Nutritional content, phenolic compounds composition and antioxidant activities of selected indigenous vegetables of Zimbabwe

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy

November, 2010

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Abstract

Foods of plant origin may contain many phytochemical compounds such as phenolic compounds in addition to commonly identified nutrients. Interest in phenolic compounds has greatly increased recently because these phytochemicals have been implicated in suppressing rates of degenerative processes such as cardiovascular disorders and cancer. The pharmacological properties of the plants may be related to their antioxidant activities and hence there was need to investigate the antioxidant potential of some indigenous vegetables in Zimbabwe. The focus of this study was to investigate the indigenous vegetables consumed in Buhera district of Zimbabwe, their nutritional content, phenolic compounds composition and antioxidant activities and the effects of processing on the nutritional composition, phenolic content and antioxidant activities. The commonly consumed vegetables, *Amaranthus hybridus, Cleome gynandra, Bidens pilosa, Corchorus olitorius, Adansonia digitata* and two exotic vegetables i.e. lettuce (*Lactuva sativa*) and rape (*Brassica napus*) were analyzed for their nutritional content, phenolic compounds composition and antioxidant activities. A questionnaire was used to gather information on the preparation, preservation methods and medicinal uses of the indigenous vegetables. Phenolic compounds were extracted from the vegetables using 50% aqueous methanol. Proximate and micronutrient analysis were done using standard analytical methods. Phenolic concentrations were determined using the vanillin, butanol, tannin binding and Folin Ciocalteau assays. The antioxidant potentials of the extracts were determined using 1, 1-diphenyl-2 picrylhydrazyl radical (DPPH), reducing power, β-carotene and inhibition of phospholipid peroxidation methods. The identification of phenolics acids and flavonoids was done using reversed phase HPLC. People in Buhera district had knowledge on a variety of edible indigenous vegetables. The mentioned vegetables are used as relish and almost all respondents came up with some medicinal uses of the indigenous vegetables. Drying was found to be the common method of preserving the vegetables. Protein content was the highest in *Cleome gynandra* (6.0 g/100 g) and the lowest in *Adansonia digitata* (4.2 g/100 g). All the leafy indigenous vegetables were found to be poor sources of carbohydrate and fat, which ranged between 8.7 to 18.0 g/100 g and 0.3 to 0.5 g/100 g respectively. The insoluble dietary fibre in the vegetables was in the range of 1.6 to 4.2 g/100 g. The Fe content of the indigenous vegetables ranged from 8.7 mg/100 g for *Corchorus olitorius* to 23.0 mg/100 g for *Adansonia digitata.* The Zn content of the vegetables ranged from 2.9 mg/100 g for *Cleome gynandra* to 22.0 mg/100 g for *Bidens pilosa.* The Cu content of the vegetables ranged from 1.8 mg/100 g for *Corchorus olitorius* to 23.7 mg/100 g for *Adansonia digitata.* The Vitamin C content varied from 18.0 mg/100 g for *Cleome gynandra* to 78.0 mg/100 g for *Corchorus olitorius*. Potassium content was the highest in *Adansonia digitata* (1090.0 mg/100 g) and lowest in *Cleome gynandra* (129.0 mg/100 g). Calcium values of the plants ranged from 120.0 mg/100 g in *Cleome gynandra* to 798.0 mg/100 g in *Amaranthus hybridus*. *Corchorus olitorius* had the highest P content (623.0 mg/100 g) and *Adansonia digitata* the lowest (14.0 mg/100 g). The total content of phenolics varied from 4.9 mg/g in lettuce to 57.5 mg/g in *B. pilosa.* The contents of flavonoids varied from 1.2 mg/g for *C. gynandra* to 8.0 mg/g for *B. pilosa.* The levels of proanthocyanidins were ranging from 1.9 mg/g for lettuce to 11.2 mg/g for *Bidens pilosa.* The tannin contents of the vegetables ranged from 5.7 mg/g for *C. gynandra* to 8.3 mg/g for *Bidens pilosa. Bidens pilosa* had the highest DPPH radical scavenging activity while *Amaranthus hybridus* had the lowest. All the vegetable extracts exhibited higher capacity in reducing ferric ions. Generally the extracts had the ability to quench peroxyl radicals formed in the β-carotene assay. The extracts showed strong activity in the phospholipid peroxidation assay. Common phenolic acids of the analysed vegetables were gallic acid and protochatechuic acid. Cooking caused significant changes in the nutritional and phenolic compounds composition of the vegetables. The present study showed that the

vegetables are valuable sources of nutrients and phenolic compounds as compared to exotic species.

Acknowledgments

I wish to give my special thanks to my supervisors Dr M. Muchuweti and Dr S. Nyagura for their invaluable help, advice and support during the progress of this project.

I also wish to give my special thanks to Mr. A. Kasiyamhuru for his guidance in the analytical skills involved in the project, especially the use of HPLC and other instruments.

I wish to thank all members of the natural products group in the department of Biochemistry for helping with some technical skills used in the project.

My sincere gratitude also goes to Professor M. A. B. Benhura for his support and feedback during the course of my studies.

Many thanks go to the academic staff in the Institute of Food, Nutrition and Family sciences for very helpful comments on my thesis and to Dr. Parawira for his comments on parts of my thesis and the Lecturers in the department for providing moral support during difficult times.

List of Figures

List Tables

TABLE OF CONTENTS

1 INTRODUCTION

About 1.02 billion people in the world do not have enough to eat and the number of undernourished people increased by 75 million in 2007 and 40 million in 2008, largely due to higher food prices. Most of the undernourished people are in Africa as 907 million people in developing countries alone are hungry (Food and Agriculture Organization, 2009). Globally, an estimated 10.6 million children under five years of age are dying every year and malnutrition is the underlying cause of 50% of these deaths. Sub-Saharan Africa, the home to 10% of the world's population, carries a burden of 41% of the global under-five mortality (WHO, 2003).

Micronutrient malnutrition also known as `Hidden Hunger' now afflicts over two billion people worldwide, resulting in poor health, low worker productivity, high rates of mortality and morbidity, increased rates of coronary heart disease, cancer, stroke, and diabetes, and permanent impairment of cognitive abilities of infants born to micronutrient-deficient mothers (Welch and Grahamn, 1999). Food based approaches are recommended for the alleviation of micronutrient malnutrition. The approaches include the use of locally available foods such as vegetables (Tontisirin *et al*., 2002). Iron deficiency, for example is the most prevalent form of malnutrition worldwide, affecting an estimated 2 billion people yet some locally available vegetables in Africa are rich sources of the mineral (WFP, 2007).

Foods of plant origin may contain many phytochemicals in addition to nutrients such as proteins, carbohydrates, vitamins and minerals. More than 900 different

phytochemicals have been identified in foods and there may be more than 100 different phytochemicals in just one vegetable (Akindahunsi and Salawu, 2005). Epidemiological studies have demonstrated that people on vegetarian diets have a reduced risk of heart diseases and obesity (Bagchi and Puri, 1998).

Some of the potential health benefits of phytochemical substances have been related to the action of the compounds as antioxidants and inhibitors of lipid peroxidation (Li-Chen *et al*., 2005). Phytochemical compounds such as phenolics are strong antioxidants against free radicals and reactive oxygen species. Free radicals and reactive oxygen species have been implicated as the major cause of chronic human diseases such as cardiovascular diseases, diabetes and degenerative diseases (Kyung-Hee *et al*., 2005; Chen and Yen, 2007).

Some vegetables in tropical Africa such as *Adansonia digitata* and *Bidens pilosa* have been reported to have medicinal properties, which include anti-bacterial, anti-rheumatic, anti-cancer and anti-malarial activity (Abbiw, 1990; Adebooye, 2001; Aguilar *et al*., 2001). Gallic, *p*-hydroxybenzoic acid and related phenolics identified in some vegetables have been found to retard or partially inhibit the growth and toxin production of *Clostridium botulinum* types A and B (Pierson and Reddy, 1982). Trompezinski *et al* in 2003 demonstrated the anti-inflammatory activity of a phenolic rich extract and the role of its major polyphenolic constituent (-) epigallocatechin gallate (EGCG). Many vegetables and fruits possess ellagic and chlorogenic acids, which serve as potential chemo-preventers against several carcinogens (Huang *et al.,* 1992). These findings demonstrate that phytochemicals have got some important

pharmacological properties and their consumption in some foods such as vegetables may lead to the amelioration of many diseases.

An enormous variety of plants has been studied for new sources of phenolic compounds but there are only a few reports about phenolic content and antioxidant activity of indigenous plants from Zimbabwe. All the studies carried out in Zimbabwe by Muchuweti *et al*., 2005a,b; Muchuweti *et al*., 2006a,b; Ndlala *et al*., 2006; 2007a,b; 2008 focused on wild fruits of Zimbabwe. Thus a need for the investigation of antioxidative phenolic compounds in leafy indigenous vegetables is of necessity to improve our understanding of their potential benefits. It is hoped that data on the antioxidant activity of the indigenous vegetables may help remove the stigma of "starvation food" for poor people and promote them as a healthy food source.

1.1 Aims of the study

The aims of the study were to determine the nutritional value, phenolic compounds content and antioxidant potential of selected indigenous vegetables of Zimbabwe and compare them to some exotic vegetables.

1.2 Specific objectives

The specific objectives of the study were to:

1. Establish the indigenous vegetables consumed in Buhera district

2. Investigate the seasonal availability, usage and methods of preparation and preservation of the established indigenous vegetables in Buhera District

3. Determine the;

(a). Micro-nutrient and macro-nutrient content of *Cleome Gynandra, Amaranthus hybridus, Bidens pilosa, Corchorus olitorius* and *Adansonia digitata* vegetable extracts

(b). Effects of cooking on the nutritional content of *Amaranthus hybridus*, *Cleome gynandra* and *Bidens pilosa* vegetable extracts

(c). Content of phenolic compounds in *Cleome gynandra, Amaranthus hybridus, Bidens pilosa, Corchorus olitorius* and *Galinsoga parviflora* vegetable extracts

(d). Antioxidant capacity of *Cleome gynandra, Amaranthus hybridus, Bidens pilosa, Corchorus olitorius* and *Galinsoga parviflora* vegetable extracts

(e). Relationship between the contents of phenolic compounds and antioxidant capacities

(f). Effects of processing and storage on the total phenolic content and antioxidant activity of *Bidens pilosa*

4. Establish the phenolic acids and flavonoids in *Cleome gynandra, Amaranthus hybridus, Bidens pilosa, Corchorus olitorius* and *Galinsoga parviflora* vegetable extracts by HPLC

2 LITERATURE REVIEW

2.1 Definition of Indigenous vegetables

The word *indigenous* is used to describe vegetables that have their natural habitat in a country and the ones which were introduced from other regions of the world. The introduced vegetables due to long use became part of the food culture in that country (Chweya and Eyzaguirre, 1999**).**

2.2 Role of African Leafy Vegetables (ALVs) in Health Promotion and Protection

Quite a large number of African indigenous leafy vegetables have long been known and reported to have health protecting properties. Several of these indigenous leafy vegetables continue to be used for prophylactic and therapeutic purposes by rural communities (Dalziel, 1937; Busson, 1965; Malgrass, 1992; Okeno *et al.*, 2003; Ayodele, 2005).

Indigenous knowledge of the health promoting and protecting attributes of ALVs is clearly linked to their nutritional and non-nutrient phytochemical properties. ALVs have long been, and continue to be reported to significantly contribute to the dietary vitamin and mineral intakes of African populations (Mulokozi *et al.*, 2004).

2.3 Nutritional importance of African leafy vegetables

Wild food plants play a very important role in the livelihoods of rural communities as an integral part of the subsistence strategy of people in many developing countries (Zamede *et al*., 2001). Locally available wild food plants serve as alternatives to

staple food during periods of food deficit, are a valuable supplement for a nutritionally balanced diet and are one of the primary alternative sources of income for many poor rural communities (Scoones *et al*., 1992).

Millions of people in many developing countries do not have enough food to meet their daily requirements and a further more people are deficient in one or more micronutrients (Cambell, 1987). In most cases rural communities depend on wild resources including wild edible plants to meet their food needs in periods of food crisis. The diversity in wild species offers variety in family diet and can contribute to household food security (Zinyama *et al*., 1990; Zamede *et al*., 2001).

Guerrero *et al* in 1998 compiled a comprehensive nutrient report of wild vegetables consumed by the first European farmers, and nearly all the species had significant amounts of several micronutrients such as copper, magnesium, zinc, iron, vitamin E, carotenoids and vitamin C. Turan *et al* in 2003 reported that the potassium, calcium, magnesium and protein contents of wild vegetables in Turkey were all higher than cultivated species. The cultivated species analysed and compared to the wild vegetables were spinach, pepper, lettuce, and cabbage. Concentrations of iron, manganese, zinc and copper were similar in both vegetable types.

Studies conducted by Booth *et al* in 1992 and Freyre *et al* in 2000 in South America have confirmed the importance of wild vegetables as sources of micronutrients. Studies conducted on wild South African vegetables by Freiberger *et al* in 1998 and Vainio-Mattila in 2000 in Tanzania underscored the wild plants' significant contribution as sources of micronutrients. However, the nutritional quality of four

wild vegetables analysed in Ghana was found to be in the same range as conventional vegetables (Wallace *et al.,* 1998).

All the researches showed that wild plants are essential components of many Africans' diets, especially in periods of seasonal food shortage. A study conducted in Zimbabwe revealed that some poor households rely on wild plant foods as an alternative to cultivated food for a quarter of all dry season meals (Kabuye, 1997).

2.4 Production of ALVs

There is very little published information on either the areas cultivated or the production levels of specific ALVs (Mirghani and Mohammed, 1997).

Spore No. 116 in 2005 indicated that the total 1998 production of leafy vegetables in Cameroon was estimated to be 93,600 tons of which 21,549 tons were of 'bitter leaf', *Vernonia amygdalina*. Such valuable production data are often dispersed and difficult to compare, given the gaps in coverage and different methodologies used.

Even the FAO database on vegetable production in sub-Saharan Africa fails to capture the indigenous and traditional vegetables that are commonly used on the subcontinent. Of the 15 vegetables documented in the FAO database, only tomatoes and mushrooms have some relevance to the diets of the majority of populations on the subcontinent (FAO, 2003). The lack of data on indigenous vegetables is a serious shortcoming because information from this database is used to inform and guide policy initiatives globally and on the subcontinent specifically (Gockowski and Ndoumbe, 2004).

Reports on the diversity of traditional leafy vegetables in sub-Saharan showed that there are more than 20 leafy vegetable species specific to Africa that are used in daily diets and are of nutritional importance (Guarino, 1997). Mirghani and Mohammed in 1997 and Okeno *et al* in 2003 reported that in contrast to cash crops, little attention has been paid to the production of indigenous leafy vegetables and so there is a lack of data on their production levels.

2.5 Patterns of consumption of ALVs

Information on the *per capita* consumption of ALVs is just as scarce as data on their production levels. It is generally believed that the introduction of exotic vegetable varieties contributed to the decline in the production and consumption of indigenous vegetables (Adedoyin and Taylor, 2000). Reports of a steady decline in dietary intakes of the African leafy vegetables with the emergence of simplified diets are based on the assumption of declining use as a result of declining availability (Okeno *et al*, 2003; Community Technology Development Trust, 2000).

Contrary to the view of declining use as a result of declining availability, Maziya-Dixon *et al* in 2004 reported that in Nigeria, leafy vegetables are relatively available and affordable particularly during the rainy seasons but were found to be among the least consumed foods. Ruel *et al* in 2004 reported the consumption of vegetables in sub-Saharan Africa and in the study the reported common vegetables included onions, carrots, tomatoes and cabbage that are really not representative of A LVs.

2.6 Importance of some micronutrients analysed

2.6.1 Iron

Iron has the longest and best described history among all the micronutrients. It is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes (Beard and Dawson, 1997; Wood and Ronnenberg, 2006).

Haeme is an iron-containing compound found in a number of biologically important molecules. Haemoglobin and myoglobin are haeme-containing proteins that are involved in the transport and storage of oxygen. Haemoglobin is the primary protein found in red blood cells and represents about two thirds of the body's iron. The vital role of hemoglobin in transporting oxygen from the lungs to the rest of the body is derived from its unique ability to acquire oxygen rapidly during the short time it spends in contact with the lungs and to release oxygen when needed during its circulation through the tissues. Myoglobin functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles (Yip and Dallman, 1996; Brody, 1999).

Cytochromes are haeme-containing compounds that have important roles in mitochondrial electron transport; therefore, cytochromes are critical to cellular energy production and thus life. They serve as electron carriers during the synthesis of ATP, the primary energy storage compound in cells. Cytochrome P450 is a family of enzymes that functions in the metabolism of a number of important biological molecules, as well as the detoxification and metabolism of xenobiotics. Non-haeme

iron-containing enzymes, such as NADH dehydrogenase and succinate dehydrogenase, are also critical to energy metabolism (Yip and Dallman, 1996).

Catalase and peroxidases are haeme-containing enzymes that protect cells against the accumulation of hydrogen peroxide, a potentially damaging reactive oxygen species (ROS), by catalyzing a reaction that converts hydrogen peroxide to water and oxygen. As part of the immune response, some white blood cells engulf bacteria and expose them to ROS in order to kill them. The synthesis of one such ROS, hypochlorous acid, by neutrophils is catalyzed by the haeme-containing enzyme myeloperoxidase (Yip and Dallman, 1996; Brody, 1999).

Inadequate oxygen, such as that experienced by those who live at high altitudes or those with chronic lung disease, induces compensatory physiologic responses, including increased red blood cell formation, increased blood vessel growth, and increased production of enzymes utilized in anaerobic metabolism. Under hypoxic conditions, transcription factors known as hypoxia inducible factors (HIF) bind to response elements in genes that encode various proteins involved in compensatory responses to hypoxia and increase their synthesis. Research indicates that an iron-dependent prolyl hydroxylase enzyme plays a critical role in regulating HIF and, consequently, physiologic responses to hypoxia (Ivan *et al.,* 2001; Jaakkola *et al.,* 2001). When cellular oxygen tension is adequate, newly synthesized HIFa subunits are modified by a prolyl hydroxylase enzyme in an iron-dependent process that targets HIFa for rapid degradation. When cellular oxygen tension drops below a critical threshold, prolyl hydroxylase can no longer target HIFa for degradation, allowing

HIFa to bind to HIFb and form an active transcription factor that is able to enter the nucleus and bind to specific response elements on genes.

Ribonucleotide reductase is an iron-dependent enzyme that is required for DNA synthesis (Beard and Dawson, 1997; Fairbanks, 1999). Thus, iron is required for a number of vital functions, including growth, reproduction, healing, and immune function.

The Recommended Dietary Allowance (RDA) for iron shown in Table 1 was revised in 2001 and is based on the prevention of iron deficiency and maintenance of adequate iron stores in individuals eating a mixed diet (Food and Nutrition Board, Institute of Medicine, 2001).

Table 1: The Recommended Dietary Allowance (RDA) for Iron

Life stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6	0.27	0.27
Infants	7-12	11	11
Children	1-3	7	7
Children	4-8	10	10
Children	9-13	8	8
Adolescents	14-18	11	15
Adults	19-50	8	18
Adults	51 years and older	8	8
Pregnancy	All ages		27
Breast-feeding	18 years and younger		10
Breast-feeding	19 years and older		9

2.6.2 Zinc

Zinc is an essential trace element for all forms of life. Clinical zinc deficiency in humans was first described in 1961, when the consumption of diets with low zinc

bioavailability due to high phytic acid content was associated with "adolescent nutritional dwarfism" in the Middle East (Prasad *et al.,* 1961). Since then, zinc insufficiency has been recognized by a number of experts as an important public health issue, especially in developing countries (Prasad, 1998).

Numerous aspects of cellular metabolism are zinc-dependent. Zinc plays important roles in growth and development, the immune response, neurological function, and reproduction. On the cellular level, the function of zinc can be divided into three categories namely, catalytic, structural and regulatory (Cousins, 2006).

Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc-dependent enzymes can be found in all known classes of enzymes (Food and Nutrition Board, Institute of Medicine, 2001).

Zinc plays an important role in the structure of proteins and cell membranes. A finger-like structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role (Food and Nutrition Board, Institute of Medicine, 2001; King and Cousins, 2006). The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function (O'Dell, 2000).

Zinc finger proteins have also been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission.

Zinc has been found to play a role in apoptosis, a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Truong-Tran *et al.,* 2000).

The RDA for zinc is listed by gender and age group in Table 2. Since a sensitive indicator of zinc nutritional status is not readily available, the RDA for zinc is based on a number of different indicators of zinc nutritional status and represents the daily intake likely to prevent deficiency in nearly all individuals in a specific age and gender group (Food and Nutrition Board, Institute of Medicine, 2001).

Table 2: The Recommended Dietary Allowance (RDA) for Zinc

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	2	2
Infants	7-12 months	3	3
Children	1-3 years	3	3
Children	4-8 years	5	5
Children	9-13 years	8	8
Adolescents	14-18 years	11	9
Adults	19 years and older	11	8
Pregnancy	18 years and younger	-	12
Pregnancy	19 years and older	-	11
Breast-feeding	18 years and younger	-	13
Breast-feeding	19 years and older	-	12

2.6.3 Calcium

Calcium is the most common mineral in the human body. About 99% of the calcium in the body is found in bones and teeth, while the other 1% is found in the blood and soft tissue. Calcium levels in the blood and fluid surrounding the cells must be maintained within a very narrow concentration range for normal physiological functioning. The physiological functions of calcium are so vital to survival that the body will demineralize bone to maintain normal blood calcium levels when calcium intake is inadequate. Thus, adequate dietary calcium is a critical factor in maintaining a healthy skeleton (Weaver and Heaney, 1999).

Calcium is a major structural element in bones and teeth. The mineral component of bone consists mainly of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals, which contain large amounts of calcium and phosphate (Heaney, 2000). Bone is a dynamic tissue that is remodeled throughout life. Bone cells called osteoclasts begin the process of remodeling by dissolving or resorbing bone. Bone-forming cells called osteoblasts then synthesize new bone to replace the bone that was resorbed. During normal growth, bone formation exceeds bone resorption. Osteoporosis may result when bone resorption chronically exceeds formation (Weaver and Heaney, 1999).

Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and the secretion of hormones like insulin (Food and Nutrition Board, Institute of Medicine, 1997). Excitable cells, such as skeletal muscle and nerve cells, contain voltage-dependent calcium channels in their cell membranes that allow for rapid changes in calcium concentrations. For

example, when a muscle fibre receives a nerve impulse that stimulates it to contract, calcium channels in the cell membrane open to allow a few calcium ions into the muscle cell. These calcium ions bind to activator proteins within the cell, which release a flood of calcium ions from storage vesicles inside the cell. The binding of calcium to the protein, troponin-c, initiates a series of steps that lead to muscle contraction.

The binding of calcium to the protein, calmodulin, activates enzymes that breakdown muscle glycogen to provide energy for muscle contraction (Weaver and Heaney, 1999).

Calcium is necessary to stabilize a number of proteins and enzymes, optimizing their activities. The binding of calcium ions is required for the activation of the seven vitamin K-dependent clotting factors in the coagulation cascade. The term, coagulation cascade, refers to a series of events, each dependent on the other that stops bleeding through clot formation (Brody, 1999).

Updated recommendations for calcium intake based on the optimization of bone health were released by the Food and Nutrition Board (FNB) of the Institute of Medicine in 1997 and are shown in Table 3. The setting of an Adequate Intake level (AI) rather than a Recommended Dietary Allowance (RDA) for calcium reflects the difficulty of estimating the intake of dietary calcium that will result in optimal accumulation and retention of calcium in the skeleton when other factors such as genetics, hormones, and physical activity, also interact to affect bone health (Food and Nutrition Board, Institute of Medicine, 1997).

Table 3: Adequate Intake (AI) for Calcium

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	210	210
Infants	7-12 months	270	270
Children	1-3 years	500	500
Children	4-8 years	800	800
Children	9-13 years	1 300	1 300
Adolescents	14-18 years	1 300	1 300
Adults	19-50 years	1 000	1 000
Adults	51 years and older	1 200	1 200
Pregnancy	18 years and younger	-	1 300
Pregnancy	19 years and older	-	1 000
Breast-feeding	18 years and younger	-	1 300
Breast-feeding	19 years and older	-	1 000

2.6.1 Magnesium

Magnesium plays important roles in the structure and the function of the human body. The adult human body contains about 25 grams of magnesium. Over 60% of all the magnesium in the body is found in the skeleton, about 27% is found in muscle, 6% to 7% is found in other cells, and less than 1% is found outside of cells (Shils, 1997).

Magnesium is involved in more than 300 essential metabolic reactions, some of which are discussed below (Spencer *et al.,* 1994).

The metabolism of carbohydrates and fats to produce energy requires numerous magnesium-dependent chemical reactions. Magnesium is required by the adenosine triphosphate (ATP)-synthesizing protein in mitochondria. ATP, the molecule that

provides energy for almost all metabolic processes, exists primarily as a complex with magnesium (Rude and Shils, 2006).

Magnesium is required for a number of steps during nucleic acid and protein synthesis. Several enzymes participating in the synthesis of carbohydrates and lipids require magnesium for their activity. Glutathione, an important antioxidant, requires magnesium for its synthesis (Rude and Shils, 2006).

Magnesium plays a structural role in bone, cell membranes, and chromosomes (Rude and Shils, 2006).

Magnesium is required for the active transport of ions like potassium and calcium across cell membranes. Through its role in ion transport systems, magnesium affects the conduction of nerve impulses, muscle contraction, and normal heart rhythm (Rude and Shils, 2006).

Cell signaling requires MgATP for the phosphorylation of proteins and the formation of the cell-signaling molecule, cyclic adenosine monophosphate (cAMP). cAMP is involved in many processes, including the secretion of parathyroid hormone from the parathyroid glands (Rude and Shils, 2006).

Calcium and magnesium levels in the fluid surrounding cells affect the migration of a number of different cell types. Such effects on cell migration may be important in wound healing (Rude and Shils, 2006).

In 1997 the Food and Nutrition Board of the Institute of Medicine increased the recommended dietary allowance (RDA) for magnesium, based on the results of tightly controlled balance studies that utilized more accurate methods of measuring magnesium (Food and Nutrition Board, Institute of Medicine, 1997). The RDAs for magnesium are shown in Table 4. Balance studies are useful for determining the amount of a nutrient that will prevent deficiency; however, such studies provide little information regarding the amount of a nutrient required for chronic disease prevention or optimum health.

