the seed.

However, the removal of such compounds depends on the type and temperature of soaking solution as well as the permeability of the cell wall. His results also showed that alkaloid content was most effectively removed through boiling processes. According to the results obtained it proved that a longer processing time was effective in reducing the alkaloid content of the species.

5.4. Oxalic acid

Oxalic acid is widely found in plant foods and is known to interfere with the absorption of calcium by forming insoluble salts of calcium (Amalraj and Pius, 2015). High oxalic acid levels have been recorded to result in kidney stones in humans (Akwaowo et al., 2000). Furthermore, oxalic acid causes gastrointestinal tract irritation, blockage of the renal tubes by calcium oxalate crystals, development of urinary calculi and hypocalcaemia (Soetan, 2009). According to (Akwaowo et al., 2000), a daily intake of 450 mg of oxalic acid has been reported to affect metabolism. The results of this study have depicted that *S. nigrum*, *A. hybridus*, *O. sinuatum* and *C. triloba* all contained oxalic acid levels above 450 mg which indicates a potential risk upon consumption. These species were only reduced to less than 450 mg after 15 minute processing. Oxalic acid toxicity levels have been estimated at 2–5 g/100g. These high levels of oxalic acid may reduce the bioavailability of metals like calcium. The exceptionally high values of oxalic acid in the 13 species at 0 minute processing in *C. triloba* correlated to work done by (Antia et al., 2006) on *Ipomoea batatas* (sweet potato leaves). This can be poisonous to humans however, it has been recorded that total oxalic acid content is reduced in leaves and vegetables through proper cooking before consumption (Akwaowo et al., 2000). This is also in accordance with work done by (Yadav and Sehgal, 2003) who reported losses of oxalic acid upon cooking and boiling. Oxalic acid content was also high in the Amaranthus species according to work done by (Gupta et al., 2005). The results obtained for the determinations of oxalic acid in Amaranthus species were also on par with work done by (Adeniyi et al., 2009). The results on *C. album* are similar to work done by (Sood et al., 2012) in which the oxalic acid content of *C. album* cultivars were between 360–2000 mg/100g. Similar work on blanched leaves was performed at 5 minutes, 10 and 15 minutes on the Amaranthus species and spinach which also correlated with the current work and showed a decrease in oxalic acid upon processing due to the plant cell walls being ruptured and soluble oxalate being leached into the cooking medium (Yadav and Sehgal, 2003).

These levels were significantly decreased with a processing time of 5 minutes and further reduced after 15 minute processing. The results obtained proved that a longer processing time was effective in reducing oxalic acid content of the species.

5.5. Cyanogenic glycoside

Cyanogenic glycosides are amino acid derived plant constituents. Hydrogen cyanide is a product of the hydrolysis in plants and is poisonous upon consumption. There has been reports of cyanide that have led to the disease "Kwashiorkor" in the African population that consume *M.esculenta* (cassava) as a dietary staple (Vetter, 2000). Cyanide ions inhibit some enzyme systems, decrease growth through interfering with certain essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Soetan, 2009). The toxic level of cyanide reported in foods is 35 g/100g (Akwaowo et al., 2000). The lethal dose of cyanide is 0.5–3 mg/kg body weight. The species contained less than the toxic levels before and after processing. Results on *A. hybridus* were slightly higher than work done by (Akubugwo et al., 2007). Similar work done by (Antia et al., 2006) on sweet potato leaves revealed a high content of cyanogenic glycoside of 30 mg/100g which correlates to the high level of cyanogenic glycoside of 33 mg/100g in *M. balsamina*. *A. gangetica* was the highest in cyanogenic glycoside content along with *M. balsamina*, *C. triloba* and *S. nigrum*. These levels were decreased with a processing time of 5 minutes and the results obtained proved that they were capable of being further reduced after 15 minute processing. A decrease in cyanogenic glycoside content with processing can be proved with similar work. According to reports by (Akubugwo et al., 2007), it was proved that processing treatments such as steaming with sun drying had the greatest reduction in the cyanide content of *S. nigrum*. The results are in accordance with work done by (Sridhar, 2006) on leguminous seeds in which it was proved that cyanide was reduced tremendously by processing methods such as boiling and soaking. Processing techniques, by means of peeling, drying, grinding, soaking, boiling or cooking, soaking and fermentation have been reported by (Bolarinwa et al., 2016) to cause a significant reduction in the cyanogenic glycoside content of processed foods. These processes have been applied to food crops such as roots, tubers, cereals and leaves, to cause significant reduction in the cyanogenic glycoside contents of the crops. Processing methods generally disintegrate cyanogenic glycoside contents of plants, which allows for the production of cyanide.

