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CENTER FOR FOOD SCIENCE AND NUTRITION

Nutritional and Phytochemical Evaluation of Anchote *(Coccinia abyssinica)* (Lam.) (Cogn.) Accessions to Promote its Contribution for Food Security and Medicinal Use

A PhD Dissertation in Food Science and Nutrition

By

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Abstract

Nutritional and Phytochemical Evaluation of Anchote *(Coccinia abyssinica)* (Lam.) (Cogn.) Accessions to Promote its Contribution for Food Security and Medicinal Use

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Anchote (Coccinia abyssinica (Lam.) (Cogn.)) is one of the important endemic crops principally grown for its edible tuber throughout the south and southwestern parts of Ethiopia. Moreover, its newly growing leaves along with tendrils are served as vegetable after cooking; making Anchote a double crop. Being one of the underutilized vegetables, there are few research efforts made to comprehensively characterize Anchote germplasm in respect to its nutritional composition, anti-nutritional factors, functional properties, phytochemical composition (qualitative and quantitative) as well as volatile organic compounds. The main objective of this study was, thus, to assess the nutritional profile and phytochemical properties of the edible parts of 44 Anchote accessions.

Significant variability was observed in nutrient composition and anti-nutrient content among the tested accessions and plant parts. Leaves were found to be rich in crude protein content (8.96±0.01% - 35.42±0.05%) compared to tubers (5.82±0.00% - 13.72±0.10 %)of 100 g dry matter. In contrast, tubers were found to be superior in utilizable carbohydrates (73.89±0.22% - 84.51±0.43%) of 100 g dry matter, and gross energy (349.14±0.10 - 368.48±0.24) of Kcal/100g dry matter. Other proximate values documented include crude fat (0.24±0.05 - 0.75± 0.07% and 2.44±0.27- 4.68±0.84%); crude fiber (3.63±0.04-6.96±0.24% and 7.89±0.03 -13.05±0.08%) as well as total ash (4.63±0.31 - 6.83±0.02% and 10.74±0.04 - 13.59±0.02%); in tubers and leaves of Anchote, respectively. Total amino acid content of accessions with high protein content ranged from 45.12 to 62.89 g/100g protein for tubers and 67.31 to 75.69 g/100g protein for leaves.

Variations in mineral contents namely Sodium (Na), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Cooper (Cu), Zinc (Zn), Manganese (Mn) and Boron (B)were also recorded among accessions and plant parts. The Ca content of tuber ranged from 80.64–372.16 mg/ 100g and for leaf, it ranged from 64.10 –226.95mg/ 100g. The Fe content ranged from 0.39–2.92 mg/100g for tuber and 1.58 – 18.65mg/100g for leaf while Zn content of tuber ranged from 0.22–0.53mg/100g and 0.32 – 3.41mg/100g for leaf. The mean antinutritional contents of tuber samples were: phytate (131.10mg/100g), tannin (112.02mg/100g) and cyanide (13.08mg/kg). For leaves, the contents were phytate (250.30 mg/100g), tannin (216.53 mg/100g) and cyanide (12.36 mg/kg). The levels of antinutrients in leaves were higher than in tubers. On the other hand, the levels of potentially toxic elements such as Cd, As, and Pb were almost negligible, with mean concentration values of 0.86, 0.83 and 7.05 ng/g, and 1.29, 2.62 and 13.53 ng/g in tubers and leaves, respectively. The mean molar ratios for phytate: calcium, phytate: iron, phytate: zinc and phytate x calcium: zinc was 0.05 and 0.11, 3.81 and 4.31, 27.79 and 22.47 and 142.20 and 90.72 in tubers and leaves, respectively.

Chemical composition and functional properties of leaf protein concentrate (LPC), tuber and leaf powder of Anchote were also analyzed. Heat coagulation at natural pH was used to obtain the LPC from the aqueous fresh leaf extract. The mean crude protein content for LPC was 47.46 g/100gand its mean total amino acid content was 99.64 g/100g protein. Lowest protein solubility of Anchote LPC (11%) obtained in pH ranges of between 6 and 10 and highest solubility (19%) recorded at pH 12. The result for in-vitro protein digestibility was 57.44±1.48 % for tuber powder, 49.46±1.68% for LPC and 40.92±0.54 % for leaf powder. Leaf powder revealed highest water (2.94 g/g) and oil (1.29 g/g) absorption capacities (WAC & OAC), and lowest value of WAC (1.61 g/g) was observed in LPC. Emulsification reduced with increase in protein concentration and increased with increase in pH in all tested samples. The foaming capacity was highest in leaf powder followed by LPC.

Anchote accessions were also tested for presence of some phytochemicals using qualitative and quantitative methods. Qualitative test was done for 12 phytochemicals using seven extraction solvents.

Secondary metabolites including total phenols, total flavonoids, crude saponins and beta-carotene were analyzed quantitatively. Positive results were observed during qualitative screening for five phytochemical compounds tested in tubers whereas only two tests were positive for leaves in all the seven solvent extracts. Water extract showed positive results for 11 phytochemicals while n-butanol extract showed positive results for six tests for both tuber and leaf samples. The water extract of Anchote showed highest number of phytochemicals in both tuber and leaf parts when compared to other solvent extracts. Anchote leaf had higher total phenol and flavonoid contents followed by fruit and the least concentration of these compounds occurred in tuber for all the tested accessions. Leaf of Anchote contained the highest percentage of saponins (27.65%) compared to other parts. The β-carotene content of Anchote leaf ranged from 25.9±0.03 to 35.2±0.16 in μg/g.

Anchote leaf and tuber powder samples were extracted by simultaneous steam distillation and solvent extraction (SDE) to determine volatile organic compounds. The extracts were characterized by gas chromatography-mass spectrometry (GC/MS). Thirty volatile compounds from leaves and 15 compounds from tubers were identified with a yield of 770.57 mg/kg and 4536.91 mg/kg, respectively.

In conclusion, the study showed that both the tuber and leaf parts of Anchote have appreciable amount of different essential nutrients. Leaves have relatively higher nutrient composition in all accessions compared to tubers, which provides a good scientific evidence to diversify the consumption habit of indigenous people who are growing Anchote mainly for its tuber, the principal edible part of the crop. The different functional properties of Anchote LPC also suggest its potential to be used as an ingredient in processed foods. Anchote is also rich in different phytochemicals and volatile organic compounds that make the plant a potential crop to be used in pharmaceuticals and food industries.

Dedication

I dedicate this work to my beloved father **Ato Ayalew Goshu Eyasu**, gone but never forgotten. I wish to have him by my side and see my accomplishments as he always encouraged me and gave everything to proceed on my academic career. I miss you my dear Dad.

May God bless his soul!

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List of Abbreviations/Acronyms

AAAs	Aromatic amino acids
AOAC	Association of Official Agricultural Chemists
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
BSA	Bovine Serum Albumin
CSA	Central Statistics Agency
Cys	Cysteine
DC	Digestibility Coefficient
DM	Dry matter
DW	Dry weight
DWB	Dry weight basis
DZARC	Debre Zeit Agricultural Research Center
EA	Emulsifying activity
EAAs	Essential amino acids
EAAI	Essential amino acid index
EHNRI	Ethiopian Health and Nutrition Research Institute
EPHI	Ethiopian Public Health Institute
ES	Emulsion stability
FAO	United Nations Food and Agriculture Organization
FC	Foaming capacity
FS	Foaming stability
FW	Fresh weight
FWB	Fresh weight basis
GC	Gas chromatography
Glu	Glutamic acid
Gly	Glycine
HCN	Hydrogen cyanide
His	Histidine
HPLC	High performance liquid chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IEP	Isoelectric point
IFAD	International Organization of Food and Agriculture Development
Ile	Isoleucine
IVPD	In vitro protein digestibility
Leu	Leucine
LPC	Leaf Protein Concentrate
Lys	Lysine
m.a.s. l	Meter above sea level
Met	Methionine
NEAAs	Non-essential amino acids
NPU	Net protein utilization

OAC	Oil absorption capacity
P-BV	Predicted biological value
P-PER	Predicted protein efficiency ratio
Phe	Phenylalanine
Pro	Proline
ppm	Parts per million
RTCs	Root and Tuber Crops
SAAs	Sulfur containing amino acids
Ser	Serine
SNNPR	Southern Nations and Nationalities Peoples Region
TAAs	Total amino acids
TEAAs	Total essential amino acids
Thr	Threonine
TNEAA	Total non-essential amino acids
TP	Total Phenol
Trp	Tryptophan
Tyr	Tyrosine
µL	Micro litter
Val	Valine
VOCs	Volatile organic compounds
WAC	Water absorption capacity
WFP	World Food Program

Chapter One: Introduction

Several agricultural crops known to be originated from Ethiopia. These include coffee (*Coffea arabica*), tef (*Eragrostis tef*), safflower (*Carthamus tinctorius*), noug (*Guizotia abyssinica*), anchote (*Coccinia abyssinica* (Lam.) Cogn.) and enset (*Ensete ventricosum*) (Winters et al. 2006; FAO 2007). It is also stated that Ethiopia is well known for its diversity of indigenous food plants, of which 27% are cultivated vegetables by traditional farmers in home gardens, and about 29% are non-cultivated vegetable species (Asfaw, 1997). However, chronic and acute food insecurity is still persisting as being prevalent in many parts of Ethiopia (GFDRE 2009). Chronic food insecurity in Ethiopia is estimated to be about 10% and this figure rises to more than 15% during frequent drought years causing acute food insecurity (Birara et al., 2015). Consequently, malnutrition in Ethiopia continues to increase, affecting primarily women and children (Salama et al. 2001; Melese 2013). The prevalence rates of stunted growth and wasting, vitamin A, iodine, iron and zinc deficiencies derived from national and localised surveys shows the magnitude of malnutrition in Ethiopia (Kaluski, Ophir, & Amede, 2002).

In Ethiopia the most common forms of malnutrition is protein-energy malnutrition (PEM), vitamin A deficiency, iodine deficiency disorders, and iron deficiency anemia (Beruk, Kebede, & Esayas, 2015). The high prevalence of malnutrition and persistent food insecurity in Ethiopia is due to the highly selective and restricted food consumption habit of the population as well as less exposure to the important wild and indigenous food plants (Getachew, 2001). As stated by Dandena (2010), malnutrition in Ethiopia still exists due to poor dietary habit regardless of the favorable highly diversified agro-ecological conditions of the country, which are suitable for the production of various types of fruit and vegetables.

Root and tuber crops are consumed by one third of the world population next to cereals as global source of carbohydrates (Chandrasekara & Kumar, 2016). They provide a substantial part of the world's food supply and are also an important source of animal feed and processed products for human consumption and industrial use (Chandrasekara & Kumar, 2016).

In many developing countries, food insecure people and subsistence farmers depend highly on root and tuber crops as food source and possibly principal food source of nutrition, and cash income (Scott, Rosegrant, & Ringler, 2000). For millions of people in the tropical humid regions of Africa root and tuber crops occupy a position of prestige among the staple foods (Lebot, 2009). Root and tuber crops provide an estimated average of 20% of the daily per capita calorie intake for the 640 million inhabitants of Sub-Saharan Africa, where with the growing population there is increasing demand for these crops both for food and for feed (Kenyon, Anandajayasekeram, Ochieng, Ave, & Uk, 2006). Hence, an improved understanding of the production, utilization, and estimated future economic importance of these crops has potentially far-reaching implication in research and development areas at both the international and, national levels.

In Ethiopia, root and tuber crops have a great contribution as part of the traditional food system and income generation specifically in Southern, South Western and Western part of the country (Andargachew, Admasu, Girma, Bjørnstad, & Appelgren, 2011; Fantaw, Nebiyu, & Mulualem, 2014). These crops are mainly used as food security plants during food shortage since they are drought-tolerant and high yielding (Wheatly, Scoot, & Wiersema, 1995). Several million people rely on root and tuber crops such as enset (*Ensete ventricosum)* as a staple or co-staple food. For instance, as many as 7 to 10 million people consume enset (Alemayehu, Dorosh, & Sinafikeh, 2011; Eastern & Terefe, 1980; Pijls, Timmer, Wolde-Gebriel, & West, 1995). Furthermore, other minor root and tuber crops such as Anchote (*Coccinia abyssinica*), Taro (*Colocasia esculenta*), Yam (*Dioscorea alata*), Cassava (*Manihot esculenta*) and Welayta dinich (*Plectranthus edulis*) are also important in some areas, mainly in South and South Western Ethiopia (Eastern & Terefe 1980; Abdissa 2000; FAO 2007). Although these crops are drought resistant and food security crops in drought prone areas, their potential as a human food and medicine has not yet been fully recognized and utilized (Gebremedhin, Endale, & Lemaga, 2008).

Anchote [*Coccinea abyssinica* (Lam.) Cogn.] is an endemic root and tuber crop of Ethiopia cultivated for human consumption (Abera, 1995; Beruk & Fikre, 2015; Habtamu, 2014; Tilahun, Sentayehu, Amsalu, & Weyessa, 2014).

It is grown widely in the Western and South-Western parts of the country. It belongs to *Cucurbitaceae* family, one of the major families from the plant kingdom encompassing about 115 genera and 960 species (Schaefer, et al., 2009). Anchote is a vine like cucurbit with a high yield and short crop cycle used as an important dietary & medicinal plant (Girma & Hailu 2007; Yassin et al. 2013). The crop is known for its tuberous root and tender leaves which are used for food (Abera, 1995).

Anchote has a significant contribution for the cultural and social values of the Oromo's (the largest tribe in Ethiopia) since long ago (Abera 1995; Desta 2011; Daba et al. 2012). The plant has been grown over a wide range of environments for a long time, and its cultivation and utilization have been passed from generation to generation through oral tradition, with very little recorded information (Abera, 1995; Girma & Hailu, 2007). The unique characteristics of the plant is the edibility of its different parts such as its tuber, leaf, and fruit which makes the plant ideal as potential food security crop (Amare 1973; Endashaw 2007; Desta 2011).

According to Daba et al. (2012) the root yield of different Anchote accessions ranges from 42-76 t ha^{-1}. There are a number of studies on genotypes (Abreham, Tileye, & Kassahun, 2014; Tilahun et al., 2014) and agronomic characteristics (Girma & Hailu 2007; Daba et al. 2012; Folla et al. 2013; Yambo & Feyissa 2013;Yassin et al. 2013) of Anchote but limited studies on its nutritional value and bioactive functionalities. The few existing studies indicate its potential for human nutrition and medicine (Desta, 2011; Habtamu, Fekadu, & Gullelat, 2013; Habtamu, 2014; Habtamu & Kelbessa, 1997). For instance, the protein content of Anchote tuber ranges from 4.6-16.4% (Desta, 2011) which is high and with wide range compared to other root and tuber crops commonly consumed in Ethiopia, with protein values ranging from 1-2% (Gebremedhin et al., 2008).

Studies indicate that Anchote has relatively higher crude protein, utilizable carbohydrate, crude fiber, energy, and ash content compared to sweet potato, potato and cassava (Habtamu et al. 2013). The calcium content of Anchote is also reportedly high (Desta, 2011; Habtamu et al., 2013).

Traditionally, Anchote is consumed to heal broken or fractured bones as well as to strengthen sick people, which could be attributed to its high its high calcium content (Abera, 1995; Habtamu & Kelbessa, 1997).

There are more than 100 accessions of Anchote in Ethiopia. However, the nutritional value and functionalities of these different accessions have not been documented. Therefore, the main objective of this study was to assess the nutritional and antinutritional profile, phytochemical properties as well as volatile compounds of the edible parts of different Anchote accessions and there-by select accessions with superior qualities that can best contribute to nutrition security and medicine.

Specifically, this study addressed the following specific objectives:

i) Determination of proximate composition and anti-nutritional factors of Anchote accessions.
ii) Determination of the macro and micro elements in of Anchote accessions
iii) Determination of functional properties and quality of protein in leaves and tubers of Anchote accessions
iv) Identification of phytochemicals in leaves and tubers of Anchote accessions
v) Identification of volatile organic compounds in leaves and tubers of Anchote accessions

This PhD thesis is organized as follows:

Chapter One: presents the introduction, statement of the problem, justification and objectives of the study.

Chapter Two: reviews on the important literatures dealing on the general characteristics of Anchote such as morphology and taxonomy followed by an overview of Anchote diversity in Ethiopia and its historical production aspects. Socio cultural importance of Anchote as food and medicinal value, its benefit for rural women, as well as the agronomic and storage practices are discussed. The importance and nutritional content, mineral element, and antinutritional factors of commonly used tubers and leafy vegetables and their contribution in plant foods, are reviewed. Leaf protein concentrate and their use in human food, as well as protein functionality and their properties in food system are also addressed. Finally, a brief description on the importance of phytochemicals, volatile organic compounds and analytical procedure for volatile compounds are presented.

Chapter Three: presents the findings of the 'Nutritional and anti-nutritional compositions of tuber and leaf of Anchote accessions from Ethiopia.'

Chapter Four: addresses the results about 'Essential and toxic metals content of Anchote tuber and leaf with estimated bioavailability of calcium, iron, and zinc.'

Chapter Five: provides findings from the 'Assessment of functional properties and protein quality of Anchote leaf protein concentrate, tuber and leaf powder.'

Chapter Six: is on the Qualitative and quantitative identification of important/major phytochemicals in different parts of Anchote.'

Chapter Seven: gives information on the 'Analysis of volatile organic compounds of Anchote.'

Chapter Eight: presents the summarized conclusion and recommendation for the study.

Chapter Two: Literature Reviews

2.1. General Characteristics of Anchote (*Coccinia abyssinica*)

2.1.1. Taxonomy of Anchote

In the major group of Angiosperms (Flowering plants), genus *Coccinia* is among the 115 genera belonging to the *Cucurbitaceae* family, one of the most economically important families of plants (Holstein & Renner, 2011; Schaefer et al., 2009). The species of *Coccinia* are about 27 in number, and most species are widespread mainly in Sub-Saharan Africa, with centers of diversity in East Africa and Southern Africa, with the exception of *C. grandis*, which is spread across in the highlands of the Arabian Peninsula, tropical Asia, Pacific Islands, and the Neotropics (Holstein & Renner, 2011). From the 27 species, *C. abyssinica* (Lam.) Cogn.; *C. megarrhiza* C. *Jeffrey*; *C. tracephylla* Gilg; *C. ogadensis* Thulin; and *C. schliebenii* Harms are reported to be geographically originated in Ethiopia, while all species in this genus are categorized under dioecious plants (Holstein & Renner, 2011). *Coccinia abyssinica* is the only species cultivated for its edible tuberous roots and young shoots which are used as leafy vegetables (Desta 2011; Habtamu 2011). Table 1 shows the scientific classification of Anchote and commonly used vernacular names.

Table 1 Scientific classification and commonly used vernacular names of Anchote

Vernacular names		Scientific classification	
		kingdom	Plantae-Plants
English	Anchote	Sub kingdom	*Tracheobionta*-Vascular plants
Amharic	-	Super division	*Spermatophyta*-Seed plants
Oromifa	Ancootee	Division	*Magnoliophyta*-Flowering plants
Tigrinya	Wushish	Sub division	Angiospermae
Welayita	Ushushe	Class	Magnoliopsida-Dicotyledons
Dawuro	Shushe/Ushushe	Subclass	*Dilleniidae*
Kafigna	Ajjo	Order	Cucurbitales
		Family	*Cucurbitaceae*- Cucumber family
		Sub family	Cucurbitoideae
		Tribe	Benincaseae
		Subtribe	Benincasinae
		Genus	*Coccinia*
		Species	*abyssinica*

Source: Abera (1995)

2.1.2. Morphology of Anchote

Members of the *Cucurbitaceae* family are herbaceous annuals or perennials with a storage root and mostly moist vines. They grow either prostrate along the ground or climb using tendrils. Their tendrils can grow branched or simple and generated at the petiole base. There are usually four arched filaments coiling with an adhesive texture. Leaves can range from simple to palmately compound (Austin & Brendan, 2008).

Anchote (*Coccinia abyssinica*), has shoots with simple tendrils, and leaves of which are palmately simple with five lobes, while the shape varies from the heart to pentagon form. Its flowers are unisexual having pistillate flowers at the nodes and staminate flowers in racemes. Fruits are red yellow at maturity, and have an oval to cylindrical shape with 8.83 cm length containing an average of 153 seeds. The stems are typically sympodial in growth (Amare, 1973).

Anchote has spherical to cone-shaped tubers which may vary with age, soil physical conditions and Anchote type. Sometimes irregular shaped tubers may result because of poor land preparation and the presence of mechanical barriers, which will result in the development of uncommon shaped tuber. The top portion of the tuber has the largest diameter with a rounded square in transverse section (Abera, 1995). In general, the plant has a runner growth habit with a trailing vine, which needs support for successful fruit development that provide sound seeds for future planting.

2.2. Ethiopian Anchote

Ethiopia is the center of origin and diversity for Anchote (*Coccinia abysinica)* (Lam.) (Cogn.) where it has been cultivated by farmers for centuries specifically in south and southwestern parts of Ethiopia (Abera, 1995; Amare, 1973). Anchote is found both as cultivated and in wild form with sporadic distribution (Endashaw, 2007; FAO, 2007; Girma & Hailu, 2007). The plant is adapted to grow in an altitudinal ranging from 1300 to 2800 meter above sea level with an estimated annual precipitation range of 762- 1016 mm (Amare, 1973). Anchote production includes method of propagation, husbandry, harvesting and postharvest handling.

It grows principally for its tuberous root named '*Ancoote*' in Oromiffa. Its leaf is edible as a cooked green vegetable, which making it a multipurpose crop. Oromo people, mainly women produce Anchote in their backyard. Despite this, adequate attention has not been given to the plant as food crop for a long period of time (Daba et al., 2012; Girma & Hailu, 2007; Yambo & Feyissa, 2013). Hence, Anchote still remains as one of the most underutilized root and tuber crops in Ethiopia.

2.2.1. Socio-Cultural Importance of Anchote as Food and Medicine

Anchote is a traditional diet in Oromo culture around Wollega province where there is also cultivation of other root crops such as Oromo potatoes, Irish potatoes, enset, sweet potatoes, yam and taro (Abera, 1995; Bula, 2008). Among all root and tubers grown in the area, Anchote holds a very special place in the customs of the Oromo people.

Anchote is served as a prestige dish in Oromo Culture during special ceremonies and on holidays (Habtamu & Kelbessa, 1997). A delicious Anchote dish is prepared with clarified butter (Traditional ghee) from the tuber (Fichtl & Admasu, 1994). It is also prepared in the form of stew locally called Anchote '*Ittoo*' on joyful events (Diriba, Mekonnen, Ashenafi, & Adugna, 2014; Habtamu & Kelbessa, 1997). Solely the '*Ittoo*' is prepared from sliced Anchote with sufficient locally made butter, butter milk and cheese by seasoning with different traditional spices and then served with '*Injera*', a round leavened bread made from tef (*Eragrostis tef*) (Zucc) Trotter which is called '*Chumbo*' in Afan Oromo (Figure 1).

Figure 1 '*Ittoo*' served with tef '*Injera*' or '*Chumbo*'

A special dish prepared from Anchote tuber is used to heal people suffering from broken or fractured bones, and also said to make lactating mothers healthier and stronger which could be due to its high content of calcium and protein (Abera, 1995; Endashaw, 2007; Habtamu & Kelbessa, 1997). The juice of Anchote tuber is said to have therapeutic effect in treatment of gonorrhea, tuberculosis and tumor cancer, among local community in the western part of Ethiopia (Dawit & Estifanos, 1991).

The tender young leaves and the new growing bud of Anchote are plucked and cooked as a leafy vegetable served with other foods in some areas of Western Oromia such as Dmbi Dollo (Abera, 1995). The leaves of Anchote that are used as a vegetable should be in a tender active growth stage but there after when the leaves become mature they are less demanded since it lost deliciousness (Desta, 2011). Therefore, commonly the succulent and tender leaves of Anchote are commonly harvested for cooking (Figure 2).

Figure 2 Plucked tender leaves of Anchote for consumption

Anchote is also a good income-generating commodity for the household in addition to food and medicinal use, and for women in particular. Women play a great role from production to food processing of Anchote (Nuri, 1993).

Women in rural households save few of the tubers at the time of harvesting and use them as planting material to establish mother plants called '*Guboo*' in Oromiffa. The mother plant will then serve as a seed source for further plantings. The stems or vines of Anchote can also be used as planting materials (Abera, 1995).

2.2.2. Anchote as Food Security Crop

In Ethiopia, Anchote is cultivated over a wide area in Western, Southeastern (Western Sidamo, Gamu Gofa, and Shewa), Central and Eastern Ethiopia (Shewa and Hararge) and Gojjam (Abera, 1995; Amare, 1973). Anchote is however cultivated mainly in Western part of Ethiopia in Oromia Region for long time and has diversified uses as a traditional and medicinal plant (Habtamu & Kelbessa, 1997). In Western Oromia Zone, Anchote is one of the major root and tuber crops cultivated on nearly 300 ha of the land yielding on average 10-15 t ha^{-1} and produced for food, cultural, social and economic purposes for the communities (Abdissa, 2000; Guma, Jane, Justus, & Kariuki, 2015). Farmers usually plant Anchote on a piece of land not more than 400-600 square meter in the backyard mainly for home consumption (Amare, 1973).

The tuber of Anchote is be ready for use within four to five months from planting depending on the environment and the accession type (Abera, 1995; Desta, 2011). According to Desta (2011), the most economical part of Anchote that is the tuberous root with diversified potentials for food, animal feed, medicinal and starch production, that will contribute towards food security, income generation, and resource base conservation. The plant produces prolific vegetative growth within two months from planting time, when, the new leaves and top growing points can be harvested and cooked (Abera, 1995).

Women also have a big role in preserving the seeds in good condition without deterioration of the quality of seeds. In Oromo society, women store the seed of Anchote in either clay or wooden pots. This will have an advantage for maintaining the shelf life of the seed as per desired level. Usually, women have an exclusive role in production and postharvest handling of the crop during harvesting, seed extraction, storage, and making it available for sowing in the next growing season (Abera, 1995).

According to the existing tradition, the suitable production area of Anchote is believed to be the home garden which strategically reduces the burden for women who are actively engaged in the cultivation of Anchote (Girma & Hailu, 2007). In general, the benefits that women in rural community get from Anchote are not only in monetary terms but also in entrepreneurial terms in decision-making and management (Abera, 1995).

2.3. Nutritional Content of Commonly Consumed Root and Tuber Crops in Ethiopia

Root and tuber crops mainly provide energy in the human diet in the form of carbohydrates and calories yielded per hectare per day than other crops (Ugwu, 2009; Wheatly et al., 1995). In most less developed countries of the tropics and sub tropics, root and tuber crops are the main staple foods (Chawanje, 1998).

Treche (1996) also indicated that most root and tuber crops have the potential to provide more dietary energy per hectare than cereals when considered on a dry weight basis. Root and tuber crops are produced with very low inputs and consumed by the poorest section of the population. Moreover, they have significant contribution to household food security and can be used as feed for animals and as raw material for processing industries. Therefore, promotion of their production and utilization for multitude of purposes is vital and yet often neglected.

Nutritional composition of any crop varies depending on the variability of climate, soil, crop variety, and other factors (Diop & Calverley, 1998). In addition, the variation of the nutritive value of root and tuber crops depend mainly on physicochemical properties of starch and protein as well as the forms and extents of anti-nutritional activities and toxic substances thereof. In most tuber crops, as compared to other crops such as cereals, protein content is low and the dietary energy is provided mainly from carbohydrates supplied by roots and tubers. However, their protein contribution is often significant considering the number of servings and quantity of crops consumed in a day, especially in developing countries like that of Ethiopia. In addition, some tuber crops contain a considerable number of vitamins and minerals.

For instance, cassava, sweet potato, potato and yam contain some vitamin C, while the yellow varieties of sweet potato, yam and cassava contain beta-carotene or pro-vitamin A, and taro is a good source of potassium. Root and tuber crops are deficient in most other vitamins and minerals but contain significant amounts of dietary fiber (FAO, 1990). Table 2 shows the nutritional composition of the commonly consumed tuber crops including cassava.

Table 2 Nutritional composition (%) of some commonly consumed root & tuber crops

Crops	Moisture	Dry matter	Total ash	Crude protein	Crude fat	Crude fiber	Total carb	Energy (Kcal/100g)
Solanum tuberosum[a]	78.90	21.90	1.45	4.74	0.71	0.77	14.20	NA
Ipomoea batatas[a]	70.57	29.43	0.97	3.13	0.79	0.90	24.54	NA
Manihot esculenta[a]	68.08	31.92	0.85	2.84	0.18	1.38	28.05	NA
Dioscorea rotundata[a]	67.13	32.87	0.78	4.45	0.15	0.67	27.49	NA
Dioscorea alata[a]	77.40	22.60	1.05	2.51	0.14	0.42	18.90	NA
Xanthosoma sagittifolium[a]	82.27	17.73	1.03	5.47	0.20	1.28	11.03	NA
Colocasia esculenta[b]	66.62	NA	4.09	6.40	0.78	1.83	86.58	378.93
Dioscorea bulbifera[c]	91.90	NA	NA	2.10	2.10	6.04	79.03	304.7
Coccinia abyssinica[d]	74.93	NA	2.19	3.25	0.19	1.73	19.44	82.12

Source: a (Odebunmi, Oluwaniyi, Sanda, & Kolade, 2007); b (Lewu, Adebola, & Afolayan, 2010); c (Vishwakarma & Dubey, 2011); d (Habtamu et al., 2013); NA: data not available

2.4. Nutritional Content of Leaves of Root Tuber and Other Green Leafy Vegetables

Fresh vegetables are important sources of nourishment and vital ingredients in healthy balanced diets. Green leafy vegetables have also bioactive phytochemicals, which perhaps protect against cardiovascular and other degenerative diseases as well as slows the aging process with powerful antioxidants (Caunii, Cuciureanu, Zakar, Tonea, & Giuchici, 2010; Kwenin, Wolli, & Dzomeku, 2011). The nutritive significance of green leafy vegetables is mainly with their richness in minerals and vitamins, which are essential in the maintenance of human health. On top of that, the tender leaves of some tuber crops such as sweet potato, cassava, taro and cocoyam are rich sources of protein, carotene and vitamins (Chandra, 1986; Diop & Calverley, 1998). Green vegetables have high water content, vitamins especially provitamin A, minerals, protein and essential amino acids, and energy value (in calories).

For example, Amaranths leaves are rich in beta-carotene, calcium, iron and vitamin C (Kwenin et al., 2011).

Table 3 Nutritional composition of leaves for commonly consumed root, tuber and green leafy vegetables

Leafy vegetables	Moisture	Total Ash	Crude Protein	Crude Lipid	Crude Fiber	Total Carb	Energy (Kcal/100g)	References
Ipomoea batatas	84.33	NA	13.50	6.40	1.42	67.80	328.10	1
Colocasia esculenta	90.00	NA	3.23	30.02	9.43	18.72	336.40	1
Moringa oleifera	77.50	NA	9.94	10.40	0.90	18.72	336.40	1
Amaranthus virdis	80.15	NA	7.95	9.00	11.66	67.78	336.60	1
Amaranthus caudatus	81.60	NA	6.36	11.00	5.92	61.03	326.70	1
Amaranth cruentus	72.93	NA	4.46	3.00	10.40	10.40	88.20	2
Xanthosoma sagittifolium	85.76	NA	4.65	3.19	10.00	6.80	71.50	2
Talinum fruticosum	91.83	NA	5.10	1.33	8.00	1.05	36.60	2
Amaranthus hybridus	83.48	13.80	17.92	4.65	8.61	52.18	268.92	3
Brassica oleracea	81.38	1.33	11.67	0.26	3.00	2.36	58.46	4
Coccinia abyssinica	NA	NA	53.00	NA	NA	NA	NA	5
Ipomoea batatas	82.21	11.10	24.85	4.90	7.20	51.95	351.30	6

References: 1,Vishwakarma & Dubey (2011); 2, Kwenin et al. (2011); 3, Akubugwo et al. (2007); 4, Emebu & Anyika (2011); 5, Desta (2011); 6, Antia et al. (2006); NA: Data not available

Vegetables have special importance in human nutrition since they enable full assimilation of vitamins in the human body, and well represent the composition of numerous minerals such as Ca, K, Fe, Na, etc. Many species of vegetables also contain high amounts of digestible carbohydrates (starch, sucrose, glucose, fructose) and non-digestible carbohydrates (cellulose, hemicelluloses, pectin, protides) (Caunii et al., 2010). Beta-carotene from leaf such as sweet potato or cassava, which contain about 800 mg/100g contribute as much as 86 percent in Asia and 80 percent in Africa (Kwenin et al., 2011).

Cassava leaves have a crude protein content of 20-35 percent on a dry weight basis but contain low crude fiber and relatively high calcium and phosphorus. Leaves of taro are also cooked and eaten as a vegetable. They contain beta-carotene, iron and folic acid, which protects against anemia. The leaves of tuber crops contain a substantial amount of beta-carotene that could contribute significantly to the daily requirement of vitamin A, especially for children (FAO & Redhead, 1990). Table 3 shows the nutritional content of leaves of some commonly consumed tuber crops and other leafy vegetables.

2.5. Mineral Elements in Plant Foods

Minerals, the inorganic elements are required for normal biological or biochemical processes of the human body including the accumulation of electrolytes (Gómez-Galera et al., 2010; Soetan, Olaiya, & Oyewole, 2010). Minerals are important essential protective nutrients in food and should be included in daily human diet (Reddy, & Bhatt 2001). Minerals are involved in the various structural and physiological functioning of the body: being the structural components of body tissues, involved in the maintenance of acid-base balance, regulation of body fluids, transport of gases and muscle contractions (Murray et al. 2000). Sodium, calcium, potassium, and magnesium are directly involved in physiological functions such as muscle contractions, and about 98% of the calcium and 80% of the phosphorus are bound up within the skeleton of the human body (Hendricks, 1998).

Minerals are broadly classified as macro (major), micro (trace) or ultra-trace elements required in less than 1 to 2500 mg per day depending on the type of mineral (Soetan et al., 2010). The macro minerals (calcium, phosphorus, sodium, potassium, magnesium, chlorine, and sulphur) are required in amounts greater than 100 mg/day, while micro minerals (iron, zinc, iodine, copper, chromium, manganese, molybdenum, fluoride, selenium, and silica) are required in amounts less than 100 mg/day (Murray et al., 2000; Nielsen, 2010). The human body requires both major and trace mineral elements (at least 22) to build a healthy wellbeing (Table 4).

Among the essential minerals, 11 of them are so abundant in foods and drinking water and their deficiency is rare. This is compared to other micronutrients such as Fe, Zn, Cu, Ca, I and Se needed in trace amounts by human body that are found in limiting amounts in many foods (Norhaizan & Norfaizadatul, 2009; White & Broadley, 2005).

Table 4 Essential mineral elements required by human body

Element	RDA	RNI	UL	SUL	Anti-nutrients	Promoters
N	NS	NS	NS	NS		
S	NS	NS	NS	NS		
K (mg)	1600–3500	3500	NS	3700b		
Cl (mg)	750–3400	2500	NS	NS		
Ca (mg)	1000–1200	700	2500	1500b	Oxalate, phytate, tannins, fiber	Inuline
P (mg)	700	550	4000	250b		
Na (mg)	500–2400	1600	< 2400	NS		
Mg (mg)	310–420	300	350b	400b	Phytate	
Fe (mg)	8.0–18.0	11.4	45.0	17.0b	Phytate, tannins, oxalate, fiber, hemaglutinins	Phytoferritin, riboflavin, ascorbate, β-carotene, cysteine, histidine, lysine, fumarate, malate, citrate
Zn (mg)	8.0–11.0	9.5	40.0	25.0b	Phytate, tannins, fiber, hemaglutinins	Palmitic acid, riboflavin, ascorbate, cysteine, histidine, lysine, methionine, fumarate, malate, citrate
Mn (mg)	1.8–2.3	1.4	11.0	4.0b		
Cu (mg)	0.9	1.2	10.0	10.0		
I (mg)	150	140	1100	500b	Goitrogens	Selenium
Se (mg)	55	75	400	450		
Mo (mg)	45	50-400	2000	NS		
Cr (mg)	25–35	25	NS	NS		
F (mg)	3–4	NS	10	NS		
B (mg)	NS	NS	20.0	9.6		
Ni (mg)	NS	NS	1000	260		
V (mg)	NS	NS	1.8	NS		
Si (mg)	NS	NS	NS	1500		
As	NS	NS	NS	NS		

Abbreviations: NS, none specified. a The US recommended daily allowances (RDA, or adequate intakes), the UK guidance daily reference nutrient intakes (RNI), the US tolerable upper intake levels (UL), and the UK guidance safe upper levels (SUL) for adults (http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf, [96]). The required amounts of N and S can be obtained if the recommended daily protein intake is achieved. b Non-food. Source: (White & Broadley, 2005)

Ultra-trace minerals (vanadium, tin, nickel, arsenic, and boron) do not have clearly defined biochemical roles, while elements such as lead, mercury, cadmium, and aluminium are toxic elements usually found in the human food chain and should be avoided in diet (Nielsen, 2010). Most mineral elements in plants occur in both soluble and insoluble forms as an organic compounds or inorganic salts. However, some essential mineral elements, such as K and Na, occur solely as soluble inorganic ions in plants.

The ability of the human body to absorb and assimilate a mineral element depends on its chemical form (Mendoza, 2002; Welch & Graham, 2004). In order to stimulate the absorption of essential mineral elements, bind by anti-nutrients promoter compounds such as certain organic acids and amino acids might be essential. Thus, when considering dietary refinement, it is important to consider not only the total amount of each element delivered but also its chemical forms and their interactions with other compounds in food (White & Broadley, 2005). Table 4 shows the recommended essential mineral elements requirement by the human body.

Almost all essential mineral and organic nutrients are of plant origin (Grusak & DellaPenna 1999; Grusak 2002). Root and tuber crops are important food crops in the tropics and subtropics providing basic food security, serving as source of income and as additional source of essential minerals (Aregahegn, Chandravanshi, & Atlabachew, 2013). Table 5 presents the major mineral composition of the commonly consumed starchy root and tuber crops.

Table 5 Mineral composition (mg/100g) of selected root and tuber crops (Raw)

Nutrients	Potatoes		Sweet potatoes	Cassava	Yam
	White flesh & skin	Red flesh & skin			
Calcium	9	10	30	16	17
Magnesium	21	22	25	21	21
Potassium	407	455	337	271	816
Phosphorus	62	61	47	27	55
Sodium	16	18	55	14	9

Source: (Chandrasekara & Kumar, 2016)

The mineral composition of plant based foods are affected by agricultural practices, climate, altitude, geochemistry, the variety of cultivars, physiological state and maturity, and post-harvest treatments (Greenfield & Southgate, 2003; Rodrı´guez, Morales, Rodrı´guez, & Romero, 2011; Yada, Huang, & Lapsley, 2013). The other factors that affect mineral concentration of foods are processing methods, handling, and food preparation (Lisiewska et al. 2009; Campo et al. 2013).

The mineral profile of certain food product can also be used for identification of a cultivar or for authentication of geographical origin (Rodrigues, 2011; Rodrı´guez et al., 2011). In practical terms, mineral malnutrition can be addressed through supplementation, food fortification, well-chosen dietary diversification, and/or increasing mineral concentrations in edible crops (bio-fortification). The bio-fortification of crops through application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements as an immediate agronomic strategy to increase mineral concentrations in edible produce and improve yields on infertile soils (White & Broadley, 2005).

Bioavailability is a general term that refers to how well a nutrient can be absorbed and used by the body. It can be affected by many factors such as the presence of anti-nutrients, including phytates, oxalates, tannins and polyphenols in foods, a person's need, fiber, competition with other nutrients and acidity of intestinal environment (Norhaizan & Norfaizadatul, 2009). Mineral bioavailability of essential elements also depends on their chemical form, the composition of the diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determinations of actual daily intake of essential elements are important problems of trace elements in nutrition (Hunt, 2003).

Mineral deficiency is the most prevalent in developing countries, since diets are based predominantly on staple foods such as milled cereals, which have a low bio-available mineral content compared to fresh foods (Christou & Twyman, 2004). An increased intake of high phytate containing plant based foods impairs iron and zinc absorption (Hunt, 2003). Hotz & Brown (2004) stated that the proportion of zinc absorption can be altered by 10-fold the dietary factors that cause a physico-chemical interaction.

The significant effect of dietary components such as phytate and dietary calcium has a negative impact on the absorption of zinc, whereas protein enhances the absorption of dietary zinc (Lönnerdal, 2000). Therefore, animal protein such as from meat and eggs, including whey protein, have further enhancing effects on zinc absorption, although casein may be inhibitory (Lönnerdal, 2000). The content of zinc itself in a meal also influences the absorption of zinc; specifically, the percent absorption decreases with increasing intake of zinc, although the absolute amount of zinc.

2.6. Antinutritional Factors in Root and Tuber Crops

Antinutritional factors (ANFs) like phytic acid, tannins, cyanides and oxalic acid are considered nutritionally undesirable since they can limit the digestibility and solubility of certain nutrient such as protein, calories, minerals and vitamins.

2.6.1. Phytic Acid

Phytic acid known as inositol hexaphosphate (IP_6) is the principal storage form of phosphorus in many plant tissues, especially in the grass family (wheat, rice, rye, barley, etc.) and beans. Phosphorus in this form is generally not bioavailable to humans because humans lack the digestive enzyme phytase, required to separate phosphorus from the phytate molecule.

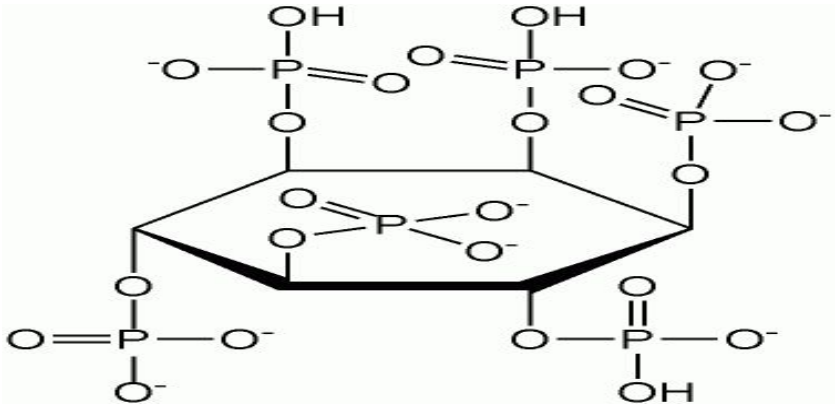


Figure 3 Basic structure of phytic acid (phytate)

In plants, phosphorus occurs mainly in two forms: phytate-phosphorus and inorganic phosphorus. Phosphorus in plants is stored in the form phytic acid (PA), or myo-inositol 1,2,3,4,5,6-hexakisphosphate (Liu, Cheng, & Zhang, 2005). The mixed salt of phytic acid is commonly known as phytate (Fowler, 2013). Phytic acid is found mainly in seeds in a storage form of phosphorus for later growth, but it has also been found to be in pollen, spores, roots, stems and leaves (Garcõâa-estepa et al. 1999; Erdal et al. 2002; Gargari et al. 2007; Seneviratne et al. 2012).

Phytate is synthesized in plant cells where it is stored, packaged by the endoplasmic reticulum, and moved to protein bodies for storage (Greenwood & Bewley, 1984). Different factors can affect phytic acid accumulation in plant parts. For example, when plants are grown in soil with higher levels of phosphorus, phytic acid accumulates in the storage organs with greater amounts (Lott, Ockenden, Raboy, & Batten, 2000). It has also been shown that cultivar, location and harvest year can affect the phytic acid content in plant storage organs (Liu et al. 2005; Dai et al. 2007; Steiner et al. 2007).

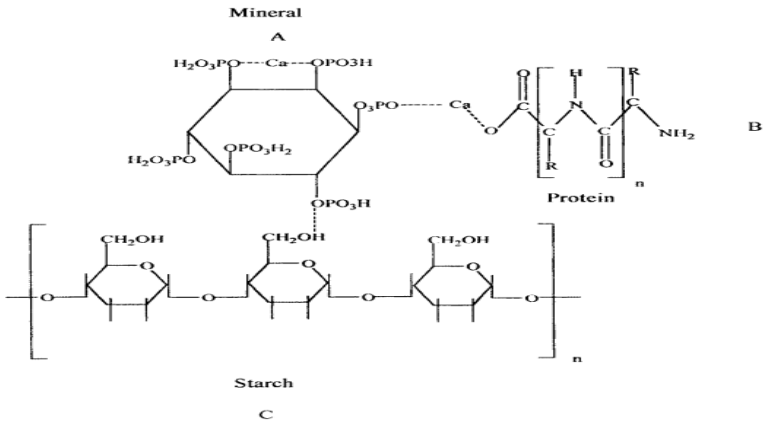


Figure 4 Interactions of phytic acid with mineral (A), proteins (B), and starch (C)

Source: (Oatway et al., 2001)

Ockenden et al. (1997) and Greiner et al. (2006) have reported that the amount of phytate in plant storage organ is positively correlated with rainfall. Phytate has been shown to bind other positively charged nutrients, mainly K and Mg but also Ca, Mn, Zn, Ba and Fe (Lott et al., 2000).

Out of all minerals, Zn appears to form the most stable and insoluble complex with phytate (Oatway, Vasanthan, & Helm, 2001). Phytate forms an insoluble complex with Ca, which can inhibit not only phosphorus availability, but also Ca availability (Lopez, Leenhardt, Coudray, & Remesy, 2002).

Phytate can also form a strong complex with some proteins and inhibit their proteolysis (Kumar, Sinha, Makkar, & Becker, 2010). Phytate anions binds protein at a pH below the protein's isoelectric point, forming insoluble complexes and inhibiting protein digestion (Rutherfurd, Chung, & Moughan, 2002). Similarly, phytate has been suggested to inhibit starch digestion by salivary amylase and *Bacillus subtilis* amylase (Thompson, Button, & Jenkins, 1987). Oatway et al. (2001) also reported that a large number of studies support the theory that phytic acid lowers the enzyme activity and the rate of starch digestion. In humans, an inverse relationship between glycemic index and phytate intake was observed (Yoon, Thompson, & Jenkins, 1983).

2.6.2. Tannins

Tannins are secondary compounds of high molecular weight polyphenolics with various chemical structures widely occurring in plant kingdom of the higher plants including many plants used as foods and feeds (Chung et al. 1998; Francis et al. 2001; Hartzfeld et al. 2002). Tannins have enormous structural diversity and they are systematically classified into two groups based on specific structural characteristics and chemical properties, hydrolysable tannins and condensed tannins (Dai & Mumper, 2010; Khanbabaee & Ree, 2001).