Table 4: Recommended Dietary Allowance (RDA) for Magnesium

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	30	30
Infants	7-12 months	75	75
Children	1-3 years	80	80
Children	4-8 years	130	130
Children	9-13 years	240	240
Adolescents	14-18 years	410	360
Adults	19-30 years	400	310
Adults	31 years and older	420	320
Pregnancy	18 years and younger	-	400
Pregnancy	19-30 years	-	350
Pregnancy	31 years and older	-	360
Breast-feeding	18 years and younger	-	360
Breast-feeding	19-30 years	-	310
Breast-feeding	31 years and older	-	320

2.6.2 Vitamin C

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. Unlike most mammals and other animals, humans do not have the ability to make their own vitamin C. Therefore, we must obtain vitamin C through our diet.

Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, and bone. Vitamin C also plays an important role in the synthesis of the neurotransmitter, norepinephrine. Neurotransmitters are critical to brain function and are known to affect mood.

In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat into cellular organelles called mitochondria, where the fat is converted to energy (Carr and Frei, 1999). Research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones (Simon and Hudes, 2000).

Vitamin C is also a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids, from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants. Vitamin C may also be able to regenerate other antioxidants such as vitamin E. One recent study of cigarette smokers found that vitamin C regenerated vitamin E from its oxidized form (Bruno *et al.,* 2006).

The recommended dietary allowance (RDA) for vitamin C was revised in 2000 upward from the previous recommendation of 60 mg daily for men and women as shown in Table 5. The RDA continues to be based primarily on the prevention of deficiency disease, rather than the prevention of chronic disease and the promotion of optimum health. The recommended intake for smokers is 35 mg/day higher than for non-smokers, because smokers are under increased oxidative stress from the toxins in cigarette smoke and generally have lower blood levels of vitamin C (Food and Nutrition Board, Institute of Medicine, 2000).

Table 5: Recommended Dietary Allowance (RDA) for Vitamin C

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	40	40
Infants	7-12 months	50	50
Children	1-3 years	15	15
Children	4-8 years	25	25
Children	9-13 years	45	45
Adolescents	14-18 years	75	65
Adults	19 years and older	90	75
Smokers	19 years and older	125	110
Pregnancy	18 years and younger	-	80
Pregnancy	19-years and older	-	85
Breast-feeding	18 years and younger	-	115
Breast-feeding	19 years and older	-	120

2.6.3 Phosphorus

Phosphorus is an essential mineral that is required by every cell in the body for normal function (Knochel, 2006). The majority of the phosphorus in the body is found as phosphate (PO_4). Approximately 85% of the body's phosphorus is found in bone (Food and Nutrition Board, Institute of Medicine, 1997).

Phosphorus is a major structural component of bone in the form of a calcium phosphate salt called hydroxyapatite. Phospholipids are major structural components of cell membranes. All energy production and storage are dependent on phosphorylated compounds, such as ATP and creatine phosphate.

Nucleic acids, which are responsible for the storage and transmission of genetic information, are long chains of phosphate-containing molecules. A number of enzymes, hormones, and cell-signaling molecules depend on phosphorylation for their activation. Phosphorus also helps to maintain normal acid-base balance by acting as one of the body's most important buffers. Additionally, the phosphorus-containing molecule 2,3-diphosphoglycerate (2,3-DPG) binds to haemoglobin in red blood cells and affects oxygen delivery to the tissues of the body (Knochel, 2006).

The recommended dietary allowance (RDA) as shown in Table 6 for phosphorus was based on the maintenance of normal serum phosphate levels in adults, which was believed to represent adequate phosphorus intake to meet cellular and bone formation needs (Food and Nutrition Board, Institute of Medicine, 1997).

Table 6: Recommended Dietary Allowance (RDA) for Phosphorus

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	100	100
Infants	7-12 months	275	275
Children	1-3 years	460	460
Children	4-8 years	500	500
Children	9-13 years	1 250	1 250
Adolescents	14-18 years	1 250	1 250
Adults	19 years and older	700	700
Pregnancy	18 years and younger	-	1 250
Pregnancy	19 years and older	-	700
Breast-feeding	18 years and younger	-	1 250
Breast-feeding	19 years and older	-	700

2.6.4 Potassium

Potassium is an essential dietary mineral and electrolyte. The term electrolyte refers to a substance that dissociates into ions in solution, making it capable of conducting electricity. Normal body function depends on tight regulation of potassium concentrations both inside and outside of cells (Peterson, 1997).

Potassium is the principal positively charged ion in the fluid inside of cells, while sodium is the principal cation in the fluid outside of cells. Potassium concentrations are about 30 times higher inside than outside cells, while sodium concentrations are more than ten times lower inside than outside cells. The concentration differences between potassium and sodium across cell membranes create an electrochemical

gradient known as the membrane potential. A cell's membrane potential is maintained by ion pumps in the cell membrane, especially the sodium, potassium-ATPase pumps. These pumps use ATP to pump sodium out of the cell in exchange for potassium. Their activity has been estimated to account for 20% to 40% of the resting energy expenditure in a typical adult (Sheng, 2000).

The large proportion of energy dedicated to maintaining sodium/potassium concentration gradients emphasizes the importance of this function in sustaining life. Tight control of cell membrane potential is critical for nerve impulse transmission, muscle contraction, and heart function (Brody, 1999; Sheng, 2000).

A limited number of enzymes require the presence of potassium for their activity. The activation of sodium, potassium-ATPase requires the presence of sodium and potassium. The presence of potassium is also required for the activity of pyruvate kinase, an important enzyme in carbohydrate metabolism (Sheng, 2000).

In 2004, the Food and Nutrition Board of the Institute of Medicine established an adequate intake level (AI) as shown in Table 7 for potassium based on intake levels that have been found to lower blood pressure, reduce salt sensitivity, and minimize the risk of kidney stones (Food and Nutrition Board, Institute of Medicine, 2004).

Table 7: Adequate Intake (AI) for Potassium

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	400	400
Infants	7-12 months	700	700
Children	1-3 years	3 000	3 000
Children	4-8 years	3 800	3 800
Children	9-13 years	4 500	4 500
Adolescents	14-18 years	4 700	4 700
Adults	19 years and older	4 700	4 700
Pregnancy	14-50 years	-	4 700
Breast-feeding	14-50 years	-	5 100

2.6.5 Copper

Copper (Cu) is an essential trace element for humans and animals. In the body, copper shifts between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) forms, though the majority of the body's copper is in the Cu^{2+} form. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reactions and in scavenging free radicals (Linder and Hazegh-Azam, 1996). Although Hippocrates is said to have prescribed copper compounds to treat diseases as early as 400 B.C. (Turnlund, 2006), scientists are still uncovering new information regarding the functions of copper in the human body.

Copper is a critical functional component for a number of essential enzymes known as cuproenzymes. Some of the physiologic functions known to be copper-dependent are discussed below.

The copper-dependent enzyme, cytochrome *c* oxidase, plays a critical role in cellular energy production. By catalyzing the reduction of molecular oxygen to water, cytochrome *c* oxidase generates an electrical gradient used by the mitochondria to create the vital energy-storing molecule, ATP (Uauy *et al.,* 1998).

Another cuproenzyme, lysyl oxidase, is required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue. The action of lysyl oxidase helps maintain the integrity of connective tissue in the heart and blood vessels and also plays a role in bone formation (Turnlund, 2006).

Two copper-containing enzymes, ferroxidase I and ferroxidase II have the capacity to oxidize ferrous iron ($Fe^{2+)}$ to ferric iron (Fe^{3+}), the form of iron that can be loaded onto the protein transferrin for transport to the site of red blood cell formation. Although the ferroxidase activity of these two cuproenzymes has not yet been proven to be physiologically significant, the fact that iron mobilization from storage sites is impaired in copper deficiency supports their role in iron metabolism (Harris, 1997; Turnlund, 2006).

A number of reactions essential to normal function of the brain and nervous system are catalyzed by cuproenzymes. Dopamine-b-monooxygenase catalyzes the conversion of dopamine to the neurotransmitter norepinephrine (Harris, 1997). Monoamine oxidase (MAO) plays a role in the metabolism of the neurotransmitters norepinephrine, epinephrine, and dopamine. MAO also functions in the degradation of the neurotransmitter serotonin, which is the basis for the use of MAO inhibitors as

antidepressants (Food and Nutrition Board, Institute of Medicine, 2001). The myelin sheath is made of phospholipids whose synthesis depends on cytochrome *c* oxidase activity (Turnlund, 2006). The cuproenzyme, tyrosinase, is required for the formation of the pigment melanin. Melanin is formed in cells called melanocytes and plays a role in the pigmentation of the hair, skin, and eyes (Turnlund, 2006).

Superoxide dismutase (SOD) functions as an antioxidant by catalyzing the conversion of superoxide radicals to hydrogen peroxide, which can subsequently be reduced to water by other antioxidant enzymes (Johnson *et al.,* 1992). Two forms of SOD contain copper: 1. copper/zinc SOD is found within most cells of the body, including red blood cells, and 2. extracellular SOD is a copper-containing enzyme found at high levels in the lungs and low levels in blood plasma (Turnlund, 2006).

Ceruloplasmin is a protein found in most tissues in the human body and may function as an antioxidant in two different ways. Firstly it binds free copper and iron ions and as a result prevents free copper and iron ions from catalyzing oxidative damage. Secondly the ferroxidase activity of ceruloplasmin facilitates iron loading onto its transport protein, transferrin, and may prevent free ferrous ions (Fe^{2+}) from participating in harmful free radical generating reactions (Johnson *et al.,* 1992).

Copper-dependent transcription factors regulate transcription of specific genes. Thus, cellular copper levels may affect the synthesis of proteins by enhancing or inhibiting the transcription of specific genes. Genes regulated by copper-dependent transcription

factors include genes for copper/zinc superoxide dismutase (Cu/Zn SOD), catalase, and proteins related to the cellular storage of copper (Uauy *et al.,* 1998).

A variety of indicators were used to establish the recommended dietary allowance (RDA) for copper, including plasma copper concentration, serum ceruloplasmin activity, superoxide dismutase activity in red blood cells, and platelet copper concentration (Food and Nutrition Board, Institute of Medicine, 2001). The RDAs for copper are shown in Table 8. The RDA for copper reflects the results of depletion-repletion studies and is based on the prevention of deficiency.

Table 8: Recommended Dietary Allowance (RDA) for Copper

Life Stage	Age	Males (mcg/day)	Females (mcg/day)
Infants	0-6 months	200	200
Infants	7-12 months	220	220
Children	1-3 years	340	340
Children	4-8 years	440	440
Children	9-13 years	700	700
Adolescents	14-18 years	890	890
Adults	19 years and older	900	900
Pregnancy	all ages	-	1 000
Breastfeeding	all ages	-	1 300

2.7 Free radicals

2.7.1 Definition and formation of free radicals

A free radical is an atom or group of atoms that has one or more unpaired electrons and can have positive, negative or neutral charge. Free radicals are formed as necessary intermediates in a variety of normal biochemical reactions. When generated in excess or not appropriately controlled, free radicals can cause damage to a wide range of macromolecules. A prominent feature of free radicals is that they have extremely high chemical reactivity, which explains not only their normal biological activities, but how they inflict damage on cells (Becker *et al.,* 2004).

Free radicals have been implicated in many disease conditions in humans, including arthritis, haemorrhagic shock, atherosclerosis, ischemia and reperfusion injury of many organs, Alzheimer and Parkinson's disease, gastrointestinal dysfunctions, tumour promotion and carcinogenesis, and AIDS (Bagchi *et al.,* 2000).

Many types of free radicals exist, but those of most concern in biological systems are derived from oxygen, and known collectively as reactive oxygen species (ROS). Oxygen has two unpaired electrons in separate orbitals in its outer shell. The unpaired electrons make oxygen susceptible to the formation of radicals. Sequential reduction of molecular oxygen, equivalent to sequential addition of electrons, leads to formation of a group of reactive oxygen species including the superoxide anion, peroxide anion, hydroxyl radical anion whose structures are shown in Figure 1, along with the notation used to denote the structures (Becker *et al.,* 2004).

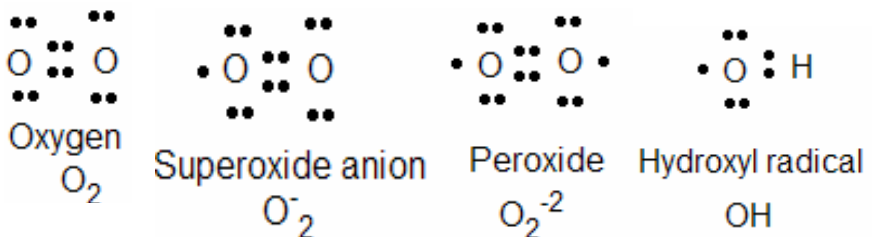


Figure 1

Reactive oxygen species and the naming notation

2.7.2 Exogenous sources of free radicals

Exposure to radiation from the environment and man-made sources causes formation of free radicals. Low-wavelength electromagnetic radiation such as gamma rays splits water in the body to generate hydroxyl radicals (OH-). The highly reactive OH- thus formed begins to react vigorously with the nearby cells (Halliwell, 1994).

It has been estimated that 1 to 3% of the oxygen we breathe in is used to make superoxide radicals (O_2-). Over 2 kg of O_2- is made in the human body every year and people with chronic inflammations may make much more to combat infection (Halliwell, 1994). The oxidants generated damage cellular macromolecules, including DNA, proteins and lipids and accumulation of such damage may contribute to ageing and age related diseases (Fraga *et al*., 1990).

2.7.3 Endogenous sources of free radicals

Enzymatically or non-enzymatically mediated electron transfer reactions are the source of free radicals produced in the cells. Electron leakage that occurs from electron transport chains, such as those in the mitochondria and endoplasmic

reticulum, to molecular oxygen are the major source of free radicals (Fridovich, 1986). Free radicals are formed in cells of our body mainly from;

- Consumption of O_2 by mitochondria during normal aerobic respiration to produce H_2O Oxidants such as oxygen free radical, H_2O_2 and hydroxyl radical are the by-products of this process
- Destroying of bacteria and virus infected cells by phagogytic cells releases nitric oxide, hydrogen peroxide and oxygen free radical
- Degradation of fatty acids and other molecules by peroxisomes, the peroxisomes produce hydrogen peroxide as a byproduct, which is then degraded by catalase
- Oxidants produced during the course of cytochrome P450 degradation of xenobiotics

2.7.4 Effects of free radicals on cells

Humans have developed many defense mechanisms to limit the level of reactive oxidants and the damage inflicted by them (Sang *et al*., 2002). Despite the cell's antioxidant defense system to counteract oxidative damage from free radicals, radical-related damage of DNA and proteins have been proposed to play a key role in the development of age-dependent diseases such as cancer, arteriosclerosis, arthritis, neuro-degenerative disorders and others (Ames, 1989).

Reactive oxygen species interact with cellular components including DNA bases and form damaged bases or strand breaks (Atoui *et al*., 2005). Oxygen radicals oxidize lipids or proteins generating intermediates that react with DNA by forming adducts.

2.8 Antioxidants

2.8.1 Definition of antioxidants

An Antioxidant is defined as a substance that when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance (Halliwell and Guteridge, 1989). For the *in vivo* situation the concept of antioxidants includes antioxidant enzymes, iron binding and transport proteins and other compounds affecting signal transduction and gene expression (Guteridge, 1989).

2.8.2 Classes of antioxidants

Antioxidants are divided into two major classes, namely endogenous antioxidants and exogenous antioxidants.

2.8.3 Endogenous antioxidants

Three groups of enzymes play important roles in protecting cells from oxidative stress (Becker *et al.,* 2004). Firstly, superoxide dismutases (SOD) are enzymes that catalyze the conversion of two superoxides to hydrogen peroxide and oxygen. Hydrogen peroxide is substantially less toxic than superoxide. The detoxifying reaction catalyzed by SOD is ten thousand times faster than the uncatalyzed reaction (Becker *et al.,* 2004).

SODs are metal-containing enzymes that depend on bound manganese, copper or zinc ion for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms are predominant in cytoplasm. SODs are inducible enzymes, with exposure of bacteria or vertebrate cells

to higher concentrations of oxygen resulting in rapid increases in the concentration of SOD.

Secondly, catalase, found in peroxisomes in eukaryotic cells, degrades hydrogen peroxide to water and oxygen, and hence completes the detoxification reaction started by SOD. Finally, glutathione peroxidase, a group of enzymes which are the most abundant contain selenium and like catalase, degrade hydrogen peroxide.

Glutathione is the most important intracellular defense against damage by reactive oxygen species. The cysteine on the glutathione molecule provides an exposed free sulphydryl group that is very reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione but the reduced form is regenerated in a redox cycle that involves glutathione reductase and the electron acceptor NADPH (Baydar *et al.,* 2007).

In addition to the three enzymes above, glutathione transferase, ceruloplasmin, hemoxygenase may participate in enzymatic control of oxygen radicals and their products.

2.8.4 Exogenous antioxidants

The three common exogenous antioxidants are vitamin E, vitamin C/ ascorbic acid and glutathione. Vitamin E is the major lipid-soluble antioxidant and plays an important role in protecting membranes from oxidative damage. The primary activity of vitamin E is to trap peroxy radicals in cellular membranes and consequently prevent lipid peroxidation of the membranes (Baydar *et al.,* 2007).

Vitamin C is a water-soluble antioxidant that can reduce radicals from a variety of sources. Vitamin C participates in recycling vitamin E radicals. Vitamin E radicals are generated when the vitamin trap peroxy radicals in cellular membranes. Vitamin C also functions as a pro-oxidant under certain circumstances and sometimes produces oxygen by-products of metabolism that can cause damage to cells (Coinu *et al.,* 2007).

In addition to vitamin E and vitamin C, phenolic compounds can function as antioxidants. The antioxidant properties of some plant extracts have been attributed partially to their phenolic compound contents (Coinu *et al.,* 2007).

2.9 Phenolic compounds

2.9.1 Definition of phenolic compounds

Phenolic compounds can be defined as naturally occurring organic species that possess at least one aromatic ring with one or more hydroxyl groups attached to the ring. Most naturally occurring phenolic compounds exist as conjugates with monosaccharides and polysaccharides linked to one or more of the phenolic groups (Harbone, 1998).

2.9.2 Classification of Phenolic compounds

There are many classes of phenolic compounds, with phenolic acids, flavonoids, and tannins as the main dietary phenolics. Phenolic acids include hydroxybenzoic and hydroxycinnamic acids. Flavonoids are a diverse group of secondary plant metabolites that include flavonols, flavanols, flavanones and flavones. The major classes of tannins in the plant kingdom are hydrolysable and condensed tannins (Harbone, 1998).

2.9.2.1 Phenolic acids

2.9.2.1.1 Hydroxybenzoic acids

The structure of hydroxybenzoic acids is based on that of *p*-hydroxybenzoic acid. Variations in the hydroxylation and methylation of the aromatic ring account for the differences in the individual hydroxybenzoic acids. The four most common acids, *p*-hydroxybenzoic, vanillic, and protocatechuic acid are shown in Figure 2.

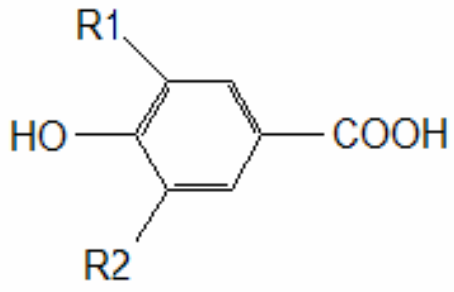


Figure 2
p-Hydroxybenzoic; R1= R2=H, vanillic; R1= Ome, R2=H, protocatechuic acid; R1= OH, R2=H and syringic acid; R1= R2 =Ome. Where Ome is a methyl group

Hydroxybenzoic acids occur as soluble forms that are conjugated to sugars or in an insoluble form, bound to lignin. Salicylic acid (2-hydroxybenzoate), whose structure is shown in Figure 3 is also a common hydroxybenzoic acid. Gallic acid, whose structure is shown in Figure 4, is a trihydroxyl derivative that participates in the formation of hydrolysable gallotannins. Hexahydroxydiphenic acid, the dimeric condensation product of gallic acid and ellagic acid shown in Figures 5 and Figure 6 respectively are common plant metabolites. Ellagic acid occurs predominantly in polymeric ellagitannins, where two gallic acid

compounds are coupled to form a hexahydroxydiphenic acid molecule shown in Figure 5 (Haddock *et al*., 1982; Haslam, 1982; Maas *et al*., 1992).

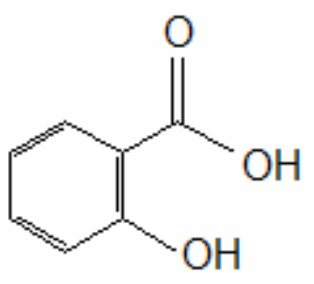


Figure 3

Salicylic acid

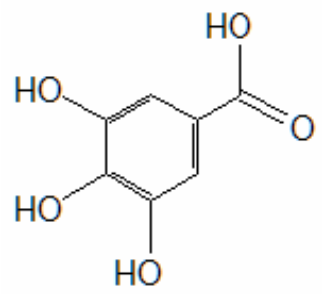


Figure 4

Gallic acid

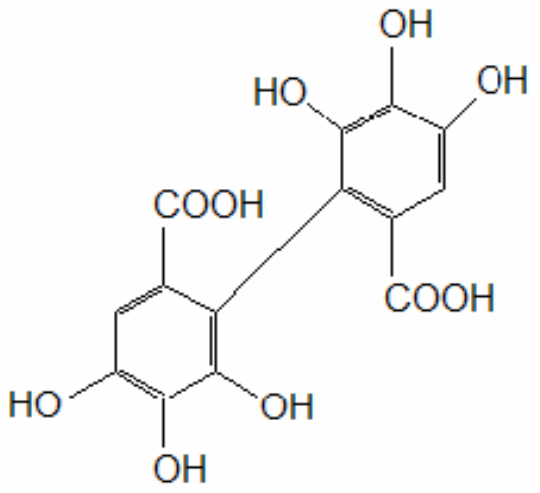


Figure 5
Hexahydroxydiphenic acid

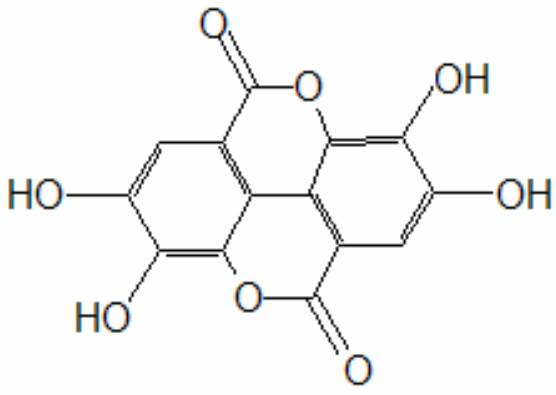


Figure 6
Ellagic acid

2.9.2.1.2 Hydrocynnamic acids

Hydroxycinnamic acids occur in various conjugated forms, with the free forms being artifacts that arise from chemical or enzymatic hydrolysis that takes place during

extraction of phenolic compounds from tissue. The conjugated forms are esters of hydroxyacids such as quinic, shikimic, tartaric acid and sugar derivatives. *p*-Coumaric, caffeic, ferulic and sinapic acids, shown in Figure 7, are the four naturally occurring acids in fruits (Galvez *et al*., 1997; Aljadi and Yusoff, 2002).

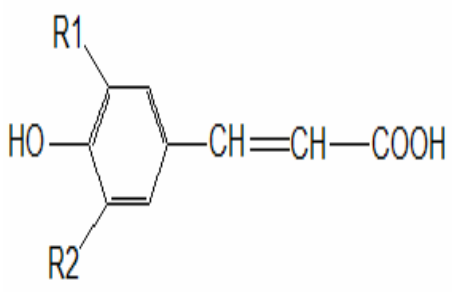


Figure 7
p-Coumaric acid; R1= R2 = H, caffeic acid; R1= OH, R2 = H, ferulic acid; R1= Ome, R2 = H and sinapic acid; R1= R2 = Ome

2.9.2.2 Flavonoids

Flavonoids are a diverse group of secondary plant metabolites that are characterized by the presence of a C15-(C6-C3-C6) flavone nucleus that is based on a heterocyclic ring systems derived from phenylalanine (ring B) and polyketide biosynthesis (ring A) linked through an oxygen containing pyran or pyrone ring (ring C) (Markam, 1982; Macheix *et al*., 1990; Harborne, 1998; Maraisi *et al*., 2006). The numbering system for the flavonoid skeleton is shown in Figure 8.

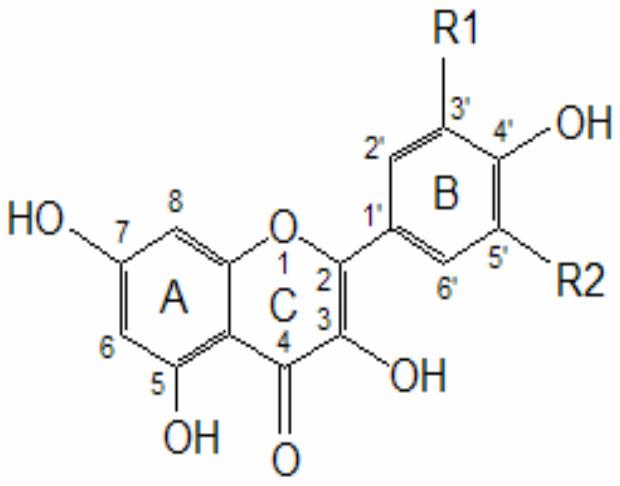


Figure 8

Flavonoid skeleton in which rings A, B and C and the numbering system are shown. Kaempferol, R1= H, R2=H; quercetin, R1= OH, R2= H; myricetin, R1=OH, R2= OH

The structures of the most common flavonoids in fruits, flowers and leaves of many plants are shown in Figure 9. Flavonoids are pale yellow compounds that are poorly soluble in water (Maraisi *et al*., 2006). They occur in foods as O-glycosides, D-glucose being the most common sugar residue although D-galactose, L-rhamnose, L-arabinose, D-xylose and D-glucuronic acid are also found. The preferred binding site for the sugar residue is C3 with binding occurring less frequently at the C7 position (Macheix *et al*., 1990).