As cyanide is volatile, additional processing techniques, like roasting and drying, will volatilize the remaining cyanide to low levels. The results obtained, prove that a longer processing time was effective in reducing cyanogenic glycoside content of the species.

6. CONCLUSION

The 13 species studied are an important source of nutrients amongst the sub-Saharan population and were found to contain substantial levels of tannins, phytic acid, alkaloid, cyanogenic glycoside and oxalic acid content without any processing techniques being applied. They are therefore nutritionally relevant. Processing methods produced significant effects on the anti-nutritional content. The effect of 5 minutes of processing time using the five techniques outlined in the objectives was adequate to reduce and in some species remove the anti-nutrient content completely however some species required a longer time period of 15 minutes. It is important to note that any longer may be at the cost of important nutrient loss. The results of this study provide evidence that these local traditional vegetables, could be important contributors in the high level of micronutrient malnutrition among the vulnerable population of developing countries and therefore be increasing the prevalence of chronic degenerative disease amongst the sub-Saharan population and can be eliminated through common processing methods aiding the problem of food insecurity such as micro-nutrient malnutrition problems faced in sub-Saharan Africa. Traditional leafy vegetables benefit sub-Saharan Africa by improving its nutritional status in vulnerable groups and therefore calls for the need of more in depth anti-nutrient studies to ensure maximum nutritional benefit of these leafy vegetables. The need for the exploration of new plants is a work in progress with more than 1700 new plants being discovered in the past year, which includes species that could help provide food in the future. Many crops have lost their genetic diversity as well as resilience due to having being bred for high yields. The discovery of new plants to foods is of great importance because they have survived thousands of years and various climatic conditions and have the genes of resilience to droughts and pests. There is a need to take these genes and breed them back into crops for the future. Approximately 28 187 species have been known to have medicinal uses, with only a mere 16% of that is actually recorded in medicinal regulatory publications and many species have different names, which causes confusion and risk. Many people do not see plants as an important thing and there needs to be more work done on plant profiling for their significance to be understood in terms of yielding new crops or drugs.

Traditional leafy vegetables may not be high in yields but have certainly survived thousands of years in many climate conditions and have the genes of resilience. Many species are recorded but there is a lack of information on their nutritional and anti-nutritional levels documented. The need for exploration of anti-nutritional information in traditional leafy vegetables is significant in overcoming nutritive disorders to contribute in health and food security in sub-Saharan Africa. It is evident that micronutrient deficiency cannot be obtained by focusing on 5 anti-nutritional factors in 13 species. Therefore, a recommendation for further studies on more anti-nutritional factors is necessary as well as the effect processing plays on the nutritional content of different traditional leafy vegetables.