Tannins that can be broken down, for example by treatment with hot water or with tannases led to the classification of such tannins as hydrolysable tannins. Hydrolysable tannins are compounds containing a central core of polyhydric alcohol such as glucose, and hydroxyl groups, which are esterified either partially or wholly by gallic acid (gallotannins) or hexahydroxy diphenic acid (ellagitannins), and have great potential to form oxidative linkages to yield more complex hydrolysable tannins (Chung, Wei, & Johnson, 1998; Ann E Hagerman, 2010; Romani et al., 2006). The great variety in the structure of these compounds is due to the many possibilities in forming oxidative linkage (Khanbabaee & Ree, 2001).

The central compound, pentagalloyl glucose, is the precursor of many complex tannin structures such as gallotannins (GT) and ellagitannins (ET) (Mueller-Harvey, 2001). Non-hydrolysable oligomeric and polymeric proanthocyanidins are classified as condensed tannins. Condensed tannins are oligomers or polymers of flavan-3-ol units linked through an interflavan carbon bond. They are also referred to as proanthocyanidins because they are decomposed to anthocyanidins through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions (Dai & Mumper, 2010). The most common condensed tannins occurring in plant tissues are procyanidins, which are derived from catechin or epicatechin and may contain gallic acid esters (Karonen, Loponen, Ossipov, & Pihlaja, 2004).

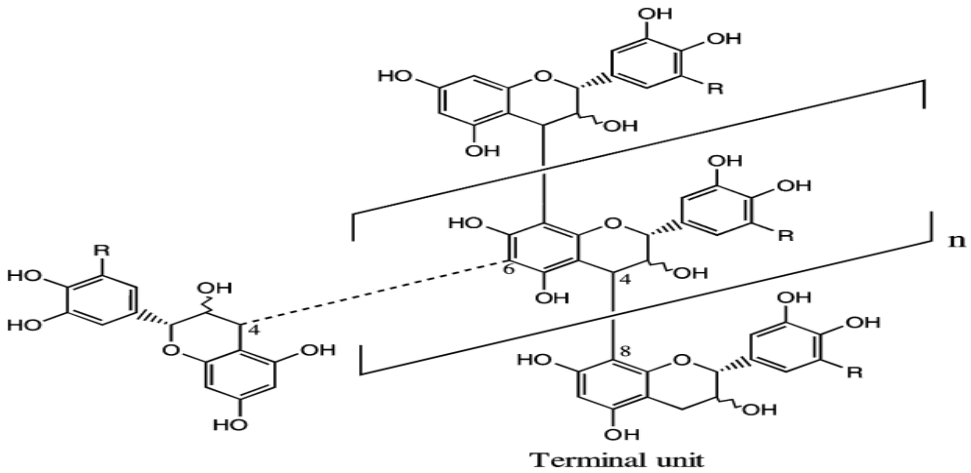


Figure 5 Model structure of condensed tannin.

R=H or OH then the structure represents a procyanidin or prodelphinidin. The 4-6 linkage (dotted line) is an alternative interflavan bond. The terminal unit is at the bottom of such a multi-unit structure.

Source: (Schofield, Mbugua, & Pell, 2001)

Tannins ingested with the diet by humans or animals may affect protein utilization by forming insoluble complexes with protein, iron utilization by complexing with iron, and biological antioxidant status by participating in redox reactions (Hagerman & Carlson, 1998). From nutritional point of view, tannins traditionally have been considered antinutrients because the presence of tannins in plant foods is usually accompanied by a reduced digestibility of protein and a subsequent increase in fecal nitrogen (Bravo, 1998).

It also affects the utilization of macronutrients such as vitamins and minerals and tannin components have also been implicated in the high levels of cheek and esophageal cancers in certain regions of the world (Chung, Wong, et al., 1998). Tannins combine with many proteins, therefore, their negative effect occurs, because the proteins of the diet are less available to the humans or animals (Mole, Rogler, & Butler, 1993; Robbins et al., 1987; Zucker, 1983). Tannins can also form complexes with carbohydrates by inhibiting the digestive enzymes and there by lowers the digestibility of most nutrients, which causes a reduction in nutritional values of foods (Chung et al. 1998 ; Mohan & Kalidass 2010). According to Chung, et al. (1998) many scholars indicated the decreased efficiency in converting the absorbed nutrients to new body substances is the major effect of tannins than their inhibition effect on food consumption or digestion.

The production of tannins seems to depend to a considerable extent on extrinsic factors, most notably soil conditions and light intensity, and their capacity to precipitate proteins varies depending on the different species; different parts of the same species as well as with the different times (Iqbal, Sajid, Abbas, & Sindhu, 2011).

2.6.3. Cyanide

Cyanoglycosides or cyanogenic glucosides (CG) are produced by about 2650 plant species, and account for approximately 90% of the wider group of plant toxins known as cyanogens (Speijers 1993; Haque & Bradbury 2002). The key characteristic of these toxins is cyanogenesis, the formation of free hydrogen cyanide, and is associated with cyanohydrins that have been caused by glycosylation (attachment of sugars) to form the cyanogenic glycosides (Vetter, 2000).

Cyanide or hydrogen cyanide (HCN) is obtained from the hydrolysis of the two CG, linamarin (93% of the total cynide content) and lotaustralin (Lebot, 2009; Omolara, 2014). These two CGs can be hydrolyzed to hydrocyanic acid by the endogenous enzyme linamarase which produce acetone cyanohydrins when the plant tissues are damaged and the linamarin present in the vacuole is brought into contact with this enzyme during harvesting or processing (Cock, 1985; Lebot, 2009).

The CG belongs to the products of secondary metabolites to the natural products of plants and it decomposes into acetone cyanohydrins under high temperature, pressure and use of enzyme linamarase or mineral acids (Vetter 2000; Umuhozariho et al. 2014). These acetone cyanohydrins spontaneously breaks down into poisonous compound, hydrogen cyanide (HCN) at pH above five or temperatures above 35°C (Siritunga & Sayre, 2004).

Cyanide has a toxic effect when converted to thiocyanate, a sulfur-containing compound by the enzyme rhodanase after entering the blood stream. This compound plays its toxic role by using up body sulfur in detoxification, thus increasing the body's demand for sulfur containing amino acids, or by interfering with the iodine uptake of the thyroid, resulting in goiter (Cock, 1985).

Chronic, low-level cyanide exposure resulting from eating poorly processed high dietary cyanide exposure like cassava results the development of goiter and tropical ataxic neuropathy (Bala Nambisan, 2011; Oluwole, Onabolu, Link, & Rosling, 2000; Tylleskär et al., 1992). The consumption of high dietary cyanide without proper processing to reduce the cyanide content will cause a number of chronic health disorders (CCDN, 2009; Nhassico, Muquingue, Cliff, Cumbana, & Bradbury, 2008).

2.7. Leaf Protein Concentrate (LPC) as Human Food

Plants can be used as an alternative sources of non-conventional protein to alleviate protein inadequacies found in most developing countries where animal proteins (milk, egg and meat) makes it unavailable to poor inhabitants resulting in kwashiorkor, marasmus, infant blindness, mortality and morbidity (Tee, 1992). Leaf protein is the most abundant and affordable protein sources that is synthesized from the direct sunlight. Leaf protein is the most available protein sources for those who are more susceptible to inadequate supply of animal protein such as weanling, pre-school children and other vulnerable groups including lactating and pregnant mothers (Adeyemi & Osubor, 2016; Agbede & Aletor, 2004). Leaf protein concentrate (LPC) was first coined in the 1960s and the idea reviewed later for human and animal consumption by Pirie Nobel Laureate (Pirie 1971; Pirie 1975; Simpson & Sanderson 2002).

Leaf protein concentrate (LPC) is prepared from disrupted plant cells by separating indigestible fiber and soluble antinutrients from much of the protein, vitamins, and minerals in a mechanical way (Kennedy 1993). Leaf protein concentrate has remarkable advantage for developing low-cost protein foods in the developing world, where there is high protein deficiency especially in children (Ghaly & Alkoaik, 2010). It can be used as a supplementary food and an alternative source of high protein specifically for vegetarians since it has high protein content, unsaturated fats, carotenes, xanthophyll, starch, including minerals such as iron, calcium and phosphorus (Virabalin, Kositsup, & Punnapayak, 1993).

2.8. Protein Functionality Properties in Food Systems

Lamsal et al. (2007) reported that functional properties of proteins are influenced by two major characteristics. The first one is water solubility, the result of surface-active properties of a protein that affects foaming, emulsification, and water and fat binding properties among others.

The second one is hydrodynamic properties that influence viscosity, gelation, thickening, and texturization. Protein solubility in water is the result of protein amino acid composition and distribution, molecular flexibility, and shape and size, whereas the hydrodynamic properties are due to shape and size of the protein (Hall, 1996).

Functional properties of protein in foods has important impact on food processing, food quality, and sensory evaluation while formulating a food product is largely determined by a protein's physicochemical and structural properties (Tang et al. 2003; Mart´inez-Flores et al. 2006). Functional properties of proteins and their applications can also be affected by their purification and/or concentration methods (Lamsal et al., 2007).

The functional properties that are very important in food processing can interact with the surrounding solvent, ions, other proteins, saccharides, lipids, and in surface phenomena (Table 6). These can affect the appearance, colour, juiciness, mouth feel, and texture of a large variety of foods, as well as cutting, mincing, mixing, formation of dough, fibers, foils, bubbles, shaping, and transporting of food materials (Sikorski, 2002).

Table 6 Functional properties of proteins displayed in interactions with different food constituents

Water	Water and Proteins	Lipids or Gases
Wet ability	Viscosity inducing	Emulsifying ability
Swelling	Gelling	Emulsion stabilization
Rehydration	Fiber forming	Foaming ability
Water holding	Dough forming	Foam stabilization
Solubility	Membrane forming	

Source: (Sikorski, 2002)

2.8.1. Protein solubility

Protein solubility is a good index of potential applications of proteins, and has a close relationship with emulsifying and foaming properties (Tang et al. 2003; Mart´inez-Flores et al. 2006). Solubility is the result of surface-active properties of a protein, which affects foaming, emulsification, and water and fat binding properties among others (Lamsal et al., 2007).

The capacity of protein to absorb water and oil is determined by its polar and non-polar amino acids composition that make up the protein, especially those amino acids on the surface of the molecule (Nielsen, 2010; Sathe, Deshpande, & Salunkhe, 1982). The thermodynamic interactions between the protein and the solvent can also determine protein solubility. The endogenous proteases that cause proteolysis also may alter protein solubility (Nielsen, 2010).

For optimal functionality of the food systems, proteins should be soluble under the conditions of use in food systems. Protein solubility can also influence other important functional attributes such as thickening (viscosity effects), foaming, emulsification, water binding, and gelation properties (Tang et al., 2003). The solubility of proteins in a food system can be influenced by the solvent polarity, pH, ionic strength, ion composition, and interactions with other food components, such as lipids or carbohydrates as well as by common food processing operations, such as heating, freezing, drying, and shearing (Nielsen, 2010).

For efficient use of various protein isolates, solubility may also be required as functional food additives in products differing in pH and salt content. The loss in solubility due to abuse treatment is often indicative of protein denaturation and subsequent cross-linking. Therefore, solubility data, if used to characterize commercial protein products, should be determined in standardized procedures.

In the methodology of solubility assays the following factors must be considered: size and disintegration of the sample, pH and ionic strength of the solvent, proportion of sample size to that of the solvent, number of extractions, foaming during blending and stirring, temperature and time of extraction, and separation of non-proteinaceous material (Kolakowski, 2001).

2.8.2. Bulk density

Bulk density indicates the behavior of a product in food formulations, for instance, high bulk density is disadvantageous for the formulation of complimentary foods as low density is required in such type of formulations. It is an important parameter that determines the packaging requirement of a product, material handling and application in wet processing in the food industry, and it is generally affected by the particle size and density of the flour (Adeleke & Odedeji, 2010; Chandi & Sogi, 2007). It can also indicates the behaviour of a product in dry mixes and it varies with the fineness of the particles (Mohamed, Zhu, Issoufou, & Fatmata, 2009).

2.8.3. Water and oil absorption capacity

Water and oil absorption capacity of protein is determined by its polar and non-polar amino acids composition (Mart´inez-Flores et al., 2006). Oil absorption is an important functional property for food product ingredients because it improves flavour retention and the sensation produced in the mouth (Mart´inez-Flores et al., 2006). The high oil absorption capacity also makes the flours suitable in facilitating enhancement in flavour and mouth feel when used in food preparations, whereas the high water absorption capacity of the flours suggests that they would be useful functional ingredients in bakery products (Appiah, Asibuo, & Kumah, 2011).

2.8.4. Emulsifying capacity

Proteins exhibit their emulsifying properties due to their amphoteric properties extended by amino acids and their building blocks, which have both hydrophobic and hydrophilic moieties or functional groups. Proteins are used as emulsifiers to lower the interfacial energy between phases to facilitate emulsion formation and to improve the stability of emulsions. Proteins migrate to the surface of a droplet during emulsion formation to form a protective layer or membrane on the surface, thus reducing interactions between the two immiscible phases. The quality of an emulsion is dictated by many factors including droplet size, droplet size distribution, density differences between the two phases, viscosity of the two phases, electrostatic and steric interactions between molecules at the interface, and thickness and viscosity of the adsorbed protein layer (McClements, 2015).

When the water and oil phases are combined by emulsification, two emulsion structures are possible. The oil phase may become dispersed throughout the aqueous phase to produce oil in water (o/w) emulsion. Alternatively, the aqueous phase may become dispersed throughout the oil phase to produce water in oil (w/o) emulsion. In general, an emulsion tends to exhibit the characteristics of the external phase. Two emulsions of similar composition can have different properties, depending on their structure. Many factors can influence the type of emulsion formed when two phases are mixed, including the type of emulsifying agent used, the relative proportions of the phases and the method of preparation employed (Brennan, 2006).

In food industries, simplified model systems that contains only a few of the most important ingredients are often used to investigate the properties of protein based emulsions, despite the fact that food emulsions usually are highly complex systems with multiple ingredients. This model system may contain only water or buffer, oil, and protein. The properties of final emulsion is usually influenced by the pH, salt concentration, temperature, type and amount of oil, protein concentration, energy input, and temperature during emulsion formation (Nielsen, 2010). Emulsifying capacity of a protein is represented by the volume of oil (cm^3) that is emulsified in a model system by one gram of protein when oil is added continuously to a stirred aliquot of solution or dispersion of the tested protein (Sikorski, 2002).

Emulsifying capacity is also closely associated with protein surface hydrophobicity. Furthermore, proteins are composed of charged amino acids, non-charged polar amino acids and non-polar amino acids, which make proteins possible emulsifiers (Mohamed et al., 2009). It is determined by measuring the quantity of oil at the point of phase inversion. The emulsifying capacity decreases with an increasing concentration of protein in the aqueous volume, and can be affected by the parameters of emulsification, depending on the equipment, as well as by the properties of the oil (Sikorski, 2002). An efficient emulsifier can prevent the breakdown or phase separation of an emulsion during storage.

Emulsions can be stable for a long period of time (months to years), so often test protocols include a destabilization step involving physical or chemical stress. Emulsion stability can be tested by centrifugation or agitation of an emulsion at a given speed and time to determine the amount of creaming or oil separation that occurs. This is a fairly rapid test, but may not adequately represent the breakdown of the emulsion during normal storage conditions. Another method involves measuring the change in particle size distribution of the dispersed phase over time (Wrolstad et al., 2005). After the initial volume has been centrifuged and standing for several hours at specified conditions the emulsion stability can be measured to obtain the final volume of the emulsion and determined as the quantity of oil or cream separated from the emulsion, or the time required for the emulsion to release a specified quantity of oil (Sikorski, 2002).

2.8.5. Foam volume and foam stability

Foams are coarse dispersions of gas bubbles in a liquid or semisolid continuous phase. Like emulsions, foams require energy input during formation and are inherently unstable. Whipping, shaking, and sparging (gas injection) are three common methods of foam formation. Proteins or other large macromolecules in the continuous phase lower the surface tension between the two phases during foam formation and impart stability to films formed around the gas bubbles (Nielsen, 2010). Foaming is responsible for the desirable rheological properties of many foods, e.g., the texture of bread, cakes, whipped cream, ice cream, and beer froth. Thus foam stability may be an important food quality criterion (Sikorski, 2002).

The two important parameters used to evaluate foams are foam volume and foam stability. Foam volume is dependent on the ability of protein to lower surface tension between the aqueous phase and gas bubbles during foam formation. Foam volume is generated during a standardized foaming process is recorded and can be compared to other foams made under identical conditions. Often the foam is formed in a blender and then transferred into a graduated cylinder for measurement. Another common approach is to measure foam over run or foam expansion. This is an indirect measure of the amount of air incorporated into the foam (Nielsen, 2010).

Foam stability depends on the properties of the protein film formed around the gas droplets. Free liquid is released as foam breaks down. More stable foam usually takes a longer time to collapse. Foam stability is often expressed as a half-life. The greater the half-life, the more stable the foam would be. Foam volume and stability are influenced by energy input, pH, temperature, heat treatment, and by the type and concentration of ions, sugars, lipids, and proteins in the foam (Aremu, Olaofe, Akintayo, & Adeyeye, 2008). A sample is whipped for a fixed time under standardized conditions. In one of the simplest methods, the foam is placed in a funnel over a graduated cylinder. The time for half of the original weight or volume of the foam to drain away from the foam is recorded (Nielsen, 2010).

2.9. Importance of Phytochemicals for Human Health

Phytochemicals are the non-nutritive bioactive compounds produced by plants through various metabolic pathways. The various phytochemical compounds commonly present in plants are such as steroids, terpenes, flavonoids, coumarins, alkaloids, xanthones, benzophenones, tannins, phenolic acids, saponins, anthocyanidin, reducing sugars and glycosides and antioxidant micronutrients (Czinner et al., 2001).

Phytochemicals are reported to have antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory potentials (Dorman & Deans 2000; Pizzale et al. 2002; Sokovic et al. 2002; Lampe 2003; Srinivasan 2005). The major classes of phytochemicals are phenolic compounds, terpenoids, and alkaloids and other nitrogen containing plant constituents (Dillard & German, 2000).

Plants commonly use these compounds for self-defense and to enhance their survival period (Williams, Stone, Hauck, & Rahman, 1989). Plants are rich sources for these phytochemicals that can protect against oxidative stress, and thus attracting attention recently due to their potential antioxidant properties and their marked effects in chemoprevention of various diseases (Abubakar et al., 2014).

The use of plants as medicine is continuously expanding throughout the world and natural products are occupying spaces in the pharmaceutical field as potential sources of new bioactive molecules (Mota et al., 2009). Plant-based foods, including fruits and vegetables are therefore potentially excellent sources of bioactive phytochemicals (Rao & Rao, 2007). Thus, for the prevention of cancer, cardiovascular diseases, diabetes and osteoporosis, dietary guidelines of plant based foods are formulated around the world (Caroline et al., 2015).

Phenolic compounds are well-known phytochemicals abundantly found in wide range of plant foods such as fruits vegetables, cereals and legumes, as well as wine, tea and coffee (Cheynier, 2005; Manach, Scalbert, Morand, Rémésy, & Jime, 2004). The synthesis of phenolic compounds in plants is mainly linked to a response to ecological and physiological pressures such as attack by pathogens and insects, ultraviolet radiation and wounding (Kennedy & Wightman, 2011).

Several thousands of phenolic compounds are described in plant foods and grouped into different classes according to their basic chemical structures such as type and number of phenolic rings, and into different subclasses, according to specific substitutions in the basic structure, association with carbohydrates and polymerized forms (Manach et al., 2004). Based on the number of phenol units in the molecule, plant phenolic compounds are classified as simple phenols or polyphenols that comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids (Soto-vaca, Gutierrez, Losso, Xu, & Finley, 2012).

Foods commonly associated with polyphenolic contents have antioxidant protection from free radicals and phytoalexins to prevent non communicable diseases such as heart disease, inflammation, cancers and diabetes (Wijngaard et al. 2009; Dai & Mumper 2010).

Phenolic compounds also have a variety of roles including colour, antimicrobial, antifungal and antimutagenic effect in human cells (Crozier et al. 2006; Pedreschi & Cisneros-Zevallos 2006; Friedman 2007; Sawadogo et al. 2012). Thus, consumption of plant products such as fruits, vegetables and legumes will provide stronger antioxidant effect mostly associated with the presence of phenolic compounds than vitamin E and C (Oboh, 2006).

Antioxidants can reduce the oxidative damage in foods and bio-molecules by delaying or inhibiting the oxidation process caused by reactive oxygen species, thus enhancing the shelf life and quality of the products as well as protecting the biological systems (Duthie, Ma, Ross, & Collins, 1996). Antioxidant compounds such as β-carotene, ascorbic acid and phenolics can also play therapeutic and preventive roles against several diseases such as aging, inflammation and certain cancers (Heliövaara et al., 1994; Vivekananthan, Penn, Sapp, Hsu, & Topol, 2003). Therefore, the natural antioxidants have recently become a major area of interest and increased consumption of plant that are rich in phenolic compounds has been recommended by various health advocates for maintaining good health (Lim, Lim, & Tee, 2007; Wong, Leong, & Koh, 2006).

2.9.1. Flavonoids

Flavonoids are the most common phenolics found in plants tissues often responsible for blue, purple, yellow, orange and red color formation together with the carotenoids and chlorophylls (Khoddami, Wilkes, & Roberts, 2013).

The flavonoid family includes flavones, flavanols, isoflavones, anthocyanins, anthocyanidins, proanthocyanidins and catechins (Ferreira & Pinho, 2012; Rong, 2010). All flavonoids are derived from the aromatic amino acids, phenylalanine and tyrosine, and have three-ringed structures (Routray & Orsat, 2012). Variation in flavonoid structure arises from the scale and pattern of hydroxylation, prenylation, alkalization and glycosylation reactions that alter the basic molecule (Stalikas, 2007). The chemical nature of flavonoids depends on their structural class; degree of hydroxylation, other substitutions and conjugations, and degree of polymerization are known to be synthesized by plants to microbial infection (Heim, Tagliaferro, & Bobilya, 2002).

Flavonoids are phenolic compounds based on C_{15} ($C_6C_3C_6$) framework. They contain a chroman ring (C-ring) with a second aromatic ring (B-ring) at the C-2, C-3, or C-4 position. The phenolic compounds have an aromatic ring bearing one or more hydroxyl groups (Chirinos et al., 2009).

Studies showed that flavonoids have health benefits against oxidative stress diseases such as Alzheimer, arteriosclerosis, cancer and aging (Corder et al., 2006; Garbisa et al., 1999; Jankun, Selman, Swiercz, & Skrzypczak-Jankun, 1997; Nunomura et al., 2001; Prasain, Carlson, & Wyss, 2011). The other beneficial effects of flavonoids are improved blood flow, the inhibition of cholesterol absorption and protection from damage by ultraviolet B radiation (Kootstra 1994; Arai et al. 2000; Schroeter et al. 2006).

2.9.2. Saponins

Saponins are a group of secondary metabolites of steroid or triterpene glycoside compounds in a variety of plants, which have many benefits. Many saponins have detergent properties, form a stable foam in aqueous solutions, show hemolytic activity and have bitter taste (Ceyhun & Artik, 2010).

It forms colloidal solutions in water, and foamy like soap if mixture is shaken. Saponins as a group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids (Konoshima, Kashiwada, Cosentino, & Hsiung, 1995). Saponins that have one sugar molecule attached at the C-3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmoside saponins (Lasztity, Hidvegi, & Bata, 1998).

Saponin can be found in the roots and leaves of plants, and possesses wide range of pharmacological properties including anti-carcinogenic, anti-inflammatory, and anti-viral activities (Yongmok & Daniel, 2009). HIV-1 replication was inhibited by triterpenoid sapogenin oleanolic acid, which probably decreased the HIV-1 protease activity (Lee et al., 2012). In addition, some triterpenoid saponins have been reported to show anti-herpes simplex virus type 1 (HSV-1) activity (Ikeda et al., 2005; Sindambiwe et al., 1998).

The presence of saponins is characterized by the existence of a stable colloidal solution (Astuti, 2011). Many saponins are known to be antimicrobial, to inhibit mould and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or Phyto protectants (Lacaille-Dubois & Wagner, 2000).

2.9.3. Carotenoids

Carotenoids as biological antioxidants are currently the focus of numerous investigations. Those carotenoids with nine or more conjugated double bonds are able to quench singlet oxygen with increasing activity depending on the number of conjugated double bonds (Yang & Min, 1994). Epidemiological studies showed that carotenoids are important under different aspects, for example, as antioxidants (Olson, 1996) and as preventing agents against cardiovascular diseases (Gerster, 1991), age-related macular degeneration (Landrum, Bone, & Kilburn, 1997), and cataracts (Heliövaara et al., 1994). Carotenoids are widely known as provitamin A, while there is an increasing interest in their role as antioxidants (Bohm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002).

Anti-cancer activity and other health benefits provided by β-carotene include the protection against cardiovascular disease or cataract prevention (Breithaupt & Bamedi, 2001). Most studies have found beneficial associations between a higher intake of nutrients such as α-carotene, β-carotene, β-cryptoxantin, and the outcomes associated with asthma and allergy (Devereux, 2006). β-Carotene is ubiquitously present in green leafy vegetables and yellow-orange fruits (Shofian et al., 2011). In recent studies, protective effects of carotenoids on bladder cancer have been reported (Hung et al., 2006).

β-carotene is important in lessening asthma symptoms, preventing certain cancers, heart disease, cataracts and age related macular degeneration and treating AIDS, alcoholism, Alzheimer's disease, Parkinson's disease and skin disorders such as psoriasis. According to Khan & Varshney (2015), β-carotene is also used in malnourished (underfed) women to reduce the chance of death and night blindness during pregnancy as well as diarrhea and fever after giving birth (Zanin, 2009).

2.9.4. Alkaloids

Alkaloids are diverse group of low molecular weight phytochemicals found in 20% of the plant species. The different subclasses of alkaloids include: betalain, indole, isoquinoline, lycopodium, pyrrolidine, pyrrolizidine and quinolone (Padwa & Zhang, 2007). The significant pharmacological activities of alkaloids reported are analgesic (morpheine and codiene), anticancer (taxol), anthiarrythmic (ajmaline), gout suppressant (colchicine), muscle relaxant (tubocurarine), and antibiotic (sanguinarine) (Cordell, Quinn-Beattie, & Farnsworth, 2001).

2.9.5. Terpenes

Terpenes are the largest classes of phytochemicals that include monoterpenoids, iridoids, sesquiterpenoids, sesquiterpene lactones, dieterpenoids, triterpenoids, phytosterols, saponins, saponins glycosides and carotenoids. These phytochemicals exist in green leafy vegetables, fruits and grains. Plants use triterpenes for carbon fixation through photosynthesis and protection from diseases involving chronic damage and growth dysregulation. Animals use these phytochemicals for hormonal and growth regulatory functions. Terpenes possess antioxidant (Milan, Dholakia, Tiku, & Vishveshwaraiah, 2008), antimicrobial (Viuda-Martos, Ruiz-Navajas, & Fernandez-Lopez, J. and Perez Alvarez, 2010), and antiviral properties (Orhan, Ozcelik, Kartal, & Kan, 2012).

2.9.6. Tannins

Tannins are water soluble phenolic compounds having molecular weights between 500-3000 and they have special properties that precipitate alkaloids, gelatin and other proteins, resulting from polymerization of flavonoids units (Bate-Smith & Swain, 1962).

Tannins have shown a wide range of pharmacological activities such as anti-helminthic (Barru, Fabre, Fouraste, & Hoste, 2005), antimicrobial (Fogliani, Raharivelomanana, Bianchini, & Hnawia, 2005), anti-proliferative, apoptotic and antioxidant activities (Seeram et al., 2005), anti-acne producing bacterial activity (Pothitirat, Chomanawang, Supabphol, & Gritsanapan, 2009), hepatoprotective (Bhattacharya, Kumar, Ghosal, & Bhattacharya, 2000), as well as diabetes mellitus (Broadhurst, Polansky, & Anderson, 2000).

2.10. Volatile Organic Compounds in Plants

Volatile flavor compounds are those chemical compounds that have molecular weights less than 300 that have a characteristic smell or odor and are sufficiently volatile to be transported to the olfactory system in the upper part of the nose (Fahlbusch et al., 2003). Flavor compounds in food can either be responsible for taste or odor. Odor is often responsible for aroma substances. However, there are compounds, which are responsible for both sensations (Belitz, Grosch, & Schieberle, 2009). The flavor compounds affect both the sense of smell and taste while those responsible for fragrance affect only smell (Fahlbusch et al., 2003). Flavor compounds can exist in food, spices, wine, perfumes, fragrance oils and essential oils, and usually formed biochemically during ripening of root bulbs, stem barks, fruits and other vegetables.

Many of the flavor compounds play a very significant role in the production of flavorants, which are used to improve and generally increase the appeal of various products to consumers, for food service industry (Fahlbusch et al. 2003; Belitz et al. 2009).

The concept of flavor substances is applied very loosely, as a compound might contribute a typical odor or taste in one food, while for another food it might cause a faulty odor or taste, or both, resulting in an off-flavor (Belitz et al., 2009). Generally, the contents of volatile flavor compounds present in foods are extremely small however; they comprise a large number of variety components. Especially foods made by thermal processes, alone such as coffee, or in combination with a fermentation process such as bread, beer, cocoa, or tea, contain more than 800 volatile compounds. However, from a great variety of volatile compounds, only a limited number are important from the aroma point of view.

Among the aroma substances, special attention is paid to those compounds that provide the characteristic aroma of the food and are called character impact aroma compounds or key odorants (Plotto, Margaria, Goodner, & Baldwin, 2008). Compounds that are aromatic substances are primarily those, which are present in food in concentrations higher than the odor and /or taste thresholds. The lowest concentration of a compound that is just enough for the recognition of its odor is called odor threshold or recognition threshold.

If the detection threshold is lower, it means that the compound concentration is detectable but it is ambiguous to establish the aroma quality. The threshold values are frequently determined by smelling (orthonasal value) and by tasting the sample (retronasal value) (Plotto et al. 2008; Belitz et al. 2009).

The analytical procedure for volatile compounds or aromas from plants or food comprises of two steps: extraction (steam distillation, hydro-distillation and simultaneous distillation-extraction etc.) and analysis usually by gas chromatography (GC), or gas chromatography coupled to mass spectrometry (GC-MS). The preferred method for volatile compound analysis is simultaneous distillation extraction (SDE) followed by GC-MS analysis (Careri et al. 1994; Swift 1999; Gan et al. 2005; Ferhat et al. 2007; Capuzzo et al. 2013).

2.10.1. Simultaneous Distillation Extraction (SDE)

Simultaneous distillation extraction (SDE), which is also known as Lickens-Nickerson method is most popular isolation technique in flavor and fragrance laboratories, for it enables combined volatility and extractability into a single step. Modification were done by many scientists to improves the original SDE device for recoveries and to use small quantities of material (Nikerson & Likens, 1966; Schultz, Flath, Mon, Eggling, & Teranishi, 1977; Swift, 1999).

An aqueous solution or slurry of the sample is steam distilled. Volatiles continuously extracted from steam condensates by a solvent reflux (Figure 6). In theory, the apparatus should allow quantitative recoveries as it is based on a double closed loop (water and solvent are continuously recycled) which can be operated for hours. In practice, components with very low volatility such as furaneol would need an infinite isolation time. However, SDE gives the most representative GC-profiles over wide a range of volatility compared to other isolation means (Reineccius, 1993).

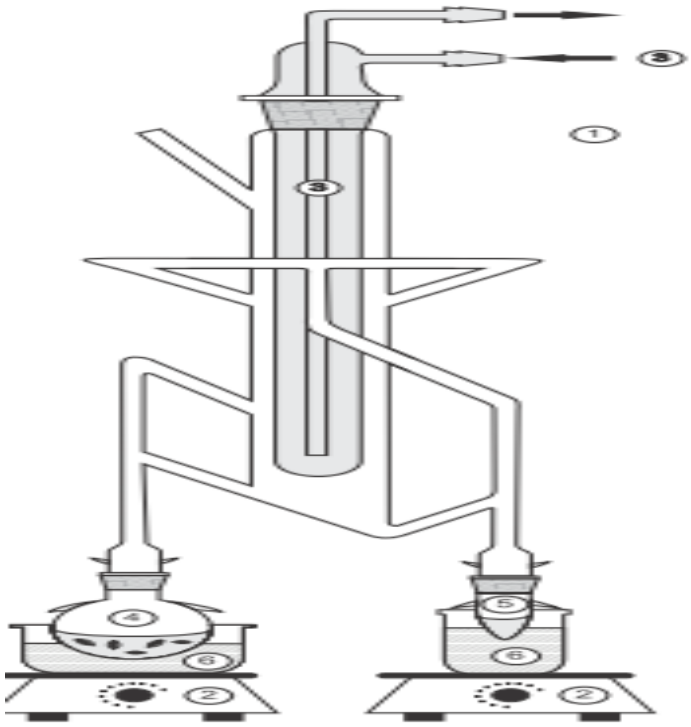


Figure 6 Schematic process of steam distillation-extraction

1. Steam distillation-extraction apparatus; 2. Stirrer/heat plate; 3. Cooling water; 4. Sample flask (30 g powder sample + 500 mL water +10 ml n-butylbenzene, heat plate: 110-130 ^{o}C); 5. Solvent flask (extraction agent, heat plate: 55-60 ^{o}C); 6. Mineral oil. Source: (Boix, Victório, Lage, & Kuster, 2010)

2.10.2. Gas Chromatography

Gas chromatography (GC) is a very important analytical technique usually employed for analysis of volatile flavor compounds in foods and other agriculture products. It has an important feature of separating and analyzing a mixture of volatile compounds in foods without decomposition.

As most flavor compounds in foods are volatile, simplified GC methods may offer an appropriate technique for the separation and characterization of volatiles in different food matrices (Azarnia, Boye, Warkentin, & Malcolomson, 2012). In GC, the mobile phase or carrier phase is an inert gas such as helium and the stationary phase is very thin layer of liquids or polymer on an inert solid support inside a column.

The volatile analytes interact with the walls of the column, and are eluted based on temperature of the column at specific retention times (Grob & Barry, 2004) and the detectors identify eluted compounds. Flame ionization and mass spectrometry are the most commonly used detectors for flavor analysis (Vas & Vekey, 2004).

2.10.3. Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that produces spectra (singular spectrum) of the masses of the atoms or molecules comprising a sample of material. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios (Hoffmann & Vincent, 2013; Sparkman, 2006). In a typical MS procedure, a sample, which may be solid, liquid, or gas, is ionized by bombarding it with electrons. This may cause some of the sample's molecules to break in to charged fragments. These ions are then separated according to their mass-to-change ratio, typically by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection (Sparkman, 2006). The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern (Hoffmann & Vincent, 2013; Sparkman, 2006).

Chapter Three: Nutritional Composition and Anti-Nutritional Factors of Tubers and Leaves of 44 Anchote (*Coccinia abyssinica*) (Lam.) (Cogn.) Accessions from Ethiopia

3.1. Abstract

Anchote (Coccinia abyssinica) (Lam.) (Cogn.) is one of the important endemic crops principally grown for tuber throughout the south and southwestern parts of Ethiopia. The objective of this study was to evaluate proximate composition, amino acid profile, and certain anti-nutritional factors of tubers and leaves for 44 Anchote accessions. The mean proximate compositions of the tubers were 7.58%, 8.50%, 0.53%, 5.05%, 6.83%, 80.52%, and 360.84Kcal/100g for moisture, crude protein, crude fat, crude fiber, total ash, utilizable carbohydrate, and gross energy value, respectively. Similarly, the proximate composition of the leaves was 8.56%, 20.50%, 3.63%, 11.42%, 11.96%, 52.56%, and 325.05Kcal/100g for moisture, crude protein, crude fat, crude fiber, total ash, utilizable carbohydrate, and gross energy value, respectively. The total amino acids content of the five selected Anchote accessions ranged from 45.12 to 62.89 g/100g protein for tubers, and 67.31 to 75.69 g/100g protein for leaves. The amino acid with the highest content value was arginine (6.50 - 9.52 g/100g protein) in tubers and glutamic acid (7.87 - 10.47g/100g protein) in leaves. The mean content values for phytates, tannins and cyanide in tubers were 131.10 mg/100g, 112.02mg/100gand 13.08mg/kg respectively, while those found in leaves were 250.30 mg/100g216.53 mg/100g and 12.36 mg/kg respectively. A significant variability was observed in nutrient and anti-nutrient contents among the accessions. Leaves were found to be significantly rich in nutrient especially in crude protein content compared to tubers. Tannins and phytates in leaves were almost double than tubers, while the cyanide content is relatively higher in the tubers which calls for better processing techniques to reduce the toxicity to safer level.

3.2. Introduction

Anchote [*Coccinia abyssinica* (Lam.) (Cogn.)] belongs to *Cucurbitaceae* family which is one of the most economically important families of plants (Schaefer et al., 2009). Among the 30-species registered under the genus *Coccinia*, about ten of them exist in Ethiopia. However only *C. abyssinica* is cultivated for human consumption (Jeffrey, 1995). Anchote is an endemic and potentially valuable crop of Ethiopia principally categorized under root and tuber crops (Amare, 1973). Its newly growing leaves along with the tendrils are also used as nutritious vegetable served after being cooked, making Anchote to have big purpose in its food use as tuber and leafy vegetable (Abera, 1995; Desta, 2011).

The tuber is prepared in different ways for consumption. In one way, it can be cooked simply and served with a traditional fermented spice '*Kochkocha*' prepared from coriander (*Coriandrum sativum*), sweet basil (*Ocimum basilium*), ginger (*Zingiber officinale*), garlic (*Allium sativum*) and salt. It is also used to prepare a soup after drying and grinding in to powder (Habtamu & Kelbessa, 1997). In jovial occasion and holydays tubers of Anchote are cooked in sliced form and pounded after mixing with plenty of butter made from cow milk and spices (Amare, 1973). Nutritionally, the crop has appreciable nutritional composition mainly of protein and calcium (Desta, 2011; Habtamu et al., 2013; Habtamu & Kelbessa, 1997).

From medicinal point of view, Anchote plays important roles in the customs and tradition of the western part of Ethiopia as it is used for healing of broken and/or fractured bones as well as dislocated joints (Abera, 1995; Amare, 1973; Endashaw, 2007). Traditionally the tuber is also used by lactating mothers and sick people to recover their health and strength (Habtamu & Kelbessa, 1997).

As stated by Amare (1973) and Tesfaye & Abebe (1988), Anchote has been grown in wide environmental conditions from dry to cooler regions of Wallagga, lllubabor, Jimma, Kafa and Sidamo. This makes the crop a potential food security in addition to its nutritional and medicinal values. However, Anchote did not get adequate attention in terms of improving its productivity and food utilization value, and hence it has remained as one of underutilized crops of Ethiopia. So far, there has been little or no efforts made to analyze varietal qualities development to identify suitable cultivars with different desirable traits and adaptable to the different agro-ecological zones of Ethiopia, and its quality utilization values for food.

Research output on Anchote is very limited as a result of which shortage of published data is a common problem (Abreham et al., 2014; Daba et al., 2012; Tilahun et al., 2014). Besides, owing to the scanty information available about the nutritional content, absence of data on the amino acids profiles for the available Anchote accessions and lack of awareness about the crop itself, there is need to carry out descriptive studies on the crop to elucidate its traits as food crop.

The collected accessions from a particular agro-climatic condition are the basic working units to conserve the genetic resource of the plant selected and adapted by the farming system. A study conducted by Desta (2011) revealed that accessions were clustered mainly based on the collection region and there is no strong evidence that shows the exact variability of the currently conserved accessions. This therefore, calls for further research works to investigate variability in the context of nutritional and other related biochemical substances among accessions. Such data is vital for further systematic selection, and improvement of the crop.

Incidentally, this baseline information will help to enhance the utilization and incorporation of Anchote, the underutilized crop in a diversified way as food and medicinal crop, and ultimately for contribution to address food and nutrition-security. Therefore, assessment of the nutritional and anti-nutritional content is a pre-requisite to further promote utilization and enhance the role of the crop in food and nutritional security. The present study was therefore conducted to evaluate proximate nutritional compositions, amino acid profiles, and selected antinutritional factors of tubers and leaves for 44 *ex-situ* conserved Anchote accessions collected from four different regions of Ethiopia.

3.3. Materials and Methods

3.3.1. Description of Sampling Site

A total of 44 Anchote accessions were obtained from Debre Zeit Agricultural Research Center (DZARC) experimental field, which is located at 47 km South East of Addis Ababa, Ethiopia (Figure 7). DZARC is geographically located at 08º44'N latitude and longitude of 38º58' E. It has an elevation of about 1860 m.a.s.l., mean annual rainfall of 851mm and mean temperature of 19 ºC. The major soil types in the area are heavy black (*koticha* in Amharic), light soil (*gomborie* in Amharic) as well as vertisols and alfisols/mollisols.

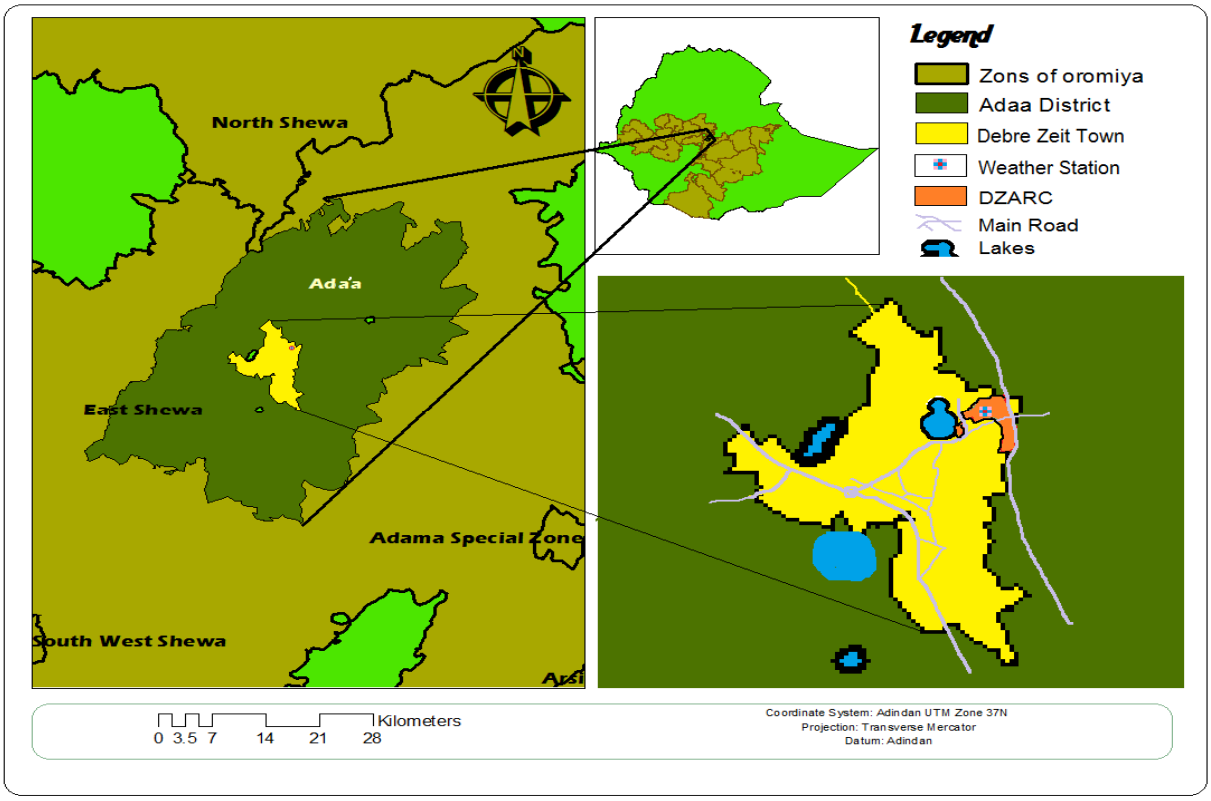


Figure 7 Map of location indicating areas of sample collection

3.3.2. Source of Plant Materials

Primarily, Anchote accessions were collected from different regions of Ethiopia having altitudes ranging from 1400 to 2560 m.a.s.l. About 86% of the accessions were collected from Oromia region of East and West Wollega, Jimma, Iluababor, Horro Guduru Wollega, and East Shoa zones. The remaining came from Southern Nations Nationalities and Peoples Regional State (SNNPRS), Amhara and Benishangul Gumuz regions. The details pertaining to the origin of the accessions is compiled in Annex A. The collected Anchote accessions were planted at the experimental field of DZARC in July 2011, and leaf and tuber samples collected at proper maturity level in November 2011 and January 2012 respectively.

3.3.3. Sample Preparation

Harvesting of tuber and leaf samples of Anchote accessions was conducted at the respective optimum maturity stage from November 2011 up to January 2012.

Three healthy tubers from each accession were harvested, washed, peeled, and sliced to small pieces and mixed thoroughly in order to prepare 400g of samples. These were placed in a paper bag and dried to a constant weight in a hot air oven (DHG- 9055A, Memment, Germany) set at 105 ^{o}C. Similarly, 200g bunches of newly growing tips of leaves were first cleaned and chopped in to small pieces and oven dried at 105 ^{o}C to a constant weight. The oven dried Anchote leaf and tuber samples were then milled using an electrical miller (FW 100, Yusung Industrial Ltd, China) to fine powder to pass a mesh size of 0.425mm. Finally, the dried and powdered samples were packed in paper bags and sealed in an airtight polyethylene bag, and labeled before storing in a refrigerator set at 4 ^{o}C for further analysis.

3.3.4. Dry Matter Content

The dry matter (DM) content of the representative tuber and leaf samples were determined according to Teye et al. (2011) by weighing a sample before and after complete drying using the following equation:

$$\text{Dry matter (\%)} = \frac{\text{Dry sample weight}}{\text{Wet sample weight)}} \text{ X } 100$$

3.3.5. Proximate Composition

i) Moisture

Moisture content was determined according to AOAC (2000), using the official method 925.09. Crucibles made of aluminium were washed and dried in drying oven and allowed to cool in desiccator (CSN-SIMAX). The mass of each dried crucibles was taken first (M_1), and about 5 g of sample was weighed in clean and dried crucible (M_2) using analytical balance (Adventurer, OHAUS, China).

