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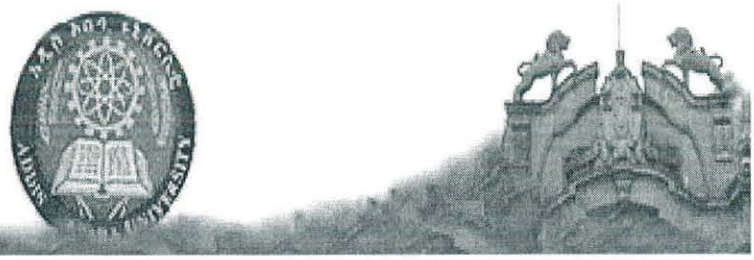

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

NUTRITIVE VALUE OF SAMMA (*URTICA SIMENS STEUDEL*) LEAVES AND EFFECT OF BOILING ON ITS COMPOSITION AND SENSORY ACCEPTABLITY

By Eskedar Getachew Assefa

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfilment of the Requirement for the Degree of Master of Science in Food Science and Nutrition

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Addis Ababa

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FOOD SCIENCE AND NUTRITION PROGRAM

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Supervisors

Dr. Gulelat Desse (Phd) .....

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Table of Contents

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List of Tables

Table **Page**

List of Figures

Acronyms

AA	Ascorbic Acid
AAS	Atomic Absorption Spectrophotometer
AAU	Addis Ababa University
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASTDR	Agency for Toxic Substances and Disease Registry
CHO	Carbohydrate
EHNRI	Ethiopian Health and Nutrition Research 1nstitute
FAO	Food and Agriculture Organization
GLVs	Green Leafy Vegetables
RDA	Recommended Daily Allowance
SPSS	Statistical Product and Service Solution

Abstract

The nutrient and antinutrient levels of raw **Samma** leaves (*Urtica Simensis*) grown in Ethiopia, specifically Deberebrhan, Fitche and Ambo were investigated using standard analytical methods. The effect of boiling at different duration on nutrient and antinutrient content of **Samma** leaves (*Urtica Semensis*) collected from Deberebrhan was also investigated. **Samma** leaves were boiled at 96°C ± 2 at different length of time such as 5min, 10 min,15 min, 20 min, 25 min and 30 min and each raw samples and boiled samples were analysed for Proximate composition, Ascorbic Acid (vitamin C), Antinutrients (Condensed Tannin and Oxalate), Minerals (Fe, Ca, Zn) and Sensory evaluation. The ranges of moisture, crude protein, crude fat, crude fibre and total ash in raw **Samma** (*Urtica Simensis*) leaves collected from the three areas were found to be 76.8-78.9 %, 25.1-26.3 %, 2.2-2.3 %, 8.5-9.4 % and 20.8-26.4 %, respectively. The values of crude fat, crude fibre and total ash reduced after boiling with ranges of (2.3-0.55%, 8.47-7.51%, 25.4-15.9 %) and increased the moisture and crude protein (78.9-89.09%, 26.3-30.95), respectively. The ranges of mineral contents of raw **Samma** leaves (*Urtica Simensis*) of iron, zinc and calcium were 38.4-47.0, 2.87-5.8 and 768.6-793.4 mg/100g, respectively and the values were reduced after boiling with ranges of (42.3-29.2, 4.90-2.09, and 768.6-392.37-492.45%), respectively. Raw Samma leaves were found to be rich in vitamin C (82.65-84.3%). The percentage loss was very high for Ascorbic Acid (vitamin C) when boiling time increases (90-100%). Raw **Samma** leaves also contained Tannin (25.3-27.0 mg/100g) and oxalate (8.59- 9.33 mg/100g) and these values reduced after boiling 25.3-9.0 in Tannin and 9.3-1.77 mg/100g in oxalate. The study showed that these indigenous leafy vegetable has high nutritive contents and their consumption need to be promoted in the various region of the country.

Key Words: *Urtica Simensis* (**Samma**), Nutrient, Antinutrent, Boiling, Ascorbic Acid (Vitamin C), Oxalate, Tannin and Mineral.

1. Introduction

1.1 Background

Wild food plants play a very important role in the lively hoods of communities as an integral parts of the subsistence strategy of people in developing countries (Zemede and Mesfin, 2001). Locally available wild food plant serves as alternatives to stable food during periods of food deficit, valuable supplement for nutritionally balanced diet and primarily alternative sources of income for many resources poor communities. Wild resource in general is often ignored and receives little recognition from the development community (Scoonnes *et al.*, 1992). Rural communities, in most cases, depend on wild resource including wild edible plants to meet their food needs in period of food crisis. The diversity in wild species offers variety in the family diet and can contribute to household food security. Numerous publications provide detailed knowledge of edible wild plants in specific location in Africa (Zemede and Mesfin, 2001).

Ethiopia is endowed with agro-climatic diversity which account for the existence of rich flora and fauna in the country. The Ethiopian flora is estimated to constitute about 6000 species of higher plants in which about 10% are endemic (Gebre Egziabher, 1991). However, Ethiopia is the country of national food deficiency, hunger and malnutrition, and is still exist in many rural and urban areas. Vegetables are of great importance in helping to alleviate this problem as they contribute significantly to the amount of other nutrients in the diet. The scarcity of vegetables, or their scarcity in the diet, is a major cause of micronutrient deficiency, which causes malnutrition and even death in young children throughout the semi-arid and arid areas of Ethiopia (Welch and Graham, 1999).

Exotic vegetable species cannot be grown under the harsh climatic and resource-poor conditions encountered in many of the rural areas where these problems occur. However, there are a number of indigenous and wild grown plant species which could help to alleviate this situation.

Nettle one of our commonest wild plant found all over the temperate areas of the world. It is the common name for 30-45 species of flowering plants which belongs to the genus *Urtica* of the family *Urticaceae.* They are often easily recognizable particularly after having experienced the sting! It can be found in a variety of habitat and soil type (Puff and Nemomissa, 2005).

Samma (*Urtica simensis*) is one of species of Nettel found only in Ethiopia. It is dark green perennial wild plant and the leaves are used as a food in a few areas of Ethiopia. This indigenous leafy vegetable has the potential to provide a valuable source of nutrition in areas with hot, dry climates (UN-EUE, 2001 and Bosch, 2004). It could fill a valuable place in the production of food in rural areas where the climate is not conducive to the production of vegetables and at a time when cultivated GLVs (Green Leafy Vegetables) are not ready for use. It could be particularly valuable in areas with a low rainfall, as these crops will produce a viable yield under these conditions, whereas most of the exotic leafy vegetables require large amounts of water for successful production. Drought-tolerant vegetable crops would be invaluable in helping to alleviate malnutrition in these areas. However, before being able to consider the plant for this purpose, more information on their nutrient composition is required. Also, careful attention should be paid to palatability if a plant is to be successfully grown as a commercial crop. However, there has been no previous study on the nutritional value, antinutrient content, effect of processing (boiling) and the sensory evaluation of *Urtica simensis* (**Samma**) leaf grown in

Ethiopia. Thus, the aim of this thesis is to investigate the nutrient content, antinutritinal factors, and effect of processing (boiling) on the nutrient content of *Urtica simensis*, locally known as **Samma** (Amharic) and Dobbi or Gulgulba (Oromeffa).

1.2 Statement of the problem

Vegetables are efficient sources of several micro nutrients, both with respect to unit cost of production and per unit of land area (Underwood, 1971; Wilson, 1989; Greenfield and Southgate, 1992; Smith *et al.*, 1995; Nordeide *et al.*, 1996; Ali and Tsou, 1997; Freiberger *et al.*, 1998; Gass *et al.*, 2000; and Tontisirin *et al.*, 2002). Wild vegetables can be promoted in to diet as the most practical and sustainable way to achieve micronutrient malnutrition or 'hidden hunger" (Chadha and Oluoch, 2003; Mnzava, 1995; Maundu *et al.*, 1999; Grutel *et al.*, 2000), Since such vegetables are efficient sources of several important micronutrients, indeed, there is increasing consensus that wild food significantly contribute to alleviating hunger and malnutrition. (Zemede and Mesfin, 2001) have also shown the important and critical roles of wild plants in supplying seasonal food needs and maintaining nutritional quality of traditional diets. Nutritional analysis of some leafy edible plants further showed that they have even better food values than cultivated vegetables (Kabuye *et al.*, 1997; FAO, 1999).

Urtica simensis (**Samma**) is one of indigenous wild edible plant in Ethiopia. Although in few area of Ethiopia *Urtica simensis* **(Samma)** have traditionally been used for food and medicinal plants, their potential contribution to food security, nutrition, health, and income generation for the well-being of mankind is still largely under exploited. But *Urtica simensis* **(Samma)** can

make important contributions to regional diets. Promoting such traditional food plants as a means of protecting food culture and enhancing food security has not been explored. The potential of spreading its use across regions and cultural groups is likewise not yet looked in to. In general in Ethiopia, the potential of wild vegetable species in food and nutrition security, health and income generation should be increased in the face of the growing environmental and socio–economic changes. One remedy for food security problems is food items diversification; *Urtica simensis* **(Samma)** can assist the majority of our society due to its agricultural and nutritional advantages, if the society acknowledges and produce it with an attention given to the other crops. However, no studies were conducted with regard to its chemical composition and nutritional values on this plant species. Thus this study aims to investigate the nutritional value, some antinutritinal factors, effect of processing (boiling) and Sensory evaluation of this indigenous wild edible plant species *Urtica simensis* **(Samma)** leaf grown in different parts of Ethiopia through nutritional studies.

1.3 Significance of the study

In Ethiopia young shoot of **Samma** (*Urtica simensis*) is edible in a few areas in different forms as green vegetable. The traditional processing practice used to convert **Samma** leaves into human consumable forms includes boiling.

The results of this study can be used to

- Promote the nutrients content and human consumption of **Samma** (*Urtica simensis*) leaves to prevent micronutrient malnutrition.
- Design an appropriate processing technique in the preparation of **Samma** (*Urtica simensis*).
- Alleviate co-existing micronutrient deficiencies in the entire household;
- Researchers, students, teachers and academicians of the field area can use findings of this work as reference material.
- The widespread interest in other *Urtica* species because of their medicinal properties might eventually attract attention from phytochemical research for **Samma** (*Urtica simensis*) leaves.
- Aware the society that **Samma** (*Urtica simensis*) is a good nutritious food crop. Instead of considering it as "poor man's food", to let the society use the crop as a basic source of micro nutrient.
- Make **Samma** (*Urtica simensis*) as an ingredient of fortification for various food items to improve their nutritional and chemical composition

- As a vegetable, **Samma** (*Urtica simensis*) will remain important not only locally but also as a commercialized food product. If the production grows as expected in the future times.
- Encourages development and expansion of **Samma** (*Urtica simensis*) based meals producing food industries

1.4 Objectives

1.4.1 General objectives

- The general objectives of this study are to investigate nutrient content of **Samma** (*Urtica simensis*) leaves and effect of processing (boiling) on nutrient content of **Samma** leaves grown in different parts of Ethiopia.