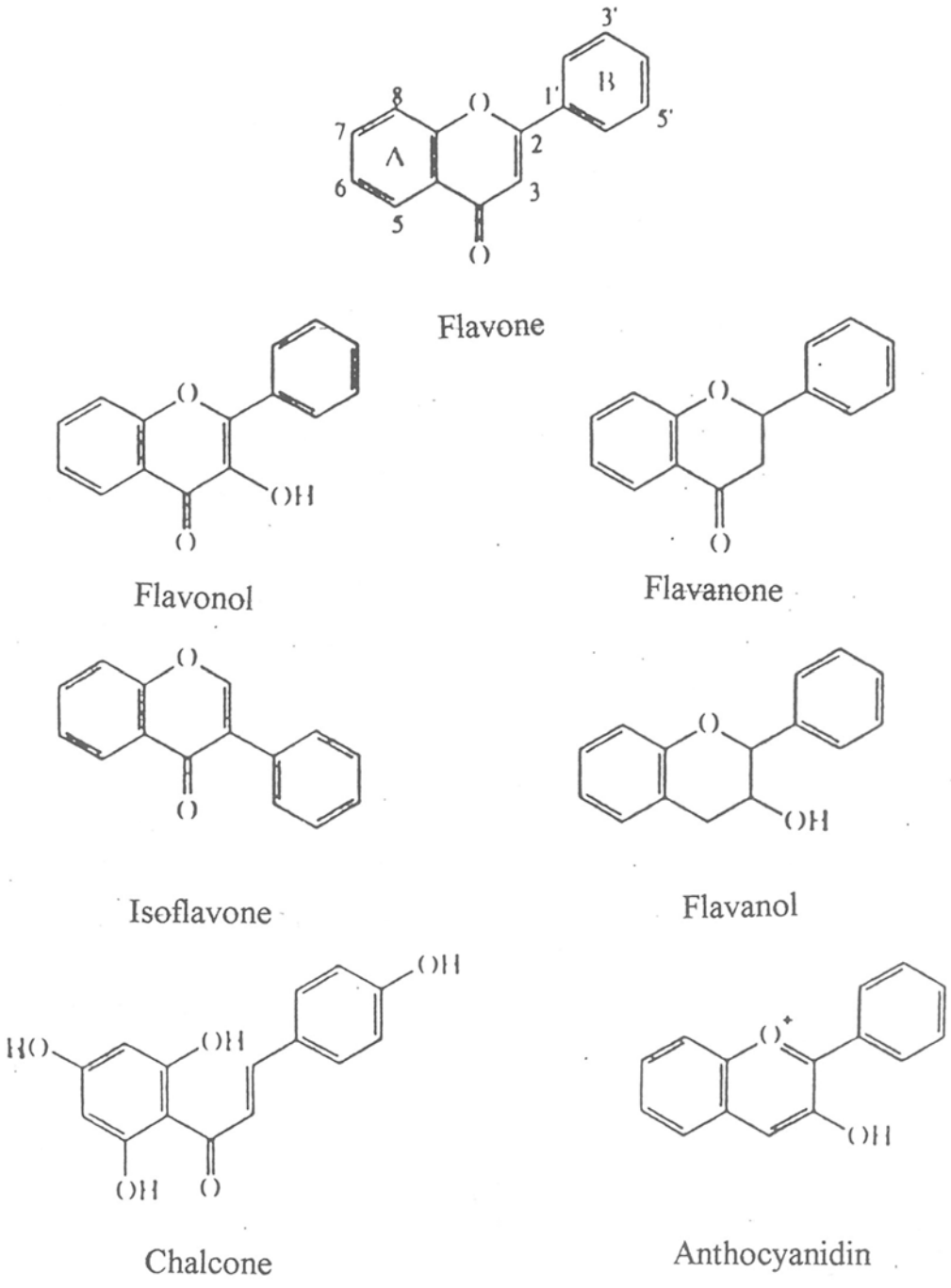


Figure 9

Major structures of flavonoids

2.9.2.2.1 Flavonols

This type of flavonoid structures is vastly distributed in the plant kingdom, forming an integral part of our daily diet. The structural composition of the flavonols depends to a large extent on the environmental factors. Over 200 aglycons have been described so far, among them quercetin, kaempferol, myricetin, and isorhamnetin as the most extensively distributed species (De Man, 1999). The structures of kaempferol, quercetin, myricetin, and isorhamnetin are shown in Figure 10.

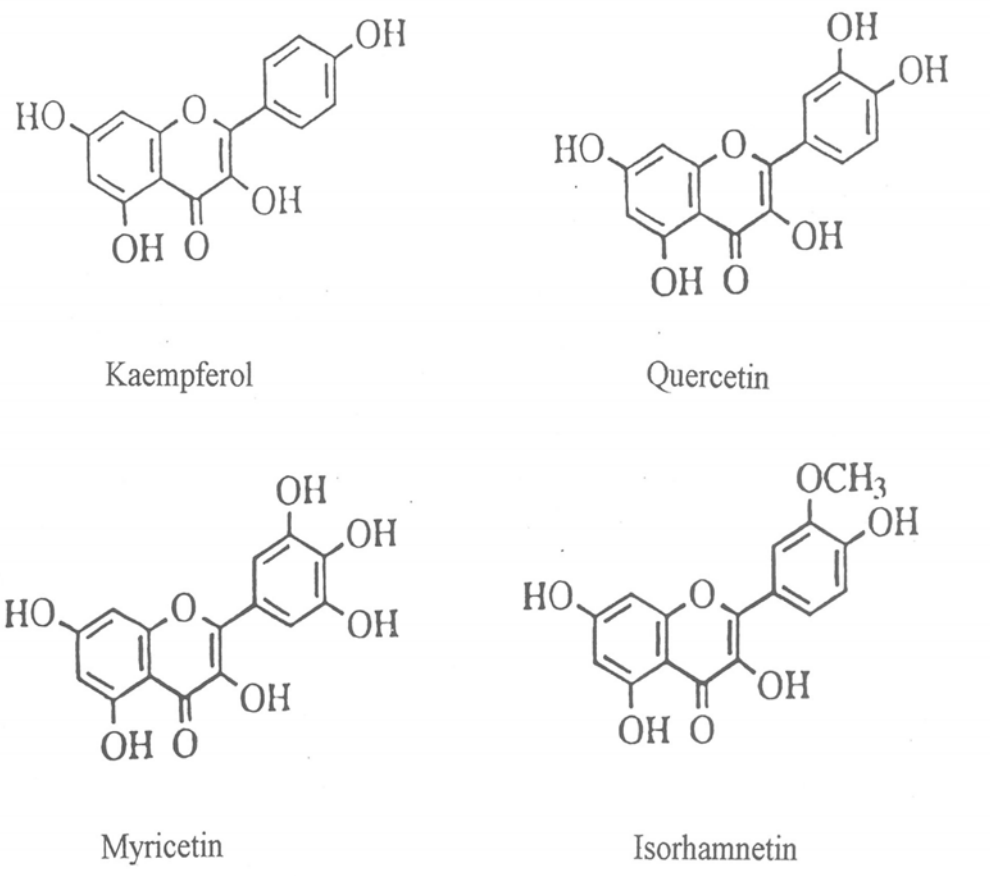


Figure 10
The structures of kaempferol, quercetin, myricetin and isorhamnetin

Quercetin - a ubiquitous compound of pharmacological importance belongs to flavonol class of flavonoids. It is found abundant in onion and apple, besides being present in other fruits and vegetables as well (Formica and Regelson, 1995; Hertog *et al.,* 1992).

Among various flavonoids, quercetin acts as a strong reducing agent, which together with other dietary reductants such as vitamin C, vitamin E, and carotenoids protects body tissues against oxidative stress. Commonly referred to as a strong antioxidant, it prevents organisms from various diseases associated with oxidative stress such as cancer, cardiovascular diseases, inflammation, and other degenerative diseases (Ames *et al.,* 1993).

Enthusiasm for quercetin in cancer prevention stems from its number of separate and independent mechanisms of antitumour action, especially through inhibiting tyrosine kinase in phase I clinical trials (David *et al.,* 1996). Similarly, high concentrations of quercetin have been found to be associated with the inhibition of growth of the malignant cells by arresting the cell cycle in the late-G1-phase or causing apoptosis of malignant cells (Yao *et al.,* 2004; Lakhanpal and Rai, 2007).

2.9.2.2.2 Flavanols

The flavan-3-ol structures constitute one of the most commonly distributed flavonoid families in nature. Within their structural framework, flavanols have got monomer units corresponding to (+) catechin and (-) epicatechin structures. Oligomer structures of (+) catechin and (-) epicatechin are known as procyanidins. The most relevant

structures are B1, B2, B3, B4 procyanidins and are shown in Figure 11 (Harborne, 1998).

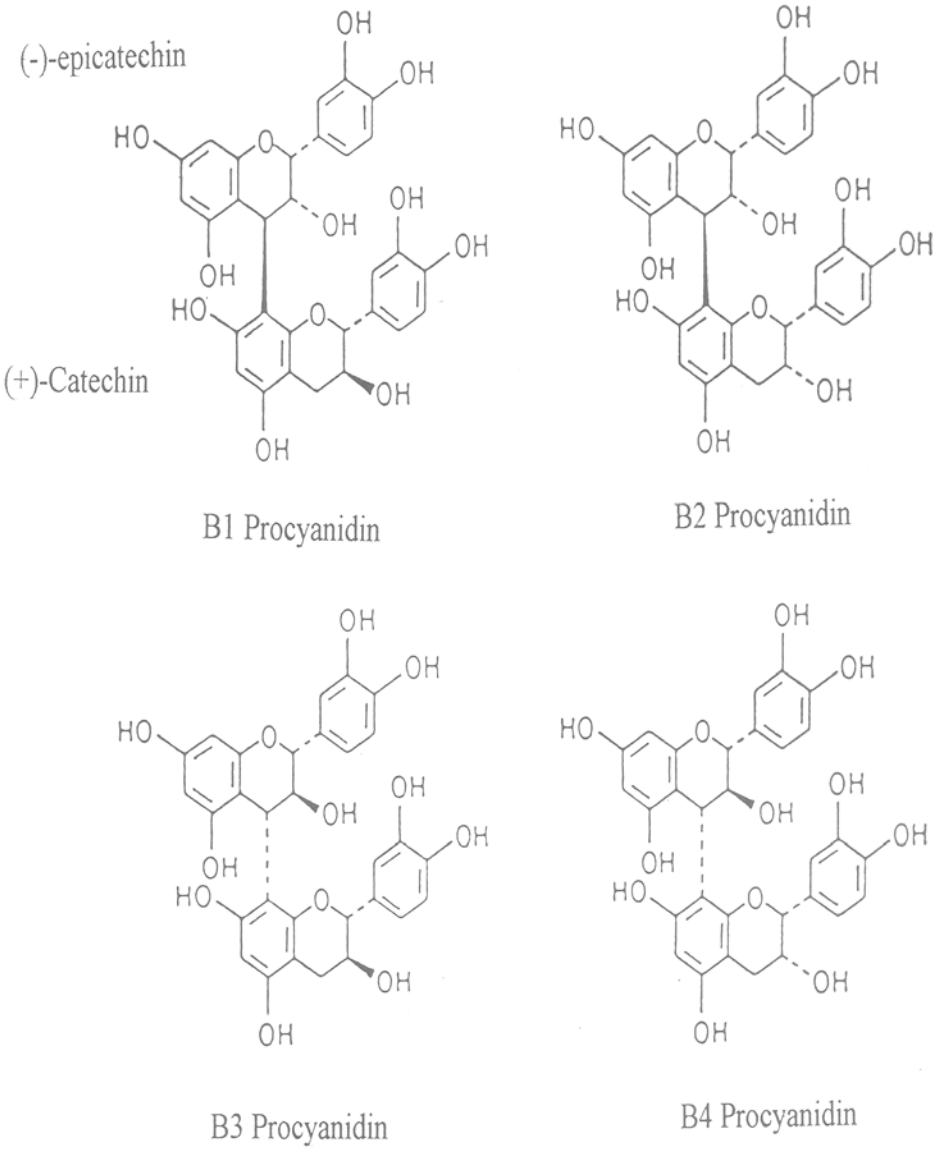


Figure 11

Representative structures of flavanols

2.9.2.2.3 Flavones

The flavones constitute the least representative polyphenolic group in foods. The most widely distributed aglycons correspond to apigenin and luteolin (De Man, 1999). The structures of some flavones are shown in Figure 12. Like all flavonoids, flavones can simply appear as glycosylated structures.

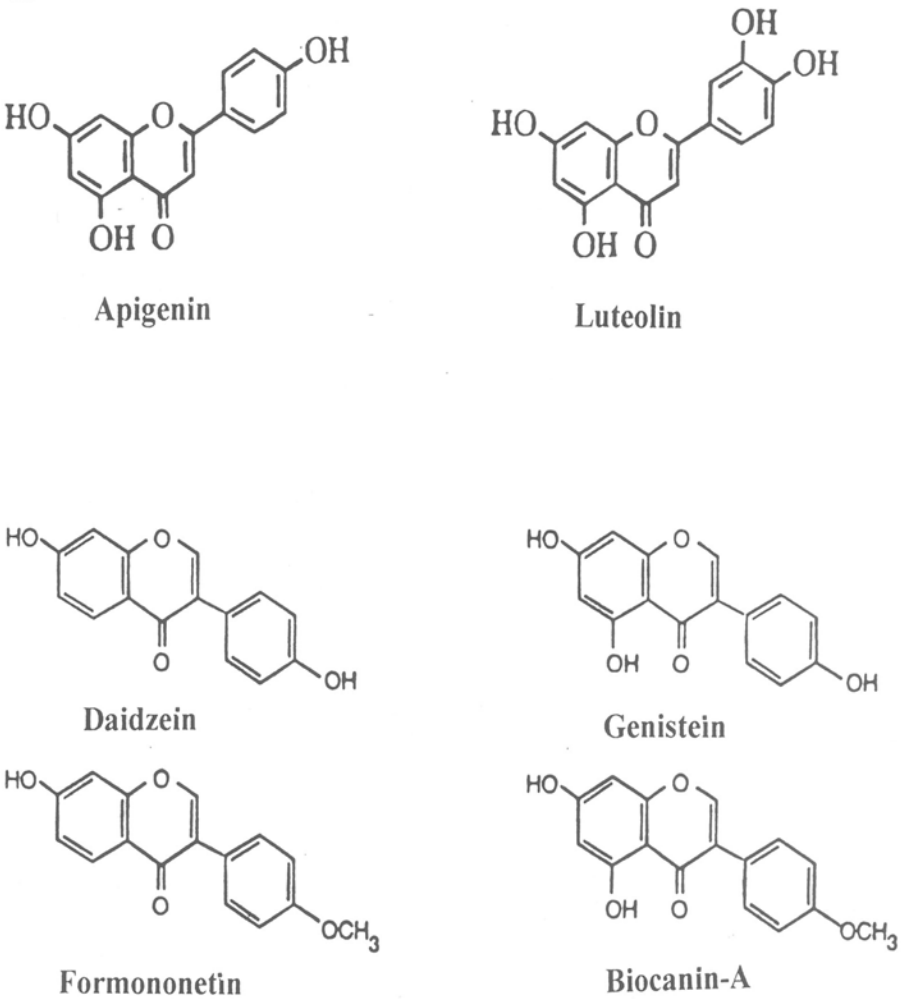
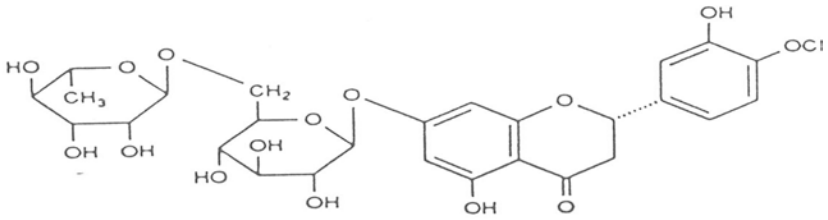


Figure 12

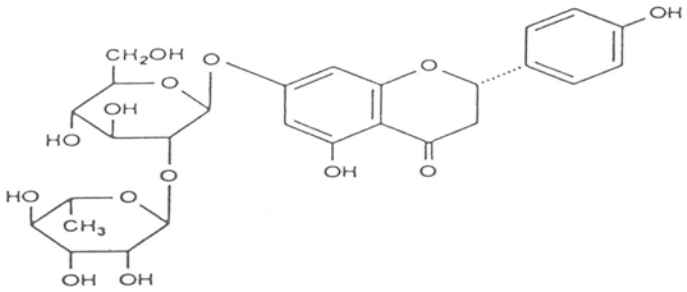
Representative structures of flavones

2.9.2.2.4 Flavanones

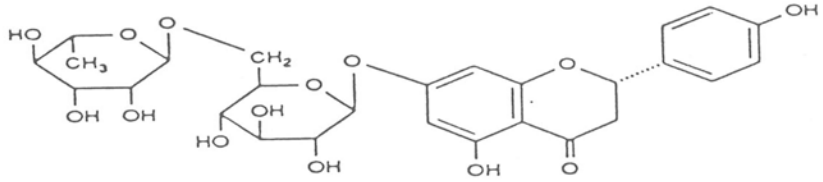
The flavanones constitute a minority group within the flavonoids, and as such are distributed in smaller quantities, except for the citric fruits, a group of fruits in which they constitute a polyphenolic majority. With respect to aglycons, the most frequent type corresponds to hesperetin, naringenin, and narirutin (Harborne, 1998), whose structures are shown in Figure 13.



Hesperidin



Naringin



Narirutin

Figure 13
Structures of some flavanones

2.9.2.3 Tannins

Tannins are naturally occurring uncrystallisable colloidal substances with pronounced astringent properties and whose main characteristic is to bind and precipitate gelatin from solution. It is the ability to form insoluble compounds with gelatin yielding tissue which enables tannins to convert raw hide and skin into leather. In the presence of tannins, the dermal network of hide is consolidated into firmer and drier structures that are thermally more stable, durable and more water resistant than the original hide (Yoshida and Hatano, 2000).

Tannins greatly affect the nutritive value of foods eaten by humans and feedstuffs eaten by animals (Mueller-Harvey, 2001). Tannins are common in foods such as grapes, persimmon, blueberry, tea, chocolate, the seeds of legumes such as *Acacia sp*., *Sesbania sp*. and in the grains of grasses such as sorghum and maize. Tannins are responsible for the astringent taste of wine or unripe fruits. Tannin products are responsible for the enchanting colours seen in flowers and in autumn leaves. Tannins are subdivided into three groups, the hydrolysable tannins (HT), proanthocyanidins (PAs) which are also called condensed tannins (CT) and mixed tannins (De Man, 1999; Jose *et al*., 2001).

2.9.2.3.1 Hydrolysable tannins

Hydrolysable tannins (HTs) are molecules that contain a polyol, generally a D-glucose as a central core as illustrated in Figure 14 and esterified with galloyl groups. In gallotannins the hydroxyl groups of the carbohydrates maybe partially or totally

esterified with phenolic groups such as gallic acid and ellagitannin (Yoshida *et al*., 1999; Hargerman, 2002).

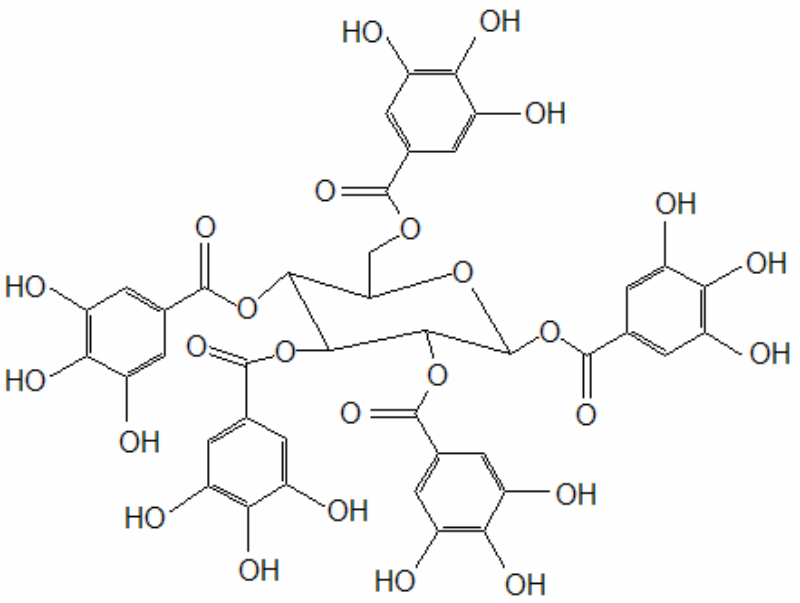


Figure 14
Penta galloyl-D-glucose

HTs have two additional groups called taragallotannins with gallic acid and quinic acid as the core and caffetannins with caffeic acid and quinic acid as the core. The structure of tannic acid has a penta galloyl-D-glucose core and five more units of galloyl linked to one of the galloyl of the core. HTs are usually present in small amounts in plants (Yoshida *et al*., 1997).

The source of gallotannins is tannic acid obtained from the twig galls of *Rhus semialata*. HTs are hydrolysed by mild acids or mild bases to yield carbohydrates and phenolic acids. HTs are also hydrolysed by hot water or enzymes such as tannase. Under the same conditions, PAs do not hydrolyse (De Man, 1999).

2.9.2.3.2 Proanthocyanidins

Proanthocyanidins (PAs) whose structure is shown in Figure 15 are widely distributed in plants than HTs and are oligomers of flavonoid units that are flavan-3-ol linked by carbon to carbon bonds, which are not susceptible to cleavage by hydrolysis.

The term proanthocyanidin is derived from the acid catalyzed oxidation reaction that produces red anthocyanidins upon heating in acidic alcohol solution, a reaction that forms the basis of the butanol-HCl assay for proanthocyanids.

The most common anthocyanidins produced by alcoholic acid oxidation are cyanidins from procyanidins and delphinidins from prodelphinidins (Hemingway *et al*., 1999). PAs carbon-carbon bonds are not cleaved by hydrolysis. Depending on the chemical structure and degree of polymerisation, PAs may or may not be soluble in aqueous organic solvents (Naczk *et al*., 1999).

Anthocyanidin pigments are responsible for the wide array of pink, scarlet, red, mauve, violet and blue colours in flowers, leaves, fruit juices and wines. The pigments are also responsible for the astringent taste of fruits and wines (Harbone, 1998; Mueller-Harvey, 2001).

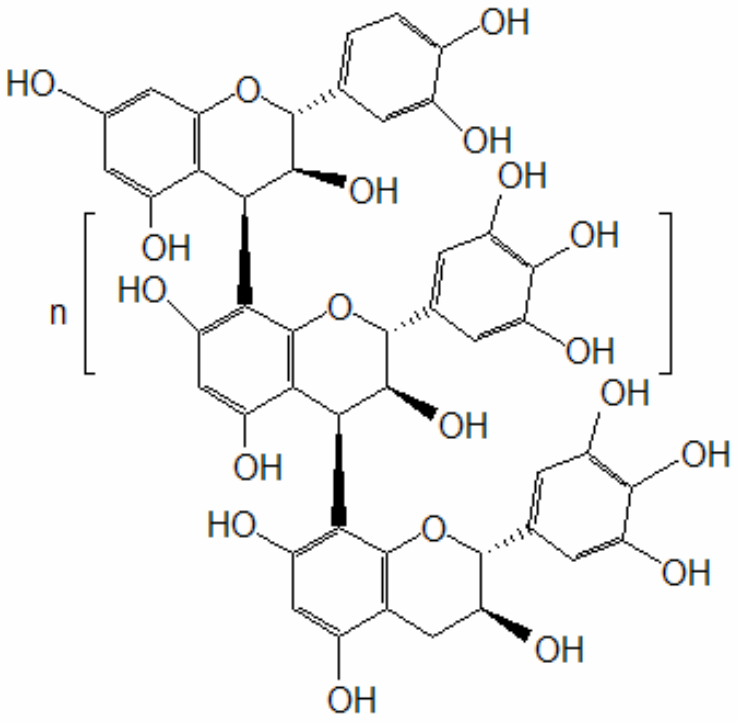


Figure 15
Proanthocyanidin. Where n is the repeating number that makes up the polymer

2.9.2.3.3 Mixed tannins

They are tannins where the building blocks of both CTs and HTs occur within the same compound. Figure 16 shows a mixed procyanidin trimer. This is a tannin molecule with two OH groups on the B ring and three OH groups on rings E and H (Cai *et al.,* 1991). Figure 17 depicts aesculitannin G from the horse chestnut tree. It is a tetrameric CT with both A- and B-type inter-flanol linkages (Morimoto *et al.,* 1987). Figure 18 shows an unusual tannin compound which contains one sugar, one gallic acid and one flavonol. It is a commonly held view that tannins must have molecular weights of at least 500 in order to precipitate proteins. This compound has a molecular

weight of 616 and just passes this threshold. However, the tanning activity of this relatively small compound is stronger than that of tannic acid (Yazaki., *et al.,* 1989).

