EVALUATION OF TRADITIONAL SOUTH AFRICAN LEAFY PLANTS FOR THEIR SAFETY IN HUMAN CONSUMPTION

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Submitted in complete fulfilment for the degree of Master of Technology: Biotechnology in the department of Biotechnology, Durban University of Technology, Durban, South Africa.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Technology, to the Durban University of Technology. It has not been submitted before for any degree or examination to any other University.

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M. MUDZWIRI

May 2007

*** FINAL COPY APPROVED FOR SUBMISSION**

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Dr. L. Reddy	Date
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Prof. B. Odhav	Date

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ABSTRACT

Eighteen traditionally leafy vegetables consumed as food or medicinal compounds by a majority of people in the KwaZulu Natal province of South Africa were analysed for the presence of potentially harmful chemicals (antinutrients) and for their toxicity and mutagenicity. The purpose of the study was to determine whether leafy vegetables were safe for human consumption.

Chemical analysis showed that none of the vegetables contained cyanogenic glycosides, however all the vegetables contained oxalic acid ranging from 24.1 mg/ml to 798.2 mg/ml with *Solanum nigrum, Portulaca oleracea* and *Mormodica balsamina* showing the highest concentrations. Most of the vegetables contained negligible amounts of phytic acid and saponins, except for *Momordica balsamina* (3.01 mg/ml and 1.83 mg/ml, respectively). Fourteen of the plants contained alkaloids with *Portulaca oleracea* having the highest content (1.53 g total alkaloids/5 g leaf material). Eight of the plants were found to inhibit trypsin activity. These chemical analyses were carried out in duplicate and the mean and standard deviation were used.

The Ames test revealed that none of the leafy vegetables produced a mutagenic frequency above 1, except 10 000 µg/ml organic extract of *Senna occidentalis* (mutagenecity considered at mutagenic frequency above 2), thus none were considered mutagenic. All 18 organic extracts did not kill off more than 50% brine shrimp and were thus considered non-toxic. On the other hand the aqueous extracts of seven vegetables, namely, *Physalis viscosa, Amaranthus dubius, Justicia flava, Bidens pilosa, Senna occidentalis, Chenopodium album and Ceratotheca triloba,* killed more than 50% of the shrimp and are thus considered toxic above 100 µg/ml. The MTT assay carried out on the organic extracts indicated that 17 vegetables did not kill off more than 50% of HepG2 cells and were thus considered non-cytotoxic. The aqueous extracts of four vegetables, namely, *Justicia flava, Asystasia gangetica, Momordica balsamin* and *Senna occidentalis, however* killed more than 50% of the shrimp and were thus considered cytotoxic above 1 000 µg/ml.

It may be concluded from the antinutrient analyses and the bioassays on the 18 vegetables that caution needs to be maintained with the consumption of certain leafy vegetables included in this study, especially *Senna occidentalis.*

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ABBREVIATIONS

ATCC	American Type Culture Collection
CCM	Complete culture Medium
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
FCS	Foetal Calf Serum
GR	Glucocorticoid receptor
HCN	Hydrogen cyanide
HPLC	High performance liquid chromatography
MTT	3-[4,4-dimethylthiazol-2ylI-2,5-diphenytle trazolium bromide
NF Kb	Neurofactor kB
TAA	Trichloro Acetic Acid

CHAPTER ONE

INTRODUCTION

Most people in Africa depend on traditional leafy plants as part of their steady daily food intake (Tukan *et al*., 1998) because these plants are believed to be high in nutrition, are of medicinal value and are readily available. There is however little information about their potential risk to human health. Based on their long-term use by humans, one might expect plants used in traditional medicine to have low toxicity.

Plants are capable of bioconverting promutagens into toxic metabolites (Grant, 1998). Elgorashi *et al*., (2003) recently reported possible mutagenic effects of 51 South African plant species used as traditional medicine. Other studies found many 'edible' and medicinal plants to be mutagenic *in vitro* (Higashimoto *et al*., 1993; Kassie *et al*., 1998). According to statistics of the Poison Unit of the Johannesburg General Hospital, about 6.5% of all poisoning cases are plant related (Van Wyk *et al.,* 2002). Antinutritional components such as oxalic acid, nitrate and erucic acid that are present in many plants (Guil *et al*., 1996; Siddhuraju *et al*., 2002)) increase the toxicity of edible plants. These substances may express cytotoxic and genotoxic activities upon consumption and show correlation with tumour development (Yen *et al*., 2001). This raises concern about the potential hazards resulting from the long-term use of such plants.

Eighteen leafy vegetable plants commonly consumed by native African South Africans and South Africans of Indian origin were selected for this study. These plants are believed

to have high medicinal and nutritional value. The 18 plants collected from Kwa-Zulu Natal, South Africa were as follows*: Solanum retroflexum, Physalis viscosa, Momordica basalmina, Amaranthus spinosus, Amaranthus dubius, Amaranthus hybridus, Asystasia gangetica, Justicia flava, Emex australis, Oxygonum sinuatum, Bidens pilosa, Galinsoga parviflora, Portulaca oleracea, Senna occidentalis, Chenopodium album, Ceratotheca triloba, Cleome monophylla and Centella asiatica.* A recent study reported these plants as having high nutritional value, especially protein, vitamins and iron (Beekrum *et al*., 2003). A few of these plants are considered famine plants and have been reported to be toxic (Russell *et al.*, 1997). In this study the safety of the above 18 traditional leafy vegetables were evaluated for their antinutrient content, and their potential toxicity, cytotoxicity and mutagenicity.

This was achieved through the following objectives :

1. Obtaining aqueous and organic crude extracts from 18 traditional leafy vegetables from Kwa-Zulu Natal.
2. Analysing the plants for the following antinutrients: phytic acid using spectrophotometry; alkaloids using precipitation; trypsin inhibitors using enzyme-substrate reaction; oxalic acid using high performance liquid chromatography (HPLC) analysis; cyanogens using picrate paper and spectrophotometry and saponins using blood agar plates.
3. Determining the mutagenicity of the plant extracts using the Ames test.
4. Determining the toxicity of the plant extracts using the Brine shrimp assay.
5. Determining the cytotoxicity of the plant extracts using the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT) assay.

CHAPTER TWO

LITERATURE REVIEW

Plants consumed as vegetables should be free of toxicity or other adverse effects on their consumer. Common indicators of unsafe plants include their toxic, cytotoxic and mutagenic potential. These harmful properties of plants are produced by the antinutrient chemicals that are produced in certain plants as defense mechanisms.

2.1 SAFETY OF THE PLANTS

Although indigenous plants offer great medicinal value, one needs to caution their use, unless they have been tested in animals and cell culture for their toxicity, cytotoxicity, mutagenicity. Cytotoxicity is the measurement of a chemical's ability to damage or kill cells whilst toxicity is the term used to describe the capacity to cause injury to a living organism or cause any diverse effects of a chemical on a living organism. The severity of toxicity produced by any chemical is directly proportional to the concentration and time of exposure. This relationship also depends on the developmental stage of an organism and its physiological status.

It may be said that in natural foods of our everyday diet there are thousands of toxic substances, which leads to the distinction between toxicity and hazard. The toxicity of a substance is its intrinsic capacity to produce injury when tested by itself. The hazard of a substance is its capacity to produce injury under circumstances of exposure. Thus many

substances have a high intrinsic toxicity but no hazard, when associated with its natural presence in foods. In spite of the multitude of toxic substances consumed daily in a normal diet by normal healthy individuals there is yet little evident hazard involved. There are three reasons for this. Firstly, the low concentrations of the toxicant present, second, because the effect of the many toxic substances present is not cumulative and lastly because of the antagonistic effect of one toxicant upon another. However, the situation could be different if 'toxic' food were regularly eaten in excessive amounts (Fox and Norwood Young, 1982).

The toxicity of the plants may be measured using the brine shrimp assay and the cytotoxicity may be measured using tests such as, MTT (3-[5,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide), flow cytometry and luciferase ATP. It can also be tested using stains such as neutral red and trypan blue (Reddy, 2005).

An herb called berberine and berberine containing plants, although non-toxic at recommended doses has been found to be harmful when used during pregnancy and higher dosages may interfere with Vitamin B metabolism. When tested in rats, the LD_{50} was 1.000 mg/kg body weight, which indicates that the toxicity is very low (Murray, 1995). Another example is the Siberian ginseng, which helps with chronic fatigue syndrome, athlerosclerosis and impaired kidney function at recommended levels. However, it can be toxic if taken at higher doses (4.5-6 ml 3 times daily) and the side effects include symptoms such as headache, irritability (Murray, 1995).

Another concern with the use of indigenous plants is their possible mutagenic nature. Mutagenicity is the ability of a chemical or physical agent to cause permanent alteration of the genetic material within the cells (Prescott *et al.*, 1999) and an agent that causes these permanent alteration within the cell other than the normal growth is called a mutagen.

There are many types of mutations. The first type is conditional mutations that are expressed under certain environmental conditions, such as lethal mutations at high temperature. The second type is biochemical mutations which cause a change in the biochemistry of a cell. Since these mutations often inactivate the biosynthetic pathway, they frequently make a microorganism unable to grow on a medium lacking an adequate supply of the pathway end (Prescott *et al.*, 1999). An example of this is a strain of *Salmonella typhimurium* which is used in Ames mutagenicity testing (Ames *et al.,* 1973) that carries a mutant gene making it unable to synthesize amino acid histidine from the ingredients in its culture medium. However, some types of mutations (including this one) can be reversed and it is then called a back mutation, with the gene regaining its function. These revertants are able to grow on a medium lacking histidine (Ferguson *et al*, 2003).

2.2 ANTINUTRIENTS

Antinutrient are chemical compounds produced in plants as a defense mechanism that inhibits the action of digestive enzymes in insects that attack plants (Lea and Leegood, 1999). These compounds may decrease the nutritional value of a plant food, usually by making an essential nutrient unavailable or indigestible when consumed by humans or

animals. A common strategy of plants is to produce foul-tasting compounds such as many bitter nitrogen containing alkaloids, glucosinolates and cyanogenic glucosides. Some of the common groups of antinutrients are discussed below.

2.2.1 Phytic Acid

Phytic acid is a natural organic plant compound (Harland and Oberleas, 1986) that is a simple ringed carbohydrate with six phosphate groups attached to each carbon. It is a major phosphate storage compound in plants and can account for 80% of total phosphorus (Lopez *et al*., 2002). It reacts with a coloured complex e.g., iron (Fe^{3+})-sulphosalicylate (Latta and Eskin, 1980) to form a colourless $Fe3^{+}$-phytate complex.

Phytic acid has long been considered as an antinutrient because of its strong ability to complex multi-charged metal ions, specially zinc (Zn^{2+}), calcium (Ca^{2+}) and iron (Fe^{2+}) (Harland and Oberleas, 1987). Most studies have shown an inverse relationship between phytate content and mineral availability, although there are great differences in the behaviour of minerals. Zn^{2+} was reported to be the essential mineral most adversely affected by phytate (Lopez *et al*, 2002; Lönnerdal, 2002). Studies on the effect of dietary phytate on mineral availability revealed that the majority of the performed investigations resulted in a reduction in mineral absorption in the presence of phytates (Konietzny *et al*, 2006).

Phytic acid inhibits iron absorption in infants to an extent similar to that in adults (Davisson *et al.,* 1994) and low iron absorption from legume and cereal-based complementary foods is a major cause of widespread iron deficiency in infants in

developing countries. Iron deficiency in infants can lead to reduced psychomotor and mental development with long term negative consequences on school performance (Davisson *et al*., 1994). The removal or degradation of phytic acid significantly improved iron absorption from soy protein and pea protein isolates. Phytic acid is a potent inhibitor of iron absorption and phytic acid in soy protein isolate had to be decreased by more than 90% to give a two-fold decrease in iron absorption and had to be degraded completely to increase the absorption by 3-4 folds (Hurrel *et al*., 1992). Consumption of large amounts of phytates may lead to mineral deficiency which will lead to health problems, for example calcium deficiency may lead to osteoporosis (Sterfontein, 2003). Although cooking deactivates them, it does not completely remove them.

There are several methods of determining phytic acid content in plants. The spectrophotometry method is based on the metal replacement reaction of phytic acid from coloured complex of Fe(III)-5-sulfosalicylic acid and reading the absorbance at 500 nm, another method reads absorbance as indicated above, followed by a chromatographic separation (Tolotti-Carneiro *et al*., 2002; Harland *et al*., 2004).

2.2.2 Saponins

Saponins are naturally occurring surface-active glycosides. They are mainly produced by plants but also by lower marine animals and bacteria (Riguera, 1997; Yoshiki *et al*., 1998). They get their names from the ability to form stable, soap-like foam in aqueous solutions. This observable character has attracted human interest from ancient times. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin)

which may be triterpenoid in nature. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances (Francis *et al*., 2001). They have been found to act as a plant's immune system.

Saponin has the ability to act like glucocorticoid, which could possibly explain the diverse effects of saponins in biological systems. Glucocorticoids, acting through the glucocorticoid receptor (GR) are involved in the regulation of numerous physiological processes (Gagliardo *et al*., 2001). Upon ligand binding GR inhibits or stimulates gene transcription. Activated GR also antagonize transcription factors, particularly neurofactor-*k*B directly and indirectly through gene transcription and protein synthesis of the NF-*k*B inhibitor (Almawi and Melemedjian, 2002). Higher glucocorticoid activity has been recorded to have negative effects on female reproductive function (Andersen, 2002). NF-*k*B negative effects of saponin on animal reproduction have long been known and have been ascribed to their aborifacient, antizygotic and anti-implantation properties (Francis *et al*., 2002). Saponins from broom weed (*Gutierreza* sp) and lechuguilla (*Agave lechuguilla)* or commercial pharmaceutical grade saponin caused abortion or death in rabbits, goats and cows when administered intravenously at concentrations above 2.3 mg/kg body weight (Francis *et al*., 2002).

Saponins are known to reduce the palatability of livestock feeds. Some saponins reduce the feed intake and growth rate of non-ruminant animals. Certain Pasteur feeds contain substantial amounts of dangerous saponins and results in life threatening toxicity for certain animal species. On injection, saponin, like all detergents cause lysis of the red

blood cells, called haemolysis and are therefore toxic. Saponins have been reported to cause bloat in ruminants consuming fresh alfalfa. It is hypothesized that the saponins either bind with bile salts or cause bile salts to bind to polysaccharides in dietary fibre, making the bile salts unavailable to bind to cholesterol (Gagliardo *et al*., 2001).

2.2.3 Alkaloids

Alkaloids are a class of "secondary" metabolites that have been traditionally classified as basic compounds derived from amino acids that contain one or more heterocyclic nitrogen atoms. They are known for their medicinal and toxic effects. There are several types of alkaloids which have diverse effects on human health; some of the examples are discussed below.

Tobacco alkaloids are derived from the amino acid lysine. There are two basic types, namely, piperidine and pyridine alkaloids. An example of piperidine alkaloid is coiine, one of several extremely toxic compounds in the hemlock plant. Hemlock was used by the Greeks to execute criminals. Coiine causes paralysis of the motor nerve endings (Dewick, 2003). An example of a pyridine alkaloid is nicotine. This alkaloid is absorbed through the mucous membrane of the lungs, mouth and nose. It acts on the central nervous system, smooth muscle of the intestine and the cardiovascular system through vasoconstriction and increase in blood pressure. The fatal dose in humans is 50 mg/kg (Van Wyk *et al.*, 2002).

Tropane alkaloids contain a tropane nucleus that is derived from the amino acid ornithine. They occur mainly in *Solanaceae* family. The alkaloids in *Atropa belladonna* have been

known for centuries as poisonous substances. The main alkaloids hyoscyamine and atropine have an effect on the central nervous system by increasing the heart rate, decreasing intestinal tone and peristalsis, and decreasing secretions such as saliva, gastric secretions and secretions from the eyes. Acute poisoning leads to hallucinations. Cocaine also falls under this group (Van Wyk *et al.,* 2002).

Of the isoquinoline alkaloids, morphine is the most well-known and occurs in the opium poppy (Dewick, 2003). It has pharmacological actions, such as a central nervous system effect of which a pain-relieving, analgestic effect is its main property, uncontrolled use leads to addiction. Heroine is the synthetic derivative of morphine (Van Wyk *et al.,* 2002).

2.2.4 Cyanogenic Glycosides

Cyanogenic glycosides can be defined chemically as glycosides of α-hydroxynitriles and belong to the secondary metabolites of plants. They are amino acid-derived plant constituents, present in more than 2 500 plant species. They are composed of an α-hydroxynitrile type aglycone and a sugar moiety (mostly D-glucose) (Vetter, 1999). Cyanohydric acid is released from the cyanogenic glycosides when fresh plant material is macerated as in chewing, which allows the enzymes and the cyanogenic glycosides to come together, releasing cyanohydric acid (HCN) and a ketone or aldehyde. The second step can also be catalyzed by the hydroxynitrile lyase, which is widespread in cyanogenic plants. In the intact plant, the enzyme and the cyanogenic glycosides remain separated, but

if the plant tissue is damaged both are put in contact and HCN is released (Francisco and Pinotti, 2000).

Cyanohydric acid is extremely toxic because of its ability to link with metals (Fe^{2+}, Mn^{2+}, Cu^{2+}) which are functional groups of many enzymes, inhibiting processes like the reduction of oxygen in the cytochrome respiratory chain, electron- transport in photosynthesis, and the activity of enzymes like catalase and oxidase (Cheeke, 1995). All cyanogenic plants show variation in the amount of HCN produced. This variation reflects on the production of cyanogenic glycosides themselves and the enzymes which degrade them.