The crucibles containing the samples were then put in an oven set at 105 ºC to dry the sample to constant weight (M_3). Finally, moisture content was calculated by using the following equation:

$$\text{Moisture (\%)} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

Where :

M1 : Mass of the crucible

M2 : Mass of the crucible and the sample before drying

M3 : Mass of the crucible and the sample after drying

ii) Crude Protein

Crude protein was determined by Kjeldahl method according to AOAC (2000) using the official method 979.09. About weighed 0.5 gm of sample was digested by heating with 6 ml of concentrated sulphuric acid (H_2SO_4) (Sigma-Aldrich, USA), and mixed with 3.5 ml of 30% hydrogen peroxide solution. 3 g of catalyst mixture prepared from 10 g of Copper Sulfate ($CuSO_4$) and 150 g of Potassium Sulfate (K_2SO_4) was added to the digestion flask and digested at 370 ^{0}C for 4 hrs. in nitrogen determination apparatus (Gerhardt vapodest, Germany), until a clear solution was obtained.

The digested samples were transferred into the fume hood for cooling, and the content in each flask diluted with distilled water and then neutralized with 35 % sodium hydroxide (NaOH) to make the solution slightly alkaline.

Finally, samples were distilled and ammonia received in flasks containing excess 2% boric acid (H_3BO_3) solution for reaction with ammonia. The reacted solution of ammonia borate was then titrated with 0.1N hydrochloric acid (HCl) (Sigma-Aldrich, USA), to determine the total nitrogen.

$$H_3 \text{ (ammonia)} + H_3BO_3 \text{ (boric acid)} \rightarrow NH_4^+ + H_2BO_3^- \text{ (borate ion)}$$

The dilution, distillation and titration steps of the digested sample were done by using Kejeltic analyzer unit (Gerhadt vapodest, Germany).

The nitrogen content was calculated using the following equation:

$$\text{Nitrogen (\%)} = \frac{(V2 - V1) \times 14}{W} \times 100$$

Where :

V1 : Volume (ml) standard HCl solution in the titration of the blank

V2 : Volume (ml) standard HCl solution in the titration of the sample

W : Sample weight

14 : The molecular weight of nitrogen

Finally, the protein content was calculated using the following equation:

$$\text{Crude Protein (\%)} = 6.25 \times \text{\% Nitrogen}$$

iii) Crude Fat

Ether extract method was used to determine the crude fat using soxhlet extraction apparatus by the official method 4.5.01(AOAC 2000). Two grams of moisture free sample was weighed in to each of the extraction thimbles (Whatman International LTD Maidstone, England) wrapped with two centimeters layer of fat free cotton. Cleaned and dried receiving beakers were first weighed filled with 70 ml of diethyl ether (Sigma-Aldrich, USA) and fitted into the soxhlet apparatus (Shanghai Qianjian Instrument Co., Ltd) for the extraction process. After four hours of extraction, the ether in the receiving beakers were allowed to evaporate in a drying oven (Cintex precision, India) at 92ºc for at least 30 minutes, and then cooled inside desiccators. Finally, the percent crude fat content was determined by using the following formula:

$$\text{Crude Fat (\%)} = \frac{(M2 - M1)}{W} \times 100$$

Where :

M1 : Mass of dried aluminium cup

M2 : Mass of aluminium cup and lipid extract

W : Sample weight

iv) Crude Fiber

Crude fiber content of the samples was determined according to AOAC (2000), using the official method 962.09. Extraction, filtration, drying and combustion were the steps involved. For extraction process, 1.5g sample was measured into each of 600 ml beakers, and 200 ml of 1.25% H_2SO_4 was added and the contents were boiled for 30 min using hot plate (Wadtech, UK Hotplate SH_3). During boiling the level of the sample solution was kept constant with hot distilled water and periodically stirred. After 30 minutes 20 ml of 28% potassium hydroxide (KOH) solution was added and further boiled for another additional 30 min following the above procedure.

After completing the extraction process the sample solution was transferred to sintered glass crucible covered with 10 mm sand layer wetted with distilled water and then filtered under vacuum. During filtration, the beaker was rinsed several times with hot distilled water and transferred into crucible. The sample residues were washed with 1% H_2SO_4 with successive rinsing using hot distilled water, followed by addition of the same volume of 1% NaOH solution with consecutive washing by hot distilled water. Once again, 1% H_2SO_4 solution was added with continuous addition of hot distilled water. At last, the residue was washed with water free acetone. Each of the crucibles with the respective contents was dried in an electric oven (DHG- 9055A, Memment, Germany) for 2 hrs. at about 130 ^oC and cooled in desiccators (CSN-SIMAX) before the weight was taken (M_1). The dried residues in each crucible were transferred in to muffle furnace (Gallenkamp, size 3) for 30 min ignition at 550 ^oC and allowed to cool in desiccators before taking the final mass of each crucible (M_2). Finally, the crude fiber content was calculated using the following equation:

$$\text{Crude fiber (\%)} = \frac{(M2 - M1)}{W} \times 100$$

Where :

M1 : Mass of the crucible and sample after drying in an oven

M2 : Mass of the crucible and sample after ashing

W : Sample weight

v) Total Ash

Total ash content was determined according to AOAC (2000), using the official method 923.03. The crucibles were first cleaned and dried in an oven at 100 ^{o}C and cooled in desiccators before the mass of each crucible was weighed by analytical balance (LA 204, Measure tech.) (M_1). By taking 2.5 gm sample (M_2) the crucibles were thoroughly charred on hot plate starting from low temperature under a hood (Nordia, London E17 6AB), and then placed in a muffle furnace (Carbolite CSF 1200) at about 550 ^{o}C until the sample changed to grayish white ash which took about five hours. To take the final mass (M_3) the crucibles that contained-ignited sample were cooled inside desiccators (CSN-SIMAX). Finally, the total ash content was calculated using the following equation:

$$\text{Ash (\%)} = \frac{(M3 - M1)}{(M2 - M1)} \times 100$$

Where :

M1 : Mass of the dried dish

M2 : Mass of the dish and the sample

M3 : Mass of the dish and the sample after ashing

vi) Carbohydrates

The utilizable carbohydrate content of the respective samples was determined on difference basis as indicated in the following formula.

$$\text{Utilizable carbohydrate (\%)} = 100 - (\text{\% Moisture} + CF + CF + CFB + \text{Ash})$$

vii) Gross Energy

The gross energy content was calculated from fat, carbohydrate and protein contents using the Atwater's conversion factors; 16.7 kJ/g (4kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in kilo calories (Nguyen et al., 2007).

The mathematical expression of gross energy is as follows:

$$\text{Gross energy Kcal/100g} = (4 \times \text{g protein}) + (4 \times \text{g carbohydrate}) + (9 \times \text{g fat})$$

Quality Control for Proximate Analyses

The quality of the analytical method for protein, fat and ash determination was tested by using Certified Reference Material (CRM) BCR-381 (Rye Flour) (IRMM, Geel, Belgium). The determined mean of the triplicate value for validation test of the analytical methods in Center for Food Science and Nutrition laboratory was in agreement with the known standard value of the CRM (Table 7).

Table 7 Validation test for methods used in proximate composition compared with certified reference material ERM-BC 381 (rye flour)

Proximate composition	Certified value (g/100 g)	Determined (g/100 g)	Recovery (%)
Kjeldahl Nitrogen	1.56 ± 0.02	1.48 ± 0.06	94.60
Fat	1.36 ± 0.16	1.25 ± 0.05	91.90
Ash	1.08 ± 0.11	1.09 ± 0.04	100.50

3.3.6. Amino Acid Analysis

The amino acid content of five-selected Anchote accessions from the tuber and leaf part were subjected to amino acid analysis. The amino acids were separated and quantified using automated amino acid analyzer (Hitachi L-8800 Amino Acid Analyzer, Tokyo, Japan). The dry powder sample (0.1g) was hydrolyzed in 6 M HCl for 24 hr. at 110 ºC in heating block. After cooling, filtering and washing, the hydrolysate was dried in a vacuum evaporator at temperature of 50 ºC. The dried residue was dissolved in a buffer at pH 3.45.

The prepared sample was analyzed using the ninhydrin method. Ninhydrin solution was buffered at pH 5.5. Buffers ranging from 3.45 to 10.85 were applied. A column 150 mm in length was filled with Ostion ANB INGOS ionex (the Czech Republic).

The temperature of the column was 57-74 ºC and that of the reactor was 120 ºC. Glutamine and asparagine were expressed as glutamic acid and aspartic acid, respectively. Sulphur-containing amino acids, methionine and cystine were determined by means of oxygenating hydrolysis, using a mixture of formic acid and hydrogen peroxide (9:1) at 110 ºC for 16 hr. before acid hydrolysis. After cooling, the sample was processed as with acid hydrolysis by using buffers of pH 2.6 and 3.0. The amino acids recovered by this method were Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, and Val. Norvalene was used as an internal standard to normalize the recovery of each amino acid from injection to injection. The method was calibrated over the range of 0.08-22.7% for each amino acid.

No analysis concerning tryptophan was carried out due to the reason that HCl hydrolysis results in partial destruction of tryptophan and thus requires an alternative hydrolysis procedure for accurate quantification.

3.3.7. Evaluation of Protein Quality

Nutritional qualities of the protein identified in the tested samples of Anchote were determined based on the observed amino acid profiles. The parameters determined included:

The proportion of total essential amino acids (TEAA) to the total amino acids (TAA) of the protein was calculated using the method of Chavan et al. (2001) as depicted in the following equation:

$$TEAA/TAA = \frac{(Ile + Leu + Lys + Met + Cys + Phe + Tyr + Thr + Trp + Val + His)}{(Ala + Asp + Arg + Gly + Glu + His + Ile + Leu + Lys + Met + Cys + Phe + Tyr + Pro + Ser + Thr + Trp + Val)}$$

Note: The essential amino acid tryptophan was not determined in our study. Therefore, tryptophan was excluded in protein quality evaluation requiring essential amino acids.

Amino acid score of the essential amino acid composition was calculated according to Chavan et al. (2001) using the following equation:

$$\text{Amino acid score} = \frac{\text{mg of amino acid per g test protein}}{\text{mg of amino acid per g of FAO/WHO standard pattern}} \times 100$$

Essential amino acid index (EAAI) was calculated according to method by Ijarotimi & Keshinro 2011 using the equation below:

$$EAAI = \sqrt{((n \,\&\, (100a \times 100b \times \ldots 100j)/(av \times bv \ldots jv))}$$

Where :

n = Number of essential amino acids

a, b …..j = Concentration of essential amino acids in the tested sample

av, bv …..jv = Content of the same amino acids in standard protein (%) (egg or casein), respectively

Predicted biological value (P-BV) was calculated according to Mune et al. (2011) using the following equation:

$$P - BV = 1.09 \times EAAI - 11.7$$

The predicted protein efficiency ratio (P-PER) was estimated according to the regression equations adopted from Mune et al. (2011) as given below:

$$P - PER = -0.468 + 0.454\,(LEU) - 0.105\,(TYR)$$

The nutritional index of the food samples was calculated according to Ijarotimi & Keshinro (2013) using the following equation:

$$\text{Nutritional Index (\%)} = EAAI \times \%protein/100$$

3.3.8. Antinutrient Analysis

i) Phytate

Phytate content of the samples was determined according to the method described by Vaintraub & Lapteva (1988). Dried flour samples (40 mg) were extracted with 10 ml of 0.2 N HCl using a mechanical shaker for an hour at an ambient temperature and centrifuged at 3000 rpm for 30 min.

Three ml of clear supernatant solution was poured into 15 ml test tube and 2 ml of Wade reagent (containing 0.03% solution of hydrated Feric chloride ($FeCl_3 .6H_2O$) and 0.3% of sulfosalicilic acid in deionized water) added and homogenized using vortex mixer (Maxi Maxi II) for 5 seconds followed by centrifugation at 3000 rpm for 10 minutes.

Preparation of Phytate Standard Solution

A series of phytic acid (analytical grade sodium phytate; phytic acid dodeca sodium salt hydrate water 10-15 % product of USA, ALDRICH) standard solution with a concentration of 0, 4.5, 9, 18, 24 and 30 mg/lt were prepared using 0.2N HCl with deionizer water. A 3 ml of aliquot standard solution was added into 15 ml of centrifuge tubes containing 3 ml of distilled water used as a zero level (blank). To each tube, wade reagent (1.0 ml) subsequently added and centrifuged as mentioned above for sample preparation to determine phytate content.

The absorbance of the solutions for both the sample and standard were measured at 500 nm using a UV-VIS Spectrophotometer (Beckman DU-64-spectrophotometer, USA). Finally, after colorimetric measurement the phytate contents were calculated by using the slope and intercept (Absorbance = -0.0042 phytic acid µg - 0. 1744, $R^2 = 0.9906$) from the calibration curve (Annex B) with the following equation.

$$\text{Phytic acid } (\mu g/g) = \frac{[(As - Ab) - \text{Intercept}] \times 10}{\text{Slope} \times W \times 3}$$

Where :
As = Sample absorbance
Ab = Blank absorbance
W = Weight of sample in gram

ii) Tannin

Tannin content was determined according to Maxson & Rooney (1972). Samples 0.5 g were weighed into a screw cap test tube and put in a mechanical shaker (Eberbach) for 24 hours extraction with 10 ml 1% HCl in methanol at room temperature.

The extracted solution was centrifuged at 1000 rpm for 5 minutes. A clear supernatant (1.0 ml) was mixed with 5 ml of vanillin HCl reagent (the combined solution of equal volume of 8% HCl in methanol and 4% vanillin in methanol.

Preparation of Condensed Tannin Standard Solution

A standard stock solution was prepared by dissolving 20 mg of (+)- Catechin in 100 ml 1% HCl in methanol. Then a series of standard solutions (0, 12, 24, 36, 48 and 60 mg/lt) were prepared from 1.0 ml stock solution by mixing with 5 ml 1% HCl in methanol. After 20 minutes, the absorbance of samples and the standard solutions were measured at 500nm using UV-VIS Spectrophotometer (Beckman DU-64-spectrophotometer, USA). The standard calibration curve was made by plotting absorbance versus concentration (Absorbance = 0.0053 catechin - 0.0012, R^2 = 0.9980) (Annex C). Finally, the slope and intercept from the calibration curve was used to calculate tannin contents using the following equation:

$$\text{Tannin mg/g} = \frac{[(As - Ab) - \text{Intercept}] \times 10}{\text{Slope} \times d \times W}$$

Where :
As = Sample absorbance
Ab = Blank absorbance
d = Density of solution (0.791g/ml)
W = Weight of sample in gram

iii) Cyanide

The level of total cyanogens in Anchote flour samples were analyzed by Picrate kit protocol (Bradbury, Egan, & Bradbury, 1999). A 100-mg sample of flour was placed on 21mm diameter Whatman 3 MM filter paper in a small flat-bottomed plastic vial (25 mm diam., 50 mm high). The filter paper had been previously loaded with 50µl of 1M phosphate buffer at pH 8 and, after airdrying linamarase solution (60 µl) that contains 1% (w/v) gelatin and 5% (w/v) polyvinylpyrrollidone-10 was added and allowed to air dry (Egan, Yeoh, & Bradbur, 1998).

The color of the picrate paper was compared with that of the color chart that contained ten colors to obtain the amount of cyanogen. The picrate paper was separated from the plastic strip and the color eluted from the filter paper in 5ml water for about 30 minutes. The absorbance of the solution was measured at 510 nm against a blank, which contained a yellow solution produced from a picrate paper not exposed to HCN. Finally, the total cyanide content (ppm) was determined as per the equation described by (Haque & Bradbury, 2002).

$$\text{Total cyanide (ppm)} = 396 \times \text{absorbance} \times 100/z$$

Where:

z = weight (mg) of ground powder (Bradbury et al., 1999)

3.3.9. Statistical Analysis

The Completely Randomized Design (CRD) was used with three replicates. Data were analyzed by one-way analysis of variance (ANOVA) using SAS, 2004 version 9.

The significance of differences in mean values of the quantitative variables among accessions were tested using Duncan's multiple range tests at 5% level of significance and reported as mean ± standard deviation (SD).

3.4. Results and Discussions

3.4.1. Moisture and Dry Matter Content

Moisture and dry matter contents (range and mean) of Anchote tubers and leaves of 44Anchote accessions are shown in Table 8. The detailed result for each accession is compiled in Annex D. Moisture content of Anchote accessions ranged from 70.44±0.35% ('223094') to 80.17±2.86% ('NJ') for tubers, and from 68.31±2.21% ('223090-1') to 75.23±0.65% ('90802') for leaves on fresh weight basis. Significant differences ($P<0.05$) were observed between the highest and lowest ranges of moisture content for both tuber and leaf parts of Anchote accessions.

Table 8 Moisture and dry matter contents (%) of tubers and leaves of Anchote accessions

Parameters	Tuber (N=44)							Leaf (N=44)						
	Max	Min	Mean	SD	CV	F-value	P>F	Max	Min	Mean	SD	CV	F-value	P>F
Moisture	80.17	70.44	75.90	2.13	2.57	2.40	0.0023**	75.23	68.31	72.34	1.17	1.82	1.59	0.0639ns
Dry matter	29.56	19.83	24.10	2.13	8.08	2.40	0.0023**	30.43	22.96	27.38	1.19	5.05	1.47	0.1025ns

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, ** and *** represent significance at $P<0.05$ and $P < 0.01$, $P < 0.001$, respectively.

The mean value of tuber moisture content (75.90 %) is in agreement with that of Habtamu et al. (2013), EHNRI (1997), and Habtamu & Kelbessa (1997) who reported values of, 74.93, 74.50, and 73.00 g/100 gm (equivalent to %) respectively for Anchote tuber. The moisture content of Anchote, also reveals similarity with other root and tuber crops. such as '*Amochi*' cultivars (*Arapaimas chimperianum* Schott, 72.43%) (Andargachew et al., 2011), potato (*Solanum tuberosum*, 76–79% and 81.53%) (Dini, Garcia, & Viña, 2012; Lewu et al., 2010), wild yam (*Dioscorea spp,* 69.5 - 80.2%) (Bhan, Kasai, & Kawabata, 2003), and American yam bean (*Pachyrhizus ahipa* 78.4–83.5%) (Cecilia Dini, Doporto, García, & Viña, 2013). However, the moisture content in this study was higher than the reported values for sweet potato varieties (*Ipomea batatas*, 62.58- 64.34%) (Rose & Vasanthakaalam, 2011), and cassava (*Manihot esculenta*, 59.68 %) (Montagnac, Davis, & Tanumihardjo, 2009).

On the other hand, the mean moisture content of leaves of Anchote (72.34%) is comparable with the reported moisture content of leaves of cassava (*Manihot esculenta*) (72.00%) (Odhav, Beekrum, Akula, & Baijnath, 2007), Aleefu (*Amaranth cruentus*) (72.93 %), *Moringa oleifera* (75.00 %) (Kwenin et al., 2011), Bengal gram (75.10%) (Singh, Kawatra, & Sehgal, 2001)and Baobab (*Adansonia digitata)* (77.00%) (FAO & Redhead, 1990).

However, higher moisture contents were reported for leaves of fresh pumpkin (96.81%), onion (93.14%) (Pedavaoh & Kavaarpuo, 2014), *Amaranthus hybridus* (88.50%) (Mepba, Eboh, & Banigo, 2007), *Amaranthus aquatica* (84.47%), *Telfaira occidentalis* (84.17%) (Gladys, 2011), sweet potato (82.21%) (Antia et al., 2006), and kale (*Brassica oleraceae*) (81.40%) (Emebu & Anyika, 2011b).

The high moisture content of leafy vegetables in general provides for greater activity of water-soluble enzymes and coenzymes needed for metabolic activities (Iheanacho & Udebuani 2009). In addition, higher moisture content has beneficial effect to remove hydrogen cyanide (HCN) content by promoting glycosidase enzymatic reaction (Feng, Shen, & Chavez, 2003). On the other hand, low moisture content confers good stability (keeping quality) and high yield of dry matter (Edem, Dosunmu, & Bassey, 2009; Ijeh, Unaegbu, & Anaga, 2004).

The dry matter content of tuber and leaf of the 44 Anchote accessions ranged from 19.83±2.86% (‘NJ’) to 29.56±0.35% (‘223094’), and 22.96±2.64% (‘DIGGA-1’) to 30.43±4.05% (‘KICHI’), respectively. The mean dry matter content of all tested accessions was 24.10% for tuber and 27.38% for leaves.

The dry matter content shows the chemical potential of the crop and reflects the true biological yield (Teye et al., 2011), and can be influenced by several factors such as the age of the plant, crop season, location, climate, day length, soil type, pest and diseases as well as efficiency of the canopy to trap sunlight (Lain, 1985; Woolfe, 1992).

3.4.2. Proximate Composition

Table 9 and 10 sows means and ranges for the proximate composition parameters including utilizable carbohydrates and gross energy that were measured in the 44 Anchote accessions. The range and mean result of the proximate composition analysis (on dry weight basis) of tuber and leaf part of 44 Anchote accessions are presented in Table 9 and Table 10. Significant differences ($p<0.05$) in nutritional compositions were observed among most of the accessions as indicated in proximate composition results for tubers and leaves (Annex E & F).

Moisture: The moisture content of the tubers was in the range between 5.36± 0.03% to 10.20±0.01% on dry matter weight basis (Table 9), where accession '223090' scored the highest value and accession 'DIGGA' the least (Annex E).

Accessions 'DIGGA', '240407G' and '223109-1' were the top three accessions with high moisture content in their tubers with values of 10.20%, 10.18% and 10.04%, respectively. However, no significant difference ($P>0.05$) was observed between the top two accessions. The overall mean moisture content for the 44 accessions considered in the present study was 7.58%.

Table 9 Proximate composition of tubers for the 44 Anchote accessions on dry weight basis

Parameters	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Moisture (%)	10.20	5.36	7.58	1.34	0.80	988.51	<0.0001***
Crude protein (%)	13.72	5.82	8.50	1.98	2.21	224.22	<0.0001***
Crude fat (%)	0.75	0.24	0.53	0.14	16.35	3.96	<0.0001***
Crude fiber (%)	6.96	3.63	5.05	0.72	4.31	21.48	<0.0001***
Total ash (%)	6.83	4.63	5.41	0.52	3.88	11.23	<0.0001***
Utilizable carbohydrate (%)	84.51	73.89	80.52	2.52	0.49	81.07	<0.0001***
Gross energy (kcal100g^{-1})	368.48	349.14	360.84	4.52	0.38	20.71	<0.0001***

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, **, and *** represent significance at $P<0.05$ and $P < 0.01$, $P < 0.001$, respectively.

Table 10 Proximate composition of leaves for the 44 Anchote accessions on dry weight basis

Parameters	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Moisture (%)	9.79	7.59	8.56	0.43	1.24	32.03	<0.0001***
Crude protein (%)	35.42	8.96	20.46	7.18	3.46	207.11	<0.0001***
Crude fat (%)	4.68	2.44	3.63	0.49	5.01	13.84	<0.0001***
Crude fiber (%)	13.05	7.89	11.33	1.14	3.26	18.40	<0.0001***
Total ash (%)	13.59	10.74	11.96	0.52	1.43	17.30	<0.0001***
Utilizable carbohydrate (%)	63.66	38.15	52.56	6.63	1.74	105.86	<0.0001***
Gross energy (kcal100g^{-1})	334.84	314.00	325.04	5.12	0.58	14.13	<0.0001***

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, **, and *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively.

The moisture content of the tubers of Anchote accessions examined in this study is in agreement with other tuber crops such as water yam (*Dioscorea alata*) (4.11-6.79%), potato yam (*Dioscorea bulbifera*) (7.02%), and bitter yam (*Dioscorea dumentorum*) (6.53%) (Ezeocha & Ojimelukwe, 2012; Ogbuagu, 2008).

With regard to the leaf of Anchote, moisture content ranged from 7.59±0.01 to 9.79±0.02% with the mean moisture content of 8.56% (Table 10). There was significant difference ($p<0.05$) between the highest (accession '223113') and the lowest (accession 'KICHI-1') leaf moisture content of Anchote accessions (Annex F). The moisture content for accession '240407B' (9.41±0.03) and for accession '223096' (9.32±0.04) had significant difference ($P<0.05$) with the rest of accessions but the difference between these two accessions was not significant ($P>0.05$). The values of moisture for leaf part of the tested accessions were comparable with the results obtained for the leaves of *Ficus asperifolia* (9.01%) (Nkafamiya, Osemeahon, Modibbo, & Aminu, 2010), *Amaranthus hybridus (*10.00%) (Asaolu, Adefemi, Oyakilome, Ajibulu, & Asaolu, 2012), pumpkin (9.45%) (Pedavaoh & Kavaarpuo, 2014), *Moringa oleifera* leaves (6.1%) (Bamishaiye, Olayemi, Awagu, & Bamshaiye, 2011) and cauliflower (9.99%) (Baloch, Xia, & Sheikh, 2015).

In the present study, highest moisture content was observed in tubers compared to leaves in fresh and dry weight basis. This is supported with the reports of Hanif et al. (2006)and Rehman et al. (2013)which confirms that moisture contents were found more in other edible parts than the leaf parts of different vegetables. The observed differences in respect of moisture content among the Anchote accessions were attributed to the difference in the genetic composition and cultivation practices (Rose & Vasanthakaalam, 2011).

The high moisture content on the one hand facilitates activities of water-soluble enzymes and co-enzymes needed for metabolic activities of vegetable crops (Iheanacho & Udebuani, 2009). On the other hand, the high moisture content exposes the leafy vegetables to deteriorate rapidly, hence there must be appropriate preservation technique in the storage to prevent deterioration due to microbial attack and excessive transpiration and thereby improve the shelf life (Kaushal, Sharma, & Attri, 2013; Kwenin et al., 2011; Nwofia, Victoria, & Blessing, 2012; Pedavaoh & Kavaarpuo, 2014).

Crude protein: The protein content of the Anchote tubers ranged from 5.82±0.00% in accession '223110' to 13.72±0.10 % in accession '223097' (Table 9). No significant difference ($P>0.05$) was observed between 13.72% in '223097' and 13.35% in '223086' accessions. However, a significant difference ($P<0.05$) was observed with the rest of the crude protein values in the other accessions and accession '223097' and '223086'. The crude protein content of Anchote tuber in the present study was stuck between the range value (4.6-16.4 %) were reported by Desta (2011), but higher than the values (3.00-3.20 %) reported elsewhere (EHNRI, 1997; Habtamu et al., 2013; Habtamu & Kelbessa, 1997).

The variations in crude protein content in tubers can be attributed to variations due to genotype, geographical sources and agronomic practices. The high crude protein content in Anchote tubers are however comparable to those reported in yam (*Dioscorea alata*) (10.27%) (Ezeocha & Ojimelukwe, 2012); taro (*Colocasia esculenta*) (11.00%) (Temesgen Melese & Negussie, 2015), and wild yam (*Dioscorea oppositifolia var. dukhumensis)* (13.80%) (Arinathan, Mohan, & Maruthupandian, 2009).

In contrast, the crude protein content in Anchote tuber were higher than injicama (*Pachyrhizus erosus),* potato *(Solanum tuberosum) and* sweet potato *(Ipomoea batatas)* having values of 1.23, 2.73 and 0.57 % respectively (Noman, Hoque, Haque, Pervin, & Karim, 2007).

The CP content in Anchote tuber was also greater than tubers of cassava (*Manihot esculenta*) 1.00 to 3.00% (Montagnac et al., 2009), '*Amochi*' (*Arisaema schimperianum*) 0.86% (Andargachew et al., 2011) and yams (*Dioscorea spp.*) 1.00–3.00% (Shewry, 2003). The high levels of crude proteins in tubers of Anchote accessions in addition to their excellent starch content make them suitable as complementary source of protein in preparation of diets for children, pregnant women and lactating mothers in rations involving mixtures of legumes and cereals. Crude protein content in leaves of the 44 Anchote accessions ranged between 8.96±0.01 % (for accession '223100') and 35.42±0.05% (for accession '223109-1') with an average crude protein content of 20.50% (Table 10). No significant difference ($P>0.05$) was observed in crude protein content of the top three accessions: '223109-1' (35.42%), '223090-1' (34.58%) and 'DIGGA-1' (34.00%), however significant difference ($P<0.05$) were observed between these three accessions and the rest of the accessions.

The mean protein content of Anchote leaves in the present study was higher than *Xanthosoma sagittifolia* (4.65 + 0.02 %), *Amaranth cruentus* (4.46 + 0.03%), *Talinum triangulare* (5.10 + 0.01%) and *Moringa oleifera* (6.60 + 0.02 %) (Kwenin et al., 2011). However, it was lower than *Moringa oleifera* leaf at different maturity stages, i.e. 10^{th} (early stage), 15^{th} (Mid stage) and 20^{th} (late stage) week after pruning (23.7±0.12 - 28.08±2.75 %) (Bamishaiye et al., 2011). Lower crude protein contents were reported for fresh leaves of pumpkin (4.58%), onion (5.30%) (Pedavaoh & Kavaarpuo, 2014), *Amaranthus aquatica* (3.50%), *Telfaira occidentalis* (4.70%) Gladys (2011), kale (*Brassica oleraceae*) (11.67%) (Emebu & Anyika, 2011b) and raw *Amaranthus hybridus* (4.3%) (Mepba et al., 2007)compared to the present crude protein contents for Anchote leaves (20.46%). However, the crude protein content recorded for Anchote leaves (20.50%) is comparable to the value reported for sweet potato leaves (24.85%) (Antia et al., 2006).

This result tends to suggest that Anchote leaves have higher protein content than tubers. Hence, it could be considered that leaves of Anchote can be good source of protein with the evidence that confirms any plant foods, which have the potential to provide about 12.00% of their calorific value from protein, are considered good source of protein (Effiong et al. 2009; Aberoumand 2010; Nwofia et al. 2012).

Crude fat: The crude fat content in tubers of the 44 accessions was ranging from 0.24±0.05% ('230566') to 0.75±0.07 % ('223112-1') and significant variations ($P<0.05$) were observed in the crude fat content of the accessions ($P<0.05$) as shown in Table 9 and Annex E. In similar parameter, the leaves of the accessions tested in the present study ranged from 2.44±0.27% ('DIGGA-2') to 4.68±0.84% ('223113') Table 10 and Annex F.

The variation in terms of crude fat content between the highest reported value in accession '223113' (4.68%) and accession '240407B' (4.45%), '223112' (4.44%), '220563' (4.37%) and '223105' (4.29%) was not significantly different ($P>0.05$), but significant differences ($P<0.05$) were observed with most of the other accessions. The mean crude fat content recorded for tubers and leaves were 0.53% and 3.63%, respectively. Higher crude fat content was recorded in leaves (3.63%) than tubers (0.53%).

Fat in food determines the amount of energy available. A diet providing 1-2% of its caloric energy as fat is said to be sufficient for human beings as excess fat consumption yields certain cardiovascular disorder such as atherosclerosis, cancer and aging (Sodamade, Bolaji, & Adeboye, 2013). In general, Anchote leaf revealed higher content of crude fat than the tuber and this vividly depicts that Anchote leaf can provide sufficient plant fat in the diet. However, low level of fat found in Anchote tuber would require dietary supplementation from a better source.

The results of the fat content of Anchote tuber in this study were comparable to other tuber crops reported elsewhere (Ekpeyong, 1984; Ezeocha & Ojimelukwe, 2012; FAO, 1990; Ogbuagu, 2008; Treche, 1996). However, the crude fat content was higher than the previous report for Anchote tuber (0.1 to 0.19 %) (EHNRI, 1997; Habtamu et al., 2013; Habtamu & Kelbessa, 1997).

Our result was also higher than cassava root (0.03 to 0.5 %) (Montagnac et al., 2009) and '*Amochi*' cultivars (*Arisaema schimperianum* Schott) (0.13 %) (Andargachew et al., 2011). They were comparable to those reported for cocoyam (0.78%), potato (0.24%) (Lewu et al., 2010) and American yam bean (0.43–0.63%) (Cecilia Dini et al., 2013). Kwenin et al. (2011) reported 1.50% fat content for leaves of *Moringa oleifera* which is lower when compared with the mean crude fat content for the Anchote leaves (3.63%) found in the present study. Bamishaiye et al. (2011) also reported lower crude fat content (2.0%) for *Moringa oleifera* than for the Anchote leaves.

In comparison, crude fat contents in pumpkin, onion, *Amaranthus aquatic, Telfaira occidentalis* and kale leaves were lower and reported to be 1.22, 1.83, 0.24, 0.14 and 0.26 % (Emebu & Anyika 2011b;H. O. Gladys 2011;Pedavaoh & Kavaarpuo 2014), but comparable to those of sweet potatoes (Antia et al., 2006).

Crude fiber: Dietary fiber has recently gained much importance since it can reduce the incidence of cardiovascular diseases and certain digestive diseases. The World Health Organization (WHO) has recommended an intake of 22-23kg of fiber for every 1000 Kcal of diet (Kanwar, Kanwar, & Shah, 1997). The presence of fiber in human diet has its own advantage for digestion by stimulating and accelerating intestinal contraction and for elimination of wastes (A. Ali, Fadimatou, Tchiegang, Saidou, & Adji, 2010; Kana, Aliyu, &Chammang, 2012; Mohan & Kalidass, 2010).

Pectin, cellulose, hemicelluloses together with lignin are classified as dietary fiber (Rose & Vasanthakaalam, 2011). The mean crude fiber content in the tubers and leaves of the 44 Anchote accessions were 5.05% and 11.33%, respectively (Table 9 and 10). The crude fiber ranged from 3.63±0.04% ('240407-1') to 6.96±0.24% ('220563-1') for tubers and 7.89±0.03% ('223109-1') to 13.05±0.08% ('223085') for leaves. Significant differences ($P<0.05$) in crude fiber were observed among tubers and leaves of the tested accessions.

The mean crude fiber content in the present study ranged from 3.63±0.04% to 6.96±0.24% and were higher than previously reported values for Anchote tuber: namely 2.58% (Habtamu et al., 2013), 1.70 % (EHNRI, 1997), and 1.60 % (Habtamu & Kelbessa, 1997).

They were also higher crude fiber content than tubers for yam species *Dioscorea oppositifolia var. dukhumensis* 3.92 % (Arinathan et al., 2009), potato (*Solanum tuberosum*) 0.3–3.67% (Burlingame, Mouillé, & Charrondière, 2009), sweet potato (*Ipomoea batatas*) (3.63–7.08% in dw) (Van Hal 2000), and '*Amochi*' cultivars (*Arisaema schimperianum*) (0.59-0.7%) (Andargachew et al., 2011). However, results in this study were lower than the values reported for yam species such as *Dioscorea oppositifolia* (8.97%) and *Dioscorea pentaphylla var. pentaphylla* (7.04%) (Arinathan et al., 2009).

The fiber content in the Anchote leaves were higher than in leaves of *Moringa oleifera* (1%) (Kwenin et al., 2011), fresh *Amaranthus aquatica* (2.01%) and fresh *Telfaira occidentalis* (2.06%) (Gladys, 2011), sweet potato (7.20%) (Antia et al., 2006), kale (*Brassica oleraceae*) (3.00%) (Emebu & Anyika, 2011b), and raw *Amaranthus hybridus* (1.6%) (Mepba et al., 2007). However, crude fiber values reported by Pedavaoh & Kavaarpuo (2014) for fresh pumpkin leaves (10.50%) and fresh onion leaves (11.02%) were comparable with the mean crude fiber content in leaves of the 44 Anchote accessions (11.33%).

Total ash: The range and mean values of total ash content of tubers and leaves for the 44 Anchote accessions are presented in Tables9 and 10. The ash contents ranged between 4.63±0.31% and 6.83±0.02% in tubers of accessions 'DIGGA' and 'NJ' and 10.74±0.04% and 13.59±0.02% in leaves of accessions 'NJ' and '240407-G'. The mean of total ash content was 6.83% and 11.96% for the tubers and leaves, respectively. The variation in the ash contents in most of the tested accessions was significantly different ($P<0.05$) in both tubers and leaves. The ash content of the top two accessions 'NJ' and '223112' in tubers was not significantly different ($P>0.05$) with values of 6.83% and 6.42%, respectively. The ash content of leaves from accession '240407-G' (13.59%) was significantly ($P<0.05$) higher than the rest of the accessions with the exception of accessions 'DIGGA' (12.79%) and '223090' (12.77%). The values obtained in the tubers of this study is higher than the findings of Habtamu et al. (2013), Habtamu & Kelbessa (1997) and EHNRI (1997) that were 2.19%, 2.00%, and 1.10%, respectively. Whereas, it has similarity with the values reported for potato (4.58%) and greater yam tubers (*Dioscorea alata*) (7.08 %) (Behera, Maharana, Sahoo, & Prusti, 2009; Lewu et al., 2010).

However, all these reports of the tubers were below the values of Anchote leaves of the present study. Lower ash contents were reported for fresh *Amaranthus aquatica* (2.17%) and fresh *Telfaira occidentalis* (1.92%) leaves Gladys (2011), kale (*Brassica oleraceae*) (1.33%) (Emebu & Anyika, 2011b) and for raw *Amaranthus hybridus* (2.2%) (Mepba et al., 2007) when compared to ash content of the leaves in this study. Comparable to the present value of leaves of Anchote (11.96%) was reported by Bamishaiye et al. (2011) for *Moringa oleifera* leaves (10.11%) and for sweet potato leaves (11.10% DM) (Antia et al., 2006). Pedavaoh & Kavaarpuo (2014) reported higher values of crude ash for fresh pumpkin leaves (21.32%) and fresh onion leaves (16.58%) than the present value for the leaf samples (11.96%).

Utilizable carbohydrate: The range and mean values of utilizable carbohydrate content in dry basis of Anchote tubers and leaves of the 44 accessions is presented in Table 9 and Table 10. The mean utilizable carbohydrate contents for the tubers and leaves were 80.52% and 52.56% with the values ranging between 73.89±0.22% (for accession '223097') and 84.51±0.43% (for accession '240407-1') in tubers. In leaves the value ranges between 38.15±0.30% (for accession '223090-1') and 63.66±0.46% (for accession '223099'). Significant difference was observed between the highest and lowest contents in both tubers and leaves. The variation in most of the accessions in utilizable carbohydrate content was significant ($P<0.05$) in both tubers and leaves.

Significant difference ($P>0.05$) was not observed among the top five tuber accessions '240407-1', '223110', '223096', '230566'and '207984' with carbohydrate contents of 84.51%, 84.41%, 84.38%, 83.90% and 83.80%, respectively. Similarly, no significant difference ($P>0.05$) was observed for the utilizable carbohydrate content of the first two top accessions ('223099' and '223100') in leaves. The dry matter content of most root crops is made up of about 60 – 90% carbohydrate and therefore, tuber and root crops are generally rich in carbohydrates (Eka, 1998; Ogbuagu, 2008). The carbohydrate content in Anchote tubers of this study were in agreement with the values reported for other root and tuber crops such as white yam (78%), African yam (83.65%), water yam (83.27%), sweet potato (83.37%), cocoyam (86.58%), and potato (83.21%) (Lewu et al., 2010; Longe, 1986; Odebunmi et al., 2007).

The carbohydrate content of Anchote leaves was higher than *Moringa oleifera* (13.5%) (Kwenin et al., 2011), and '*Amochi*' cultivars (*Arisaema schimperianum*) (25.61 %) leaves (Andargachew et al., 2011). At different maturity stages comparable results was observed for *Moringa oleifera* leaves (52.56%) (Bamishaiye et al., 2011) and sweet potato leaves (51.95 %) (Antia et al., 2006).

Energy: The highest calorific value on dry matter basis was observed in accession '240407-1' (368.48±0.24 kcal/100g) and '223105-1' (334.84±0.41 kcal/100g) for tubers and leaves, respectively (Table 9 and Table 10). In accession '220563-1' (349.14±0.10 kcal/100g) for tubers, and in accession '240407-G' (314.00±1.35 kcal/100g) for leaves lowest energy contents were recorded. Significant difference ($P<0.05$) was observed between the highest and lowest energy values in both the tuber and the leaf part. However, no significant difference ($P>0.05$) was observed in energy values 368.48, 367.21, 366.71, 366.67, 366.06 and 365.48 kcal/100g among six accessions that are '240407-1', '223094', '207984', '223096', '223101' and '223110' in tubers, respectively. Similarly, there was no significant difference ($P>0.05$) in energy values of leaves among the top five accessions: '223105-1' (334.84 kcal/100g), '223097-1' (333.61 kcal/100g), '223100' (332.90 kcal/100g), '223112-1' (332.34 kcal/100g) and 'KICHI' (331.15 kcal/100g). The mean energy contents in the tubers and leaves were 360.84 kcal/100g and 325.05 kcal/100g, respectively showing that the tuber part was relatively higher than leaf part in energy value. The energy values of Anchote tuber (349.14-368.48 kcal/100g) observed in this study are comparable with the value reported from energy-rich tubers of cocoyam (*Colocasia esculenta*) 378.93 kcal/100 g, potato (*Solanum tuberosum*) 376.30 kcal/100 g and water yam (*Dioscorea alata*), 357.65 kcal/100 g, (Ezeocha & Ojimelukwe, 2012; Lewu et al., 2010). However, the values obtained in the leaf are lower than these literature values. The energy content of Anchote leaves in this study was much higher than the energy content of *Moringa oleifera* (90.20 kcal/100g) as reported by Kwenin et al. (2011), and kale (*Brassica oleraceae*) (58.46 kcal/100g) (Emebu & Anyika, 2011b)but it is comparable with sweet potato leaves (351.30 kcal/100g) (Antia et al., 2006). Generally, roots and tubers contribute a lot by providing the cheapest source of energy for the poor in developing nations (Temesgen Melese & Negussie, 2015).

3.4.3. Amino Acid Composition of Five Selected Anchote Accessions

Proteins are composed of different amino acids and hence the nutritional quality of a protein is determined by the essential amino acids content, proportion and availability of the amino acids (Becker 2007). The uppermost five Anchote accessions in crude protein content out of 44 accessions were selected in both tubers and leaves to determine the amino acid profile (Table 11). Seventeen amino acids namely alanine (Ala), arginine (Arg), aspartic acid (Asp), cysteine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylamine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val) were analyzed for this study.

Tryptophan was not analyzed for the reason that acid hydrolysis results to its complete destruction and therefore requires an alternative hydrolysis procedure for accurate quantification (Wathelet, 1999). The amino acids profile for proteins in Anchote tuber showed that arginine (6.50 - 9.52 g/100g protein) was highest in content, while methionine (0.30-0.40 g/100g protein) was lowest in four accessions, namely ‘223097’, ‘223087-1’, ‘223085’, and ‘223090-1’.

Table 11 Amino acid composition in tubers and leaves of five selected accessions (g/100g protein dry weight basis)

Amino acids	Tuber					Leaf				
	223097	223087-1	223085	223090-1	NJ	223109-1	223090-1	DIGGA-1	KICHI	240407-1
Essential amino acids										
Histidine	0.62	0.56	0.63	0.89	0.70	1.34	1.44	1.36	2.39	1.62
Isoleucine	2.73	2.48	2.71	3.45	4.31	3.23	3.39	3.24	5.06	3.56
Leucine	3.59	3.12	3.43	4.34	5.32	5.21	5.49	5.15	5.42	5.65
Lysine	2.42	1.92	2.35	3.05	3.61	3.61	3.74	3.57	4.07	4.01
Methionine	0.31	0.40	0.36	0.30	1.10	0.89	0.94	0.87	0.98	0.96
Phenylalanine	1.72	1.52	1.62	2.17	2.61	2.96	2.85	2.87	3.42	3.39
Threonine	2.81	2.40	2.71	3.35	4.01	3.26	3.39	3.21	3.82	3.63
Valine	2.89	2.48	2.98	3.55	4.51	4.03	4.25	4.02	4.12	4.42
TEAA	17.10	14.88	16.78	21.09	26.18	24.53	25.50	24.29	29.28	27.25
Conditionally essential amino acids										
Arginine	8.51	9.52	8.03	6.50	7.02	3.12	3.43	5.07	6.28	3.75
Cystine	1.41	1.36	1.62	1.68	1.81	3.55	3.29	3.64	2.92	2.97
Glycine	2.73	2.56	2.80	3.25	4.51	5.84	6.18	5.35	4.69	6.39
Proline	0.94	0.80	0.90	0.69	0.60	1.78	2.01	2.66	2.30	2.05
Tyrosine	0.86	0.72	0.90	1.08	1.50	1.49	1.56	2.18	1.27	1.67
TCEA	14.44	14.96	14.26	13.20	15.45	15.78	16.47	18.90	17.46	16.83
Non-essential amino acids										
Alanine	3.43	3.12	3.43	3.94	5.92	5.58	5.88	5.03	5.17	6.22
Aspartic acid	4.76	4.08	4.60	5.71	7.42	8.39	9.21	7.49	9.35	10.28
Glutamic acid	5.62	5.36	5.23	3.84	3.31	8.86	9.21	7.87	8.17	10.47
Serine	3.20	2.72	3.16	3.74	4.61	4.17	4.33	4.32	4.47	4.64
TNEA	17.02	15.28	16.42	17.24	21.26	27.00	28.63	24.71	27.14	31.61
TAA	48.55	45.12	47.46	51.53	62.89	67.31	70.60	67.89	73.89	75.69

TEAA: Total essential amino acid; TCEA: Total conditionally essential amino acid; TNEAA: Total non-essential amino acid; TAA: Total amino acid

Aspartic acid with 7.42 g/100g protein was highest, followed by arginine at 7.02 g/100g protein while proline was the least at 0.60 g/100g protein in accession 'NJ'. For the leaves, glutamic acid was highest in content (7.87 - 10.47g/100g protein) in accessions '223109-1', '223090-1', 'DIGGA-1' and '240407-1' with the exception of accession 'KICHI' in which aspartic acid (9.35 g/100g protein) was the most abundant of all the tested amino acids; while the limiting amino acid in all the tested leaves was methionine. Similar to our result high amount of glutamic acid was also observed in previously reported plant based protein (Adeyeye, 2004; Ijarotimi & Keshinro, 2013; Ogungbenle, 2011; Olaofe, Adeyemi, & Adediran, 1994; Oshodi, Esuoso, & Akintayo, 1998). The most abundantly found amino acids in Anchote tuber (Arg, and Asp) were in close agreement with the reported literature for *Dioscorea* species and cassava tubers (Babu, Nambisan, Sundaresan, & Abraham, 1990; Montagnac et al., 2009). In parallel observation, the abundant amount of Glu and Asp in Anchote leaves was in accordance with the observations reported for *Amaranths hybridus* leaves (Akubugwo et al., 2007).