1. 4.2 Specific objectives

The specific objectives of this study include:

- Determining the proximate composition (moisture, total ash, crude protein, crud fiber, crud fat and carbohydrate) of raw **Samma** (*Urtica simensis*) leaves
- Analyzing the level of selected mineral content of raw **Samma** (*Urtica simensis*) leaves
- Determining some antinutrient content (oxalate and condensed tannin) of **Samma** (*Urtica simensis*) leaves.
- Analyzing vitamin C content (Ascorbic acid) of **Samma** (*Urtica simensis*) leaves.
- Determining the effect of boiling at different length of time on nutrient and antinutrient contents of **Samma** (*Urtica simensis*) leaves.
- Evaluating sensory acceptability of **Samma** (*Urtica simensis*) leaves which are boiled at different duration.

2. Literature Review

2.1 Status and Uses of Wild Edible Plants

Between 60 % and 70 % of the population in developing countries dwell in rural areas. Although the total population that rely on edible wild plants is difficult to estimate accurately, about 200 to 300 million rural people are partially or totally dependent on forests, particularly wild plants (Pimentel *et al.*, 1997). Research in different parts of Africa has shown that wild plant and animal species are quite extensively used at time of food shortage (Lepofsky *et al.*, 1985; Ogle and Grivetti, 1985; Zinyama *et al.*, 1990). Wild plants in different parts of Africa also indicate that they are integral in the diet of the people (Maundu *et al.*, 1999, Ogle and Grivetti, 1985; Pimentel *et al.*, 1997; Zinyama *et al.*, 1990). Ogle and Grivetti (1985) reported that wild plants contributed a greater share of the food for 39% of the Swaziland people than domestic cultivars, and 18% reported an even balance of the wild plants and domesticated crops.

Ethiopia's topographic, climatic, ethnic, linguistic, and religious diversity and its long history, mosaic environment and social diversification have led to varied plant lore. It has been indicated that there are about 378 types of wild edible plants commonly consumed in the country (Demel, 2010). Among the edible wild plant species, *Balanites aegyptiaca, Berchemia discolour, Leptadenia hastate, Cordia africana, Dovyalis abyssinica, Ficus sur, Rosa abyssinica, Urtica simensis, Rubus apetalus, and R. steudneri* were consumed in all the study districts.

Indigenous vegetables play an important role in human diets. They supply the body with minerals, vitamins, fibre, quality protein, colour, flavour and are recognized for their therapeutic value and certain hormone precursors in addition to protein and energy (Gupta *et al.*, 2005).

Increase in consumption of vegetables is recommended for better health and management of chronic diseases such as cardiovascular disease, diabetes, and cancer (Thompson, 1993). There are also concerns that the high intake of these foods may concurrently increase intake of antinutrients that mainly impair mineral and protein absorption. Some of these antinutriernts directly affect human health and even can reach lethal dose, or indirectly reduce bioavailability of nutrients and cause micro nutrient malnutrition. Some of these antinutrients have also beneficial effect on health (Thompson, 1993).Despite the consumption of exotic vegetables; some indigenous vegetables have been reported to be more nutritious and less expensive than the exotic ones. Many indigenous vegetables are collected from the wild.

Consumption of vegetables is common in Ethiopia especially by the rural farming communities. There are commonly grown and consumed vegetables in the country. Apart from these domesticated vegetables (wild and semi wild origin) that are common but consumed with less frequency in different areas or confined to certain locations. Due to fast regeneration of most vegetables under limited moisture, they are extensively used to bridge the gap during food shortage and famine by the rural communities (Getahun, 1974; Guinand and Dechasa, 2001; Zemede and Mesfin, 2001; Addis *et al.*, 2005 and Wondimu, 2007).

The nutritional importance, health promoting factor, and antinutritional property of edible plants in general is partly or entirely dependent on their processing history. Processing affects content, activity, and bioavailability of the compounds (Nicoli *et al.*, 1999; Zhang and Hamauzu, 2004; Yadav and Sehgal, 2003). Despite the actual and potential benefit, research in to the green leafy vegetables has to a large extent been ignored in Ethiopia. Therefore, the present investigation

was designed to (1) identify the nutrient content of edible wild plant species **Samma** (*Urtica simensis*) in different areas of the country; (2) possible effects of processing (boiling) and (3) Sensory evaluation of **Samma** (*Urtica simensis*) leaves which are boiled for different duration.

2.2 Utilization of Wild Edible Plant in Ethiopia

The use of wild plants in Ethiopian diet has been under reported. However, observations and few studies on the field indicate that a number of wild plant species are consumed by the rural population (Getahun, 1974; Engels and Gottesch, 1991; Mengestu, 1995; Guinand and Dechassa 2001; Zemede and Mesfen, 2001). The knowledge, tradition and opportunity of using wild plants by different communities as supplements to the dietary intake have been described as wide. The number of species and plant parts used for food by all age and gender groups increase at times of famine or food shortage.

Studies on the utilization of wild plants in Ethiopia indicate that they are mainly used for house building and household utensils, clothing, food, soap, medicine, and magic and ritual purposes (Abbink, 1993). Since antiquity, pastoral groups, farming communities, monks, nuns, in isolated monasteries and churches in rural areas have used wild plants as a source of food both at times of plenty and food shortage (Getahun, 1974). Some studies indicate that consumption of wild plants is widespread in food insecure areas of the country as compared to relatively food sufficient areas (Abbink, 1993; Bekele *et al.*, 1993 and Kloos and Lindtjrn, 1993). In 2003, Ethiopian government officially announced that 13.2 million people were exposed to famine and requested 1.8 million tonnes of food assistance (Anonymous, 2003). In a situation where millions of rural people are still unable to feed themselves and are in need of food assistance, the need to promote

utilization of climatically adapted and nutritious edible wild plants is of paramount importance. In spite of the role of edible wild plants in bridging periods of food shortages and providing dietary variety, very little attention has been given to the inventory and conservation of species. Moreover, in view of the sizable area, and immense social, cultural, and geographical diversity of the country, documentation and preservation of local knowledge and assessments of the nutritional composition of wild plants as well as health problems caused by their consumption are very scanty (Abbink, 1993; Zemede and Mesfin, 2001; Getahun, 1974). Under such circumstances, not only the knowledge and skills of identifying nutritious and/or climatically adapted edible wild plants but also the plants themselves will be irreversibly lost.

Literature on the nutritional composition of Ethiopian edible wild plant species is limited. However, literature from other African countries shows that the potential to enhance the diet and health status of populations by encouraging the use of selected edible wild plants cannot be overestimated. Wild plants are comparable and can be superior in some respects (such as nutrition range, and availability) to some contemporary agricultural crops (Maundu *et al.*, 1999; Ogle and Grivetti, 1985). Moreover, both cultivated and wild edible plants may contain elements that interfere with the absorption of nutrients (Kebede *et al.*, 1995; Ogle and Grivetti, 1985), so a study on the nutritional composition and antinutrients of edible wild plants is warranted.

In Ethiopia *Urtica Simensis* (**Samma)** grows up land grassland more easily in rural subsistence farming systems especially in North Gonder, Gojam, Shoa, Bale and Arsi provinces and eaten in few areas as pot herbs (Puff and Nemomissa, 2005; Friis, 1989; Bosch, 2004). It is commonly considered as a weed among rural communities in most parts of Ethiopia. Although the vegetable

has the potential to be developed as a valuable crop, very little is known about its role in the overall food acquisition system in different parts of Ethiopia especially in relation to its contribution to the intake of important micronutrients (Getahun, 1974 and Wondimu 2007). The nutritive value, as well as antinutrient content of *Urtica Simensis* **(Samma)** has not been well researched in Ethiopia .The importance of vegetables as a source of dietary zinc, calcium and iron depends upon the total mineral content and other constituents in the diet like oxalate and tannins that affect bioavailability.

2.3 Nettle (*Urtica dioica*) (Stinging nettle)

Nettle is any of numerous plants having stinging hairs that cause skin irritation on contact. It is the common name for 30-45 species of flowering plants of the genus *Urtica* and in the family *Urticaceae,* with a cosmopolitan though mainly temperate distribution. They are mostly herbaceous perennial plants, but some are annual and a few are shrubby. **Samma** (*Urtica simensis*) is one of species of Nettel found only in Ethiopia (Friis, 1989 and Bosch, 2004).

2.3.1 Food from Nettle

Nettles can be used in a variety of recipes, such as polenta and pesto. Nettle soup (Nässelsoppa in Swedish) is a common use of the plant, particularly in Scandinavian countries. Young nettle leaves are similar in texture to spinach and other leafy greens, and can be substituted for or mixed with other greens in recipes. The high protein content of nettles makes them nutritionally valuable for vegetarians. From a cooking point of view the Nettle has an old reputation. It is one of the few wild plants still gathered each spring by country-folk as a pot-herb. It makes a healthy vegetable, easy of digestion (Wheeler, 2002 and Yarnell, 1998).

Nutritionally the nettle is an excellent source of vitamin C, calcium, magnesium, iron and numerous trace elements (Nordeide *et al.*, 1996 and Pearson, 1971). The stings disperse when you cook them and the young shoots can be used for soups and stews in place of spinach. It is not only humans that have benefited from the consumption of the nettle. Dried nettle when loses its sting, becomes palatable to livestock. In Sweden the nettle is sometimes cultivated for this purpose and fed to milk cattle thereby the production milk increased (Wheeler, 2002).

The most commonly consumed part of nettle plant is the leaves (young shoot) and to date reports indicate that different country of the world used different nettle recipe to be used as a main course or accompany with other food. Soaking nettles in warm water will remove the stinging chemicals from the plant, which allows them to be handled and eaten without incidence of stinging (Bremness and Lesley, 1984). The young tops should be gathered when 6 to 8 inches high. Gloves should be worn to protect the hands when picking them. They should be rubbed through a hair-sieve either served plain, or warmed up in the pan and then put into a saucepan, dripping, and cooked with the lid on for about 10 minutes. Then chopped or warmed up in the pan again, with a little salt, pepper and butter, or a little gravy, and served with or without poached eggs. They thus form a refreshing dish of spring greens, which is slightly laxative. By earthling up, Nettles may be blanched in the same way as sea kale and eaten in a similar manner. They also make a good vegetable soup, and in Scotland are used with leeks, broccoli and rice to make Nettle pudding, a very palatable dish (Hill, 1998 and Healthy Life Magazine, 2007).

Analyses of nettle have revealed the presence of more than fifty different chemical constituents (Pearson, 1971; Peterson and Jensen, 1986 and Pollard and Brings, 1984). Nettle's leaves have

been found to contain histamine, acetylcholine (Emmelin, and FeldBerg, 1947). Formic acid, tannins, 5-hydroxytrypatamine (Collier, and Chesher, 1956), vitamins A, C and D; mineral salts, calcium, potassium, silicon, iron, manganese and sulphur (Nordeide, 1996).