Hydrogen cyanide after oral administration is readily absorbed (it is also readily absorbed after inhalation and through skin and eyes) and is rapidly distributed in the blood. The concentration of cyanide is higher in erythrocytes than in plasma. The cyanide level in different human tissue in fatal cases of HCN poisoning has been reported as follows: gastric content, 0.03; blood, 0.50; liver, 0.03; kidney, 0.11; brain, 0.07 and urine, 0.20 mg/10g (Environmental Protection Agency, 1990). The lethal dose of cyanogenic glycosides has been reported as 0.5-3 mg/kg body weight (Cheeke and Schull 1985).

Cassava and sorghum are important staple foods containing cyanogenic glycosides (Cheeke, 1995). There are approximately 25 cyanogenic glycosides that have been characterized so far. Table 2.1 lists some of the cyanogenic glycosides found in commonly eaten foods.

Table 2.1 Cyanogenic glycosides in common plants (Conn, 1979b)

Cyanogenic glycoside	**Plant common name**
Amygdalin	Almonds
Dhurrin	Sorghum
Linamarin	Cassava
Lotaustralin	Lima beans, Cassava
Prunasin	Stone fruits
Taxaphyllin	Bamboo shoots

Shragg *et al.* (1982) reported that a 67 year old woman collapsed after consumption of slurry of 12 ground bitter almonds mixed with water. She recovered after treatment in hospital. The average cyanide content was 6.2 mg HCN/ bitter almond. A study to conduct possible association of high cyanide low sulfur intake in cassava-induced spastic paraparesis was conducted in Mozambique. During drought roots were eaten after only a few days of sun drying. The study revealed 1102 cases of spastic paraparesis. After a year the samples were collected 30 village children that were said to be healthy and 30 Swedish children of the same age were used as control as well as the cassava-eating children from the neighboring village where there was no case of spastic paraparesis. The children from the village had increased thiocyanate and decreased inorganic sulphate excretion, indicating high cyanide and sulphur-containing amino acid intake. The children from the neighboring village had lower cyanide and high sulphate excretion. This supported the hypothesis that the epidemic was due to the combined effects of high dietary cyanide exposure and sulphur deficiency (Cliff *et al*., 1985).

There are different possibilities for the qualitative, semi-qualitative or quantitative determinations of the compounds. The first group of the estimations contains direct methods, the second group is based on the preliminary hydrolysis and the third group is the quantification of HCN (Vetter, 1999). Kawamura *et al.* (1993) suggested a method for direct estimation of linamarin in beans, bean paste and cassava flour.

2.2.5 Trypsin inhibitors

Seed proteins such as cereals grains, legumes and oil seeds are important sources of dietary proteins in many areas of the world but some contain enzyme inhibitors. The most widely studied of these is trypsin inhibitors. Trypsin is an enzyme involved in protein digestion and trypsin inhibitors can result in a decreased availability of protein. Trypsin is a proteolytic enzyme which converts proteases and peptones into polypeptides and amino acids (Claus *et al.*, 1971). Dewick (2003) describes trypsin as an enzyme that is found in bovine pancreas, it hydrolyses proteins and it is essential in wound and ulcer cleansing.

Trypsin inhibitors, according to Venter and Van Eyssen (2001) are compounds that interfere with protein digestion. They cause pancreatic enlargement and enhance chemically induced pancreatic tumors. They constitute a unique class of proteins with the ability to react in a highly specific manner with a number of proteolytic enzymes in the digestive secretions of animals. The trypsin inhibitors of soybean are now well characterized and are an important determinant of nutritive value. Proteinase inhibitors are also present in other leguminous seeds such as field beans, winged beans, pigeon pea and cowpea. Effects in animals include reduced protein digestion and endogenous loss of

amino acids, with the overall result that performance is impaired (Venter and Van Eyssen, 2001).

2.2.6 Oxalic acid

Oxalic acid or ethanedioic acid is a colourless, poisonous solid soluble in water, alcohol and ether (ARS phytochemical database, 2000). Oxalic acid crystallizes in colourless, transparent, oblique, quadrilateral prisms with two-sided summits. The crystals are odourless, have a strong acid taste and faintly effloresce in a dry atmosphere. It reddens the litmus paper, when pure it is completely volatized by heat, without becoming blackened.

Oxalic acid is also found in rhubarb leaves and its salts (oxalates) occur in wood sorrel (*Oxalis oxalidaceae*) and other plants. Although purslane (*Portulaca oleracea*, Portulacaceae family) is an excellent source of omega-3 fatty acids and antioxidant vitamins, its consumption as a green vegetable, is limited by high concentrations of Oxalic Acid which is 1.31 g/100g (Palaniswamy *et al*., 2004). Oxalic acids are found in commonly consumed vegetables as listed in Table 2.2.

Table 2.2 Vegetables containing oxalic acid and the amount they contain per 100g (Agriculture handbook, 1984)

Vegetable	**Oxalic acid (g/100g)**
Amaranth	1.09
Asparagus	0.13
Cabbage	0.10
Carrot	0.50
Cassava	1.26
Chives	1.48
Garlic	0.36
Lettuce	0.33
Parsely	1.70

Oxalic acid reduces iron compounds, and is therefore used in metal polishes, stain removers, and writing inks. When it absorbs oxygen, it is converted to a volatile carbon dioxide and water ([HOOC.COOH]. $2H_2O$) and it is used as a bleaching agent in detergents and as a mordant in dyeing processes (Cameselle *et al*., 2004).

Oxalic acid is very poisonous; it is similar to Epsom salt and for this reason has been mistaken for this substance, which in many cases, has fatal results. Oxalates are readily decomposed on heating, leaving a residue of carbonate or oxide of metal. The human body synthesizes oxalic acid from ascorbic acid (vitamin C). Oxalic acid may combine with calcium, iron, sodium, magnesium or potassium to form oxalates. Since it binds to important nutrients, making them inaccessible to the body, regular consumption of large

amounts of foods high in oxalic acid over a period of weeks to months may result in nutrient deficiencies, most notably calcium (Duke, 2000)

Oxalic acid is a strong acid and it is irritating to the tissue. Oxalates form tiny insoluble crystals with sharp edges, which are also irritating to tissue. High levels of oxalic acid/oxalates in diet lead to irritation of the digestive system, particularly the stomach and kidneys. Oxalates may also contribute to the formation of kidney stones. One must avoid food high in oxalic acid or oxalates if they suffer from kidney stones, rheumatoid arthritis or gout (Duke, 2000).

2.2.6 Phytoestrogens

Phytoestrogens are naturally occurring phenolic plant compounds, present in foods such as beans, cabbage, soyabean, grains and hops. They are structurally similar to the mammalian oestrogen, oestradiol, and have oestrogenic properties. However, their oestrogenic activity is generally much less than that of human oestrogens (oestrogenic activity ranges from 1/500 to 1/1000 of the activity of oestradiol). Hence phytoestrogens can act as anti-oestrogenic agents by blocking the oestrogen receptors and exerting a much weaker oestrogenic effect compared with the hormone. As a consequence it has been suggested that they might partly suppress or inhibit normal oestrogenic activity in oestrogen-responsive tissues such as breast tissue. They may, in addition to their endocrine effects, have action on cellular targets which are independent of oestrogen, thereby complicating the prediction of their properties in humans (Thompson *et al*., 2005)

Phytoestrogens are found in the seeds, stems, roots or flowers of plants, serving as natural fungicides and acting as part of the plant's defense mechanism against microorganisms. They also are the molecular signals that emanate from the root of leguminous plants that attract specific nitrogen-fixing soil bacteria. The main classes of phytoestrogens are the isoflavones, coumestans, Prenylated flavonoids and lignans (Cassidy, 1999).

Oestrogen toxicity has been associated with plant exposures. Phytoestrogens have been shown to induce infertility and developmental toxicity in certain animals, and coumesterol has caused "sheep clover disease", inhibiting fertility (Sheehan and Medlock, 1995). Exposure to phytoestrogen may be detrimental as they have mutagenic, tetratogenic and carcinogenic potential.

2.2.7 Coumarins

The derivatives of coumarin usually occur as secondary metabolites present in seeds, root, and leaves of many plant species. They are competitive inhibitors of Vitamin K in the synthesis of prothrombin (Desai, 2004). Liver disease or injury, or treatment with the medication warfarin, may cause an abnormal prothrombin synthesis time.

Warfranin is a synthetic coumarin-type which was designed using discoumarol as a model. Discoumarol is toxic through its anti-coagulant properties and it is used to kill rats (WebMD, 2005). Xanthotoxin, an example of coumarins, causes severe light-induced dermatitis (a severe allergic reaction that may result in painful blistering of skin).

Coumarins may be found in essential oils because they are sufficiently volatile to be extracted through the process of steam distillation.

Coumarins are mainly used in rat poisoning. Other coumarin rich sources are lace flower fruits, cabbages and wild parsley. These may cause dermatitis in sensitive people (Van Wyk *et al.*, 2002).

2.2.8 Phenolic compounds

Phenolic compounds consist of a hydroxyl functional group (OH) which is attached to an aromatic hydrocarbon group. These compounds are available in all vegetables. Polyphenols are extracts of various plants which have the ability to react with protein in animal skins to produce leather. Tannins are an example of polyphenols (Kallanmeyer, 2003). Phenols are known to inhibit bone mineralization.

2.2.9 Summary of the effects of common antinutrients

Some examples of antinutrients and their effects on humans and animals are listed in Table 2.3 below.

Table 2.3 Common antinutrients found in plants and their effects on humans and animals.

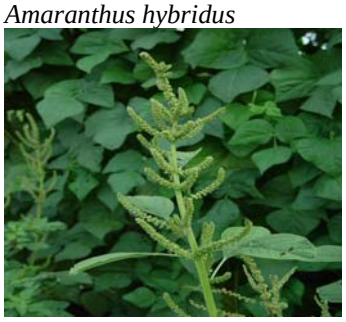
Antinutrients	**Plant source**	**Effects on humans and animals** **(Francis *et al.*, 2001; Duke, 2000)**
Cyanogenic glycosides	Sweet potatoes, stone fruits, lima beans, cassava and sorghum	Gastro-intestinal inflammation and inhibition of cellular respiration
Oxalates	Spinach, rhubarb, tomato	Reduces solubility of Calcium, Zinc and Iron
Phenols	Most fruits and vegetables, cereals, soybean, potato, tea, coffee	Destroys thiamine, raises cholesterol and mimic estrogen
Phytates/ phytic acid	Legumes and cereals	Prevents digestion. Inhibits the uptake of calcium and can cause problems with metabolic bone disease, renal failure, shell pyramiding and bladder stones
Saponins	Alfalfa, afombrilla	Binding of bile acids to dietary fiber, cause bloating in animals, speed up haemoglobin degradation
Alkaloids	Potato, tomato, tobacco	Depressed central nervous system; kidney inflammation; carcinogenic; birth defects; reduced iron uptake
Trypsin inhibitors	Cereal grains, legumes and oilseeds	Interfere with protein digestion
Phytoestrogens	Soya	Induce infertility
Coumarins	Celery, parsley, parsnips, figs	Light-activated carcinogens; skin irritation
Lectins	Most cereals, soybeans, other beans, potatoes	Intestinal inflammation; decreased nutrient uptake/absorption

2.3 TRADITIONAL LEAFY PLANTS USED IN THIS STUDY

Various traditional plants are consumed in South Africa as part of their daily diet, as medicine or as famine food. Table 2.4 below identifies these plants and lists their present uses.

Table 2. 4 Plant classification, edible uses, medicinal uses and toxicity

Plant classification (scientific, English and Zulu name, picture of plant)	**Edible uses**	**Medicinal uses**	**Toxicity**
Solanum retroflexum 	Leaves used as potherb relish. Ripe fruit is eaten (Fox and Norwood Young, 1982).	Antiphlogistic, diaphoretic, diuretic, emollient, antiperiodic, febrifuge, narcotic, purgative, sedative (Duke and Ayensu, 1985), Poultice in treatment of cancerous sores, leucoderma, wounds.	Toxicity is variable. Green berries contain solanine causing degrees of poisoning (Tredgold, 1986).
Physalis viscosa 	Fruit and berries are edible (Beekrum, 2003).	The plant is used as a tonic, laxative, diuretic and sedative. The juice of the berries is beneficial in several urinary disorders. Used to treat inflammatory diseases	No toxicity reports available.
Momordica balsamina 	Cooked as spinach, with nuts or mielie meal, relish, soup (Fox and Norwood Young, 1982).	Leaves used for liver deficiencies, blood cleanser, ulcers of the stomach and duodenum, disorders. Used as a purgative, emetic, bitter stomachic and as a wash for fever.(Beekrum, 2003)	No toxicity reports available

Amaranthus spinosus 	Eaten raw or cooked as spinach. Famine food (Fox and Norwood Young, 1982).	Astringent, diaphoretic, diuretic, emollient, febrifuge, purgative. Used to treat snake bites, ulcerated mouths, vaginal discharges, nosebleeds, wounds (Beekrum , 2003).	No members of this genus are known to be poisonous (Duke, 2000)
Amaranthus hybridus 	Relish, mixed with mielie meal (Fox and Norwood Young, 1982). Cooked as spinach, added to soups, eaten raw .	Tea made from leaves is astringent. Used in treatment of intestinal bleeding, diarrhoea, excessive menstruation.	No members of this genus are known to be poisonous (Duke, 2000).
Amaranthus dubius 	Potherb, cooked and eaten as spinach. Seeds are nutritious (Beekrum , 2003).	The whole plant is used to alleviate stomach pains (Beekrum, 2003).	No members of this genus are known to be poisonous (Duke, 2000).
Asystasia gangetica 	Leaves are edible occasionally (Beekrum , 2003).	To ease childbirth pains, facilitate labour, stomach aches, fever aches, epilepsy, heart pains (Beekrum , 2003).	No toxicity reports available.
Justicia flava 	Semi cultivated and eaten as a vegetable (Beekrum, 2003).	Stomach ache, diarrhea. Treats fevers and yaws. Leaves used as emetics and eye lotions (Beekrum , 2003).	No toxicity reports available.

Emex australis 	Cooked as spinach (Fox and Norwood Young, 1982).	Used to treat gastrointestinal disorders, colic, biliousness and dyspepsia (Beekrum *et al*., 2003).	Contains oxalic acid (Beekrum *et al*., 2003).
Oxyagonum sinuatum 	Potherb, spinach	Leaf sap used for cough and bronchial catarrh Used for gastric ulcers, malaria and hepatitis (Beekrum, 2003).	No toxicity reports available.
Bidens pilosa 	Potherb, tea, salads, soups, stews Relish, peanut butter added (Tredgold, 1986).	Leaves are anti-inflammatory, styptic, anti-rheumatic. Substances isolated are bactericidal, fungicidal. A juice made from leaves used to dress wounds (Beekrum , 2003).	Phototoxic. Substances isolated from leaves can destroy human skin in the presence of sunlight at concentrations as low as 10 ppm (Beekrum, 2003).
Galinsoga parviflora 	Eaten as a potherb, raw or cooked. Flavouring in soups (Beekrum , 2003).	When rubbed onto the body, the plant is useful in treating nettle stings (Chopra *et al*., 1986).	No toxicity reports available.

Portulaca oleracea 	Eaten as salad and vegetable Can be eaten raw (Beekrum , 2003)	Whole plant to be bactericidal in bacillary dysentry, diarrhoea, haemorrhoids and enterorrhagia., antibacterial, antiscorbutic, depurative, diuretic, febrifuge (Beekrum , 2003).	9% oxalic acid. Incoordination of gait and tetanic conditions in sheep (Beekrum , 2003).
Senna occidentalis 	Young leaves are eaten. Regarded as a famine food. Roasted seed is used as coffee substitute (Fox and Norwood Young, 1982).	Used for stomach pains, biliousness, fevers, jaundice, ringworms, sore throats and. Fresh leaves are used to treat edemas, abscesses and skin diseases (Beekrum , 2003).	Unroasted seeds are toxic (Fox and Norwood Young, 1982). Leaves are toxic only if large quantities are consumed (Beekrum , 2003).
Chenopodium album 	Cooked as spinach, mielie meal added, eaten as porridge, relish Seeds dried, ground into a meal and used as gruel, eaten raw, added to salads (Beekrum, 2003).	Leaves are anthelmintic, antiphlogistic, anti-rheumatic, mildly laxative, odontalgic . Applied as a wash or poultice to bug bites, sunstroke, rheumatic joints, Contains small amounts of saponins. Cooking the plant reduces the oxalic acid content (Beekrum , 2003).	Contains small amounts of saponins. Cooking the plant reduces the oxalic acid content . Raw leaves consumed in large quantities causes problems (Beekrum , 2003).
Ceratotheca triloba 	Cooked as spinach. Unpleasant scent disappears with boiling. Sweet tasting and used a relish (Tredgold, 1986).	Used in treatment of painful menstruation, stomach cramps, nausea, fever and diarrhoea. To relieve gastric disorders (Tredgold, 1986).	No toxicity reports available

Centella asiatica 	Eaten as spinach, mixed with mielie meal. Leaves are dried and used as famine food (Fox and Norwood Young, 1982). Used in salads and curries.	Rejuvenating diuretic herb that clears toxins, reduces inflammations and fevers, improves healing and immunity, improves the memory. Used in treatment of wounds, chronic skin conditions, venereal diseases, malaria, varicose veins, ulcers, nervous disorders and senility (Beekrum , 2003).	In large doses, this plant is a stupefying narcotic, sometimes producing cephalagia or vertigo with a tendency to coma (Beekrum , 2003).
Cleome monophylla 	Cooked with other herbs and nuts, cowpeas or tomatoes (Fox and Norwood Young, 1982).	Leaves applied to sores, roots chewed for coughs and the whole plant is used externally for swellings. Used as an anthelmintic.	No toxicity reports

Three plants namely, *Emex australis*, *Portulaca oleraceae* and *Chenopodium album* contain oxalic acid, a colourless poisonous crystalline dicarboxylic acid which occurs naturally in many plants. Oxalic acid may combine with calcium, iron, sodium, magnesium, or potassium to form less soluble salts known as oxalates. High levels of oxalic acid/oxalates in the diet lead to irritation of the digestive system, and particularly of the stomach and kidneys. They may also contribute to the formation of kidney stones (Agricultural Research Service, 1998).