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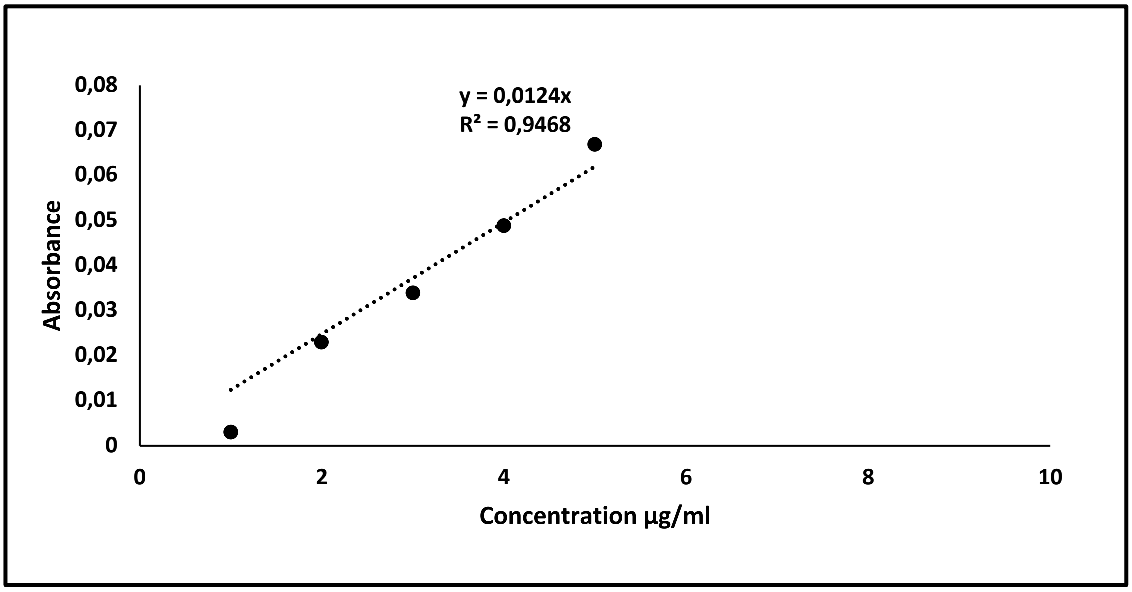
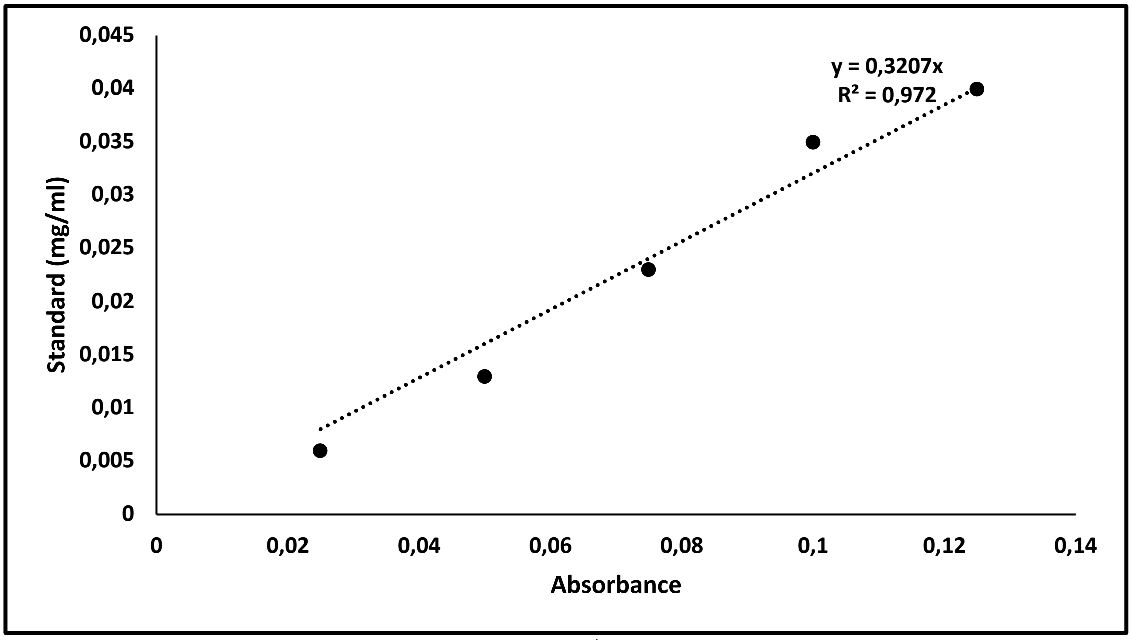
8. APPENDICES