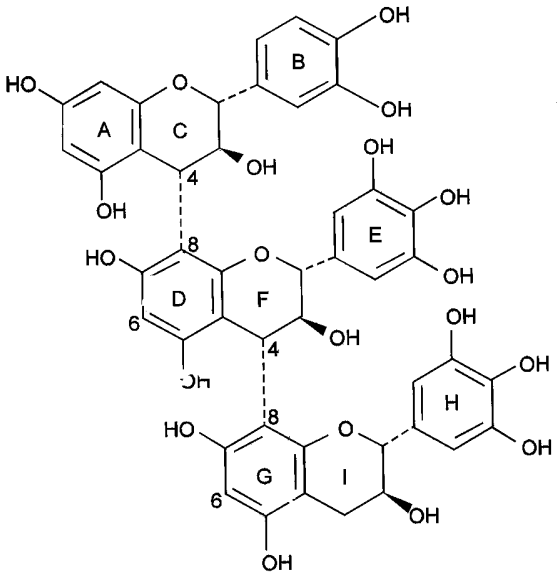


Figure 16

A mixed procyanidin-prodelphinidin trimer

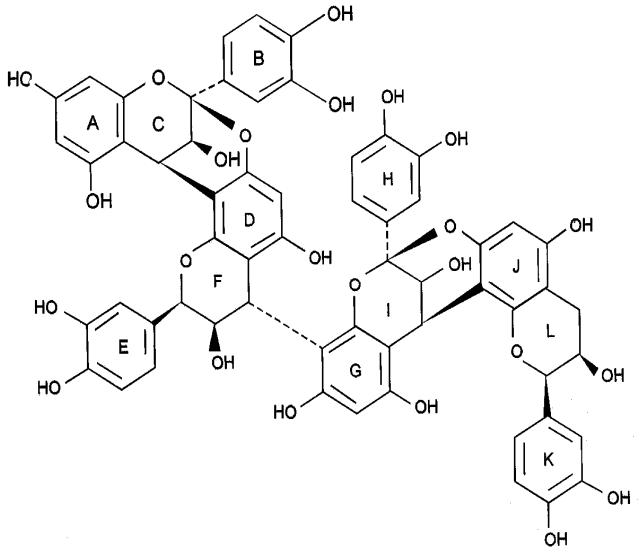


Figure 17
Aesculitannin G

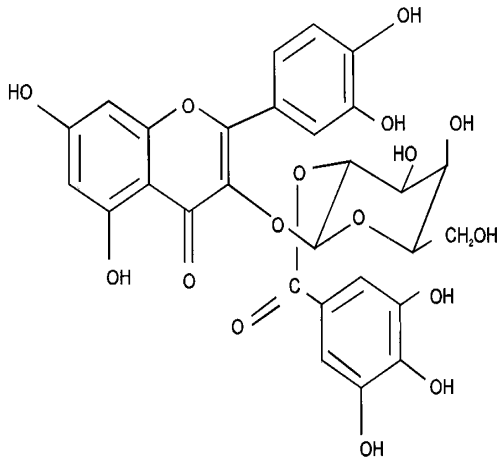


Figure 18

Hyperin-2”-O-gallate

2.10 Resveratrol

Resveratrol (3,4,5-trihydroxystilbene) belongs to a class of polyphenolic compounds called stilbenes (Langcake and Pryce, 1976; Yoshiaki *et al.,* 2002). Resveratrol and its most common conjugates and analogs are shown in Figure 19. The stilbene originates in at least 72 species of plants distributed amongst 31 genera and 12 families (Creasy and Coffee, 1988. All of the families found to contain resveratrol belong to the spermatophytes division: *Vitaceae, Myrtaceae, Dipterocarpaceae, Cyperaceae, Gnetaceae, Leguminosae, Pinaceae, Moraceae, Fagaceae,* and *Liliaceae* (Siemann and Creasy, 1992; Sanders *et al.,* 2000). As a dietary source, it is a naturally occuring antioxidant found in grapes (Soleas *et al.,* 1997), grape products such as wine (Tokusoglu *et al.,* 2005) and some other botanical sources like peanuts (Wang *et al.,* 2002), pistachio (Callemien *et al.,* 2004), strawberries, currants, cranberries and cranberry juice (Ehala *et al.,* 2005). Langcake and Pryce in 1977 detected *trans*-

resveratrol in hop, suggesting that stilbenes might also be found in beer. Quantitative amounts of *trans*-resveratrol were found as 0.03 to 7.17 ppm in grapes, grape vine, and red wines; they range from 0.01 to 1.79 ppm in peanuts (Callemien *et al.,* 2004).

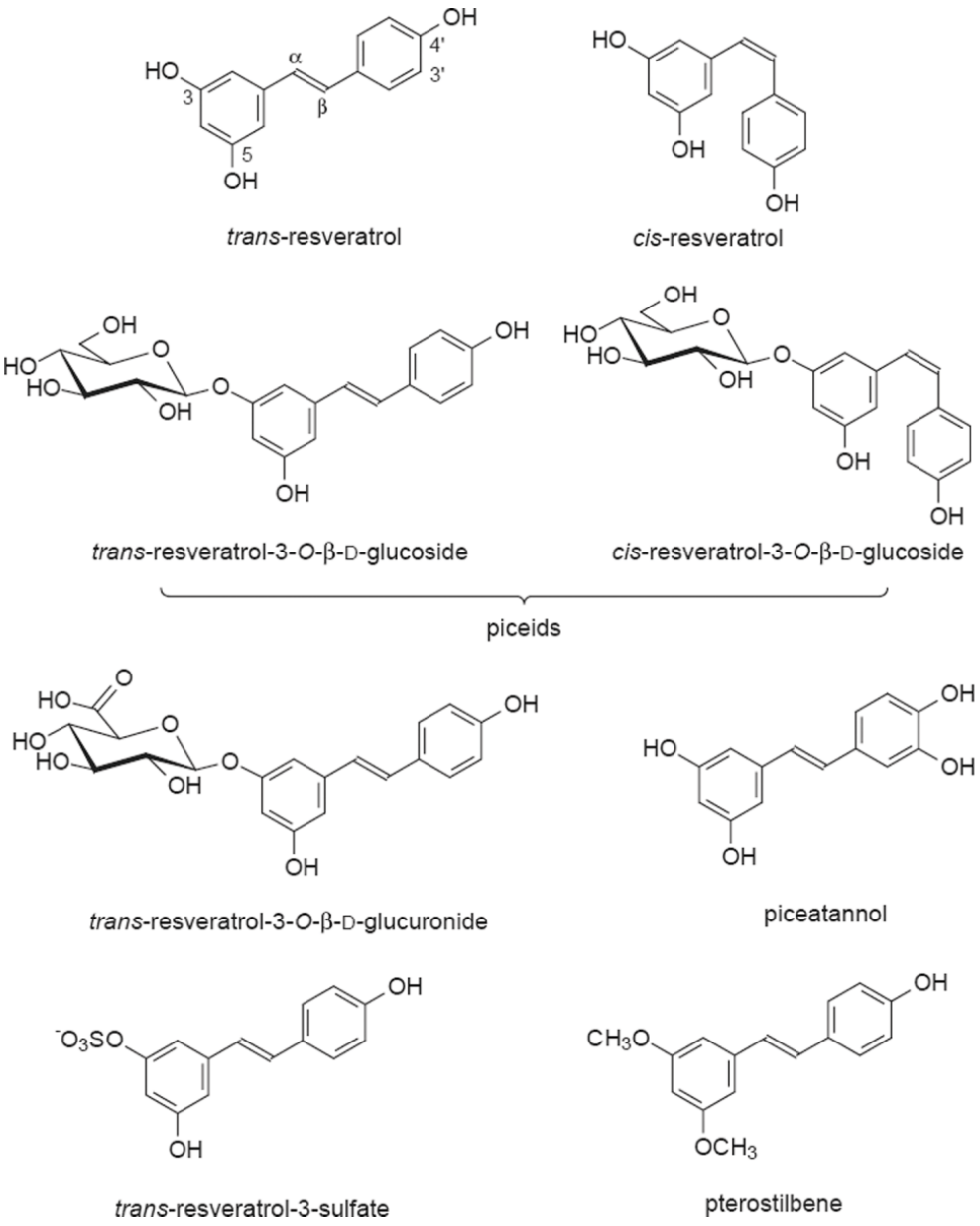


Figure 19

Resveratrol and its most common conjugates and analogs

The plant acting as a self-defense agent against fungal infections, injuries, stresses, UV irradiation, chemicals and climatic conditions produces *trans*-resveratrol (Soleas *et al.,* 1997; Dixon, 2001; Stervbo *et al.,* 2007). Resveratrol is toxic to plant pathogens, but some parasites such as fungi overcome this toxicity through the action of membrane proteins (ABC transporters) that transport the compound out of the cellular compartment (Jang *et al.,* 1997). Over production of stress response molecules in plants can trigger hypersensitivity reaction and this can lead to cell death when the stress cannot be counteracted (Nakaune *et al.,* 2002).

Resveratrol is of prime interest due to its antioxidant properties and other physiological effects in humans, including anti-platelet, anti-inflammatory, estrogenic, cardioprotective, anti-tumor and anti-viral action (Heath, 2000). In addition to this, resveratrol aids in the inhibition of lipid peroxidation, chelation of copper, free-radical scavenging activity and the modulation of lipid metabolism (Heath, 2000).

Scientists became interested in exploring potential health benefits of resveratrol in 1992, when its presence was first reported in red wine leading to the speculation that resveratrol might help explain the "French paradox," which is the low incidence of heart disease amongst people in France whom consume relatively high amounts of fat and red wine (Tokusoglu *et al.,* 2005).

2.11 Importance of phenolic compounds

2.11.1 Contribution to Flavour

Phenolic compounds may contribute to the aroma and taste of numerous food products of animal and plant origin. Singleton and Nobel in 1976 related the presence of chlorogenic acid, other hydroxycinnamates and, in particular, oligomeric proanthocyanidins to the bitterness and astringency of wine and cider. Similarly, Dadic and Belleau in 1973 associated bitterness to the presence of phenolics.

2.11.2 Phenolics as Natural Food Pigments

A large and diversified group of phenolic substances known as flavonoids is responsible for the color of fruits and vegetables. For example, anthocyanins are responsible for pink, scarlet, red, mauve, blue, and violet colors of vegetables, fruits, fruit juices, and wines (Mazza and Miniati, 1994). Other flavonoids also may contribute to the color of food products. Some of the groups of yellow or ivory flavonoid pigments that can be found in plants include flavonols, flavones, chalcones, aurones, flavanones, isoflavanones and biflavonyls (Mazza and Miniati, 1994).

2.11.3 Contribution to Stress Resistance

In response to stress, plants may use phenolics already present in cells, phenolics formed after stress from compounds existing in cells as a result of hydrolysis or oxidation or initiate biosynthesis of phenolics that contribute to the healing process. This last group of phenolics that possess antimicrobial properties also is known as phytoalexins (Macheix *et al.,* 1990). The usual response of plant to stress is an

increase in total phenolics content, especially chlorogenic acid (Rhodes and Wooltorton, 1978). Immediately after injury, there is an oxidation of existing phenolics, their subsequent degradation and decrease in phenolics content (Rhodes and Wooltorton, 1978).

2.11.4 Antimicrobial Properties

Many preservatives are added to foods either as antioxidants or as antimicrobial agents. Phenolic compounds are known to possess both of these properties. However, the lipophilic nature of phenols may reduce their antimicrobial properties (Baranowski and Nagel, 1984). Gallic, *p*-hydroxybenzoic acid and related phenolics have been found to retard or partially inhibit the growth and toxin production of *Clostridium botulinum* types A and B and the inhibitory activity increases with decrease in the bacterial concentration (Pierson and Reddy, 1982). Mold growth has been found to be inhibited by naturally occurring ethyl *p*-methoxycinnamate at concentrations of 10 to 50 ppm (Gupta and Banerjee, 1976). *p*-coumaric acid at a 100 ppm level increases the lag phase of *Saccharomyces cerevisiae* and at concentrations > 250 ppm, the inhibition after 72-hour growth is proportional to its concentration, whereas ferulic acid at 50 ppm brings about an increase in the lag phase and even complete inhibition is achieved at low concentrations of 250 ppm (Baranowski *et al.,* 1980).

2.11.5 Anti-viral Activity

A number of flavonoids present in foods of plant origin possess antiviral activity. For example, tannins from strawberries have the ability to inactivate polio, enteric, and herpes viruses (Konowalchuk and Spiers, 1976). Quercetin, a flavonol aglycone,

found in a number of fruits such as apple, apricot, fig, plum, strawberry, and tomato, has shown antiviral activities against herpes simplex virus type 1, Para influenza virus type 3, and polio virus type 1 both in the *in vivo* and *in vitro* studies (Middleton, 1986; Musci, 1986).

2.11.6 Anti-inflammatory Activity

Phenolic compounds extracted from bilberry juice, *Vaccinium myrtillus*, after fermentation constitute the active principal of a drug used for vascular protection. Anthocyanins present in the extract act on capillary permeability and fragility (Wagner, 1985). In skin inflammation, tumour necrosis factor (TNF) plays an important role as a potent activator of normal human keratinocytes (NHK) and as an inducer of pro-inflammatory mediators including Vascular Endothelial Growth Factor (VEGF) and interleukin-8 (IL-8).

Trompezinski *et al* in 2003 demonstrated that green tea extract and (-) epigallocatechin gallate (EGCG) inhibited in a dose-dependent manner the up-regulation of both VEGF and IL-8 in TNFα-stimulated keratinocytes. Their results confirm the anti-inflammatory activity of green tea extract and the role of its major polyphenolic constituent EGCG.

2.11.7 Anti-tumour Activity

Phenolic compounds have been reported to have antitumor activities, especially the flavonoids. The (-) epigallocatechin-3-gallate, a polyphenolic component of green tea, has been found to reduce the incidences of spontaneously and chemically induced

tumours in experimental animals, as observed for tumors of liver, stomach, skin, lungs, and esophagus (Huang *et al.,* 1992). Resveratrol has been shown to inhibit ribonucleotide reductase and certain other cellular events associated with initiation, promotion, and progression of carcinogenesis (Jang *et al.,* 1997). Administration of 25 μM of resveratrol reduced the number of skin tumours in mice by 98% and reduced the number of mice with tumours by 88% (Jang *et al.,* 1997).

2.11.8 Anti-cancer Activity

Many vegetables and fruits possess ellagic and chlorogenic acids, which serve as potential chemopreventers against several carcinogens (Huang *et al.,* 1992). Quercetin and rutin have the capability of inhibiting colonic neoplasia induced by azoxymethanol (Deschner, 1992). It has been suggested that regular consumption of tannins may induce the development of a defensive mechanism by animals and human beings to lower the risk of cancer. This may include utilization of very specialized tannin-binding proteins. For example, the mammalian herbivores produced salivary proteins with a high affinity for tannins (Salunkhe *et al.,* 1989). On the other hand, some tannins may exert anti-carcinogenic effects by acting as free radical scavengers.

2.11.9 Reduction of Coronary Heart Disease Risk

In a cohort study conducted in Finland (Knekt *et al.,* 1996) with 5 133 men and women, aged 30 to 69 years, onions and apples, rich sources of dietary flavonoids, were associated with a reduction in coronary heart diseases mortality. Individuals in the highest quartile for apple intake had an approximately 50% reduction in coronary mortality. Likewise, a similar reduction was reported for individuals in the highest

quartile of onion consumption. In a prospective study of 34 492 post-menopausal women in Iowa (Yochum *et al.,* 1999), total flavonoid intake was associated with a decreased risk in the group with the highest flavonoid intake. Thus, much of the epidemiologic evidence suggests that flavonoids have a protective effect against coronary mortality. For those studies that have reported an association, putative mechanisms of action include inhibition of low-density lipoprotein (LDL) oxidation and inhibition of platelet aggregation and adhesion (Frankel *et al.,* 1993).

2.12 Antioxidant extraction processes

Plants contain a wide spectrum of metabolites, as many as 200 000 different compounds (Fiehn *et al.,* 2002), although not every metabolite occurs in every species. These metabolites represent many different classes of compounds and their derivatives such as amino acids, fatty acids, carbohydrates, and organic acids. The physical-chemical properties of the metabolites are highly variable, therefore appropriate extraction protocols have to be chosen, as the optimum extraction conditions differ widely for different types of compounds.

2.12.1 Homogenization and solvent extraction

The plant tissue must be homogenised properly in order to extract plant metabolites efficiently. Various techniques such as grinding with a mortar and pestle together with liquid nitrogen, milling in vibration mills with chilled holders, homogenization with a metal pestle connected to an electric drill (Edlund *et al*., 1995) and ultra-turrax devices (Orth *et al*., 1999) are available.

The degree of homogenization determines the efficiency at which the solvent can penetrate the tissue, and therefore strongly influences the length of time required for solvent extraction. The most common way to extract metabolites is to shake the homogenized plant tissue at low or high temperatures in organic solvents, or mixtures of solvents (Fiehn *et al*., 2000). Methanol, ethanol, and water are the solvents mostly used for extracting polar metabolites, whereas chloroform is the most common solvent for non-polar ones (Roger, 1999).

2.12.2 Alternative extraction techniques

They include sub-critical water extraction (Ozel *et al.,* 2003), pressurized liquid extraction (Rostagno *et al*., 2004), microwave-assisted extraction (Shu *et al*., 2003) and supercritical fluid extraction (Roger, 1999). Different extraction techniques, such as Soxhlet, microwave assisted extraction and supercritical fluid extractions (Lopez-Sebastian *et al*., 1998) have been used to isolate antioxidants from the plants.

2.13 Analytical methods for phenolic compounds from plants

The analysis of phenolic compounds in plants has been reviewed by several authors (Van Sumere *et al.,* 1978; Markam, 1982; Waterman and Mole, 1994; Harborne, 1998; Makkar, 1999; Stobiecki and Kachlicki, 2006) and in plant based foods (Macheix *et al.,* 1990; Lee and Widmer, 1996). Herrmann in 1989 and Waksmundzka-Hajnos in 1998 reviewed the analysis of hydroxycinnamic and hydroxybenzoic acids in plants and plant-based foods. The analysis of flavonoids has been reviewed extensively by Markham in 1982 and 1989, Harborne in 1994, 1998 and Robards and Antolovich in 1997.

The analysis of phenolic compounds in raw or processed food matrix begins with extraction. The extraction procedure depends on the type of food to be analysed, the phenolic compound being investigated and the analytical procedure to be used (Lee and Widmer, 1996). The first step is to crush, mill, macerate, or grind the sample in order to increase the surface area of the material, allowing for more effective contact of the extracting solvent with the sample (Waterman and Mole, 1994; Makkar, 1999). Crushing, milling, macerating, or grinding helps in mixing the sample to ensure that the extracted portion is representative of the entire sample. Because many phenolic compounds occur as glycosides or esters, the sample preparation may include alkaline, acid or enzymatic hydrolysis in order to facilitate release of the bound phenolics, with the hydrolysis step being omitted if the phenolics are to be analysed as derivatives.

2.13.1 Analysis techniques of phenolic compounds

Quantitative analysis of individual phenolic compounds is difficult because most standards are not commercially available. It is necessary to use a standardized testing protocol for the evaluation of phenolic compounds. A four step procedure has been developed (Becker *et al.,* 2004).

The first step (I) in analysis of phenolic compounds involves the quantification and identification of the phenolic compounds in the material to be assayed for antioxidant activity. The second step (II) involves the quantification of the radical scavenging activity using more than one method and taking into account the effects of the solvent on antioxidant mechanism. The third step (III) is the evaluation of the ability of the antioxidants to inhibit or halt lipid oxidation in model systems. The last step (IV) is

divided into step IVa and IVb depending on the aim of the study. Step IVa is used for evaluation of food applications, where storage experiments are mandatory, while step IVb is required for evaluation of dietary antioxidant effect in the human body (Becker *et al.,* 2004).

Step I: Quantification and identification of phenolic compounds

One of the methods that have been developed for quantification of phenolic compounds is the Folin Ciocalteu (Folin C.) method, which is based on the reduction of phosphomolybdic acid in acid by phenols in aqueous alkali. The method is used to determine the total free phenolic groups (HTs and PAs). The method does not differentiate between tannins and many other phenolics that are not tannins. Interfering substances such as ascorbic acid, tyrosine and glucose are also measured (Singleton and Rossi, 1965; Makkar, 1999).

Commercial tannic acid which used to be the common standard for the Folin C method comprises a mixture of gallotannins. Tannic acid has been replaced by gallic acid in its use as a standard because of its heterogeneous structure which consists of a mixture of galloyl esters (Hargerman, 2002).

The butanol-HCl assay, used for the determination of PAs, involves the HCl catalysed depolimerization of PAs in butanol to yield red anthocyanidins that can be detected spectrophotometrically. A drawback of the butanol-HCl method is that it can lead to underestimation of phenolic compounds when PAs are hydrolyzed to dimmers and trimers instead of monomers (Makkar, 1999).

Vanillin-HCl assay is used to determine the concentration of PAs. Vanillin reacts with the meta-substituted A-ring of flavanols to form a chromophore that absorbs at a wavelength of 500 nm. The concentration of the flavanols is proportional to the absorbance of the solution. A drawback of the vanillin reaction is that low molecular weight flavanols over react and large polymers under react. In this reaction, catechin is used as a standard (Butler *et al.,* 1982; Porter *et al.,* 1986; Makkar, 1999).

The rhodanine assay is used for quantification of gallotannins. The sample is subjected to hydrolysis to release gallic acid which reacts with the dye rhodanine to produce intense colour that is measured spectrophotometrically at 520 nm (Makkar, 1999).

Tannin binding assay is based on the fact that polyvinyl polypyrolidone binds to all polyphenolic compounds and precipitates them. Folin C. method will determine the amount of simple phenolic compounds only. The disadvantage of this assay is that some of the polyphenolic compounds may fail to bind; hence the obtained value of simple phenolic compounds may include those of polphenols. This shortcoming is however minimal (Kuda *et al.,* 2005).

2.13.1.1 Chromatographic techniques for detecting polyphenolic compounds

In the 1950s and 1960s paper chromatographic methods were developed for phenolic compounds detection and quantification (Markham, 1982; Robards and Antolovich, 1997). Paper chromatographic techniques were largely replaced by thin–layer chromatography (TLC) in the 1970s providing an inexpensive and useful technique for the simultaneous analysis of several samples (Robards and Antolovich, 1997;

Harborne, 1998). Selection of a suitable stationary phase and solvent depends on the types of flavonoids to be examined. Flavonoids, such as flavonols, can be readily separated by TLC on polyamide or microcrystalline cellulose (Wildanger and Herrmann, 1973; Robards and Antolovich, 1997).

TLC is still in common use for preparative separations (Lee and Widmer, 1996) and as a rapid, low-cost screening method for determining the phenolic compounds present in fruits (Fernandez de Simon *et al.,* 1992) and honey (Sabatier *et al.,* 1992).

Gas chromatography (GC) has only a limited applicability in the analysis of phenolic compounds due to their limited volatility, which makes it necessary to include an extra step to prepare volatile derivatives of the phenolics (Lee and Widmer, 1996; Robards and Antolowich, 1997). GC analysis, combined with mass spectrometric (MS) detection, has been used for the analysis of phenolic compounds in black tea (Finger *et al.,* 1991) and cabbage (Nielsen *et al.,* 1993). Advantages of GC analysis include an improved separation of closely related isomers and simple coupling to MS detectors for identification through the fragmentation pattern (Mouly *et al.,* 1993, Schmidt *et al.,* 1994).

High-performance liquid chromatography (HPLC) has been the most widely used chromatographic technique in phenolic compounds analysis during the past 20 years (Robards and Antolowich, 1997; Harborne, 1998; Merken and Beecher, 2000). Advantages of HPLC include the improved resolution of flavonoid mixtures compared to other chromatographic techniques, the ability to obtain both qualitative and

quantitative data in one operation, and the great speed of analysis (Markham, 1989; Harborne, 1998).

Normal-phase chromatography has been used for the separation of flavone, flavonol and flavanone aglycones in orange juice (Galensa and Herrmann, 1980). Flavonoid acetates were separated isocratically on LiChrosorb Si60 using benzene-acetonitrile, benzene-ethanol or iso-octane-ethanol-acetonitrile solvent systems and detected at 312 or 270 nm. For normal-phase systems, it is possible that highly polar materials may be irreversibly retained in the column (Van de Casteele *et al.,* 1983), with the result that the separation characteristics of the column could be gradually altered.

Reversed-phase (RP) chromatography has been the method of choice for the separation of flavonols and other flavonoid groups in fruits. The typical methods of separation use a C18-column of particle size 3–5 mm, together with aqueous mobile phases and methanol or acetonitrile as an organic modifier. Small amounts of acetic acid, formic acid or phosphate buffers incorporated in the mobile phase may markedly improve separations of flavonoids and other phenolic compounds (Lamuela-Raventos and Waterhouse, 1994; Merken and Beecher, 2000).

Other modern separation systems such as capillary zone electrophoresis have been applied to only a limited extent, mainly for the analysis of proanthocyanidins in berries (Bridle and Garcia–Viguera, 1997; Costa *et al.,* 1998) and of polyphenolics in wine (Andrade *et al.,* 1998; Arce *et al.,* 1998; Prasongsidh and Skurray, 1998).

2.13.2 Chromatographic techniques for phenolic acids

TLC for the quantitative analysis of phenolic acids is usually carried out using normal phase chromatography on cellulose or silica layers and with the compounds being separated with a mixture of toluene, dioxane or benzene as hydrocarbon carriers and acetone, butanol, ethanol or acetic acid as polar organic modifiers (Azar *et al.,* 1987; Regnault-Roger *et al.,* 1987; Srisuma *et al.,* 1989; Agbor-Egbe and Rickard, 1990). The advantages of screening the sample extract by TLC prior to HPLC includes the detection of contaminants that may absorb to the stationary phase in the HPLC column and the determination of solvent conditions necessary for a successful separation of phenolic compounds (Fernandez de Simon *et al.,* 1992; Lee and Widmer, 1996).

2.13.2.1 Detection of phenolic compounds

Phenolic compounds absorb in the UV region and the most commonly used detector for HPLC is a variable-wavelength UV or UV-vis detector (Lee and Widmer, 1996; Robards and Antolowich, 1997). Because the compounds can display absorbance maxima at different wavelengths no single wavelength is ideal for monitoring all classes of phenolic compounds (Harborne, 1990). Most benzoic acid derivatives display maxima at 246 to 262 nm, except for gallic acid and syringic acid, which have absorption maxima at 271 and 275 nm, respectively (Torres *et al.,* 1987). Hydroxycinnamic acids absorb in two UV regions, the first maximum being in the range of 225 to 235 nm and the other in the range of 290 to 330 nm (Ribereau-Gayon 1972). At 320 nm, cinnamic acid derivatives can be detected without interference from benzoic acid derivatives, which have a higher absorptivity at 254 nm. Detection at 280 nm is the most effective alternative for the determination of both classes of phenolic

compounds (Pussayanawin and Wetzel, 1987). The range 350 to 370 nm has been widely used for flavonol aglycones and 280 nm for flavan-3-ol and flavonol glycosides (Robards and Antolowich, 1997).

Photo-diode array detection in the analysis of phenolic compounds has been successfully used and has led to improvement in the HPLC analysis (Jaworski and Lee, 1987; Mazza and Velioglu, 1992; Fernandez de Simon *et al.,* 1992; Hertog *et al.,* 1992). UV detection has been widely used in detecting phenolic compounds (Lee and Widmer, 1996; Roussef *et al.,* 1992; Hollman and Venema, 1993; Giachetti *et al.,* 1999; Dragovic-Uzelac *et al.,* 2007).