Solanum retroflexum, *Senna occidentalis* and *Centella asiatica* have been reported to be toxic if consumed in large amounts whilst the fruit of *Cucumis metuliferus* contains cucurbitacines that are harmful to both humans and animals. The quantity of consumption of these plants by humans should be minimized, and measures should be taken to ensure

that these plants, which are dangerous to animals, do not grow excessively in grazing areas so that the risk of poisoning is reduced.

Literature does not cover the toxicity levels of the 18 leafy vegetables discussed above. These plants have been found to be toxic but no reasons for their toxicity have as yet been given nor their toxic compounds identified. This study was undertaken to establish a comprehensive safety profile of 18 traditionally consumed plants in South Africa by analyzing the plants for potential toxic antinutrient compounds and carrying out toxicity and cytotoxicity studies on the plant extracts using established methods.

CHAPTER THREE

MATERIALS AND METHODS

3.1 SAMPLING

Eighteen leafy vegetables were selected for this study based on the nutritional status of leafy vegetables commonly consumed by the indigenous community of KwaZulu Natal, South Africa (Odhav *et al*., 2006). The plants were identified and collected from across the eastern seaboard of KwaZulu Natal and voucher specimens are housed in the Ward Herbarium, University of KwaZulu Natal. Biodata on the plants are listed in Table 3.1 below.

Table 3.1 Biodata on the traditional leafy vegetables used in this study.

Scientific name	Family	English Name	Zulu Name	Source
Cleome monophylla	Capparaceae	Spindle-pod	Isiwisa	Reservoir Hills
Senna occidentalis	Fabaceae	Cassia senna	Isinyembane	Reservoir Hills
Justicia flava	Acanthaceae	Yellow justicia	Impela	Reservoir Hills
Asystasia gangetica	Acanthaceae	Hunter's spinach	*	Reservoir Hills
Centella asiatica	Apiaceae	Marsh pepperwort	Icukudwane	Reservoir Hills
Amaranthus hybridus	Amaranthaceae	Cockscomb	Imbuya	Reservoir Hills
Amaranthus spinosus	Amaranthaceae	Spiny pigweed	*	Reservoir Hills
Ceratotheca triloba	Pedaliaceae	Wild foxglove	Udonqa	Reservoir Hills
Portulaca oleracea	Portulacaceae	Purslane	Amadilika	Verulam
Amaranthus dubius	Amaranthaceae	Wild spinach	*	Reservoir Hills
Oxygonum sinuatum	Polygonaceae	*	*	Reservoir Hills
Galinsoga parviflora	Asteraceae	Gallant soldier	Ushukeyana[7]	Reservoir Hills
Physalis viscosa	Solanaceae	Grape ground-cherry	Uqadolo	Park Rynie

Solanum nigrum	Solanaceae	Black nightshade	Umsobo	Reservoir Hills
Momordica balsamina	Cucurbitaceae	Balsam apple	Inkaka	National Botanical Institute, Durban
Chenopodium album	Chenopodiaceae	Fat hen	Imbikilicane	Reservoir Hills
Bidens pilosa	Asteraceae	Blackjack	Ilenjane	Reservoir Hills
Emex australis	Polygonaceae	Devil's thorn	Inkunzane	Reservoir Hills

* Not recorded

3.2 ANTINUTRIENT SCREENING OF SELECTED LEAFY PLANTS

The plants were assayed for the following antinutrients: phytic acid, saponins, alkaloids, cyanogenic gycosides, trypsin inhibitors and oxalic acid. In all antinutrient assays, 5 g of oven dried, crushed leafy material were used, except for oxalic acid analysis where 0.5 g of crushed leaves were used. All assays were carried out in triplicate. The mean and standard deviations in all assays were calculated and the results were presented graphically. A composite table of results was also compiled to indicate antinutrient profiles of each plant. Particular methods used for each antinutrient are decried below.

3.2.1 Quantitation of phytic acid

The phytic acid content in the plants was determined using the spectrophotometric method described by Omotoso (2006) and by Wheeler and Ferrel (1971) with minor modifications. Phytic acid reacts with a coloured complex such as Fe (III)-sulphosalicylate to form a colourless Fe (III)-phytate complex. This method thus measures the Fe (II) content which correlates 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 (II) in the range 0.025-2 mg/ml (Appendix B). Five grams of the 18 different leafy materials were added to 50 ml of 3% trichloroacetic acid (TAA). The samples were then placed in a shaking incubator (Labcon, USA) for 30 min at constant speed (156 rpm). The suspensions were then centrifuged (Eperndorf, USA) at 10 000 rpm for 15 min 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. All eighteen tubes containing the samples were heated for 45 min in a water bath (90-100ºC). The solutions were then centrifuged (10 000 rpm for 15 min) and the supernatants poured off. The pellets were then washed twice by adding 10-15 ml 3% TAA solution, heating for 5-10 min and centrifuging (10 000 rpm for 15 min). The resulting pellet was washed once with distilled water and resuspended in 1 ml distilled water and 1.5 ml of 1.5N NaOH solution and stirred. The volume was brought up to 15 ml with distilled water (dH_2O), heated in boiling water for 30 min and centrifuged (10 000 rpm for 15 min). The solution was filtered while hot with Whatman No. 2 filter paper. The precipitate was washed with 30-40 ml of hot dH_2O and the filtrate discarded. The precipitate left in the paper was dissolved with 20 ml 3.2N solution of HNO_3 transferring it to a 50 ml volumetric flask. The sample was cooled at room temperature (RT) and calibrated with dH_2O. A 2.5 ml sample was transferred to a volumetric flask and diluted to 35 ml with dH_2O. Thereafter 10 ml of 1.5 M potassium thiocyanate (KSCN) solution was added and the solution calibrated to 50ml with dH_2O. The absorbance of Fe (II) in the samples was read within 1 min at 480 nm using a spectrophotometer (UltraspecII-LKB, Biochrom, England).

3.2.2 Quantitation of saponins

Saponins were quantified by a disc diffusion method outlined by Makkar (2004) with minor modifications. Saponins have a haemolytic property and their presence in blood agar produces haemolytic zones which are proportional to the amount of saponins in the plate.

The dried crushed leafy materials were dissolved in 25 ml of 80% methanol for 24 h and filtered (Whatman no.1). The filtrate was then concentrated by rotary evaporation. The extracts were reconstituted in 1 ml of dH_2O when required. Saponin standards (Sigma, USA) were prepared in the range 1-4 mg/ml in dH_2O. Whatman no. 1 filter discs were immersed into each of the standards and the samples and placed onto the blood agar plates which were made up according to standard procedures. The plates were incubated at 37^oC for 24 h. The diameter of the haemolytic zones were measured in mm and used to plot a standard curve. The unknown concentrations of saponins in the 18 leafy extracts were determined from the standard curve (Appendix B).

3.2.3 Alkaloid precipitation

The presence of alkaloids was determined using a precipitation method outlined by Harborne (1973) and Edeoga *et al*. (2005) with minor modifications. Ammonium hydroxide added to plant extracts was found to precipitate alkaloids.

The dried leafy material was treated with 200 ml of 10% acetic acid in ethanol (v/v) for 4 h at RT. The extract was filtered and concentrated on a rotary evaporator (60°C) to 50 ml. One ml concentrated ammonium hydroxide was added drop wise to the extract until the

precipitation was complete. The solution was allowed to stand for the precipitate to settle. The precipitate was then collected and washed with dH_2O : ammonium hydroxide (5ml: 5ml) (v/v) and then filtered. The residue was dried at RT and weighed. The results were recorded in grams per 5 g dried leaves. The control used was a local tobacco leaf extract as suggested by Rai *et al*. (1994).

3.2.4 Detection of cyanogenic glycosides

Cyanogenic glycosides were detected using the technique of the picrate-impregnated paper according to Tan and Yeoh (1997). When the cyanogenic glycosides release cyanogenic hydroxides, they change yellow picrate paper to brownish-red.

Dried plant material was added to 1.5 ml dH_2O and six drops of chloroform. A strip of sodium picrate paper was suspended in the test tube and incubated at ambient temperature for 2 h. The strips were examined for a colour change from yellow to brown-red within 24 h indicating the presence of cyanogenic glycosides. A brown-red colour appearing within 24 h indicated that the cyanogenic glycosides spontaneously released HCN without the action of an enzyme. The control used was 1.5 ml dH_2O with six drops of chloroform.

3.2.5 Quantitation of trypsin inhibitors

Trypsin activity of plant material was first determined using the reaction: tyrosine produces trypsin in the presence of a casein substrate, as outlined by Jayaraman (1981). Trypsin inhibition by the plant extract was indicated by trypsin activity falling below the tyrosine standard activity.

As a control 0.9 ml of the casein bovine substrate and 0.1 ml of 0.25% trypsin were added to a test tube, mixed well and incubated for 30 min at RT. Then 1.5 ml of 10% TAA was added to stop the reaction. After 15 min, the test tube was put into boiling water for 10 min and filtered using Whatman no. 1 filter paper. The filtrate (0.5 ml) was added to the test tube together with 1 ml of dH_2O, 5 ml 0.5 N NaOH and 1.5 ml of diluted Folin Ciocalteau (FC) reagent.

Five grams of each of the plant extracts was added to 200 ml of dH_2O and allowed to shake for 24 h at 37^{o}C. After 24 h, the extracts were filtered with Whatman no.1 filter paper. Each of the 18 extracts (0.1 ml) was added to 0.9 ml of the substrate and 0.1 ml of 0.25% trypsin. Tyrosine (Sigma, USA) was used as a standard to measure trypsin activity. A blank was prepared by adding 1.5 ml of water to 5 ml of 0.5 N NaOH together with 1.5 ml of the FC reagent. Absorbance (Abs) was read at 650 nm.

The activity of trypsin was calculated as follows:

$$\text{Activity of trypsin} = \frac{\text{Abs of test} - \text{Abs of (+ve) control}}{\text{Abs of the standard} - \text{Abs of the blank}} \times \text{dilution factor}$$

3.2.6 Quantitation of oxalic acid

The oxalic acid content was determined using isocratic reverse phase high performance liquid chromatography (HPLC) analysis modified method of Miller and Woodrow (2004). A standard curve was used to determine the concentration of the unknown oxalic acid in the plant extracts.

Oxalic acid standards (Sigma, USA) were prepared in the range 1-20 mg/ml and run chromatographed on an isocratic reverse phase HPLC system (D7000 Lichrom Merck-Hitachi, Germany). The parameters included the following: C18 column (250 x 4 mm id, particle size 5 µm Luna 5µ C-18 (Phenomenex, USA) at RT, 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 was found to 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 from the 18 plants using 4 ml of 0.025 M HCl. The above extract was centrifuged at 10 000 rpm for 20 min at 25ºC. 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 calculated from the standard curve using the formula $y = mx + c$ (Appendix B). The experiment was done in duplicate.

3.3 BIOASSAYS ON SELECTED LEAFY PLANTS

The bioassays used to determine the safety of the plants for human consumption were the following: the brine shrimp assay for toxicity, the MTT cytotoxicity assay and the Ames test for mutagenicity. All assays were carried out in triplicate. The mean and standard deviations were calculated in all assays and presented graphically.

3.3.1 Preparation of plant extracts for bioassays

Fifty grams of each of the 18 dried leafy plant materials were used to prepare the plant extracts. The aqueous extracts were prepared using 200 ml of dH_2O and 200 ml of 80% methanol was used to prepare the organic extracts. The extracts were shaken at 156 rpm for 24 h at 37^{o}C in a shaking incubator. The extracts were then filtered with Whatman no. 1 filter and the organic extracts were concentrated to approximately 3 g using a rotary evaporator (60^{o}C) and kept in a fridge (5^{o}C). The aqueous extracts were kept in a biofreezer for 24 h and thereafter freeze-dried (Virtis, USA) for 24 h to 48 h and gave a final dry weigt of approximately 1 g..

3.3.2 Mutagenicity assay

The Ames test was performed by the method of Maron and Ames (1979) and as revised by Elgorashi *et al*. (2003). The objective of this assay was to evaluate if any of the plants display a mutagenic potential by studying its effect on a histidine-requiring strain of *Salmonella typhimurium* in the absence of a liver metabolising system. When the cultures are exposed to a mutagen some of the bacteria undergo genetic changes due to chemical interactions resulting in reversion of the bacteria to a non-histidine-requiring state. The reverted bacteria are then grown in the absence of exogenous histidine thus providing an indication of the potential of a chemical to cause mutation. *Salmonella typhimurium* TA 100 tester strain was used in this assay (ATCC, USA).

Preparation of stock cultures of S. typhimurium

From the frozen disc cultures of *S. typhimurium* TA 100 tester strain (Elgorashi *et al*., 2005) broth cultures were made. One disc culture was aseptically removed from the

container and inoculated into a 250 ml Erlenmeyer flask containing 25 ml of nutrient broth and 78 µl of 10 mg/ml of ampicillin. The culture was incubated at 37°C in a shaker at 150 rpm for 16 h until an absorbance of between 1.2 and 1.4 was obtained. An aliquot of the cultures (100 µl) was then transferred to a sterile test tube containing 2 ml of 0.05 mM histidine in 0.05 mM biotin top agar (Appendix C). This was then vortexed and plated onto minimal glucose agar plates (Appendix C) which were incubated at 37°C for 48 h. Well-separated colonies were used for initial broth cultures.

Preparation of plant extracts

Each of the plant extracts (10 mg) were dissolved in 1 ml of DMSO for organic extracts and in 1 ml sterile dH_2O for aqueous extracts, yielding a concentrations of 10 000 µg/ml. Tenfold dilutions were of this plant stock solution were made (100 µg/ml and 1000 µg/ml).

Ames test procedure

Broth cultures of *S. typhimurium* were made by inoculating nutrient broth with the master plate colonies. These cultures were incubated on a rotary shaker (150 rpm) at 37°C for 24 h.

One hundred µl of each of the plant extracts (100 µg/ml, 1 000 µg/ml and 10 000 µg/ml) was added to 900 µl soft agar (Appendix C). Sodium azide (NaN_3), a highly mutagenic compound (Elgorashi *et al.,* 2005) was dissolved in DMSO and used as a positive control (5µg/ml, 10µg/ml and 20 µg/ml). Sterile dH_2O and DMSO were used as negative controls. All tests were carried out in duplicate.

In a sterile test tube 100 µl of bacterial culture, 50 µl of test sample and 2.9 ml of top agar were added. This was mixed and poured onto minimal glucose agar plates. Once the agar solidified, the plates were inverted and incubated for 48 h at 37°C, after which the number of revertant colonies were counted and the mutant frequency determined. The mutant frequency is expressed as the quotient of the number of revertant colonies over the number of colonies in the negative control. This was expressed using the following formula:

Mutant frequency = Revertant number of colonies / Negative control

A mutagenic potential of a test compound is assumed if the mutant frequency is 2.0 or higher. A possible mutagenic potential is assumed if the quotient ranges between 1.7 and 1.9. No mutagenic potential is assumed if all quotients range between 1.0 (or lower) and 1.6.

3.3.3 Toxicity assay

The brine shrimp assay (Carballo *et al*., 2002) was used to determine the toxicity of the aqueous and organic extracts of the 18 leafy plants on brine shrimp larvae (*Artemia salina*).

Brine shrimp eggs were hatched in a shallow rectangular dish (22 x 32 cm) filled with sea water. This was left to stand for 48 h.

Preparation of plant extracts

The freeze-dried aqueous or concentrated organic plant extracts (10 mg) were dissolved in 1 ml of DMSO for organic extracts and 1 ml dH_2O water for aqueous extracts and this was

called solution A. Solution B was prepared by adding 0.5 ml solution A in 9.5 ml of either water for aqueous or DMSO for organic extracts. The following volumes of solution B were added to the filter discs (20 µl , 200 µl and 2 ml) giving the following concentrations of plant extracts used: 10 µg/ml, 100 µg/ml and 1 000 µg/ml.

Brine Shrimp toxicity assay

A set of controls was prepared using DMSO for the organic extracts and dH_2O for the aqueous extracts. The discs impregnated with the above extracts were dried at RT to evaporate the DMSO, leaving only the test compound on the disc; the discs for water extracts were not dried. Five ml of sea water was added to each of the vials and 10 live shrimp added to each vial containing sea water and filter paper discs. A drop of yeast suspension was supplied to each vial (3 mg in 5 ml sea water) as food source for the shrimp. The vials were incubated at 27ºC for 24 h. After the incubation period, live shrimp were counted.