The most prominent member of the genus *Urtica* is stinging nettle (*Urtica dioica*), native to Europe, North Africa, Asia, and North America. The genus *Urtica* comprises many species and is almost cosmopolitan, with most species in temperate regions of the northern hemisphere and about 5 in Africa, 2 of which are introduced weeds. *Urtica massaica* is also used as a vegetable in East Africa; it can be distinguished by larger stipules and usually double-serrate leaf margins, and it is more robust. *Urtica simensis* has been reported from tropical Africa (Ethiopia), probably as an introduced weed in gardens and its presence is confirmed by herbarium specimens (Friis, 1989 and Lemordant, 1971). However, a large number of species names that will be encountered in this genus in the older literature (about 100 species have been described) are now recognized as synonyms of *Urtica dioica*. Some of these taxa are still recognized as subspecies (Friis, 1989). Most of the species share the property of having stinging hairs, and can be expected to have very similar medicinal and culinary uses to the stinging nettle (*Urtica dioica*). Only a relative few of this family member's sting but some of these can be even more fearsome than our own familiar nettle. Studies indicate that, one species from Java, *Urtica urentissima* causes a burning sensation and symptoms like lockjaw, this can last for days or weeks, whilst *Urtica crenulato* and *Urtica heterophylla*, both of India, are also most virulent. Furthermore, the stings of *Urtica ferox*, the ongaonga or tree nettle of New Zealand, produces effects which last for a year, and are even said to cause death. The stinging hairs of most nettle species contain formic acid, serotonin

and histamine; however recent studies of *Urtica thunbergiana* (Fu *et al*, 2006) implicate oxalic acid and tartaric acid rather than any of those substances, at least in that species.

Chemical Constituents found in *Urtica dioica* (Stinging nettle) Leaf

Stinging nettle's (*Urtica dioica*) leaves is a powerhouse of nutrients. It contains on average 21-23% protein, 4% fats, 9-21% fibre, and 24% ash and 30% Carbohydrate. In another study the dried leaf of nettle contains 40 % protein. They are one of the highest known sources of protein in a leafy green, and of superior quality than many other green leafy vegetables, and it was reported to be used by vegetarian (Yarnell, 1998; Healthy Life Magazine, 2007 and Hill, 1998)

Reports also indicate that, the fresh herb contains 85 g water and 3.55 g mineral in 100 g of the herb. It is also reported that the plant contains 1050 mg calcium, 613 mg potassium, 340 mg silicon, 50-265 mg phosphorus, 2-200 mg iron, 180 mg chloride, 175 mg magnesium, 58 mg sodium, 8 mg manganese, 4 mg boron, 2.7mg titanium, 1.3 mg cuprum, 0.03 mg nickel. On the other hand, nettle's main plant chemicals in the fresh leaves contain vitamin A, C, D, E, K and B-complexes as well as thiamine, riboflavin, niacin, and vitamin B-6, all of which were found in high levels, and act as antioxidants. Samples of dried leaves have been analyzed for nutrient content. They were found to be rich in alpha-tocopherol, riboflavin, iron, zinc, calcium, phosphorous, and potassium. However, the analyses indicated that as a result of the drying and storage process, total loss of vitamin C and a substantial loss of Beta-caroten had been incurred (Nordeide et al., 1996 and Pearson, 1971). .

Uses of Stinging Nettle (*Urtica dioica*)

Because of the above constituents, stinging nettle (*Urtica dioica*) is known to have many therapeutic applications or medicinal purposes (Anderberg and Kirsten), pesticide and as foliar fertilizers other than cooking as avegetables for many years. As Pesticide, Kraus and Spiteller (1991) found *Urtica dioca* to be effective as aphid repellents. Bozsik (1996) also carried out studies on aphicidal efficacy of different stinging nettle extracts fermented on plum (*Prunus domestica*), red currant (*Ribes rubrum*) and (*Spiraea vanhouttei*) and found it to reduce infestation and in another studies in Poland showed that water extracts of *Urtica dioca* was more active as a natural pesticide against aphids than synthetic pesticides (Achremowicz and Ciez, 1992). As foliar fertilizers, Studies carried out in Germany noted that plants treated with nettle water had positive effects such as increased plant growth, dark green leaves and better resistance against pests and diseases. Studies by Peterson and Jensen (1985) reported that water extracts of *Urtica dioca* had growth stimulating effect on plants..

2.4 Description of *Urtica simensis* Steudel.

Local Name **Samma**

Species Name ***Urtica simensis***

Family Name **Urticacea**

In Ethiopia one species of nettle, *Urtica simensis* locally called **Samma** is used as food crop. Reports indicate that, it is endemic in Ethiopia and the young leaves are edible, eaten as a pot herb in some areas (Friis, 1989; Puff and Nemomissa, 2005; UN-EUE, 2001; Lemordant, 1971 and Bosch, 2004).The plant characteristic of species of *Urtica simensis* **(Samma)** described as

herbs. It is an erect, pale green, non-branched, wild-growing nettle plant. Leaves are oval and coarsely toothed. The whole plant is covered with stinging hairs. The plant grows all year round and therefore can be harvested whenever there is a need (Friis, 1989 and Bosch, 2004). It is dioeciously, erect, perennial herb up to 1 m tall, almost none branched; rhizome creeping; petioles, leaf blades and inflorescences with 2.5 mm long stinging hairs. Leaves opposite, simple; stipules fused, interpetiolar, 0.5–1cm long (Friis, 1989; Puff and Nemomissa; Lemordant, 1971).

Ecologically *Urtica simensis* found in Upland grassland areas most common in disturbed localities, often plentiful near houses. It is found at 1500–3500 m altitude. Tigray, Gonder, Gojam, Showa, Ambo, Fitche, Arsi, Bale, Debrebrehane, Borena, and Sidama. It considered a weed in fields and pastures. The plant is also used as a medicine to ease aching joints and for liver complaints (Puff and Nemomissa, 2009; Westphal, 1975; UN-EUE, 2001; Lemordant, 1971 and Bosch, 2004).

The parts used for cooking are the leaves (young shoot). **Samma** leaves (the young shoot) should be gathered from the field and crushed between the two hides by either stamping on them or by using the hands to rub them through sieve to avoid the burning sensation of the leaves Acetylcholine, histamine and 5-hydroxytryptamine have been implicated in itching from the stinging hairs of other *Urtica* species.
and leaves sorted, stalks removed, washed in running water, drained and boiled for approximately 20 minutes, cooking water removed and coiled between palms. Then chopped and boiled with the lid on again for 10 minutes with barley flour. When boiled the leaves have to be

grid one more time to become a smooth puree, a bit like shero wot. The puree can be salted and cooled and eaten either by its own or together with injera or bread. The plant is eaten by everybody before cultivated crops are harvested and in times of food shortage (Friis, 1989; Lemordant, 1971 and Bosch, 2004).

Fig.1 Edible Samma (*Urtica simensis*) leaves grown in Ethiopia

2.5 Effects of Processing (Boiling) on Nutrient and Antinutrient Contents of GLVS

The nutritional importance, health promoting factor, and antinutritional property of edible plants in general is partly or entirely dependent on their processing history. Processing method that may affect their content, activity, and bioavailability of the compounds (Nicoli *et al.*, 1999; Zhang and Hamauzu, 2004; Yadav and Sehgal, 2003). Vegetables are usually processed by the application of dry or moist heat to improve their organoleptic properties or extend their shelf life. This technique has been known from time immemorial to reduce the quantity of nutrients in foods, especially the heat labile vitamins. Hence, consumer demand for nutritious foods, which are minimally and naturally processed, has led to interest in some non thermal technologies. These non thermal technologies are not commonly used by industries in developing countries. Therefore, in most of the processing unit operations heat is applied. When subjected to heat treatment vegetables are affected differently.

Vegetable processing such as blanching, boiling, canning, sterilising and freezing, as well as cooking is expected to affect the yield, composition and sensory accessibility and some nutritional antioxidants such as the heat labile vitamin C. During vegetable processing, qualitative changes, antioxidant breakdown and their leaching into surrounding water may influence the antioxidant activity of the vegetables. Some antioxidant compounds like ascorbic acid and carotenoids are very sensitive to heat and storage and are lost during different vegetable processing steps (Amin et al., 2006; Zhang and Hamauzu, 2004; Turkmen, Sari, and Velioglu, 2005; Nicoli, Anese and Parpinel, 1999)..

2.6 Antinutritional Factors

Antinutritional factors are substances occurring in the diet which acts antagonistically towards one or multiple nutrients, reducing bioavailability. This is usually done through complex formation which reduces nutrient absorption and directly inflicting health problems of the consumer (Liener 1980; Thompson, 1993). Despite their possible detrimental effect, many of those considered being antinutritional factors including polyphenols and protease inhibitors are also known to have health benefits. Some of the health benefits explained by Thompson (1993) include lowering blood glucose and hormonal responses, reduction of blood lipids and reduction of risk of cancer.

Tannin

Tannins are polyphenol components prevalent in plants. Studies have shown that tannins interact with proteins and form tannin-protein complexes, which decrease protein digestibility and protein solubility. This decrease in protein digestibility may be caused by either the inactivation of digestive enzyme or the reduction of the susceptibility of the substrate proteins after forming the complex. Polyphenols are found to interact with proteins and cause either inactivation of enzyme or make protein insoluble (Thompson, 1993). In addition to this, tannins inhibit the absorption of minerals such as iron if ingested in excessive quantities which leads to anaemia. This is because tannins are metal ion chelators, and tannin-chelated metal ions are not bioavailable.

Although there are fragmentary reports on the effect of processing on the above chemicals, detailed and systematic report on the effects of home based processing methods on vegetables is lacking. Therefore, there is a need for comprehensive study the effect of convectional cooking

methods on the individual chemicals so as to retain or optimize the important constituents and effects and reduce or eradicate detrimental ones.

In most studies on the effects of heat treatment on the total phenolic content, the results are contradicting. Some researchers reported an increase in the phenolic content whilst others observed a decrease. In some researches an attempt was made to simulate the actual cooking conditions and as a result in some papers the cooking conditions were not explicitly specified. The data generated using the actual cooking conditions is beneficial when included in food composition databases as it will enable the users to evaluate the actual amounts of bioactive compounds consumed.

Oxalates

Oxalates are anti nutritional factors that precipitate dietary calcium and inhibit its absorption. They precipitate as insoluble salts and accumulate in the renal glomeruli and can be a cause for renal disorders (Curhan, 1999). So far, there is no verification on the benefit of oxalates to human health.

Oxalates are naturally occurring substances found in plants, animals, and in humans. In chemical terms, oxalates belong to a group of molecules called organic acids, and are routinely made by plants, animals, and humans (Prakash, 1993). Our bodies always contain oxalates, and our cells routinely convert other substances into oxalates. For example, vitamin C is one of the substances that our cells routinely convert into oxalates. In addition to the oxalate that is made inside of our body, oxalates can arrive at our body from the outside, from certain foods that contain them (Sienera, 2006). It is a highly reactive molecule that is abundant in many plant foods (Prakash,

1993). But in human cells, when it is present in high amounts, it can lead to oxidative damage, depletion of glutathione, the igniting of the immune system's inflammatory cascade and the formation of crystals which seem to be associated with pain and prolonged injure (Low and Stoller, (2005). It seemed reasonable to see if lowering the dietary supply of oxalates could be beneficial (Curhan, 1999 and Thompson, 1993).