Electrochemical detection (EC) is very sensitive for compounds that can be oxidized or reduced at low-voltage potentials. EC detection is mainly used for the determination of small amounts of sample, as the technique shows enhanced sensitivity and selectivity than the other detection methods (Van Sumere *et al.,* 1978). EC detection has been applied for the detection of flavonols and phenolic acids in vegetables (Chiavari *et al.,* 1988), beverages and plasma (Lunte, 1987).

HPLC-MS is a fast and reliable method for structural analyses of non-volatile phenolic compounds, since techniques have been developed for the removal of the liquid mobile phase before ionization (Careri *et al.,* 1998).

Thermospray liquid chromatography LC-MS has been reported to be an excellent technique for the analysis of flavonol glycosides from medicinal plants (Pietta *et al.,*

1994; Meagher *et al.,* 2004). Flavan-3-ols (Lin *et al.,* 1993) and various groups of polyphenols including flavonol glycosides (Kiehne and Engelhardt, 1996) in tea have been studied using thermospray LC-MS (Meagher *et al.,* 2004).

Step II: Quantification of the radical scavenging activity

The radical scavenging assays involve direct measurement of hydrogen atom donation or electron transfer from the potential antioxidant to free radical molecules in lipid free systems. Such assays are available as commercial kits and or a laboratory can prepare their own reagents (Becker *et al.,* 2004). The assays for detection of antioxidant activity, scavenging of stable radicals and scavenging of short lived radicals are outlined below.

(a). Classical assays for detection of antioxidant activity

The electron transfer antioxidant activity is usually quantified by the ferric reducing antioxidant power (FRAP) assay which measures antioxidant capacity by the reduction of the ferric tripyridyltriazine complex to the blue ferrous complex (Becker *et al.,* 2004). A second assay for detecting antioxidants is the trolox equivalent antioxidant capacity (TEAC) which relies on the reduction of the coloured cation radical of 2,2-azonobis (3-ethylbenzothiazoline-6-sulphonate) ($ABTS^{+\cdot}$). The draw back of TEAC assay is use of the synthetic $ABTS^{+\cdot}$ radical, which does not occur in biological systems (Becker *et al.,* 2004).

(b). Assays for detection of scavenging of stable radicals

Assays on the scavenging effects of phenolic compounds is based on the stable chromogenic radical 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) quantified

spectrophotometrically at 516nm (ε=12.500 M^{-1} cm^{-1}) in the presence of the phenolic compound under investigation. The decrease in colour has been correlated to a dose response curve with a standard antioxidant as in TEAC assay. Other stable radicals such as Fremy's salt (potassium notrosodisulphonate) and galvinoxyl (2,6-di-tert–butyl-α-(3,5 –di-tert-butyl-4-oxo-2,5-cyclohexadienl-ylidene)-ptolyloxy radical) have been used for the same reaction (Becker *et al.,* 2004).

(c). Assays for the detection of scavenging of short-lived radicals

The oxygen radical absorbance capacity assay (ORAC) has been developed. ORAC assay is based on the quenching of fluorescence from the protein- β- phycoerythrin by radicals. β-phycoerythrin is not photostable and interacts with phenolic compounds. The assay has been modified to include fluorescein (ORAC-FL) instead of β-phycoerythrin (Becker *et al.,* 2004).

Step III: Evaluation for the ability to inhibit or halt lipid peroxidation in model systems

Assays based on the inhibition of lipid peroxidation include model systems which involve oxidation substrates. The evaluation of antioxidants in model systems is based on measuring changes in the concentration of compounds being oxidized, on depletion of oxygen or on the formation of oxidation products (Becker *et al.,* 2004; Kumazawa *et al.,* 2004).

Quantification of the loss of reactants, the formation of radicals and the formation of primary or secondary oxidation products is the mostly used marker for lipid

peroxidation depending on the stage of oxidation. Lipid oxidation involves concomitant formation and degradation of several products.

The depletion of oxygen and the ESR detection of radicals, either directly or indirectly by spin trapping, can be used to follow the initial steps of oxidation. Frankel in 1993 criticized the standard methods for the measurement of lipid oxidation as peroxide value (POV) which is based on the formation of lipid hydroperoxides, the thiobarbituric acid reactive substances (TBARS) based on formation of secondary oxidation products and the electrical conductivity caused by short-chained acids (Rancimat).

Two different methods, an iodometric method and an iron/thiocynate method are standard for POV, which often give different results. The TBARS test is often criticized for being unspecific and measures the formation not only of malondialdehyde, but also of other oxo-compounds. The Rancimat has the disadvantage of using high temperatures, which due to marked differences in energies of activation of different processes may result in oxidation levels of the peroxides far in excess. For most applications, detection of oxidation products is the more sensitive method compared to measurement of decrease in oxidation substrate concentration (Becker *et al.,* 2004; Schnitzer *et al.,* 2007).

2.14 Principles of some antioxidant assays

2.14.1 DPPH radical scavenging assay

DPPH is a relatively stable free radical. It is deep purple coloured reagent. Hydrogen donation to this compound results in it being decolourized to a yellow colour. Decolourization of the DPPH depends on the ability of the reducing agent to exert its function. The disadvantage of this assay is that other reducing agents, which are not phenolic compounds, can exert their effect (Kuda *et al.,* 2005).

2.14.2 Reducing power

Reducing power assay is based on the potential of phenolic compounds to donate their electrons to an oxidized substrate. The ferric state will be reduced to a ferrous state. The disadvantage of this assay is that other reducing agents in the extract such as ascorbic acid can also reduce the ferric state iron and hence the reducing power is cumulative activity of all reducing agents in the extract (Becker 2004).

2.14.3 β-carotene bleaching assay

Linoleic acid is easily oxidized and in the presents of oxidized lipids, β-carotene will donate its electrons and results in it being bleached. Phenolic compounds will inhibit the formation of oxidized lipids hence minimal bleaching is noticed (Kuda *et al.,* 2005). This assay may have shortcomings in that it is carried out at relatively high temperatures hence there could be oxidation of phenolic compounds and consequently, loss of activity. There could also be a cumulative antioxidant activity as a result of other reducing agents in the extracts.

2.14.4 Phospholipid peroxidation assay

In biological systems, lipid peroxidation generates a number of degradation products such as MDA. MDA is found to be the most important cause of cell membrane destruction and cell damage (Singh and Arora, 2007). The formation of MDA products has been used as an index of lipid peroxidation and oxidative stress. In this assay peroxidation is induced by addition of $FeCl_2$-H_2O_2. The rate at which MDA was produced decreased with increase in the concentration of sample extract.

3 MATERIALS AND METHODS

3.1 Samples procurement and preparation

The commonly used traditional vegetables that were mentioned by respondents during focus group discussions are shown in Table 9. The vegetables were of Zimbabwean origin and collected from Buhera district of Manicaland province, between 2007 and 2008. A composite sample comprising at least 3 vegetables of the same species was made. The vegetables in the composite sample were from ecological regions 3, 4 and 5 of Buhera district. Rape (*Brassica napus*) and Lettuce (*Lactuva sativa*) samples were bought in a Spar Borrowdale retail outlet. The leaves were transported in polythene bags and stored in a refrigerator at $4^{o}C$ upon arrival in our laboratory and dried the following day.

3.2 Reagents

All the chemical reagents used were of analytical grade. Caffeic acid, catechin, coumaric acid, ferulic acid, gallic acid, linoleic acid, protochatechuic acid, vanillic acid, butylated hydroxyl anisol (BHA), Kjedahl catalyst tablets, chloroform, dichlorophenol-indophenol (DCPIP), diethyl ether, 1,1-diphenyl-2 picrylhydrazyl radical (DPPH), ethyl acetate, acetonitrile, ethyl ether, $FeCl_3$, ferric ammonium sulphate, $FeSO_4$, Folin C reagent, Na_2SO_4, potassium ferricyanide, sodium carbonate, sodium hydrogen carbonate, sodium hydroxide, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tween 80, β-carotene, vanillin reagent were obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Other chemical reagents for the analyses were obtained locally.

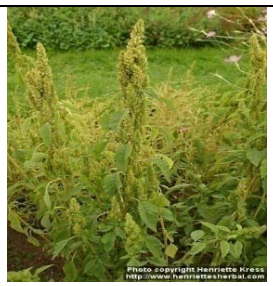
3.3 Identification of the edible plants

Most of the plants were recognized by the villagers *in situ* during short field walks and were collected for scientific identification. Identification of some of the plants was done by reference to Tredgold of 1986 and further confirmation was carried out at the National Botanical Gardens. Voucher samples were prepared and deposited in our laboratory in the department of Biochemistry at the University of Zimbabwe.

3.4 Collecting information on the seasonal availability, usage and methods of preparation of the edible plants

Information on the wild plants seasonal availability, part used as food and the methods of preparation and preservation methods was collected during a survey in 28 accessible wards of the 32 wards of Buhera district. A questionnaire (Appendix 1) was administered to 1 550 respondents. The ages of the respondents were ranging from 10 years to above 70 years. Of the 1 550 respondents, 995 were males and 655 were females. Meetings that were organized by the local councilors were attended mostly by men with administrative and political responsibilities. Focus group discussions were conducted before the questionnaire was administered to gather general information on the consumption of vegetables in Buhera. The respondents were asked about the recipes and ingredients used in the preparation of some dishes. Information on the medicinal uses of some vegetables was also gathered.

Table 9: Vegetables used in the study

Latin name	Vegetable	English name	Local *Shona* name	Family
Amaranthus hybridus		Amaranth	*Mowa*	Amaranthaceae
Bidens pilosa		Black jack	*Tsine*	Asteraceae
Cleome gynandra		Cats' whiskers	*Nyevhe*	Capparaceae
Corchorus olitorius		Bush okra	*Derere munda*	Tiliaceae
Galinsoga parviflora		Gallant soldier	*Teketera*	Asteraceae

3.5 Proximate composition

For proximate composition, the samples were assayed according to the modified methods of the (Association of Official Analytical Chemists) AOAC of 1990.

3.5.1 Crude fat content

In duplicate, 2 g of sample was weighed into a cellulose extraction thimble and the tube was plugged with glass wool before being placed into a Soxhlet extraction chamber. Sixty five milliliters of anhydrous ethyl ether was carefully added to a 100 mL round bottomed flask. The 100 mL round bottomed flask was attached to an extraction chamber and a condenser. The 100 mL round bottomed flask with ether was gently heated to boiling and ether condensed and collected back into the extractor chamber for 7 hr. After extraction the 100 mL round bottomed flask containing ether and dissolved lipid was removed and the traces of ether removed on a rotary evaporator. The flask was heated in a 100^{o}C oven for 1 hr and cooled in a desiccator. The flask with the dissolved lipid was weighed. The crude fat was calculated as follows:

% Fat on dry weight basis = (g of fat in sample / g of dried sample) x 100

3.5.2 Crude protein

Five grams of the sample was weighed into a 1 L Kjeldahl flask with 10 g potassium sulphate, copper sulphate catalyst tablets and 25 mL concentrated sulphuric acid. The mixture was digested until frothing had ceased. After clearing, the mixture was heated for an additional 60 min. The 1 L Kjeldahl flasks with digested samples were cooled and 400 mL distilled water added. After addition of 80 mL of 40% sodium hydroxide

into the digested sample the 1 L Kjeldahl flask was heated in order to liberate ammonia. The ammonia was collected into a boric acid solution (40 mg/L) that contained an indicator made up of 0.5 g bromosocresol green and 0.1 mL methyl red in 100 mL of 95% ethanol. Heating continued until 50 mL distillate was collected. The boric acid mixture was titrated against 0.1 M HCl until a faint pink colour persisted. The blank contained reagents only and the titre value was subtracted from the sample value. The crude protein was calculated as follows:

% Nitrogen of sample = [(volume of acid titrated – volume of blank titrated) x (Acid M x 0.14 x 100)] / weight of sample (g).

% Crude protein = % Nitrogen x 6.25.

3.5.3 Crude fibre

The sample from the fat extraction was transferred from the extraction thimble to a 250 mL Erlenmeyer flask. Two hundred millimeters of boiling sulphuric acid (1.25%) was added to the sample and the mixture was allowed to boil for 30 min. The digested sample was filtered using a cheese cloth and the residue was washed with distilled water several times. The washed residue was transferred into a 500 mL beaker and 200 mL boiling sodium hydroxide (1.25%) was added and boiled gently for 30 min. The residue was filtered using a cheese cloth and washed with boiling distilled water. The filter contents were transferred to a Gooch crucible and ignited in a muffle furnace at 550^{o}C for 60 min. The crucible was allowed to cool for 30 min and was reheated, cooled as many times as necessary to a constant weight. The amount of crude fibre was calculated as loss in weight between digested and ashed sample on a dry weight basis.

3.5.4 Carbohydrates

Carbohydrate content was determined by difference. The percentage matter remaining after moisture, ash, fat, and protein were determined was considered to be the carbohydrate content.

3.5.5 Determination of ascorbic acid content

3.5.5.1 Extraction of samples

Five grams of the vegetable sample was extracted with 100 mL of 1% acetic acid. The sample was centrifuged at 3000 rpm for 10 min and left to stand for 20 min. The supernatant was collected for further analysis.

3.5.5.2 Standardization and titration

Twenty milliliters of Dichlorophenol-indophenol (DCPIP) solution (26 mg dissolved in 100 mL distilled water containing 21 mg of sodium hydrogen carbonate) was titrated against ascorbic acid (50 mg in 50 mL metaphosphoric acid). The filtered solution (5 mL) was pippeted into a volumetric flask and acetic acid (20 ml, 1%) was added. The samples were titrated against Dichlorophenol-indophenol (DCPIP). The ascorbic acid content of the extracts was determined by interpolation from a standard graph of ascorbic acid titrated against DCPIP.

3.5.6 Mineral elements analyses

Phosphorous was determined photometrically using the molybdate-vanadate method (AOAC, 1990). Minerals (potassium, calcium, magnesium, iron, zinc and copper)

were determined in the ash from incineration of the leaves. The ash was dissolved in mixtures of concentrated HNO_3 and diluted with distilled water according to AOAC of 1990. Concentrations were determined with a Perkin-Elmer 2280 atomic absorption spectrophotometer.

3.6 Effects of cooking on nutritional composition

3.6.1 Cooking vegetables

Five hundred grams of the sun dried leaves were boiled for 2 hr, changing the cook water after 30 min. The leaves were left on a wire mesh to drain off surface water for 10 min. The sample was dried under direct sunlight for two weeks. Moisture content of the samples was determined before analysis of the nutrients.

3.6.2 Data analysis on the effects of cooking on nutrient content

The Students *t* test packaged in the Statistical Package for Social Sciences (SPSS) for Windows Standard Version 8.0.0 was used for the statistical evaluation with $P < 0.05$ considered statistically significant.

3.7 Determining the content of phenolic compounds

3.7.1 Extraction method for phenolic compounds

Phenolic compounds were extracted from the plant material following the method described by Makkar in 1999. The finely ground sample (2 g) was suspended in cold 50% aqueous methanol (10 mL) in a 50 mL conical flask suspended in ice. The suspended extract was subjected to ultrasonication for 20 min. Centrifugation of the

extract was done at 3000 rpm for 10 min. The supernatant was transferred into 25 mL small sample bottles for analysis.

3.7.2 Total content of phenolic compounds

Total phenolic compounds were determined following the method by Makkar of 1999. To a sample (50 μL), distilled water (950 μL) was added to make up to 1 mL, followed by 1 N Folin Ciocalteau reagent (500 μL) and 2% sodium carbonate (500 μL). After incubating the reaction mixture for 40 min at room temperature, absorbance at 725 nm was read on a Spectronic 20® Genesys™ spectrophotometer against a blank that contained 50% methanol. Total content of phenolic compounds were expressed in terms of equivalent amounts of gallic acid.

3.7.3 Vanillin assay for proanthocyanidins

The sample (5 μL) was made up to 1 mL with distilled water in a test tube before adding 1:1v/v methanol-HCl (2.5 mL) and finally 0.5g/25 mL vanillin reagent (2.5 mL). The mixture was vortexed and allowed to stand. After 20 min absorbance at 500 nm was read using a Spectronic 20® Genesys™ spectrophotometer against a blank of 50% methanol. The proanthocyanidins were expressed as catechin equivalence, as suggested by Porter *et al* in 1986.

3.7.4 The butanol-HCl assay for proanthocyanidin

To the sample (500 μL), 95:5 v/v butanol-HCl (3 mL) reagents was added, followed by 100 μL of ferric reagent prepared with 2% ferric ammonium sulphate in 2 M HCl. After mixing the sample, butanol-HCl and ferric reagent, the tubes were placed in a

boiling water bath. After 60 min, absorbance at 550 nm was measured against a blank prepared by mixing the extract (500 μL) and butanol reagent (3 mL) and ferric reagent (100 μL), but without heating. Condensed tannin (% dry matter) as leucocyanidin equivalent was calculated by the formula: (A_{550nm} x 78.26 x Dilution factor) / (% dry matter) as described by Makkar in 1999.

3.7.5 Tannin binding assay for tannins

The tannins were determined following the method of Makkar and Goodchild (1996). Polyvinyl-polypyrolidine (PVPL) (1 g) was dissolved in distilled water (1 mL). Samples (1 mL) were added to the mixture. The mixture was vortexed and left for 15 min at 4ºC. The mixture was centrifuged at 3000 rpm and the supernatant obtained was determined for total content of phenolic compounds. The tannin content was calculated as follows:

Tannin content (mg /g) = Total content of phenolic compounds before binding with PVPL - Total content of phenolic compounds after binding with PVPL.

3.8 Determining antioxidant capacities of the extracts

3.8.1 DPPH radical scavenging activity

The radical scavenging activity was determined following the method by Kuda *et al* of 2005. Methanolic solutions of DPPH (1.5 mL, 1 mM) containing up to 80 μL sample were incubated at room temperature. After 20 min absorbance was read at 517 nm on a Spectronic 20® Genesys™ Spectrophotometer. Ascorbic acid (0.1 M) was used as a positive control. The scavenging activity was reported as percentage decrease in absorbance with time.

3.8.2 Reducing power effects

Reducing power effects were determined following the method by Kuda *et al* of 2005. Up to 80 µL sample or 0.1 M ascorbic acid control solution was mixed with phosphate buffer (0.2 mL, 0.2 M pH 7.2) and 1% potassium ferricyanide (0.2 mL). The mixture was incubated at 50^{o}C for 20 min. After which TCA (0.2 mL, 10%) was added. After transferring an aliquot of the mixture (0.125 mL) into a microtitre plate, distilled water (0.125 mL) and $FeCl_3$ (0.02 mL, 0.1%) was added. The mixture was vortexed and the absorbance was measured at 655 nm on a Spectra MAX 340 microtitre plate spectrophotometer. The reducing power effects were recorded by plotting absorbance of the extracts against sample volumes.

3.8.3 β-carotene-linoleic acid system assay

β-carotene (2 mg) was dissolved in chloroform (10 mL). An aliquot (1 mL) of the solution was taken and chloroform was removed by vacuum on a rotary vaporizer. Linoleic acid (40 mg) was added to the almost dry dissolved sample followed by Tween 80 (400 mg) and aerated distilled water (100 mL) with vigorous shaking. Aliquots (3 mL) of the linoleic acid mixture were transferred into test tubes containing extracts of vegetables up to 80 µL, 5% BHA was used as a positive control. As soon as the mixture was added to each test tube, the absorbance at the start was measured at 470 nm using a Spectronic 20® Genesys™ Spectrophotometer. Measurement of absorbance at 470 nm was continued at 5 min intervals until the colour of β-carotene disappeared (2 hr). A blank without β-carotene but with all the other reagents was prepared. The β-carotene-linoleic acid system assay activity was reported as percentage decrease in absorbance with time.

3.8.4 Inhibition of phospholipid peroxidation

Female Sprague Dawley rats (*Rattus norvegicus*) were obtained from the animal house, University of Zimbabwe and dissected in the Physiology department to obtain the brains which were stored at -85^{o}C until required. Rat brain (2 g) was homogenized in chloroform-methanol mixture (2:1, v/v) followed by centrifugation at 3000 rpm for 5 min. The supernatant was used as the source of phospholipids. The test run contained the phospholipids solution (50 µL), the sample extract (0.5 mL), 50% methanol (0.2 mL), 10mM $FeSO_4$ (0.5 mL), thiobarbituric acid (1% TBA) (0.5 mL) and trichloroacetic acid (10 % TCA) (4 mL). Ascorbic acid (5%) was used as the positive control. After incubation at 37^{o}C for 30 min, 1% TBA and 10% TCA were added. The solution was heated in a boiling water bath for 15 min. After cooling the solution on ice, absorbance at 532 nm was read on a Spectronic 20® Genesys™ Spectrophotometer. The inhibitory activity was recorded by plotting absorbance of the extracts against sample volumes.

3.9 Correlation studies

3.9.1 DPPH and total phenolic content

Pearson's product moment correlation test packaged in the Statistical Package for Social Sciences (SPSS) for Windows Standard Version 8.0.0 was used for the statistical evaluation with $P < 0.05$ considered statistical significant.

3.9.2 Total content of phenolic compounds and other assays

The total content of phenolic compounds was ranked from 1 to 5. The vegetable with the highest content was assigned a rank value of 1 and the vegetable with the lowest content was assigned a rank value of 5. The ranks of the other assays were computed as in the total content of phenolic compounds. The ordinal data generated from the ranks of the extracts was analyzed using the Spearman's correlation coefficient test packaged in the Statistical Package for Social Sciences (SPSS) for Windows Standard Version 8.0.0 with $P < 0.05$ considered statistical significant.

3.10 Effects of processing and storage on the total content of phenolic compounds and antioxidant activity of *Bidens pilosa*

3.10.1 Unblanched sample

Two kilograms of *Bidens pilosa* leaves which were young and tender were collected in polythene bags. The sample was stored for 12 hr at 4°C and then analyzed for total content of phenolic compounds and antioxidant activity. For storage studies the leaves were frozen for 30 days.

3.10.2 Blanching

Five hundred grams of the unblanched leaves were immersed in boiling distilled water for 5 min. The leaves were left on a wire mesh for 10 min in order to drain off surface water. Phenolic compounds were extracted and the antioxidant activity determined.

3.10.3 Drying in the sun

One hundred grams of the unblanched and 300 g blanched leaves were spread on stainless steel trays for five days at ambient temperature to dry. After which total content of phenolic compounds and antioxidant activity were determined. The samples were stored under ambient conditions with an average temperature of about 19±3°C for 30 days.

3.10.4 Drying in the Oven

The unblanched and blanched leaves were dried in an oven cabinet for 24 hr at 100°C followed by determination of the phenolic content and antioxidant activity. The dried samples were stored for 30 days under ambient conditions with an average temperature of about 19±3°C.

3.11 Phenolic acids and flavonoids in the vegetables

3.11.1 Extraction method for simple phenolic acids for HPLC

Simple phenolic acids were extracted according to the procedure described by Pena-Neira *et al* in 2000. The ground sample (5 g) was extracted 3 times with diethyl ether (10 mL) and 3 times with ethyl acetate (10 mL) and the organic fractions combined. After 30 min of drying with anhydrous Na_2SO_4, the extract was evaporated to dryness under vacuum. The residue was redissolved in 50% methanol in water (2 mL) and the dissolved sample was transferred into 3 mL vial bottles ready for HPLC analysis.

3.11.2 HPLC analysis of phenolic acids and flavonoids

A Shimadzu HPLC system with a SCL-6B Shimadzu system controller, C-R AX Shimadzu Chromatopac, Shimadzu SPD-10 AV UV-Vis detector equipped with a Dynamax 60 Å C18 column was used for analysis of phenolic compounds. Five microlitres of sample were injected and the flow rate set at 1 mL/min. All samples, in duplicate were, filtered through a 0.22 μm filter unit (Millex® -GV, Molsheim, France) before injection and the solvents were filtered through a 0.45 μm filter (Whatman, Maidstone, England). Two mobile phases, A, which contained water-acetic acid (98:2 v/v), and B, which contained water-acetonitrile-acetic acid (78:20:2 v/v/v), were used. The gradient profile used had 0% Solvent B at the start, rising to 80% within 15 min, remaining at 80% up to 20 min and falling back to 0% after 30 min. Detection of the phenolic acids and flavonoids was carried out by reading absorbance at 280 nm according to Pena-Neira *et al* in 2000. After each run, the system was reconditioned for 15 min before analysis of the next sample.

4 RESULTS AND DISCUSSION

4.1 Edible vegetables identified, their availability, preparation and Usage

4.1.1 Edible indigenous vegetables mentioned by respondents

Seventy nine traditional vegetables were mentioned by the respondents and 20 of them were classified according to their species as shown in Appendix 2. The local names assigned are the ones commonly used by people in Buhera as the vegetables might have other vernacular names in some regions of the country.

The characterized vegetables belonged to 13 different families. Asteraceae was the frequently encountered botanical family. Ethnobotanists have often argued that a high rate of useful species in a family is a direct indicator of a family's importance (CBD, 2001). In this study the high species diversity of the Asteraceae family implied that the taxa or family as a whole was of general dietary relevance to the local people.

In contrast to the results of the present study, Chweya and Eyzaguirre in 1999 reported *Amaranthus hybridus* which belongs to the Amaranthaceae family to be the most consumed indigenous vegetable in West and Southern Africa. Information from focus group discussions revealed that people tend to use preferably the plants that are easily available to them, excluding those that are toxic or noxious. As Bonet and Valles in 2002 affirm, the more common a plant (family or species) is in an area, the greater is the probability of its popular use.

4.1.2 Availability of the vegetables

The vast majority of indigenous vegetables were reported to be available during the wet season as shown in Appendix 3, with only a few vegetables such as *Cleome gynandra* being available during the hot dry season. *Cleome gynandra* may grow in home gardens during the hot dry season and thus can become available for use. During focus group discussions the respondents revealed that the frequency of consumption of the indigenous vegetables during the wet season was higher as compared to the dry months.

Although our study was rather qualitative, the results are in agreement with a study carried out in Uganda where an average consumption of 160 g/day during the rainy season was reported while 12 g/day was consumed during the hot dry season (Rubaihayo, 1997). Hart *et al* in 2005 also reported adult *per capita* consumption of 59 to 130 g/day during the months of May to July, the peak season of vegetable production in the study area. During the hot dry season people in Buhera consume dried vegetables, although drying vegetables inevitably results in loss of some nutrients, the method ensures availability when fresh vegetables are scarce.