3.3.4 Cytotoxicity assay

Cell culture

A human hepatoblastoma cell line, HepG2 (HB-8065) was obtained from the American Type Culture Collection (Rockville,MD). These cells exhibit a relatively normal hepatocyte phenotype, including many inducible enzymes and an aromatic hydrocarbon receptor. The cells were maintained in tissue culture flasks with Dulbecco's modified Eagle's medium (DMEM), containing 10% foetal calf serum (FCS) and supplemented with 1% antibiotics (penicillin: 10 000 U/ml, streptomycin sulphate 10 000 U/ml) and 1 mM sodium pyruvate. All cell culture media and reagents were tissue culture grade and

obtained from Highveld Biological (South Africa). Cells were incubated in a humidified incubator containing 5% CO_2 at 37°C and passaged weekly. When the cells were 80% confluent, they were trypsinized using 0.1% ethylenediaminetetraacetic acid (EDTA) and 0.25% trypsin (1:1 *v/v*) (Reddy, 2005).

Cell treatment

Two hundred µl of $3x10^4$ of cells/ml was added into each well and was incubated in a 96 well plate for 24 h and 2 µl of each of the 18 aqueous and organic extracts was added to the wells in triplicate. Three untreated cultures in wells were used as control with carrier medium. The plate was incubated for 24 h in a 37ºC humid chamber.

MTT assay

Cytotoxicity was quantitated using the MTT assay (Hanelt *et al*., 1994) which measures mitochondrial activity within cells. After 24 h incubation, 20 µl of MTT reagent (Appendix C) was added to each well and incubated for 3 h in a 37ºC humid chamber. The formazon crystals produced were dissolved in 100 µl DMSO for 30 min. The absorbance was then read at 578 nm (with a reference wavelength 630 nm) on an ELISA reader according to Reddy (2006). The percentage cell viability then calculated.

$$\% \text{ cell viability} = \frac{\text{no. of treated cells}}{\text{no of untreated cells}} \times 100$$

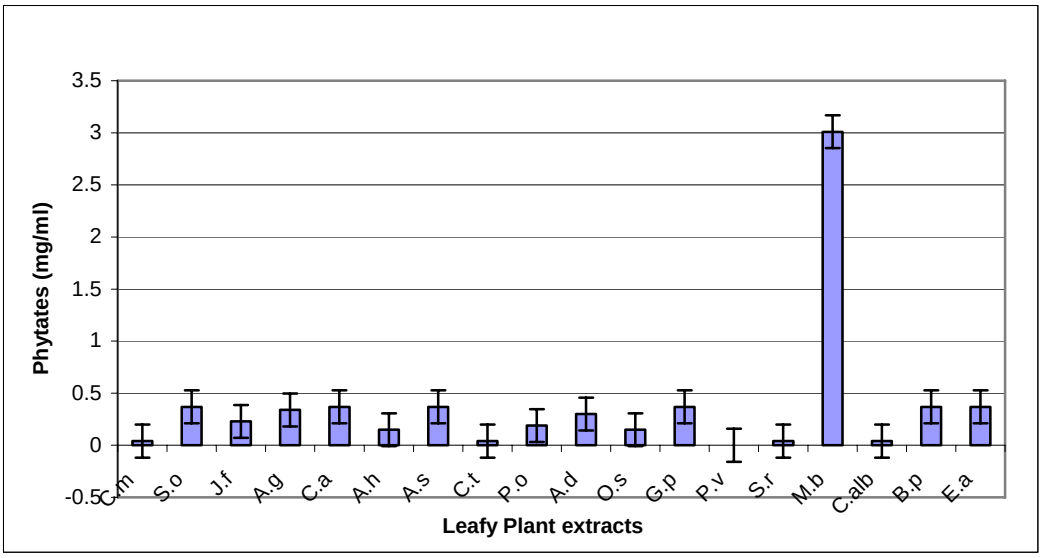
% Cell death = 100% - % Cell viability

CHAPTER FOUR

RESULTS

4.1 PHYTIC ACID

The concentration of phytic acid was determined spectrophotometrically in 18 plant samples as shown in Figure 4.1. The concentrations of phytic acid were obtained from 5 g dried leafy material. All the plants contained phytic acid, except for *Physalis viscosa* which did not contain any phytates (detection limit 0.025 mg/ml) in this study. This may have been due to the fact that there was a limitation in terms of the detection concentration since concentrations less than mg was not tested. *Momordica balsamina* was found to contain the highest concentration of phytates with a concentration of 3.01 mg/ml. *Chenopodium album, Solanum retroflexum, Ceratotheca triloba* and *Cleome monophylla* contained phytates even though it was detected at a low concentration of 0.04 mg/ml. Other plants such as *Senna occidentalis, Centella asiatica, Amaranthus spinosus, Galinsoga parviflora, Bidens pilosa* and *Emex australis* had a concentration of 0.37 mg/ml.



Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*

Figure 4.1 Concentration of phytic acid in 18 plant extracts

4.2 SAPONINS

Saponins were detected by the presence of haemolytic zones on blood agar plates using the disc diffusion method (Figure 4.2). The concentrations of saponins were obtained from 5 g dried leafy material.A standard curve was drawn and used to determine concentration of saponins in the 18 leaf extracts. These zones were used to draw up a standard curve and the unknown concentrations of saponins in the 18 leafy plants were determined from the curve (Figure 4.3). Saponins were not found in *Galinsoga parviflora, Physalis viscosa, Senna occidentalis, Asystasia gangetica, Justicia flava, Chenopodium album* and O*xygonum sinuatum.* All the other plants contained varying amounts of saponin ranging

from 0.58 to 1.8 mg/ml. *Momordica balsamina* contained the highest concentration (1.8 mg/ml) which is very close to the saponin standard (2 mg/ml). *Solanum retroflexum*, *Emex australis*, *Amaranthus dubius* and *Cleome monophylla* also contained high concentrations of saponins ranging from 1.33 to 0.857 mg/ml (Appendix B).

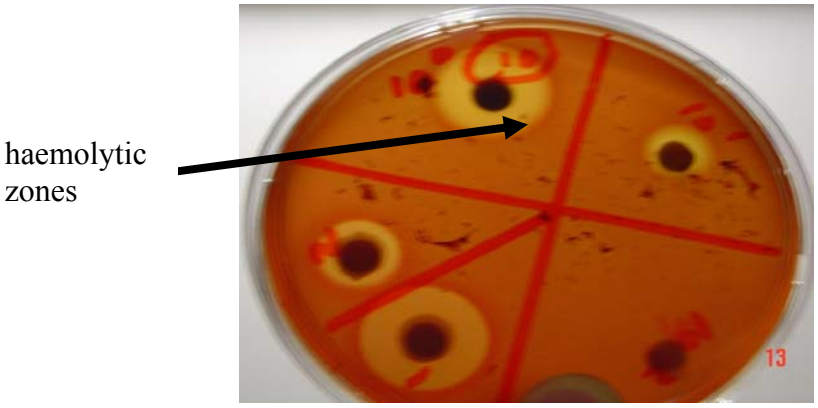
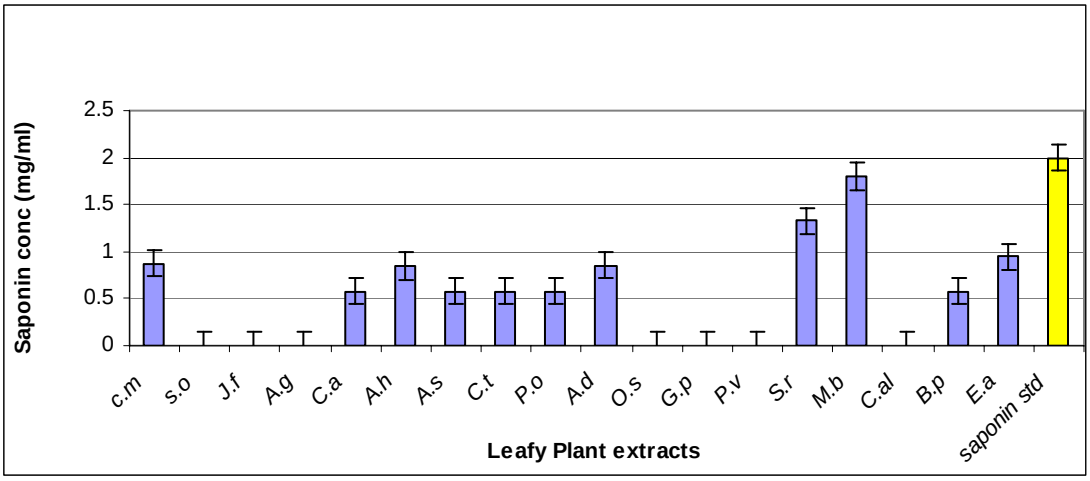


Figure 4.2 Haemolysis produced on blood agar plates by saponins from leaf extracts

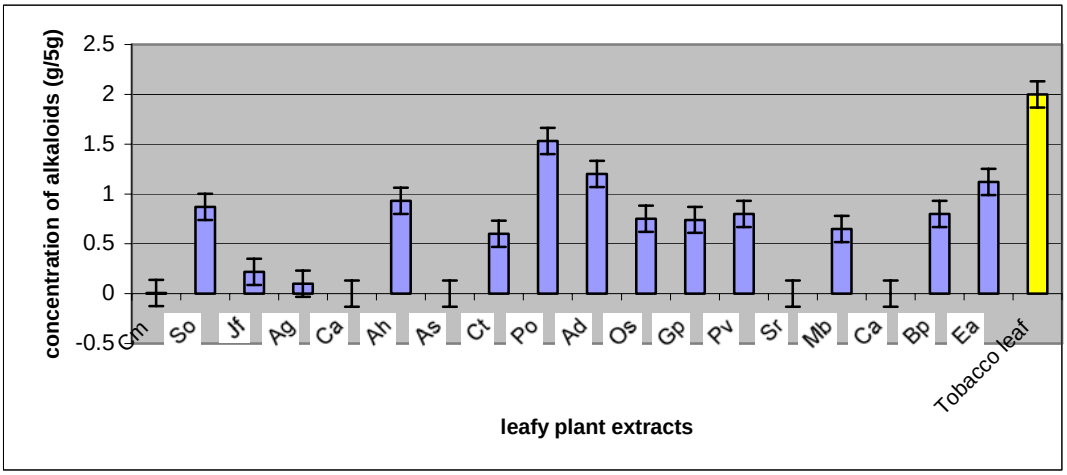


Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*
Standard=saponin		

Figure 4.3 Saponin content of 18 leafy plants

4.3 ALKALOIDS

Alkaloid precipitates measured in the 18 plants are shown in Figure 4.4 below. *Cleome monophylla, Centella asiatica, Solanum retroflexum* and *Chenopodium album* were negative for the presence of alkaloids as there was no precipitation. *Portulaca oleracea, Amaranthus dubius* and *Emex australis* had high concentrations of alkaloids ranging from1.53, 1.2 to 1.12 g/5g respectively. *Portulaca oleracea* had alkaloid concentration higher than that of the standard as the standard only had 1.5 g/5g alkaloid concentration as indicated in Figure 4.4. Plants such as *Amaranthus hybridus, Senna occidentalis, Physalis viscosa* and *Chenopodium album* had fairly high concentrations of 0.93, 0.87, 0.8 and 0.8 g/5g respectively. Low levels (less than 0.5 g/5g) were found in *Cleome monophylla, Justicia flava* and *Asystasia gangetica*. Their concentrations ranged from 0.006, 0.22 and 0.1 g/5g respectively.



Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*
Standard=Tobacco leaf		

Figure 4.4 Alkaloid content in 18 leafy plants

4.4 CYANOGENIC GLYCOSIDES

In this study, all the plants did not release hydrogen cyanide gas and thus the 18 plants were negative. Picrate paper remained yellow in all 18 plant extracts, thus none of the plants contained cyanogenic glycosides as indicated in Figure 4.5.

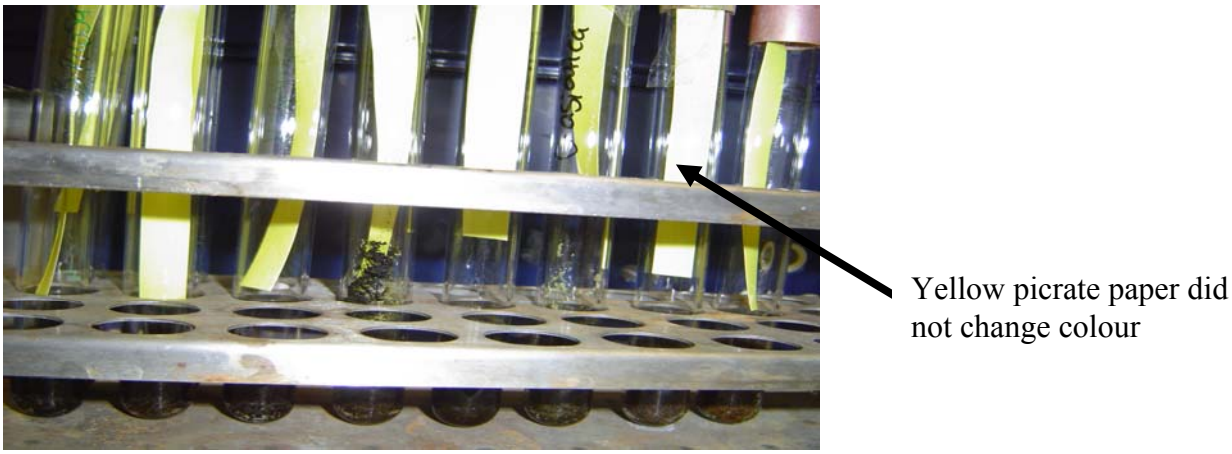


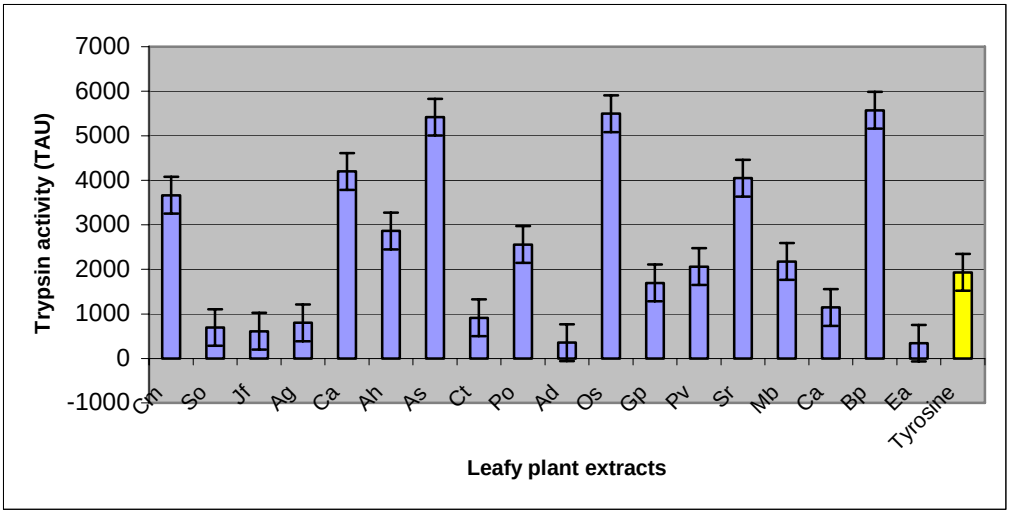
Figure 4. 5: Detection of cyanogenic glycosides in 18 leafy plants using sodium picrate paper

4.5 TRYPSIN INHIBITION

Figure 4.6 illustrates the results of the trypsin inhibitors. *Emex australis, Amaranthus dubius, Justicia flava, Senna occidentalis, Asystasia gangetica* and *Ceratotheca triloba* had reduced activity of trypsin (inhibition effect) compared to the tyrosine standard. Their activity ranged from 343, 354.3, 610, 694, 801 to 912 (TAU) respectively (Figure 4.6).

Oxygonum sinuatum, Bidens pilosa, Amaranthus spinosus, Centella asiatica and Solanum retroflexum had high trypsin activity even when compared to the standard, they had activity which indicates the absence of trypsin inhibitors their activity was 5496, 5573,

5419,4 198 and 4045 (TAU) respectively. A few others such as *Cleome monophylla, Amaranthus hybridus, Portulaca olerace* and *Momordica balsamina* also had trypsin activity.