Cooking has a relatively small impact on the oxalate content of foods. Repeated food chemistry studies have shown no statistically significant lowering of oxalate content following the blanching of green leafy vegetables. A lowering of oxalate content by about 5-15% is the most you should expect when cooking a high-oxalate food. It does not make sense to overcook oxalate-containing foods in order to reduce their oxalate content. Because many vitamins and minerals are lost from overcooking more quickly than are oxalates, the overcooking of foods (particularly vegetables) will simply result in a far less nutritious diet that is minimally lower in oxalates (Kelsay, Prather, 1983 and Kikunaga, Arimori and Takahashi, 1988).

2.7 Minerals

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well being (FAO and WHO, 1998). Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals, and with a well-balanced diet that includes mixed sources of grains, fruits, vegetables, roots and tuber crops, plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle (Dwyer, 1994).

Many individuals both in developed and developing countries are failing to attain recommended mineral intakes (Dwyer, 1994). Whereas an increased consumption of plant food products would be beneficial, it appears that behavioural and/or environmental factors will continue to limit their consumption (Baranowski *et al.*, 1999). Thus, as an alternative strategy, efforts are underway to increase the nutrient composition of those plant foods which people do eat, as an attempt to ensure adequate attainment of dietary nutrients in all individuals (Baranowski *et al.*, 1999 and Bouis, 1996).

Currently, the achievable densities of minerals in our existing agricultural crops means that few individual plant foods are able to supply the daily recommended intake for any given mineral in an average or reasonable serving size. This problem of low mineral density is particularly troublesome in staple foods, such as cereal grains tuber crops and root crops, which make up a large proportion of daily food intake in the developing world (FAO and WHO, 1998,; Dwyer, 1994; Baranowski, 1999 and Bouis, 1996).

Diet-related factors have a greater influence on the bioavailability of the micronutrients in plant foods, particularly Ca, Fe and Zn, than on the macronutrients. The absorption of Ca, Fe and Zn is particularly affected. The net effect on the nutrient bioavailability depends on the balance between factors that either inhibit or enhance nutrient absorption and/or utilization in the whole diet (Sandstrom, 2001). The adverse effects of some of the organic component in plant foods on nutrient bioavailability can be reduced by household food processing and preparation practices.

In general wild Plant foods are good sources of minerals. The most important minerals contained in plants are calcium, phosphorus, magnesium, iron, copper, zinc, sodium and potassium. Being a good source of minerals, wild GVS fulfil dietary requirements of human in adequate manner among different food groups. GVS plants get minerals from their soil environment and deposit these to their leaves. Roots utilize specific and/or selective transport to obtain minerals that are essential for plant growth and development including calcium (Ca), iron (Fe) and zinc (Zn). (Grusak and Dellapenna, 1999). This study focused only on iron (Fe), zinc (Zn) and calcium (Ca).The physiological role of Ca, Fe and Zn are briefly described below.

Calcium (Ca)

Calcium forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. It also participates in glycogen metabolism (Bosco, 1980). Inadequate intake of calcium increases the risk of osteoporosis (bone loss with no apparent cause). Excess intake of calcium may cause kidney stones and reduces mineral absorption in general (Smith, *et al.*, 1995). Calcium (Ca) is an essential nutrient for humans, but is quite often limited in diets of low-income sectors and is of particular concern for pre-school children, adolescents, and pregnant and lactating women.

Iron (Fe)

Iron is involved in many vital functions in the human body. First, iron is important for oxygen transport. Further, iron is essential to brain function and development and severe iron deficiency can cause retarded mental development, which may be irreversible (Cowan, *et al.,* 1967). Dietary iron is present in foods in two main forms; haem iron only in foods of animal origin (high

amounts in liver and red meat) and non-haem iron in both animal and plant foods, mostly in the ferric state. Haem iron and non-haem iron are absorbed through different mechanisms. Haem iron is transported into the enterocyte by the haem receptor, while non-haem iron uses the divalent metal transporter (DMT), which means that dietary ferric iron (Fe^{3+}) must be reduced to ferrous iron (Fe^{2+}) before uptake (Mackenzie *et al.*, 2005) .

Absorption of non-haem iron can be enhanced or inhibited by various dietary components and thus depends on the meal composition. The absorption of haem iron is much higher than the absorption of non-haem iron; about 25% for haem iron and less than 10 for non-haem iron. Great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health (Turna *et al.*, 2003).

Zinc (Zn)

Zinc is an essential element found in the tissue of animals and plants even at normal ambient concentrations. However, if plants and animals are exposed to large concentrations of bioavailable Zn, significant bioaccumulations can result, with possible toxic effects (ASTDR, 1995).

Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function. If zinc is removed from the catalytic site, activity is lost; replacement of zinc restores activity. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including

transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with zinc mal-absorption resulting in severe deficiency, have provided much insight into the functional outcomes of zinc deficiency. These include impairments of dermal, gastrointestinal, neurologic and immunologic systems (ASTDR, 1995). Thus Zinc (Zn) is an essential nutrient for all forms of life. Zinc deficiency is a global micronutrient deficiency in humans. Worldwide, about 3 billion people, especially in developing countries, are affected by micronutrient deficiencies including Zn deficiency. Zinc deficiency in human results in a number of health problems, such as impairments in linear growth, sexual maturation, learning ability, immune functions and the central nervous system, susceptibility to infection, impaired wound healing, etc (Brown *et al.*, 2001).

Based on national food balance data, approximately 20.5% of the world's population is estimated to be at risk of inadequate Zn intake, with the percentage of individuals at risk highest in South East Asia (33.1%), Sub Saharan Africa (28.2%), South Asia (26.7%) and Latin America and the Caribbean (24.8%) (Wuehler *et al.*, 2005).

3. Materials and Methods

3.1 Chemicals, Reagents and Apparatus

All chemicals used for analysis are analytical grade. Similarly, the glasswares were cleaned to make them free from any possible contamination prior to analysis. The required chemicals for each parameter analysis are given under each experimental section. The main equipments used during the analysis are listed below:

Drying oven (DHG- 9055A, Memment Germany), Analytical balance (ARZ 140, N315, SNR, 1203290469, USA), Digest stove, Crude protein analyzer, Muffle furnace (Carbolite, Aston Lane, Hope Sheffield 30 ZRR, England), Centrifuge (DYNAC II, Clay Adams, division of Becton and Dikinson Company, USA), Flame Atomic Absorption Spectrophotometer (Varian spectra AA – zoplus, Varian Austeralia, ltd, Austeralia), UV-Vis Spectrophotometer (Beckman, Du-64 Japan), Fat determinator (Soxhlet extractor), Kjeldahl apparatus, and refrigerator (Hitachi, Tokyo, Japan), Blending device (Moulinex, France).

3.2 Sample Collection

3.2.1 Plant material:

The leaves of raw **Samma** (*Urtica simensis*) were collected in March 2002 from the country-sides near Addis Ababa, specifically Fitche, Ambo and Debrebrehan where its consumption in these area high. The voucher specimen was then deposited in the specialized herbarium of EHNRI.

3.2.2 Sample preparation

About 2kg of Plant material was collected from each area in the morning and placed in isothermal box with icepacks and brought to the laboratory for analysis. In the laboratory the edible part of raw **Samma** leaves was sorted, crushed between the two hides and rubbed them to avoid the stinging hairs which are responsible for the burning sensation of the leaves. After that the leaves of the plant materials were thoroughly mixed, the non edible part is removed, washed with tap water and rinsed with de-ionized water. The residual moisture on the surface of the vegetable was soon dried with blotting paper. Moisture and Vitamin C contents of the raw **Samma** leaves were analyzed on the same day of collection. The remaining sample was dried in an oven maintained at 45^0c and ground to fine powder using pestle and mortar. The powder then sieved through a 2.0 mm mesh.

3.2.3 Boiling

De-ionized water (500 ml) was added in to a stainless steel pan and allowed to boil at 96°C $\pm$ 2. Fresh cleaned edible portions (150gm) of **Samma** leaves was added and boiled for different duration such as 5min, 10min, 15 min, 20 min, 25 min and 30 min. Each boiled portions were evaluated for the acceptance of human consumption and the remaining sample were dried under the Oven at 45^0c and ground into fine powder using pestle and mortar, and sieved through a 2.0 mm mesh that was used for all the analyses.

3.3 Proximate Analysis

Moisture content, total ash, crude protein, crude fibres, and crude fat of the leaves of **Samma** were determined according to AOAC (2000) using the official methods 925.09, 923.03, 979.09, 962.09, and 4.5.01, respectively.

3.3.1 Determination of Moisture Content (AOAC 925.09, 2000)

The amount of water present in a sample is considered to be equal to the loss of weight after drying the sample to constant weight at a temperature near the boiling temperature of water. Empty dishes and lids (made of porcelain) where dried using air drying oven (Memment, Germany) for 1 hour at 100^0C, transferred to the desiccators (with granular silica gel), cooled for 30 minutes, and weighed 5.000g of fresh sample was weighed and transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 24 hr at 50^0C, and then the dishes and their contents were cooled in desiccators to room temperature and reweighed.

Calculation

$$\textbf{Moisture (\%)} = \frac{M2 - M3}{M2 - M1} \text{x} 100$$

$\mathbf{M_1}$=mass of the dish,

$\mathbf{M_2}$=mass of the dish and the sample before drying, and

$\mathbf{M_3}$=mass of the dish and the sample after drying

3.3.2 Determination of Crude Protein (AOAC 979.09, 2000)

The Protein content was determined by the Kjeldahl method. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulphuric acid and concentrated orthophosphoric acid containing copper sulphate and potassium sulphate as a catalyst. The ammonia released after alkalinisation with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

Digestion: About 0.5000g of fresh samples were taken in a Tecator tube and 6ml of acid mixture (5parts of concentrated orthophosphoric acid and 100 parts of concentrated sulphuric acid) was added, mixed, thoroughly and 3.5ml of 30% hydrogen peroxide was added step by step . As soon as the violet reaction had ceased, the tubes were shaken for a few minutes and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000g of copper sulphate with 100 g of potassium sulphate) was added into each tube, and allowed to stand for about 10 min before digestion. When the temperature of the digester reached 370^0C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1 hr. The tubes in the rack was transferred into the fume hood for cooling, a 15ml of de ionized water was added, and shaken to avoid precipitation of sulphate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml de-ionized water and the rinses were added into the

solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, stopper and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate. The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few millilitre of water before the receiver was removed.

Mg nitrogen in the sample = VxNx14

$$g\ nitrogen/100\ g\ sample = \frac{mg\ of\ nitrogen}{mg\ of\ sample} x\ 100$$

Total nitrogen (%) = (V-Vb) xNx14/W

Crude protein (%) = total nitrogen (%) x6.25

Where: **V** = volume of hydrochloric acid consumed to neutralize the sample;

Vb= the volume of acid consumed to neutralize the blank;

N = normality of the acid;

14=Eq. wt of Nitrogen;

6.25 = conversion factor from total nitrogen to crude protein.

3.3.3 Determination of Crude Fat Content (AOAC 4.5.01, 2000)

Crude fat was determined by exhaustively extracting a known weight of sample in diethyl ether (boiling point, 55 °C) in a soxhlet extractor. The ether was evaporated from the extraction flask.