The essential amino acids (His, Ile, Leu, Lys, Met, Phe, Thr, Try and Val) tested in this study ranged from 32.98 – 41.63% in tubers with mean value of 37.22%, and from 35.78 – 39.63% in leaves with mean value of 36.79%. Among the essential amino acids, leucine was found to be the dominant amino acid in all accessions tested with values ranging from 3.12 to 5.32 g/100g protein for tubers and from 5.15 to 5.65 g/100g protein for leaves. The highest leucine values were recorded in accession 'NJ' (5.32 g/100g sample) and '240407-1' (5.65 g/100g sample) for tubers and leaves, respectively. The least dominant essential amino acid was methionine in both tubers and leaves. The limited amount of methionine observed in our study was in agreement with Babu et al. (1990) for the germplasm accessions of *Dioscorea* species, and Van Hal (2000) for sweet potato cultivars.

Total percentage for conditionally essential amino acids (Arg, Cys, Gly, Pro and Tyr) ranged from 24.56 to 33.16 % with mean value of 28.62% for tubers, while for the leaves it was 22.24 to 27.83 % with mean value of 24.10%. The non-essential amino acids (Ala, Asp, Glu and Ser) percentage values were in a range from 33.87 to 42.92 % (mean= 34.16%) and 36.39 to 41.76% (mean=39.11%) for tubers and leaves, respectively.

Arginine was the most abundant amino acid from the conditionally essential amino acids in all the studied accessions for tubers and in one of the accession evaluated for leaves ('KICHI') with values ranging from 6.28 to 9.52 g/100g protein, whereas, glycine was the highest amino acid for the rest of the accessions tested for leaves. With regard to non-essential amino acids, glutamic acid was dominantly found in tubers (5.23 – 5. 62 g/100g protein) and leaves (7.87 – 10.47 g/100g protein) except in accession '223090-1' and 'NJ' for tubers, and in accession 'KICHI' for leaves in which aspartic acid was the highest value. These results are common with most vegetable protein (El-Adawy, Rahma, El-Bedawey, & Gafar, 2001; Mune et al., 2011; Ogunlade, Olaifa, Adeniran, & Ogunlade, 2011; Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999).

Out of the total amino acids in our result, the mean percentage value of dispensable/non-essential amino acids was found to be higher in concentration (62.78% and 63.21%) compared to indispensable/essential amino acids (37.22% and 36.79%) for tubers and leaves, respectively. Similar observations were also reported in previous studies (Akubugwo et al., 2007; Aremu, Olaofe, & Akintayo, 2006; Hassan & Umar, 2006). The total amino acid (TAA) ranged between 45.12 to 62.89 g/100 g protein in tubers and 67.31to 75.69 g/100g protein in leaves with the absence of the essential amino acid tryptophan that was not analyzed in the present study. The TAA values in all the tested accession for the leaves and accession 'NJ' (62.89 g/100 g protein) for tubers were higher than the value of 56.6 g/100g crude protein of the reference egg protein (Paul, Southgate, & Russell, 1980).

In general, the average amino acid content was higher in leaves (71.08g/100g protein) compared to the tubers (51.11g/ 100g protein). This is associated with protein content of the samples as highest crude protein content was recorded in leaf part (35.42%) compared to the tuber higher protein content (13.72%). This observation is in close agreement with the report of Kenyon et al. (2006) that states leaf and vine of sweet potato are high in total amino acids than the tubers. A balanced or high-quality protein contains essential amino acids in ratios commensurate with human needs that can be determined by comparing the amino acid contents of various proteins with the FAO reference pattern.

The FAO reference pattern based on the essential amino acid requirements of young children (1-2 years) is considered the preferred reference protein (Cheftel, Cuq, & Lorient, 1985). The average proportions of the essential amino acid profile of Anchote tubers and leaves are compared with the FAO/WHO (2007) reference pattern (Table 12).

Table 12 Comparison of mean (n=5) essential amino acid composition of Anchote tuber and leaf with the FAO/WHO standard reference pattern

EAAs (g/100 g protein)	Tuber	Leaf	FAO/WHO* (1-2 yrs. age children)
His	0.68	1.63	1.80
Ile	3.14	3.70	3.10
Leu	3.96	5.38	6.30
Lys	2.67	3.80	5.20
Met	0.49	0.93	-
Phe	1.93	3.10	-
Thr	3.06	3.46	2.70
Trp	-	-	0.74
Val	3.28	4.17	4.20
SAAs	2.07	4.20	2.60
AAAs	2.94	4.73	4.60

Source: * FAO/WHO (2007), Essential amino acids (EAAs), Sulphur amino acids (SAAs), Aromatic amino acids (AAAs,)

Our study revealed that all the essential amino acids used for analysis (except tryptophan which is not tested in this study) were presented in both tuber and leaf of Anchote. With few exceptions, the essential amino acids of Anchote had adequate quality of essential amino acids compared to the references. In our study, methionine and histidine were low in amount for both tubers and leaves. This could be due to two possible reasons; they might be denaturized during analysis or might be found in limited amount in Anchote protein. The low availability of methionine is in accordance with previous studies (Van Hal 2000; Montagnac et al. 2009). To compensate this limitation in Anchote, additional consumption of animal or plant proteins such as milk, egg, lentils and pulses are highly recommended (Andini, Yoshida, & Ohsawa, 2013).

Essential amino acids such as isoleucine (3.70 g/100g protein), threonine (3.46 g/100g protein), sulfur amino acids (4.20 g/100g protein) and aromatic amino acids (4.73 g/100g protein) in leaf part and isoleucine (3.14 g/100g protein) and threonine (3.06 g/100g protein) in tuber part were found in higher amount.

These essential amino acids were relatively higher than the reported values by FAO/WHO (2007) set as reference standards (isoleucine: 3.10 g/100g protein, threonine: 2.70 g/100g protein, sulfur amino acids: 2.60 g/100g protein and aromatic amino acids: 4.60 g/100g protein). These results suggest that Anchote can be exploited for those essential amino acids which are found in adequate amount in either of its edible part to enhance protein quality especially when preparing complimentary food products.

3.4.4. Protein Quality

The nutritional quality of a food protein depends on the kinds and amounts of amino acids it contains, and represents a measure of the efficiency with which the body can utilize the protein (Chawanje et al. 2001). Our results on the nutritional parameters of Anchote tuber and leaf were determined based on their amino acid profile and presented in Table 13. While it is known that cystine can spare part of the requirement for methionine, the total of the two (methionine and cystine) amino acids should be identified for more satisfactory scoring purposes (FAO/WHO 2007). The Sulphur containing amino acids (methionine and cystine) in Anchote leaves were found in a relatively higher quantity (4.20 g/100g protein) than the required reference pattern (2.2 - 2.8 g/100g protein or 22 -28 mg/g protein) set for all age group by FAO/WHO (2007), whereas the tubers were found to be below the recommended value. This might be because of the reason that Anchote leaf protein contains substantially more cystine than methionine that is in close agreement with many vegetable proteins, especially in legumes (FAO/WHO, 2007). The aromatic amino acids of Anchote tuber and leaf were 2.94 and 4.73 g/100 g protein. Aromatic amino acids (phenylalanine and tyrosine) content of the leaves fall within the ideal range (3.8 -4.6 g/100g protein or 38 – 46 mg/g protein) of amino acids requirement suggested by FAO/WHO (2007) for different age groups except for ideal infant (5.2 g/100g protein or 52 mg/g protein) requirement.

Table 13 Estimated nutritional quality of protein for Anchote tubers and leaves based on their amino acid profile

Nutritional quality of Anchote proteins	Tuber	Leaf
TSAA(Met+Cys) (g/100g protein)	2.07	4.20
TArAA (Phe+Tyr) (g/100g protein)	2.94	4.73
Leu/Ileu ratio	1.26	1.46
TEAA/TAA%	37.57	36.82
TNEAA/TAA%	62.43	63.18
TEAA/TNEAA ratio	0.60	0.58
P-PER	1.22	1.80
EAAI (%)	35.28	53.93
P-BV (%)	26.76	47.09
Nutritional index (%)	4.11	17.71
Amino acid score	73	108

Total aromatic amino acids (TArAA), Total Sulphur amino acids (TSAA), Total essential amino acids (TEAA), Total non-essential amino acids (TNEAA), Total amino acids (TAA), Histidine (His), Arginine (Arg), Leucine (Leu), Isoleucine (Ile), Protein efficiency ratio (PER), Essential amino acid index (EAAI), Biological value (BV)

The leucine/isoleucine ratios of Anchote tubers (1.26) and leaves (1.46) were lower than the reported literature values of Bambara bean flour (2.10) and protein concentrate (2.21) by Mune et al. (2011) which is in the ideal range of FAO/WHO (1991) recommendation. Deosthale et al. (1970) showed that excess leucine in foods interfered with the utilization of isoleucine and lysine. The percentage ratio of essential to total amino acids (TEAA/TAA) was 37.57% and 36.82% for tubers and leaves, respectively. This value is higher than 26% for children and 11% for adults but slightly lower than 39% for infants that considered as an adequate ideal protein quality (Oyarekua & Eleyinmi, 2004).

The average predicted protein efficiency ratios (P-PER) for tubers and leaves of Anchote were 1.22 and 1.80. These values were higher than P-PER reported for sorghum ogi (0.27) (Oyarekua & Eleyinmi, 2004) and *L. sativum* negative to 0.03 (Salunkhe & Kadam, 1989).

However, these values were lower than the P-PER value reported for whole hen's egg (2.88) (Paul et al., 1980), reference casein (2.50) and modified corn ogi (4.06) (Oyarekua & Eleyinmi, 2004); but favorably comparable to cowpea (1.21) and pigeon pea (1.82) (Salunkhe & Kadam, 1989), as well as millet ogi (1.62) (Oyarekua & Eleyinmi, 2004). The essential amino acid index (EAAI) of Anchote tuber (35.28%) were higher than fermented popcorn-African locust bean (29.19%) and lower than fermented popcorn-bambara groundnut (40.72%) and fermented popcorn-African locust bean-bambara groundnut (47.38%),whereas Anchote leaf (53.93%) was higher than the EAAI in the blended flours samples (Ijarotimi & Keshinro, 2013). According to Ijarotimi & Keshinro (2011) EAAI value can be useful as a rapid tool to evaluate the protein quality for food formulations.

The Predicted Biological Value (P-BV) of Anchote tuber sample (26.76%) was lower than Anchote leaf sample (47.09%). The P-BV for Anchote tuber was more than that reported for fermented popcorn-African locust bean flour blend (20.13%) (Ijarotimi & Keshinro, 2013), *Citrullus colocynthis* (12.83%) (Ogundele, Oshodi, & Amoo, 2012), fermented popcorn (3.15%) and germinated popcorn (10.53%) (Ijarotimi & Keshinro, 2011). The P-BV of the leaf samples was higher than that of beach pea protein isolates (36.5-40.13%) (Chavan et al., 2001), raw popcorn flour samples (36.45%) (Ijarotimi & Keshinro, 2011), flour blends made from fermented popcorn-bambara groundnut (32.69%) and fermented popcorn-African locust bean-bambara groundnut (39.94%) (Ijarotimi & Keshinro, 2013). The P-BV values in Anchote leaf samples were in agreement with those for plant based proteins (45%) (Huge, 2011; Ogundele et al., 2012). The nutritional index was 4.11% and 17.71% for the Anchote tuber and leaf samples, respectively.

Formulated complementary food samples of plant based protein (5.98–12.73%) reported by Ijarotimi & Keshinro (2013) were lower than leaf sample but higher than tuber sample of Anchote in our study. The amino acid score is the ratio of the amino acid content in the sample protein to the content of the same amino acid in the requirement pattern. The amino acid score of Anchote tuber (73) was lower when compared to beach pea protein isolates (108-110), while the content in Anchote leaf (108) had a similarity with this report (Chavan et al., 2001).

3.4.5. Anti-Nutritional Factors

Increasing the consumption of root and tuber crops has raised concern about the effects of the anti-nutritional factors on human health, which interfere with the absorption of nutrients. For example, phytate forms insoluble complexes with minerals such as calcium, zinc, iron and copper. Tannins chelate metals such as iron and zinc and hence the absorption of these nutrients will be reduced (Sarkiyayi & Agar, 2010). They also inhibit digestive enzymes and may precipitate proteins (Beecher, 2003). In the present study among the anti-nutritional factors, tannin, phytate and cyanide were analyzed and results discussed.

Tannin: The tannin content in the tested 44 Anchote accessions varied from 9.83±5.74 mg/100g to 329.92±4.39mg/100g for tubers and 54.11±6.84 mg/100g to 385.63±6.27 mg/100g for leaves as shown in Table 14. Accession '223087-1' and 'NJ' had the highest tannin contents while '220563-1' and '240407-1' had the lowest tannin levels both in tubers and leaves (Annex G). Significant differences ($P<0.05$) were observed among most of the tested accessions. The mean tannin content for tubers and leaves of Anchote were 112.02 mg/100g and 216.53 mg/100g, respectively. Accessions '223087-1', '223090' and '223092' were the top three accessions with high levels of tannin in tubers and the difference among these three accessions was significant ($P<0.05$). However, accessions '220563', 'DIGGA' and 'KICHI-1' were with the least tannin content with values of 9.83 mg/100g, 14.33 mg/100g and 17.19 mg/100g, respectively without significant (>0.05) difference among the values. Similarly, accessions 'NJ', '223101' and '223099' recorded the top three values of tannin content in leaves of the tested accessions with significant difference ($P<0.05$) between the first and the latter two accessions. On the other hand, accession '240407-1', '223109-1' and '223113' were the bottom three accessions with relatively low content of tannin with values of 54.11 mg/100g, 95.17 mg/100g and 113.57 mg/100g, respectively. The tannin content of some of our accessions (about 8 of them) was higher than that reported by Habtamu et al. (2013) for an Anchote tuber which had a tannin content of 173.55 mg/100g. On the other hand, the tannin content of all our accessions was lower than uncooked tubers of cocoyam (4216.00 mg/100g) and potato (4001.33 mg/100g) (Lewu et al., 2010).

Lower tannin content was also reported for taw taro flour (47.69 mg/100g) (Adane, Shimelis, Negussie, Tilahun, & Guleleat, 2013) and for sweet potato leaves (0.21 mg/100g) (Antia et al., 2006) than Anchote tubers and leaves. The tannin content of *Hibiscus cannabinus* (2.74mg/100g) and *Haematostaphis barteri* (4.92 mg/100g) leaves have lower tannin content compared to Anchote leaves (Kubmarawa, Andenyang, & Magomya, 2009).

These authors also proved that boiling and fermentation significantly reduced the values of antinutritional factors. The tannin content of *Amaranthus hybridus* leaves (0.49 mg/100g) (Akubugwo et al., 2007) was also much lower than the present values in both tubers and leaves.

Phytate: Among the studied Anchote accessions, the lowest level of phytate was recorded in accession 'DIGGA-1' (20.38±0.95mg/100g) while the highest was in accession '223086' (325.03 mg/100g) for tubers on dry matter basis with significant difference between the two extremes (Table 14). Similarly, the higher phytate content was recorded for accession 'DIGGA-2' (295.98±4.41 mg/100g) while the lowest for accession '223101' (183.25±6.27 mg/100g) in Anchote leaves (Table 14 and Annex G). Accession 'DIGGA-1', '229702' and '229702-1' registered relatively lower contents of phytate in their tubers with values of 20.38 mg/100g, 36.39 mg/100g and 37.73 mg/100g, respectively. Similarly, accession '223101', '223105-1' and '223097' had relatively the least phytate contents with values of 183.25 mg/100g, 203.09 mg/100g and 211.11 mg/100g, respectively with significant difference ($P<0.05$) among each other. The mean phytate content in tubers and leaves were 131.10 mg/100g and 250.30 mg/100g, respectively. The present finding revealed that both tannin and phytate contents were higher in leaves than in tubers.

In the study of Habtamu et al. (2013) the value of raw Anchote tuber contained 389.30 mg/100g phytate on fresh weight basis that was higher than the value obtained for both tubers and leaves of Anchote in the present study in dry weight basis. The reported value of phytate in raw yam tubers ranged from 46 to 72 mg/100g (Bhandari & Kawabata, 2006), in fresh weight basis, which is relatively low when compared with the mean phytate contents of both the leaves and tubers.

In the present study, the phytate content for both tubers and leaves of Anchote is higher than the value reported for uncooked potato tuber (37.56±0.10 mg/100g) and uncooked cocoyam tuber (87.48±1.36 mg/100g) on dry basis (Lewu et al., 2010). Adane et al. (2013) also reported a relatively lower (115.43 mg/100g) phytate content for raw taro flour than the present values for the tuber and leaves of Anchote. Kubmarawa et al. (2009) reported lower phytic acid content for leaves of *Hibiscus cannabinus* (19.78 mg/100g) and *Haematostaphis barteri* (17.80 mg/100g).

Table 14 Anti-nutritional content (mg/100g dry weight basis) of tubers and leaves of Anchote accessions

Parameters	Tuber (N=44)							Leaf (N=44)						
	Max	Min	Mean	SD	CV	F-value	P>F	Max	Min	Mean	SD	CV	F-value	P>F
Tannin	329.92	9.83	116.31	76.23	16.21	32.27	<0.0001***	385.63	54.11	216.53	76.31	2.59	370.36	<0.0001***
Phytate	325.03	13.22	126.64	66.85	19.81	14.35	<0.0001***	295.98	183.25	250.30	27.48	1.37	129.12	<0.0001***

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, ** and *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively.

Generally, the present values revealed that both tuber and leaf samples of Anchote accessions constituted of higher phytate content when compared with other root and tuber crops. Reduction of phytate with different processing techniques such as cooking, fermentation and drying is expected to enhance the bioavailability of proteins and dietary minerals and at the same time, the lower level of phytate have some health promotional activities. Currently there is evidence that dietary phytate at low level may have beneficial role as an antioxidant, anticarcinogens and likely play an important role in controlling hypercholesterolemia and atherosclerosis (Harland & Morris, 1995; Phillippy, Lin, & Rasco, 2004).

Cyanide: Cyanide was among the anti-nutritional factors considered in the present study and the result of the cyanide analysis of the selected eleven Anchote accessions based on their high protein content is presented in Table 15.

The residual cyanogens, linamarin and acetone cyanohydrins, are the apparent source of cyanide toxicity to animals when converted to cyanide inside the body (Abebe, 2013). Cyanide is very poisonous because it binds itself to an enzyme called cytochrome oxidase and stops its action in respiration, which is a key energy generating process in the body. The hydrogen cyanide (HCN) toxicity is mostly due to its ability to combine reversibly with enzymes associated with cellular respiration thus suppressing natural respiration and causing cardiac arrest (Rosling 1993; Francis et al. 2001; Conn 2002).

The value of cyanide contents in tubers of the evaluated eleven Anchote accessions ranged between 11.78±1.56 mg/kg (‘223086-1’) and 15.93±1.03 mg/100kg (‘223093’) with average cyanide content of 13.08 mg/kg. Similarly, the cyanide content in leaves of the tested accessions ranged between 10.65±0.47 mg/kg (‘240407-1’) and 14.78±0.70 mg/kg (‘223087-1’) with the mean cyanide content of 12.36 mg/kg. Significant difference ($P<0.05$) was observed between accessions having the highest and lowest cyanide content. Of the tested accessions, about 64% and 55% were below the mean cyanide content for the tested accessions in the tubers and leaves, respectively.

Processing methods could be effective to reduce the amount of HCN down to tolerable levels. In line with this there is an evidence of reducing the cyanide content in cassava root by 70 to 80% by using different processing techniques such as effective cooking, washing, drying and fermentation (Guédé, Traoré, & Brou, 2013; Kobawila, Louembe, Keleke, Hounhouigan, & Gamba, 2005). The mean cyanide content of the present study (13.08 mg/kg in tuber and 12.36 mg/kg in leaves) was much lower than the average cyanide content found in cassava accessions (>500 mg/kg having an average of 30–50 mg HCN/kg) (Cardoso et al., 2005; Dufour, 1994; Mlingi & Bainbridge, 1994; B. Nambisan, 1994).

The values of the present study are also much lower than the values reported for cassava leaves of different species and processing techniques ranged between 352.4 mg/kg and 2179.7 mg/kg (Umuhozariho et al., 2014) and for sweet potato leaves (302.4 mg/kg) (Antia et al., 2006).

Although the permissible limit of cyanogens set by WHO is 10 mg HCN/kg (FAO/WHO 1991; Omolara 2014), there are research reports that state the lethal dose for humans is 50 mg HCN/kg and the acceptable limit could reach up to 40 mg HCN/kg (Damardjati, Widowati, & Rachim, 1993; Djazuli & Bradbury, 1999; Guédé et al., 2013).

In view of the present findings, the cyanide content of the tested Anchote accessions falls far below the lethal dose of cyanide content that is assumed to be 0.5-3.5 mg HCN/kg body weight for human intake by ingesting through the mouth, which amounts 30-210 mg HCN for 60 kg adult (Montgomery, 1980; Solomonson, 1981b). The lethal doses of HCN are generally reported to be between 0.66 and 15 mg HCN/kg body weight for various species of animals (Speijers, 1993).

Despite the fact that cyanide content of Anchote tubers and leaves were relatively lower than the lethal dose, both tubers and leaves of the plant need to be processed properly in order to reduce the cyanide and other anti-nutritional factors before using it for human consumption.

Table 15 Total cyanide levels (mg HCN /kg) in tubers and leaves of 11 selected Anchote accessions

Tuber Accessions	HCN	Leaf Accessions	HCN
223086-1	11.78±1.56^{c}	240407-1	10.65±0.47^{d}
223097	11.51±0.09^{c}	223094	11.32±1.17^{cd}
223087	11.58±1.30^{c}	34.1	11.65±0.64^{cd}
223096-1	12.13±0.63^{bc}	DIGGA-2	11.65±1.28^{cd}
223087-1	12.85±0.51^{bc}	223109-1	11.66±0.28^{cd}
223098	12.90±0.18^{bc}	223088	11.80±1.26^{cd}
223100	12.96± 0.89^{bc}	223097	12.53±1.11^{bcd}
NJ	13.17±1.40^{abc}	223090-1	12.74±1.31^{abcd}
223090-1	14.02±0.86^{abc}	DIGGA-1	12.93±0.26^{abc}
GM	15.03±2.51^{ab}	KICHI	14.24±0.29^{ab}
223093	15.93±1.03^{a}	223087-1	14.78±0.70^{a}
Mean	13.08	Mean	12.36

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$)

3.5. Conclusion

In conclusion, the present study pertaining to the nutritional and anti-nutritional composition of different accessions showed that both the tuber and leaf parts of Anchote (*Coccinia abyssinica*) have appreciable amount of calorific value, carbohydrate, crude protein, crude fiber, ash content, and essential amino acids such as leucine, valine, isoleucine, threonine, and lysine. Comparison between the leaf and tuber parts of Anchote depicted that the leaves demonstrate relatively higher nutrient composition, in all accessions, which provide a good scientific evidence to diversify the consumption habit of indigenous people who are growing Anchote mainly for its tuber, the principal edible part of the crop. Moreover, Anchote could serve as a potential plant food to alleviate protein-energy malnutrition by providing useful nutrients to the diet. Based on the existing evidence, both tubers and leaves are nutritious which makes Anchote a double purpose crop and hence can serve as a potential plant food for the indigenous inhabitants of Anchote growing areas to ensure food and nutrition security. Anchote is also rich in quality protein and can provide essential amino acids as source of protein supplement for all age groups in order to meet the recommendations of WHO/FAO/UNU. There was also a great variability in protein levels and amino acid composition among the Anchote accessions in tuber and leaf parts, which makes possible the selection of protein-rich accessions to overcome low level of protein through selection and hybridization. Genetic improvement in terms of modification of the amino acid composition and amounts can be envisaged in order to improve the availability and quality of protein. Considering the significant variation that exists among the different Anchote accessions, the present study opens up an opportunity for exploiting genetic potential of the accessions that are characterized by low anti-nutritional content. Moreover, among alternatives that can reduce the toxicity of anti-nutrients to the minimum level and ensure safety to consumers, the use of effective processing methods including cooking, fermentation, soaking and drying is quite viable.

Chapter Four: Essential and Toxic Metal Contents of Tubers and Leaves of 44 Anchote (*Coccinia abyssinica*) (Lam.) (Cogn.) Accessions and Estimated Bioavailability of Calcium, Iron, and Zinc

4.1. Abstract

Tubers and leaves of 44 Anchote accessions were analyzed for major (sodium, phosphorus, potassium, calcium, and magnesium), minor (iron, zinc, copper, manganese, and boron) and 3 potentially toxic trace (arsenic, cadmium, and lead) mineral elements using inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-optical emission spectrometry (ICP-OES) following standard procedures. The mineral contents of tuber samples in mg per 100 g dry weight were Na (42.78–98.78), P (14.09–48.64), K (13.63-95.28), Ca (80.64–372.16), Mg (9.33–59.36), Fe (0.39–2.92), Cu (0.10–0.21), Zn (0.22–0.53), Mn (0.14–0.35) and B (2.14 – 7.04). As for leaf samples, minerals detected included Na (30.45 – 92.24), P (42.46 –79.15), K (107.72-178.91), Ca (64.10 –226.95), Mg (29.72 – 70.65), Fe (1.58 – 18.65), Cu (0.47–1.60), Zn (0.32 – 3.41), Mn (0.97 – 1.85), and B (2.14 – 7.04) (mg/100g). The heavy metals Cd, As and Pb were found in mean concentrations of 0.86, 0.83 and 7.05 ng/g, respectively in tubers, and 1.29, 2.62 and 13.53 ng/g in leaves. The mean phytate: calcium molar ratios in tubers and leaves were 0.05 and 0.11, respectively. Similarly, the mean phytate: iron, phytate: zinc and phytate x calcium: zinc molar ratios for tubers and leaves were 3.81 and 4.31, 27.79 and 22.47 and 142.20 and 90.72, respectively. Concentration of phytate phosphorous was in the range of 5.71 to 91.01mg/100g (mean 36.71 mg/100g) for tubers and 42.46 to 79.15 mg/100g (mean 64.63 mg/100g) for leaves. The mean phytate: calcium molar ratios in the tuber and leaf samples in the present study were 0.05 and 0.11, respectively. Similarly, the mean phytate: iron, phytate: zinc and phytate x calcium: zinc molar ratios for tubers and leaves were 3.81 and 4.31, 27.79 and 22.47 and 142.20 and 90.72, respectively. The study shows that Anchote is a rich source of essential minerals with negligible amount of toxic metals. The bioavailability of calcium, iron and zinc were satisfactory. Hence, cognizant of the rich essential nutrients and low level of anti-nutrients, it is pertinent to recommend wider consumption of Anchote tubers and leaves as standalone food or blended with other types of foods.

4.2. Introduction

In Ethiopia, root and tuber crops have a great contribution in the traditional food system and income generation specifically in Southern, South Western and Western part of the country (Solomonson 1981; Andargachew et al. 2011). Anchote [*Coccinia abyssinica* (Lam.) Cogn.] is among the root and tuber crops which are grown in the Western and South-Western parts of Ethiopia. Anchote is a vine like cucurbit used as food ingredient and medicinal item (Girma & Hailu, 2007; Yassin et al., 2013).

The nutritional value of minerals such as calcium, magnesium, potassium, sodium, iron, manganese, copper, and zinc is an important aspect of a balanced diet. Iron being required for the hemoglobin; calcium for relaxing the central nervous system; magnesium to prevent muscle spasms; potassium and sodium for electrolyte balance; manganese and copper are linked to superoxide dismutase (SOD) and zinc is important in the healing of wounds (Millikan, 2012). According to Stawarz et al. (2007) copper, zinc, and manganese are also essential for normal metabolism, growth and development. In addition, mineral elements are mostly cofactors of many enzymes and thus have very important role in several physiological functions of humans and other animals (Khan, 2014). Minerals are classified as major, minor (trace) and toxic elements according to their essentiality and amount required for the human body.

Approximately 4 to 6% of human body weight is composed of mineral elements, which are essential in the diet (Khan et al., 2014). There would be severe health problems such as anemia, rickets, osteoporosis and diseases of the immune system due to the deficiency of minerals (Frontela, Ros, & Martínez, 2011; Norhaizan & Norfaizadatul, 2009). Among the essential minerals iron, zinc and calcium are often lacking in human diets, either due to poor absorption or due to insufficient intake (Ma et al., 2007). In developing countries, zinc and iron are two of the trace nutrients that are usually deficient (Melaku, West, & Habtamu, 2005).

Antinutritional components like tannins, phenols and phytic acid (myoinositol hexaphosphate), are known to have adverse effects on human nutrition by inhibiting iron and zinc absorption. According to Frontela et al. (2011) it is not the intake of a mineral that is important in maintaining the mineral balance, but the amount that is available to be absorbed by the body. Phytate (inositol hexakisphosphate), is a phosphorus containing molecule/compound that inhibits mineral absorption through binding with them and hence its presence in foods is associated with reduced mineral absorption (Norhaizan & Norfaizadatul, 2009). Phytates are strong chelators of divalent metals such as copper, calcium, magnesium in biological systems or they block the absorption of zinc and iron in the intestinal tract and thus decrease their bioavailability (Ma et al., 2007).

It is possible to improve mineral bioavailability through reducing the phytate content and adding extra minerals during food fortification process (Norhaizan & Norfaizadatul, 2009). In addition, advanced techniques such as breeding through selection of low phytate varieties, optimization of fertilizer use, genetic engineering and use of exogenous phytate degrading enzymes that are traceable or fungal phytases can be used to reduce the phytate content of foods effectively. In many parts of Ethiopia, diets are mainly of plants origin especially cereals, legumes and starchy roots and tubers, which are rich in phytate content, since the consumption of animal origin foods is low. Thus the consumption of high phytate plant based foods without dephytinization or other strategies such as including the addition of organic acids (especially ascorbic acid), ethylenediamine tetra acetic acid (EDTA) complexes will cause a negative effect on the bioavailability of certain minerals such as iron, zinc and calcium (Gibson, Bailey, Gibbs, & Ferguson, 2010; Siddhuraju & Becker, 2001). Such deficits in iron, zinc, and calcium can have far-reaching adverse consequences on growth, health, and cognitive development during childhood (Gibson et al., 2010).

Apart from the reduction of bioavailability, toxic metals such as Cd, As, and Pb could be accumulated in plant parts due to increase of urban, agricultural and industrial emissions (Khan et al., 2014). Migration of these contaminants into non-contaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge could be the main contributor towards contamination of the ecosystems (Tangahu et al., 2011). The presence of these metal pollutants may facilitate their entry into the food chain and thus increases the possibility of having toxic effects on humans and animals(Ali, Khan, & Sajad, 2013). Plant species is one of the several factors that can affect accumulation and distribution of heavy metals in the plant (Cheng & Leong, 2016).

According to Habtamu (2011) Anchote is rich in crude protein, utilizable carbohydrate, crude fiber, energy and ash contents. In addition, Anchote is a rich source of calcium, which is very high when compared to other root crops such as cassava, potato, sweet potato, taro, and yam (Desta, 2011; Habtamu, 2011). Based on knowledge of the heavy metal accumulation in plants, it is possible to select those species of crops, which accumulate fewer heavy metals, for food cultivation and fodder for animals.

Thus, determination of toxic metal contents in food substrates is very helpful in identification of plant species that can hyperaccumulate heavy metals which will be used for phytoremediation technique that is less disruptive than the current techniques of physical and chemical process used for reduction of heavy metals contamination (Tangahu et al., 2011). Therefore, in this section of the study, the concentrations of five major elements namely sodium (Na), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), five minor elements; iron (Fe), cupper (Cu), zinc (Zn), manganese (Mn) and boron (B) and three toxic arsenic (As), cadmium (Cd) and lead (Pb) metals were analyzed. The relative bioavailability of Ca, Fe and Zn were also determined by calculating their corresponding Phytate: calcium, phytate: iron, phytate: zinc and Phytate x calcium: zinc molar ratios in both tuber and leaf samples of 44 Anchote accessions.

4.3. Materials and Methods

4.3.1. Determination of Major, Minor/Trace and Toxic Metals in Anchote accessions

4.3.2. Sample Acquisition

Leaf and tuber samples of 44 Anchote accessions were collected from experimental field of Debre Zeit Agricultural Research Center (DZARC) in November 2011 and January 2012.The harvested leaf and tuber samples from each accession were cleaned and prepared before dried in hot air oven (DHG- 9055A, Memment, Germany) at 105 ^{o}C to a constant weight. The dried samples were then powdered using an electrical miller (FW 100, Yusung Industrial Ltd, China). Finally, the dried and powdered samples were packed in paper bags and sealed in an airtight polyethylene bag, and labeled before storing in a refrigerator set at 4 ^{o}C for further analysis.

4.3.3. Preparation of Samples for Analysis

Anchote powder samples (0.5 g) were weighed into 300 mm long Pyrex glass digestion tubes (Foss, USA). For digesting the samples 25 mL concentrated HNO3 (70%) and 2.0 mL H2O2

catalyst were used and digested using a heating block (Tecator Co., Hoganas, Sweden) (Khan et al., 2014).

The temperature increased gradually, starting from 50 oC and increasing up to 150–160 oC. The digestion process was completed in about 10–12 h, as determined by the appearance of approximately 5.0 ml colorless solution, just like water. The mixture was then left to cool down and the contents of the tubes transferred to 50 ml self-standing polypropylene volumetric tubes with plug seal caps (Corning NY, Mexico). The volumes made up to 7.0 ml with concentrated HNO3 and then diluted to 25.0 g with ultrapure deionized water, labeled accurately and used for analysis. At regular intervals during analysis, calibration standards were analyzed as samples to monitor instrument drift. Furthermore, ultrapure deionized water blanks were also frequently analyzed alongside samples to check for any loss or cross contamination. Blanks were prepared by completion of the full analytical procedure without samples.

4.3.4. Chemicals and Reagents

Analytical reagent grade concentrated HNO_3 (70%) and hydrogen peroxide (30-32%) were obtained from Dong Woo Fine-Chem Co., Ltd Iksan, Korea. The ultrapure deionized water with resistivity of 18.2 MΩ cm, was obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). The calibration standard solutions were prepared from 10 mg/L multi-element standard solution (AnApure KRIAT Co, Ltd. Daejeon, Korea). The Standard Reference Material (NIST- 1570a), Spinach Leaves, was obtained from National Institute of Standards and Technology, Gaithersburg MD, USA. The plastic/glass containers were soaked in 10% v/v HNO_3 for at least 24 h, and then rinsed extensively with Milli-Q water prior to use. All containers, polypropylene flasks, pipette tips, and reagents that came into contact with samples or standards were checked for contamination.

4.3.5. Analysis using ICP-MS and ICP-OES Instrumentation

Minor mineral elements (Zn, Mn, Cu, and B) and toxic metals (As, Cd and Pb) analysis were conducted by using the quadruple Inductively Coupled Plasma Mass Spectrometer (ICP-MS) Elan DRC II (Perkin–Elmer SCIEX, Norwalk, CT, USA).

It was combined with a high-efficiency sample introduction and dissolving system equipped with a quartz cyclonic spray chamber having mixing chamber to homogenize and stabilize the sample aerosol stream. This was essential to have a stable signal from the ICP-MS and a PFA-400 nebulizer operating with peristaltic pump (APEX-IR, Omaha, NE, USA). The purity of the argon gas was 99.98%. Elan 6100 DRC Sensitivity Detection Limit Solution (Perkin Elmer Pure (N8125034) USA) was used to check the performance of the instrument before the actual experiment has been done. The instrumental settings and operative conditions are reported in Table 16.

Table 16 ICP-MS operating conditions and measurement parameters

Spectrometer	Elan 6100 DRC II (Perkin-Elmer SCIEX, Norwalk, CT, USA)
Nebulizer	Meinhard
Spray chamber	Cyclonic
RF power (kW)	1.35
Plasma	16
Auxiliary	1–1.3
Nebulizer	1.0–1.07
Lens voltage (V)	6.25
Torch horizontal alignment (mm)	0.5–1.0
Torch vertical alignment (mm)	0.2–0.5
Scanning mode	Peak hopping
No. of replicates per sample	3
Resolution (amu)	0.7
Dwell time (ms)	50
Sweeps/reading	20
Sampling depth (mm)	6.0–8.0
Sample uptake rate (mL/min)	0.24
Minor / trace elements	^{55}Mn, ^{63}Cu,^{66}Zn, ^{11}B,
Toxic trace elements	^{208}Pb, ^{112}Cd, ^{75}As

For the analysis of Na, P, K, Ca, Mg, and Fe a Varian Model 730-ES simultaneous CCD, inductively coupled plasma-optical emission spectrometer (ICP-OES) (Wyndmoor, PA, USA) having a Sea Spray concentric nebulizer (Glass Expansion, Pocasset, MA, USA) and cyclonic spray chamber was used.

After scanning a blank, a standard solution and a sample solution in the programmed wavelength range, the background correction wavelengths were selected manually at appropriate background positions for each analyte peak. Instrument configuration and general experimental conditions are summarized in Table 17.

Table 17 ICP-OES operating conditions and measurement parameters applied determination of metals

Spectrometer	ICP-OES (730-ES simultaneous CCD, Varian, USA)
RF generator	27.12 MHz
RF power (kW)	1.3
Nebulizer	Sea Spray
Spray chamber	Cyclonic
Plasma viewing	Axial
Processing mode	Area
Plasma	16
Auxiliary	1.5
Nebulizer	0.94
Read delay (sec)	30
Rinse (sec)	30
Replicates	3
Elements, wavelengths (nm)	589.592nm (Na), 213.617nm (P), 766.490nm (K), 317.933nm (Ca), 285.213nm (Mg), 238.204nm (Fe)

4.3.6. Calibration Procedure and Quality Assurance

For the quantitative analysis of the samples, external calibration technique was followed. Standard solutions were prepared in 19.6% (w/w) HNO_3 (the same percentage of acid present in the samples) by diluting a multi-element standard solution containing all the elements.

The calibration curves for all the analytes were built on six different concentrations, from the limit of detection (LOD) of the corresponding element up to 200 ng/g. All measurements were carried out using the full quantitative mode analysis.

The correlation coefficients for all the calibration curves were at least 0.99, showing good linear relationship throughout the ranges of concentrations studied. The absence of polyatomic interferences was then checked by measuring several isotopes of the elements and checking the isotopic ratio in the digested solution of the samples.

The analytical method followed for the determination of major, minor and toxic elements in Anchote accessions were validated by measuring several quality parameters including sensitivity, linearity, precision, accuracy and spike recovery. Sensitivity of the instrument was estimated through the determination of detection limits of all elements studied. The limits of detection (LOD) and limits of quantification (LOQ) were calculated with three and ten times the standard deviation of the blank divided by the slope of the analytical curve respectively. Linearity was established by preparing the calibration curves of all analyte elements using a non-weighted least-squares linear regression analysis method. All calibration curves were prepared with eight standard solutions including the blank. These were prepared in such a way that the concentrations all analyte elements in the samples were within the linear range of calibration curves and above the established lower linearity limit (Khan et al., 2013).

Precision is the degree of variability given by the expression of results, not taking into account the influence of the sample (sample variability). It can be evaluated by using relative standard deviation of 10 repeated determinations of one sample(Khan et al., 2013). Following this method, the present coefficient of variation (CV %) were obtained for all analyte elements. The accuracy of the method was checked by analyzing the NIST- 1570a, Spinach Leaves, for the determination of all elements and the analytical quality control was also verified via recovery experiments by spiking at two selected concentrations of 1000 µg/kg and 100 µg/kg (Khan et al., 2014).

4.3.7. Determination of Molar Ratio of Phytate/Mineral

The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/ mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals (Norhaizan & Norfaizadatul, 2009).

4.3.8. Statistical Analysis

Data were reported on dry weight basis (mean ± standard deviation). Significantly different ($P<0.05$) means of the same mineral elements among different Anchote accessions were analyzed by one-way analysis of variance (ANOVA), following Duncan multiple range test (DMRT) for significant difference test in the statistical package of SAS Software Version 9.

4.4. Results and Discussions

4.4.1. Major Mineral Elements Concentration in Anchote Tubers

The range and mean values of major mineral elements (Na, P, K, Ca and Mg) for 44 Anchote tuber samples are compiled in Tables 18 and the comprehensive results for each accession is presented in Annex F. The mean contents of Na, P, K, Ca and Mg in the tubers were 67.38, 29.5, 51.46, 223.19 and 28.77 mg/100g on dry matter basis, respectively. The Na content of the analyzed tubers was in the range of 42.78 ± 0.51 – 98.78 ± 12.96 mg/100g on dry basis. Similarly, P, K, Ca and Mg were in the range of 14.09 ± 1.81 – 48.64 ± 1.22, 13.63 ± 1.70 – 95.28 ± 3.65, 80.64 ± 0.66 – 372.16 ± 1.39, and 9.33 ± 0.04 – 59.36 ± 4.24 mg/100g on dry basis, respectively. The highest concentrations of Na (98.78 ± 12.96 mg/100g), P (48.64 ± 1.22 mg/100g), K (95.28 ± 3.65 mg/100g), Ca (372.16 ± 1.39 mg/100g) and Mg (59.36 ± 4.24 mg/100g) were recorded for accessions ‘223112-1’, ‘223112’, ‘223093’, ‘220563-1’ and ‘NJ’, respectively. The Na content of accession ‘223112-1’ and ‘DIGGA-2’ were significantly ($P<0.05$) higher than the rest of accessions considered in the present study. Accessions ‘2231112’ and ‘223104’ had significantly ($P<0.05$) higher P content than the other accessions.

Accessions '223093', 'NJ' and '223092-1' were the top three accessions in respect of their K content as compared to the rest of the accessions but the values were significantly different ($P<0.05$) among these three accessions. Similarly accessions '220563-1', '223088' and '240407-G' had significantly ($P<0.05$) higher Ca content than the remaining accessions.
Accessions 'NJ' and 'DIGGA-1' were found to have significantly ($P<0.05$) higher Mg content than the other accessions and the difference in Mg content between these accessions were significant ($P<0.05$).

The mean concentration of Na (67.38 mg/100g) in tuber part of this study was higher than values reported for peeled (16.3 mg/100g FW) and whole (16 mg/100g FW) Anchote tubers FW (Habtamu & Kelbessa, 1997). Our result was also higher than other tuber crops such as *Alocasia indica* (14.40 g/100g DM), wild yam (4.15–17.8 mg/100g FW) and raw cassava (14 mg/100g FW) (Basu, Das, Sen, Choudhury, & Datta, 2014; Bhandari, Kasai, & Kawabata, 2003; Montagnac et al., 2009).

However, the result of Na was lower than the mean value (192 mg/100g) reported for sweet potato cultivars (Velmurugu Ravindran, Ravindran, Sivakanesan, & Rajaguru, 1995). Sodium regulates body fluid and maintain electric potential in the body tissue (Alinnor & Oze, 2011). The mean phosphorus content in this study was less than the reported values of Anchote tuber by Habtamu & Kelbessa (1997) and Habtamu et al. (2013) as well as other tuber crops such as cassava, sweet potato and potato (Montagnac et al., 2009; Velmurugu Ravindran et al., 1995; Sarkiyayi & Agar, 2010). Phosphorus together with calcium required in largest quantity for the formation of body and bones structure (Onwordi, Ogungbade, & Wusu, 2009).

Potassium contents of Anchote tubers in this study were lower than the finding of Habtamu & Kelbessa (1997) on Anchote and other root and tuber crops such *Ipomoea batatas, Dioscorea spp*, *Xanthosoma sagittifolium* and *Hippocratea welwitschii, Manihot esculenta* (Montagnac et al., 2009; Okoh-Esene, Okogun, Okwute, & A., 2012; Velmurugu Ravindran et al., 1995; Senanayake, KKDS, Bamunuarachchi A, & Gunarathne A, 2012). Potassium is important in the regulation of heart beat, neurotransmission and water balance of the body (Alinnor & Oze, 2011).

The Ca content of whole (344 mg/100g) and peeled (327 mg/100g) Anchote tuber reported by Habtamu & Kelbessa (1997) were comparable with the values obtained from accession '223088' (343.01 mg/100g) and '240407-G' (325.09 mg/100g) but lower than the highest value (372.16 mg/100g) obtained in accession '220563-1' of the present study. Habtamu et al. (2013) also reported lower value (119 mg/100g) of Ca content for raw Anchote tuber as compared to the average value (223.18 mg/100g) noted in this study.

Table 18 Major mineral elements concentration (mg/100g dry basis) in tubers of 44 Anchote accessions compared with RDA/AI values

	N=44								
Major minerals	Max	Min	Mean	SD	CV	F-value	P>F	RDA/AI* (mg/day)	UL (mg/day)
Na	98.78	42.78	67.38	11.99	5.71	18.61	<0.0001***	120*-1500*	-
P	48.64	14.09	29.50	7.77	5.34	48.12	<0.0001***	100*-1,250	3,000-4,000
K	95.28	13.63	51.46	19.71	3.75	210.55	<0.0001***	400*-5100*	-
Ca	372.16	80.64	223.18	82.27	1.19	1935.80	<0.0001***	200* -1300	1000-3000
Mg	59.36	9.33	28.77	11.44	6.32	79.09	<0.0001***	30*-420	65-350[ab]

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, **, and *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively. **RDA**/AI* = Recommended Dietary Allowances (RDA) in **bold type** / Adequate Intakes (AI) in ordinary type followed by an asterisk (*); UL = upper tolerable level of daily nutrient intake; a represents the values for the tolerable upper intake level is not represents infants 0 to 12 months life stage groups; b The ULs for Mg represent intake from a pharmacological agent only and do not include intake from food and water.

Source: Dietary reference intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary reference intakes, applications in dietary assessment (2000); Dietary Reference Intakes for Calcium and Vitamin D (2011).

Anchote tuber has higher value of Ca than that of cassava and sweet potato (Sarkiyayi & Agar, 2010). Yewelsew et al. (2007) also reported a lower Ca (52 mg/100g) of '*Kocho'* fresh pulp (Enset) on fresh weight basis. According to Habtamu & Kelbessa (1997), Anchote has 26 fold higher Ca content than potato, 10 fold higher than sweet potato and 7 fold higher than taro.

Calcium is good for growth and maintenance of bones, teeth and muscles (Dosunmu, 1997; Turan, Kordali, Zengin, Dursun, & Sezen, 2003), therefore Anchote tuber could provide our body with veritable sources of calcium. The Mg content (9.33-59.36 mg/100g) of tuber samples in all the tested accessions in our study were lower than the previously reported values for tubers of Anchote, 79.73 mg/100g by Habtamu et al. (2013) and 124 mg/100g by Habtamu & Kelbessa (1997), sweet potato, 86 mg/100g by Ravindran et al. (1995) and *Alocasia indica,* 86.05 mg/100g.