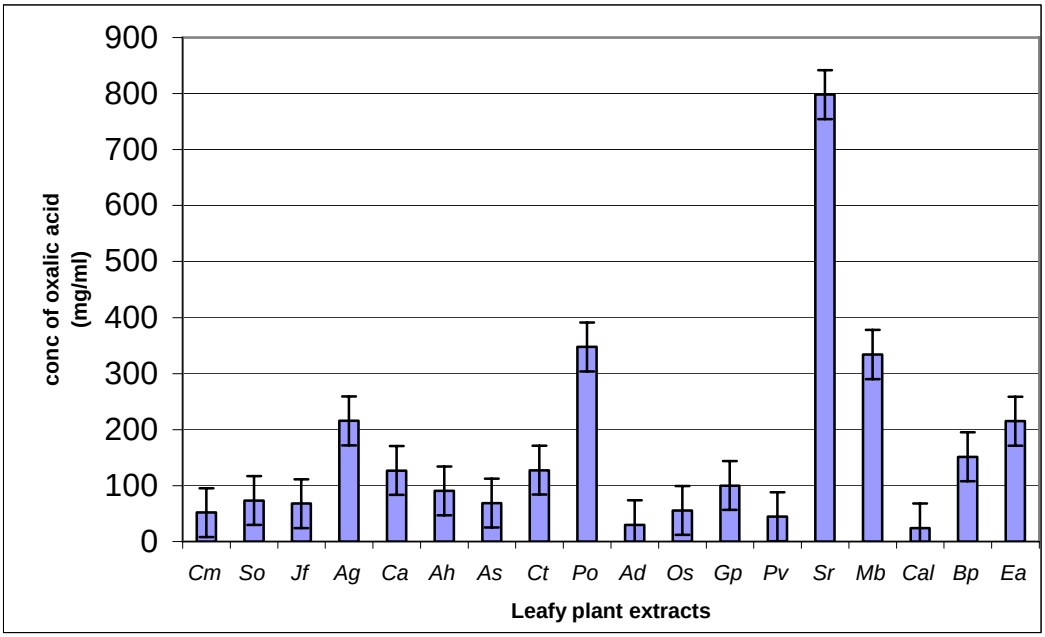
Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*
Standard=Tyrosine		

Figure 4.6 Trypsin activity of 18 leafy plants

4.6 OXALIC ACID

Oxalic acid was quantified using the HPLC. The concentrations of oxalic acid were obtained from 0.5 g dried leafy material. All the plants were positive for this antinutrient with S*olanum retroflexum* having the highest concentration followed by *Portulaca oleracea* and *Momordica balsamina* with concentrtations ranging from 798, 347.5 to 334.05 mg/ml respectively (Figure 4.7). *Chenopodium album* had the lowest concentration of 24.1 mg/ml followed by *Amaranthus dubius* with a concentration of 29.7 mg/ml. Other

plants such as *Cleome monophylla, Senna occidentalis, Justicia flava, Amaranthus hybridus, Amaranthus spinosus, Oxygonum sinuatum* and *Physalis viscosa* also had concentrations lower than 100 mg/ml.



Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*

Figure 4.7 Oxalic acid content in 18 leafy plants

4.7 ANTINUTRIENT PROFILE

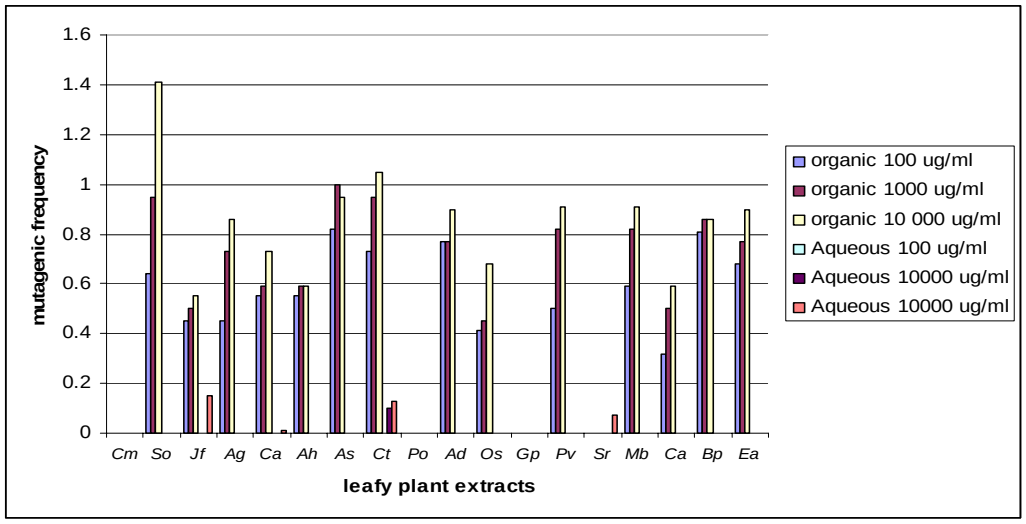
A composite table of results (Table 4.1) was also compiled to indicate antinutrient profiles of each plant. *P.oleracea, M.balsamina, E.australis, A.hybridus* and *A.dubius* contained high levels of all the antinutrients particularly trypsin inhibitors.

Table 4. 1 Antinutrient profile of the 18 traditional leafy vegetables determined in this study.

Vegetables	Cyanogenic glycosides	Alkaloids (g/5g)	Phytates (mg/ml)	Saponins (mg/ml)	Trypsin activity	Oxalic acid (mg/ml)
Standards	0	1.5		2	1931.2	
Cleome monophylla	0	0.01	0.04	0.86	3664	51.9
Senna occidentalis	0	0.87	0.37	0	694.6	73.19
Justicia flava	0	0.22	0.23	0	610	67.7
Asystasia gangetica	0	0.12	0.34	0	801	215.7
Centella asiatica	0	0	0.37	0.58	4198	127.03
Amaranthus hybridus	0	0.93	0.15	0.85	2862	90.7
Amaranthus spinosus	0	0	0.37	0.58	5419	68.8
Ceratotheca triloba	0	0.6	0.04	0.58	912.2	127.5
Portulaca oleracea	0	1.53	0.19	0.58	2557.2	347.5
Amaranthus dubius	0	1.2	0.30	0.86	354.3	29.7
Oxygonum sinuatum	0	0.75	0.15	0	5496	55.6
Galinsoga parviflora	0	0.74	0.37	0	1694.6	100.01
Physalis viscosa	0	0.8	0	0	2061	44.5
Solanum retroflexum	0	0	0.04	1.33	4045	798.2
Momordica balsamina	0	0.65	3.01	1.8	2175.5	334.05
Chenopodium album	0	0	0.04	0	1145	24.1
Bidens pilosa	0	0.8	0.37	0.58	5573	151.6
Emex australis	0	1.12	0.37	0.95	343	215.1

4.8 Mutagenicity

Aqueous and organic extracts of each of the 18 leafy plants were tested and their mutagenic frequencies are shown in Figure 4.8. Mutagenic frequency of *Cleome monophylla, Portulaca oleracea* and *Galinsoga parviflora* was zero for both aqueous and organic plant extracts. *Asystasia gangetica, Amaranthus hybridus, Senna occidentalis, Amaranthus dubius, Amaranthus spinosus, Senna occidentalis, Physalis viscosa, Momordica balsamina, Centella asiatica, Bidens pilosa and Emex australis, Oxygonum sinuatum* and *Chenopodium album* gave a mutagenic frequency of zero for their aqueous extracts. The organic extracts of *Senna Occidentalis, Amaranthus spinosus, Amaranthus dubius, Ceratotheca triloba, Physalis viscosa, Momordica balsamina, Bidens pilosa* and *Emex australis* had low mutagenic frequency since they were above 0.50. a quotient of 2.0 is considered a potential carcinogen, and none of the plants in this study displayed mutagenic potential at concentrations assessed.



Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*

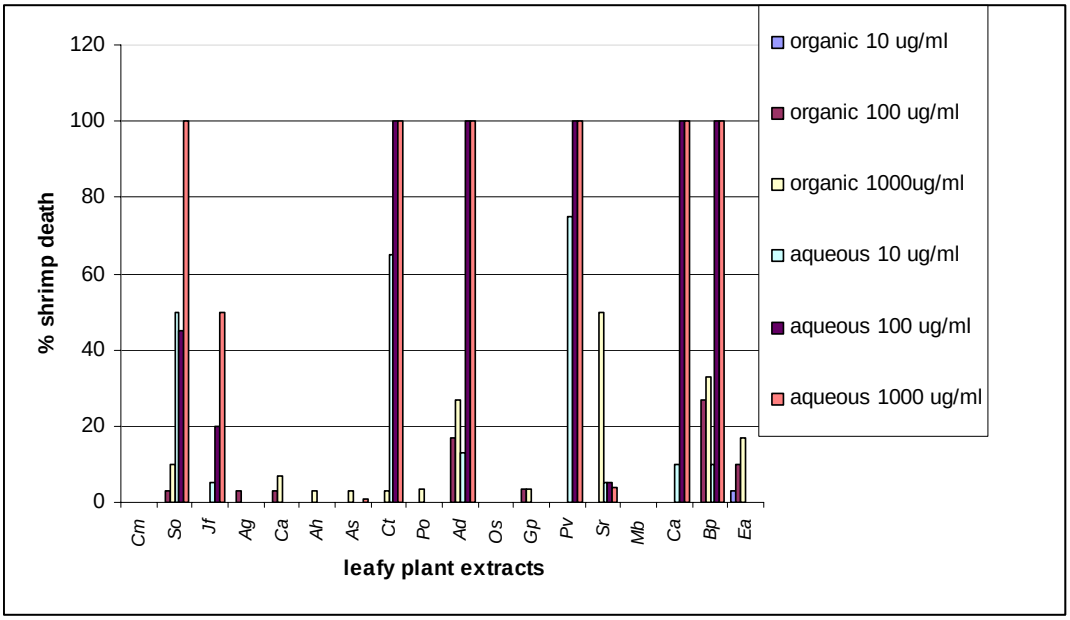
Mutagenic frequency of >2 - considered potential mutagenic

Figure 4.8 Mutagenic frequency of organic and aqueous extracts of leafy plants administered to *Salmonella typhimurium* strain TA 100 at different concentrations

4.9 Toxicity

In this study toxicity was assumed if an extract caused 50% or more shrimp death. The percentage of brine shrimp killed by the various concentrations of aqueous and organic extracts of the 18 plants are shown in Figure 4.9. While aqueous extracts of *Asystasia gangetica, Centella asiatica, Amaranthus hybridus, Portulaca oleracea, Galinsoga parviflora* and *Emex australis* did not kill any shrimp while *Momordica balsamina, Oxygonum sinuatum* and *Cleome monophylla* did not kill any shrimp on both the aqueous and organic extracts. The organic extracts of *Chenopodium album, Physalis viscosa* and

Justicia flava were non-toxic towards the shrimp at concentrations 100, 1000 and 10 000 µg/ml (appendix B) Aqueous extracts of *Amaranthus dubius*, *Ceratotheca triloba*, *Physalis viscosa*, and *Bidens pilosa* were toxic towards the shrimp since all the shrimp died at concentrations 100 and 1000 µg/ml when exposed for 24 h. Aqueous extracts of *Senna occidentalis* killed all the shrimp at 1000 µg/ml.

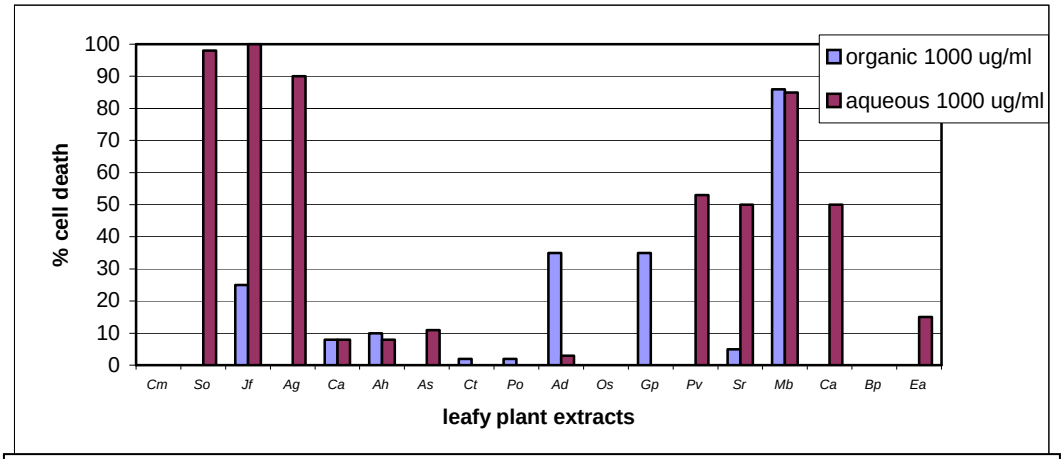


Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*

Figure 4.9 Plant toxicity on brine shrimp

4.10 Cytotoxicity

The cytotoxicity of the plant extracts was determined using MTT cytotoxicity assay. The percentage cell death for all 18 aqueous and organic plant extracts are shown in Figure 4.10 *wrt* to the HepG2 cell line which was used due to it being a liver cell line. Knowing the toxicity to these hepatocytes would be appropriate as most toxic syndrome manifest in the liver. Although most of the plants were found to have lower levels of cytotoxicity, aqueous extracts of *Justicia flava, Asystasia gangetica, Senna occidentalis* and *Momordica balsamina* were found to have high cytotoxicity levels with *Justicia flava* having 100% cell death at 1000 µg/ml. *Momordica balsamina* was found to have high levels of percentage cell death on both aqueous and organic plant extracts, with 85% on aqueous and 86% on organic extracts. *Bidens pilosa* and *Oxygonum sinuatum* were found to be non-cytotoxic since none of the cells died.



Cm-*Cleome monophylla*	S.o-*Senna occidentalis*	J.f-*Justicia flava*
A.g-*Asystasia gangetica*	C.a-*Centella asiatica*	A.h-*Amaranthus hybridus*
A.s-*Amaranthus spinosus*	C.t-*Ceratotheca triloba*	P.o-*Portulaca oleraceam*
A.d-*Amaranthus dubius*	O.s-*Oxygonum sinuatum*	G.p-*Galinsoga parviflora*
P.v-*Physalis viscosa*	S.r-*Solanum retroflexum*	M.b-*Momordica balsamina*
C.alb-*Chenopodium album*	B.p-*Bidens pilosa*	E.a-*Emex australis*

Figure 4.10 Plant cytotoxicity on HepG2 cell line

CHAPTER FIVE

DISCUSSION

There are more than 900 different phytochemicals that have been identified as components of food. It is estimated that there may be more than 100 different phytochemicals in one vegetable (Fowler, 2002). Some of these phytochemicals are antinutrients, and are said to reduce the bioavailability of essential nutrients (Aletor and Adeogun, 1995) and also cause toxicity and mutagenicity.

5.1 ANTINUTRIENTS

Phytic acid is a natural organic plant compound (Harland and Oberleas, 1986) that is a simple ringed carbohydrate with six phosphate groups attached to each carbon. It is a major phosphate storage compound in plants and can account for 80% of total phosphorous (Lopez *et al*., 2002). The presence of phytic acid in plants consumed may inhibit iron absorption in infants to an extent similar to that in adults. Iron deficiency in infants can lead to reduced psychomotor and mental development with long term negative consequences on school performance (Davisson *et al*., 1994). All the plants were found to have low concentrations of phytic acid. *Momordica balsamina* was found to have the highest concentration of phytic acid of 3.01 mg/ml. Only *Physalis viscosa* was found to contain no phytic acid. Some plants such as *Cleome monophyla, Ceratotheca triloba, Solanum retroflexum* and *Chenopodium album* had the same concentration of 0.04 mg/ml. Other plants such as *Justicia flava* were amongst the plants that contain phytic acid with a

concentration of 0.19 mg/ml. There is no information in literature regarding the relationship between the plants in this study and phytic acid, however, Sirkka (1997) reported that phytic acid markedly reduced calcium bioavailability and formed calcium-phytate complexes that inhibit Fe and Zn. Phytic acid intake of 4-9 mg/100g DM is said to decrease absorption of iron by 4-5 fold in human (Hurrel *et al*., 1992). Depending on the amount of plant-derived foods in the diet and the grade of food processing, the daily intake of phytic acid can be as high as 4 500 mg (Reddy, 2002). In average, Reddy *et al.* (1982) found the daily intake of phytic acid to be an estimated 2000-2600 mg for vegetarian diets as well as diets of inhabitants of rural areas of developing countries, and 150-1400 mg for mixed diets.

Saponins are glycoside compounds often referred to as "natural detergents" because of their foamy texture. They are chemical structures consisting of triterpenoidal or steroidal aglycones with various carbohydrate moieties that are found in many plants (MacDonald *et al.,* 2005). On injection, saponin, like all detergents cause lysis of the red blood cells, called haemolysis and are therefore toxic (Davidson, 2005). *Solanum nigrum* and *Momordica balsamina* had the highest concentrations of saponin, they were found to have concentrations of 1.33 and 1.8 mg/ml respectively.

Other plants that had saponins were *Emex australis, Bidens pilosa, Portulaca oleracea* and all the *Amaranth* plants amongst others. *Senna occidentalis, Physalis viscosa* and *Oxygonum sinuatum* were some of the plants that did not have any saponins. The high amount of saponin in *Solanum retroflexum* correlates with a study by Ferreira *et al*. (1996) that reported that the *Solanaceae* family is known to contain saponins. Saponins such as

ophiopogonins and ginsenocides haemagglutinated human, rabbit, and sheep erythrocytes but were not haemolytic. It was therefore concluded that these saponins were able to bind to the membrane lipids of erythrocytes and form bridges between the cells. Steroid and triterpenoid saponins with a single sugar chain were found to have strong haemolytic activity (Fuduka *et al*., 1985).

Alkaloids were not detected at levels assessed in this study in the following vegetables, namely, *Amaranthus spinosus, Centella asiatica, Solanum nigrum* and *Chenopodium album*. *Cleome monophyla* had the lowest alkaloid concentration of 0.01 g/5g of extract while *Portulaca oleracea, Emex australis* and *Amaranthus dubius* had the high concentration of 1.53, 1.12 and 1.2 g/5g respectively. There were other plants that exhibited concentrations lower than 1 g/5g of alkaloids for example *Amaranthus hybridus, Senna occidentalis, Physalis viscosa* and *Bidens pilosa* amongst others. Plant extracts of *Asystasia gangetica* and *Justicia flava* exhibited fairly low concentrations of 0.12 and 0.22 g/5g extract respectively. The absence of alkaloids in *Solanum retroflexum* however, contradicts with a report by Van Wyk *et al*. (2002) that suggested that tropane alkaloids occur in the *Solanaceae* family. This report was however not conclusive. The contradiction could also be due to the methods used and the concentrations assessed.. The fatal dose of alkaloids in humans has been reported as 50 mg/kg (Van Wyk *et al.,* 2002).