The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

The extraction flasks were cleaned, dried in drying oven (Memmert, Germany) at 70^0C for 1hour, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2.00 gram of fresh samples were added into the extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber. The cooling water was switched on, and a 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for about 3 hrs. The extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 70^0C for about 1hr, cooled to room temperature in the desiccator for about 30 minutes and re-weighed.

$$W=W_2-W_1$$

$$Fat\ g/100\ g\ fresh\ sample = (Wx100]/W_O$$

Where: **W** = weight of fat;

W2 =weight of extraction flask after extraction of flask and fat;

W1 = weight of extraction flask before extraction (wt. of flask);

WO = weight of fresh Sample.

3.3.4 Determination of Crude Fibres Content (AOAC 962.09, 2000)

Crude fiber was determined after digesting a known weight of sample by refluxing 1.25% boiling sulphuric acid and 28% boiling potassium hydroxide.

Digestion: About 1.6000g of fresh sample was placed into a 600ml beaker, 200ml of 1.25% H_2SO_4 was added, and boiled gently exactly for 30 minutes placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring.

Filtration: The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a little distilled water. The solution was poured from beaker into sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to crucible, and filtered

Washing: The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H_2SO_4 and filtered, and then washed with hot distilled Water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% H_2SO_4 and filtered. Finally the residue was washed with water- free acetone.

Drying and combustion: The crucible with its content was dried for 2 hours in an electric drying oven at 130^0C and cooled for 30 min in the desiccators (with granular silica gel), and then Weighed. The crucible was transferred to a muffle furnace (Gallenkamp, size 3) and incinerated for 30 min at 550^0C. The crucible was cooled in the desiccators and weighed.

Then the fibber was calculated as a residue after subtraction of the ash.

$$Crude\ fibre\ g\ /100\ g = \frac{W_1 - W_2}{W_3} x\ 100$$

Where: **W1** = weight of (crucible +sample) after drying;

W2 = weight of (crucible +sample) after ashing;

W3 = weight of fresh sample

3.3.5 Determination of Total Ash (AOAC 923.03, 2000)

Ash was determined by incineration of known weights of the samples in a muffle furnace at 550^0C (Gallenkamp, size 3) until a white ash was obtained. Organic matter was burned off and the inorganic material remaining is cooled and weighed. Heating was carried out in stages, first to derive the water, then to char the product thoroughly and finally to ash at 550^oC in a muffle furnace. The ashing dishes (made of porcelain) were placed into a muffle furnace for 30 min at 550^0C. The dishes were removed and cooled in desiccators (with granular silica gel) for about 30 minutes to room temperature; each dish was weighed to the nearest g. About 2.000g of sample was added into each dish. The dishes were placed on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550^0C for 4 hours, and removed from the muffle and then placed in desiccators for 1hr to cool. The ash was clear white in appearance. When cooled to room temperature, each dish. Weight of total ash was calculated by difference and expressed as percentage of sample.

Calculation:

$$Total\ ash\ (\%) = \frac{W2-W}{W1-W} x\ 100$$

Where **W**= weight in grams of empty dish

$\mathbf{W_1}$ =weight in grams of the dish plus the dried test material

$\mathbf{W_{2=}}$ weight in grams of the dish plus ash

3.3.6 Determination of Total Carbohydrate

Total carbohydrate (CHO) content was determined by difference. It was determined by subtracting the crude protein, crude fibre, total ash and fat from the total dry weight of the sample.

3.3.7 Determination of Gross Energy

Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors; 16.7 kJ/g (4 kcal/) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories (Guyot et al., 2007).

3.4 Analysis of Ascorbic Acid

Determination of total Ascorbic Acid (Vitamin C) spectrophotometrically was done at wave length of 515nm by Methods of Vitamin Assay (Freed 1966). Extract 5g of fresh leaf sample with 100ml of 6%TCA (Tri Chloro Acetic Acid) by mortar & paste for 2-5 minute and remove the suspended solids by centrifuging or filtration. In a conical flask containing sample solution add 1-2 drops of saturated Bromine solution & aeration to 10ml aliquot add 10ml of 2% thiourea. Pipette 4ml from sample solution into each of the test tube and set one tube aside to serve as

blank and add to each of the remaining tubes 1ml of 2, 4-DNPH (Di NitroPhosphate Hydrazin). Let them put all test tubes in water bath at 37^0C for 3 hour and cool in an ice bath for approximately 5 min. Add slowly 5ml 85%H_2SO_4 while the tubes are in an ice bath and add 1ml of 2%DNPH to the blank and mix all tube and standing all tubes at room temperature for 30 min. Then read the absorbance of the standards, blank and test samples at 515 nm.

The Vitamin C content was calculated using the formula;

$$\frac{mg\ AA}{100\,g} = \frac{(A_s - A_b)\,x10}{A_{10\,\mu g\ Std} - A_b}$$

Where: $\mathbf{A_s}$ = Absorbance of samples

$\mathbf{A_b}$ = Absorbance of blank

$\mathbf{A_{10\ \mu g\ Std}}$ = Absorbance of 10 µg AA (Ascorbic Acid) standard

3.5 Mineral Analysis

3.5.1. Equipments and Reagents

A drying oven, a blending device, a Kjeldahl apparatus, and a refrigerator were used and atomic absorption spectrophotometer was used for the analysis of metals (Ca, Fe and Zn,) using air–C_2H_2 flame. All the reagents used were of analytical grade. 69–72% HNO_3 (Spectrosol, BDH, England) and 70% $HClO_4$ Aldrich, A.C.S. Reagent Germany) were used for digestion of **Samma** samples. Lanthanum nitrate hydrate (98%, Aldrich, USA) was used to avoid refractory interference (for realizing calcium). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Ca, Fe and Zn were used for preparation of calibration standards and in the

spiking experiments. Distilled and deionised water was used throughout the experiment for sample preparation, dilution, and rinsing the apparatus prior to the analysis.

3.5.2 Prevention of Contamination

To minimize the risk of contamination, all glassware used for the analytical methods was washed with deionized water followed by acid-wash, and sterile disposable powder-free plastic gloves were worn when handling the foodstuffs during the sampling and analyses stages. The digested solutions were kept in the refrigerator until analysis.

3.5.3 Digestion of Samma samples

For the extraction of metals from the plant, wet digestion method reported by Atlabachew and Chandravanshi (2008) was used. In fact, before applying to the real sample analysis, the applicability of the method was evaluated and found to be efficient to destruct the organic matters from **Samma** samples.

Exactly 0.5 g of dried and homogenized **Samma** samples was transferred into a 250 mL round bottomed flask. To this was added 4 mL of a mixture of HNO_3 (69–72%) and $HClO_4$ (70%) with a volume ratio of 1:1 and the mixture was digested on a micro-Kjeldahl digestion apparatus by setting the temperature first to dial at 4 (120 ^{0}C) for 30 min and then increased to dial 6 (180^{0}C) for the next 30 min and then increased to 9 (approximately 270 ^{0}C) for the remaining 1 h. The digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution, 15 mL of deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of the

filter paper by the digest residue while filtering with whatmans, (110mm; diameter), filter paper. The round bottom flask was rinsed subsequently with 5 mL deionized water until the total volume reached around 45 mL. To this final solution, 1%lanthanum nitrate solution was added and the solution was filled to the mark (50 mL) with deionized water. Triplicate digestions were carried out for each sample. Six blank solutions were prepared following the same digestion procedure as the sample. All the digested samples were kept in the refrigerator until analysis.

Method Detection Limit

Method detection limit was determined from standard deviation of 3 blank samples prepared under the same conditions as of the samples. The method detection limit was taken as three times standard deviation of the blank. Method detection limits (µg/g) of Ca, Fe and Zn for **Samma** samples are 4.4, 1.9 and 0.5 respectively.

Recovery test

The efficiency of the procedure was evaluated using recovery experiment, i.e., by adding known concentration of each metal to 0.5 g of sample. The procedure was as follow: 0.2 mg of Fe; and 0.04 mg of Zn were spiked at once in to 0.5 g of **Samma** sample from Debrebrehan and the remaining metal (1.5 mg of Ca) was spiked in to another digestion flask containing 0.5 g of the sample. After digesting the spiked samples following the above procedure, each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer. Recovery test was performed in triplicates.

3.6 Determination of Antinutrient

3.6.1 Condensed Tannin

Tannin content was determined by the method of Burns (1971) as modified by Maxson Rooney (1972). About 0.1gram of **Samma** leaves were weighed in a screw cap test tube. The 0.1g dried **Samma** was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). D-catechin was used as standard for condensed tannin determination. A 40mg of D-catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm, and the calibration curve was constructed from the series of standard solution using SPSS-15.

Calculation:

$$Tannin\ in\ mg/100g = \frac{Absorbance - \text{intercept}}{slope\ x\ density\ x\ weight\ of\ sample\ x\ 10}$$

3.6.2 Determination of Oxalate Content

The oxalate content was determined using the method in Manual for Nutrition Surveys (Freed 1966). The procedure involves three steps: digestion, oxalate precipitation and permanganate titration.

Digestion: At this step, 2 g (db) of flour was suspended in 190 ml of distilled water contained in a 250-ml volumetric flask; 10 ml of 6M HCl was added and the suspension digested at 100°C for 1 h, followed by cooling, and then made up to 250 ml before filtration.

Oxalate Precipitation: Duplicate portions of 125 ml of the filtrate were measured into a beaker and four drops of methyl red indicator added, followed by the addition of concentrated NH_4OH solution (drop wise) until the test solution changed from its salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90°C and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 5°C. The solution was then centrifuged at a speed of 2500 rev/min for 5 min. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4, solution.

Permanganate Titration: At this point, the total filtrate resulting from digestion of 2 g of flour was made up to 300 ml. Aliquots of 125 ml of the filtrate were heated until near-boiling, and then titrated against 0.5M standardized $KMnO_4$, solution to a faint pink colour which persisted for 30 s. The calcium oxalate content was calculated using the formula.

$$\textbf{Oxalate} = \frac{T\times(Vme)(DF)\times 105}{(ME)\times mf}\ \textbf{(mg/100g)}$$

Where **T** is the titre of $KMnO_4$ (ml),

Vme is the volume - mass equivalent (i.e. that $1cm^3$ of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid),

DF is the dilution factor VTA (2.4, where VT is the total volume of filtrate (300ml) and A is the aliquot used (125 ml)),

ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$, redox rxn)

mf is the mass of flour used.

3.7 Sensory Evaluation of Boiled Samma Leaves

Appearance, colour, taste, texture, and over all acceptability of processed **Samma** leaves were evaluated with 10 sensory panellists comprising staff (EHNRI) and students from food science and nutrition program of AAU in standard sensory analysis both at EHNRI following standard procedures (Eddy *et al.*,2007, Ouyoun *et al.*, 2010). Panellists were trained in the use of sensory evaluation procedures and the meaning of the descriptive terms used as stated in Eddy *et al.* (2007). Panellists were also trained concepts of the hedonic scale and how to score their sensory evaluation.