4.1.3 Preparation and usage of the indigenous vegetables

Within the edible plants, leaves are plant parts most widely used as shown in Appendix 3. The usage of leaves implies that the plants may be used throughout the rain season as the shoots will be harvested continuously. The leaves are used as relish and in most cases eaten fresh or dried with the staple meal of *sadza* which is prepared from available cereals. Kimiywe *et al* in 2007 also reported the consumption of

various indigenous leafy vegetables in Kenya that included cowpea leaves, Jute, pumpkin leaves, *Amaranthus*, *Bacella*, spider plant, black night shade and *Crotolaria*. Awobajo *et al* in 2010 also reported the use of leafy traditional vegetables in Nigeria as relish as well as folklore medicine.

Information from focus group discussions revealed that some of the indigenous vegetables consumed are bitter. Hence preparation of the bitter vegetables always involves boiling for long periods to tone down the bitterness. Singleton and Nobel in 1976 related the presence of chlorogenic acid, other hydroxycinnamates and, in particular, oligomeric proanthocyanidins to the bitterness and astringency of some foods. To tone down the bitterness of some vegetables, composite dishes are usually prepared by some households in Buhera. Composite dishes are prepared by mixing vegetables with relatively bland tastes with those ones with bitter tastes. One example of such composite dish is prepared by mixing *Amaranthus hybridus* which has a relatively bland taste with *Bidens pilosa* which is bitter.

Two procedures are used for drying vegetables. When drying leaves that are used to prepare *derere*, a mucilaginous type dish, and the leaves are typically dried in the shade without prior cooking. For other vegetables, the common method of drying involves, boiling, draining of water and drying in the sun for a period that depends on the amount of vegetable and environmental conditions.

Nowadays, people do not spend so much time outside in the natural environment eating raw vegetables, and therefore, since they are influenced by contemporary

dietary trends of cooked food, edible plants collected from the wild are brought home for more elaborate cooking recipes. In general young and tender leaves and tender shoots are used in the preparation of the dishes. For all the indigenous vegetables, cooking may be done with peanut butter or cooking oil. The use of peanut butter might increase the protein content of a meal as vegetables are poor sources of protein. Some households reported the use of oils from wild plant seeds such as *mapfura* (*Sclerocarya birrea)*.

The cooking and drying processes should be carefully considered when studying the nutritional properties of food resources because cooking and drying influences the bio-availability of specific classes of natural constituents, and consequently beneficial effects of food, such as their antioxidant activity (Pieroni, 2002). Kimiywe *et al* in 2007 reported that the use of some methods such as chopping before washing, repeated boiling and addition of sodium bicarbonate should be discouraged as they have deleterious effects on the nutrient content of vegetables. Focus group discussions revealed that some people in Buhera use these methods of preparation which could lead to a decrease of the nutritive value of cooked food.

Vegetables are sometimes fried with some additives such as tomatoes, onions and salt. Frying is considered to have almost the same or even less effect on nutrient losses compared with other cooking methods (Bognar, 1998). The oil used might increase the nutritive value of the vegetables as it is rich in vitamin E and unsaturated fatty acids (Fillion and Henry, 1998). Other additives such as tomatoes and onions might contribute to the nutritional value of the cooked vegetables.

Some plants were reported to be used for food as well as for medicine. The medicinal uses of the vegetables are shown in Table 10 and compared to some uses reported in literature. The reported diversity of indigenous vegetables in Buhera district might offer broad health benefits as some of the vegetables had various medicinal uses. Studies have further shown that countries that retain indigenous vegetable diets and had high consumption of these vegetables are much less likely to be affected by cardiovascular diseases, diabetes and other adverse consequences of the nutrition in transition (Johns and Sthapit, 2004). These findings concur with those of Musinguzi *et al.,* 2000 which found out that there was a potential relationship of indigenous vegetables and the ability to treat diabetes, gout, hyperlipidemia, gastro-intestinal tract infections, protozoan parasites, amongst others in Kenya and Tanzania. This is encouraging for intervention geared towards motivating individuals to increase the consumption and utilization of indigenous leafy vegetables. Hence there is need for further investigation to establish the basis of the above-mentioned perceptions.

Table 10: Reported medicinal uses of the indigenous vegetables

Vegetable	Reported medicinal uses	Medicinal uses from literature	References
Corchorus olitorius	treats dysentery, fevers, gonorrhoea.	treats abscesses, swellings, stomachache, anaemia, diabetes	Egharevba and Ikhatua, 2008; Kimiywe *et al.,* 2007; Ediriweera, 2007
Cleome gynandra	cure diseases such as bleeding of gums, headaches, epileptic fits, chest pain, constipation, worm infection and stomachaches	relieve pain, constipation, epileptic fits, improve eyesight, cure marasmus, ease child birth	Ediriweera, 2007
Adansonia digitata	treats fever, diarrhoea, small pox, measles. Coughs, malaria, sores	taken to treat fevers, gastric complaints, malaria, haemoptysis	Shukla *et al.,* 2001; De Caluwé, 2009
Amaranthus hybridus	treats intestinal bleeding, diarrhoea and excessive menstruation	treat sores, malaria, colds, coughs, AIDS, stomachache, diarrhoea, skin rashes, diabetes, backache	Kimiywe *et al.,* 2007
Bidens pilosa	treats sore gums, mouth sores, thrush ulcers dysentery, skin diseases, poisonous bites	has antibacterial, anti-inflammatory, antiallergic, antimalarial, anti-virus and antibiotic properties	Bairwa *et al.,* 2009; Etkin, 1994 Etkin and Ross, 1982; Pieroni, 2002; Johns, 1990; Etkin, 1994

4.2 Nutritional composition of the indigenous vegetables

Results for proximate analyses of the indigenous vegetables are shown in Table 11 and compared to values reported by Chitsiku in 1991 as shown in Table 12. Protein content was the highest in *Cleome gynandra* (6.0±1.0 g/100 g) and the lowest in *Adansonia digitata* (4.2±0.7 g/100 g). Protein content of all the wild vegetables was higher than the content of *Brassica napus* (rape). The results in this study are in agreement to those reported by Odhav *et al* in 2007, where the authors reported the protein contents of *Amaranthus hybridus*, *Bidens pilosa* and *Cleome spp* to be 6.0 g/100 g, 5.0 g/100 g and 5.0 g/100 g respectively.

Some indigenous vegetables were also reported in literature to have higher protein content than exotic vegetables. (FAO, 1990; Odhav *et al.,* 2007) reported that the crude protein content of both *Senna occidentalis* and *Manihot esculenta*, was 7.0 g/100 g, which is greater than that reported for *Brassica oleracea* subsp. *capitata* (Mosha and Gaga, 1999) and *Spinacea oleracea* (Kruger *et al.,* 1998) with values of 1.0 g/100 g and 3.0 g/100 g respectively. However, compared to legumes, indigenous vegetables are not very good sources of protein.

All the leafy indigenous vegetables were found to be poor sources of carbohydrate and fat, which ranged between 8.7±0.9 to 18± 2.1 g/100 g and 0.3± 0.1 to 0.5±0.5 g/100 g respectively. Odhav *et al* in 2007 reported the carbohydrate content of *Amaranthus hybridus*, *Bidens pilosa* and *Cleome spp* to be 6.1 g/100 g, 3.7 g/100 g and 3.4 g/100 g respectively. Differences in the agro-climatic conditions might account for the variation in carbohydrate content for *Cleome gynandra* observed in this study and that

reported by Odhav *et al* in 2007. Uusiku in 2008 also reported low fat contents of *Adansonia digitata*, *Amaranthus spp*, *Bidens pilosa* and *Cleome spp*. and therefore people on high vegetable diets should be encouraged to obtain their essential fatty acids from other sources such as oils from indigenous seeds.

Table 11: Nutrient composition on dry weight basis of the indigenous vegetables as determined by AOAC methods.

Nutrient	*Cleome gynandra*	*Amaranthus hybridus*	*Bidens pilosa*	*Corchorus olitorius*	*Adansonia digitata*
Protein[1]	6.0 ± 1.0	4.70 ± 0.5	4.4 ±0.8	5.1 ±0.5	4.2 ± 0.7
Fibre[1]	1.6± 0.3	1.5 ± 0.1	1.7 ±0.5	4.2 ± 0. 8	3 ± 0.5
Carbohydrate[1]	8.9 ±0.1	8.7 ±0.9	8.84±1.0	14.0 ±1.8	18.0 ± 2.1
Fat[1]	0.4 ± 0.1	0.4 ± 0.3	0.5 ±0.5	0.3 ± 0.1	0.4 ± 0.3
Iron[2]	13.1 ± 2.0	11.4 ± 0.8	17.5±3.0	8.7 ±1.8	23.0 ± 3.7
Zinc[2]	2.9 ± 0.7	5.8 ± 1.0	22.0 ± 3.0	4.5 ±0.9	20.1 ± 1.1
Copper[2]	10.0±2.4	7.65± 0.8	10.6±2.0	1.8 ± 0.8	23.7±3.3
***A/acid**[2]	18.0 ± 3.0	64.0 ± 6.0	70.0 ± 7	78.0 ± 12.0	55 ± 8
Potassium[2]	129.0 ± 60	550.0 ± 100	600±53	370.0 ±50	1090 ± 100
Calcium[2]	120.0± 60	798.0 ± 100	370 ± 67	380.0 ±70	400.0 ± 100
Magnesium[2]	97.0±20	440.0± 100	600±126	290.0± 130	370.0±100
Phosphorous[2]	66.0 ±11	550.0 ±117	500 ± 109	623.0 ±123	66.0 ± 11

*ascorbic acid, [1] units are g/100g, [2]units are mg/100g

Table 12: Nutrient composition of some exotic vegetables commonly found in Zimbabwe

Nutrient/Species	Lettuce *Lactuca sativa*	Spinach *Spinacia oleracea*	Cabbage *Brassica oleracea*	Rape *Brassica napus*
Crude protein (g)	1.2	2.9	1.2	4.1
Crude fiber (g)	1.4	2.7	1.8	NI
Carbohydrate (g)	2.4	3.5	5.43	6.2
Fat(g)	0.2	0.35	0.2	0.4
Potassium (mg)	158	557	233	NI
Calcium (mg)	19	97	219	330
Magnesium (mg)	9	77	21	NI

Adapted from Chitsiku, 1991
NI: No information was given for the nutrient

The insoluble dietary fibre in the vegetables was in the range of 1.6±0.3 to 4.2±0. 8 g/100 g. Odhav *et al* in 2007 reported the fibre content of *Amaranthus hybridus*, *Bidens pilosa* and *Cleome spp* to be 2.8 g/100 g, 2.9 g/100 g and 2.1 g/100 g respectively. The observed variations may be due to differences in stages of plant maturity, seasonal variation, fertilizers or chemicals used, variety of plant, geographical location and the method used for analysis (Aletor *et al.,* 2002; Punna and Parachuri, 2004). Furthermore, cooking of plant tissues alters the physical and chemical properties of plant cell walls, which in turn affects their performance as dietary fibre and as a result the dietary fibre content of extracts might be underestimated (McDougall *et al.,* 1996).

The iron content of the indigenous vegetables ranged from 8.7±1.8 mg/100 g for *Corchorus olitorius* to 23±3.7 mg/100 g for *Adansonia digitata*. Kruger *et al* in 1998

reported the iron content of *Amaranthus spp*, *Bidens pilosa* and *Cleome spp* to be 105.0 mg/100 g, 79.0 mg/100 g and 44.0 mg/100 g respectively. The values reported by Kruger *et al* in 1998 are relatively high as compared to the values obtained in this study. The differences might have been influenced by factors such as soil type and pH, water availability to the plant, climatic conditions, plant variety (Khader and Rama, 2003), plant age (Gupta *et al.,* 1989) and the use of fertilizers (Guil Guerrero *et al.,* 1998).

The zinc content of the vegetables ranged from 2.9±0.7 mg/100 g for *Cleome gynandra* to 22.0±3.0 mg/100 g for *Bidens pilosa*. The zinc content within the same species was reported by Orech *et al* in 2007 to be highly variable and ranges from 0.02 to 8.4 mg/100 g for several *Amaranthus* species, from 1.4 to 18.5 mg/100 g for *Chenopodium album* and 0.03 to 3.1 mg/100 g for *Ipomoea batatas.* The same author suggested that the variations might be due to differences in stages of plant maturity, seasonal variation, fertilizers or chemicals used, variety of plant, geographical location and the method used for analysis.

The copper content of the vegetables in the present work ranged from 1.8±0.8 mg/100 g for *Corchorus olitorius* to 23.7±3.3 mg/100 g for *Adansonia digitata.* Odhav *et al* in 2007 reported the Cu content of *Amaranthus hybridus*, *Bidens pilosa* and *Cleome spp* to be 2.0 mg/100 g, 10.0 mg/100 g and 2.0 mg/100 g respectively. The values obtained from this study were relatively higher than those reported by Odhav *et al* in 2007. Variations in the chemical composition of leafy vegetables, including the quantities of compounds that are useful and detrimental to humans, are influenced by farming

practices and prevailing environmental conditions. Often sources of differences could be attributed to the age of plants at harvest, which affects their genetic composition (Nordeide *et al.,* 1996).

Large variations were found in the vitamin C content of the wild vegetables. The Vitamin C content varied from 18.0±3.0 mg/100 g for *Cleome gynandra* to 78.0±12.0 mg/100 g for *Corchorus olitorius*. The vitamin C contents in this study are within the range reported by other researchers (Kruger *et al.,* 1998; FAO, 1990; Steyn *et al.,* 2001; Mosha and Gaga, 1999; Ejoh *et al.,* 2007). Kruger *et al* in 1998 reported ranges of between 2.0 to 311.0 mg/100 g in selected unprocessed traditional vegetables. Similar ranges were observed because the DCPIP method is commonly used in the analysis of vitamin C.

Potassium content was the highest in *Adansonia digitata* (1090±100 mg/100 g) and lowest in *Cleome gynandra* (129.0±60 mg/100 g). Yazzie *et al* in 1994 reported a potassium content of 1541.0 mg/100 g in baobab leaves. Sena *et al* in 1998 reported a value of 583 mg/100 g. Comparisons between published data on potassium show wide variations in content (Sidibe and Williams, 2002). The plant state of maturation, genetic variances, and environmental factors are all possible explanations for the reported discrepancies (Boukari *et al.*, 2001). Moreover, mineral composition in food may vary greatly depending on where the food was grown (Boukari *et al.*, 2001), on seasonal variations (Yazzie *et al.*, 1994), and on the used analytical method (Boukari *et al.*, 2001).

Calcium values of the plants ranged from 120.0±60 mg/100 g in *Cleome gynandra* to 798.0±100 mg/100 g in *Amaranthus hybridus*. Magnesium levels were found to be highest in *Bidens pilosa* (600±126 mg/100 g) and lowest in *Cleome gynandra* (97.0±20 mg/100 g). Compared with cultivated vegetables, wild plant Mg content was higher than plants such as *Spinacea oleracea*, *Lactuva sativa* and *Brassica oleracea*. The calcium contents in this study are within the range reported by other researchers (FAO, 1990; Odhav *et al.,* 2007; Kruger *et al.,* 1998). FAO in 1990 reported the calcium content of *Adansonia digitata* to be 410.0 mg/100 g. Odhav *et al* in 2007 and Kruger *et al* in1998 reported the calcium content of *Amaranthus spp* to be in the range of 253.0 to 425.0 mg/100 g. Odhav *et al* in 2007 reported the magnesium content of *Amaranthus hybridus*, *Bidens pilosa* and *Cleome spp* to be 1317.0 mg/100 g, 658.0 mg/100 g and 371.0 mg/100 g respectively. Some of the analysed vegetables such as *Amaranthus hybridus* and *Corchorus olitorius* could potentially contribute significantly towards the dietary requirements of these two minerals.

Corchorus olitorius had the highest phosphorous content (623.0±123 mg/100 g) and *Cleome gynandra* the lowest (66.0±10 mg/100 g). Odhav *et al* in 2007 reported the phosphorus content of *Amaranthus hybridus*, *Bidens pilosa* and *Cleome spp* to be 604.0 mg/100 g, 504.0 mg/100 g and 784.0 mg/100 g respectively. Hence the relatively higher levels of phosphorous in the indigenous vegetables may contribute significantly to the phosphorous requirements of especially the elderly who are likely to suffer from osteoporosis.

The micro-nutrient content of the indigenous vegetables was higher than that of lettuce and rape vegetables with the exception of lettuce which had high magnesium content as compared to the indigenous vegetables. For instance, *Amaranthus hybridus* had 7, 13 and 20 times more vitamin C, calcium and iron respectively than lettuce. All the five vegetables in this study were found to have higher than expected iron content. Iron is important in the diet of both pregnant and nursing mothers as well as infants, the convalescent and the elderly (Manzocco *et al*, 2001). Similarly zinc, a trace mineral that is especially important for the normal functioning of the immune system, was abundant in *Bidens pilosa* compared to other indigenous analyzed and exotic vegetables.

From the focus group discussions it was evident that many people living in Buhera District of Zimbabwe rely on farming and gathering for their food requirements. As in most diets with a significant proportion of vegetables consumed, protein, carbohydrate and fat quality and quantity are major nutritional concerns.

Most plants do not supply all the nutrients required by humans and as a result combining different plant foods may improve the situation. The data in the present study provide evidence of the potential nutritional value of indigenous vegetables of poor populations who are compelled to rely on such plant foods for sustenance when exotic plants are in short supply.

4.3 Effect of cooking on the nutrient content of some indigenous vegetables

The nutrient content of the cooked indigenous vegetables and the corresponding *p* values are shown in Table 13. Cooking caused significant changes ($p < 0.05$) in all the twelve nutrients of *Amaranthus hybridus*. The potassium content of *Cleome gynandra* was the only nutrient not reduced significantly ($p > 0.05$). Cooking also caused significant changes ($p < 0.05$) in all the twelve nutrients of *Bidens pilosa.*

A significant decrease in protein content of all the indigenous vegetables was observed. Thermal processing was reported to enhance the digestibility of proteins (Gibson *et al.,* 2006), but can also lead to protein degradation that lowers the protein content of traditional vegetables. Thus the observed significant decrease in the protein content might have been caused by protein degradation.

A significant increase in the fibre content of all the indigenous vegetables was observed. Cooking of plant tissues might have altered the physical and chemical properties of plant cell walls, which in turn affects their performance as dietary fibre (McDougall *et al.,* 1996). The increased temperature during cooking may have led to breakage of weak bonds between polysaccharides and the cleavage of glycosidic linkages, which may result in solubilization of the dietary fibre (Svanberg *et al.,* 1997). There seems to be very little known information about the effect of cooking on total dietary fibre (TDF) content of indigenous vegetables. However, it may be hypothesized that the effects noted above could also have occurred in the indigenous vegetables analysed. Kala and Prakash in 2004 reported similar results to the results we got. The authors reported that cooking caused a slight increase in the total dietary

fibre (TDF) content of Indian indigenous vegetables, which was attributed to the hydration or polymerization of TDF fractions. In contrast to our present results, Puupponen-Pimiä *et al* in 2003 reported no significant change in soluble, insoluble and total dietary fibre contents of blanched and freezer stored spinach. This could be due to the stability of dietary fibre components found in this vegetable species.

In this study vitamins and minerals were reduced significantly with the exception of potassium. Cooking was reported to have variable effects on micronutrients in indigenous vegetables ranging from no effect on iron and zinc content, increases in β-carotene bioavailability, and reduction in vitamin C due to leaching (Uusiku *et al.,* 2008). In comparison to vitamins, minerals were reported to have greater stability and their contents do not change significantly due to cooking (Kala and Prakash, 2004).

Vitamin C was found to be the most heat sensitive nutrient. Hence it might be difficult to calculate vitamin C contribution towards dietary vitamin C requirements. A decrease in ascorbic acid by 19% in cooked amaranth, 61% in dried *Vernonia amygdalina* and by almost 100% in dried *Adansonia digitata* has been reported (FAO, 1990). The storage of dehydrated vegetables should be at low temperature because it effectively reduces degradation of ascorbic acid and browning (Negi and Roy, 2001). Losses of ascorbic acid from vegetables are large during blanching procedures and relatively small during frozen storage, suggesting that losses during blanching occur primarily by leaching rather than by chemical degradation (Howard *et al.,* 1999). Mepba *et al* in 2007, Sreeramulu *et al* in 1983 and Wallace *et al* in 1998 found a significant reduction in ascorbic acid of several indigenous vegetables after thermal

processing. Steam blanching, followed by dehydration have been reported as the most effective preservation methods in retaining ascorbic acid (Schippers, 2000). Freeze-drying also retains most ascorbic acid in comparison with shade-, sun-, and vacuum-drying of vegetables (Shitanda and Wanjala, 2006).

Table 13: Nutrient content of uncooked and cooked *Amaranthus hybridus, Cleome Gynandra* and *Bidens pilosa* per 100 g dried portion with the corresponding *p*-values.

Vegetable	*A. hybridus*			*C. gynandra*			*B. pilosa*		
Nutrient	**Uncooked**	**Cooked**	***P*-value**	**Uncooked**	**Cooked**	***P*-value**	**Uncooked**	**Cooked**	***P*-value**
Crude protein (g)	4.70 ± 0.5	4.66 ± 0.33([a])[sd]	*P*<0.0001	6.0 ± 1.0	4.70 ± 0.50[sd]	*P*<0.0001	4.4 ±0.8	3.7 ± 0.30[sd]	*P*=0.0006
Crude fiber (g)	1.5 ± 0.1	1.7 ± 0.19[sd]	*P*=0.0132	1.6± 0.3	1.7 ± 0.49[sd]	*P*<0.0001	1.7 ±0.5	2.1 ± 0.09[sd]	*P*<0.0001
Carbohydrate (g)	8.7 ±0.9	6.9 ± 0.68[sd]	*P*<0.0001	8.9 ±0.1	7.58 ± 1.9[sd]	*P*<0.0001	8.84±1.0	6.40 ± 0.77[sd]	*P*=0.5185
Fat(g)	0.4 ± 0.3	1.0 ± 0.46[sd]	*P*=0.0011	0.4 ± 0.1	1.3 ± 0.23[sd]	*P*=0.2879	0.5 ±0.5	0.6 ±0.37[sd]	*P*<0.0001
Potassium (mg)	550.0 ± 100	530 ± 57[sd]	*P*<0.0001	129.0 ± 60	131 ±69[(b)ns]	*P*=0.0001	600 ± 53	580 ± 95[sd]	*P*<0.0001
Calcium (mg)	798.0 ± 100	530±88[sd]	*P*<0.0001	120.0± 60	115 ± 99[sd]	*P*<0.0001	370 ±67± 3.0	300 ±79[sd]	*P*<0.0001
Magnesium (mg)	440.0± 100	343 ±118[sd]	*P*<0.0001	97.0±20	70 ± 23[sd]	*P*<0.0001	600 ±126	570 ±119[sd]	*P*<0.0001
Phosphorus (mg)	550.0 ±117	450 ± 134[sd]	*P*<0.0001	66.0 ±11	10 ±5[sd]	*P*<0.0001	500 ± 109	480 ± 115[sd]	*P*=0.0030
Iron (mg)	11.4 ± 0.8	7.3 ± 0.99[sd]	*P*<0.0001	13.1 ± 2.0	9.5 ± 1.09[sd]	*P*<0.0001	17.47 ± 3.4	15 ± 2.7[sd]	*P*=0.0007
Zinc (mg)	5.8 ± 1.0	4.90 ± 0.73[sd]	*P*<0.0001	2.9 ± 0.7	1.73 ± 0.55[sd]	*P*<0.0001	22 ± 2.65	19.12 ± 2.3[sd]	*P*<0.0001
Copper (mg)	7.65± 0.8	5.12±0.78[sd]	*P*<0.0001	10.0±2.4	9.77 ± 1.17[sd]	*P*<0.0001	10.61±1.94	9.06 ±0.79[sd]	*P*<0.0001
Ascorbic acid (mg)	64.0 ± 6.0	46± 7[sd]	*P*<0.0001	18.0 ± 3.0	10 ± 2[sd]	*P*<0.0001	70 ± 7	40 ± 9[sd]	*P*<0.0001

[a]significant difference
[b] no significant difference
*p < 0.05 was considered significant

4.4 Content of phenolic compounds

4.4.1 Total content of phenolic compounds

The total content of phenolic compounds varied from 4.9±0.06 GAE mg/g in lettuce (*Lactuva sativa*) to 51.1±5.56 GAE mg/g in *B. pilosa* as shown in Figure 20. The exotic and indigenous vegetables showed significant ($p < 0.05$) differences in the total content of phenolic compounds. All the indigenous vegetables had higher total content of phenolic compounds than rape (*Brassica napus*) and lettuce (*Lactuva sativa*) extracts.

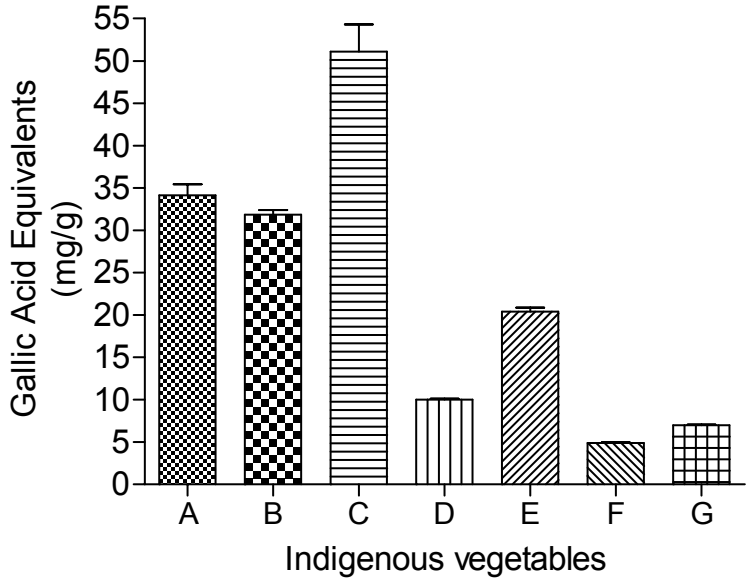


Figure 20
Total contents of phenolic compounds of *A. hybridus* (A), *C. olitorius* (B), *B. pilosa* (C), *C. gynandra* (D), *G. parviflora* (E), Lettuce (F) and Rape (G) expressed as gallic acid equivalents. Values are means of triplicate determinations ± standard deviation

The total contents of phenolic compounds in this study are within the range reported by other researchers (Morrison *et al*, 1995; Capecka *et al*, 2003; Ismail *et al*, 2004) and hence the vegetables might be an important source of antioxidant components.