Cyanide is one of the most rapidly poisons known with a lethal dose of 0.5-3mg/kg body weight. This is due to its ability of linking with metals (Fe^{2+}, Mn^{2+}, and Cu^{2+}) that are functional groups of many enzymes, inhibiting processes like the reduction of oxygen in cytochrome respiratory chain, electron chain transport in photosynthesis and the acting of

enzymes such as catalase, and oxidase (Cheeke, 1997). All the plants investigated did not have any cyanogenic glycosides at the levels tested. The reason may be that the plant material was dried and other researchers such as Tan and Yeoh (1997) use fresh material. The dried plant material was used because the study focuses on the dried plants and not the fresh material. The analysis in this study concentrated on dried material.

Trypsin inhibitors, according to Venter and Van Eyssen (2001) are compounds that interfere with protein digestion, they cause pancreatic enlargement and enhance chemically induced pancreatic tumors. All the plant extracts tested were found to have trypsin activity. Some of the plants inhibited the trypsin activity while the others enhanced it. Extracts of *Bidens pilosa, Solanum retroflexum, Oxygonum sinuatum, Amaranthus spinosus* and *Centella asiatica* enhance the trypsin activity to more than double. Other plants such as *Amaranthus dubius, Senna occidentalis, Justicia flava, Asistacia gangentica, Ceratotheca triloba, Galinsoga parviflora, Chenopodium album and Emex australis* inhibited the activity of trypsin. *Emex australis* and *Amaranthus dubius* had the lowest trypsin activity of 343 and 354.3 respectively. However the trypsin inhibitors are heat-labile in nature and this suggests that they can be inactivated by cooking (Prathibha *et al*., 1995). And this also shows that if the vegetable is properly cooked, the trypsin inhibitors may not interfere with digestion (Bhandari and Kawabata, 2004).

Oxalic acid is one of the antinutritional factors which are widely distributed in plant foods (Gupta, *et al.*, 2005). A lethal dose of oxalic acid has been reported in *D. teltoida* (high oxalate containing yams) as 1 kg of fresh material (at once) as it contains 2 g oxalic acid, which is thought to be a lethal dose in humans (Libert, *et al*., 1987). Massey *et al*., (2001) advised patients who suffer from kidney stones to limit their oxalates intake to 50-60 mg

per day. *Centella asiatica* was reported by Gupta *et al*. (2005) as containing oxalic acid, it was found to contain 60 mg/100g fresh vegetable total oxalates and 20 mg/100 g soluble oxalates which contradicts with 127.03 mg/ml which was reported in this study.

Portulaca oleracea commonly known as purslane was reported by Palaniswamy *et al*. (2004) as containing high concentrations of oxalic acid (1.31 g/100g) and this has contributed to its limited consumption. Despite its nutritive value in human diet, the acceptance of purslane as a green leafy vegetable is limited to a large extent because of reported accumulation of large amounts of oxalic acid (Palaniswamy *et al*. 2004).

Results still need to be extrapolated to the amounts of these vegetables consumed by the average person. Thus we cannot categorically suggest these plant not be consumed due to the antinutrient concentrations found.

5.2 BIOASSAYS

The Ames test revealed that none of the leafy vegetables produced a mutagenic frequency above 1, except 10 000 µg/ml organic extract of *Senna occidentalis* (mutagenecity considered at mutagenic frequency above 2), thus none were considered mutagenic (Elgorashi *et al*., 2003). All 18 organic extracts did not kill off more than 50% brine shrimp and were thus considered non-toxic. On the other hand the aqueous extracts of seven vegetables, namely, *Physalis viscosa, Amaranthus dubius, Justicia flava, Bidens pilosa, Senna occidentalis, Chenopodium album and Ceratotheca triloba,* killed more than 50% of the shrimp and are thus considered toxic above 100 µg/ml. Bidebs pilosa which

was found to be toxic to Brine shrimp has been reported in literature to be phototoxic (Duke and Ayensu, 1985). *Chenopodium album* has also been reported as being toxic as it contains saponins and oxalic acid (Beekrum, 2003). The MTT assay carried out on the organic extracts indicated that 17 vegetables did not kill off more than 50% of HepG2 cells and were thus considered non-cytotoxic. The aqueous extracts of four vegetables, namely, *Justicia flava, Asystasia gangetica, Momordica balsamin* and *Senna occidentalis,* however killed more than 50% of the shrimp and were thus considered cytotoxic above 1 000 µg/ml. It may be concluded from the antinutrient analyses and the bioassays on the 18 vegetables that caution needs to be maintained with the consumption of certain leafy vegetables included in this study, especially Senna occidentalis.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

The plants evaluated in this study are well endowed with all of the essential nutrients required for human nutrition. A recent study showed that *Momordica balsamina*, *Amaranthus spinosus*, *Amaranthus hybridus*, *Cleome monophylla*, *Bidens pilosa*, *Chenopodium album*, *Physalis viscosa, Senna occidentalis* and *Centella asiatica* provide excellent sources of protein and minerals (Odhav *et al.,* 2007; Beekrum, 2003). Of these 9 nutrition-rich plants, *Bidens pilosa*, *Momordica balsamina*, *Chenopodium album*, *Physalis viscosa* and *Senna occidentalis* have shown potential toxicity and cytotoxicity. Their consumption should therefore be cautioned. Consumption of other plants evaluated in this study, such as *Amaranthus dubius, Emex australis, Momordica balsamina, Solanum retroflexum* and *Portulaca oleracea,* should also be cautioned as they have indicated high antinutrient content especially trypsin inhitors. These plants may predispose toxic effects and / or reduce the bioavailability of nutrients in humans when consumed. The fact that the aqueous extract were generally found to be more toxic suggests that the method of cooking may have an effect on the potential toxicity of the vegetables. The effects of cooking on the toxicity of the vegetables should be carried out in future.

Most of the 18 plants contained at least one antinutrient in varying concentrations. Consumption of plants with low concentrations of antinutrients may appear safe at face value, but it should be noted that consumption of these plants over a long period as staple food in the case of the traditional South African may contribute to adverse accumulative

effects in the consumer. Thus further investigation with respect to the accumulative effect and toxic doses need to be established.

A database outlining the antinutrient, toxicity, cytotoxicity and mutagenicity is provided in Table 6.1. This will be added onto a website for broad access by the community.

Table 6.1 Database of the antinutrient content, toxicity and mutagenicity of 18 leafy vegetables from Kwa-Zulu Natal.

Vegetable names	Saponin (mg/ml)	Trypsin inhibitors	Oxalic acid mg/ml	Alkaloids (g/5g)	Phytic acid (mg/ml)	Cyanogenic glycosides	Mutagenicity	Toxicity	Cytotoxicity
Cleome monophyla	+	-	+	+	+	-	-	-	n/t
Senna occidentalis	-	+	+	+	+	-	-	+	+
Justicia flava	-	+	+	+	+	-	-	+	+
Asistacia gangentica	-	+	+	+	+	-	-	-	+
Centella asiatica	+	-	+	-	+	-	-	-	-
Amaranthus hybridus	+	-	+	+	+	-	-	-	-
Amaranthus spinosus	+	-	+	-	+	-	-	-	-
Ceratotheca triloba	+	+	+	+	+	-	-	+	-
Portulaca oleracea	+	+	+	+	+	-	-	-	-
Amaranthus dubius	+	+	+	+	+	-	-	+	-
Oxygonum sinuatum	-	-	+	+	+	-	-	-	-
Galinsoga parviflora	-	-	+	+	+	-	-	-	-
Physalis viscosa	-	+	+	+	-	-	-	+	+
Solanum retroflexum	+	-	+	-	+	-	-	-	+
Momordica balsamina	+	-	+	+	+	-	-	-	+
Chenopodium album	-	+	+	-	+	-	-	+	+
Bidens pilosa	+	-	+	+	+	-	-	+	-
Emex australis	+	+	+	+	+	-	-	-	-

(+) indicates the presence and a (-) indicates the absence of either an antinutrient, mutagenicity, toxicity or cytotoxicity

(+) for mutagenicity indicates that the plant produced a mutagenic frequency greater than 2.

(+) for trypsin inhibitors indicates that the plant contains trypsin activity below that of the standard tyrosine

(+) for cytotoxicity means that the plant extract caused 50% or more cell death

(+) for toxicity means that the plant extract caused 50% or more shrimp death

n/t means that the extract was not tested

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APPENDIX A

NUTRIENT AND ANTINUTRIENT PROFILE OF 18 PLANTS USED IN THIS STUDY

Previous published results of the nutritional composition of the 18 plants used in the study indicate their nutritional value in terms of diet (Beekrum *et al.*, 2003; Odhav *et al*., 2007). In order to put the antinutrient properties of these plants in perspective, complete profiles of each plant has been compiled.

Solanum retroflexum

Solanum retroflexum (Figure 5.1) belongs to the family *Solanaceae.* It is consumed regularly by the local community who use the leaves as potherb relish and the fruit is eaten when young. It is also used as antiphlogistic, diaphoretic, diuretic, emollient, antiperiodic, febrifuge, narcotic, purgative, sedative and is also as poultice in treatment of cancerous sores, leucoderma wounds (Beekrum, 2003). Its toxicity is not clear but the green berries contain solanine causing degrees of poisoning (Tredgold *et al.*, 1986).

Figure 5.1 *Solanum retroflexum*

Nutritional value (per 100 g fresh weight ± SD)

Energy (kJ), 229.31 ± 4.86; Moisture (%), 84.84 ± 0.17; Protein (g), 3.27 ± 0.21; Fat (g), 0.62 ± 0.03; Fibre (g), 2.42 ± 0.03; Ash (g), 2.24 ± 0.16; Carbohydrates (g), 9.03 ± 0.57

Micronutrient content studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}); 2067.48 ± 7.25; P (mg 100 g^{-1}), 478.01 ± 0.39; Na (mg 100 g^{-1}), 431.34 ± 2.21; Cu (mg 100 g^{-1}), 6.30 ± 0.14; Zn (mg 100 g^{-1}), 23.49 ± 1.87; Mg (mg 100 g^{-1}), 276.86 ± 1.15; Mn (mg 100 g^{-1}), 2.77 ± 0.17; Fe (mg 100 g^{-1}), 84.53 ± 0.35 (Beekrum, 2003).

Antinutrients (per 5 g dry weight)

Phytic acid (0.04 mg/ml); Saponin (1.33 mg/ml); Oxalic acid (798.2 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0 mg/ml); Trypsin inhibitors (4045 TIU).

Toxicity (100-10 000 µg/ml)

Aqueous (100 µg/ml-5%; 1000 µg/ml-5%; 10 000 µg/ml-4%); Organic (100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-50%)

Cytoxicity *(100-10 000 µg/ml)*

Aqueous extract (> 50% HepG2 cell death; Organic 5%

Mutagenic frequency *(100-10 000 µg/ml)*

Aqueous (100 µg/ml-0; 1000 µg/ml-007; 10000 µg/ml- 0); Organic (100 µg/ml- 0; 1000 µg/ml-0; 10 000 µg/ml-0)

Physalis viscosa

Physalis viscosa (Figure 5.2) also belong to the family Solanaceae. It is regarded as a famine food but is an important food supplement in the Transkei region. Important food supplement in Transkei where the fruit and berries are edible. The plant is used as a tonic, laxative, diuretic and sedative. The juice of the berries is beneficial in several urinary disorders. Used to treat inflammatory diseases (Beekrum *et al.,* 2003). No toxicity data for this plant is reported.

Figure 5.2 *Physalis viscosa*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 289.63 ± 6.86; Moisture (%),81.49 ± 0.3; Protein (g), 5.62 ± 0.06; Fat (g), 0.83 ± 0.06; Fibre (g), 1.97 ± 0.07; Ash (g), 2.25 ± 0.18; Carbohydrates (g), 9.81 ± 0.59

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1166.97 ± 9.52; P (mg 100 g^{-1}), 616.25 ± 6.46; Na (mg 100 g^{-1}), 363.79 ± 1.51; Cu (mg 100 g^{-1}), 3.60 ± 0.21; Zn (mg 100 g^{-1}), 14.48 ± 1.71; Mg (mg 100 g^{-1}), 535.21 ± 0.64; Mn (mg 100 g^{-1}), 1.73 ± 0.07; Fe (mg 100 g^{-1}), 19.82 ± 0.65 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0 mg/ml); Saponin (0 mg/ml); Oxalic acid (44.5 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.8 mg/ml); Trypsin inhibitors (2061 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-75%; 1000 µg/ml-100%; 10000 µg/ml-100%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 53% ; Organic 0%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.5; 1000 µg/ml-0.82; 1000 µg/ml-0.91]

Momordica balsamina

The leaves and the ripe fruit of *Momordica balsamina* (Figure 5.3) are consumed regularly amongst the African population. Cooked as spinach, with nuts or mielie meal, relish, soup (Fox and Norwood Young, 1982). Leaves used for liver deficiencies, blood cleanser, ulcers of the stomach and duodenum, inflammations, insomnia, marsh fever, urinary tract infections, and bile disorders. Used as a purgative, emetic, bitter stomachic and as a wash for fever and yaws (Beekrum *et al*., 2003). No toxicity reports available.

Figure 5.3 *Momordica balsamina*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 222.15 ± 4.09; Moisture (%), 85.28 ± 0.29; Protein (g), 5.34 ± 0.31; Fat (g), 0.49 ± 0.04; Fibre (g), 2.75 ± 0.04; Ash (g), 2.07 ± 0.1; Carbohydrates (g), 6.82 ± 0.16

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 2688.20 ±153.24; P (mg 100 g^{-1}), 355.90 ± 3.24; Na (mg 100 g^{-1}), 376.24 ± 1.51; Cu (mg 100 g^{-1}), 2.68 ± 0.27; Zn (mg 100 g^{-1}), 11.83 ± 1.73; Mg (mg 100

g^{-1}), 612.68 ± 1.78; Mn (mg 100 g^{-1}), 9.60 ± 0.51; Fe (mg 100 g^{-1}), 22.84 ± 2.33 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (3.01 mg/ml); Saponin (1.8 mg/ml); Oxalic acid (334.05 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.65 mg/ml); Trypsin inhibitors (2175 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-3.3%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 85% ; Organic 86%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.59; 1000 µg/ml-0.82; 1000 µg/ml-0.91]

Amaranthus spinosus

*Amaranthus spinosus (*Figure 5.4) belong to the Amaranthacaeae family, eaten raw or cooked as spinach. Famine food (Fox and Norwood Young, 1982). Astringent, diaphoretic, diuretic, emollient, febrifuge, purgative. Used to treat snake bites, ulcerated mouths, vaginal discharges, nosebleeds, and wounds. No members of this genus are known to be poisonous (Duke, 2000).

Figure 5.4 *Amaranthus spinosus*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 111.02 ± 1.12; Moisture (%), 91.36 ± 0.25; Protein (g), 4.12 ± 0.06; Fat (g), 0.60 ± 0.01; Fibre (g), 2.48 ± 0.08; Ash (g), 2.76 ± 0.34; Carbohydrates (g), 1.16 ± 0.16

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 3930.58 ± 15.3; P (mg 100 g^{-1}), 628.60 ± 7.23; Na (mg 100 g^{-1}), 392.92 ± 3.03; Cu (mg 100 g^{-1}), 3.36 ± 0.34; Zn (mg 100 g^{-1}), 15.47 ± 0.86; Mg (mg 100 g^{-1}), 1165.64 ± 2.57; Mn (mg 100 g^{-1}), 3.02 ± 0.07; Fe (mg 100 g^{-1}), 31.92 ± 2.46 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.37 mg/ml); Saponin (0.58 mg/ml); Oxalic acid (68.8 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0 mg/ml); Trypsin inhibitors (5419 TU).

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-1%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-3%)

Cytoxicity(Aqueous 11% ; Organic 0%)

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml-1]; Organic [100 µg/ml-0.82; 1000 µg/ml-1; 1000 µg/ml-0.95]

5.3.5 Amaranthus hybridus

Amaranthus hybridus (Figure 5.5) belong to the Amaranthacaeae family. Relish, mixed with mealie meal (Fox and Norwood Young, 1982). Cooked as spinach, added to soups, eaten raw . Tea made from leaves is astringent. Used in treatment of intestinal bleeding, diarrhoea, and excessive menstruation. No members of this genus are known to be poisonous (Duke, 2000).