Appendix 1: Tannic Acid standard curve



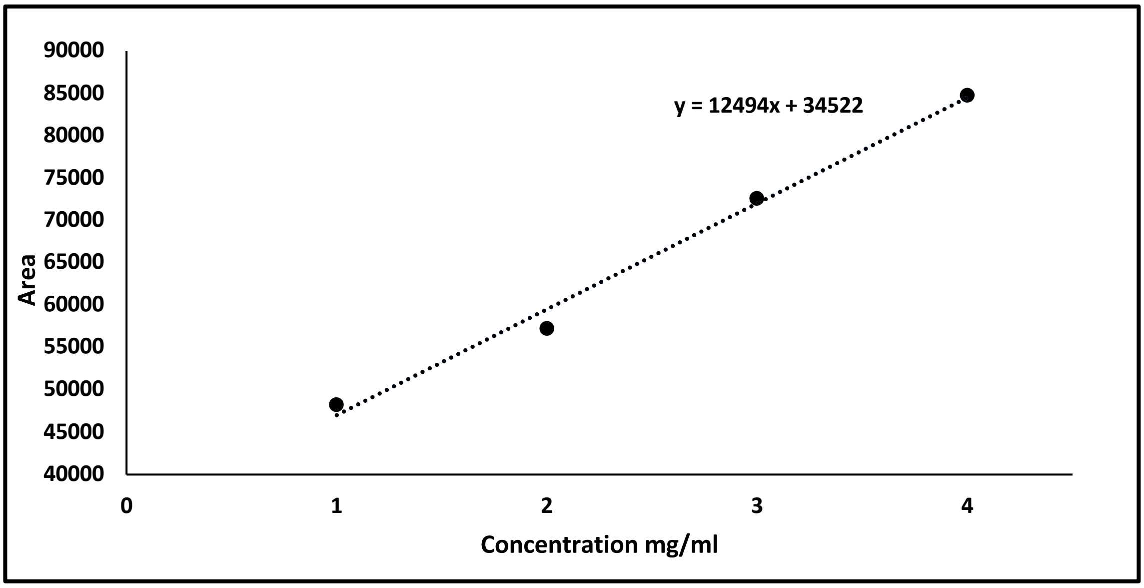
(Standards range from 10 –50 µg/ml). Each point represents mean (n=3).

Appendix 2: Phytic Acid standard curve



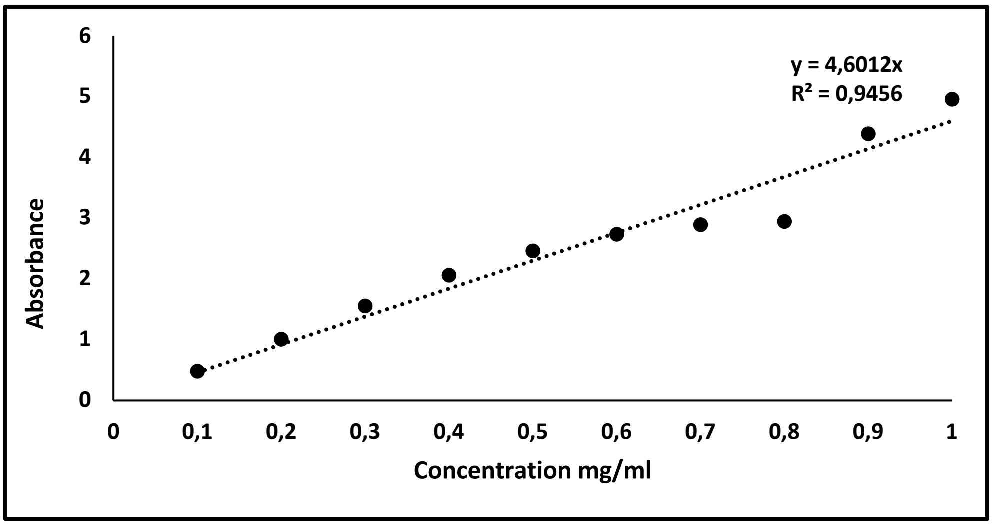
(Fe(II) Standards range from 0.025–2 mg/ml). Each point represents mean (n=3).

Appendix 3: Oxalic Acid standard curve



(Oxalic acid Standards range from 1–4 mg/ml). Each point represents mean (n=3).

Appendix 4: Cyanogenic Glycoside content standard curve



(Potassium Cyanide Standards range from 0.1–1.0 mg/ml). Each point represents mean (n=3).