Morrison *et al* in 1995 reported the concentration of phenolic compounds in cowpea (*Vigna unguiculata)* seed coats as 0.6 mg/g after extraction with methanol. Ismail *et al* in 2004 reported ranges of between 11.1 to 71.7mg/g in selected vegetables. Capecka *et al* in 2003 reported between 11.1 mg/g and 14.1 mg/g phenolic compounds concentration in some herbs.

There are wide variations between the total content of phenolic compounds of different vegetables, or even for the same vegetables reported by different authors. The differences might be due to the different groups of phenolic compounds in the plants and the methods of extraction and analysis (Bravo, 1998; Kalt, 2001). Variation in the phenolic contents of the vegetables studied might have been caused by the species differences and the environment, as the plants were collected from different ecological regions i.e. ecological regions 3, 4 and 5 of Buhera district (Rapisarda, 1999; Tomas-Barberan and Espin, 2001).

4.4.2 Content of flavonoids by the vanillin–HCl assay

The contents of flavonoids varied from 1.2$\pm$0.01 mg/g for *C. gynandra* to 8.0$\pm$0.14 mg/g for *B. pilosa* with the contents being significantly different ($p < 0.05$) for all the vegetables as shown in Figure 21 *A. hybridus* and *G. parviflora* were the only extracts

that had statistically the same ($p > 0.05$) contents of flavonoids. The content of flavonoids in rape was higher than the contents in *G. parviflora*, *C. olitorius,* C. *gynandra* and *A. hybridus* and lower than *B. pilosa*.

The content of flavonoids in lettuce was lower than all the vegetables with the exception of *C. gynandra*. Catechin, a common flavonoid was detected by HPLC analysis in *Bidens pilosa* and *Galinsoga parviflora* extracts only. Other flavonoids could not be identified because of the limited number of standards that were used.

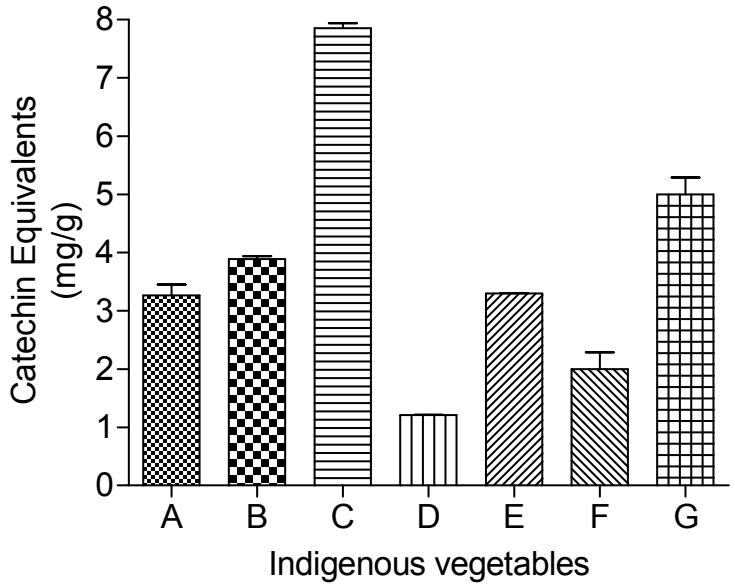


Figure 21

Flavonoid concentrations of *A. hybridus* (A), *C. olitorius* (B), *B. pilosa* (C), C. *gynandra* (D), *G. parviflora* (E), Lettuce (F) and Rape (G) expressed as catechin equivalents. Values are means of triplicate determinations ± standard deviation

The contents of flavonoids of the studied vegetables were higher than the contents of exotic vegetables analysed by Bahorum *et al* in 2004. The author reported the total flavonoids content of cabbage (*Brassica chinensis* L), onion (*Allium cepa* L), mugwort (*Artemisia vulgaris* Cantley) and broccoli (*Brassica oleracea* L var *botrytis* L subvar *cymosa*) to be between 0.05 mg/g and 1 mg/g on wet weight basis.

Variation in the composition of flavonoids might be attributed to genetic factors as the plants belonged to different genera. The environment effects were perceptible because the plants were collected from different ecological regions characterized by diverse weather patterns (Masa *et al*., 2007).

Consumption of vegetables rich in flavonoids is beneficial as they have anti-inflammatory, antibacterial, antihistaminic, antimutagenic and angioprotective properties and potent phosphodiesterase inhibition (Della Loggia *et al*., 1989; Du Toit *et al*., 2007; Mlambo *et al*., 2007).

4.4.3 Proanthocyanidins content by the butanol-HCl assay

The levels of proanthocyanidins were ranging from 1.9$\pm$0.21 mg/g for lettuce to 11.2$\pm$1.55 mg/g for *Bidens pilosa* as shown in Figure 22. All the indigenous vegetables had higher proanthocyanidins content than the rape and lettuce extracts.

The levels of proanthocyanidins of the evaluated vegetables were higher than the levels of the exotic species reported by Bahorum *et al* in 2004. Proanthocyanidins were detected at relatively low levels in the exotic vegetables which included cabbage

(*Brassica chinensis* L), onion (*Allium cepa* L), mugwort (*Artemisia vulgaris* Cantley) and broccoli (*Brassica oleracea* L var *botrytis* L subvar *cymosa*).

Some wards in Buhera district are in areas that are characterized by recurrent droughts. The stressful conditions induced by the drought will entail the synthesis of high amounts of proanthocyanidins as a defense mechanism against environmental stress by the plants (Alonso-Amelot *et al*., 2007; Oszmianski *et al*., 2007).

The disadvantage of consuming plants with high levels of proanthocyanidins is that the proanthocyanidins might inhibit various bacterial enzymes involved in the metabolism of carbohydrates including cellulose, amylase and galactosidase (Butler, 1982). Besides the disadvantage of inhibiting enzymes, proanthocyanidins are reported to reduce soil nematode populations and have got antihelmintic effects in sheep (Taylor and Murant, 1966; Iqbal *et al*., 2007).

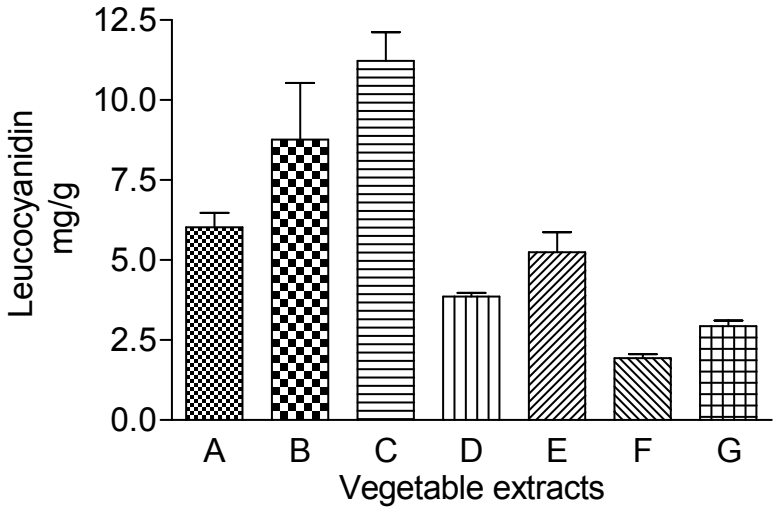


Figure 22

Proanthocyanidin contents of *A. hybridus* (A), *C. olitorius* (B), *B. pilosa* (C), *C. gynandra* (D), *G. parviflora* (E), Lettuce (F) and Rape (G) expressed as leucocyanidin equivalents. Values are means of triplicate determinations ± standard deviation

4.4.4 Tannin content

The tannin contents of the vegetables ranged from 1.90±0.30 mg/g for *C. gynandra* to 8.3+-2.04 mg/g for *Bidens pilosa* as shown in Figure 23. There was no significant difference ($p > 0.05$) among the condensed tannin contents of *Bidens pilosa*, *Corchorus olitorius* and *Galinsoga parviflora* and between that of *Amaranthus hybridus* and *Cleome gynandra*. All the indigenous vegetables had higher tannin contents than the rape and lettuce extracts.

The tannin contents reported by Das *et al* in 2005 are relatively high than the values obtained in this study. The author reported the tannin content of *Amarathus* species, *Spinacia aleracea* and *Basella alba* to be 198.0, 185.0 and 192.0 mg/100g respectively. Various reasons might have contributed to the observed variations and

they include the methods used for extraction and analysis and the environmental conditions in the different ecological regions (Masa *et al*., 2007).

People consuming high tannin foods might get protective effects elicited by some phytochemicals in the vegetables. Foods with high tannin content are also known to reduce the bioavailability of some essential nutrients (Aletor and Adeogun, 1995).

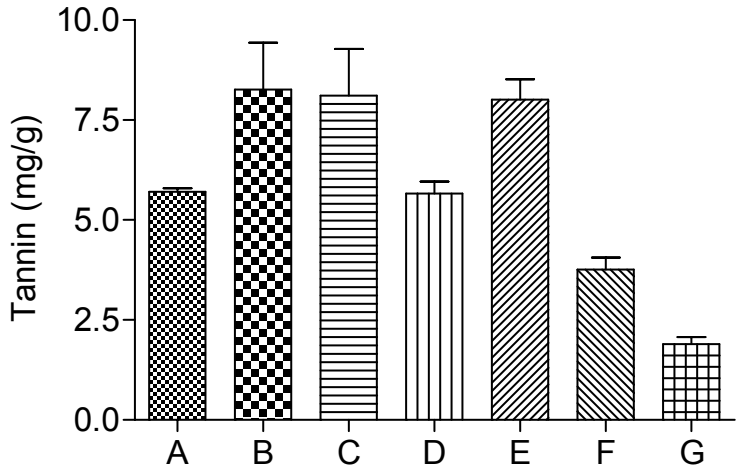


Figure 23

Tannin contents of *A.hybridus* (A), *C. olitorius* (B), *B. pilosa* (C), *C. gynandra* (D), *G. parviflora* (E), Lettuce (F) and Rape (G) expressed as gallic acid equivalents. Values are means of triplicate determinations ± standard deviation

4.5 Antioxidant capacities of the extracts

4.5.1 DPPH radical scavenging activity

There were significant differences ($p < 0.001$) in the activities of the seven vegetables as shown in Figure 24. *Bidens pilosa* had the highest activity while rape had the lowest. The activities of the extracts were comparable to ascorbic acid and the flavonoid standard of catechin. Rape had the lowest activity than all vegetables and lettuce had a higher activity than *Amarathus hybridus* extracts only.

Some of the vegetables which had high concentrations of phenolic compounds also had higher capacities to scavenge for DPPH radicals. The key role of phenolic compounds as scavengers of free radicals is emphasised in some reports (Komali *et al*., 1999; Moller *et al*., 1999). Polyphenolic compounds have an important role in preventing lipid oxidation and are associated with antioxidant activity (Yen *et al*., 1993; Gulcin *et al*., 2003). Polyphenolic compounds may contribute directly to antioxidative action (Duh *et al*., 1999). Tanaka *et al* in 1998 suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g of polyphenols are daily ingested from a diet rich in fruits and vegetables.

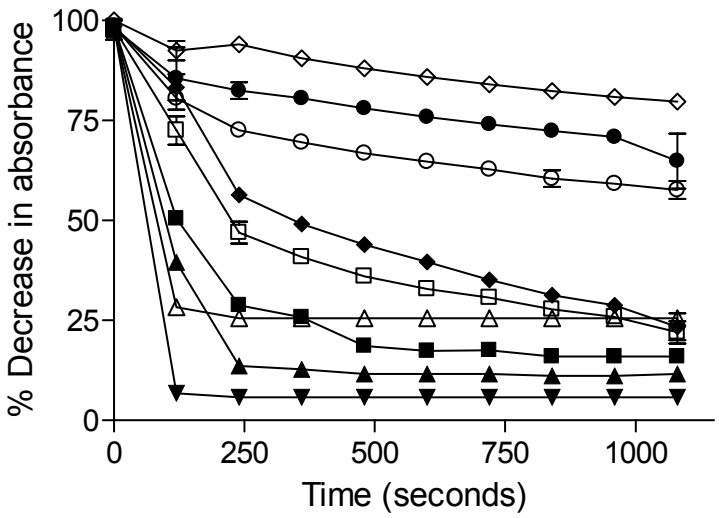


Figure 24

Scavenging effects of methanolic extracts of *A. hybridus* (●), *C. gynandra* (♦), *G. parviflora* (□), *C. olitorius* (■) *B. pilosa* (▲), ascorbic acid (▼), catechin (Δ), lettuce (○) and rape (◊) on DPPH activity as reflected by % decrease in absorbance. Values are means of triplicate determinations ± standard deviation

4.5.2 Reducing power of extracts

The ability of the extracts to reduce ferric ions increased with the concentration of the extract as shown in Figure 25. All vegetable extracts were capable of reducing ferric ions. The reducing power of *Bidens pilosa* was the highest at 80 mg/mL sample concentration.

Lettuce had a low activity even at 80 mg/mL sample concentration. There was no significant difference ($p > 0.05$) in the reducing power of ascorbic acid and *Bidens pilosa* which had the highest activity. Lettuce and rape had lower activities than all the indigenous vegetables.

The findings are in agreement with published results (Yi *et al*., 2007; Zhenbao *et al.,* 2007). Yi *et al* in 2007 obtained reducing powers on extracts of *Citri Reticulatae* that increased with an increase in the concentration of the samples. Zhenbao *et al* in 2007 reported an increase in reducing power as the concentration of the extract of *Cassia tora L* increased. In the reducing power assay, the presence of reductants in the test samples would result in reduction of Fe^{3+} to the Fe^{2+} form. The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue colour at 655 nm (Singh and Arora, 2007).

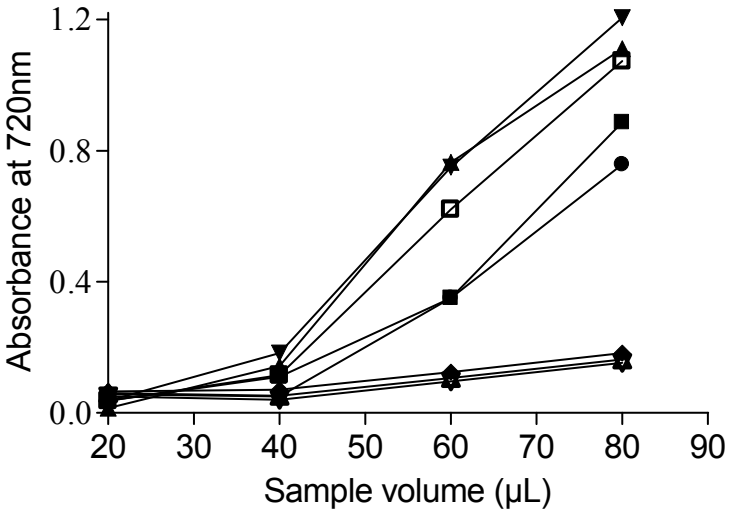


Figure 25

Reducing power of methanol extracts of *A. hybridus* (●), *C. gynandra* (♦), *G. parviflora* (□), *C. olitorius* (■), *B. pilosa* (▲), lettuce (○), rape (◊) and ascorbic acid (▼). Increased absorbance of the reaction mixture indicated the increased reducing power. Values are means of triplicate determinations ± standard deviation

The reducing power of the vegetable extracts was probably due to the electron donating abilities of the phenolic compounds (Shon *et al*., 2007). The phenolic compounds could react with free radicals to convert them to more stable products and terminate radical chain reactions.

4.5.1 Antioxidant activity by β- carotene assay

The extracts tested had the ability to quench peroxyl radicals formed from oxidation of linoleic acid thereby retarding the decolorization of the β-carotene by the peroxyl radical. *Corchorus olitorius* had the highest activity and *rape* had the lowest as shown in Figure 26. The activities of *Corchorus olitorius*, *Bidens pilosa* and *Galinsoga parviflora* were not significantly different ($p > 0.05$) and higher than that of *Amaranthus hybridus* and *Cleome gynandra.* Lettuce and rape had lower activities than all the indigenous vegetables.

The difference in the ability of the vegetable extracts to bleach β-carotene could be due to the different phenolic compounds found in the vegetables (Kalt, 2001). The ability of phenolic compounds to delay the bleaching of β-carotene depends on the reducing capabilities of the different phenolic compounds in the extracts, for example flavonoids have greater ability to delay bleaching of β-carotene than other compounds with similar structures (Böhm *et al*., 2001). Tepe and Sokmen in 2007 reported correlation between the ability to delay or stop the bleaching of β-carotene and the phenolic compound concentration.

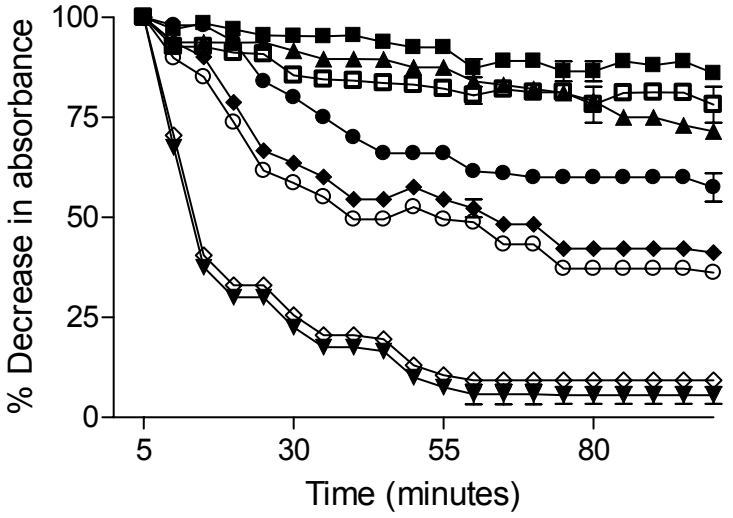


Figure 26

Capacity to retard β-carotene oxidation by extracts of *A. hybridus* (●), *C. gynandra* (♦), *G. parviflora* (□), *C. olitorius* (■), *B. pilosa* (▲), lettuce (○), rape (◊) and Methanol (▼). Values are means of triplicate determinations ± standard deviation

4.5.1 Inhibition of Phospholipids Peroxidation

Lipid peroxidation generates a number of degradation products such as malonaldehyde (MDA). The rate at which MDA was produced decreased with an increase in the concentration of sample extract.

There were significant differences in the ability of the extracts to inhibit the peroxidation of phospholipids as shown in Figure 27. The order of activity was ascorbic acid > *Bidens pilosa* > *Amaranthus hybridus* > *Corchorus olitorius* > *Galinsoga parviflora* > *Cleome gynandra*.

Inhibition of the peroxidation of phospholipids by extracts of the vegetables was an indication of the presence in the extracts of components that might prevent the peroxidation chain reaction. Chain reaction breaking properties are as a result of hydrogen and electron donation which was observed in the reducing power effects and the DPPH radical quenching abilities.

Shon *et al* in 2007 reported that substances that can chelate iron ions can inhibit the peroxidation of lipids. Extracts of the vegetables could inhibit the peroxidation of phospholipids by chelating iron ions.

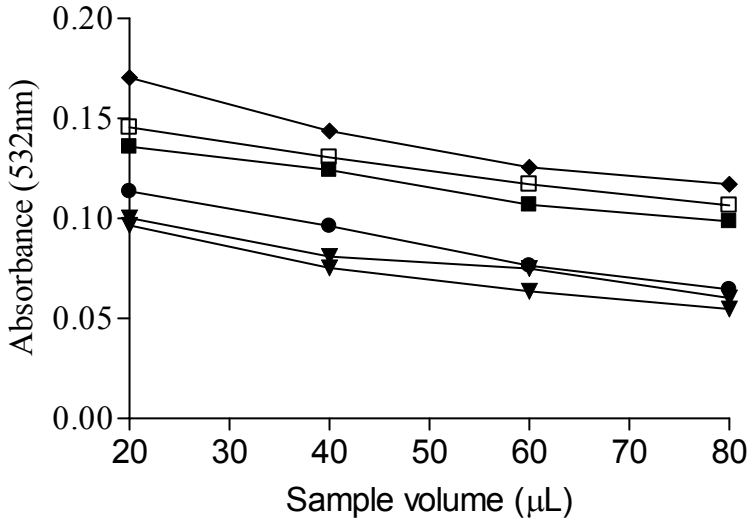


Figure 27

Inhibition of phospholipids peroxidation by the methanolic extracts of *A. hybridus* (●), *C. gynandra* (♦), *G. parviflora* (□), *C. olitorius,* (■), *B. pilosa* (▲) and ascorbic acid (▼). Values are means of triplicate determinations ± standard deviation

4.6 Correlation between the content of phenolic compounds and antioxidant capacities

4.6.1 DPPH activity and total content of phenolic compounds

A relationship was established between the antioxidant activity of the vegetables and the total content of phenolic compounds. Total content of phenolic compounds and activity was weakly correlated (r^2=0.49) and the relationship was significantly nonlinear (runs test). Turkmen *et al* in 2005 reported no correlation between the total content of phenolic compounds and antioxidant activity of 92 plants extracts they studied. The weak correlation can be explained by the fact that the phenolic compounds present in the vegetable extracts might not be potent in scavenging the DPPH radical.

Response of phenolic compounds in Folin-Ciocalteu assay depends on the phenolic compounds chemical structure and the radical scavenging capacity cannot be predicted on the basis of total phenolic compound content (Makkar, 1999; Shahidi *et al*., 1994). According to Katalinic *et al* in 2005 various phenolic groups have different responses in this assay. The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate (Frankel *et al.*, 1995).

4.6.2 Total content of phenolic compounds and other assays

The r^2 values of the tests and the p values were computed and tabulated in Table 14. The phospholipids peroxidation assay had the strongest correlation (r^2 = 1, p = 0.02)

than all the six assays. The beta carotene content had the weakest correlation ($r^2 = 0.2$, $p = 0.78$) than all the six assays.

The different correlation coefficients from the various assays compared to the total content of phenolic compounds showed that the amount of phenolic compounds in an extract is not the sole predictor of antioxidant activity. The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity (Rice-Evans *et al*., 1996; Robards, 1999).

Table 14: Different assays correlated with the total content of phenolic compounds

Assay	r^2	*p-Value
Tannin binding	0.5	0.45
Butanol - HCl	0.9	0.08
Vanillin - HCl	0.9	0.08
Reducing power	0.6	0.35
Beta carotene	0.2	0.75
Phospholipid	1	0.02

*P < 0.05 was considered to be significant

4.7 Effects of processing and storage on the total content of phenolic compounds and antioxidant activity of *Bidens pilosa*

4.7.1 Total content of phenolic compounds

4.7.1.1 Unblanched and blanched *Bidens pilosa*

As shown in Figure 28, there was a significant decrease ($p < 0.05$) from 30.0 GAE mg/g (unblanched) to 7.8 GAE mg/g (blanched) in the total content of phenolic compounds. After storing for 30 days, no significant change ($p > 0.05$) in the total content of phenolic compounds was observed.

The reduction in the total content of phenolic compounds after blanching could arise from the thermal decomposition of phenolics (Hunter and Fletcher, 2002). Leaching of water-soluble antioxidants into the liquor was also found to cause some significant changes in the total content of phenolic compounds (Hunter and Fletcher, 2002).

Various researchers reported contradicting results on the effect of blanching on the polyphenol content of legume seeds and vegetables (Kataria *et al*., 2003; Vallejo *et al*., 2003; Obor, 2005).

Obor in 2005 reported an increase in the total phenolic content of Nigerian leafy vegetables after blanching. Kataria *et al* in 2003 reported a decrease in polyphenol content of *Vigna mungo* seeds.

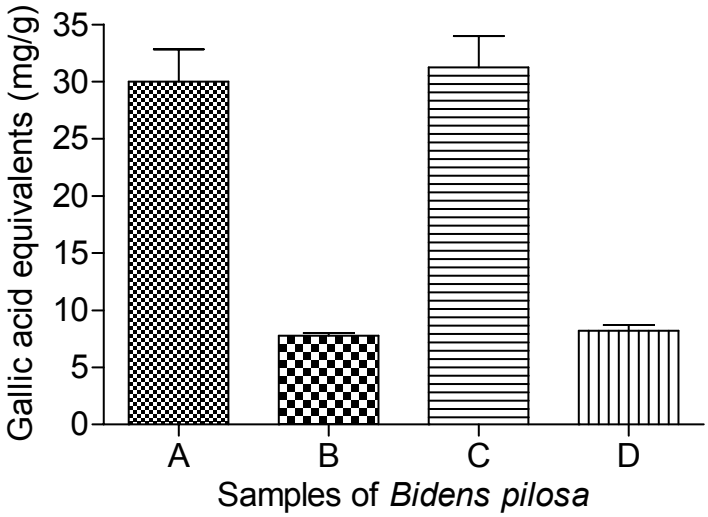


Figure 28

Effect of blanching and storing on total content of phenolic compounds on unblanched (A), blanched (B), unblanched and stored (C) and blanched and stored (D) *Bidens pilosa* extracts expressed as gallic acid equivalents. Values are means of triplicate determinations ± standard deviation

4.7.1.2 Unblanched and blanched sun dried *Bidens pilosa*

The total content of phenolic compounds ranged from 35.1 GAE mg/g for the unblanched sun dried sample to 37.3 GAE mg/g for the blanched sun dried sample as shown in Figure 29. The sample which was sun dried had a higher content than the unblanched sample. The slight decrease in the content of phenolic compounds when samples were stored at room temperature was not significant ($p > 0.05$).

The blanched extracts had higher phenolic content than the unblanched samples. Ansari *et al* in 2005 suggested that heat processing may change the chemical composition of the plant extracts, leading to an increase in the amount of antioxidant

components by decomposition. Heat changes bound phenolic compounds to free phenolic compounds that are easily extracted and ultimately quantified.