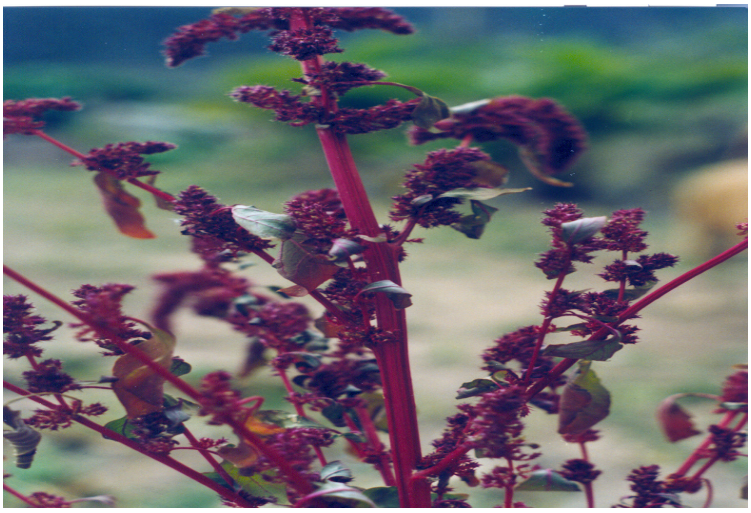

Figure 5.5 *Amaranthus hybridus*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 221.07 ± 1.29; Moisture (%), 82.55 ± 0.07; Protein (g), 5.92 ± 0.04; Fat (g), 0.53 ± 0.08; Fibre (g), 2.81 ± 0.03; Ash (g), 4.91 ± 0.11; Carbohydrates (g), 6.09 ± 0.31

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 2363.26 ± 0.41; P (mg 100 g^{-1}), 603.94 ± 1.00; Na (mg 100 g^{-1}), 427.10 ± 0.18; Cu (mg 100 g^{-1}), 2.38 ± 0.33; Zn (mg 100 g^{-1}), 17.93 ± 0.28; Mg (mg 100 g^{-1}), 1316.88 ± 2.28; Mn (mg 100 g^{-1}), 24.38 ± 0.91; Fe (mg 100 g^{-1}), 21.2 ± 0.54 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0 mg/ml); Saponin (0 mg/ml); Oxalic acid (44.5 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.8 mg/ml); Trypsin inhibitors (2061 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-0%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-3%); Cytoxicity(Aqueous 8% ; Organic 10%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.55; 1000 µg/ml-0.59; 1000 µg/ml-0.59].

Amaranthus dubius

Amaranthus dubius (Figure 5.6) belong to the Amaranthacaeae family and are very popular spinaches. These species are harvested in summer as many times as possible. They are not stored for later use and therefore cooked immediately after harvesting. Potherb, cooked and eaten as spinach. Seeds are nutritious. The whole plant is used to alleviate stomach pains (Beekrum *et al.*, 2003). They are known toxicity effects

Figure 5.6 *Amaranthus dubius*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 205.79 ± 5.68; Moisture (%), 84.59 ± 0.38; Protein (g), 3.89 ± 0.08; Fat (g), 0.24 ± 0.06; Fibre (g), 2.87 ± 0.04; Ash (g), 3.42 ± 0.03; Carbohydrates (g), 7.86 ± 0.55

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1686.20 ± 0.44; P (mg 100 g^{-1}), 487.40 ± 0.24; Na (mg 100 g^{-1}), 346.99 ± 1.06; Cu (mg 100 g^{-1}), 2.76 ± 0.17; Zn (mg 100 g^{-1}), 56.12 ± 1.47; Mg (mg 100 g^{-1}), 805.63 ± 2.14; Mn (mg 100 g^{-1}), 81.92 ± 0.92; Fe (mg 100 g^{-1}), 25.11 ± 0.34 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0 mg/ml); Saponin (0 mg/ml); Oxalic acid (44.5 mg/ml); Cyanogenic glycosides
(0 mg/ml); Alkaloids (0.8 mg/ml); Trypsin inhibitors (2061 TU).

Bioassays per 50 g dry weight

Toxicity : (aqueous [100 µg/ml-12%; 1000 µg/ml-100%; 10000 µg/ml-100%]; organic [100 µg/ml-0%; 1000 µg/ml-17 %; 10000 µg/ml-27%)

Cytoxicity (Aqueous 3% ; Organic 35%)

Mutagenic frequency: (aqueous [100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml-0]; organic [100 µg/ml- 0.0.77; 1000 µg/ml-0.77; 1000 µg/ml-0.9]

Asystasia gangetica

Asystasia gangetica (Figure 5.7) belong to the Acanthaceae family. It is indigenous to Africa and is rarely consumed but this plant serves as a soap substitute. To ease childbirth pains, facilitate labour, stiff neck, nose bleeding, stomach aches, fever aches, epilepsy, heart pains and urethral discharge (Beekrum *et al*., 2003). Not toxic.

figure 5.7 *Asystasia gangetica*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 207.63 ± 4.03; Moisture (%), 85.36 ± 0.35; Protein (g), 3.05 ± 0.06; Fat (g), 0.48 ± 0.06; Fibre (g), 1.63 ± 0.04; Ash (g), 2.84 ± 0.04; Carbohydrates (g), 8.27 ± 0.31

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 2565.89 ± 0.81; P (mg 100 g^{-1}), 813.75 ± 0.61; Na (mg 100 g^{-1}), 932.81 ± 0.96; Cu (mg 100 g^{-1}), 3.59 ± 0.17; Zn (mg 100 g^{-1}), 6.99 ± 0.17; Mg (mg 100 g^{-1}),

960.56 ± 1.19; Mn (mg 100 g^{-1}), 18.07 ± 0.23; Fe (mg 100 g^{-1}), 20.77 ± 1.19 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.34 mg/ml); Saponin (0 mg/ml); Oxalic acid (215.7 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.1 mg/ml); Trypsin inhibitors (801 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-0%]; Organic [100 µg/ml-0%; 1000 µg/ml-3 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 90% ; Organic 0%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.45; 1000 µg/ml-0.73; 1000 µg/ml-0.86]

Justicia flava

Justicia flava (Figure 5.8) belong to the Acanthaceae familty. It is semi-cultivated and eaten as a vegetable . Stomach ache and diarrhoea . Treats fevers and yaws. Leaves used as emetics and eye lotions (Beekum *et al.,* 2003). No toxicity reports available.

Figure 5.8 *Justicia flava*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 215.52 ± 1.78; Moisture (%), 84.31 ± 0.1; Protein (g), 3.20 ± 0.03; Fat (g), 0.40 ± 0.03; Fibre (g), 1.39 ± 0.01; Ash (g), 3.32 ± 0.04; Carbohydrates (g), 8.77 ± 0.2

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 2072.53 ± 0.95; P (mg 100 g^{-1}), 292.30 ± 0.27; Na (mg 100 g^{-1}), 580.96 ± 0.83; Cu (mg 100 g^{-1}), 5.54 ± 0.24; Zn (mg 100 g^{-1}), 10.68 ± 1.13; Mg (mg 100 g^{-1}), 1408.86 ± 3.8; Mn (mg 100 g^{-1}), 8.4 ± 0.18; Fe (mg 100 g^{-1}), 16.31 ± 0.08 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.23 mg/ml); Saponin (0 mg/ml); Oxalic acid (67.7 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.22 mg/ml); Trypsin inhibitors (610 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-5%; 10000 µg/ml-20%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 100% ; Organic 25%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0.15]; Organic [100 µg/ml- 0.45; 1000 µg/ml-0.5; 1000 µg/ml-0.55]

Emex australis

Emex australis (Figure 5.9) belong to the family Polygonaceae. *Emex australis* is widespread in South Africa and available throughout the year and therefore there is no need to store this plant. Cooked as spinach (Fox and Norwood Young, 1982). Used to treat gastrointestinal disorders, colic, biliousness and dyspepsia . Contains oxalic acid .

Figure 5.9 *Emex australis*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 148.61 ± 4.97; Moisture (%), 89.18 ± 0.21; Protein (g), 4.93 ± 0.0; Fat (g), 0.54 ± 0.06; Fibre (g), 1.57 ± 0.07; Ash (g), 2.62 ± 0.01; Carbohydrates (g), 2.73 ± 0.17

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1601.79 ± 17.17; P (mg 100 g^{-1}), 290.43 ± 8.19; Na (mg 100 g^{-1}), 332.39 ± 0.41; Cu (mg 100 g^{-1}), 0.77 ± 0.2; Zn (mg 100 g^{-1}), 20.38 ± 2.52; Mg (mg 100 g^{-1}), 1017.64 ± 2.59; Mn (mg 100 g^{-1}), 30.59 ± 2.29; Fe (mg 100 g^{-1}), 15.21 ± 0.51 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.37 mg/ml); Saponin (0.95 mg/ml); Oxalic acid (215.1 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (1.12 mg/ml); Trypsin inhibitors (801 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-0%]; Organic [100 µg/ml-3%; 1000 µg/ml-10 %; 10000 µg/ml-17%); Cytoxicity(Aqueous 15% ; Organic 0%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.68; 1000 µg/ml-0.77; 1000 µg/ml-0.9]

Oxygonum sinuatum

Oxygonum sinuatum (Figure 5.10) belong to the family Polygonaceae. Leaf sap used for cough and bronchial catarrh. Used for gastric ulcers, malaria and hepatitis (Beekrum *et al*., 2003. No toxicity data of this plant is available.

Figure 5.10 *Oxygonum sinuatum*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 108.63 ± 7.40; Moisture (%), 91.94 ± 0.66; Protein (g), 2.58 ± 0.16; Fat (g), 0.47 ± 0.1; Fibre (g), 1.68 ± 0.01; Ash (g), 2.16 ± 0.1; Carbohydrates (g), 2.85 ± 0.51

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1474.33 ± 0.25; P (mg 100 g^{-1}), 472.81 ± 1.26; Na (mg 100 g^{-1}), 1459.78 ± 5.64; Cu (mg 100 g^{-1}), 3.82 ± 0.11; Zn (mg 100 g^{-1}), 7.49 ± 0.49; Mg (mg 100 g^{-1}), 520.95 ± 3.3; Mn (mg 100 g^{-1}), 4.38 ± 0.35; Fe (mg 100 g^{-1}), 39.39 ± 1.51 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.15 mg/ml); Saponin (0 mg/ml); Oxalic acid (100.1 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.74 mg/ml); Trypsin inhibitors (1694.6 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-0%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 0% ; Organic 0%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.41; 1000 µg/ml-0.45; 1000 µg/ml-0.68]

Bidens pilosa

Bidens pilosa (Figure 5.11) belong to the family Asteraceae. Both plants are weeds of cultivated areas. The leaves of these plants are harvested in summer by the local community and stored during the winter months. Potherb, tea, salads, soups, and stews. Relish, peanut butter added (Tredgold, 1986). Leaves are anti-inflammatory, styptic, and anti-rheumatic. Substances isolated are bactericidal, fungicidal. A juice made from leaves used to dress wounds (Duke and Ayensu, 1985). Phototoxic. Substances isolated from leaves can kill human skin in the presence of sunlight at concentrations as low as 10 ppm (Duke and Ayensu, 1985).

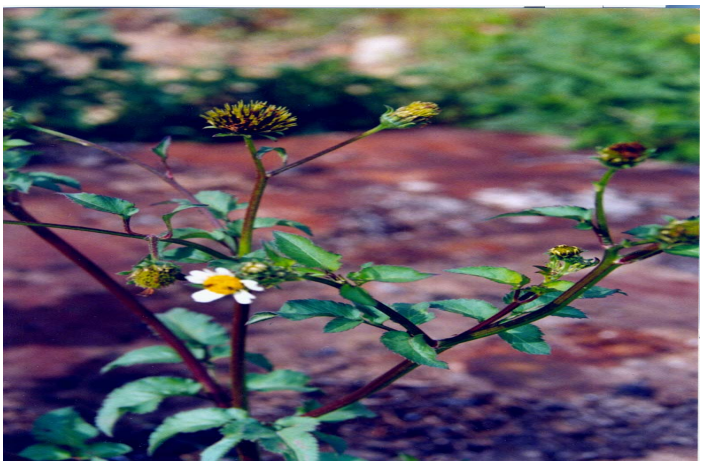

Figure 5.11 *Bidens pilosa*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 163.85 ± 1.18; Moisture (%), 88.12 ± 0.1; Protein (g), 4.76 ± 0.08; Fat (g), 0.58 ± 0.06; Fibre (g), 2.92 ± 0.13; Ash (g), 2.82 ± 0.04; Carbohydrates (g), 3.72 ± 0.28

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1353.61 ± 191.11; P (mg 100 g^{-1}), 504.42 ± 7.98; Na (mg 100 g^{-1}), 289.99 ± 1.23; Cu (mg 100 g^{-1}), 9.67 ± 1.06; Zn (mg 100 g^{-1}), 22.64 ± 3.1; Mg (mg 100 g^{-1}), 657.78 ± 0.44; Mn (mg 100 g^{-1}), 20.83 ± 1.12; Fe (mg 100 g^{-1}), 17.33 ± 0.1 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.37 mg/ml); Saponin (0.95 mg/ml); Oxalic acid (215.1 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (1.12 mg/ml); Trypsin inhibitors (801 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-10%; 1000 µg/ml-100%; 10000 µg/ml-100%]; Organic [100 µg/ml-0%; 1000 µg/ml-27 %; 10000 µg/ml-33%); Cytoxicity(Aqueous 0% ; Organic 0%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.81; 1000 µg/ml-0.86; 1000 µg/ml-0.86]

Galinsoga parviflora

Galinsoga parviflora (Figure 5.12) belong to the family Asteraceae. Eaten as a potherb, raw or cooked. Flavouring in soups. When rubbed onto the body, the plant is useful in treating nettle stings (Chopra *et al*., 1986). An extract of the fresh leaves is used to dress wounds and cuts. No toxicity reports available.

Figure 5.12: *Galinsoga parviflora*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 170.58 ± 7.88; Moisture (%), 88.71 ± 0.4; Protein (g), 3.75 ± 0.04; Fat (g), 0.51 ± 0.04; Fibre (g), 1.24 ± 0.08; Ash (g), 1.74 ± 0.13; Carbohydrates (g), 5.29 ± 0.61

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 161.82 ± 1.68; P (mg 100 g^{-1}), 38.02 ± 0.68; Na (mg 100 g^{-1}), 35.78 ± 1.15; Cu (mg 100 g^{-1}), 3.41 ± 0.27; Zn (mg 100 g^{-1}), 13.65 ± 0.11; Mg (mg 100 g^{-1}), 681.42 ± 1.68; Mn (mg 100 g^{-1}), 43.97 ± 1.32; Fe (mg 100 g^{-1}), 27.3 ± 0.35 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.37 mg/ml); Saponin (0mg/ml); Oxalic acid (100.1 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.74 mg/ml); Trypsin inhibitors (1694.6 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-0%]; Organic [100 µg/ml-0%; 1000 µg/ml-3.3 %; 10000 µg/ml-3.3%); Cytoxicity(Aqueous 0% ; Organic 35%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0; 1000 µg/ml-0; 1000 µg/ml-0]

5.3.13 Cleome monophylla

Cleome monophylla (Figure 5.13) belongs to the family Capparaceae and the older leaves are harvested as many times as possible by the local community. This plant is usually cooked with other herbs and nuts. Potherb. Leaves cooked with groundnuts, cowpeas or tomatoes (Fox and Norwood Young, 1982). Leaves applied to sores, roots chewed for coughs and the whole plant is used externally for swellings. Used as an anthelmintic. No toxicity reports.

Figure 5.13 *Cleome monophylla*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 162.80 ± 7.22; Moisture (%), 88.08 ± 0.35; Protein (g), 4.86 ± 0.1; Fat (g), 0.65 ± 0.08; Fibre (g), 2.14 ± 0.03; Ash (g), 3.01 ± 0.03; Carbohydrates (g), 3.40 ± 0.14

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 3203.29 ± 0.62; P (mg 100 g^{-1}), 784.18 ± 0.37; Na (mg 100 g^{-1}), 25.35 ± 0.04; Cu (mg 100 g^{-1}), 2.17 ± 0.3; Zn (mg 100 g^{-1}), 5.43 ± 0.65; Mg (mg 100 g^{-1}), 370.59 ± 1.06; Mn (mg 100 g^{-1}), 10.16 ± 0.16; Fe (mg 100 g^{-1}), 23.57 ± 1.27 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.04 mg/ml); Saponin (0.857 mg/ml); Oxalic acid (51.9 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.06 mg/ml); Trypsin inhibitors (3664 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-0%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous n/d ; Organic n/d); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0; 1000 µg/ml-0; 1000 µg/ml-0]

5.3.14 Portulaca oleracea

Portulaca oleracea (Figure 5.14) belongs to the Portulacaceae family and is either consumed raw or cooked by the local community. Eaten as salad and vegetable Can be eaten raw . Whole plant to be bactericidal in bacillary dysentry, diarrhoea, haemorrhoids

and enterorrhagia. antibacterial, antiscorbutic, depurative, diuretic, febrifuge. Nine percent oxalic acid. Incoordination of gait and tetanic conditions in sheep (Beekrum *et al.*,2003).

Figure 5.14: *Portulaca oleracea*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 99.38 ± 1.96; Moisture (%), 92.63 ± 0.13; Protein (g), 2.52 ± 0.01; Fat (g), 0.34 ± 0.04; Fibre (g), 1.21 ± 0.04; Ash (g), 1.86 ± 0.04; Carbohydrates (g), 2.65 ± 0.23

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1360.61 ± 0.75; P (mg 100 g^{-1}), 333.07 ± 0.45; Na (mg 100 g^{-1}), 148.24 ± 0.16; Cu (mg 100 g^{-1}), 3.38 ± 0.27; Zn (mg 100 g^{-1}), 34.10 ± 0.54; Mg (mg 100 g^{-1}), 1036.59 ± 1.02; Mn (mg 100 g^{-1}), 23.76 ± 0.82; Fe (mg 100 g^{-1}), 42.06 ± 0.33 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.19 mg/ml); Saponin (0.58 mg/ml); Oxalic acid (347.5 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (1.53 mg/ml); Trypsin inhibitors (2557.2 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-3.3%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 0% ; Organic 2%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0; 1000 µg/ml-0; 1000 µg/ml-0]

5.3.15 Senna occidentalis

Senna occidentalis (Figure 5.15) belongs to the Fabaceae family and is considered as a famine food by the local community. Young leaves are eaten . Regarded as a famine food . Roasted seed is used as coffee substitute (Fox and Norwood Young, 1982). Used for stomach pains, biliousness, fevers, jaundice, ringworms, sore throats and wounds . Dried leaves are used for lumbago and haemorrhoids whilst fresh leaves are used to treat edemas, abscesses and skin diseases. Unroasted seeds are toxic (Fox and Norwood Young, 1982). Leaves are toxic only if large quantities are consumed .