However, the mean Mg content of Anchote tuber (28.77 mg/100g DW) in our study was found to be higher than the reported values of raw cassava root (21mg/100g FW) and raw potato tuber (23mg/100g) (Montagnac et al., 2009); wild yam tubers (18.3-27.3 mg/100g DW)(Bhandari et al., 2003). Anchote is remarkably rich in Ca and Mg contents as compared to other major nutrients considered in the present study.

4.4.2. Minor/Trace Mineral Elements Concentration in Anchote Tubers

Similar to the major elements, tubers samples, taken from same number of Anchote accessions, were analyzed in order to determine the contents of trace minerals and the maximum, minimum and mean values are presented in Table 19. The concentrations of Fe, Cu, Zn, Mn and B from all 44 accessions tuber samples depict that the range of values for Fe, Cu, Zn, Mn and B were 1.58±0.00-18.65±4.15, 0.47±0.00-1.60±0.01, 0.32±0.37-3.41±0.33, 0.97±0.01-1.85±0.02, and 2.14±0.06-7.04±0.04 mg/100g on dry basis, respectively. Similarly, the mean values were 3.65 mg/100g, 0.73 mg/100g, 0.64 mg/100g, 1.29 mg/100g, and 3.62 mg/100g for Cu, Zn, Mn and B respectively. Accessions with the highest concentration of Fe, Cu, Zn, Mn and B were ‘223092’, ‘223093’, ‘240407-G’, ‘NJ’ and ‘223112-1’ respectively. These accessions were significantly ($P<0.05$) different with all the remaining accessions.

The mean values of Fe, Cu, and Mn content observed in the present study are within the range of RDA daily recommendation. However, only Zn contents in accession ‘240407-G’ (3.41± 0.33mg/100g) and ‘223112’ (2.79± 0.35 mg/100g) were found to be within the range of the daily recommended adequate intake level while its average content, 0.64mg/100g was lower.

Out of the 44 accessions, 14 for Fe (3.65 mg/100g), 11 for Cu (0.73 mg/100g), 9 for Zn (0.64 mg/100g), and 17 for B (3.62 mg/100g) had values greater than the mean values, and respectively. The detailed result of the analyses for the trace minerals in tubers of the 44 accession is compiled in Annex G. Five of our accessions were superior in their Fe content than the value reported for raw Anchote tuber (5.49 mg/100g) by Habtamu et al. (2013), four accessions exceeded the whole Anchote tuber (5.5 mg/100g).

Peeled tubers of eight Anchote accessions (4.6 mg/100g) reported by Habtamu & Kelbessa (1997) on fresh weight basis. The high mean Fe content (18.65 mg/100g) of Anchote tuber is greater than the mean (3.64 mg/100g DW) and range (4.2-6.3 mg/100g DW) values of sweet potato cultivars (Velmurugu Ravindran et al., 1995; Senanayake, Ranaweera, Gunaratne, & Bamunuarachchi, 2013) and fresh *kocho* pulp (1.1 mg/100g FW) from Enset (Yewelsew et al., 2007). The Fe content of bitter cassava variety (18 mg/100g) is in agreement with Anchote tuber (Sarkiyayi & Agar, 2010). Iron is needed for formation of blood and equally important for normal functioning of the central nervous system (Adeyeye & Fagbohun, 2005). It is also an important trace element that binds oxygen in hemoglobin (Geissler & Powers, 2005).

Table 19 Minor mineral elements concentration (mg/100g dry basis) in tubers of 44 Anchote accessions compared with RDA/AI values

Minor minerals	N=44								
	Max	Min	Mean	SD	CV	F-value	P>F	RDA/AI* (mg/day)	UL (mg/day)
Fe	18.65	1.58	3.65	2.61	18.53	29.03	<0.0001***	0.27*- 27	40-45
Cu	1.60	0.47	0.73	0.24	2.68	305.06	<0.0001***	0.2*-1.3	1-10
Zn	3.41	0.32	0.64	0.58	15.26	69.70	<0.0001***	2*-13	4-40
Mn	1.85	0.97	1.29	0.22	15.72	93.01	<0.0001***	0.003*- 2.6*	[a]2-11
B	7.04	2.14	3.62	1.20	26.11	4.86	<0.0001***	-	[a]3-20

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, **, and *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively. **RDA**/AI* = Recommended Dietary Allowances (RDA) in **bold type**/Adequate Intakes (AI) in ordinary type followed by an asterisk (*); UL = upper tolerable level of daily nutrient intake that is likely to pose no risk of adverse effects; a represents the values of tolerable upper intake level is not represents infants 0 to 12 months life stage groups.

Source: Dietary reference intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary reference intakes, applications in dietary assessment (2000); Dietary Reference Intakes for Calcium and Vitamin D (2011).

This study shows that the mean Cu content (0.73 mg/100g) was higher than the previously reported values of 0.4 and 0.5 mg/100g FW for whole and peeled Anchote tubers, respectively (Habtamu & Kelbessa, 1997).It is in agreement with the mean value for sweet potato (0.73 mg/100g DW) and yellow yam (7.34 mg/kg FW) cultivars (Akin-Idowu, Odunola, Asiedu, Maziya-Dixon, & Uwaifo, 2008; Velmurugu Ravindran et al., 1995). Copper is vital for many biological systems and forms parts of at least 13 different enzymes, and its presence is needed for each if they are to function properly (Altundag & Tuzen, 2011).

Zn content in accession '240407-G' (3.44 mg/100g) and '223112' (2.79 mg/100g) was relatively higher than the reported values of 2.23 mg/100g FW by Habtamu et al. (2013) and 1.8 mg/100g FW by Habtamu & Kelbessa (1997)for raw Anchote tuber. Moreover, the Zn content of these two accessions were higher than other starchy roots and tubers such as *Alocasia indica* (2.00 m/100g DW), cassava (0.34 mg/100g FW) and potato (0.29 mg/100g FW) (Basu et al., 2014; Montagnac et al., 2009). The contents of Mn and B in Anchote tubers in this study were higher than the values reported for potato and cassava tubers (Montagnac et al., 2009; Wang, Li, & Malhi, 2008).

4.4.3. Major Mineral Elements Concentration in Anchote Leaves

The mean concentrations of major minerals in Anchote leaves were Na (54.46 mg/100g), P (64.63 mg/100g) K (139.82 mg/100g), Ca (147.8 mg/100g) and Mg (45.04 mg/100g). The range values are 30.45±1.96 - 92.24±14.12, 42.46±0.12 - 79.15±1.57, 107.72±1.50 - 178.91±3.94, 64.10±1.12 - 226.95±4.09, and 29.72±0.29 - 70.65±7.47 for Na, P, K, Ca and Mg, respectively (Table 20). The concentration of leaf major minerals for each of the 44 accessions were presented in Annex H. Accessions '223087-1' (92.24 mg/100g), '223109-1' (86.29 mg/100g), and '223101' (79.18 mg/100g) were the top three accessions with significantly ($P<0.05$) higher Na content than the other accessions but the difference among these three accessions was significant ($P<0.05$). Accession '223112-1' showed significantly highest ($P<0.05$) content of P and K. Accessions '223112-1' (178.91 mg/100g) and '223101' (175.43 mg/100g) had significantly higher K content than the rest of the accessions.

Similarly accessions '220563-1' (226.95 mg/100g), '223104' (226.93 mg/100g) and '220563' (218.55 mg/100g) had significantly (P<0.05) higher Ca content than the other accessions; however, the difference among these three accessions was not significant (P>0.05). Accession '223088' (70.65 mg/100g) had significantly higher Mg content than the other accessions.

Table 20 Major mineral elements concentration (mg/100g dry basis) of leaves of 44 Anchote accessions compared with RDA/AI values

Major minerals	N=44								
	Max	Min	Mean	SD	CV	F-value	P>F	RDA/AI* (mg/day)	UL (mg/day)
Na	92.24	30.45	54.46	13.28	5.15	44.41	<0.0001***	120*-1500*	-
P	79.15	42.46	64.63	8.28	4.39	16.19	<0.0001***	100*-1,250	3,000-4,000
K	178.91	107.72	139.82	18.94	1.81	112.58	<0.0001***	400*-5100*	-
Ca	226.95	64.10	147.80	39.72	4.58	68.71	<0.0001***	200* -1300	1000-3000
Mg	70.65	29.72	45.04	8.50	5.09	26.76	<0.0001***	30*-420	65-350[ab]

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, **, and *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively. **RDA**/AI* = Recommended Dietary Allowances (RDA) in **bold type**/Adequate Intakes (AI) in ordinary type followed by an asterisk (*); UL = upper tolerable level of daily nutrient intake that is likely to pose no risk of adverse effects; a represents the values of tolerable upper intake level is not represents infants 0 to 12 months life stage groups; b The ULs for Mg represent intake from a pharmacological agent only and do not include intake from food and water.

Source: Dietary reference intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary reference intakes, applications in dietary assessment (2000); Dietary Reference Intakes for Calcium and Vitamin D (2011).

Accession '223112-1' had significantly (P<0.05) higher P (79.15 mg/100g) and K (178.91 mg/100g) contents than the rest of the accessions. The mean concentrations of P, K and Mg in the leaf samples of the different accessions were higher than that of the tuber samples. However, the mean concentrations of Na and Ca were higher in the tubers than leaves.

The recommended Ca intake for infants and children, adolescents and adults are 300-700mg/day, 1300 mg/day and 1000-1300 mg/day respectively(FAO/WHO, 1998). Both leaf and tubers of Anchote (*Coccinia abyssinica)* found to have high Ca content with a range of 80.64 – 372.16 mg/100g (tuber) and 64.10 – 226.95 mg/100g (leaf) and it would be possible to meet the FAO/WHO recommended dosage with consumption of small quantity of Anchote leaf or tuber.

FAO/WHO (1998) recommended a daily intake of 26-240 mg Mg for different age groups and Anchote had 9.33 – 59.36 mg/100g Mg in tuber and 29.72 – 70.65 mg/100g Mg in leaf samples. Therefore, Anchote can be considered as a good source of Mg in human nutrition.

In the present study the Na, P, K, and Ca content were found to be higher than kale (*Brassica oleracea*), sweet potatoes (*Ipomoea batatas*) and *Amaranthus hybridus* leaves (Akubugwo et al., 2007; Antia et al., 2006; Emebu & Anyika, 2011b). However, the Mg content in sweet potato (340 mg/100g) and *Amaranthus hybridus* (231.22 mg/100g) were found to be higher than the Mg content (29.72-70.65 mg/100g) in our result (Akubugwo et al., 2007; Antia et al., 2006). In addition, lower level of sodium to potassium is recommended in the body to lower blood pressure, and food with Na/K value lesser than one is considered good for consumption (Oyelude, Gli, & Amafo, 2012). In present study, calculated value of Na/K for Anchote tuber and leaf was 1.31 and 0.39 respectively. Therefore, this notifies that Anchote leaf has the potential of lowering blood pressure.

4.4.4. Minor /Trace Mineral Elements Concentration in Anchote Leaves

Results of analyses conducted to determine concentrations of trace elements in leaves of 44 Anchote (*Coccinia abyssinica)* accessions is presented in Table 21, and the detailed result of each accession is shown in Annex I. The mean and range of Fe, Cu, Zn, Mn and B concentrations were 8.54 (range 1.85 – 45.18), 0.99 (range 0.47 – 2.18), 1.95 (range 0.48 – 4.68), 0.95 (range 0.12 – 2.44) and 5.02 (2.64-10.72) mg/100g on dry basis. The highest Fe (45.18±0.15 mg/100g), Cu (2.18 mg/100g), Zn (4.68±0.31mg/100g), Mn (2.44±0.08 mg/100g) and B (10.75±0.05 mg/100g) were recorded for accessions '223109-1', '223087-1', '240407-G', '223099', and '90801' respectively. The difference among accessions '223109-1' and '223090-1' and the rest of accessions in respect of Fe content was significant ($P<0.05$) However, the difference between the two accessions ('223109-1' and '223090-1') was not significant ($P>0.05$). Accession '223087-1' had significantly ($P<0.05$) highest Cu content (2.18 mg/100g) than the other accessions. Similarly accessions '240407-G' (4.68 mg/100g) and '90801' (4.36 mg/100g) had significantly higher Zn concentrations than the rest of accessions, and the difference between these accessions was also significant ($P<0.05$).

With regard to content of Mn and B, accession '223099' and '90801' respectively had significantly ($P<0.05$) highest values than the other 43 accessions considered in the present study. The result of this study showed that the mean content of Fe in Anchote leaf (8.54 mg/100g) was in agreement with the Fe content (8.94 mg/100g) of kale. Green leafy vegetables contain iron needed for hemoglobin formation (Ladan, Bilbis, & Lawal, 1996), and hence recommended for anemic convalescence when there is iron deficiency (Emebu & Anyika, 2011b). Therefore, Anchote leaf can be recommended for iron deficiency anemia. Anchote leaf Fe content was superior to other leafy vegetables such as fresh cauliflower (2.83 mg/100g) (Baloch et al., 2015), fresh *Phaseolus vulgaris* (4.59 mg/100g) (Oyelude et al. 2012) and *Moringa oleifera* (1.00 mg/100g FW) (Kwenin et al., 2011), spinach (0.8 mg/100g) and lettuce (0.86 mg/100g) (Montagnac et al., 2009).

Exceptionally, two accessions, '223109-1' (45.18±0.15 mg/100g) and '223090-1' (44.27±1.11 mg/100g) were found to be superior to the RDA value (27 mg/day). This implies that consumption of iron rich Anchote leaf could assist to meet the daily requirements of Fe (0.425 mg/g) (FAO/WHO, 2001). Iron is essential in the diet of pregnant women, nursing mothers, infants and elderly to prevent anemia. According to WHO (2008), Africa has the highest proportion of persons affected by anemia and the RDA for iron in adult men and children is 10 mg/100g while it is 15 mg/100 g for adult females.

The levels of Cu in Anchote leaf (0.47-2.18 mg/100g) were much higher than the value reported for other leafy vegetables including: *Phaseolus vulgaris* (0.22 mg/100g FW) (Oyelude et al., 2012), spinach (0.093 mg/100g) and lettuce (0.029 mg/100g) on fresh weight basis (Montagnac et al., 2009). The RDA value for copper is 1 to 3 mg for adult (Khan et al., 2013). Copper contributes a role in hemoglobin formation as well as in iron and energy metabolism (FAO, 2001).

The Zn content of about 20 accessions (2.19 – 4.68 mg/100g) in the present study were comparable to the reported values for green leafy vegetables such as *Amaranthus tricolor* (2.4 mg/100g), *Mentha spicata* (4 mg/100g), *Coriandrum sativum* (2.7 mg/100g) on dry weight basis (Singh et al. 2001).

Table 21 Minor mineral elements concentration (mg/100g dry basis) of leaves of 44 Anchote accessions compared with RDA/AI values

Minor minerals	N=44								
	Max	Min	Mean	SD	CV	F-value	P>F	**RDA**/AI* (mg/day)	UL (mg/day)
Fe	45.18	1.85	8.54	8.94	8.01	345.27	<0.0001***	0.27*- **27**	40-45
Cu	2.18	0.47	0.99	1.44	4.51	184.07	<0.0001***	0.2*-**1.3**	1-10
Zn	4.68	0.48	1.95	0.42	3.22	1052.93	<0.0001***	2*-**13**	4-40
Mn	2.44	0.12	0.91	0.52	5.72	204.12	<0.0001***	0.003*-2.6*	[a]2-11
B	10.75	2.64	5.02	1.49	3.30	325.76	<0.0001***	-	[a]3-20

Min: minimum; Max: maximum; S.D: Standard Deviation; N: number of samples; n.s.: not significant; *, **, and *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively.

RDA/AI* = Recommended Dietary Allowances (RDA) in **bold type**/Adequate Intakes (AI) in ordinary type followed by an asterisk (*); UL = upper tolerable level of daily nutrient intake that is likely to pose no risk of adverse effects; a represents the values of tolerable upper intake level is not represents infants 0 to 12 months life stage groups.

Source: Dietary reference intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary reference intakes, applications in dietary assessment (2000); Dietary Reference Intakes for Calcium and Vitamin D (2011).

The mean concentration of Zn (1.95 mg/100g) was higher than the reported values for sweet potatoes (0.08 mg/100g) (Antia et al., 2006), and *Phaseolus vulgaris* (0.30 mg/100g FW & 1.38 mg/100g DW) (Oyelude et al., 2012).

The highest Mn content in accession '223099' (2.44 mg/100g) favorably agreed with the reported values of Indian spinach (2.54 mg/100g) (Asaolu et al., 2012). In the present study, we also observed that the mean content of Mn (0.95 mg/100g) was higher than spinach (0.639 mg/100g) and lettuce (0.25 mg/100g) (Montagnac et al., 2009), but lower than the values documented for other leafy vegetables (Antia et al., 2006; Singh et al., 2001). The levels of Mn concentration in all the tested accessions were found to be within the range of RDA and hence the leaf of Anchote can be considered a good source of this mineral. The content of Cu in our study (2.64-10.75 mg/100g) was within the range of the upper tolerable level of daily nutrient intake (3-20 mg/day) that is likely to pose no risk of adverse effects.

Besides, the RDA for Cu levels (900 µg/day) set by EU (2003) indicates that Anchote leaf is good source of this element for its nutritional benefit. Copper is a component of a number of enzymes that are involved in reducing molecular oxygen. Moreover, copper is a constituent of enzymes that oxidize ferrous iron and facilitate the binding of iron to transferrin, and part of cytochrome c oxidase, a critical component of energy production(Dickinson, 2000). In general, the concentrations of Fe, Cu, Zn, and B in Anchote were greater in leaves than in tubers and the mean values were 8.54 mg/100g (Fe), 0.99 mg/100g (Cu), 1.95 mg/100g (Zn) and 5.02 mg/100g (B) (Table 21).

4.4.5. Toxic Mineral Elements Concentration in Anchote Tubers and Leaves

Plants can take up heavy metals by their roots, or even via their stems and leaves, and accumulate them in their organs. Plants take up elements selectively. Accumulation and distribution of heavy metals in the plant depends on the plant species, element species, chemical and bioavailability, redox, pH, cation exchange capacity, dissolved oxygen, temperature and secretion of roots (Cheng, 2003).

Cadmium (Cd), arsenic (As) and lead (Pb) have well known toxic roles in various biochemical reactions (Fraga, 2005; Karadaş & Kara, 2012). In this study, the concentrations (ng/g) of these toxic trace elements for the analyzed 44 Anchote accessions were found to be in the range of Cd (0.07– 6.26), As (0.16 –1.87), and Pb (0.09–62.60) in tubers (Table 22), whereas, in leaves the range was 0.33- 7.04, 0.49-6.90 and 4.07-95.41 for Cd, As and Pb, respectively (Table 23).

In general, the mean concentrations of these potentially toxic metals in our study were higher in the leaf part compared to the tuber part of Anchote. Similarly, (Dowdy & Larson, 1975)had also reported vegetative tissue were higher than those of the fruiting, root, and tuber tissues. In tuber samples, the order of toxic metal mean concentrations was Pb>Cd>As whereas in leaf samples, the concentrations were in a decreasing order of Pb>As>Cd. The highest concentration of toxic metals for tuber samples were observed in accession ‘223085’ (6.26 ng/g) for Cd, ‘90802’ (1.87 ng/g) for As, ‘NJ’ (62.60 ng/g) for Pb.

On the other hand, in leaf samples the highest values of the tested toxic metals were observed in accession '230566' (7.04 ng/g) for Cd, '223090-1' (6.90 ng/g), '230566' (95.41 ng/g). Significant variations ($P<0.05$) were detected in terms of toxic metal accumulation among the tested accessions of Anchote, both in tuber and leaf parts (Annex J & Annex K). Several possible explanations could be forwarded for the apparent variation in the concentration of toxic metals.

Bioavailability and bioaccumulation rates of metals are influenced by difference in metabolic rates of the species, exposure routes, metal mobility values, bioavailability, chelators present in water, the environmental factors of pH, temperature, salinity, nutrients, organic matter, organic carbon, and conditions of an ecosystem (Girum et al., 2015; Gokoglu, Yerlikaya, & Gokoglu, 2008; Vinodhini & Narayanan, 2008).

Excessive dietary Cd intake can accumulate in the kidney cortex and cause renal tubular dysfunction (Fanconi syndrome), a disease in which low molecular weight proteins are excreted in urine (Dudka & Miller, 1999). Cd has different bioavailability depending on the presence of different nutrients in the same crop (Chaney, 1992). Cadmium content in Anchote were formed to be 0.07- 6.26 ng/g and 0.0001-0.0063 mg/kg in tubers and 0.33 -7.04 ng/g and 0.0070 – 0.0003 mg/kg in leaves. Our results are lower than the values reported for plant species e.g., dried fruits (0.65–1.34 μg/g dw) (Sattar, Wahid, & Durrani, 1989), apricot (0.09–0.21 mg/kg) (Zahoor, Jaffar, & Saqib, 2003), legumes (0.16–0.24 mg/g dw) (Yebra & Cancela, 2005), vegetables (0.002–0.05 mg/kg) (Radwan & Salama, 2006), and apricot (0.02–0.72 μg/g) (Saracoglu, Tuzen, & Soylak, 2009).

Arsenic is a carcinogenic element and has harmful effects on the digestive tract, heart, vascular system and central nervous system and even causes death up to 60 mg/L in drinking water (Javadi, Khatibi, Khakpour, Ganjali, & Ghorbanpour, 2009). Arsenic undergoes some accumulation in soft tissue organs such as the liver, spleen, kidneys, and lungs, once absorbed, into the body. However, the major long-term storage site for arsenic is keratin-rich tissues, such as skin, hair, and nails making the measurement of arsenic in these biological specimens useful for estimating total arsenic burden and long-term exposure under certain circumstances (Howard, 2002).

Lead is toxic and has adverse effects on human health (WHO, 1993). It causes rise of blood pressure, kidney damage, miscarriage, subtle abortion, brain damage, decline in fertility of men through sperm damage and diminishes learning abilities due to neuron damaging actions (Gafar & Itodo, 2011).

According to the WHO (1993), a provisional tolerable weekly intake for lead is 0.025 mg/kg of body weight, and this implies that the Pb contents in our samples are below this level and are safe for consumption. The reported Pb contents in investigated plant species were 1.91 µg/g as average value in apricot samples (Saracoglu et al., 2009), 6.6 - 9.2 µg/g in spices, dry fruit and plant nuts from Pakistan (Sattar et al., 1989) and 1.66 mg/kg in apricot of Pakistan (Zahoor et al., 2003). In our study, the content of Pb was 0.0001-0.0626 and 0.0041-0.0954 in mg/kg for tubers and leaves, respectively that is by far lower than the above reported literatures.

Table 22 Toxic mineral elements concentration (ng/g dry basis) of tubers of 44 Anchote accessions

Toxic mineral elements	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Cd	6.26	0.07	0.86	0.98	9.50	289.44	<0.0001***
As	1.87	0.16	0.83	0.40	5.38	161.71	<0.0001***
Pb	62.60	0.09	7.05	10.74	1.56	19015.5	<0.0001***

Table 23 Toxic mineral elements concentration (ng/g dry basis) of leaves of 44 Anchote accessions

Toxic mineral elements	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Cd	7.04	0.33	1.29	1.04	13.45	71.64	<0.0001***
As	6.90	0.49	2.62	1.02	7.44	54.54	<0.0001***
Pb	95.41	4.07	13.53	13.59	2.39	3532.21	<0.0001***

Considering the Provisional Tolerable Weekly Intake Levels (PTWI) reported by FAO/WHO (1982, 1996) and Food & Board (2001), 0.007 for Cd, 0.015 for As, and 0.025 Pb mg/kg, respectively. It can easily be explained that the levels of these toxic trace elements detected were very low in all Anchote accessions of leaves and tubers tested in this study and there is no health concern to consumers.

4.4.6. Relative Bioavailability of Calcium, Iron and Zinc

Tubers

Molar ratios of phytate to calcium, iron, zinc and phytate x calcium: zinc for tuber samples of the 44 Anchote accessions were calculated based on the results of the analysis of these minerals considered in the present study and the result for each of the accessions is presented in Annex L. The mean and range values for phytate to minerals is summarized in Table 24. The highest phytate to calcium molar ratio (0.18) was recorded for accession '223085' and the difference with the rest of accessions was significant ($P<0.05$).

Table 24 Molar ratio between phytate and minerals analyzed in tubers of 44 Anchote accessions

Molar ratio of phytate & Mineral	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Phytate/Ca	0.18	0.01	0.05	0.04	6.56	332.69	<0.0001***
Phytate/Fe	16.3162	0.4710	3.81	2.97	11.72	88.57	<0.0001***
Phytate/Zn	89.539	3.688	25.32	16.94	7.99	140.92	<0.0001***
Phytate x Ca/Zn	481.36	22.22	127.53	86.74	9.38	105.35	<0.0001***

Similarly, the highest phytate to iron (16.32 ± 0.62) and phytate to zinc (89.54 ± 5.65) molar ratios were recorded for the same accession '223085' and the difference of these ratios with the rest of accessions was significant ($P<0.05$). Significant difference ($P<0.05$) was also observed among accessions in phytate x calcium: zinc molar ratio and the highest ratio was recorded for accession '223086' and the difference with the rest of accessions was significant.

This accession also has the second highest value for phytate: iron and phytate: zinc molar ratios. The highest phytate: calcium molar ratio in tuber samples of Anchote in the present study was less than 0.24 and this implies that the bioavailability of calcium in tuber samples is good and less than the critical value (0.24) as indicated by Morris & Ellis (1985). The phytate x calcium: zinc molar ratio in the tuber samples of most accessions was <200 and this was good in terms of bioavailability (Bindra, Gibson, & Thompson, 1986).

However, the bioavailability of both iron and zinc in tuber samples was not in the normal range as the molar ratio of phytate: iron and phytate: zinc for most accessions was above the critical limits (>1 for iron and >15 for phytate for zinc) (Hallberg, Brune, & Rossander, 1989; Sandberg, Andersson, Carlsson, Sandstro, & Sandström, 1987).

Leaves

Table 25 shows the mean and range values of molar ratios of phytate to calcium, iron, zinc and phytate x calcium: zinc in leaves of 44 Anchote accessions. The detailed phytate to mineral ratio of these accessions is given in Annex M. The highest (0.22±0.01) phytate to calcium molar ratio was recorded for accession '223109-1' and this ratio was significantly higher than the remaining ratios calculated for the 43 accessions. This ratio was in the range (0.06 - 0.22). On the other hand, calculated phytate to iron ratio was significantly ($P<0.05$) higher for accession 'KICHI-1' (10.64±0.40) than the rest of accessions and the range of this ratio lies between 0.43-10.64.

The highest phytate to zinc and phytate x calcium to zinc molar ratio was recorded for accession '230565' and '229702', respectively. However, the difference in phytate to zinc molar ratio for accessions '229702', '230565' and 'KICHI-1' was insignificant ($P>0.05$). Accession 'KICHI-1' had the highest phytate to iron and phytate to zinc molar ratio. Similarly, accession '229702' had significantly ($P<0.05$) higher (223.13±4.84) phytate x calcium to zinc molar ratio than the rest of the accessions. The highest phytate: calcium molar ratio in the leaf samples was lower than the critical limit (0.24); this showed the bioavailability of calcium in Anchote leaf was within the acceptable limit (Morris & Ellis, 1985).

Like that of the tuber samples, the bioavailability of iron and zinc in the leaf samples of most accessions was found to be more than the critical limits of 1 for iron and 15 for zinc (Hallberg et al., 1989; Morris & Ellis, 1985; Sandberg et al., 1987; Turnlund, King, Keyes, Gong, & Michel, 1984). The phytate: zinc molar ratio reported in the present study is higher than the report of Melaku et al. (2005) who reported a lower (<12) molar ratio than the critical limit.

However, the molar ratio of phytate x calcium: zinc in the leaf samples of most accessions was less than the critical limit 200 (Bindra et al., 1986). The mean phytate: calcium molar ratios in the tuber and leaf samples in the present study were 0.05 and 0.11, respectively. Similarly, the mean phytate: iron, phytate: zinc and phytate x calcium: zinc molar ratios for tuber and leaf samples were 3.81 and 4.31, 27.79 and 22.47 and 142.20 and 90.72, respectively.

Table 25 Molar ratio between phytate and mineral analyzed in leaves of 44 Anchote accessions

Phytate/ mineral	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Phytate/Ca	0.22	0.06	0.11	0.03	7.08	36.47	<0.0001***
Phytate/Fe	10.64	0.43	4.31	2.79	9.41	94.49	<0.0001***
Phytate/Zn	45.05	5.62	22.47	14.63	4.36	449.89	<0.0001***
Phytate x Ca/Zn	15.83	223.13	90.72	73.08	6.06	356.72	<0.0001***

Unlike to the present finding Melaku et al. (2005) reported a lower (12.6±2.1) phytate: zinc, a higher phytate: calcium (20.3±3.2) and a lower phytate: iron (1.0±0.1) molar ratios for Anchote stew on fresh weigh basis. Lower phytate: iron (0.9) and phytate: zinc (8.6) molar ratios were reported for '*Kocho'* fresh pulp (Yewelsew et al., 2007) than the present values for tuber and leaf samples of Anchote. The phytate: calcium molar ratio of both tuber (0.18) and leaf (0.22) samples in the present study was relatively higher than the reported values for sweet potato based complementary foods (0.0 & 0.02) by Gibson et al. (2010).

However, these authors reported a very low phytate: iron (0.3 & 0.6) and phytate: zinc (1 & 3) molar ratios than the present values. Comparable phytate: iron molar ratio (8.06) was reported for wheat flour (Norhaizan & Norfaizadatul, 2009) however, the molar ratio of phytate: calcium (30.7) reported by these authors is a bit higher than the present values both for tuber and leaf samples. On the other hand, the same authors reported lower phytate: zinc (21.5) molar ratio. According to Gibson et al. (2010), high levels of phytate, a potent inhibitor of iron, zinc, and calcium absorption is usually found in plant based food ingredients.

However, root and tuber crops and most of leafy vegetables and fruits contain very low amounts of phytate, Molar ratios of phytate: calcium, phytate: iron, and phytate: zinc were calculated and presented to provide an estimate of the relative bioavailability of these minerals in tuber and leaf samples of 44 Anchote (*Coccinia abyssinica)* accessions (Morris & Ellis, 1989).

According to Melaku et al. (2005) though there is uncertainty on the critical molar ratio above which calcium absorption is compromised by phytate, there are suggestions by some investigators that phytate: calcium molar ratios less than 0.17 are desirable. For foods with phytate: zinc molar ratio >18 the estimated zinc absorption is 18% to 25%, whereas, for those foods with phytate: zinc molar ratios between 4 and 18 the estimates are between 26% and 34% (Hotz & Brown, 2004).

Though the inhibitory effect of phytic acid on iron absorption is also dose-dependent, the phytate: iron molar ratio should be less than 1.0:1.0 in order to have a two fold increase of iron absorption as would zinc absorption (Hurrell, 2004).

On the other hand, critical values have been calculated and reported for the ratio of phytate: calcium, phytate: iron, phytate: zinc and phytate: calcium/zinc by different researchers. Thus, the critical values are >0.24 for phytate: calcium (Morris & Ellis, 1985), > 1 for phytate: iron (Hallberg et al., 1989), >15 for phytate: zinc (Sandberg et al., 1987) and >200 for phytate x calcium/zinc (Bindra et al., 1986).

4.4.7. Phytate Phosphorus and Non-Phytate Phosphorus

Phytate is a form of P used for storage in plants. Organic P or phytate makes up around two-thirds of the total amount of P in grains and is also present in smaller amounts in forages (Eeckhout & De Paepe, 1994). A few factors can hinder the bioavailability of P to be absorbed. The presence of phytate-P is one of the factors most likely has the greatest negative influence on phosphorus bioavailability (Fowler, 2013). The range and mean concentration of phytate phosphorus, non-phytate phosphorus and phosphorus as phytate in tuber and leaf samples of Anchote is presented in Table 26 and Table 27.

Table 26 Maximum, minimum and range of phytate P, non-phytate P and P as phytate in tuber samples of 44 Anchote accessions

Forms of P	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Phytate P	91.01	5.71	36.71	18.70	6.92	108.54	<0.0001***
Non-phytate P	57.98	-30.86	7.21	18.05	45.38	60.51	<0.0001***
P as phytate	2.76	0.21	1.28	0.63	10.26	46.33	<0.0001***

Table 27 Maximum, minimum and range of phytate P, non-phytate P and P as phytate in leaf samples of 44 Anchote accessions

Forms of P	N=44						
	Max	Min	Mean	SD	CV	F-value	P>F
Phytate P	79.15	42.47	64.63	8.28	4.39	16.19	<0.0001***
Non-phytate P	35.13	23.87	5.46	12.25	52.12	36.48	<0.0001***
P as phytate	1.83	0.68	1.11	0.21	3.95	47.80	<0.0001***

Significantly (P<0.05) high phytate phosphorus concentration was recorded for tuber samples of accessions '223086' (91.01±2.37mg/100g) and '223085' (88.36±0.44 mg/100g) **(**Annex N) whereas the highest concentration of phytate phosphorus (79.15±1.57 mg /100g) for leaf samples was recorded for accession '223112-1' when compared with the rest of the accessions (Annex O).

Concentration of phytate phosphorous was in the range of 5.71 to 91.01mg/100g (mean 36.71 mg/100g) for tuber samples and 42.46 to 79.15 mg/100g (mean 64.63 mg/100g) for leaf samples. The average contents of phytate phosphorus in our study for both tuber and leaf samples were higher than the reported phytate phosphorus for various roots and tubers (21–25%) of the total phosphorus (Ravindran, Ravindran, & Sivalogan, 1994).

It was also reported that the phytate phosphorus content of cereal grains, oilseeds and grain legumes is the range 60-82% of total P. In another research, there was also report on the content of phosphorus in phytate, as a percentage of total phosphorus for wheat (77.6%), oats (74.3%), corn meal (78.3%), bran (77.7%), and extracted soybean (53.9%) (Morse, Head, & Wilcox, 1992).

The highest (57.98±3.74 mg/100g) concentration of non-phytate phosphorus for tuber samples was recorded for accession '223086'. However, the difference with accession '223085' (56.40±0.49 mg/100g) was not significant (P>0.05). Similarly, the highest (35.13±1.04 mg/100g) concentration of non-phytate phosphorus for leaf samples was recorded for accession 'DIGGA-1' and the difference with other accessions was significant.

The mean non-phytate phosphorus concentrations for tuber and leaf samples were 7.21 mg/100g and 5.46 mg/100g, respectively. The highest phosphorus as phytate percentage was 2.76 % for '223085' and '223086' accessions in tuber and 1.83% for 'DIGGA-1' accession in leaf samples with the mean concentrations of 1.28 % and 1.11%, respectively.

4.5. Conclusion

Though the difference in terms of the contents of major and trace minerals among the 44 analyzed samples was significant, Anchote was found to possess high levels of both major and trace minerals especially of calcium and iron. On the other hand, the levels of potentially toxic elements such as Cd, As, and Pb are almost negligible. The bioavailability of calcium, iron and zinc was noted to be satisfactory. Specifically, the bioavailability of calcium in both tubers and leaves was below the critical limit. Due to its high contents of minerals, both in the tubers and leaves, and their relative bioavailability, Anchote can be considered as a food ingredient in the food processing and food fortification interventions and can be included in the development of complementary foods for different age groups. However, a detailed further study is indispensable to identify the effect of processing on the bioavailability of the minerals. In Ethiopia, most of the complementary foods are based on cereal and/or legumes; there is a possibility of enriching them using Anchote.

Chapter Five: Functional Properties and Protein Quality of Anchote (*Coccinia abyssinica*) (Lam.) (Cogn.) Leaf Protein Concentrate, Whole Tuber and Leaf Powders

5.1. Abstract

*Chemical composition and functional properties of leaf protein concentrate (LPC) and, powder from tubers and leaves of Anchote were assessed to establish their potential for food use. Heat coagulation at natural pH was used to obtain the LPC from the aqueous fresh leaf extract. Composition of the LPC was analyzed using a standard method of AOAC. Amino acid content was analyzed by High performance liquid chromatography (HPLC) and in vitro protein digestibility (IVPD) test was done using pepsin-pancreatin enzyme mixture. The protein and carbohydrate contents of the LPC were 47.46 g/100g and*40.05 *g/100g, respectively. Moisture, fat, ash and energy contents were 7.36 g/100g, 7.97 g/100g, 4.53 g/100g, and 421.73 Kcal/100 g, respectively. The total amino acids content (TAAs) of Anchote LPC was 47.29 in g/100g sample and 99.64 in g/100g protein. The highest percentage of protein digestibility was recorded for whole tuber (57.44±1.48 %) followed by leaf protein concentrate (49.46±1.68) and leaf powder (40.92±0.54 %). The protein solubility of Anchote LPC was minimum (11%) when the pH ranged between 6 and 10 while maximum solubility (19%) was observed at pH 12. Anchote LPC exhibited relatively high bulk density compared to tuber and leaf powder. Water Absorption Capaccity (WAC) and Oil Absorption Capacity (OAC) were highest in leaf powder (i.e., 2.94 g/g and 1.29 g/g, respectively) and lowest value of WAC was observed in LPC (1.61 g/g), whereas tuber powder (0.81 g/g) registered the least OAC. Emulsification was reduced at the expense of increasing protein concentration and increased with the increase in pH in all tested samples. However, emulsion stability varied with changes in protein concentration and pH. The highest foam expansion was found in leaf powder, followed by LPC, and tuber powder. Foam stability for leaf and LPC powder was constant irrespective of the change in protein concentration unlike powder from tuber which showed a different trend with increasing protein concentration. On the other hand, foam stability was almost constant over a wide range of pH values. The results revealed that, owing to their positivefunctional properites and protein quality, LPC and powder of tuber and leaf of Anchote can be used as an ingredient for different food formulations.*

5.2. Introduction

With the increasing rate of world population, it is predictable to face food shortage since the demand for food consumption is likely to outpace food production. The increase in population mainly occurred in lesser-developed countries in the humid lowland tropics, and hence high-grade animal products, cereal grains, and animal feeds are expensive.

The use of leaf protein concentrate to counter malnutrition is becoming an excellent alternative to fill the gap of ever widening food shortage (Mune et al., 2011). As an additional source of protein, leaf protein concentrates (LPCs) should be given serious attention because leaves are abundant all year round in the tropics and many have high protein content (Kennedy 1993).

Leafy vegetables are the cheapest and most abundant source of proteins because of their ability to synthesize amino acids from a wide range of virtually available primary materials such as water, carbon dioxide, and atmospheric nitrogen as in legumes (Aye, 2012). Leaf proteins can be considered as the world most abundant protein source which is synthesized with a direct and efficient utilization of solar energy (Fasuyi & Aletor, 2005; Kung et al., 1980). Leaf protein concentrates (LPCs) also have a favorable amino acid composition which could be used effectively to supplement traditional cereal based diets used in most developing countries (Dewanji et al. 1997).

Anchote has appreciable amount of protein in its leaf, and can be good source for production of leaf protein concentrate for human food as well as animal feeds. In addition, the tuber and leaf of Anchote also can be used as a food ingredient and for new food product development since the nutrient composition of Anchote showed us its potential for different food formulation, specifically with its high protein content. Therefore, the objective of this study was to determine the functional properties of Anchote LPC, whole tuber and leaf powders, and *in vitro* protein digestibility of LPC for possible application in food processing.

5.3. Materials and Methods

5.3.1. Preparation of Anchote Leaf and Tuber Samples

Freshly harvested Anchote tuber and leaf samples from Debre Zeit Agricultural Research Center (DZARC) experimental field were first cleaned and sliced into small pieces before dried in a hot air oven (Cintex precison, India) set at 105°C to a constant weight. Next, the dried slices of leaf and tuber samples were milled into powder using electric grinder (FW 100, Yusung Industrial Ltd, China) and were sieved (0.425 mm).

Finally, the powder samples were sealed in plastic bags until analysis. The steps for sample preparation of both tuber and leaf powder samples are presented in flow chart (Figure 8).

5.3.2. Preparation of Anchote Leaf Protein Concentrate (LPC)

Anchote leaf protein concentrate was prepared according to the method described by Pandey & Srivastava (1993). Fresh plucked leaves of Anchote (100g), were thoroughly washed and crushed using distilled water in stoneware pestle and mortar, followed by filtration and pressing through double-layered muslin cloth, to obtain 300 ml leaf extract. Then the proteins were coagulated at pH 7 by heating the extract to 80 ºC for 20 to 30 minutes and allowed to settle for two hours. Separation of the coagulated protein was done by gravity filtration using muslin cloth stockings and dried in an air oven set at 60 °C and ground in to powder using pestle and mortar. The flow chart for processing steps of Anchote LPC illustrated in Figure 9.

5.3.3. Proximate Compositions of Anchote LPC

Proximate analysis of LPC for moisture content, dry matter, crude protein, crude fat, carbohydrates and ash were done according to AOAC methods (AOAC, 2000). Energy was calculated by Atwater's conversion factors 17 kJ/g (4kcal/g) for protein, 37 kJ/g (9 kcal/g) for fat and 17 kJ/g (4 kcal/g) for carbohydrates and expressed in calories (Nguyen et al., 2007).

5.3.4. Analysis of Free and Total Amino Acids for Anchote LPC

5.3.4.1. Extraction Procedure

a) Free Amino Acids

Free amino acids were extracted following the method used by Moore et al. (1958) with some modifications. Two g of sample and 100 ml of ultrapure water was added in a vacuum tube, sealed and shaken for 60 minutes on a rotational shaker. Two g of 5-Sulfosalicylic acid were then added to the solution and shaken for additional 60 minutes. The solution was decanted and the supernatant passed through 0.45µm membrane filter.

b) Total Amino Acids

Extraction of TAA involved weighing approximately 2 g of LPC adding50 ml of 6N HCl to a test tube and acid digest at 100 ˚C for 22 hrs. After evaporation (volatilization), 10 ml of 6N H_2O was added and then the tube was flushed with nitrogen closed and heated at 150˚C for two hrs. After cooling 450 µL of 4 M NaOH was added to the hydrolysate, and then diluted to 20 ml with a citrate buffer at pH 2.2. Finally, the extract was filtered using 0.45µm membrane filter.

5.3.4.2. Amino Acid Analysis

The extract samples for both free and total amino acid analysis were injected into the rugged HPLC analysis protocol using Agilent 1100 HPLC according to the method of Henderson et al. (2000). All total and free amino acid contents were determined except total tryptophan, which is the most fragile amino acid that can have a possibility to be destroyed by the extraction procedure. Amino acid standards were also run in a similar way as the samples.