The Panellists were instructed to evaluate each sample in the following order appearance, colour, taste, texture, and over all acceptability. The three samples were presented turn by turn in identical containers coded with three digit random numbers. A nine point hedonic scale with 1 representing the least score (dislike extremely) and nine the highest score (like extremely) was

used. Water was provided to rinse the mouth between evaluations and covered expectoration cups were also provided when panelists did not wish to swallow the samples (Olaoye).

3.8 Data analysis

The analysis was carried out in three triplicates for all determinations. The mean and standard deviation of means were calculated. The data were analyzed by one way analysis of variance (ANOVA) in SPSS 15.0 for windows evaluation version computer programme was used to analyze the results. A multiple comparison procedure of the treatment means was performed by Duncan's new multiple range test (Duncan, 1955). Significance of the differences was accepted at $P < 0.05$

4. Result and Discussion

Edible wild plants are useful in filling the gap of food deficit during hungry periods and providing dietary variety to peoples in developing countries including Ethiopia. Although documented study on the nutritional composition of Ethiopian edible wild plant species are lacking, investigation in other African countries reflect that a number of them supply much if not most of the required vitamins (especially A, B and C), protein, carbohydrate, micronutrients, trace metals of nutritional importance and fibre (Ogle and Grivetti, 1985; Maundu, 1999). Hence, in Ethiopia there is a potential for enhancing the diet and health status by encouraging the use of nutritionally rich edible wild plants. In this study, the nutritional and antinutritional compositions of raw **Samma** leaves as well as the effect of processing (boiling) on nutritional, and antinutritional content and Sensory evaluation of boiled **Samma** leaves at different duration of time were investigated.

4.1 Nutrient Content of Raw Samma Leaves

Proximate Composition of Raw Samma Leaves

As indicated elsewhere, **Samma** leaves were collected from three areas (Debreberhan, Fitche and Ambo) to see if there exists variation in the nutrient composition of the vegetable with respect to geographical location. However, it was found that, except the mineral and ash content of the leaves, there is no significant difference ($P<0.05$) in moisture content and other nutrients analyzed in this study (Table 1).

Moisture Content

The moisture content of fresh **Samma** leaves (*Urtica Simensis*) collected from Debreberhan; Fitche and Ambo were 78.9 g/100g, 79.0 g/100g and 76.8 g/100g respectively. These values were found to be lower compared with commonly cultivated green leafy vegetables in Ethiopia such as Spinach (*Spinacea oleracea*) (83.30 g/100g), Lettuce (*Lactuca sativa*) (95.50g/100g), Swiss chard (*Beta vulgaris*) (91.5g/100g) and Kale (*Brassica carinata*) (87.60 g/100g) (Food Composition Table for use in Ethiopia, 1997). This indicted that **Samma** leaves contained relatively lower content of water in its leaf tissue than the corresponding similar vegetables mentioned above.

Protein

Samma (*Urtica Simensis*) leaf samples collected from Debreberhan, Fitche and Ambo areas had more or less similar protein contents with mean value of around 26g/100g (Table 1). This value was higher than the protein content of green leafy vegetable reported for Spinach (*Amaranthus Virids*), Lagose Spinach (*Celosia argentea*), Lenghui (*Urtica dioica*), Malabar Spinach (*Basella rubra*) and Bonongwe, mowa (*Amaranthus hybridus)* where their protein content was 10, 8.1, 19.5, 20.3 and 23.1 g/100g, respectively (Nordiede *et al.*, 1996 and Bhardwaj *et al.*, 2009). The protein content of raw **Samma** was also found to be higher compared to commonly consumed vegetables in Ethiopia such as Lettuce (*Lactuca sativa*) (15.5 g/100g), Swiss chard (*Beta vulgaris*) (12.2 g/100g), Kale (*Brassica carinata*) (8.0 g/100g) and Spinach (*Spinacea oleracea*) (18.6 g/100g) (Food Composition Table for use in Ethiopia, 1997). This indicates that the leaves of **Samma** may be another cheap source of plant protein for marginal resource communities of Ethiopia.

In addition to that, the level of protein content of raw **Samma** is comparable with Pulses (Gupta and Wagle, 1988; Gupta *et al.*, 1989; Yadav and Sehgal, 2003). The higher protein content of this green leafy vegetable can complement the limiting protein obtained from the intake of small quantity of Pulses, Milk and other products that may not meet the daily requirement set by FAO/WHO (1998). Use of this leafy vegetable can therefore be taken as means of alleviating the low intake of protein rich foods.

Crude Fat

Fat contents of **Samma** leaves (*Urtica Simensis*) collected from Debrebrehan, Fitche and Ambo areas were 2.2g/100g, 2.4g/100g and 2.3g/100g respectively (Table 1). Like that of the protein content, the fat content of raw **Samma** leaves was also found to be higher than spinach (*Spinacea oleracea*) (0.8/100g), lettuce (*Lactuca sativa*) (0.2g/100g), Swiss chard (*Beta vulgaris*) (0.4g/100g) and Kale (*Brassica carinata*) (0.80 g/100g) (Food Composition Table for use in Ethiopia, 1997) commonly consumed in Ethiopia. Similarly, the fat content of **Samma** leaves was also found to be higher than the fat contents in Malabar Spinach (*Basella rubra*) (0.86g/100g), Bonongwe, mowa (*Amaranthus hybridus)* (0.4g/100g) (Bhardwaj *et al.,* 2009) collected from overseas. The results of this study are also lower when compared with the average for vegetables consumed in South Africa. However, these results are in the lower range when compared with reported values (8.3 – 27.0% DW) in some vegetables consumed in Nigeria and Republic of Niger (Ifon and Bassir, 1980; Sena *et al.*, 1998). This shows that leave of **Samma** studied have moderate lipid contents, which further agrees with the findings that leafy vegetables are low lipid containing food, and this may be an advantage for people suffering from obesity (Lintas, 1992).

Crude fibre

The crude fibre content of raw **Samma** leaves (*Urtica Semensis*) collected from Debreberhan, Fitche and Ambo was around 9 g/100g (Table 1). This value was higher than that reported for Bonongwe, mowa (*Amaranthus hybridus)* and Malabar Spinach (*Basella rubra*) which were 1.5 and 1.57g/100g respectively (Muchuweti *et al.,* 2009). The crude fibre content of raw **Samma** leaves is also high compared to cultivated green leafy vegetables consumed in Ethiopia such as spinach (*Spinacea oleracea*) (4.60 g/100g), lettuce (*Lactuca sativa*) (3.7g/100g), Swiss chard (*Beta vulgaris*) (6.10g/100g) and Kale (*Brassica carinata*) (7.50 g/100g) (Food Composition Table for use in Ethiopia, 1997). The Relatively higher content of crude fibre in the leaves investigated in this study confirms that non-starchy vegetables are the richest sources of dietary fibre (Agostoni *et al.*, 1995). It is therefore suggested that the leaves of the species may be employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Saldanha, 1995). High levels of fibre in foods help in digestion and prevention of colon cancer (Saldanha, 1995; UICC/WHO, 2005).

Ash

The ash content of the studied **Samma** leaf samples was found to be 20.8, 23.8 and 25.4 g/100g in samples from Debreberhan, Fitche and Ambo respectively (Table 1). Looking at the table, Sample from Debreberhan was found to contain relatively higher contents than Fitche and Ambo. The result revealed that ash content in the studied samples significantly differs ($P < 0.05$) to each other. These obtained values were higher than that reported for Amaranth and Spinach (Agbo *et al.* 2009). The significant difference in ash content within the studied samples might be

due to variation in climatic condition, soil type and age of harvested vegetable (Kabata-Pendias and Pendias, 2001).

Total Carbohydrate

In this study, total carbohydrate of Samma leaves collected from the three areas was evaluated and it is indicated in Table 1. The leaves of Samma leave low in carbohydrate. A possible explanation may be that the species deposit most of their carbohydrate food reserve in the tuberous root; and the leaves are therefore consumed for their mineral and other nutrient contents (Duru and Uma, 2002). The carbohydrate contents obtained in the raw samples ranged from 31.8 to 37.7 % while Ambo Samma leave had the highest carbohydrate content. This is slightly less than 38.8% reported for another cultivar of raw *C. esculenta* leaves in Nigeria (Mepba *et al.*, 2007).

Table 1: Proximate composition of raw **Samma** (*Urtica simensis*) leaves per 100 g dried portion

Location	Moisture* (g/100g)	Ash (g/100g)	Crude protein (g/100g)	Crude fat (g/100g)	Crude fiber (g/100g)	Total CHO (g/100g)	Energy Kcal (g/100g)
Debrebrehan	78.9±0.80^{a}	25.4±0.06^{a}	26.3±0.61^{a}	2.2±0.5^{a}	8.5±0.51^{a}	31.8±0.75	252.2
Fitche	79±1.20^{a}	23.8±0.82^{b}	25.6±0.90^{a}	2.4±0.1^{a}	9.4±0.15^{a}	33.3±0.30	257.2
Ambo	76.8±0.61^{a}	20.8±1.71^{c}	25.1±0.65^{a}	2.3±0.1$_{a}$	9.1±0.20^{a}	37.7±1.10	271.9

All are edible portion of young shoot of **Samma** leaves and the values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at ($P< 0.05$)

* The moisture content is in fresh wet portion per 100 g

Ascorbic acid (Vitamin C)

Samma samples collected from Debreberhan, Fiche and Ambo areas had ascorbic acid content of 84.28 mg/100gm, 86.64mg/100gm and 82.65mg/100gm, respectively in wet base. Ascorbic acid (Vitamin C) concentration did not differ statistically in the studied area (Table 2). This value was comparable with that of *Basella rubra* (83.7 mg/100gm) (Bhardwaj *et al.* 2009) and higher than that of Bonongwe, mowa (*Amaranthus hybridus)* (64%) and Lenghui (*Urtica dioica*) (45.3mg/100gm) (Muchuweti *et al,* 2009; Nordiede *et al.* 1996). Ascorbic acid content of raw **Samma** leaves was also higher compared to cultivated green leafy vegetables consumed in Ethiopia spinach (*Spinacea oleracea*) (32.00g/100g), lettuce (*Lactuca sativa*) (6.00g/100g) and Swiss chard (*Beta vulgaris*) (18.00g/100g) and Kale (*Brassica carinata*) (20.00 mg/100g) (Food Composition table for use in Ethiopia, 1997).

Table 2: Ascorbic acid (Vitamin C) and mineral content of Samma (*Urtica simensis)* leaves.

Location	Ascorbic Acid (mg/100gm)	Fe (mg/100gm) (DW)	Ca (mg/100gm) (DW)	Zn (mg/100gm) (DW)
Debrebrhan	84.3 ± 1.2^{a}	42.3 ± 2.47^{a}	768.6 ± 112^{a}	4.90 ± 0.73^{a}
Fitche	86.6 ± 1.0^{a}	38.4 ± 3.68^{b}	787.7 ± 120^{b}	2.87 ± 1.09^{b}
Ambo	82.65 ± 0.9^{a}	47.0 ± 2.92^{c}	793.4 ± 132^{c}	5.8 ± 0.55^{c}

All are edible portion of young shoot of **Samma** leaves and values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at ($P< 0.05$)

Minerals

Recovery test

The efficiency of digestion procedure was evaluated using recovery experiment. Result of the analysis is given in table 3. Looking at the table, the percentage recoveries of **Samma** sample are between 90.83 % and 106.67 %, which are within the acceptable range.