Figure 5.15 Senna occidentalis

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 353.93 ± 4.14; Moisture (%), 77.40 ± 0.19; Protein (g), 6.79 ± 0.07; Fat (g), 2.21 ± 0.06

Fibre (g), 2.58 ± 0.03; Ash (g), 4.23 ± 0.13; Carbohydrates (g), 9.37 ± 0.45

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 2230.44 ± 13.38; P (mg 100 g^{-1}), 417.35 ± 5.74; Na (mg 100 g^{-1}), 346.72 ± 1.05; Cu (mg 100 g^{-1}), 2.09 ± 0.33; Zn (mg 100 g^{-1}), 9.14 ± 0.57; Mg (mg 100 g^{-1}), 854.14 ± 2.62; Mn (mg 100 g^{-1}), 6.73 ± 0.07; Fe (mg 100 g^{-1}), 10.88 ± 0.04 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.37 mg/ml); Saponin (0 mg/ml); Oxalic acid (73.19 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.87 mg/ml); Trypsin inhibitors (694.6 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-50%; 1000 µg/ml-45%; 10000 µg/ml-100%]; Organic [100 µg/ml-0%; 1000 µg/ml-3 %; 10000 µg/ml-10%); Cytoxicity(Aqueous 98% ; Organic 0%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.64; 1000 µg/ml-0.95; 1000 µg/ml-1.41]

Chenopodium album

Chenopodium album (Figure 5.16) belongs to the Chenopodiaceae family. Cooked as spinach, mielie meal added, eaten as porridge, relish Seeds dried, ground into a meal and used as gruel, eaten raw, added to salads. Leaves are anthelmintic, antiphlogistic, anti-rheumatic, mildly laxative, odontalgic . Applied as a wash or poultice to bug bites, sunstroke, rheumatic joints, swollen feet (Duke and Ayensu, 1985). Contains small amounts of saponins. Cooking the plant reduces the oxalic acid content. Raw leaves consumed in large quantities causes problems.

Figure 18 *Chenopodium album*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 245.31 ± 1.07; Moisture (%), 83.36 ± 0.06; Protein (g), 4.60 ± 0.03; Fat (g), 0.76 ± 0.03

Fibre (g), 1.92 ± 0.06; Ash (g), 2.94 ± 0.04; Carbohydrates (g), 8.34 ± 0.16

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 1489.65 ± 25.1; P (mg 100 g^{-1}), 797.31 ± 0.65; Na (mg 100 g^{-1}), 682.68 ± 0.64; Cu (mg 100 g^{-1}), 3.58 ± 0.55; Zn (mg 100 g^{-1}), 108.72 ± 2.91; Mg (mg 100 g^{-1}), 1238.56 ± 1.53; Mn (mg 100 g^{-1}), 26.99 ± 1.65; Fe (mg 100 g^{-1}), 12.6 ± 0.25 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.04 mg/ml); Saponin (0 mg/ml); Oxalic acid (24.1 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0 mg/ml); Trypsin inhibitors (1145 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-10%; 1000 µg/ml-100%; 10000 µg/ml-100%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-0%); Cytoxicity(Aqueous 50% ; Organic 0%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0]; Organic [100 µg/ml- 0.32; 1000 µg/ml-0.5; 1000 µg/ml-0.59]

5.3.17 Ceratotheca triloba

Ceratotheca triloba (Figure 5.17) belongs to the family Pedaliaceae and respondents revealed that this plant has an unpleasant scent when harvested, however when cooked the scent disappears. Cooked as spinach. Unpleasant scent disappears with boiling. Sweet tasting and used a relish (Tredgold, 1986). Used in treatment of painful menstruation, stomach cramps, nausea, fever and diarrhoea. To relieve gastric disorders (Tredgold *et al*., 986). No toxicity reports available.

Figure 5.17 *Ceratotheca triloba*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 258.74 ± 4.36; Moisture (%), 84.99 ± 0.27; Protein (g), 2.29 ± 0.11; Fat (g), 2.17 ± 0.07; Fibre (g), 2.07 ± 0.06; Ash (g), 2.27 ± 0.07; Carbohydrates (g), 8.28 ± 0.52

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 705.22 ± 0.51; P (mg 100 g^{-1}), 222.67 ± 1.34; Na (mg 100 g^{-1}), 114.76 ± 0.54; Cu (mg 100 g^{-1}), 3.36 ± 0.21; Zn (mg 100 g^{-1}), 2.66 ± 0.2; Mg (mg 100 g^{-1}), 427.79 ± 1.71; Mn (mg 100 g^{-1}), 8.03 ± 0.38; Fe (mg 100 g^{-1}), 18.55 ± 0.33 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.04 mg/ml); Saponin (0.58 mg/ml); Oxalic acid (127.5 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0.6 mg/ml); Trypsin inhibitors (912.2 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-65%; 1000 µg/ml-1000%; 10000 µg/ml-100%]; Organic [100 µg/ml-0%; 1000 µg/ml-0 %; 10000 µg/ml-3%); Cytoxicity(Aqueous 0% ; Organic 2%);

Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0.1; 10000 µg/ml- 0.13]; Organic [100 µg/ml- 0.73; 1000 µg/ml-0.95; 1000 µg/ml-0.05]

Centella asiatica

Centella asiatica (Figure 5.18) belongs to the Apiaceae family. This plant is cooked as spinach with mealie meal by the local community. Eaten as spinach, mixed with mielie meal. Leaves are dried and used as famine food (Fox and Norwood Young, 1982). Used in salads and curries. Rejuvenating diuretic herb that clears toxins, reduces inflammations and fevers, improves healing and immunity, improves the memory and has a balancing effect on the nervous system, used in treatment of wounds, chronic skin conditions, venereal diseases, malaria, varicose veins, ulcers, nervous disorders and senility (Chopra *et al*., 1986). In large doses, this plant is a stupefying narcotic, sometimes producing cephalagia or vertigo with a tendency to coma (Beekrum *et al*., 2003).

Figure 5.18 *Centella asiatica*

Nutritional value of plants (per 100 g fresh weight ± SD)

Energy (kJ), 219.01 ± 0.54; Moisture (%), 87.78 ± 0.03; Protein (g), 3.15 ± 0.24; Fat (g), 2.72 ± 0.04; ibre (g), 1.92 ± 0.04; Ash (g), 2.54 ± 0.06; Carbohydrates (g), 3.81 ± 0.37

Micronutrient content of plants studied (per 100 g dry weight ± SD)

Ca (mg 100 g^{-1}), 2425.27 ±0.13; P (mg 100 g^{-1}), 326.94 ± 1.16; Na (mg 100 g^{-1}), 15.78 ± 0.08; Cu (mg 100 g^{-1}), 6.69 ± 0.44; Zn (mg 100 g^{-1}), 19.86 ± 1.37; Mg (mg 100 g^{-1}), 271.38 ± 0.65; Mn (mg 100 g^{-1}), 22.81 ± 0.23; Fe (mg 100 g^{-1}), 18 ± 0.34 (Beekrum, 2003).

Antinutrients (per 5g dry weight)

Phytic acid (0.37 mg/ml); Saponin (0.58 mg/ml); Oxalic acid (127.03 mg/ml); Cyanogenic glycosides (0 mg/ml); Alkaloids (0 mg/ml); Trypsin inhibitors (4198 TU).

Bioassays per 50g dry weight

Toxicity :(Aqueous [100 µg/ml-0%; 1000 µg/ml-0%; 10000 µg/ml-3.3%]; Organic [100 µg/ml-0%; 1000 µg/ml-3 %; 10000 µg/ml-7%); Cytoxicity(Aqueous 8% ; Organic 8%); Mutagenic frequency: (Aqueous[100 µg/ml-0; 1000 µg/ml-0; 10000 µg/ml- 0.01]; Organic [100 µg/ml- 0.55; 1000 µg/ml-0.59; 1000 µg/ml-0.73]

APPENDIX B

RESULTS

Table B1 Evaluation of mutagenicity, cytotoxicity and toxicity of aqueous and organic extracts of 18 leafy vegetables.

Plant Names	Mutagenic frequency of revertants						% shrimp death						% Cytotoxicity	
	Organic extracts			Aqueous extracts			Organic extracts			Aqueous extracts			Organic extracts	Aqueous extracts
	100µg/ml	1000µg/ml	10000 µg/ml	100µg/ml	1000µg/ml	10000 µg/ml	10µg/ml	100µg/ml	1000 µg/ml	10µg/ml	100µg/ml	1000 µg/ml	10000 µg/ml	10000 µg/ml
Cleome monophylla	0	0	0	0	0	0	0	0	0	0	0	0	n/t	n/t
Senna occidentalis	0.64	0.95	1.41	0	0	0	0	3	10	50	45	100	0	98
Justicia flava	0.45	0.5	0.55	0	0	0.15	0	0	0	5	20	50	25	100
Asystasia gangetica	0.45	0.73	0.86	0	0	0	0	3	0	0	0	0	0	90
Centella asiatica	0.55	0.59	0.73	0	0	0.01	0	3	7	0	0	0	8	8
Amaranthus hybridus	0.55	0.59	0.59	0	0	0	0	0	3	0	0	0	10	8
Amaranthus spinosus	0.82	1	0.95	0	0	0	0	0	3	0	0	1	0	11
Ceratotheca triloba	0.73	0.95	1.05	0	0.1	0.13	0	0	3	65	100	100	2	0
Portulaca oleracea	0	0	0	0	0	0	0	0	3.3	0	0	0	2	0
Amaranthus dubius	0.77	0.77	0.9	0	0	0	0	17	27	13	100	100	35	3
Oxygonum sinuatum	0.41	0.45	0.68	0	0	0	0	0	0	0	0	0	0	0

Galinsoga parviflora	0	0	0	0	0	0	0	3.3	3.3	0	0	0	35	0
Physalis viscosa	0.5	0.82	0.91	0	0	0	0	0	0	75	100	100	0	53
Solanum retroflexum	0	0	0	0	0	0.07	0	0	50	5	5	4	5	50
Momordica balsamina	0.59	0.82	0.91	0	0	0	0	0	0	0	0	0	86	85
Chenopodium album	0.32	0.5	0.59	0	0	0	0	0	0	10	100	100	0	50
Bidens pilosa	0.81	0.86	0.86	0	0	0	0	27	33	10	100	100	0	0
Emex australis	0.68	0.77	0.9	0	0	0	3	10	17	0	0	0	0	15

nt - not tested

Table B2 Fe(II) concentrations for the determination of phytic acid

Fe(II) concentration (mg/ml)	Absorbance		
	1	2	Average
2	0.046	0.040	0.043
1	0.033	0.033	0.033
0.01	0.025	0.02	0.022
0.025	0.011	0.011	0.011

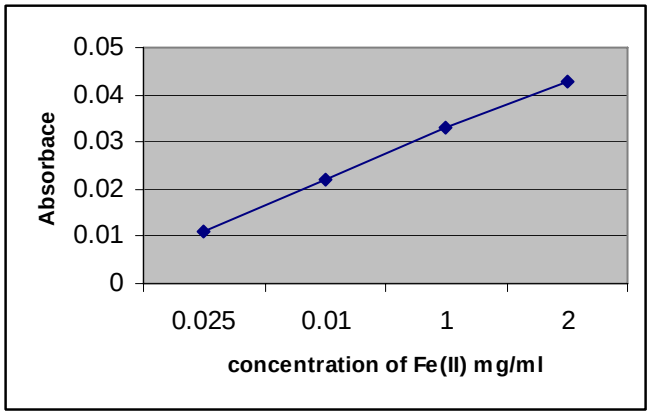

Figure B1 A standard curve for Fe(II) for the determination of phytic acid

Table B3 Haemolytic zones produced by saponin standards

Saponin concentration	Haemolytic zones		
	Zone 1	Zone 2	Average
2	22.5	21.5	22
1	17.5	16	17
0.5	12	12	12
0.25	6.5	7.5	7

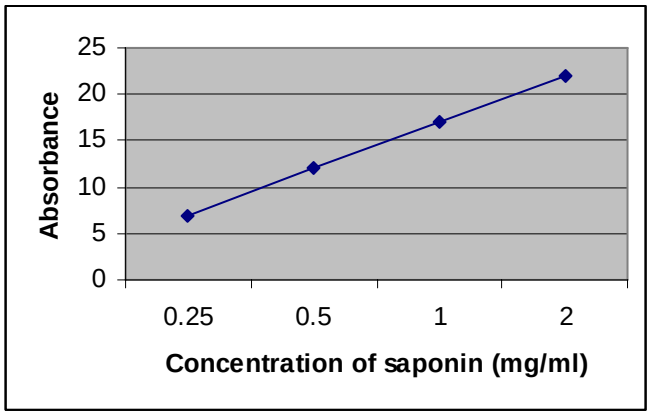

Figure B2 A standard curve for the determination of saponin content

Table B4 HPLC analysis of oxalic acid standards.

Concentration (mg/ml)	Area		Average ±SD
0.1	10630	11304	10967 ±476.5
1	23717	32372	28090 ± 6120
5	410549	406186	40336 ±10156.17
10	76116	30658	53387 ±32143.6

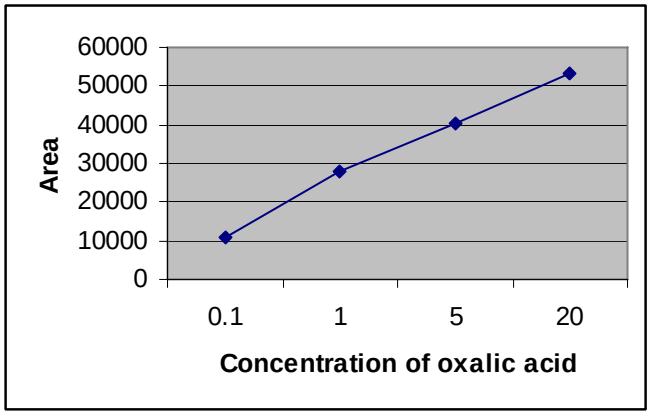

Figure B3 Standard curve of oxalic acid

APPENDIX C

METHODOLOGY

A1. MUTAGENICITY

5mM histidine/0.5mM biotin solution for top agar

Ingredient	per 250 ml
D-Biotin	30.5 mg
L-Histidine	26.2 mg
DH_2O	250 ml

Dissolve biotin in hot water first, filter sterilize and store at 4ºC in glass bottle

Vogel-Bonner (VB) medium E (50 x strength stock) for minimal agar base

Ingredient	per 1000 ml
Warm ddH_2O	670 ml
$MgSO_4.7H_2O$	10 g
Citric acid monohydrate	100g
$K2HPO_4$	500 g
$NaHNH_4PO_4.4H_2O$	175 g

Add salts in the order indicated above to warm water in a flask placed on a magnetic stirring hot plate. Allow each salt to dissolve completely before adding the next. Adjust the volume to 1 litre. Distribute into two 1 litre glass bottles. Autoclave for 20 min in 121ºC.

40% glucose (sterile)

Ingredient	per 1000 ml
Glucose	400 g
dd H_2O	1000 ml

Minimal glucose agar plates

Ingredient	per 1000 ml
Agar	15 g
dd H_2O	930 ml
VB medium E stock	20 ml
40% Glucose	50 ml

add agar to dd H_2O in a 2 litre flask. Place a large magnetic stirrer bar inside for mixing later. Autoclave at 121 ºC for 20 min using slow exhaust. When the solution has cooled slightly, add sterile VB medium E stock and sterile 40% glucose. Stir and mix thoroughly. Pour ± 30 ml to each petri dish.

Top agar

Ingredient	per 1000 ml
Agar	6 g
NaCl	5 g
dd H_2O	1000 ml

Mix the above ingredients and microwave to dissolve the agar. Mix thoroughly and make 100 ml aliquots. Autoclave at 121 ºC for 20 min. Cool the agar.

Add 1/10 of the 0.5mM histidine/ 0.5mM biotin solution to the molten agar top agar. Mix thoroughly by swirling.

A.2 CYTOTOXICITY

Media and reagents for continuous cell culture

Dulbecco's modified Eagle's medium (DMEM) with 4.5g/l glucose, 0.110/l sodium pyruvate with L-glutamite (Highveld biological, South Africa).

Foetal calf serum(FCS) was filter sterilized and gamma irradiated at 25-28 kGy under conditions which preserve the biological integrity of the serum. The serum was aliquoted into 50 ml sterile flasks and kept frozen (-20 ºC) until use (Highveld biological, South Africa).

0.25% (w/v) trypsin in Ca++ and Mg++ free phosphate buffered saline (PBS) (Highveld biological, South Africa).

0.1% (w/v) EDTA in Ca++ and Mg++ free PBS(Highveld biological, South Africa)

3-[5,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) reagent was prepared by dissolving 5 mg MTT in 1 ml PBS (pH 7.5). The solution was filter sterilized and kept in the fridge.

Maintenance of cells lines

The cells were maintained in 10%Complete Culture Medium {(10ml FCS and 1ml pen/strep in 100 ml DMEM (Dulbecco's modified eagle's medium)} with glucose and stored in a humidified chamber at 37°C with 5% CO_2.

Trypsinization

When the cells were 80% confluent, they were trypsinized using 0.1% EDTA and 0.25% trypsin (1:1). Add 5 ml of the trypsin/EDTA solution to the flask and wait for 5 min. after 5 min, tap the flask gently against the palm of the hand until all cells are in solution.