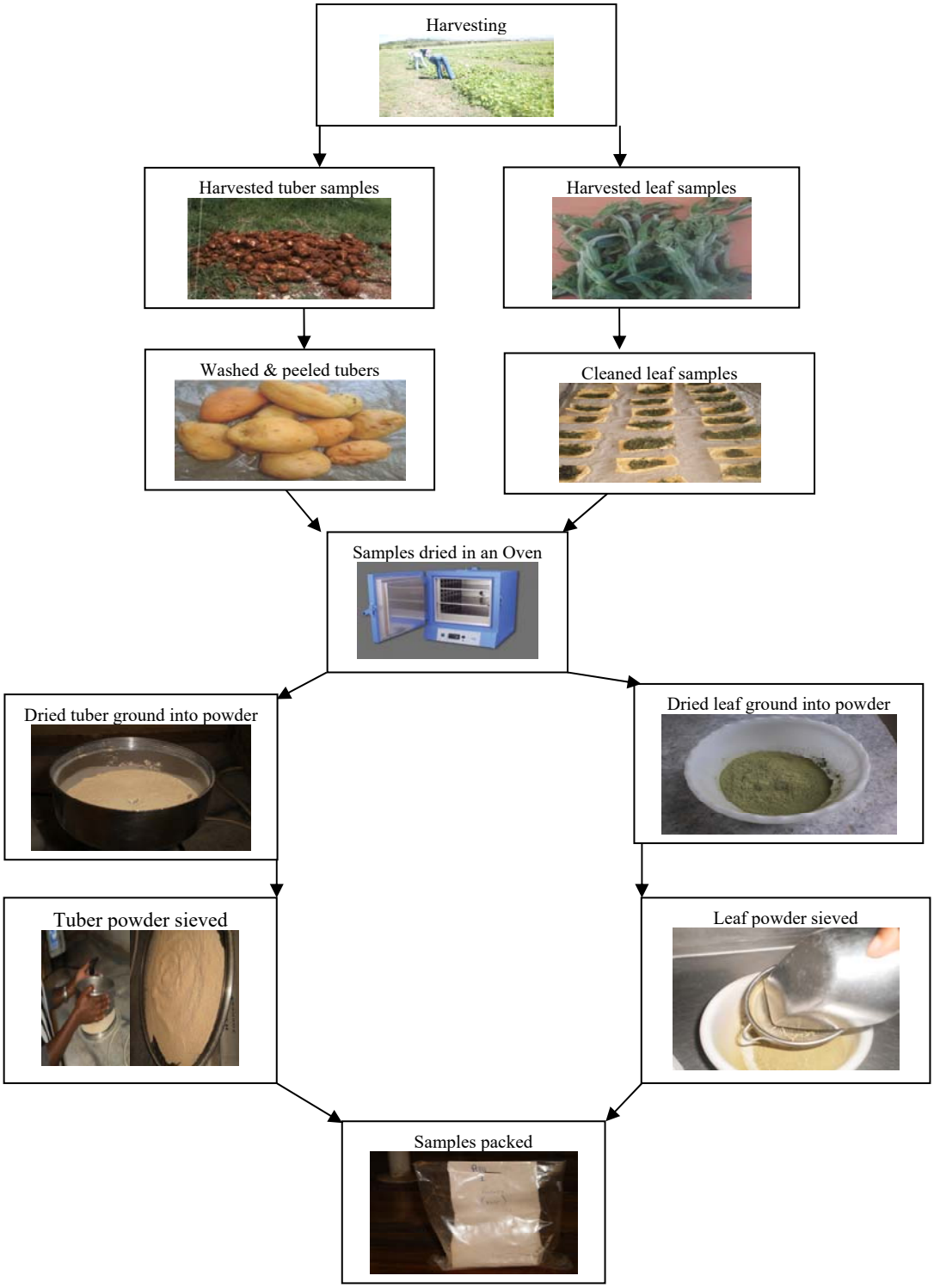


Figure 8 Flow diagram for whole tuber and leaf powder preparation

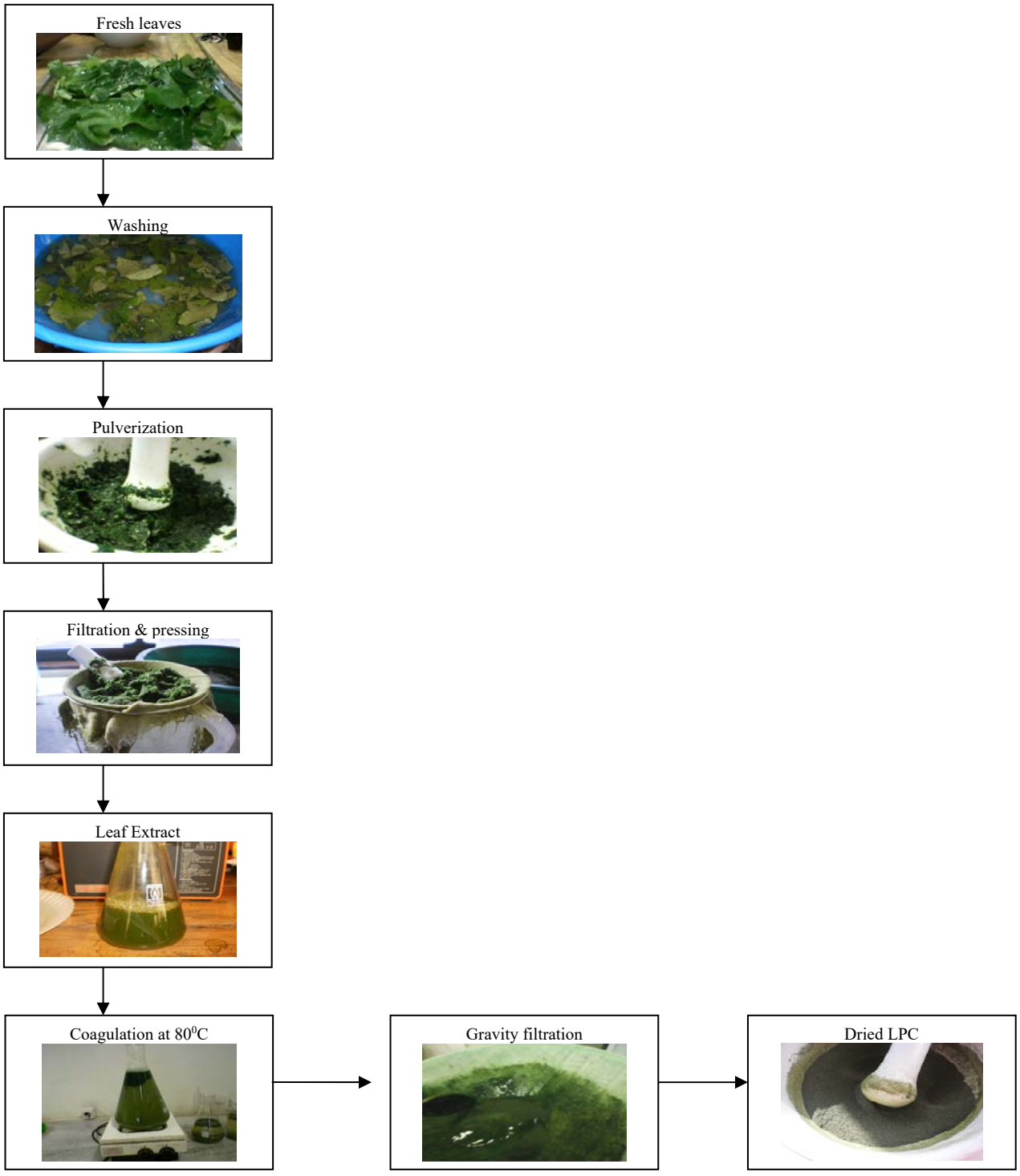


Figure 9 Flow diagram of Anchote leaf protein concentrate preparation

5.3.5. *In-Vitro* Protein Digestibility

In vitro protein digestibility was determined for Anchote LPC using Pepsin-Pancreatin enzyme according to Manukumar et al. (2014) with little modification. Anchote LPC (1g) was added to 15 ml of 0.16M HCl containing 1.5 mg of pepsin (Sigma, P-7000, 14900 u/ml) and incubated for 2 hrs. at 37˚C in a shaking water bath. The resulting suspension was neutralized with 7.5 ml of 0.2 M NaOH and treated with 4 mg of pancreatin (Sigma, P-7545, 8 * USP specifications) in 7.5 ml of 0. 2 M phosphate buffer (pH 8).

The mixture then incubated for an additional 2 h at 37˚C. By omitting the sample enzyme blank was prepared under the same conditions described above for the sample preparation. After incubation, the sample was treated with 10 ml of 10% trichloroacetic acid (TCA) (Sigma) to remove undigested protein and larger peptides and centrifuged at 5000 g for 20 min at room temperature. The supernatant solution was filtered using Whatman No. 1 filter paper and the protein in the supernatant was then determined using Kjeldahl method (AOAC, 2000). Finally, percentage of protein digestibility was calculated according to (Kataria, Chauhan, & Punia, 1992) as follows:

$$\text{Protein digestibility } (\%) = \frac{\text{Digestible crude protein}}{\text{Total crude protein}} \text{ X } 100$$

5.3.6. Protein Solubility

Protein solubility was determined using the method of Bera & Mukherjee (1989) with slight modification. Anchote LPC (100 mg) was directly weighed into a conical flask and dispersed with 10 ml of deionized water. The suspensions were adjusted to pH ranging from 2.0 - 12.0 using either 0.1 N HCl or 0.1 N NaOH.

Dispersed samples were shaken in an orbital shaker for 30 min at room temperature and then centrifuged at 3000rpm for 30 min. Duplicate aliquots of the supernatant were analyzed for protein content using the Kjeldahl method (AOAC, 2000). Finally, the protein solubility was calculated and expressed in percentage as follows:

$$\text{Protein solubility (\%)} = \frac{A}{B} X\ 100$$

Where :
A : Total protein content (mg) of the supernatan t
B : Total protein content (mg) of the sample

5.3.7. Functional Properties of Anchote LPC, Whole Tuber and Leaf Powder

i) **Bulk Density**

The method used by Udensi & Okaka (2000) was adopted for determination of the bulk density of the samples. Fifty ml graduated cylinder containing 5 g of Anchote powder sample was tapped continuously against the palm of hand until a constant volume was obtained. The volume was used to calculate the bulk density with the following formula:

$$\text{Bulk density } (g/cm3) = \frac{\text{Weight of sample}}{\text{Volume of sample}}$$

ii) Water and Oil Absorption Capacity (WAC and OAC)

Water and oil absorption capacity were determined according to Gandhi & Srivastava (2007). One gram of sample was mixed with 10 ml distilled water and sunflower oil respectively in centrifuge tubes, and then allowed to stand for 30 min. Samples were centrifuged at 3000rpm for 30 min. Weight of the tube was measured after discarding the supernatant. Results were reported by taking the average of 10 measurements.

The WAC (grams of water per gram of sample) and OAC (grams of oil per gram of extract) was calculated using the following equations:

$$\text{Water Absorption Capacity (WAC)} = \frac{(W2 - W1)}{W0}$$

Where :
W0 : Weight of dry sample (g)
W1 : Weight of tube plus dry sample (g)
W2 : Weight of tube plus sediment (g)

$$\text{Oil Absorption Capacity (OAC)} = \frac{(F2 - F1)}{F0}$$

Where :
F0 : Weight of dry sample (g)
F1 : Weight of tube plus dry sample (g)
F2 : Weight of tube plus sediment (g)

iii) **Emulsifying Properties**

a) Effect of Protein Concentration on Emulsifying Properties

The emulsifying properties of Anchote LPC was determined by the turbidometry method according to Agyare et al. (2009). LPC emulsions were prepared containing 10 to 50mg/ml LPC in 0.01 M sodium phosphate buffer in a 15 ml plastic tubes, stirred at room temperature for 30 min followed by addition of 5 ml of pure sun flower oil. The mixtures in each sample solution were then shaken together and homogenized with a polytron homogenizer at speed of 12,000rpm for one minute. 50 µl aliquots of freshly prepared emulsion were taken 0.5 cm above from the bottom of the plastic tube at 0 and 10 minutes, and dispersed into 5 ml of 0.1% sodium dodecyl sulfate (SDS) solution (1:100 dilution), and shaken briefly in a vortex mixer.

The absorbance for the dispersed emulsion samples were then measured at 500 nm against 0.1% SDS solution blank in UV/VIS/NIR spectrophotometer (Perkin Elmer, USA). The indexes of emulsifying activity, EAI (m^2/g) and emulsion stability, ESI (%) of the homogenized samples were calculated as follows:

$$EAI\ (m2/g) = \frac{(2 \times 2.303)}{C \times (1-\phi) \times 104} \times A500 \times Dilution$$

Where :

A500 : The absorbance at 500 nm

C : Protein concentrat ion (g/ml) before emulsion

ϕ : Oil volume fraction (v/v) of the emulsion (ϕ = 0.20)

$$ESI\ (\%) = 100 * A10/A0$$

Where :

A10 : The absorbance after 10 min at 500 nm

A0 : The absorbance at time zero at 500 nm

b) Effect of pH on Emulsifying Properties

The effect of pH on the emulsifying activity and stability was determined according to Qi et al. (1997). Sample solutions (0.1%, w/v) were prepared and adjusted to pH 2–12 (0.1M HCl or NaOH) with final volume 15 ml. Emulsifying activity and stability were then determined as described above.

iv) Foaming Properties

a) Effect of Protein Concentration on Foaming Properties

The effect of protein concentration on foaming capacity (FC) and foam stability (FS) of Anchote LPC, whole tuber and leaf powder and egg albumin (reference protein) was determined according to Agyare et al. (2009) and Arogundade (2006). Protein dispersions (10 to 50 mg/ml) stirred at room temperature for 60 min followed by homogenization for 1 minute at high-speed velocity (12000 rpm). The resulting foam was then poured into a 100-ml cylinder to record total foam volume. Finally, the foam capacity was expressed as the percent increase in volume by calculating with the following formula:

$$FC\ (\%) = \frac{\text{Volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} X\ 100$$

Foam stability was reported as foam volume standing up to the end of a holding time (60 min) after whipping and calculated as follows:

$$FS = \frac{\text{Foam volume after 60 min}}{\text{Initial foam volume}}\ X\ 100$$

b) Effect of pH on Foaming Properties

To determine the effect of pH on the foaming capacity and stability of LPC, whole tuber and leaf powder and egg albumin, 0.5 g of sample was dissolved in 50 ml of buffer solutions, with different pH values (2, 4, 6, 8 10, 12). The suspensions were then whipped at 12000 rpm for 1 min at room temperature. Foaming capacity and foaming stability were then determined using the equation described above.

5.3.8. Statistical Analysis

The average of triplicate measurements was analyzed using one-way analysis of variance (ANOVA). Means were compared by Duncan multiple range test (DMRT) with mean square error at 5% probability using SAS, 2004 version 9. Mean ± standard deviation is used to express the data.

5.4. Results and Discussions

5.4.1. Proximate Composition of Anchote Leaf Protein Concentrate (LPC)

Proximate composition of Anchote LPC is shown in Table 28. Moisture content of Anchote leaf protein concentrate was 7.36±0.08 g/100g, and falls in the range of 6.6±0.6 to 7.6±0.6 g/100g reported for *Telfairia occidentalis* and *Amaranthus hybridus* leaf protein concentrates, respectively (Adeyeye & Omolayo, 2011).

On the other hand, the moisture content of our sample was higher than that reported for LPC values of 6.49±0.10 to 6.58±0.36 % (g/100g) for *Pongamia pinnata* under varied optimized process parameters by Khan & Varshney (2015). Moisture in food determines the rate of food absorption and assimilation within the body as well as the keeping quality of food (Sodamade et al., 2013). The moisture content of Anchote LPC was within the recommended value (≤10%) for leaf protein concentrates (Khan & Varshney, 2015).

Anchote LPC contains high amount of crude protein (47.46±0.40 g/100g DM) compared to the values reported for *Amaranth hybrids* (35.2±0.9 g/100g DM), *Manihot esculenta* (41.7±0.4 g/100g DM) LPC (Aletor & Adebayo, 2012) and (46.1±1.2 g/100g DM) *Solanum Africana* (Aletor et al. 2002). On the other hand, the protein content of Anchote leaf concentrate was lower than that reported for *Telfairia occidentalis* (554.8±1.1 g/ kg DM which was 55.48 g/100g) (Agbede, Adegbenro, Onibi, Oboh, & Aletor, 2008), *Vernonia amygdalina* (52.2±2.4) and *Telfaria occidentalis* (54.9±1.3) in g/100g DM (Aletor et al., 2002).

The presence of high quantity crude protein in Anchote LPC shows its potential to be used as nutritionally valuable ingredient in areas where there is high prevalence of protein malnutrition (Aletor & Adebayo, 2012). The proteins in LPC of most green vegetables are indispensable to ensure the restoration of the amino-acids required for the synthesis of the structural and functional cells of the body (Khan & Varshney, 2015). Therefore, with high level of protein intake and amino acid supplementation, it is possible to substitute a large proportion of animal protein requirement by these vegetable proteins (Aletor et al., 2002).

Crude fat content of Anchote LPC (7.97±0.02 g/100g) was in agreement with the reported average value for four leafy vegetables LPC powder which was 7.9 g/100 g DM (range 5.6-11.9 g/100g DM) (Aletor et al., 2002). On the other hand, the fat content of our sample was higher than *Amaranthus hybridus* (5.6±0.3), *Vernonia amygdalina* (5.6±0.37) and *Moringa oleifera* (2.43 ± 0.47) but lower than *Solanum africana* (8.1±0.1), *Telfaria occidentalis* (11.9±0.2), and *Pongamia pinnata* (12.54±0.31-12.82±0.49) in g/100g (%) leaf protein concentrates (Aletor et al., 2002; Khan & Varshney, 2015; Sodamade et al., 2013).

The ash content of Anchote LPC was 4.53±0.31 g/100g. This value was higher than the value reported for *Pongamia pinnata* (3.66±0.19 to 3.97±0.69)(Khan & Varshney, 2015). The ash content of Anchote LPC was lower than the reported LPC values such as *Moringa oleifera* (6.00 ± 0.63) (Sodamade et al., 2013), *Amaranth hybridus* (5.6±0.4) and *Manihot esculenta* (8.1±0.2) (Aletor & Adebayo, 2012), *Amaranthus hybridus* (17.2 ± 0.01) and *Telfaria occidentalis* (12.3 ± 0.01) (Adeyeye & Omolayo, 2011) as well as *Manihot esculenta* (6.00-8.74) (Castellanos, Altamirano, & Moretti, 1994)in (g/100g).

The high ash content in the food determine largely the extent of mineral matters likely to be found on food substance and the intake of those LPC with high ash content expected to contribute a large proportion of mineral requirement of the body.

Table 28 Proximate analyses of Anchote leaf protein concentrate (dry basis[a])

Chemical composition	Contents
Moisture (g/100g)	7.36±0.08
Crude protein (g/100g)	47.46±0.40
Crude fat (g/100g)	7.97±0.02
Ash (g/100g)	4.53±0.31
Total carbohydrate (g/100g)	40.05±0.62
Energy (Kcal/100g)	421.73±1.14

a Means ± standard deviation of triplicate determination.

The data obtained in this work shows that Anchote LPC has total carbohydrate content of 40.05±0.62 g/100g. The value obtained in this study is comparable with the carbohydrate content of LPCs isolated from lower, middle and upper canopy of *Pongamia pinnata* which ranged from 39.18±0.83 to 39.83±0.38 (g/100g) (Khan & Varshney, 2015).

On the other hand, the carbohydrate content of Anchote LPC was higher than the LPC obtained from *Manihot esculenta* (33.59-36.03 g/100g) in different treatments (Castellanos et al., 1994)and for *Amaranthus hybridus* and *Telfairia occidentalis* (29.0±0.4 and 33.4±2.4 g/100g) (Adeyeye & Omolayo, 2011).

The total energy content of Anchote LPC was 421.73±1.14Kcal/100g.This value is lower as compared to the total energy values reported for *Amaranth hybridus* (635 Kcal/100g) and *Manihot esculenta* (474 Kcal/100g) (Aletor & Adebayo, 2012). The average energy value (439 kcal/100g DM) of LPC for four leafy vegetables (*Vernonia amygdalina, Solanum africana, Amaranthus hybridus,* and *Telfaria occidentalis*) (Aletor et al., 2002) is also higher than our result.

5.4.2. Free and Total Amino Acid Analysis of Anchote LPC

Table 29 shows the total and free amino acid contents in Anchote leaf protein concentrate. The percentage contribution of essential amino acids (EAAs) for Anchote LPC was 45%, of which leucine (Leu) had the highest value (26%) while histidine (His) showed the lowest (6%). The non-essential amino acid content (NEAAs) comprised 55% of which alanine (Ala) score the highest amino acid taking a share of about 28% followed by tyrosine (Tyr) which contains 18 %.

The ratio of EAAs to NEAAs was 0.83%. The total amino acids content (TAAs) of Anchote LPC was 47.29 g/100g sample. On the other hand, the free amino acids comprised of 0.67 g/100g sample. The free essential amino acids were better concentrated (0.37g/100g sample or 0.77 g/100g protein) than that of non-essential amino acids (0.30g/100g sample or 0.64 g/100g protein).

The efficiency of plant protein could be greatly increased if mechanical power and technology were used to separate plant protein from fiber and concentrate .into consumable forms (Kennedy, 1993b). The leaf protein concentrate is the potential source of protein which is relatively cheap and most abundant as compared to animal source proteins since it is a cellulose-free protein it can supply 10-20 g protein/person/day (Oke, 1973). According to Aletor et al. (2002), taking a high level of LPC amino acid supplements is expected to replace the animal protein requirement which is expensive for most of the poor inhabitants in the developing countries.

Table 29 Free and total amino acid profile of Anchote leaf protein concentrate (LPC) on dry weight basis

Amino acids	g/100g sample		g/100g protein	
			Protein (g/100g) = 47.46	
	Free	Total	Free	Total
Essential Amino Acids (EAAs)				
His	0.02	2.12	0.05	4.46
Ile	0.04	2.90	0.09	6.12
Leu	0.10	4.83	0.20	10.17
Met	0.04	2.25	0.09	4.73
Phe	0.05	4.25	0.11	8.96
Thr	0.04	2.63	0.09	5.55
Val	0.06	2.50	0.14	5.28
Total EAAs	0.37	21.48	0.77	45.26
Non-Essential Amino Acids (NEAAs)				
Ala	0.09	3.03	0.18	6.39
Arg	0.02	4.00	0.05	8.44
Asp	0.03	4.35	0.07	9.17
Cys	0.00	0.11	0.00	0.23
Glu	0.03	4.40	0.07	9.28
Gly	0.02	2.70	0.05	5.69
Pro	0.02	2.15	0.05	4.53
Ser	0.03	2.34	0.07	4.94
Tyr	0.05	2.72	0.11	5.73
Total NEAAs	0.30	25.81	0.64	54.38
SAAs	0.04	2.35	0.09	4.96
AAAs	0.11	6.97	0.23	14.69
TAAs	0.67	47.29	1.41	99.64

In addition, the LPC obtained from green plants is sufficiently high quality, and can be used as a dietary supplement to help eliminate the world's protein malnutrition problem and this justifies increased research to solve problems of LPC production and utilization (Gerloff et al. 1965). From a dietary standpoint, the leaf protein concentrate has a favorable balance of essential and non-essential amino acids, and it contains nutritional potential that could find application in food ingredient, infant formula, food supplement and food formulation (Sodamade et al. 2013).

5.4.3. *In-Vitro* Protein Digestibility of LPC and Powder Samples

Determination of the nutritive value of protein by enzyme solubilization *in vitro* is widely used as rapid method to estimate protein quality (Buchanan, 1969). The *in vitro* protein digestibility (IVPD) was therefore determined for Anchote leaf protein concentrate, whole powder made from oven dried leaf and tuber to evaluate digestibility of protein that is the primary indicator of the availability of amino acids. The highest percentage of protein digestibility was recorded for tuber powder (57.44±1.48 %) followed by leaf protein concentrate (49.46±1.68) and leaf powder (40.92±0.54 %) (Figure 10).

Low digestibility of protein is a factor which undermines nutritional quality of food and this might be caused by antinutritional factors like protease inhibitors, haemagglutinins, phytic acid and polyphenols (Kataria et al. 1992). Improvement of protein digestibility are achieved in various research findings. Some of them are degradation by proteolytic enzymes, hydrolysis of proteins and tannins, heat processing to destroy heat labile protease inhibitors and denature globulins that are highly resistant to proteases, as well as soaking to facilitate leaching of antinutrients (Hassan & Tinay 1995; El Hag et al. 2002; Duodu et al. 2003; Pranoto et al. 2013; Amare et al. 2015).

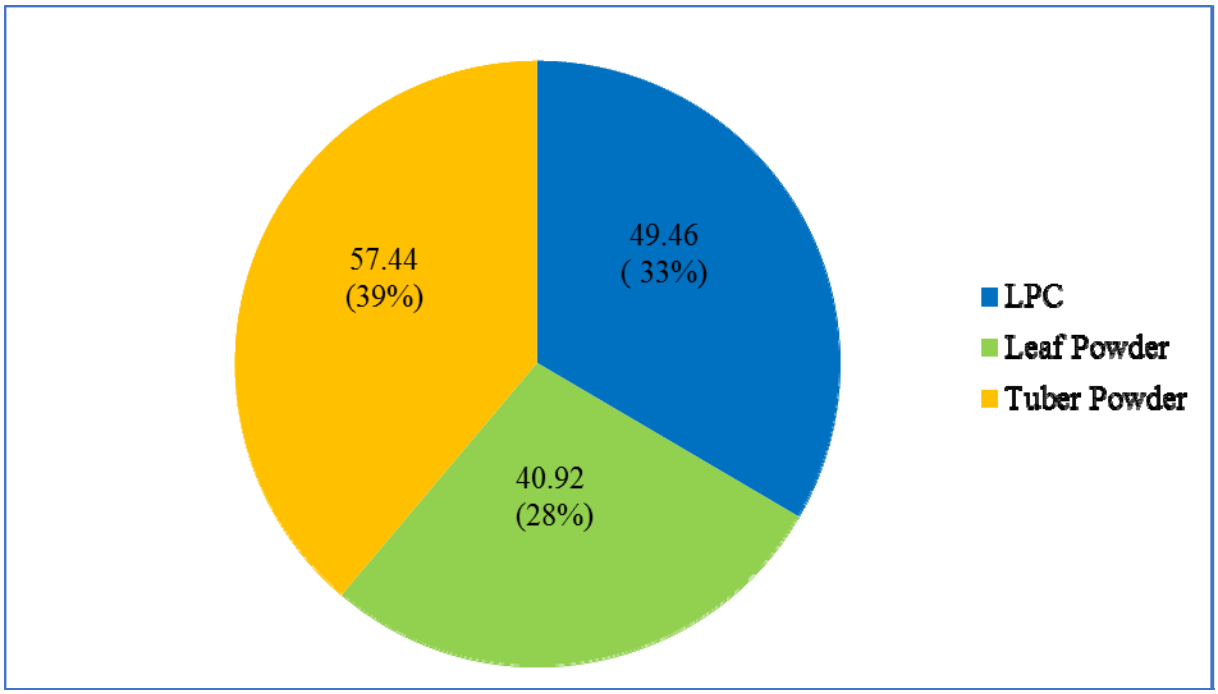


Figure 10 *In vitro* protein digestibility percentage of Anchote LPC and whole powder of leaf and tuber

5.4.4. Protein Solubility for Anchote LPC

Protein solubility is an important property and is directly related to functional properties such as emulsifying, foaming and gel forming abilities of a given food protein (Ortiz & Wagner, 2002; Schwenzfeier, Wierenga, & Gruppen, 2011). Figure 11 shows the protein solubility profile of Anchote LPC with a change in pH. There was a significant difference ($p < 0.05$) in protein solubility as a function of pH. From the result it is revealed that protein of LPC had shown higher solubility at pH 2 and 7, with a decreasing trend in between these two pH peaks and then increased as the sample pH increased till it reached the maximum peak at pH 12. It is evident from these results that, greater amounts of soluble proteins were extracted at alkaline pH, and is also possible that there was a greater denaturation of protein with increasing pH, which increased solubility (Alobo, 2004; Mao & Hua, 2012; Sze-Tao & Sathe, 2000).

Minimum protein solubility which represents the isoelectric point (IEP) was reached at pH 8 (11%). This helped to indicate the decrease in solubility as the pH decreases until it reaches the IEP and then increases (Mao & Hua, 2012). Anchote LPC showed greater solubility in acid, neutral and alkaline media (Figure 11). The solubility pattern suggests Anchote LPC as being useful in the formulation of both acid and alkaline foods in addition to the neutral media.

The result of protein solubility in this study is comparable with the findings reported by Fasuyi & Aletor (2005) for cassava leaf meal and protein concentrates, and by Alobo (2004) for defatted papaya kernel flour. A higher protien solubility of Anchote LPC might have facilitated diffusion and spreading at oil/water interfaces, since the diffusion results from a concentration gradient of protein at the interface (Qi et al., 1997).

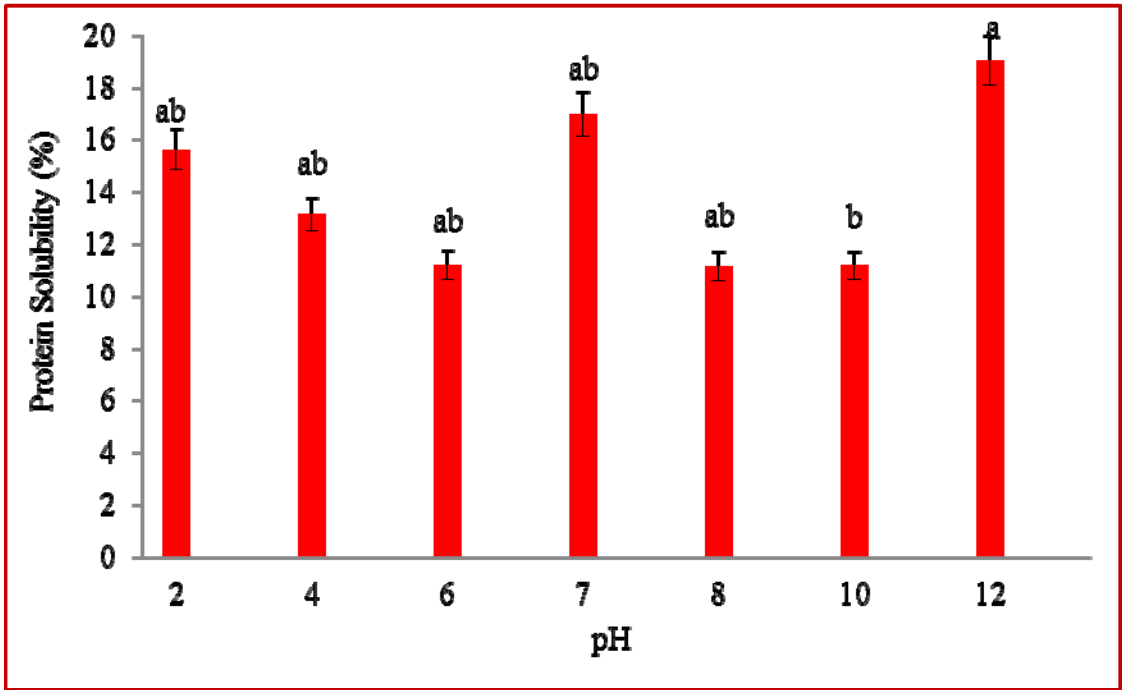


Figure 11 Effect of pH on protein solubility of Anchote leaf protein concentrate (LPC)

5.4.5. Functional Properties of Anchote LPC, Tuber and Leaf Powder

i) Bulk Density

The bulk density for the powder sample of Anchote was 0.85±0.02, 0.71±0.02, and 0.91±0.01g/ cm^3 for tuber, leaf, and LPC, respectively (Table 30). The LPC exhibited the highest value of bulk density and the lowest value recorded for leaf powder. The bulk density in our study was found to be higher than the value reported for rice bran protein concentrate (Chandi & Sogi, 2007) and *Moringa peregrina* and soy products (Al-Kahtani & Abou-Arab, 1993). The bulk density of Anchote tuber (0.85g/ml) and LPC (0.91g/ml) were comparable with the bulk density of casein (0.89 g/ml) (Appiah et al., 2011). The bulk density of Anchote leaf powder was within the range of cowpea varieties 0.69 - 0.80 g/dm^3 (Appiah et al., 2011).

Bulk density depends on the combined effects of interrelated factors such as the intensity of attractive inter particle forces, particle size, and number of contact points (Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009). Higher bulk density is desirable for greater ease of dispersibility of flours by increasing the sink ability of powdered particles (Padmashree et al. 1987; Udensi & Okaka 2000; Ekwu et al. 2005). In addition, the density of the flour is important in determining the packaging requirement and material handling (Ezeocha, Omodamiro, Oti, & Chukwu, 2011). High bulk density and large diameter of the aggregates also result in precipitation of protein (Singh, Kaur, & Kawaljit, 2005). In contrast, low bulk density would be an advantage in the formulation of complementary foods since it helps to reduce the paste thickness which is an important factor of complimentary food formulations (Akpata & Akubor, 1999; Appiah et al., 2011).

ii) Water and Oil Absorption Capacity

a) Water Absorption Capacity

Water and oil absorption behaviors of food systems have been shown to be dependent on the content of lipophilic and hydrophilic groups of proteins and carbohydrates (Kinsella, 1976; Shanmugasundaran & Venkatamaran, 1989).

The ability of protein to bind water is indicative of its water absorption capacity, since protein has both hydrophilic and hydrophobic properties and so can interact with water in foods (Appiah et al. 2011). The water absorption capacity was highest in leaf powder (2.94 g/g) and lowest in LPC (1.61 g/g) as shown in Table 30. The observed high water absorption capacity could be attributable to the presence of hydrophilic proteins which suggests that they would be useful functional ingredients in bakery products (Appiah et al. 2011). High water absorption of proteins also helps to reduce moisture loss in packed bakery goods, and is required to maintain freshness and moist mouth feel of baked foods (Aletor et al. 2002).

On the other hand, the lower water absorption capacity might be due to less availability of polar amino acids found in the powder (Kuntz, 1971). The overall observed variation in water absorption among the tested powder samples may be due to different protein concentrations, their degree of interaction with water and their conformational characteristics (Butt & Batool, 2010). The water absorption capacity of Anchote tuber (1.88 g/g) was comparable to cowpea variety (1.89 g/g) reported by Appiah et al. (2011) in Ghana. Aletor et al. (2002) reported average water absorption capacity of 2.66 (g/g) for different leaf protein concentrates. Water absorption capacity values ranging from 1.49 to 4.72 (g/g) are considered critical in viscous foods such as soups and gravies (Aletor et al. 2002).

b) Oil Absorption Capacity

Oil absorption is an important functional property for food product ingredients because it improves flavor retention and the sensation produced in the mouth (Mart´inez-Flores et al., 2006).The highest oil absorption capacity was observed in Anchote leaf powder (1.29 g/g), and Anchote tuber powder exhibited the lowest (0.81 g/g) value (Table 30).

The value of oil absorption capacity obtained from leaf powder and LPC of Anchote were higher compared to egg albumin (1.08 g/g), and casein (1.72 g/g) (Chandi & Sogi, 2007). Oil absorption capacity is attributed to the physical entrapment of oil and to the number of non-polar side chains on proteins that bind hydrocarbon chains on fatty acids (Al-Kahtani & Abou-Arab, 1993).

The difference between oil absorption capacity of different proteins could be related to the variation in amino acid compositions and several parameters such as hydrophobicity, degree of denaturation and the size and flexibility of protein (Onsaard, Pomsamud, & Audtum, 2010).

Table 30 Bulk density, water and oil absorption capacity, and water solubility of Anchote tuber, leaf and leaf protein concentrate (LPC) powder

Parameters	Tuber Powder	Leaf Powder	LPC	Egg albumin
Bulk density (g/ cm^3)	0.85±0.02	0.71±0.02	0.91±0.01	-
Water absorption capacity (g water/g sample)	1.88±0.54	2.94 ±0.15	1.61±0.08	1.70±0.53
Oil absorption capacity (g oil/g sample)	0.81±0.04	1.29±0.12	1.23±0.01	1.08±0.05

iii) Emulsifying properties

The emulsifying property (EA) is one of the important functional properties of protein in foods that affects applications of the protein in food formulation and food additive (Singh, Ye, & Horne, 2009; L. Tang et al., 2012). Emulsions are formed due to presence of hydrophobic and hydrophilic groups of proteins (Chandi & Sogi, 2007).

Emulsifying property reflects the ability of proteins to rapidly adsorb at the water/oil interface during the formation of emulsion, while ES reflects the ability of the proteins to maintain a stable emulsion over a period by preventing flocculation and coalescence of the oil globules (Shevkani, Singh, Rana, & Kaur, 2014). The emulsifying properties for LPC, leaf and tuber powder of Anchote compared over a range of protein concentrations and pH values.

a) *Effect of Protein Concentration on Emulsifying Properties*

The effect of protein concentration on the emulsifying activity (EA) and emulsion stability (ES) of Anchote LPC, anchote tuber and leaf powder are compared to the standard bovine serum albumin (BSA) as shown in Figure 12 (a) and (b).

Emulsion activity (EA) of BSA has a significant difference compared to Anchote samples in which the EA of BSA increased with the increase in protein concentration. Emulsifying activity decreased in a similar trend in all the three samples with the expense of increasing protein concentration until it reached the level of 30 mg/ml concentration (Figure 12 (a)). The trend of decreasing in EA with an increase in concentration continued until the final concentration level of 50 mg/ml in case of tuber powder.

The decreasing trend of EA as protein concentration increased in Anchote tuber was comparable with cashew nut protein concentrate and isolate (Ogunwolu et al., 2009); soy protein isolate (Chove, Grandison, & Lewis, 2001); winged bean protein concentrate (Sathe et al., 1982) and sunflower protein isolate(Lin, Humbert, & Sosulski, 1974). In contrast, the EA of leaf powder continues to increase and the level of EA kept constant from 30 mg/ml upward in the case of the LPC. Emulsion capacity is linked to soluble protein content of a substance (Shanmugasundaran & Venkatamaran, 1989). In general, high emulsion activity and stability of the powder suggests its possible use in cakes, sausages and other colloidal food systems (Alobo, 2004).

According to Phillips (1981) the dependence of emulsifying activity on concentration of protein has been explained based on adsorption kinetics in which at low protein concentrations, protein adsorption at the oil water interface is diffusion-controlled whereas at high protein concentration, activation energy barrier does not allow protein migration in a diffusion dependent manner. Further increase in sample concentration may lead to accumulation of peptides in the aqueous phase, a development that result in decrease in emulsifying activity (Agyare et al., 2009). Regarding emulsion stability (ES), at initial stage of protein concentration (10 mg/ml) leaf powder and BSA were higher in ES than that of tuber powder and LPC as shown in Figure 12 (b).

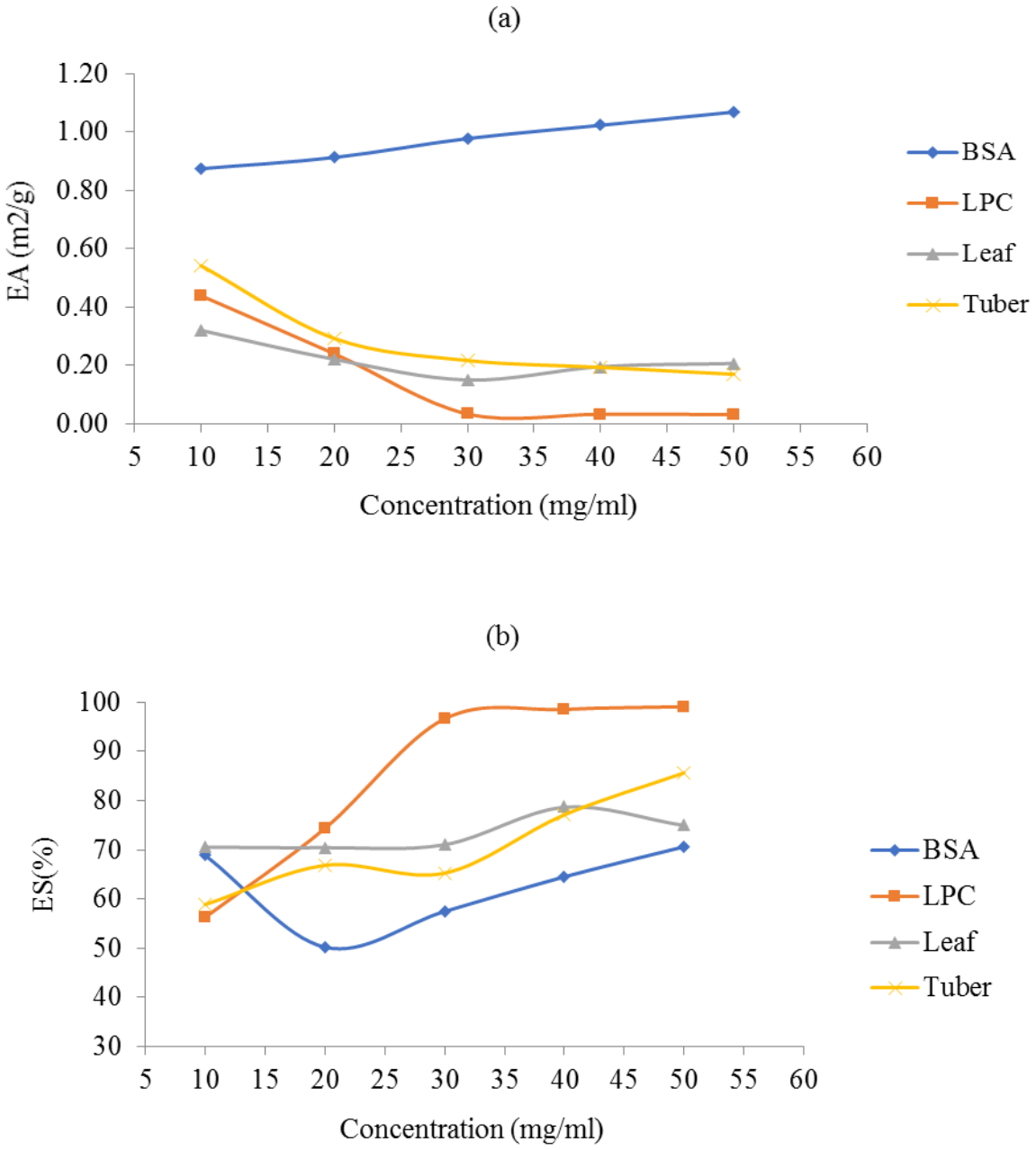


Figure 12 Effect of protein concentration on emulsifying activity (EA) (a), and emulsion stability (ES) (b)

A gradual decrease of ES was observed for leaf powder and BSA at 20 mg/ml, followed by concentration, again there was a slight increase in ES at 30 and 40 mg/ml concentration level but the percentage of ES decreased at 50 mg/ml in case of leaf powder while the BSA increased at this level. On the other hand, ES of LPC increased with an increase in concentration and became more stable whereas the tuber powder showed an increment at a concentration level of 20 mg/ml with a slight reduction at 30 mg/ml; and increase again with the increase in concentration.

For LPC and tuber powder, with increasing protein concentration, the EA was reduced, while the ES increased. This trend is in close agreement with the result obtained for peanut protein and peanut peptide (Tang et al., 2012). Solubility and surface hydrophobicity are known as important properties for maintaining a stable emulsion (Galazka, Dickinson, & Ledward, 1996). The decrease in emulsifying stability may be attributed to the decrease in molecular flexibility of proteins resulted from aggregation, and ES increases when there is unfolding of protein which leads to a decrease in surface tension (Kato & Nakai, 1980; Qin et al., 2013). Thus it can be concluded that the negative effect of protein aggregation is capable of counteracting the positive effect of an increased surface hydrophobicity on emulsifying capacity, leading to a decrease in ES (Qin et al., 2013).

b) Effect of pH on Emulsifying Properties

The effect of pH on the emulsifying activity (EA) and emulsion stability (ES) of Anchote LPC, tuber and leaf powder samples as compared to the standard bovine serum albumin (BSA) presented in Figure 13 (a) and (b). The EA of all the three Anchote samples were minimal at pH 2 that is the isoelectric point (IEP) and increased with the increase in pH Figure 13 (a). The EA values of BSA were significantly higher than the three Anchote samples showing an increase in EA with the increase in pH. The EA of LPC constantly increased when the pH shifts from acidic to alkaline and the highest emulsification capacity observed at pH 12. However, in the case of the leaf and tuber powder samples, reduction in EA observed at pH 10, which again increased at pH 12.

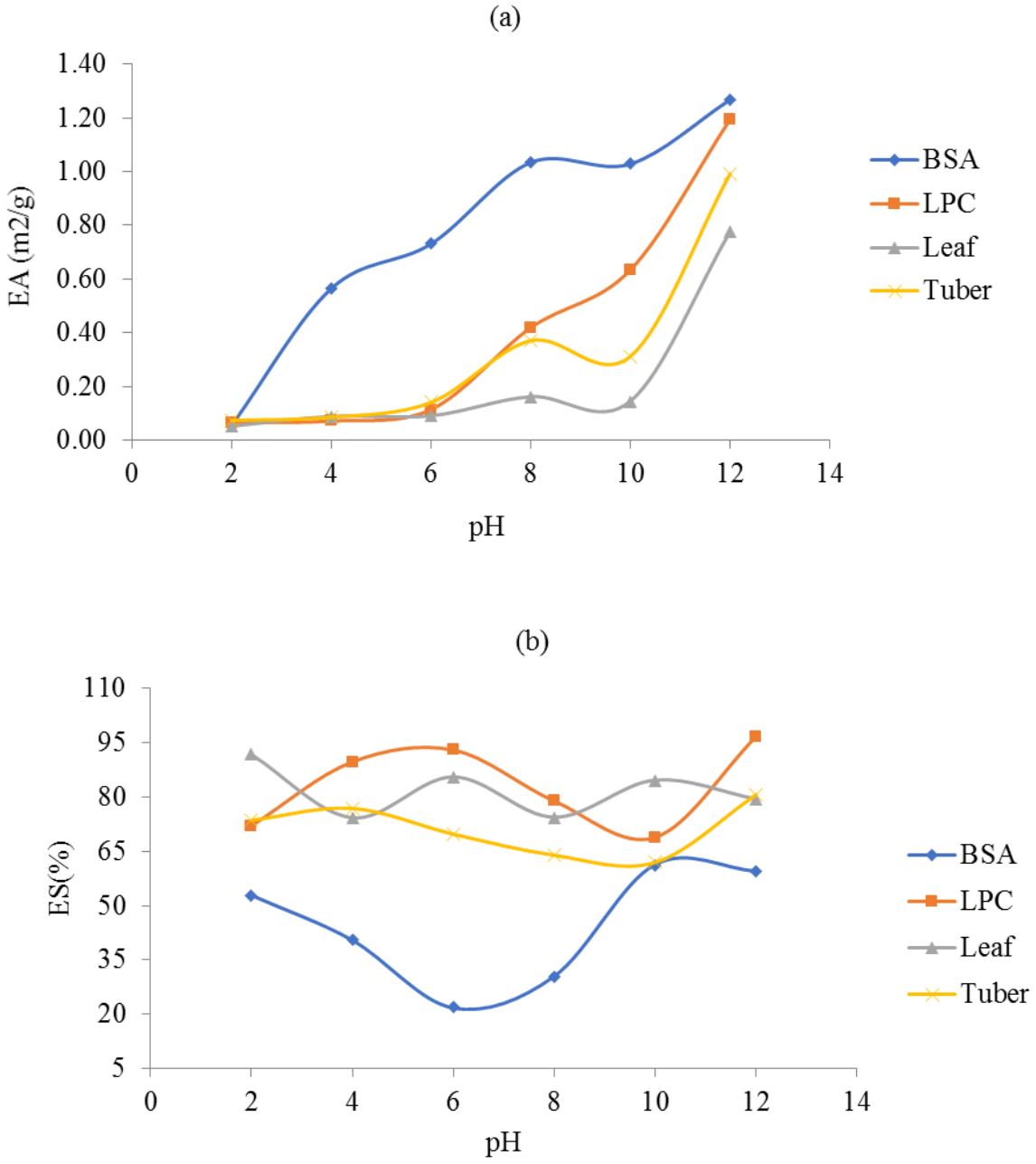


Figure 13 Effect of pH on emulsifying activity (EA) (a), and emulsion stability (ES) (b)

It is suggested that the effects of pH on EA are similar to those on protein solubility and the net charge on the protein molecule is zero at its IEP and the absorbed protein is rarely at the interfacial of hydrated layer and fat globules, so the EA became minimal at this point (Tang et al., 2012).

The pH had pronounced effect on the emulsifying activity because soluble proteins emulsifying activity depend upon the hydrophilic-lipophilic balance which in turn is affected by pH (Wu, Wang, Ma, & Ren, 2009). At the oil water interface, the protein oriented lipophilic residues to the oil phase and hydrophilic residue to the aqueous phase, thus reducing surface tension at the interface (Mao & Hua, 2012).

The effects of pH on emulsion stability (ES) of LPC, leaf and tuber powder are shown in Figure 13 (b). All the three tested samples showed varying emulsion stability with changes in the pH. In leaf powder, the highest ES observed at pH 2 whereas the lowest ES found in BSA at pH 6. The ES of Anchote LPC increased rapidly from pH 2 to pH 6, and then decreased until it reaches its IEP at pH 10; after wards, it increased up to the maximum point of ES at pH 12. On the other hand, the proteins in leaf powder generally had multiple maximum and minimum ES with the change in pH.

The protein in tuber powder had maximum ES at pH 12 and minima at pH 10. As it is stated by Martínez-Flores et al. (2006) most food proteins, including vegetable proteins, at their isoelectric pH are low in solubility and lack electrostatic repulsive forces, they are poor emulsifiers at this pH. The low ES at low pH attributed to increased interaction between the emulsified droplets. As the pH increased, the repulsion increased between neighboring droplets, coupled with increased hydration of the charged protein molecules (Mao & Hua, 2012). These factors resulted in reduction of interface energy and combination of emulsion droplet, which might account for the higher ES obtained (Chavan et al., 2001).