Table 3 Recovery Test Results

Metal	Conc. in sample (mg/g)	Amount added (mg/g)	Conc. in spiked sample (mg/g) (mean ± SD)	% Recovery
Ca	7.685	3.0	10.51 ± 0.45	94.17
Fe	0.423	0.4	0.805 ± 0.016	95.67
Zn	0.08	0.08	0.153 ± 0.01	90.83

Determination of Minerals

Results of the studied nutrients of the three samples showed the ability of **Samma** to accumulate high amounts of both macro- (Ca) and micronutrient elements (Zn and Fe) (table 3). With regard to the ranges of concentrations of the studied metals, it could be arranged according to their levels in the **Samma** samples of all the sampling sites in the following order in dry weight basis in mg/100g : Ca(768.6 –793.4) > Fe(38.4 – 47.0) > Zn(2.87-5.8) for Debreberhan, Fitche and Ambo sites respectively.

The higher level of Ca in the leaves according to Marschner (1995) was due to the broad range of Ca-bearing minerals in soil and water and usually abundant in ground water and surface water which can easily be absorbed by the plant.

Looking at Table 2, it is also clear that the concentration of Ca and Fe were relatively higher followed by Zn in all the samples. Since most soil types of Ethiopia are moderately acidic to slightly basic with the pH ranges from 5.6 to 7.3 (Beyene, 1988), the plant is expected to have a better accumulation of micronutrients like iron and zinc (Kabata-Pendias, 2004). Furthermore, these elements are main elements that plant could accumulate in their tissue. Therefore, the detection of the high concentration of Zn from trace metals next to iron in **Samma** may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in the leaves of the plant (Kabata-Pendias and Pendias, 2001). Thus, **Samma** could be good alternative source for Zn and Iron.

Results revealed that there is significant difference in metals accumulation within the sampling sites for the three metals ($p< 0.05$). The presence of significant difference might be due to the fact that difference in the climatic condition of the regions and physicochemical property of the soil.

Comparing with other similar vegetables found in Ethiopia and elsewhere, the Fe and Zn content of **Samma** leaves are high. The respective amounts of iron and Zn in Lagose Spinach (*Celosia argentea*), Lenghui (*Urtica dioica*) and Spinach (*Amaranthus Virids*) were 28.3 and 0.2 mg/100gm, 8.9 and 0.15 mg/100gm and 8.8 and 0.25 mg/100gm respectively. The range of Fe

content of the tested sample is comparable with Bitter Letuce (*Launae cornuta*) (44.60 mg/100gm) and African spider flower (Gynandropsis gynandra) (47.01 mg/100gm) (Noor, 2008; Msuya and Mamiro, 2009). Thus green leafy wild **Samma** contains significantly high concentration of both macro and micronutrients. Hence **Samma**, if is included in the daily diet of a person, will have significant contribution to the RDA (Recommended Daily Allowance) of a person.

4.2 Antinutrient content of raw Samma leaves

Condensed Tannin

The antinutritional factors are the major factors limiting the wide use of many plants as they are present in the plants naturally and capable of eliciting deleterious effects in man and animals (Marshal et al., 1967; Liener 1980 and Thompson, 1993). The results for anti-nutritional factors for **Samma** leaves are presented in Table 4. The tannin content of raw **Samma** leaves collected from Debrebrehan, Fitche and Ambo areas were 25.3, 28.2 and 27.0 mg/100gm respectively in dry weight basis. These values were very low compared to other indigenous wild vegetables reported by Addis (2009). In fact this low concentration of Condensed Tannin is an advantage for not lowering the bioavailability of other nutrients. There is no significant difference ($P<0.05$) in Tannin content of the three areas.

Oxalates

With regard to the antinutritional content, the content of oxalate in raw Samma leaves collected from Debrebrehan, Fitche and Ambo areas were 9.33, 9.01 and 8.59 mg/100gm (Table 4).This value was higher than the oxalate content reported for Lagos Spinach (7.65 mg/100gm) and

Amaranth (5.92mg/100gm) but it is comparable with Spinach (8.72 mg/100gm) (Agbo *et al.*, 2009). Oxalic acid is regarded as compound that could reduce the different constituent in the diet especially it reduces the absorption of calcium by forming insoluble salts of calcium (Gupta et al., 2005).

Table 4: Antinutritional content of raw samma leaves

Sample area	Tannin (mg/100gm DW)	Oxalate (mg/100gm DW)
Debrebrhan	25.3±4.4[a]	9.33±6.1[a]
Fitche	28.2±8.1[a]	9.01±4.8[a]
Ambo	27.0±6.5[a]	8.59±8.0[a]

[a] Any two means in the same column not followed by the same letter are significantly difference ($P<0.05$).

4.3 Effect of Boiling on Nutrient Content of Samma Leaves

4.3.1 Proximate Compostion

In Ethiopia, **Samma** leaves are usually eaten as a pot herb. Hence, in this study we tried to determine the nutritional and antinutritional content of boiled **Samma** leaves by boiling at different duration (5min, 10min, 15min, 20min and 25min) at 96°C±2. The purpose was to select the best boiling time in maintaining the nutritional content of **Samma** as well as identifying the most acceptable treatment in terms of sensory attributes of the food product.

Moisture Content

The moisture content of boiled **Samma** leaves had increased after boiling compared to raw samples (Table 5). The increase in moisture content could be as a result of water absorption by the fibres and other natural chemical component of the vegetables. The increase in moisture content is in agreement with boiled spinach (*Spinacea oleracea*) (88.40/100g) (Food Composition Table for use in Ethiopia, 1997).

Protein

The protein content of **Samma** leaves increased after boiling (Table 5) and the results significantly different ($p< 0.05$) compared to raw samples. Muchuweti, (2009) also reported that cooking can increase the protein content. More levels of protein after boiling could be linked to the break down in tannin which is well known to form complexes with protein thereby, inhibiting protein availabilty (Bello *et al.*, 2008).

Ash

The ash content is a measure of the nutritionally important mineral contents present in the food material. In this case, the observed decrease in ash content after boiling implies that the potential ability of these leafy vegetables to supply essential mineral has been reduced. This is in accordance with the observation of Onyeike and Oguike (2003) on boiled groundnut (*Arachis hypogaea*) seeds. According to the authors, this may be due to water absorption during boiling leading to dilution, and hence, low amount of the ash. Nevertheless, the results of the ash contents obtained in this study after boiling and in the raw **Samma** leaves, are still considered high enough when compared with commonly consumed leafy vegetables.

Fat

During boiling, the fat content of **Samma** leaves was reduced and shows significant difference ($p< 0.05$) compared to the fresh samples (Table 5). Similar results were also reported by Muchuweti *et al.* (2009) for *Amaranthus hybridus* and *Telfairia Occidentalis*. According to Muchuweti *et al.* (2009), with boiling the fat must have melted into the boiling water thus causing a reduction in the fat content of *Amaranthus hybridus* and *Telfairia Occidentalis*. The fat content of boiled **Samma** was also found to be comparable with commonly consumed vegetables in Ethiopia such as Swiss chard (*Beta vulgaris*) and Kale (*Brassica carinata*) (0.5 g/100g) (Food Composition Table for use in Ethiopia, 1997).

Crude fibre

In the present study, boiling of **Samma** leaves at different time intervals resulted in the decrease of the crude fibre content and insignificant difference ($p>0.05$) compared to the fresh samples (Table 5). Similar finding was also reported by Bhardwaj *et al.* (2009). The decrease in fibre content during boiling could be attributed to the partial solubilisation of cellulose and hemicellulosic type of material by heat.

Table 5 Effect of boiling on the proximate Compostion of Samma Leaves

Sample code	Moisture (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total CHO (%)	Energy Kcal (%)
R	78.9 ±0.8[a]	25.4±0.6[a]	26.3±0.6[a]	2.3±0.02[a]	8.5±0.5[a]	31.8±0.75	252.2
001	86.80±0.1[b]	21.4±0.1[b]	30.95±0.4[b]	0.61±0.1[b]	8.54±0.3[a]	37.70±0.9	256.09
002	88.01±1.2[b]	20.02±0.2[b]	30.3±0.2[b]	0.59±0.2[b]	8.47±0.2[a]	41.42±0.68	269.79
003	86.98±0.2[b]	15.9±0.6[c]	30.68±0.5[b]	0.59±1.0[b]	8.34±0.5[a]	44.69±0.51	282.79
004	89.09±0.3[b]	16.4±0.3[c]	29.5±0.8[b]	0.59±0.8[b]	8.02±0.1[a]	44.69±1.5	282.76
005	88.50±0.0[b]	17.09±0.5[c]	29.34±0.5[b]	0.55±0.3[b]	7.89±0.3[a]	43.73±0.18	277.23
006	87.90±0.5[b]	17.5±0.8[c]	29.01±0.3[b]	0.55±0.5[b]	7.51±0.2[a]	43.63±0.15	275.47

All are boiled portion of young shoot of Samma leaf collected from Debereberhan

[a-c] :Values are means of triplicates (± SD). Means not sharing a common superscript letter in a column are significantly different at ($P< 0.05$) as assessed by Duncan's multiple range tests.

Key: R: Raw **Samma** leaf

001: Boiled Samma leaf for 5min;

002: Boiled Samma leaf for 10minutes;

003min: Boiled Samma leaf for 15min;

004: Boiled Samma leaf for 20min

005: Boiled Samma leaf for 25min

006: Boiled Samma leaf for 30min

;

4.3.2 Effect of boiling on Ascorbic Acid (Vitamin C) content of Samma Leaves

The effect of different boiling time on ascorbic acid content of **Samma** leaves collected from Debrebrhane is listed in Table 6. Before boiling Samma leaves had highest Ascorbic acid (vitamins C) content far above the values of *Amaranthus hybrid* and *Urtica dioica* as reported by Muchuweti *et al.* (2009) and Nordiede *et al.* (1996). A number of studies have been carried out to evaluate the effect of heat treatment on the fate of Ascorbic acid (Vitamin C). In this study, the Ascorbic acid (Vitamin C) content of **Samma** sample decreased during boiling. The leaves that are boiled for 5min exhibits considerable loss of Vitamin C followed by a 10 min boiled sample. When boiling time increases, total loss of Vitamin C was observed. This result was in agreement with Gottlieb *et al.*, (1984), which indicate boiling decreased the level of Ascorbic acid (Vitamin C) content of GVs. In general Ascorbic acid is highly unstable; thus a high level of the acid is lost during heat treatment (Fafunso, *et al.*, 1987). The reported losses of ascorbic acid during blanching or cooking are enormous and may vary between 40 and 70% in some cooked vegetables when processed at 100°C for 15 min (Bassir, and Umoh, 1976). Other researchers observed losses up to 66% 63%to 73% and 62 to 93% ascorbic acid in cooked vegetables (Oteng *et al.*, 1987).These values support the results obtained for this studies. The high solubility of ascorbic acid in water and the relative ease with which it is oxidized makes this vitamin particularly susceptible to processing conditions.