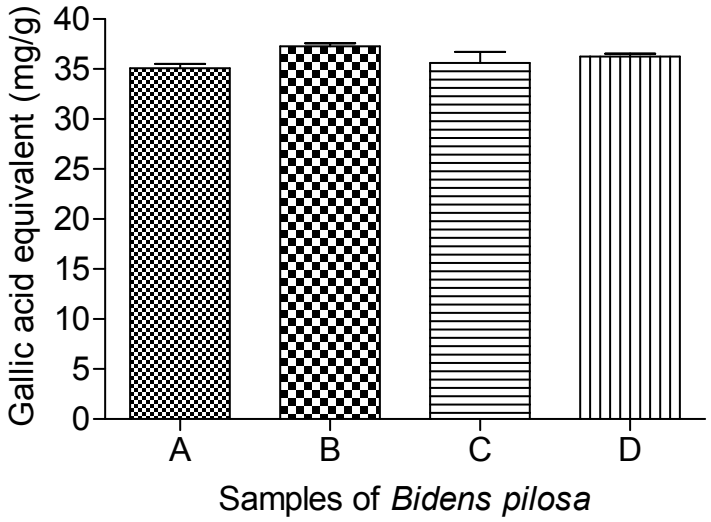


Figure 29

Effect of sun drying and storing on total content of phenolic compounds on unblanched (A), blanched sun dried (B), unblanched sun dried and stored (C) and blanched sun dried and stored (D) *Bidens pilosa* extracts expressed as gallic acid equivalents. Values are means of triplicate determinations ± standard deviation

4.7.2 Unblanched and blanched oven dried *Bidens Pilosa*

As shown in Figure 30 the total content of phenolic compounds of unblanched oven dried *Bidens pilosa* extract (19.6 mg/g) was significantly different ($p < 0.05$) from the content of blanched oven dried sample (26.1 mg/g). The relatively high total content of phenolic compounds of the blanched oven dried sample might be attributed to the degradation of phenolic compounds to compounds with enhanced reducing potential (Davey *et al*., 2000).

For all the extracts there was no significant change ($p > 0.05$) observed in the total content of phenolic compounds after 30 days storage. The observation might be attributed to the stability of flavonoids and some phenolic compounds over long periods of storage (Vallejo *et al*., 2003).

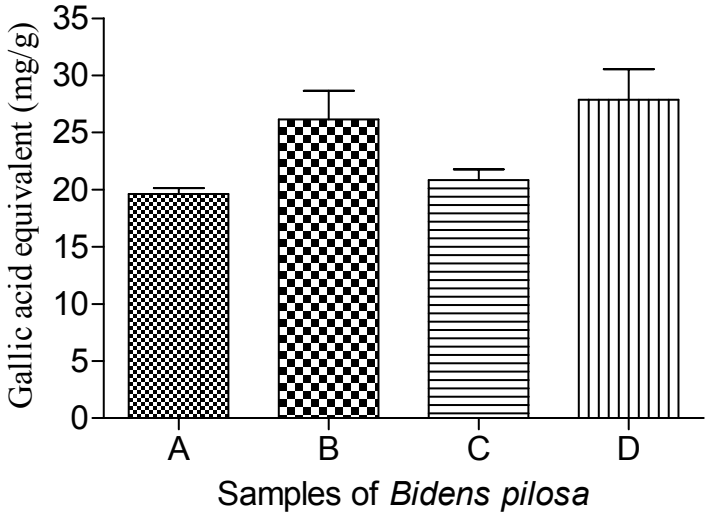


Figure 30
Effect of oven drying and storing of *Bidens pilosa* on total content of phenolic compounds on unblanched oven dried (A), blanched oven dried (B), unblanched oven dried and stored (C) and blanched oven dried and stored (D) expressed as gallic acid equivalents. Values are means of triplicate determinations ± standard deviation

4.7.3 DPPH antioxidant activity

4.7.3.1 Unblanched and blanched *Bidens pilosa*

The DPPH radical scavenging activity of extracts of *B. pilosa* after various treatments is shown in Figure 31. The unblanched extracts had the highest activities irrespective of the method of treatment. Storage for 30 days caused an increase in the antioxidant activities of the blanched and unblanched extracts.

The loss in antioxidant activity after blanching could have arisen due to the decrease in heat sensitive vitamin C and partly by phenolic compounds (Parr and Bolwell, 2000). Oboh in 2005 reported 50.4 to 82.4% loss in the contents of ascorbic acid after blanching of some tropical green leafy vegetables.

The increase in DPPH radical scavenging activity after storage might be attributed to the fact that the pre-cooking or cooking treatments might have bruised the tissue and exposed ascorbic acid and some phenolic compounds, thereby resulting in a higher reducing power. Lin and Chang in 2005 reported that reducing power of some Chinese vegetables increased after cooking treatments.

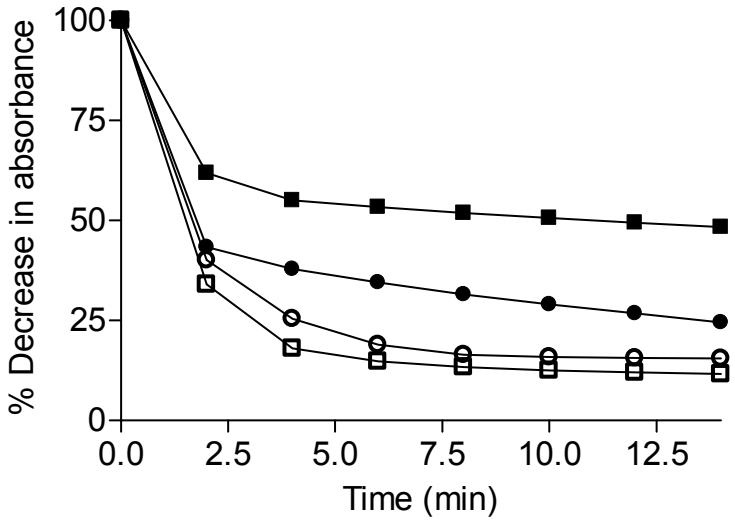


Figure 31

Effect of blanching and storing methanolic extracts of unblanched (□), blanched (■), unblanched and stored for 30 days (○) and blanched and stored for 30 days (●) *Bidens pilosa* on DPPH activity as reflected by % decrease in absorbance. Values are means of triplicate determinations ± standard deviation

4.7.3.2 Unblanched and blanched sun dried *Bidens pilosa*

Drying in the sun or storing for 30 days at room temperature had no effect on the ability of extracts from *Bidens pilosa* to quench DPPH as shown in Figure 32. The blanched sun dried sample was relatively more stable on storage than the unblanched sun dried sample.

Though the differences in the scavenging activities were insignificant, the blanched sun dried extract had a higher activity than the unblanched sun dried sample. The relatively high value of the blanched sun dried extract might have been caused by the breakdown of condensed tannins to simple reactive molecules (Oboh, 2005).

Manzocco *et al* in 2001 reported that processing and storage did not affect the antioxidant potential of fruits and vegetables. The same author reported enhanced antioxidant capacity as a result of storage and attributed the phenomenon to the formation of compounds such as Maillard reaction products with enhanced antioxidant capacities.

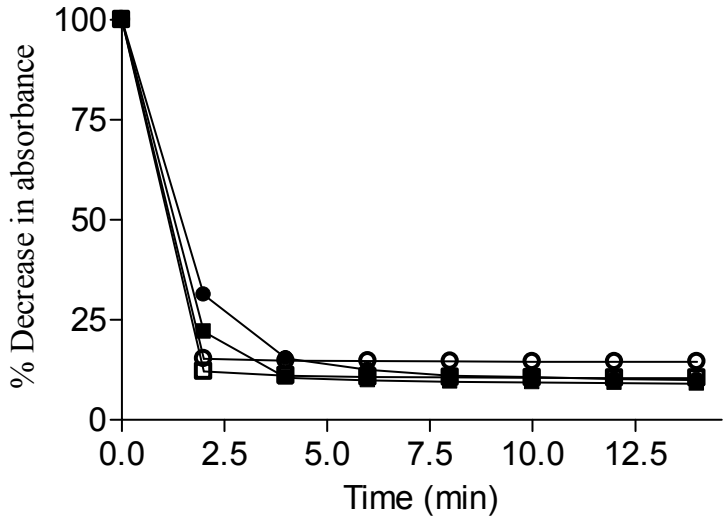


Figure 32

Effect of sun drying and storing methanolic extracts of unblanched sun dried (□), blanched sun dried (■), unblanched sun dried and stored for 30 days (○) and blanched sun dried and stored for 30 days (●) Bidens pilosa on DPPH activity as reflected by % decrease in absorbance. Values are means of triplicate determinations ± standard deviation

4.7.3.3 Unblanched and blanched oven dried *Bidens pilosa*

The ability to quench DPPH by *Bidens pilosa* extracts that had been blanched and oven dried was significantly ($p < 0.05$) higher than that of unblanched oven dried leaves as shown in Figure 33. Storing the extracts did not cause significant changes in the activities of the samples.

The blanched oven dried sample had a higher activity than the unblanched oven dried sample. Ansari *et al* in 2005 suggested that dry heat processing might change the phenolic composition of the plant extracts, leading to an increase in the amount of antioxidant components. A study by Turkmen *et al* in 2005 revealed that dry heating methods increased the antioxidant activities of some vegetables.

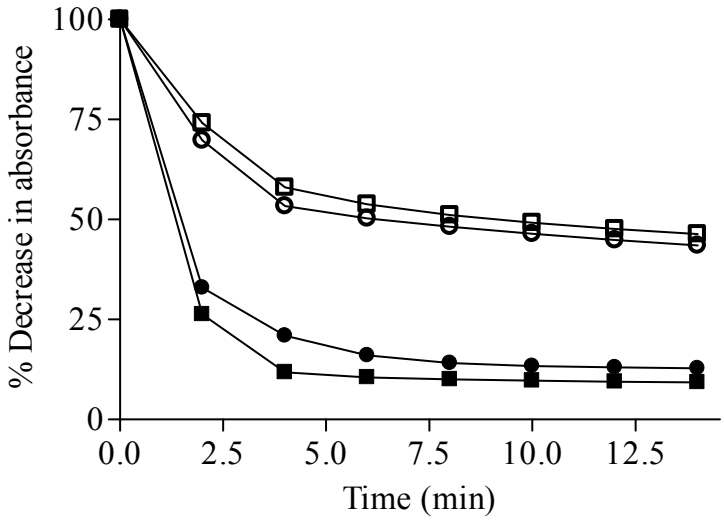


Figure 33

Effect of oven drying and storing methanolic extracts of unblanched oven dried (□), blanched oven dried (■), unblanched oven dried and stored for 30 days (○) and blanched oven dried and stored for 30 days (●) *Bidens pilosa* on DPPH activity as reflected by % decrease in absorbance. Values are means of triplicate determinations ± standard deviation

4.1 Phenolic acids and flavonoids in the vegetables

The results of the HPLC analyses are shown in Table 15. Common phenolic acids of the analysed vegetables were gallic acid and protochatechuic acid. The vast majority of the peaks on the chromatograms could not be identified because a limited number of standards were used. The standards used should not be limited to phenolic acids only, but include other plant phytochemicals such as catechins, flavonoids and some tannic acids. Most of the phenolic compounds eluted were non-polar as they were eluted when solvent B was between 80 to 100%.

The phenolic compounds identified in this study are widely found in plants. Shahidi *et al* in 1994 identified gallic acid, protochatechuic acid, ferulic acid and vanillic acid in tomatoes. Phenolic acids identified might have contributed to the antioxidant activities of the vegetables observed in this study. Caffeic and ferulic acids, which are well recognized food phenolics with antioxidant activity, prevented photo-oxidative stress in skins, as evaluated in two model experiments (Saija *et al.,* 1999). Abdel-Wahab *et al* in 2003 carried out studies that proved the beneficial effect of the natural phenolic acid *p*-coumaric acid in protecting animals against doxorubicin (DOX)-induced cardiac oxidative damage. The protecting potential of *p*-coumaric acid could be due to its free radical scavenging capability observed in the phospholipid peroxidation assay.

Table 15 Phenolic acids identified when vegetable extracts were analysed by HPLC.

Vegetable	*Phenolic acids identified
Bidens pilosa	G, Pr, Hb, Ct, Cf
Cleome gynandra	G, Pr
Amaranthus hybridus	G, Pr, Hb, Fr
Corchorus olitorius	G, Pr
Galinsoga parviflora	G, Pr, Hb, Ct, Cf, Fr

*The available standards were gallic acid (G), protochatechuic acid (Pr), hydroxybenzoic acid (Hb), catechin (Ct), caffeic acid (Cf), vanillic acid (V), coumaric acid (Cr) and ferulic acid (Fr)

5 CONCLUSIONS, RECOMMENDATIONS AND FUTURE WORK

5.1 Conclusions

People in Buhera district had knowledge on a variety of edible indigenous vegetables as seventy nine traditional vegetables were mentioned by the respondents and 20 of them were classified according to their species. The characterized vegetables belonged to 13 different families and Asteraceae was the frequently encountered botanical family. The vast majority of indigenous vegetables were reported to be available during the wet season and consequently the local people consume the vegetables during this period more often than the dry months.

Within the edible plants, leaves are plant parts most widely used. The leaves are used as relish and in most cases eaten fresh or dried with the staple meal of *sadza* which is prepared from available cereals. Some of the indigenous vegetables consumed are bitter and as a result preparation of the bitter vegetables always involves boiling for long periods to tone down the bitterness. For all the indigenous vegetables, cooking may be done with peanut butter or cooking oil and additives such as tomatoes, onions and salt may also be used. The vegetables are sometimes dried for future use and two procedures are often used for drying. The procedures include drying in the shade without prior cooking or boiling and then drying in the sun.

Some plants were reported to be used for food as well as for medicine. The reported medicinal uses of the vegetables include their use in treating diseases such as dysentery,

fevers, gonorrhoea, headaches, epileptic fits, chest pain, constipation, worm infection and stomachaches.

The results in this study revealed that the indigenous and exotic vegetables assayed are poor sources of macro-nutrients such as protein, fat, fibre and carbohydrate. However, compared to some exotic vegetables, the indigenous vegetables had higher macro-nutrient content. The micro-nutrient content of the indigenous vegetables was higher than that of lettuce and rape vegetables with the exception of lettuce which had high magnesium content as compared to the indigenous vegetables. For instance, *Amaranthus hybridus* had 7, 13 and 20 times more vitamin C, calcium and iron respectively than lettuce. All the five vegetables in this study were found to have higher than expected iron content. Similarly, zinc a micronutrient of public health significance was abundant in *Bidens pilosa* compared to other analyzed indigenous and exotic vegetables.

Cooking caused a significant decrease in the contents of protein, vitamin C and minerals of the indigenous vegetables assayed. However a significant increase in the fibre content of all the indigenous vegetables was observed.

Phenolic compounds were present in all vegetables assayed though varietal differences in the contents of total phenolic compounds, flavonoids, proanthocyanidins and tannins were observed among indigenous and exotic vegetables assayed. All the indigenous vegetables had higher total content of phenolic compounds than rape

(*Brassica napus*) and lettuce (*Lactuva sativa*) extracts. The content of flavonoids in rape was higher than the contents in *G. parviflora*, *C. olitorius,* C. *gynandra* and *A. hybridus* and lower than *B. pilosa*. All the indigenous vegetables had also higher proanthocyanidin and tannin contents than the rape and lettuce extracts.

All the indigenous and exotic vegetables were found to have antioxidant activities. The ability of the vegetable extracts to exert antioxidant power increased with the amount of extract added but differed depending on the type of vegetable used. Rape had the lowest DPPH activity than all vegetables and surprisingly lettuce had a higher activity than *Amarathus hybridus* extracts. All vegetable extracts were capable of reducing ferric ions and the ability of the extracts to reduce ferric ions increased with the concentration of the extract. Lettuce and rape had lower reducing power than all the indigenous vegetables. The extracts tested had the ability to quench peroxyl radicals formed from oxidation of linoleic acid and in this assay lettuce and rape had lower activities than all the indigenous vegetables. The indigenous vegetables also showed some activities in the lipid peroxidation assay.

The total content of phenolic compounds and DPPH activity was weakly correlated and the relationship was significantly nonlinear. When all other assays were correlated to each other, the phospholipids peroxidation assay had the strongest correlation and the beta carotene content assay had the weakest. The present results indicate that there was no consistent correlation between the amounts of phenolic compounds in the vegetable extracts and antioxidant capacities.

Blanched and oven dried *Bidens pilosa* extracts had lower phenolic compounds content and consequently lower antioxidant activity as compared to sun dried extracts. Storage time of up to 30 days had no effect on the phenolic compounds content and the antioxidant activity of *Bidens pilosa*.

Common phenolic acids of the analyzed vegetables were gallic and protochatechuic acids. Hydroxybenzoic, caffeic, vanillic, coumaric, ferulic acids and catechin varied in their distribution among the vegetables.

5.2 Recommendations

Indigenous vegetables were found to be rich sources of micronutrients and some phenolic compounds. Therefore, the consumption of indigenous vegetables should be promoted by researchers, non-governmental organizations and other relevant government/non-governmental departments at all levels of society. Consumption may be promoted by enhancing the selection and breeding of the vegetables by agriculturalists and also organizations working with indigenous vegetables should conduct food festivals with the aim of preserving the indigenous knowledge of these vegetables. In addition to all these promotional activities, the government should be engaged by researchers towards the implementation of policies that will promote research on indigenous vegetables.

From the present results it is hypothesized that most losses might be due to leaching of nutrients and phenolic compounds from the indigenous vegetables into the cooking

water during prolonged exposure to water and heat. Therefore, it is vital for researchers to come up with better cooking methods that will minimize these losses.

5.3 Future work

Studies are required to find out the best preparation methods of the indigenous vegetables as the cooking of the vegetables was found to be deleterious to the nutrients and phenolic compounds in the extracts. Research should also be carried out to elucidate some structures of the phenolic acids and flavonoids not identified on the HPLC chromatograms. Furthermore, some studies should be carried out to establish the antioxidant potentials of the vegetables in food models.

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7. APPENDICES

Appendix 1

Questionnaire: Surveys of wild plant foods used in Buhera district of Manicaland Province

1. What vegetables are available during the hot dry season?

Name of vegetable	Parts of plant that are used as food				
	Leaves	Flower	Seed	Preparation	Usage

2. What vegetables are available during the hot wet season?

Name of vegetable	Parts of plant that are used as food				
	Leaves	Flower	Seed	Preparation	Comment

3. What vegetables are available during the winter season?

Name of vegetable	Other parts of plant that are used as food				
	Leaves	Flower	Seed	Preparation	Usage

Appendix 2:

Edible indigenous vegetables mentioned by respondents

Local Shona Name	Latin Name	Family
Bonongwe	*Amaranthus hybridus*	Amaranthaceae
Booragomo	NC	NC
Borewore	NC	NC
Bupwe	*Cratotheca spp*	Tiliaceae
Chiboorahari	NC	NC
Chidumbutumbu	NC	NC
Chigwanda	NC	NC
Chimbiya	NC	NC
Chirakaraka	NC	NC
Chikadaya	NC	NC
Chipondamasvinya	NC	NC
Chirevereve	*Senecio erubescence*	Asterecea
Chitupatupa	NC	NC
Damba	NC	NC
Dhinda	NC	NC
Desvu	NC	NC
Derere gusha	*Corchorus olitorius*	Tiliaceae
Derere muuyu	*Adansonia digitata*	Bombacaceae
Derere nyenje	NC	NC
Derere rename	NC	NC
Derere nyamwenda	*Sesamum angustifolium*	Pedaliaceae
Derere feso	NC	NC
Ngaka	NC	NC
Furanondo	NC	NC
Garara	NC	NC
Goche	NC	NC
Gungwe	*Oxalis spp*	Oxalidaceae
Hambakadare	NC	NC
Jakara	NC	NC
Kasongo	NC	NC
Kasunika	NC	NC
Kwangwe	NC	NC
Mhururwa	NC	NC
Monja	NC	NC
Mowa dongi	*Amaranthus spinosus*	Amaranthaceae
Mowa guru	NC	NC
Mubhongopongo	NC	NC

Mubvunzandadya	*Chenopodium album*	Chenopodiaceae
Mundochicha	NC	NC
Mudyambi	NC	NC
Mudyamvuu	NC	NC
Mudyandota	NC	NC
Mufandichimuka	NC	NC
Mugakawembwa	*Cucumis metuliferus*	Cucurbitaceae
Mugakawenyoka	NC	NC
Mujachacha	*Cucumis anguria*	Cucurbitaceae
Mujakari	*Cleome monophylla*	Capparaceae
Mukawekawe	NC	NC
Murupwe	NC	NC
Mukono	NC	NC
Mundawarara	*Celosia trigyna*	NC
Munhenzva	*Asclepias densiflora*	Asclepiadaceae
Muninga	NC	NC
Munhuwenhuwe	*Chenopodium ambrosioides*	Chenopodiaceae
Mupari	NC	NC
Murere	*Sterculia africana*	Sterculiaceae
Murovamhodzi	NC	NC
Musvosvanyoka	*Cassia singueana*	Leguminosae
Mushamba	NC	NC
Mushinga	NC	NC
Musungusungu	*Solanum nigrum*	Solanaceae
Mutangaya	NC	NC
Mhuuyu	*Bidens pilosa*	Asteraceae
Muvhidiko	NC	NC
Muzhamharare	NC	NC
Mwenda	NC	NC
Ndakupuka	NC	NC
Ngaka	NC	NC
Nhindiri	NC	NC
Nyevhe	*Cleome gynandra*	Capparaceae
Rukukurume	NC	NC
Rwambura	NC	NC
Sosoori	NC	NC
Teketera	*Galinsoga parviflora*	Asteraceae
Tsatsashindi	NC	NC
Tsikiri	NC	NC
Tsvatsva	NC	NC
Vavangwe	NC	NC
Zumbani	NC	NC

NC-Not classified

Appendix 3

Seasonal availability of the vegetables

Local Shona Name	Latin Name	Part used as food	Season when available
Bonongwe	*Amaranthus hybridus*	Leaves and seeds	Hot wet season
Booragomo	NC	Leaves	Hot wet season
Borewore	NC	Leaves	Hot wet season
Bupwe	*Cratotheca spp*	Leaves	Hot wet season
Chiboorahari	NC	Leaves	Hot wet season
Chidumbutumbu	NC	Leaves	Hot wet season
Chigwanda	NC	Leaves	Hot wet season
Chimbiya	NC	Leaves	Hot wet season
Chirakaraka	NC	Leaves	Hot wet season
Chikadaya	NC	Leaves	Hot wet season
Chipondamasvinya	NC	Leaves	Hot wet season
Chirevereve	*Senecio erubescence*	Leaves	Hot wet season
Chitupatupa	NC	Leaves	Hot wet season
Damba	NC	Leaves	Hot wet season
Dhinda	NC	Leaves	Hot wet season
Desvu	NC	Leaves	Hot wet season
Derere gusha	*Corchorus olitorius*	Leaves	Hot wet season
Derere muuyu	*Adansonia digitata*	Leaves/fruits/seed	Hot wet season
Derere nyenje	NC	Leaves	Hot wet season

Derere rename	NC	Leaves	Hot wet season
Derere nyamwenda	*Sesamum angustifolium*	Leaves	Hot wet season
Derere feso	NC	Leaves	Hot wet season
Ngaka	NC	Leaves	Hot wet season
Furanondo	NC	Leaves	Hot wet season
Garara	NC	Leaves	Hot wet season
Goche	NC	Leaves	Hot wet season
Gungwe	*Oxalis spp*	Leaves	Hot wet season
Hambakadare	NC	Leaves	Hot wet season
Jakara	NC	Leaves	Hot wet season
Kasongo	NC	Leaves	Hot wet season
Kasunika	NC	Leaves	Hot wet season
Kwangwe	NC	Leaves	Hot wet season
Mhururwa	NC	Leaves	Hot wet season
Monja	NC	Leaves	Hot wet season
Mowa dongi	*Amaranthus spinosus*	Leaves	Hot wet season
Mowa guru	NC	Leaves	Hot wet season
Mubhongopongo	NC	Leaves	Hot wet season
Mubvunzandadya	*Chenopodium album*	Leaves	Hot wet season
Mundochicha	NC	Leaves	Hot wet season
Mudyambi	NC	Leaves	Hot wet season
Mudyamvuu	NC	Leaves	Hot wet season
Mudyandota	NC	Leaves	Hot wet season

Mufandichimuka	NC	Leaves	Hot wet season
Mugakawembwa	*Cucumis metuliferus*	Leaves	Hot wet season
Mugakawenyoka	NC	Leaves	Hot wet season
Mujachacha	*Cucumis anguria*	Leaves	Hot wet season
Mujakari	*Cleome monophylla*	Leaves	Hot wet season
Mukawekawe	NC	Leaves	Hot wet season
Murupwe	NC	Leaves	Hot wet season
Mukono	NC	Leaves	Hot wet season
Mundawarara	*Celosia trigyna*	Leaves	Hot wet season
Munhenzva	*Asclepias densiflora*	Leaves	Hot wet season
Muninga	NC		Hot wet season
Munhuwenhuwe	*Chenopodium ambrosioides*	Leaves	Hot wet season
Mupari	NC	Leaves	Hot wet season
Murere	*Sterculia africana*	Leaves	Hot wet season
Murovamhodzi	NC	Leaves	Hot wet season
Musvosvanyoka	*Cassia singueana*	Leaves	Hot wet season
Mushamba	NC	Leaves	Hot wet season
Mushinga	NC	Leaves	Hot wet season
Musungusungu	*Solanum nigrum*	Leaves	Hot wet season
Mutangaya	NC	Leaves	Hot wet season
Mhuuyu	*Bidens pilosa*	Leaves	Hot wet season
Muvhidiko	NC	Leaves	Hot wet season
Muzhamharare	NC	Leaves	Hot wet season

Mwenda	NC	Leaves	Hot wet season
Ndakupuka	NC	Leaves	Hot wet season
Ngaka	NC	Leaves	Hot wet season
Nhindiri	NC	Leaves	Hot wet season
Nyevhe	*Cleome gynandra*	Leaves	Hot wet season/ hot dry season
Rukukurume	NC	Leaves	Hot wet season
Rwambura	NC	Leaves	Hot wet season
Sosoori	NC	Leaves	Hot wet season
Teketera	*Galinsoga parviflora*	Leaves	Hot wet season
Tsatsashindi	NC	Leaves	Hot wet season
Tsikiri	NC	Leaves	Hot wet season
Tsvatsva	NC	Leaves	Hot wet season
Vavangwe	NC	Leaves	Hot wet season
Zumbani	NC	Leaves	Hot wet season