According to Mao & Hua (2012) the differences in ES of LPC, leaf and tuber powder might be due to their differences in protein content and the surface hydrophobicity of samples. In addition, the difference might come due to differences in their composition, solubility, structure, and interaction with other compounds. An extensive protein–protein interaction, caused by hydrophobic interaction on the surface of the protein, would form a strong oil–water interface, resulting in a stable emulsion (Wasswa, Tang, & Gu, 2008).

iv) Foaming Properties

Formation of foam is a function of the configuration of protein molecules (Alobo, 2004). The proteins should solubilize in the aqueous phase and rapidly unfold to form a cohesive layer of protein around gas/air droplets to form a foam (Tang et al., 2003). When there is a flexible protein molecule there will be good foamability, and to the reverse when there is highly ordered globular molecules there will be low foamability due to lack of reduction of the surface tension of the air-water interface (Diwakar, Kushwah, & Kushwah, 1996).

These two properties are important in foods that require a high foaming capacity and stability such as cake mixes and frostings (Alobo, 2004). To have foam stability, protein molecules should form continuous intermolecular polymers enveloping the air bubbles, since intermolecular cohesiveness and elasticity are important to produce stable foam (Tang et al., 2012). Anchote LPC, leaf and tuber powder compared for foaming properties and pH levels with different protein concentration to understand their potential applications in food processing.

a) Effect of Protein Concentration on Foaming Properties

Figure 14 (a) and (b) shows the effect of protein concentration on foaming capacity and stability of Anchote LPC, leaf and tuber powder. As shown in the figures, the highest value of foam expansion found in leaf powder, followed by LPC, and the lowest in tuber powder.

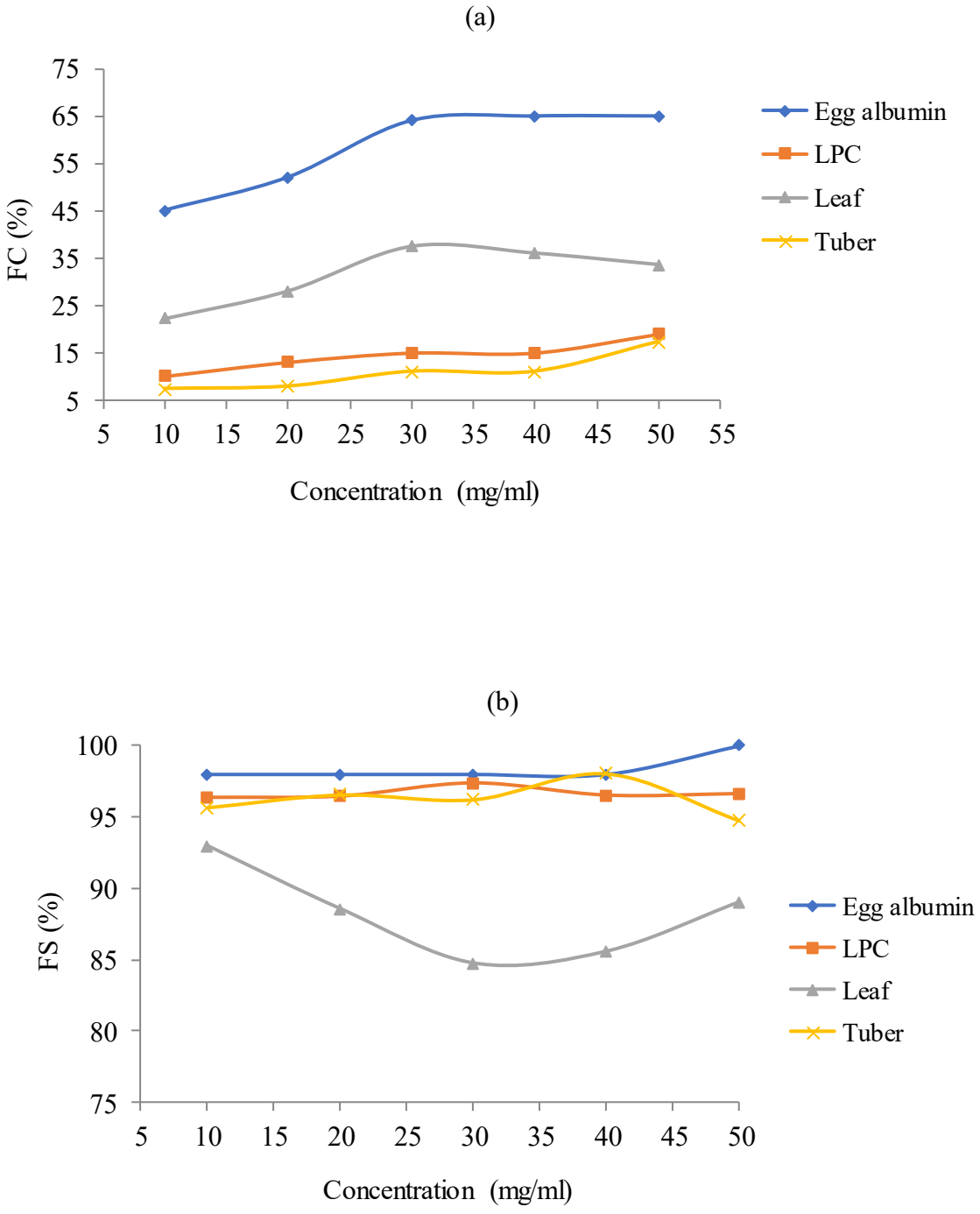


Figure 14 Effect of protein concentration on foaming capacity (FC) (a), and Foaming stability (FS) (b)

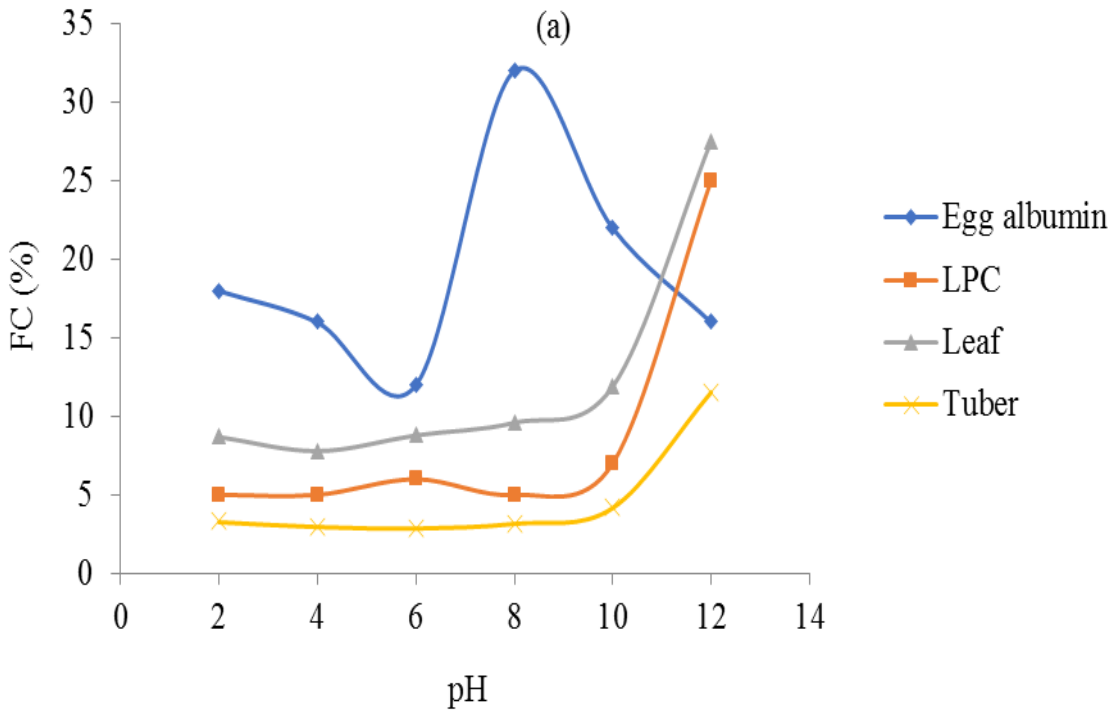
As described by Damodaran (1990), foaming capacity is favored by more flexible random coiled structure of proteins. Bera & Mukherjee (1989) also described that soluble proteins are essential to form good foam, whereas the poor foam expansion might be because of the presence of bound lipids, which depressed foaming. The LPC of Anchote contained 8% of crude fat which might be responsible for poor foaming formed compared to the leaf powder that constitute about 4% of fat. The increase in protein dispersion improved the foaming capacity of the LPC and tuber powder samples, but it reduced in leaf powder sample as shown in Figure 14 (a). The protein concentration affects the properties of the solution. The higher the protein concentration the thicker, more cohesive, and viscoelastic are the gas bubbles (Tang et al., 2012). The foam stability for leaf and LPC powder were constant throughout the change in protein concentration and this trend observed for the commonly used foaming agent, egg albumin. The tuber sample showed somewhat different trend of foam stability with the increase in protein concentration (Figure 14 (b)).

The foaming stability is important since the success of whipping agents depend on their ability to maintain whip as long as possible (Aletor et al., 2002). When the concentration of protein is high, protein molecules form thicker adsorbed films which are beneficial to FS (Akintayo, Oshodi, & Esuoso, 1999). In general, high protein concentration enhanced the foam capacity and stability by increasing the viscosity and facilitates the formation of a multilayer, cohesive protein film at the interface (Damodaran, 1997).

*b) **Effect of PH on Foaming Properties***

The effect of pH on foaming capacity and stability of Anchote LPC, leaf and tuber powder, presented in Figure 15 (a) and (b). Foaming capacity of leaf powder was higher than that of LPC at all pH values (2–12). LPC were in turn higher than that of tuber powder, and all the three samples of Anchote which followed similar trend of increasing FC with the change in pH from acidic to alkaline state. However, the foaming capacity of the reference protein (egg protein) had different pattern compared to our samples, in which the FC decreased from pH 2-6 with sharp increase towards pH 8 where the highest FC observed, whereas in the alkaline region, there was a decrease in FC.

Foaming stability (FS) for all tested samples of Anchote and the reference egg albumin showed almost constant stability between 97-100% over a wide range of pH values (2 to 11) except a slight decrease was observed at pH 12 for LPC and leaf powder samples (Figure 15 (b)). This result was in agreement with findings reported for soluble leaf proteins (SLPs) from alfalfa herbage (Lamsal et al., 2007), in which the initial volumes were found to be higher for high-pH foams even though they were not stable compared to low-pH foams. It has been stated by Tang et al. (2012) the formation of more compact structure of protein at pH values near to IEP, results in low FC. On the other hand, because of the increasing of static charge near IEP, the surface hydrophobicity weakened, causing an increased flexibility of the protein and thereby increased FS.



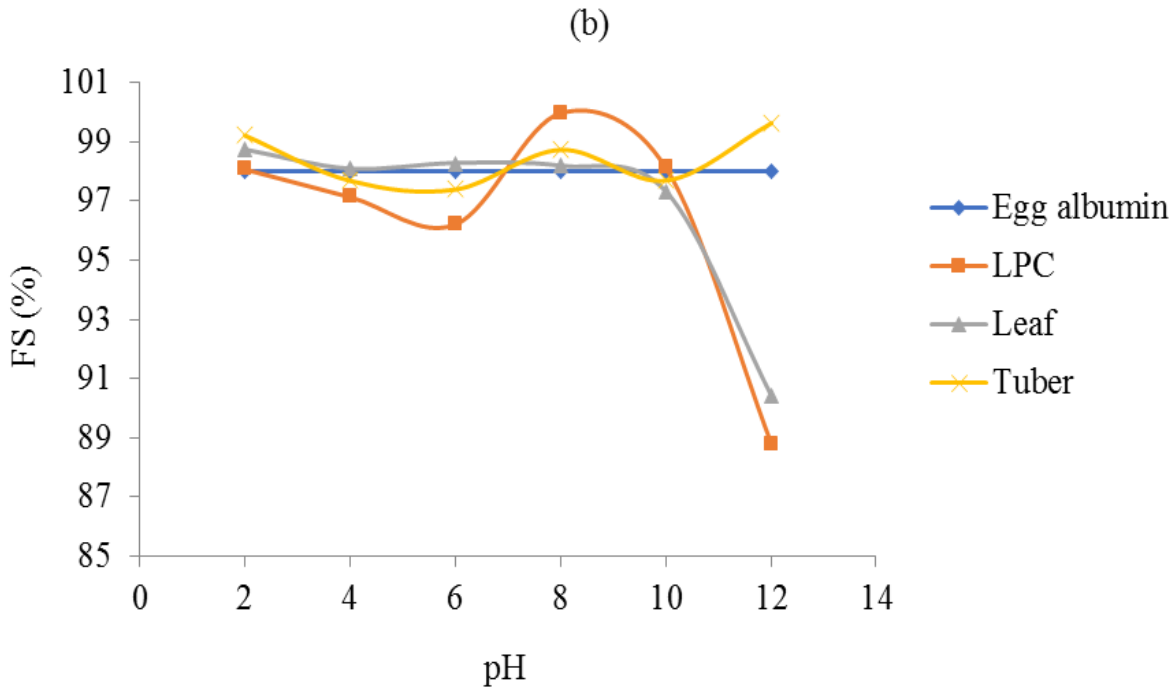


Figure 15 Effect of pH on foaming capacity (FC) (a), and foaming stability (FS) (b)

5.5. Conclusion

The results of this study showed that Anchote leaf protein concentrate is reach in protein and can be a good source of other essential nutrients, indicating that Anchote LPC can potentially use as nutritionally valuable ingredient in areas where there is high prevalence of protein malnutrition. Leaf protein concentrate is relatively cheap and most abundant as compared to animal source proteins and have a favorable amino acid profile to use it as a supplement to cereal-based diets. Anchote LPC also showed greater solubility on acid, neutral and alkaline media suggesting that it is useful for the formulation of various food products. In addition, it is worth mentioning to use Anchote LPC, whole tuber and leaf powder as an ingredient in processed foods since our result for the functional properties revealed multiple advantages that maximize its usage into suitable food product. Therefore, this study suggests Anchote LPC, whole tuber and leaf powder has good prospect as an alternative raw material for the development of a functional food.

Chapter Six: Phytochemical Constituents in Edible Parts of Anchote (*Coccinia abyssinica*) (Lam.) (Cogn.) Accessions

6.1. Abstract

Leaf, tuber and fruit parts of different Anchote (Coccinia abyssinica) accessions were used to test the presence of major phytochemicals by qualitative and quantitative methods. The qualitative tests were performed for 12 phytochemicals from seven solvent extracts by using standard methods. As for quantitative study, the important secondary metabolites analyzed were total phenols, total flavonoids, crude saponins and beta-carotene. In qualitative screening tests, all the seven extracts revealed positive results for five phytochemical compounds in tubers whereas, only two phytochemicals responded positively in all the extracts in leaves out of the 12 tested phytochemicals. Water extract showed positive results in 11 tests while n-butanol in six tests out of the 12 phytochemicals for both tuber and leaf. The water extract of both parts of Anchote reveals the presence of most phytochemicals when compared with the other solvent extracts. From the quantitative analysis, Anchote leaves had higher total phenol and flavonoid contents followed by fruits and the least concentration occurred in tubers for all the tested accessions. Leaves of Anchote contained the highest percentage of saponins (27.65%) whereas the minimum value (14.65%) was recorded in tubers. The β-carotene content in five accessions of Anchote leaves ranged from 25.9±0.03 to 35.2±0.16 in µg/g. In conclusion, the plant has potential to improve the health status of the consumers owing to the presence of various phytochemicals that are anti-cancer and anti-aging factors vital for good health. This study has shown a way forward to further screen phytochemicals from leaves, fruits and tubers of Anchote to identify some active compounds that could have potential medicinal purposes.

6.2. Introduction

Plants are valuable sources of nutrients and bioactive compounds for treatment of common and infectious diseases (Aliyu, Musa, Oshanimi, Ibrahim, & Oyewale, 2008; Hossain, Uddin, Salim, & Haque, 2014; R. Khan, Zakir, Afaq, Latif, & Khan, 2010). Bioactive compounds in fruits, vegetables, herbs, spices, pulses, cereals, and starchy plant foods also provide the best protection against the development of chronic illness such as cancer, cardiovascular diseases, type II diabetes, hypertension, cataract, and impaired cognitive function (Halvorsen et al. 2002; Del Rio et al. 2013). Because of their therapeutic effect, plants are used to synthesize many useful drugs, in which about 80% of the medicines are directly or indirectly obtained from the so called medicinal plants (Yadav, Mishra, & Tiwari, 2010).

To identify the bioactive chemical constituents belonging to different classes, it is essential to conduct a preliminary test in order to screen out different phytochemical constituents derived from plant sources. Natural products have long been a thriving source for the discovery of new drugs because of their chemical diversity (Selvam, Sumathy, & Kumuthakalavalli, 2014).

Anchote (*Coccinia abyssinica*)is the only tuberous cucurbit belonging to the family *Cucurbitaceae* in the genus *Coccinia* (Girma & Hailu, 2007). According to the report by PGRC/E (1988) Anchote is an indigenous root and tuber crop widely produced in South and Southwestern parts of Ethiopia. It is also regarded as medicinal plant due to the fact that it is used traditionally to cure people having bone-fracture or joint dislocation as well as women during giving birth or lactating (Abera, 1995; G. Amare, 1973; Endashaw, 2007; Girma & Dereje, 2015; F. Habtamu & Kelbessa, 1997). Anchote is commonly produced for its tuber to be used as food in south and southwestern Ethiopia (Abera, 1995; G. Amare, 1973). According to Abera (1995)Anchote become popular in the custom of Oromo and non-Oromo's because of its medicinal role, gained from practical experience than the rest of its uses. The high medicinal value of Anchote tuber is possibly because of its high calcium and protein content (Amare, 1973). The over matured Anchote tuber "*guboo*", though no scientific research has proved it, is presumed to contain high nutrient concentration especially calcium and iron of the tuberous root when the harvesting date is delayed or stored *in-situ* and thus used for healing many maladies (Girma & Dereje, 2015). There was also a report by Dawit & Estifanos (1991) which revealed that the juice prepared from Anchote tuber can be used to treat gonorrhea, tuberculosis and cancer with the assumption that the juice contains saponin as an active ingredient. However, comprehensive study would be required to prove such claims. In view of all the merits that Anchote has in the traditional medicine, it is imperative to investigate the phytochemical constituents of Anchote.

Therefore, the aim of this study was to quantify bioactive compounds such as total phenolics, total flavonoids and β-carotene contents, and major phytochemical constituents including alkaloids, carbohydrates, glycosides, cardiac glycosides, steroids, coumarins, oxalate and saponins in Anchote accessions that are potentially associated with therapeutic value.

6.3. Materials and Methods

6.3.1. Sample Collection and Preparation

Edible parts of Anchote from different accessions were collected from the experimental field of Debere Zeit Agricultural Research Center, Ethiopia. They were washed with running tap water and rinsed with distilled water. The cleaned tubers and fruits were cut into small pieces followed by drying in an oven (DHG- 9055A, Memment Germany) at 105 ^{o}C constant weight.

Leaves were air dried under shade. The dried samples were then ground to a fine powder using an electric grinder (FW 100, Yusung Industrial Ltd, China) and sieved to pass through 0.425 mm mesh. Finally, each powder sample was sealed using airtight plastic bags, labeled and kept under refrigerated condition at 4 ^{o}C for analysis.

6.3.2. Extraction of phytochemical compounds

a) Extraction for qualitative screening test

The extraction of Phytochemicals was executed according to the procedures described by Ugochukwu et al. (2013). Seven different extracting solvents namely n-hexane, n-butanol, acetone, methanol, ethyl acetate, ethanol and water were used for qualitative screening of phytochemicals in Anchote tubers and leaves. Five gram of powder sample was taken from 10 randomly selected accessions of each leaf and tuber part separately. The powder samples were dissolved in 50 ml of extracting solvents.

The extraction solutions were left to stand for two hours at room temperature, heated for 60 ^{o}C for 30 minutes and the supernatant filtered with Whatman filter paper No. 1. The filtrates were then centrifuged at 2500 revolution per minute (rpm) for 15 minutes, and the filtrates were used for qualitative screening for presence of phytochemicals.

b) Extraction for phytochemical quantification

Anchote fruits, leaves& tuber powder were extracted according to Barros et al. (2007) and Ferreira et al. (2007) with some modification. Each plant material (0.1gm) was separately extracted in 20 ml of 75% methanol by stirring at 150 rpm at room temperature for 24 hr. using shaker incubator (ZHWY-103B). The recovered supernatant and the residue were extracted two times as described above. The resulting extracts were combined and filtered through Whatman No. 2 filter paper. The combined methanolic extracts were evaporated at 40 °C to dryness using rotary evaporator (Rikakikai Co. Ltd., Tokyo, Japan), re-dissolved with methanol and adjusting the volume to 20 ml. The final extract was then kept in a refrigerator at 4 ºC until used to determine total phenol and flavonoid content.

6.3.3. Qualitative Test of Phytochemicals

For preliminary identification of phytochemical constituents, the crude extracts using n-hexane, n-butanol, acetone, methanol, ethyl acetate, ethanol and water were screened for the detection of alkaloids, carbohydrate, coumarins, fatty acids, flavonoids, glycosides, oxalate, phenolic compounds, saponins, steroids, tannins and terpenoids according to standard procedures as follows.

1) Terpenoids (Salkowski's test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. Formation of a reddish brown coloration at the interface was considered as a positive indicator for the presence of terpenoids (Edeoga, Okwu, & Mbaebie, 2005).

2) Steroids

Steroids test was conducted according to Kumar et al. (2009).One ml of the crude extract was dissolved in 10 ml of chloroform and equal volume of concentrated H_2S0_4 carefully added by the side of the test tube.

Finally, the presence of steroids was confirmed by the change of the upper layer to red color and theH_2S0_4 layer (next to the upper layer) to yellow color with green fluorescence.

3) Fatty Acids

A sample of crude extract (0.5 ml) mixed with 5 ml of ether, and a filter paper immersed into the mixture. Then the socked filter paper was allowed to evaporate and the appearance of transparence on the filter paper was taken as an indicator for the presence of fatty acids (Savithramma, Rao, & Suhrulatha, 2011).

4) Flavonoids

To a filtrate of 2 ml extract, dilute ammonia (5 ml) and concentrated H_2SO_4 (1 ml) were added slowly and the development of a yellow color that disappears on standing was considered as an indicator for the presence of flavonoids (Alagesaboopathi & Sivakumar, 2011; Ayoola et al., 2008).

5) Tannins

To a 2 ml of extract, few drops of 1% lead acetate was added and formation of a yellowish precipitate was taken as indication for the presence of tannins (Savithramma et al., 2011).

6) Alkaloids

According to Ugochukwu et al. (2013)crude extracts (2 ml) were treated with 3-5 drops of Wagner's reagent (1.27 gm of iodine and 2 gm of potassium iodide in 100 ml of water) and observed for the formation of reddish brown precipitate or coloration.

7) Carbohydrates (Molisch's test)

To 2 ml of the extract, few drops of Molisch's reagent was added followed by addition of 2ml of concentrated H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes and checked for the formation of a red or dull violet color at the interface of the two which indicates the presence of carbohydrates (Ugochukwu et al., 2013).

8) Phenols (Ferric Chloride test)

Two ml of the extract treated with 5 % aqueous ferric chloride and observed for formation of deep blue or black color to confirm the sample constitutes phenols (Ugochukwu et al., 2013).

9) Glycosides (Salkowski's test)

About 3 ml of crude extract was mixed with 2ml of chloroform, and then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. Appearance of reddish brown color was taken as an indicator for the presence of steroidal ring, i.e., glycone portion of the glycoside (Yadav & Agarwala, 2011).

10) Oxalate

A few drops of ethanoic acid glacial were added to 3ml portion of extracts. A greenish black coloration was checked to prove the presence of oxalates (Ugochukwu et al., 2013).

11) Saponins (Foam test)

To 2 ml of the filtered extracts, 5 ml of distilled water was added to a test tube. The mixture was shaken vigorously to observe for the appearance of stable persistent froth on warming, as preliminary evidence for the presence of saponins (Abba, Inabo, Yakubu, & Olonitola, 2009).

12) Coumarins

Three ml of 10% NaOH was added to 2 ml of the extract and formation of yellow colour indicates the presence of coumarins (Savithramma et al., 2011).

6.3.4. Quantitative Determination of Phytochemicals

1) Total Phenols

Total phenol content were determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965). Diluted extract (40 µl) was added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent followed by addition of 800 µl of saturated sodium carbonate (75 gm/lt) after 4 min.

After two hrs. of incubation at room temperature, the absorbance was measured at 765 nm. Gallic acid (G7384; Sigma-Aldrich) with the concentration range of 0, 1, 10, 25, 50, 100 and 250 mg/lt was used for the standard calibration curve Annex R (Absorbance = 0.0024 catechin µg - 0.0010, R^2 = 0.9989). The results were expressed as gallic acid equivalent (GAE)/gm sample on dry weight basis and calculated as mean value ± SD (n = 3).

2) *Total Flavonoids*

Total flavonoid was determined by a colorimetric method (Xu & Chang, 2007). Briefly, 0.25 ml of the extract was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture and incubated at room temperature for 5 minutes. Following incubation, 0.5 ml of 1 M NaOH and 2.5 ml of deionized water was added to the mixture and then thoroughly vortexed. Finally, the absorbance of the pink colour was measured at 510 nm against the blank. For calibration curve (+)-Catechin was used with a concentration of 10, 20, 40, 60, 80 and 100 mg/lt (Absorbance = 0.0037 catechin mg - 0.0008, R^2 = 0.9984) Annex S. Results were expressed as mg (+)-catechin equivalent (CE)/g of extract.

3) *Crude Saponins*

Crude saponins determination was done according to Obadoni & Ochuko (2002)and Edeoga et al. (2005). Briefly, 20g of powder sample was mixed with 100ml 20% aqueous ethanol in a conical flask. The mixture was then heated over a hot water bath for four hr. with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. After concentrating the combined extracts to 40 ml at about 90 °C over water bath, the concentrated extract was then transferred into a 250-ml separatory funnel and 20 ml of diethyl ether was added followed by vigorous shaking until the aqueous, and ether layer separated. By discarding the ether layer, the aqueous layer was collected repeatedly until no more layer formation observed. To the collected aqueous extract, 60 ml n-butanol was added and washed twice with 10 ml of 5% aqueous sodium chloride. Finally, the remaining solution was evaporated in a water bath and dried in an oven until constant weight and the saponin content calculated as percentage.

4) *Beta Carotene*

Beta carotene content of Anchote leaf were determined following the method described by Zakaria et al. (1979). Briefly, 2 g of leaf powder was extracted repeatedly with petroleum ether until the residue was colorless. By decanting the extract into a separating funnel, the aqueous-acetone phase removed by repeated washing using distilled water. The upper petroleum ether layer then collected and dried over anhydrous sodium sulphate.

The petroleum ether phase was then transferred to drying flask and evaporated to dryness on a rotary evaporator followed dissolving it in about 1ml of petroleum ether and subjected to chromatographic column on neutral alumina. β-carotene eluted using petroleum ether was collected in a flask and the volume was measured in a measuring cylinder. The optical density (OD) was finally read by UV/visible spectrophotometer at 450 nm. Consequently, the β -carotene content was calculated using the following equation:

$$\beta\text{-carotene } (\mu g/g) = \frac{(A \times V(ml) \times 10^4)}{A_{1\%_{1cm}} \times W}$$

Where:

A = Absorbance

$A_{1\%\ 1cm}$ = Absorption coefficient of carotenoid in solvent used PE is 2592

V(ml) = Volume of the solution that gives an absorbance of A at a specified wavelength

W = Weight of sample in gram

6.4. Results and Discussions

6.4.1. Qualitative Phytochemical Screening

The qualitative test for phytochemicals in Anchote leaf and tuber is presented in Table 31. All the 12 tested phytochemicals were present in leaf. Coumarins and flavonoids gave a positive result in all seven extracts, and glycosides, tannins and terpenoids found in six of the extracts out of the seven. Of all the tested phytochemicals saponins was positive only in water, methanol and ethanol extracts.

In case of tuber alkaloids, carbohydrate, glycosides, tannins and terpenoids, responded positively in all seven extracts, whereas coumarins revealed its presence in five of the extracts, fatty acids in four, as well as saponins and steroids in three of the seven extracts. Flavonoids and phenolic compounds were present only in a water extract, and oxalate was absent in all extracts. During the present screening test, diverse types of results observed in different solvents for both the leaf and tuber. The presence or absence of phytochemicals in one or another solvent provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds (Gujjeti & Mamidala, 2013). According to the same author, the selection of appropriate solvent system for a particular plant extracts could be known from the retention factor values of the compounds in different solvent system using thin layer chromatographic studies. Tiwari et al. (2011) and Ugochukwu et al. (2013) also reported that successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure, and this logic is validated in our study. According to Tiwari et al. (2011) there are factors affecting the choice of solvent such as quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the extractant.

In preliminary phytochemical evaluation of our study, water extract revealed a positive result in eleven of the twelve tested phytochemicals with the exception of oxalate which was absent in both leaf and tuber extract. These showed that water extract is more effective than other solvent extracts in this study. Anchote leaves showed wider percentage of phytochemicals (73%) compared to tubers (62%). Our result suggests that Anchote leaves and tubers possess several known and unknown bioactive compounds, which are a potential source of useful drugs. Thus, by isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders. In addition, consumption of the Anchote leaves can also improve the health status of the consumers because of the various compounds thereof that are vital for good health.

The phytochemicals identified in leaf and tuber part of Anchote have various medicinal properties as follows: alkaloids represent a class which affects the central nervous system, reduces appetite and behaves as diuretic (Haller & Benowitz, 2000). Carbohydrates, coumarins and glycosides are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements (Yadav, Chatterji, Gupta, & Watal, 2014). Coumarins can be suggested to be beneficial for hyper proliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects (Theis & Lerdau, 2003). Glycosides also have vast therapeutic efficacy, as they are existing in almost every medicinal plant. Tannins have amazing stringent properties known to hasten the healing of wounds, and inflamed mucous membranes. Flavonoids are used as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Salah et al. 1995; Benavente-García et al. 1997). In addition it helps in managing diabetes induced oxidative stress (Yadav et al., 2014). Terpenoids are useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, tihyperglycemic, anti-inflammatory and immunomodulatory properties (Wagner & Elmadfa 2003; Rabi & Bishayee 2009). Moreover, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Sultana & Ata, 2008). Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells (Sodipo et al. 2000; Okwu 2004). Steroids are responsible for cholesterol-reducing properties and also help in regulating the immune response (Shah, Qazi, & Taneja, 2009).

Table 31 Phytochemical screening of tuber and leaf parts of Anchote (*Coccinia abyssinica*) in different solvents

Leaf extracts (n=10)							
Phytochemicals	Water	Methanol	Ethanol	Acetone	Ethyl acetate	n-hexane	n-butanol
Alkaloids	+	-	-	+	+	+	-
Carbohydrate	+	+	+	+	+	-	-
Coumarins	+	+	+	+	+	+	+
Fatty acids	+	+	-	+	+	-	-
Flavonoids	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	-	+
Oxalate	-	-	-	+	+	+	+
Phenolic compounds	+	+	+	+	-	-	-
Saponins	+	+	+	-	-	-	-
Steroids	+	+	+	-	-	+	+
Tannins	+	+	+	+	+	+	-
Terpenoids	+	+	+	+	-	+	+
Tuber extracts (n=10)							
Alkaloids	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+
Coumarins	+	+	+	+	-	-	+
Fatty acids	+	-	-	+	+	+	-
Flavonoids	+	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+
Oxalate	-	-	-	-	-	-	-
Phenolic compounds	+	-	-	-	-	-	-
Saponins	+	+	+	-	-	-	-
Steroids	+	+	+	-	-	-	-
Tannins	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+

+ = present; – = absent; n = number of samples used in each test

6.4.2. Quantitative Analysis of Phytochemicals

1) Total Phenols

Total phenol (TP) content of Anchote fruits, leaves & tubers were expressed as gallic acid equivalent (GAE) in mg/g. The results showed that TP content of fruit, leaf and tuber of Anchote in the tested accessions varied significantly ($p < 0.05$) with values ranging from 3.02±0.86 to 59.90±0.56 mg GAE/100 g sample (Table 32). The fruit TP concentrations ranged from 14.50±0.06 to 57.33±0.03 mg GAE/g in which 'NJ' presented the highest TP concentration, whereas '223093' was found to have the lowest TP content. These values are higher than fruit TP content reported by Petridis et al. (2012) for some olive cultivars which ranged from 8.03 to 17.96 mg/g (FW).

On the other hand, the values of TP content reported by Kähkönen et al. (1999) for berries which was in the range of 12.4±0.6 - 50.8±1.0 mg/g GAE and by Babbar et al. (2011) for different fruit residues (17.5-37.4 mg GAE/g DW) were found to be within the range of our result. Accession '240407-1' (59.90±0.56 mg GAE/g DW) was found to have the highest, whereas accession '223090-1' (26.42±0.03 mg GAE/g DW) contained the lowest TP content in leaves. The value in Anchote leaves were comparably lower than the TP content in leaf extracts of *Adhatoda vasica* which varied from 63.95±2.1 to 92.4±0.14 mg/g (Maurya & Singh, 2010) but higher than different herb extracts with values ranging from 9.1±0.8 to 23.1±0.8 mg/g GAE (Kähkönen et al., 1999). The TP content in *Moringa oleifera* leaf extracts were found to be within the range of the result obtained in leaf (Sreelatha & Padma, 2009).

In tubers, the highest level of TP was found in accession '223086' (30.02±2.12 mg GAE/100 g), while the lowest was in '223097' (3.02±0.86 mg GAE/100 g). The TP content in Anchote tuber were higher than the sweet potato genotypes with distinctive flesh color (Teow et al., 2007). In general, the TP content in Anchote were found to be higher than the TP content reported for cereals (0.2±0.0-1.3±0.1 mg/g GAE) and vegetables (0.4±0.0 - 6.6±0.1mg/g GAE) (Kähkönen et al., 1999).

However, in this study Anchote leaves had higher TP concentration followed by fruits in all the tested accessions. Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Geetu Singh et al., 2007) and primarily responsible for the hydrophilic antioxidant activity (Teow et al., 2007).

Table 32 Total phenol contents in Anchote (*Coccinia abyssinica*) fruit, leaf and tuber extract (dry weight)

Accessions	Parts used	Total phenolic (mg GAE/g)
90802-1	Fruit	19.03 ± 0.21^{b}
223093	Fruit	14.50 ± 0.06^{c}
NJ	Fruit	57.33 ± 0.03^{a}
229702	Fruit	15.06 ± 0.37^{c}
223090-1	Leaf	26.42 ± 0.03^{e}
223109-1	Leaf	58.04 ± 0.13^{b}
DIGGA-1	Leaf	46.61 ± 0.73^{c}
240407-1	Leaf	59.90 ± 0.56^{a}
KICHI	Leaf	36.02 ± 0.88^{d}
223085	Tuber	3.66 ± 0.32^{d}
223086	Tuber	30.02 ± 2.12^{a}
223087-1	Tuber	8.66 ± 1.65^{c}
223097	Tuber	3.02 ± 0.86^{d}
NJ	Tuber	19.71 ± 0.32^{b}

Means followed by different superscript letters in the same column of the same plant part are significantly different ($p< 0.05$); Data are mean±SD of triplicate measurements (n=3); GAE: Gallic acid equivalent

2) Total Flavonoids

Phenolic compounds comprise of a large diversity of compounds among which flavonoids, the most important groups of secondary metabolites and bioactive compounds in plants may function as effective natural antioxidants in our daily diet (Kim, Jeong, & Lee, 2003; Ndhlala, Moyo, & Van Staden, 2010).

Some of the proven biological activities of flavonoids include antimicrobial, antiviral, anti-inflammatory, antiallergic, vasodilatory effects and inhibition of lipid peroxidation (Cook & Samman, 1996). Total flavonoid contents of Anchote fruits, leaves and tubers are shown in Table 33.

The total flavonoid content of Anchote leaves in the tested accessions were superior ranged from 14.28±3.38 to 19.23±3.27 mg CE/g followed by fruits from 1.65±0.27 to 4.16±1.80 mg CE/g. and tuber samples that consisted of the least in total flavonoids content ranging from 0.41±0.21 to 0.63±0.17 mg CE/g). Of all tested accessions, 'DIGGA-1' exhibited the maximum concentration of total flavonoid whereas the minimum was observed in the leaf and tuber samples of 'NJ', respectively.

Table 33 Total flavonoid contents in Anchote (*Coccinia abyssinica*) fruit, leaf and tuber extract (dry weight)

Accessions	Parts used	Total flavonoid (mg CE/g)
90802-1	Fruit	1.65±0.27^{b}
223093	Fruit	4.16±1.80^{a}
NJ	Fruit	1.94±0.20^{b}
229702	Fruit	1.88±0.64^{b}
223090-1	Leaf	14.28±3.38^{a}
223109-1	Leaf	15.74±2.15^{a}
DIGGA-1	Leaf	19.23±3.27^{a}
240407-1	Leaf	17.99±3.45^{a}
KICHI	Leaf	18.87±2.45^{a}
223085	Tuber	0.63±0.17^{a}
223086	Tuber	0.47±0.20^{a}
223087-1	Tuber	0.61±0.16^{a}
223097	Tuber	0.59±0.09^{a}
NJ	Tuber	0.41±0.21^{a}

Means followed by different superscript letters in the same column of the same plant part are significantly different ($p< 0.05$); Data are mean±SD of triplicate measurements (n=3); CE: Catechin equivalent

Higher level of total flavonoid was observed in mature leaf extractof *Moringa oleifera* (27±0.03 mg CE/g), but the tender leaf extract of *Moringa oleifera* (15±0.02 mg CE/g) was found to be in agreement with the result obtained in our study (15.74±2.15 mg CE/g) in leaf of accession '223109-1' (Sreelatha & Padma, 2009).

On other hand Atanassova et al. (2011) had reported lesser content of total flavonoid for the medicinal herbs of lemon balm (0.45 mg CE/g), sage (0.28 mg CE/g) and mint (0.25 mg CE/g) compared to the leaf samples in our study. The reported values of total flavonoids by Marinova et al. (2005) for blueberries fruit (190.3 mg CE/100g = 1.903 mg CE/g) were in close agreement with the result obtained for Anchote fruit of accessions 'NJ' and '229702' in the present study. However, all the other fruit species tested by same authors have lesser flavonoid concentration compared to Anchote fruits in the tested accessions.

3) *Crude Saponins*

Saponins could be present in different plant parts such as root, tuber, bark, leaf, seed, and fruit (Yongmok & Daniel, 2009). The content of saponins in Anchote leaf and tuber were ranged from 16.54±0.06 to 27.65±0.27% and 14.65±0.05 to 17.42±0.06%, respectively (Table 34). Anchote leaf contained the highest percentage crude yield of saponins (27.65%) and this is in close agreement with leaves of *Anredera cordifolia* (Binahong) (28.14±0.22%) (Astuti, 2011). While minimum yield was recorded in tuber (14.65%) which had a similarity with the values reported by Unekwu et al. (2014) for wild edible Nigerian mushroom (*Cantharelle cibarius*) (150.41±0.50 mg/g = 15.04%). Our result was higher than the reported values for medicinal plants from Nigeria (1.12±0.22 - 3.92±0.11 %) (Edeoga et al., 2005), leaf and stem of *Andrographis neesiana* (1.05±0.10 - 3.40±0.80%) (Alagesaboopathi & Sivakumar, 2011), seeds of selected weed plants (0.48±0.06 - 1.29±0.03%) (Abbas, Rana, Shahid, Rana, & Hussain, 2012), and stems of *Anredera cordifolia* (Binahong) (3.65±011%) (Astuti, 2011). However, it is lower than tubers saponin content of *Anredera cordifolia* (Binahong) (43.15±0.10%) (Astuti, 2011). Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid glycoside (Wina, Muetzel, & Becker, 2005).

They are reported to have a wide range of beneficial pharmacological properties, such as producing inhibitory effect on inflammation, precipitating and coagulating red blood cells, having anti-diabetic, antitumorigenic and antiviral activities (Just et al. 1998; Madar & Stark 2002; Shi et al. 2004; Singh 2011; Lee et al. 2012). Saponins also has a characteristics of foam formation in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sudip et al. 2000; Okwu 2004). According to Dini et al. (2009) the qualitative and quantitative saponins composition of food plants can vary considerably due to factors such as variety/cultivar, geographic effects/climate, season, stage of maturity and plant part used.

Table 34 Percentage of saponin content from crude aqueous extracts of Anchote leaves and tubers (dry weight)

Accessions	Parts used	Saponin (%)
90801	Leaf	27.65±0.27a
223087-1	Leaf	25.20±0.11b
223090-1	Leaf	24.95±0.08[b]
223104	Leaf	16.54±0.06[d]
DIGGA	Leaf	22.39±0.10[c]
223110	Tuber	14.65±0.05[c]
229702-1	Tuber	15.10±0.06[c]
220563-1	Tuber	16.23±0.10[b]
230565	Tuber	17.42±0.06[a]
240407-1	Tuber	14.57±0.05[c]

Means followed by different superscript letters in the same column are significantly different ($p < 0.05$); Data are mean±SD of triplicate measurements (n=3)

4) *Beta Carotene*

Carotenes contain mainly β-carotene, the main precursor of vitamin A, which constitute about 80% of carotenoid (Jeszka, 1997), and it has antioxidant potential being used as anti-cancer activity and other health benefits including the protection against cardiovascular disease or cataract prevention (Bohm et al., 2002; Khan & Varshney, 2015).

β-carotene, is also involved in cell differentiation, synthesis of glycoprotein, reproduction and overall growth and development (Vimala, Thushara, Nambisan, & Sreekumar, 2011). As shown in Table 35, the β-carotene content of Anchote leaf in the tested five accessions was ranged from 25.9±0.03 to 35.2±0.16 in µg/g.

The result of the present study revealed that accession 'DIGGA-1' had the least β-carotene content which varied significantly ($p < 0.05$) from those of three accessions ('223087-1', '223109-1', 'KICHI') while no significant difference was observed with accession '223090-1'. Our result is higher than the reported β-carotene content for white-fleshed sweet potato (0.18 µg/g), pumpkin (578 µg/100 g) and tomato (365 µg/100 g) but lower than that found in carrot (6769 µg/100 g) and dark orange-colored sweet potato clones (167 and 226 µg /g fw) (Tee & Lim, 1991; Teow et al., 2007). As it is stated by Shofian et al. (2011) β-Carotene is ubiquitously present in green leafy and yellow-orange fruits and vegetables and its content can be influenced by the growing conditions, maturity index, post-harvest handling conditions, as well as variety or cultivar.

Table 35 Content of beta-carotene in the leaf part of Anchote accessions

Accessions	Parts used	β-carotene (µg/g)
223087-1	Leaf	33.1 ± 0.19^{a}
223090-1	Leaf	30.7 ± 0.16^{ab}
223109-1	Leaf	35.2 ± 0.16^{a}
DIGGA-1	Leaf	25.9 ± 0.03^{b}
KICHI	Leaf	34.9 ± 0.36^{a}

Means followed by different superscript letters in the same column are significantly different ($p< 0.05$); Data are mean±SD of triplicate measurements (n=3)

6.5. Conclusion

Phytochemicals screened in Anchote plant parts in this study pointed out the potential of the plant to be used as a source of a pharmaceutical product. Therefore, an in-depth investigation is vital to provide concrete information through further isolation, identification, and characterization of the phytochemicals.

Chapter Seven: Characterization of Anchote (*Coccinia abyssinica*) (Lam.) (Cogn.) Tuber and Leaf for Volatile Organic Compounds

7.1. Abstract

Volatile organic compounds of Anchote (Coccinia abyssinica) leaf and tuber powder were extracted by simultaneous steam distillation and solvent extraction (SDE)and characterized using gas chromatography-mass spectrometry (GC-MS) to identify volatile organic compounds. Thirty volatile compounds from leaf and fifteen from tuber were identified with the yield of 770.57 mg/kg and 4536.91 mg/kg, respectively. The SDE extraction and GC-MS analysis in Anchote leaf and tuber successfully identified various volatile flavor compounds. Therefore, Anchote could be used as a food flavoring agent.

7.2. Introduction

Research findings showed more than 1,000 low M_r organic compounds are emitted from floral and vegetative parts of many plant species (Dudareva, Pichersky, & Gershenzon, 2004).Some of the compounds emitted may have physiological significance without any distinctive smell to humans whereas other substances have distinctive smells. The release of volatiles from many vegetative organs have anti-microbial or anti-herbivore activity and so could also act to protect valuable reproductive parts of plants from enemies(Friedman, Henika, & Mandrell, 2002; Hammer, Carson, & Riley, 2003)

The volatile substances released by plants have great importance to several fields of basic and applied research in various disciplines. Plant parts such as leaves, flowers and fruits release the volatile organic compounds (VOCs) into the atmosphere, and roots into the soil as a defense mechanism against herbivores and pathogens or as an attraction of pollinators and seed dispersers. In some plants, released VOCs may also act as wound sealers(Maffei, Gertsch, & Appendino, 2011). In addition, VOCs is one of the most important factors to influence the flavor, taste, and sensorial quality of food, in which the aroma is formed by a complex group of chemical substances such as aldehydes, alcohols, ketones, esters, lactones, terpenes etc. (Riu-Aumatell, Castellari, López-Tamames, Galassi, & Buxaderas, 2004). Over 90% of the natural emission of volatile organic compounds (VOCs) is due to plant species (Maffei et al., 2011).

Aroma results from the interplay of these emitted VOCs (Liu et al., 2015). Identification of VOCs for fragrance and pharmacologically active ingredients is also important for potential multi-purpose functional use (Gyawali & Kim, 2012). Flavor compounds can be found in food, spices, wine, perfumes, fragrance oils and essential oils (Fahlbusch et al., 2003). They are usually formed biochemically during ripening of root bulbs, stem barks, fruits and other vegetables (Khan, 2014). Among the volatile compounds, only a limited number are important for the characteristic aroma of the food, which are called key odorants (Anne Plotto, Margaria, Goodner, Goodrich, & Baldwin, 2004).

Anchote (*Coccinia abyssinica*) has been used as traditional medicine to treat various illnesses for long period of time (Dawit & Estifanos, 1991; Endashaw, 2007). Knowing the volatile flavor of Anchote would be very important in discovering the key compounds responsible for the health effects and aroma characteristics. Three *Coccinia abyssinica* accessions, '223090', '220563' and '230566' were randomly selected for volatile flavor profile studies to find out the active components and their contribution towards the overall aroma impacts and medicinal values of Anchote leaf and tuber. This would indirectly confirm the phytochemicals constituents and other bioactivities of Anchote plant parts that are already determined in previous section of this dissertation.