Table 6 Effect of Boiling on Ascorbic Acid (Vitamin C) Content of Samma Leaves

Sample code	Ascorbic acid (Vitamin C) content
R	84.3±1.2 mg/100gm
001	8.44± mg/100gm
002	1.74± mg/100gm
003	ND
004	ND
005	ND
006	ND

R: Raw **Samma** leaf

001: Boiled **Samma** leaf at 5minutes;

002: Boiled **Samma** leaf at 10minutes;

003: Boiled **Samma** leaf at 15minutes;

ND: Not detected

004: Boiled **Samma** samma leaf at 20min

005: Boiled **Samma** leaf at 25minutes;

006: Boiled **Samma** leaf at 30 minutes

4.3.3 Effect of boiling on Minerals content of Samma Leaves

The effect of different boiling times on the mineral content of the plant has been evaluated. As it is mentioned elsewhere, consumers usually decant the water after boiling **Samma** leaves. Thus, the same procedure was followed in this work. In general, there was a reduction in the mineral content of boiled **Samma** leaves compared to the raw ones (Table 7). The mineral content of the leaves shows decrement till 15 minutes. However, it exhibits a slight increment when cooking

time is prolonged. In general decreasing in metal content due to leaching or extraction of the metals from the leaves in to the water and gets disposed off during decantation (Sheela et al., 2004). The increase in metals after 15 min might be due to the fact that, as boiling time increases, water is evaporating while concentrating the metals on the leaf tissue. Hence more metals will remain in the leaves after the filtrate has been disposed off.

Table 7 Effect of Boiling on Mineral Content of Samma Leaves

Boiling time	Fe (mg/100gm(DW))	Zn (mg/100gm(DW))	Ca (mg/100gm(DW))
Raw	42.3±2.47[a]	4.90±0.73[a]	768.6±12[a]
5min	34.75 ± 2.3[b]	3.33±0.66[b]	492.45±88[b]
10min	31.62±2.5[c]	3.01±0.73[b]	455.37±117[c]
15min	29.2±2.9[c]	2.09±0.8[c]	392.37±79[d]
20min	30.71±3.1[c]	2.13±0.6[c]	398.08±99[d]
25min	31.07±2.5[c]	2.22±0.5[c]	405.48±120[d]
30min	31.82±2.3[c]	2.27±0.73[c]	411.82±109[d]

All are boiled portion of young shoot of Samma leaf collected from Debereberhan

[a-d] Any two means in the same column not followed by the same letter are significantly different (P< 0.05), R: Raw **Samma** leaf

4.3.4. Effect of Boiling on the Antinutritional Content of Samma Leaves

There was a stasticall difference between tannin and oxalic acid content of raw **Samma** leaves compared with boiled **Samma** leaves at different duration. The tannin content of **Samma** leaves showed a slight increment with an increase in boiling time (Table 8). The increment in tannin

might be due to the fact that increasing in boiling time may enhance the interaction or condensation of other phenolic compounds mainly flavonoids to form tannins. Though no conclusive report has been made, some reports indicated that higher temperature may facilitate polymerization of flavonoids to form tannins (Turkmen *et al.*, 2005). Increase in boiling time could enhance evaporation of water from the cooking material and results in pre-concentration of tannins compared to boiling for short period of time. In fact there is also tannin to be lost during decanting the water after cooking but this loss may be compensated by reformation of Tannins by the above mechanism.

Reports with regard to the effect of heat treatment on the tannin content of vegetables are conflicting. Amin and Lee (2005) reported that during cooking the phenolic content of asparagus increased by 23%. On the other hand, some researchers reported a decrease in the phenolic content of green vegetables during heat treatment. For instance, total phenolic content of selected vegetables (peas, carrot, spinach, cabbage, cauliflower, yellow turnip and white turnip) was found to be generally decreased by boiling, frying and microwave cooking. Numerous analyses also reported that convectional and steam cooking caused significant reduction in total phenolic content of red cabbage and decreasing cooking water and time by half led to better retention of the phenolic compounds. Onu *et al.*, (2001) was also reported that cooking *Solanum nigrum* and *Solanecio biafrae* for 15 minutes exhibits a significant reduction in tannin content.

There was also a dramatic loss of phenolic content on conventional and organic grown food as a result of thermal treatment (Turkmen, *et al.*, 2005). . Blanching up to 15 min was also found to cause loss of phenolic content, depending on the species of spinach (Amin *et al.*, 2006).

Unlike to the condensed tannins, oxalic acid content of boiled **Samma** leaves decreases with boiling time. This might be due to the fact that the soluble oxalates like sodium oxalate might be lost significantly with increment in billing time. The finding of the present study on the effects of boiling methods is in agreement with the report of Marshal *et al.* (1967).

Table 8 Effect of Boiling on the Antinutritional Content of Samma Leaves

Boiling Time (min)	Tannin (mg/100gm)	Oxalate (mg/100gm)
Raw	25.3 ± 4.4^{a}	9.33 ± 6.1^{a}
5 min	19.5 ± 0.4^{b}	4.985 ± 0.05^{b}
10 min	16.8 ± 0.1^{c}	3.991 ± 0.02^{b}
15min	9.0 ± 0.5^{d}	2.031 ± 0.01^{c}
20min	9.51 ± 0.2^{d}	1.986 ± 0.03^{c}
25min	10.2 ± 0.3^{d}	$1.824\pm.0.04^{c}$
30min	11.5 ± 0.1^{d}	1.771 ± 0.05^{c}

$^{a-d}$ Any two means in the same column not followed by the same letter are significantly difference ($P< 0.05$).

: Raw and boiled portion are young shoot of Samma leaf collected from Debereberhan

R: Raw **Samma** leaf

4.3.5 Sensory Analysis of Boiled Samma Leaves

Appearance, colour, taste, texture, and over all acceptability of boiled **Samma** leaves were analysed using a nine point hedonic scale and the results are indicated in Table 9.

Table 9 Effect of boiling on the Sensory quality of Samma leaves grown in Ethiopia

Boiling time	Appearance	Color	Texture	Taste	Overall acceptability
5min	8.1[a]	8.9[a]	8.8[a]	6.04[a]	5.8[a]
10min	7.9[a]	8.5[a]	8.5[a]	8.9[b]	8.9[b]
15min	7.7[a]	7.7[b]	8.2[a]	7.7[b]	8.0[b]
20min	7.7[a]	7.4[b]	7.5[b]	7.5[b]	7.7[b]
25min	7.3[a]	7.1[b]	7.3[b]	7.4[b]	7.5[b]
30min	7.0[a]	6.9[b]	6.8[b]	7.1[b]	7.3[b]

□,[b] any two means in the same column not followed by the same letter are Significantly difference (P>0.05).

Appearance

The visual appearance of any food is the first of organoleptic sense that a consumer experiences. Therefore, if the food has had any changes in colour and appearance, the consumer is more likely to reject the product. In the study, the condition was boiling **Samma** leaves at different duration; hence any changes occurring on the leaves is be due to the increment of boiling time. As it can

be seen from table 9 appearance has been observed to be none significantly affected ($p > 0.05$) by the increment of boiling time of **Samma** leaves.

Colour

Colour is an important parameter in judging the quality and/ or acceptability of a food sample besides reflecting raw material used for preparation. The preference of boiled Samma leaves is also highly dependent on its colour. As it can be seen from table 9 Colour of **Samma** leaves boiled for 15, 20, 25 and 30min has been observed to be significantly affected. A decrease in colour of the **Samma** leaves was observed with an increase in the amount of boiling time. **Samma** leaf boiled at 5min and 10 min were found superior scoring 8.9 and 8.5, respectively than the rest of the boiled sample. These result significantly different from the rest of the boiled samples and brings a difference on the acceptability with respect to colour.

Taste

Taste is the main criterion that makes the product to be liked or dislike. It is evident from the result (table 9) that taste of **Samma** leaves boiled for 5min were significantly different ($p > 0.05$) and achieves less acceptability than the rest of boiled samples which do not have significant difference with one another.

Texture

Surface texture is also another important sensory attribute that could properly explain the property of a food sample. As it can be seen from table, mean texture scores are significantly affected ($p > 0.05$) with the increment in boiling time 20, 25 and 30min. **Samma** leaf boiled at 5

min and 10 min and 15 min were found to be superior scoring 8.8, 8.5 and 8.2 respectively than the rest of the boiled sample.

Over all Acceptability

Over all acceptability was not significantly affected by boiling **Samma** leaves at different time except boiled **Samma** leaves treated for 5 min. However, more than two thirds of the panellists responses revealed that boiled **Samma** leaves at 5 min and 10 min were found to be superior in appearance, taste, colour, texture and no significant difference ($P>0.05$) in the sensory perception except taste of **Samma** leaves treated at 5 min (table 9). Thus, in general appearance, colour, tastes and texture of boiled **Samma** leaves for 10 min achieve better acceptability.

5. Conclusion and Recommendation

5.1 Conclusion

In our study, it was found that **Samma** has high nutritional value compared to many green leafy vegetables commonly cultivated and consumed in Ethiopia. Its protein and mineral content is exceptionally high which makes this vegetable as an inexpensive but high quality nutrition source especially for the poor segment of the population where malnutrition is prevalent. However, in this study it was also observed that the traditional processing of **Samma** which involves boiling for long period of time can be detrimental as it may lead to the loss of heat labile vitamins and other nutrients. In general it can be said that, if processed appropriately, **Samma** could be valuable particularly in areas with a low rainfall, as it will produce a viable yield under these conditions, whereas most of the exotic leafy vegetables require large amounts of water for successful production.

5.2 Recommendation

The following recommendations are made based on a holistic view of the subject area:

- Appropriate methods should be sought for the processing of **Samma** to retain its nutrient components at maximum level.
- Although **Samma** has high nutritional value, it is a neglected plant in Ethiopia. Promotion of the vegetable is important among people as it can contribute substantially to improving their diet and thus combat protein and micronutrient malnutrition. In other words, the plant should be adapted to the food system of the country.

- New products can be developed by using the plant as a protein and mineral supplement either in locally consumed food items in Ethiopia or other food items.
- Cultivation of the vegetable should be encouraged.
- Further studies are required to evaluate the presence of toxicants (toxic compounds) in the plant.
- The health benefit (medicinal value) of this plant should be also studied.

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A score test for acceptance (HEDONIC) test.

Please evaluate the samples you have provided and indicate how much you like or dislike for acceptance appearance, color, taste, texture and over all acceptability by a right score. Rinse your mouth with water after evaluate sample and before you start the next one.

Panellist code..... Date.....

Sample code.....

gree of liking or disliking	Sensory characteristics attributes				
	Appearance	Color	Taste	Texture	Over all acceptability
ike extremely					
ike very much					
like moderately					
ike slightly					
nether like nor dislike					
dislike slightly					
dislike moderately					
lislike very much					
lislike extremely					

DECLARATION

I, the under signed, declare that this thesis is my original work and that all the sources of materials used for the thesis have been correctly acknowledged.

Name: Eskedar Getachew _____

The thesis has been submitted with my approval as a supervisor.

Dr.Gulelat Desse _____

Dr.Getachew Addis _____

Place and date of submission: School of Graduate Studies, Addis Ababa University, Addis Ababa, Ethiopia,