7.3. Materials and Methods

7.3.1. Reagents

All the reagents used in this experiment were purchased from Sigma Co. (St. Louis, USA) and Fisher Scientific (Waltham, Massachusetts, USA). Organic solvents; diethyl ether and n-pentane were redistilled using a wire spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany). Milli-Q water was generated through a water purification system (Millepore Corporation, Bedford, USA). Anhydrous Na_2SO_4 was used for dehydration of organic solvents after burned overnight at 650 ^oC in a furnace (F 6000, Barnstead Thermolyne Co., IA, USA) and allowed to cool down in desiccators.

7.3.2. Analytical Apparatus

Distilling apparatus: Wire spiral packed distilling apparatus (Normschliff Geratebau, Wertheim, Germany)

Blender: multi mixer (Braun MR 550 CA, Braun, Spain)

pH meter: pH meter (HM-30P, DKK-TOA Corp., Tokyo, Japan)

Extraction apparatus: Simultaneous steam distillation and extraction (SDE), Likens and Nickerson type simultaneous steam distillation and extraction apparatus, (Normschliff Geratebau, Wertheim, Germany)

Concentration Column: Vigreux column (250 mL, Normschliff, Werthein, Germany)
GS-MS: GCMS- QP2010, equipped with mass spectrum libraries Willey 7, NIST 05 and FFNSC 2.0 (Shimadzu, Japan)

Capillary column: DB-Wax (60 m Length, 0.25 mm Diameter, 0.25 µm film thickness, J & W, USA)

7.3.3. Extraction of Volatile Organic Compounds

Anchote leaf and tuber samples (30 g) were homogenized in a blender (MR 350CA, Braun, Spain) and mixed with 500ml of distilled water which is adjusted to pH 7 using 1N NaOH and 1N HCl. Then, n-butyl benzene (10 mL) was added as an internal standard. The resultant slurry was used for extraction of volatile organic compound (VOCs) with 100 ml redistilled n-pentane: diethyl ether (1:1, v/v) mixture, using simultaneous distillation-extraction (SDE) apparatus under atmospheric pressure according to Nickerson & Likens (1966) as modified by Schultz et al. (1977) for three hrs. The solvent, containing compound extract, was dehydrated for 12 hrs. using 10 g anhydrous Na_2SO_4 and then concentrated to approximately 1.5 ml using the vigreux column. This extract was further concentrated to 0.5 ml under gentle stream of N_2 gas and used for gas chromatography-mass spectrometry (GC-MS) analysis (Figure 16).

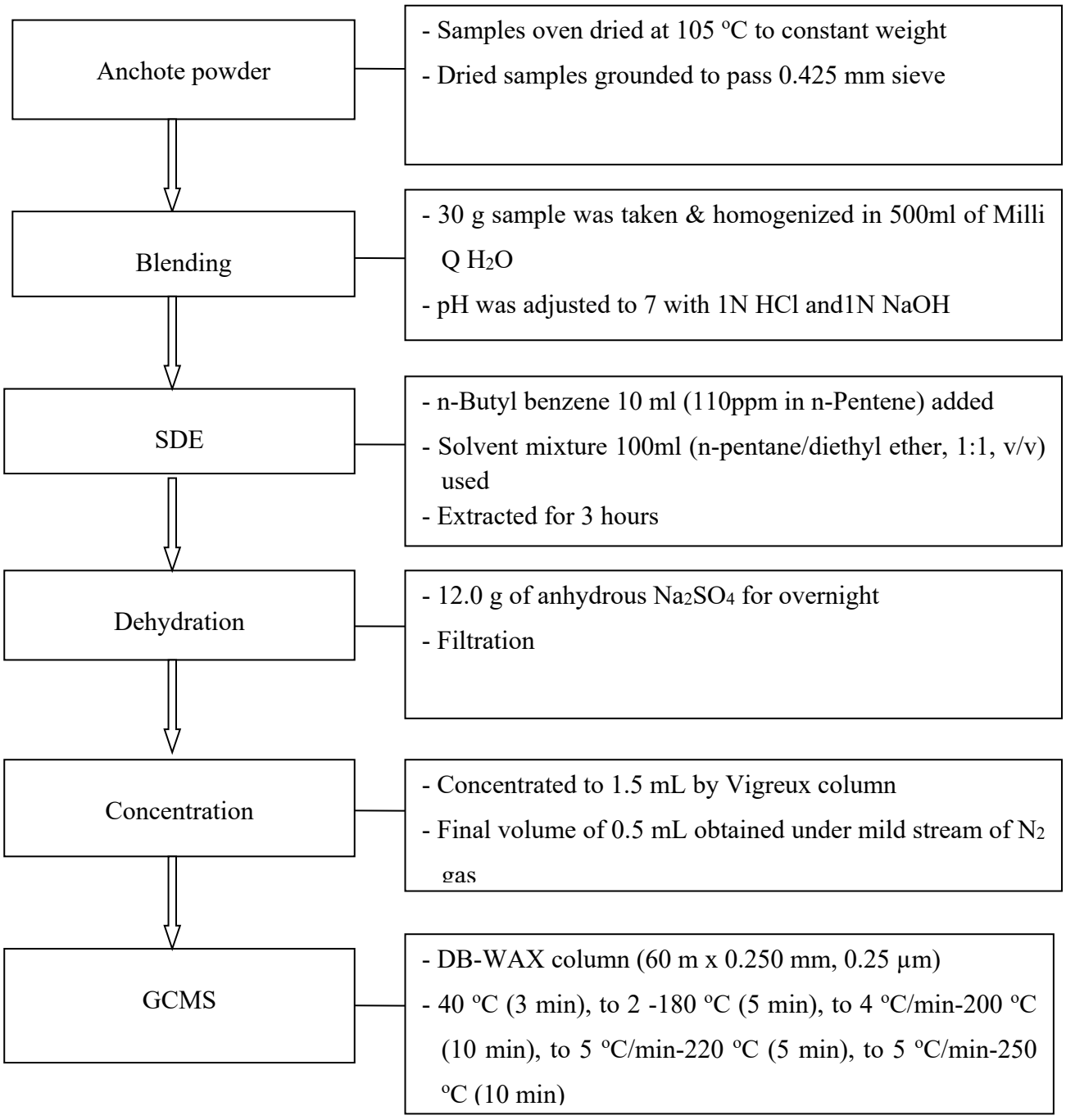


Figure 16 Schematic diagram for analysis of volatile flavor compounds of Anchote leaf and tuber samples

7.3.4. Establishment of Retention Index

Retention index or Kovats index is a suitable indication rule for retention indication that was indicated by the same compound to retention time for the standard alkane. Retention index was used as parameter for checking of a solute from chromatogram by comparing the retention time of both alkane that appeared above and below the solute.

$$RIi = 100Z + 100\{(\text{Log VR}(i) - \text{Log VR}(Z))/(\text{Log VR}(Z+1) - \text{Log VR}(Z))\}$$

Where :
RIi = Retention index of compound i
VR(i), VR(Z), VR(Z+1), = Retention time of standard alkanes (alkanes eluted before and after the substance of interest) which bracket the substance of interest.
Factor Z = Factor Z contains the number of carbon eluted e.g Z+1, Z+2...etc.

By definition, retention time of alkane is the value as multiple of carbon number that the compound has to be unrelated with column solid phase, the temperature of separation and requirements of other chromatography. Therefore, n-alkane was indicated as standard index for CH_4 (RI=100), C_2H_6= (RI=200) ……...C_nH_{2n+2} ($RI=100_n$), and even anything in analysis column. In order to obtain a scaled retention time (RT) of standard sample of known hydrocarbon mixture of n-alkane mixture (C7~ C22) was used as standard. 1µL mixture was taken to determine the RT of the internal standard by GC-MS. Retention index (RI) of each peak was established by a basic program that substituted the RT of each peak of n-alkane confirmed at GC chromatogram.

7.3.5. Chromatographic Analysis of Volatile Flavour Compounds

Chromatographic analysis was carried out using a Shimadzu GC-MS (Model QP-2010, Shimadzu Co., Kyoto, Japan) in EI (electron impact ionization) mode. The ionization voltage was 70eV and temperatures of ion source and injector were 230ºC and 250ºC, respectively. The mass spectrometer scanned from 50 to 400 m/z. The separation was done by capillary column, DB-WAX (60 m length × 0.25 mm diameter, 0.25 µm film thickness, Agilent J&W, USA).

The program of oven temperature was initially started at 40 ºC (Isothermal for 3 min) which was ramped to 180 ºC (isothermal for 5 min) at 2 ºC/min. Subsequently it increases to 200 ºC (isothermal for 10 min) at 4 ºC/min, to 220 ºC (isothermal for 5 min) at 5 ºC/min. Finally, it reaches to 250 ºC (isothermal for 10 min) at 5 ºC/min. Helium was used as the carrier gas at a flow rate of 1mL/min, and the sample injector volume was 1µL using 1:100 split ratio (Table 36).

Table 36 GC-MS conditions for analysis of volatile flavor compounds in Anchote samples

GC-MS	GCMS-QP2010, Shimadzu, Japan
Column	DB-WAX (60 m length, 0.25 mm Diameter, 0.25 µm film thickness)
Carrier gas	He (1.0mL/min)
Temperature program	40 ºC (3 min), to 2 ºC-180 ºC (5 min), to 4 ºC/min-200 ºC (10 min), to 5 ºC/min-220 ºC (5 min), to 5 ºC/min -250 ºC (10 min)
Injector	250 ºC
Ion source	230 ºC
Ionization	Electron Impact(EI)
Ionization voltage	70 eV
Mass range	50 ~ 400 (m/z)
Injection volume	1 µL

7.3.6. Identification and Quantification of Volatile Flavour Compounds

Mass spectra of volatile organic compounds were identified with the aid of our own mass spectral data and those contained within the Willey 7, NIST 05 and FFNSC 2.0 spectral libraries of the GCMS instrument. In addition, by the comparison of retention indices to the reference data (Zhu et al. 2008; Gyawali & Kim 2009; Shim et al. 2009; Jerković et al. 2010; Jerković et al. 2012; D'Auria & Racioppi 2015). The quantitative analysis was carried out with the help of peak area percent of internal standard (n-butylbenzene) using the following formula:

$$\text{Component Content (mg/kg)} = \frac{(C \times 1000\ g)}{(A \times B\ g)}$$

Where :
A = Peak area of each sample of internal standard
B g = Amount of sample
C = Peak area of each component in sample

7.4. Results and Discussions

7.4.1. Establishment of Retention Index of N-Alkane

The standard value of Retention Index (RI) was determined by n-alkane mixture (C7~ C22) considering as standard. 1µL mixture of n-alkanes was analyzed to find out the retention time (RT) by GC-MS analysis following the same conditions as mentioned in Table 37. The RI of each peak was established by basic program that substituted the RT of peak of n-alkane confirmed at GC-MS chromatogram.

Table 37 Retention time of n-alkane mixture for GC-MS retention index

Alkanes	Retention time
C_7	8.405
C_8	13.095
C_9	19.775
C_{10}	27.423
C_{11}	35.188
C_{12}	42.689
C_{13}	49.808
C_{14}	56.528
C_{15}	62.875
C_{16}	68.877
C_{17}	74.637
C_{18}	81.002
C_{19}	86.341
C_{20}	92.825
C_{21}	98.567
C_{22}	104.059

7.4.2. Volatile compounds of Anchote leaf

Volatile compounds (VOC's), percentage of their relative peak area and concentration are shown in Table 38. Having a peak area above 0.5% was used for identification of VOCs. In Anchote leaf 30 compounds were identified, among which 16 compounds were found in all the three of the tested accessions. These compounds are ethyl acetate, acetoin, 1, 1-Diethoxyethane, 3-Methyl-1-butanol(E)-2-Hexenal, (Z)-3-Hexen-1-ol, benzaldehyde, benzyl-alcohol, phenylacetaldehyde, phenethyl-alcohol, butyrophenone, 2-Methoxy-4-vinylphenol, phytone, methyl-palmitate, dibutyl-phthalate, methyl-linolenate. Ethyl acetate 90.47% (697.13 mg/kg) detected in a higher amount followed by phenylacetaldehyde 1.88 % (14.51 mg/kg) and (E)-Hex-2-enal 1.62 % (12.47 mg/kg), by exhibiting > 1% peak area. They accounted together 93.97 % (724.11 mg/kg). The rest 6.03% (46.46 mg/kg) were the minor quantities (< 1 %) of the total (770.57 mg/kg) volatile flavor fraction.

The VOCs identified in Anchote leaf have various applications. A monoterpene compound linalool was presented in very low concentration in accession '220563'. It is one of the major volatile components of several aromatic species used in foodstuffs as food additives, and pharmacologically to cure a variety of ailments being a sedative effect inducer, glutamatergic neurons inhibitor, anti-inflammatory, anti-carcinogenic and antiseptic (Crowell, Lin, Vedejs, & Gould, 1992; Elisabetsky, Marschner, & Souza, 1995; JECFA, 1999; Mazzanti, Battinelli, & Salvatore, 1998; Peana et al., 2002; Sugawara et al., 1998).Furfural has an aroma of almond and is one of the components found in vanilla. Furfural has a wide variety of uses such as for flavoring food, as herbicide, fungicide, and have an effect on yeast survival and biochemical enzyme activities (Gyawali & Kim, 2012). Nonanal, 1-hexanol, (Z)-3-hexenol, linalool and benzaldehyde, were considered as important contributors to the aroma of fresh plums fruit (Pino & Quijano, 2012). 3-methyl-1 butanol is a main ingredient in the production of banana oil, an ester found in nature and produced as flavoring in industry. (*Z*)-3-hexen-1-olis a very important aroma compound used in fruit and vegetable flavors as well as in perfumes. 1-Hexanol used in perfume industry. Benzyl alcohol produced naturally by many plants and commonly found in fruits and teas. It is also found in a variety of essential oils (O'Neil, 2013).

Hexanol occurs naturally after hydrolysis or enzymatic reduction reactions and used in the flavor industry to produce fruity flavors. Benzaldehyde has a characteristic almond like odor and is the primary component of bitter almond oil. Benzaldehyde is commonly employed to confer almond flavor to foods and scented products, and sometimes used in cosmetics products (Andersen, 2005). Acetol or 1,1-Diethoxyethane is a major flavoring component of distilled beverages, especially malt whisky (Maarse, 1991).Phenylacetaldehyde is used as an ingredient to fragrances as well as in flavored cigarettes and beverages; its aroma is described as honey-like, sweet, rose, green, and grassy (Kohlpaintner et al., 2014). The aroma of ethyl acetate contributes towards the general perception of fruitiness. Dihydroactinidiolide has a sweet, tea-like odor and used as a fragrance. Acetoin used as a food flavouring in baked goods and as a fragrance. It is therefore a common ingredient in flavors and perfumery, particularly when the odor of rose is desired (Fahlbusch et al., 2003).Acetoin also contribute to the flavor of beef fats (Watanabe & Sato 1971). At very low concentrations, indole has a flowery smell and is a constituent of many flower scents such as orange blossoms and perfumes. 2-Methoxy-4-vinylphenolis an aromatic substance used as a flavoring agent and is known as one of the compounds responsible for the natural aroma of buckwheat (Janeš, Kantar, Kreft, & Prosen, 2009). Caryophyllene oxide which is an oxygenated terpenoid, is also well known for its preservative characteristics in foods, drugs and cosmetics (D. Yang, Michel, Chaumont, & Millet-Clerc, 2000), as well as it has a significant central and peripheral analgesic, along with anti-inflammatory activity (Chavan, Wakte, & Shinde, 2010).

The identified volatile organic compounds (VOC's) in Anchote leaves so far belong to chemical classes of alcohol (7), aldehyde (3), alkane (2), carbonyl compound (4) ester (3), hydrocarbon (1), ketone (1), terpene (1) and miscellaneous (8) (Table 39). Esters were dominant with highest proportion of relative peak area (91.34 %) from the emitted volatile organic compounds. Ethyl acetate accounted 99.05 % among the ester content (703.84 mg/kg), whereas the remaining percentage is shared by methyl palmitate (0.25 %) and dibutyl phthalate (0.71%). Carbonyl compounds were the second major group accounting 2.53% of relative peak area.

Table 38 Volatile flavor compounds identified in Anchote leaf

No.	Compound name	MF[a]	223090				220563				230566			
			Area%	mg/kg	RT[b]	RI[c]	Area%	mg/kg	RT[b]	RI[b]	Area%	mg/kg	RT[b]	RI[b]
1	Ethyl acetate	$C_4H_8O_2$	4.52	19.58	6.12	606	66.58	335.49	14.43	824	4.13	18.17	6.11	606
	Ethyl acetate	$C_4H_8O_2$	0.10	0.45	19.66	899	-	-	-	-	73.50	322.97	14.22	820
	Ethyl acetate	$C_4H_8O_2$	-	-	-	-	-	-	-	-	0.11	0.47	19.65	898
2	Acetoin	$C_4H_8O_2$	0.10	0.44	8.79	710	0.11	0.53	8.77	710	0.08	0.35	8.77	710
3	1,1-Diethoxyethane	$C_6H_{14}O_2$	0.19	0.81	9.48	727.22	0.17	0.87	9.48	727	0.25	1.10	9.48	727
4	3-Methyl-1-butanol	$C_5H_{12}O$	0.28	1.19	9.86	736	0.19	0.98	9.86	736	0.31	1.36	736	736
5	Hexanal	$C_6H_{12}O$	-	-	-	-	-	-	-	-	0.07	0.32	13.13	801
6	Furfural	$C_5H_4O_2$	0.18	0.79	15.23	837	0.15	0.78	15.26	837	-	-	-	-
7	(E)-2-Hexenal	$C_6H_{10}O$	0.98	4.25	16.52	856	0.79	3.96	16.57	857	0.97	4.26	16.52	856
8	(Z)-3-Hexen-1-ol	$C_6H_{12}O$	0.46	1.98	16.64	858	0.27	1.38	16.71	859	0.31	1.35	16.65	858
9	Ethyl benzene (EB)	C_8H_{10}	-	-	-	-	0.05	0.24	16.99	863	-	-	-	-
10	1-Hexanol	$C_6H_{14}O$	0.08	0.35	17.63	872	-	-	-	-	-	-	-	-
11	Benzaldehyde	C_7H_6O	0.11	0.47	24.47	965	0.10	0.53	24.46	965	0.11	0.50	24.46	965
12	Benzyl alcohol	C_7H_8O	0.10	0.41	30.011	1036	0.13	0.68	30.01	1036	0.31	1.37	29.997	1036
13	Phenylacetaldehyde	C_8H_8O	0.60	2.60	30.82	1047	1.49	7.49	30.83	1047	0.77	3.40	30.82	1047
	Phenylacetaldehyde	C_8H_8O	-	-	-	-	0.20	1.01	31.11	1051	-	-	-	-
IS[d]	Butylbenzene	$C_{10}H_{14}$	7.70	33.33	31.77	1059	6.61	33.33	31.77	1059	7.57	33.33	31.77	1059
14	4-Nonanol	$C_9H_{20}O$	-	-	-	-	0.06	0.29	34.62	1093	-	-	-	-
15	Linalool	$C_{10}H_{18}O$	-	-	-	-	0.05	0.27	35.10	1099	-	-	-	-
16	Nonanal	$C_9H_{18}O$	0.08	0.35	35.51	1105	-	-	-	-	0.12	0.55	35.50	1105
17	Phenethyl alcohol	$C_8H_{10}O$	0.11	0.49	36.08	1113	0.34	1.71	36.07	1113	0.35	1.54	36.06	1113
18	Butyrophenone	$C_{10}H_{12}O$	0.09	0.40	46.47	1255	0.16	0.79	46.46	1255	0.09	0.38	46.46	1255
19	Indole	C_8H_7N	-	-	-	-	-	-	-	-	0.05	0.23	49.23	1292
20	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.07	0.30	50.46	1310	0.14	0.70	50.45	1310	0.11	0.47	50.45	1310
21	beta-Ionone	$C_{13}H_{20}O$	-	-	-	-	0.09	0.46	61.59	1481	0.06	0.27	61.60	1481
22	Dihydroactinidiolide	$C_{11}H_{16}O_2$	-	-	-	-	0.05	0.25	64.78	1533	0.10	0.44	64.79	1533
23	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	-	-	-	-	0.12	0.60	68.12	1588	-	-	-	-
24	Phytone	$C_{18}H_{36}O$	0.05	0.23	83.13	1841	0.12	0.59	83.12	1840	0.09	0.39	83.13	1799
25	Methyl palmitate	$C_{17}H_{34}O_2$	0.10	0.43	87.75	1922	0.18	0.93	87.73	1922	0.09	0.38	87.74	1900
26	Dibutyl phthalate	$C_{16}H_{22}O_4$	0.28	1.21	89.53	1950	0.41	2.08	89.51	1950	0.38	1.69	89.53	1899
27	Hexadecanoic acid <n->	$C_{16}H_{32}O_2$	-	-	-	-	0.40	2.02	89.82	1955	0.79	3.46	89.85	1899
28	Eicosane <n->	$C_{20}H_{42}$	-	-	-	-	-	-	-	-	0.05	0.20	104.04	2000
29	Methyl linolenate	$C_{19}H_{32}O_2$	0.19	0.81	98.38	2097	0.23	1.18	98.36	2096	0.29	1.28	98.37	1999
30	Docosane <n->	$C_{22}H_{46}$	-	-	-	-	0.06	0.31	104.03	2200	-	-	-	-

a: Molecular formula, b: Retention time, c: Retention index, d: Internal standard

From the identified carbonyl compounds phenylacetaldehyde accounts the highest amount 14.51 mg/kg (74.35 %) followed by 1,1-Diethoxyethane2.78 mg/kg (14.27%), while acetoin and nonanal were quantified as 1.32 mg/kg (6.77%) and 0.90 mg/kg (4.60%), respectively. Alcohol group constituted 1.51% of the identified volatile compounds that were specified as 3-Methyl-1-butanol (0.46%), [Z]-3-hexen-1-ol (0.61%), 1-Hexanol (0.05%), Benzyl alcohol (0.32%),4-Nonanol (0.04%) and linalool (0.04%).

The remaining four functional groups such as alkane, hydrocarbon, ketone and terpene detected at levels lower than 0.4%. Beside the identified functional groups, nine other volatile compounds were with no identified functional groups and categorized as miscellaneous. Among the miscellaneous (E)-2-Hexenal (1.62%) was occupied the major position with >1% and the remaining compounds in content order were as follows: hexadecanoic acid <n->, phenethyl alcohol, methyl linolenate, butyrophenone, 2-methoxy-4-vinylphenol, phytone, (-)-Caryophyllene oxide and indole.

Table 39 Content of functional groups in identified volatile components from Anchote leaf

Functional groups	No. of compounds	mg/kg	Relative peak area%
Alcohol	6	1.51	11.62
Aldehyde	3	0.44	3.39
Alkane	2	0.07	0.51
Carbonyl compound	4	2.53	19.51
Ester	3	91.34	703.84
Hydrocarbon	1	0.03	0.24
Ketone	1	0.09	0.73
Terpene	1	0.09	0.69
Miscellaneous	9	3.90	30.04
Total	30	100	770.57

7.4.3. Volatile compounds of Anchote tuber

The identified VOC's in three accessions of Anchote tuber that are listed according to their elution order on DB-WAX column with their amount of concentrations are shown in Table 40. Fifteen volatile compounds were identified in Anchote tuber from three accessions. Among the 15 identified compounds, ethyl acetate, was the only major compound that accounted together for 99.15% (4498.33 mg/kg) of the total volatile flavor fraction (4536.91 mg/kg) and 0.85% (38.58 mg/kg) being reported in minor quantities (< 1 %).

Hexanal content is directly related to oxidative off-flavours, and the compound is easily detected because of its low odor threshold (5 ppb) (Buttery, Turnbaugh, & Ling, 1988). Propyl acetate is commonly used in fragrances and as a flavor additive. Propyl acetate are synthesized via alcohol or acetic acid having a clear, volatile, mobile liquid with a characteristic odour reminiscent of acetone and pears, and commonly used in fragrances and as a flavor additive (Fellman & Mattheis, 1995). Watanabe & Sato (1971) reported that typical odor of pyrazine compounds is responsible for the nut-like and peanut butter like flavors, which are found in roasted barley, coffee, potato chips and cocoa. 2,3,5-trimethylpyrazine is categorized as cosmetic, flavor and fragrance agents and tetramethylpyrazine is an inhibitor of phosphodiesterase, has been widely used for treatment of cardiovascular diseases (Liao, Wu, & Yen, 1998).

Tetramethylpyrazine (TMP) has also significant vascular protective properties which have been used widely for the treatments of ischemic neural disorders and cardiovascular diseases (Jiang et al., 2015).Butyrophenones are widely used drugs for treatment of psychoses and are frequently encountered in forensic chemistry and clinical toxicology (Shim, Grant, Singh, Gallagher, & Lynch, 1999). Dibutyl phthalate is an artifact extracted from plastic which often is present in extracted samples from fruit (Niedz, Moshonas, Peterson, Shapiro, & Shaw, 1997). It is also identified from buckwheat honey (Wolski & Tambor, 2006).

Table 40 Volatile flavor compounds identified in Anchote tuber

No.	Compound name	MF[a]	223090				220563				230566			
			%	mg/kg	RT[b]	RI[c]	%	mg/kg	RT[b]	RI[c]	%	mg/kg	RT[b]	RI[c]
1	2-Pentanol	$C_5H_{12}O$	0.20	8.09	8.45	701	0.19	1.98	8.45	701	-	-		-
2	Heptane	C_7H_{16}	-	-	-	-	-	-	-	-	0.14	0.76	8.38	699
3	Acetoin	$C_4H_8O_2$	0.12	4.62	8.78	710	0.12	1.26	8.77	710	0.11	0.61	8.77	709
4	Pyrrole	C_4H_5N	-	-	-	-	-	-	-	-	0.05	0.26	10.49	750
5	1,1-Diethoxyethane	$C_6H_{14}O_2$	0.13	5.15	9.48	727	0.25	2.53	9.47	727	0.34	1.86	9.47	727
	1,1-Diethoxyethane	$C_6H_{14}O_2$	0.06	2.23	9.92	737	0.05	0.54	9.92	737	-	-	-	-
	1,1-Diethoxyethane	$C_6H_{14}O_2$	0.06	2.35	20.81	916	-	-	-	-	-	-	-	-
6	Hexanal	$C_6H_{12}O$	-	-	-	-	-	-	-	-	0.12	0.67	13.13	801
7	Ethyl acetate	$C_4H_8O_2$	81.77	3224.81	14.35	822	80.47	823.19	14.34	822	79.16	433.32	14.31	822
	Ethyl acetate	$C_4H_8O_2$	0.08	3.10	25.35	976	0.22	2.30	19.67	899	0.14	0.79	19.66	899
	Ethyl acetate	$C_4H_8O_2$	0.27	10.81	26.89	994	-	-	-	-	-	-	-	-
8	Unknown	$C_9H_{20}O_4$	-	-	-	-	0.05	0.48	20.02	904	-	-	-	-
9	2-Pentyl furan	$C_9H_{14}O$	-	-	-	-	-	-	-	-	0.10	0.56	26.59	991
10	Benzaldehyde	C_7H_6O	-	-	-	-	-	-	-	-	0.10	0.54	24.47	965
11	n-Propyl acetate	$C_5H_{10}O_2$	-	-	-	-	0.07	0.68	25.32	976	-	-	-	-
12	2,3,5-trimethyl pyrazine	$C_7H_{10}N_2$	-	-	-	-	-	-	-	-	0.06	0.30	27.47	1001
13	Tetramethyl pyrazine	$C_8H_{12}N_2$	-	-	-	-	-	-	-	-	0.38	2.11	33.83	1084
I.S[d]	Butylbenzene	$C_{10}H_{14}$	0.84	33.33	31.74	1059	3.25	33.33	31.75	1059	6.08	33.33	31.77	1059
14	Butyrophenone	$C_{10}H_{12}O$	-	-	-	-	-	-	-	-	0.08	0.45	46.47	1255
15	Dibutyl phthalate	$C_{16}H_{22}O_4$	-	-	-	-	0.05	0.55	89.52	1950	-	-	-	-

a Molecular formula, b Retention time, c Retention index, d Internal standard

The identified VOC's in Anchote tubers so far belong to chemical classes of alcohol (1), aldehyde (1), alkane (1), carbonyl compound (3), ester (2), heterocyclic compound (1), unknown (1) and miscellaneous (5) are presented in Table 41. The order of concentration for the identified functional groups is as follows: Esters > carbonyl compounds > alcohols > alkanes > aldehydes > heterocyclic compounds. Most esters have a fruity and floral flavor and may contribute to the aroma and flavor (Young et al. 1996; Echeverría et al. 2008; Song et al. 2012). Carbonyl compounds are widely found in food products, such as fried foods and beverages and its caused by the oxidation of fatty acids and higher alcohols, Strecker degradation, aldol condensation, or Millard reactions (Osorio & Cardeal, 2013).2-Pentanol was the only compound belonging to alcohol group constituting 0.22% that is lower than 1% that is consider as a minor compound.

Aldehydes are particularly important in relation to flavour alteration and from a toxicological perspective (Frankel, 1980, 1983; Frankel, 1991). A particular property of the aldehyde volatile oils is their insect repellent activity due to very strong scent (Gyawali & Kim, 2012).

Table 41 Content of functional groups in identified volatile components from Anchote tuber

Functional group	Number of compounds	mg/kg	Relative area%
Alcohols	1	0.22	10.07
Aldehydes	1	0.01	0.67
Alkanes	1	0.02	0.76
Carbonyl compounds	3	0.48	21.69
Esters	2	99.16	4499.01
Heterocyclic compounds	1	0.01	0.26
Unknown	1	0.01	0.48
Miscellaneous	5	0.09	3.97
Total	15	100	4536.92

Alcohols, aldehydes, alkanes, carbonyl compounds and esters were found in both the leaf and tuber samples. Comparative profile of VOCs showed that the group with the highest percentage of compounds was detected in ester group accounting 91.34% for leaves and 99.16% for tubers followed by carbonyl compounds and alcohols in both leaf and tuber samples.

7.5. Conclusion

The study of volatile organic compounds in leaf and tuber part of the three Anchote accessions showed that there are several bioactive volatile components present, which could be isolated and used for various purposes. In general, this study confirms the potential of Anchote for various biochemical applications in foods and in folk medicines. Therefore, further studies on the extraction and structure elucidation of the various important VOCs from the different parts of Anchote are essential in order to promote effective utilization of under exploited genetic resources for more specific and valuable applications.

Chapter Eight: General Conclusion and Recommendation

8.1. Conclusion

The present study showed that both the tuber and leaf parts of Anchote have appreciable amount of calorific value, carbohydrate, crude protein, crude fiber, ash content, and essential amino acids such as leucine, valine, isoleucine, threonine, and lysine. As compared to tubers, the leaves in all accessions were found to be rich in all nutrients capitalizing that Anchote is a double purpose crop. Anchote is also rich in quality protein and can provide essential amino acids for all age groups. There was a great variability with respect to the protein levels and amino acid composition among accessions of Anchote, which may be important for the selection of protein-rich accessions for breeding purpose. Anchote was also found to have high levels of both major and trace minerals especially calcium and iron and safe in terms of toxic metals such as Cd, As, and Pb. The bioavailability of calcium, iron and zinc was also found to be satisfactory especially the bioavailability of calcium both in the tuber and leaf samples was good. The leaf protein concentrate (LPC) of Anchote is rich in protein and can be a good source of other essential nutrients, indicating that Anchote LPC can be used as a nutritionally valuable ingredient in areas where there is high prevalence of protein-energy malnutrition. The concentrate also showed greater solubility on acid, neutral and alkaline media suggesting that it can be a useful ingredient for the formulation of various food products. Phytochemicals screened in the different parts of the plant pointed out the potential of the plant to be used as an ingredient in pharmaceutical industries. Analysis of volatile organic compounds from Anchote leaf and tuber extracts showed the presence of several bioactive volatile components that can be isolated and used for various purposes (food flavoring, aroma, etc.).

8.2. Recommendation

The finding of this study has indicated the potential of Anchote for food and medicinal use. The variability of accessions in terms of their nutrient, anti-nutrient and physicochemical properties paves the way to select accessions with the compound of interest. In general, Anchote edible parts (tuber and leaf) have high protein and calcium contents compared to other root and tuber crops which make the crop to be a potential crop to be used in infant foods. Promotion of Anchote leaf to be eaten by the local community is also recommended as the leaf was found to be more nutritious especially in terms of its protein content than the tuber for almost all accessions.

Perspectives for further research are also recommended on the following areas:

- Characterization and quantification of vitamins in tubers, leaves and fruits of Anchote;
- Effective Isolation and detailed characterization of the different phytochemicals identified in both tubers and leaves to discover new metabolites with health benefits;
- Development of new products from Anchote tubers and leaves and evaluating their nutritional and sensorial characteristics;
- Determination of quality and quantity of starch in the local cultivars of Anchote;
- Development of appropriate post-harvest management options for proper handling, storage, and processing of Anchote tubers and leaves;
- Processing techniques to reduce cyanide and other anti-nutritional factors of Anchote tuber and leaves;
- Comprehensive study to determine the bioavailability of minerals in human body by using techniques such as *in vitro*, *in vivo*, isotope labeling and efficacy studies;
- Selection, improvement and multiplication of accessions with good nutritional and phytochemical potential;
- Further investigations on the nutritional and health benefits of other untapped Anchote accessions; and
- Intensive collection and conservation system to avoid genetic erosion and better harness the genetic potential of the crop in ensuring food and nutrition security.

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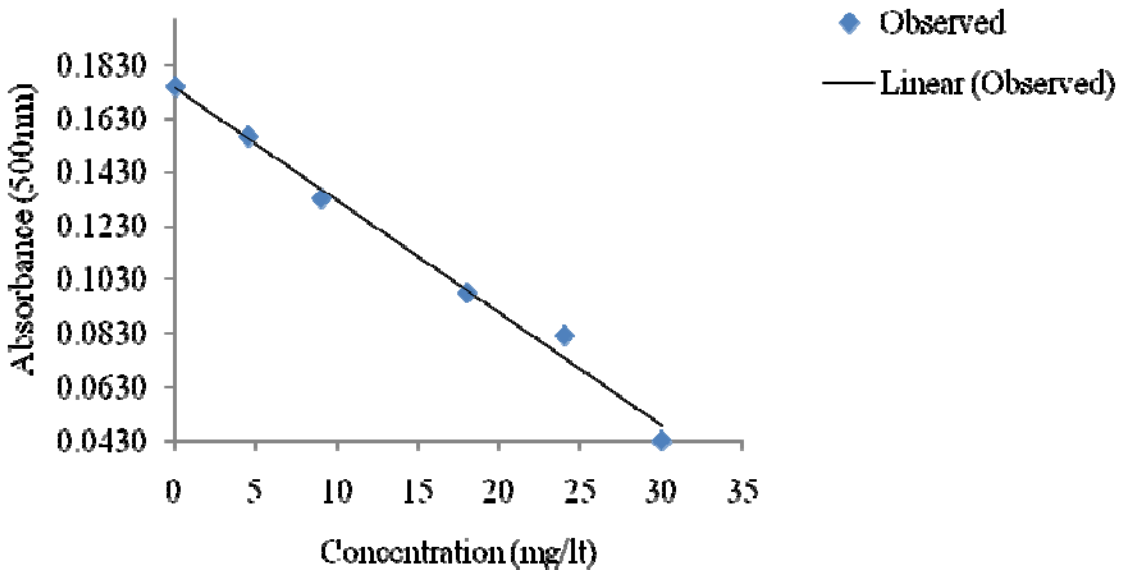
Annexes

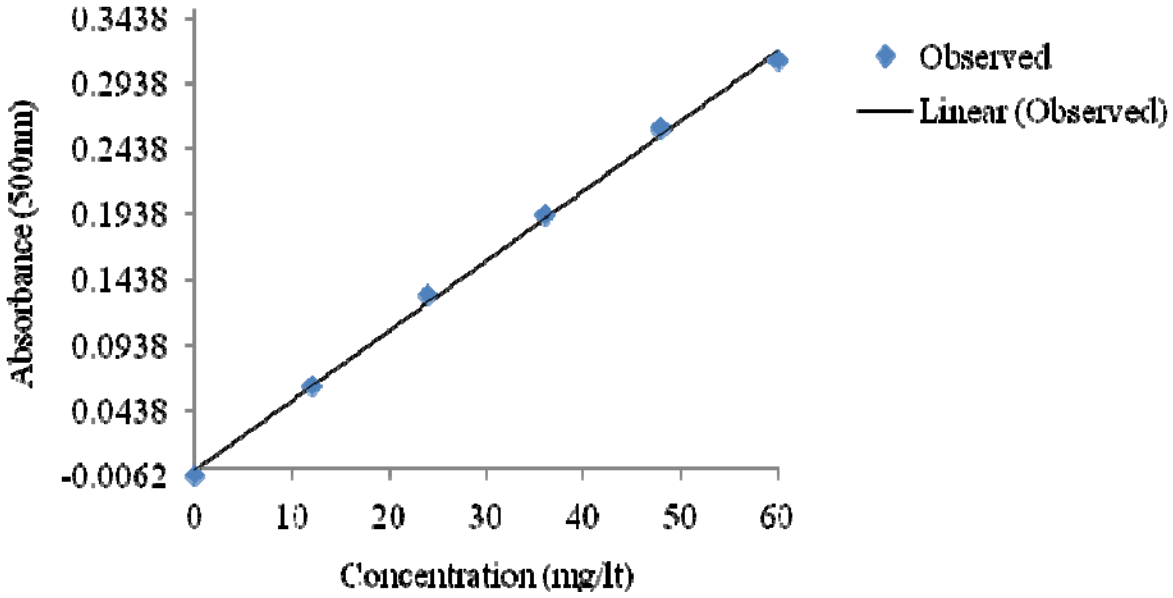
Annex A-Description of collection areas for Anchote (*Coccinia abyssinica*) accessions

S. No.	Accession	Origin		
		Region	Zone	Woreda/ District
1	90801	Oromia	Horro Guduru Wollega	Abbay Chomen
2	90802	Oromia	Horro Guduru Wollega	Abbay Chomen
3	90802-1	Oromia	Horro Guduru Wollega	Abbay Chomen
4	207984	Benishangul Gumuz	Asosa	Asosa
5	223085	Oromia	East Wollega	Digga Leka
6	223086	Oromia	East Wollega	Digga Leka
7	223087-1	Oromia	East Wollega	Digga Leka
8	223088	Oromia	West Wollega	Gimbi
9	223090	Oromia	West Wollega	Gimbi
10	223090-1	Oromia	West Wollega	Gimbi
11	223092	Oromia	East Wollega	Sibu Sire
12	223092-1	Oromia	East Wollega	Sibu Sire
13	223093	Oromia	East Wollega	Sibu Sire
14	223094	Oromia	East Wollega	Sibu Sire
15	NJ	Oromia	West Wollega	Nejo
16	223096	Oromia	East Wollega	Guto Wayu
17	223097	Oromia	East Wollega	Guto Wayu
18	223097-1	Oromia	East Wollega	Guto Wayu
19	223099	Oromia	East Wollega	Jimma Arjo
20	223100	Oromia	East Wollega	Jimma Arjo
21	223101	Oromia	East Wollega	Jimma Arjo
22	223104	Oromia	Jimma	Dedo
23	223105	Oromia	Jimma	Dedo
24	223105-1	Oromia	Jimma	Dedo
25	223109-1	Oromia	Ilu Ababor	Ale
26	223110	Oromia	Ilu Ababor	Ale
27	223112	Oromia	Ilu Ababor	Bedelle
28	223112-1	Oromia	Ilu Ababor	Bedelle
29	223113	Oromia	Jimma	Manna
30	229702	Amhara	Misirak Gojam	Hulet Iju Enese
31	229702-1	Amhara	Misirak Gojam	Hulet Iju Enese
32	220563	Oromia	East Shoa	Bako Tibe
33	220563-1	Oromia	East Shoa	Bako Tibe
34	DIGGA	Oromia	East Wollega	Digga
35	DIGGA-1	Oromia	East Wollega	Digga
36	DIGGA-2	Oromia	East Wollega	Digga
37	230565	Oromia	East Wollega	Guto Wayu
38	230566	Oromia	West Wollega	Gimbi
39	240407-1	SNNP	Keficho Shekicho	Decha
40	240407-B	SNNP	Keficho Shekicho	Decha
41	240407-G	SNNP	Keficho Shekicho	Decha
42	KICHI	Oromia	East Wollega	Gute
43	KICHI-1	Oromia	East Wollega	Gute
44	KUWE	Oromia	East Wollega	Sibu Sire

Annex B- Standard curve for the determination of phytate concentration (R2 =0.9906)



Annex C- Standard curve for the determination of tannin concentration (R2=0.9980)



Annex D- Moisture and dry matter content of fresh and oven dried tubers and leaves of Anchote accessions (%)

Accessions	Tuber		Leaf	
	Moisture	Dry Matter	Moisture	Dry Matter
90801	77.52±0.55^{a-e}	22.48±0.55^{e-i}	72.15±0.12^{a-e}	27.85±0.12^{a-d}
90802	78.23±2.89^{abc}	21.77±2.89^{ghi}	75.23±0.65^{a}	24.77±0.65^{de}
90802-1	76.69±3.98^{a-h}	23.31±3.98^{c-i}	71.92±0.63^{b-e}	28.08±0.63^{a-d}
207984	75.26±0.37^{b-h}	24.74±0.37^{b-h}	72.86±0.45^{a-d}	27.14±0.45^{a-d}
223085	77.07±1.15^{a-g}	22.93±1.15^{d-i}	72.34±0.19^{a-e}	27.66±0.19^{a-d}
223086	75.24±0.66^{b-h}	24.76±0.66^{b-h}	72.46±1.01^{a-e}	25.65±1.65^{b-e}
223087-1	72.37±3.96^{ghi}	27.63±3.96^{abc}	72.18±1.68^{a-e}	27.82±1.68^{a-d}
223088	72.68±0.69^{f-i}	27.32±0.69^{a-d}	72.90±0.12^{a-d}	27.10±0.12^{a-d}
223090	76.19±2.33^{a-h}	23.81±2.33^{b-i}	71.95±0.30^{b-e}	28.05±0.30^{a-d}
223090-1	72.53±0.94^{ghi}	27.47±0.94^{a-d}	68.31±2.21^{f}	28.30±2.58^{abc}
223092	77.67±0.35^{a-e}	22.33±0.35^{f-i}	70.49±1.27^{def}	27.57±1.48^{a-d}
223092-1	77.40±0.72^{a-f}	22.60±0.72^{e-i}	71.83±0.59^{b-e}	28.17±0.59^{a-d}
223093	76.82±0.00^{a-g}	23.18±0.00^{c-i}	71.80±0.31^{b-e}	28.20±0.31^{abc}
223094	70.44±0.35^{i}	29.56±0.35^{a}	72.19±2.34^{a-e}	27.81±2.34^{a-d}
NJ	80.17 ±2.86^{a}	19.83±2.86^{i}	73.89±2.12^{abc}	26.11±2.12^{bcd}
223096	74.73±2.16^{b-i}	25.27±2.16^{a-h}	72.17±0.61^{a-e}	27.83±0.61^{a-d}
223097	76.17±0.10^{a-h}	23.83±0.10^{b-i}	73.24±1.54^{a-d}	26.76±1.54^{bcd}
223097-1	78.03±3.55^{a-d}	21.97±3.55^{ghi}	72.31±0.10^{a-e}	27.69±0.10^{a-d}
223099	78.16±3.57^{abc}	21.84±3.57^{ghi}	72.17±0.33^{a-e}	28.38±0.45^{abc}
223100	75.83±0.30^{a-h}	24.17±0.30^{b-i}	72.67±0.08^{a-e}	27.33±0.08^{a-d}
223101	79.231±1.17^{ab}	20.77±1.17^{hi}	73.14±0.19^{a-d}	26.86±0.19^{bcd}
223104	76.28±1.41^{a-h}	23.72±1.41^{b-i}	72.56±0.89^{a-e}	26.15±0.94^{bcd}
223105	72.95±0.73^{e-i}	27.05±0.73^{a-e}	73.64±2.10^{a-d}	26.36±2.10^{bcd}
223105-1	76.47±1.71^{a-h}	23.53±1.71^{b-i}	71.53±0.10^{b-e}	28.47±0.10^{abc}
223109-1	78.45±0.10^{abc}	21.55±0.10^{ghi}	70.97±3.23^{c-f}	29.03±3.23^{ab}
223110	75.73±2.48^{a-h}	24.27±2.48^{b-i}	74.45±0.06^{ab}	25.55±0.06^{cde}
223112	76.09±1.88^{a-h}	23.91±1.88^{b-i}	71.78±0.78^{b-e}	28.22±0.78^{abc}
223112-1	75.31±3.63^{b-h}	24.69±3.63^{b-h}	72.52±0.33^{a-e}	27.48±0.33^{a-d}
223113	75.29±0.53^{b-h}	24.71±0.53^{b-h}	72.30±1.34^{a-e}	27.70±1.34^{a-d}
229702	78.28±1.80^{abc}	21.72±1.80^{ghi}	73.04±0.17^{a-d}	26.96±0.17^{bcd}
229702-1	77.03±0.44^{a-g}	22.97±0.44^{d-i}	71.58±0.34^{b-e}	28.42±0.34^{abc}
220563	73.21±1.26^{d-i}	26.79±1.26^{a-f}	70.59±0.97^{def}	27.67±1.49^{a-d}
220563-1	74.84±1.98^{b-i}	25.16±1.98^{a-h}	73.22±0.38^{a-d}	27.98±1.32^{a-d}
DIGGA	76.70±3.17^{a-h}	23.30±3.17^{c-i}	71.93±0.52^{b-e}	28.07±0.52^{a-d}
DIGGA-1	76.70±2.26^{a-h}	23.30±2.26^{c-i}	73.42±2.47^{a-d}	22.96±2.64^{e}
DIGGA-2	77.37±1.09^{a-f}	22.63±1.09^{e-i}	72.84±0.78^{a-d}	27.16±0.78^{a-d}
230565	71.97±0.15^{hi}	28.03±0.15^{ab}	73.47±2.14^{a-d}	26.53±2.14^{bcd}
230566	72.97±0.84^{e-i}	27.03±0.84^{a-e}	72.04±0.97^{a-e}	27.96±0.97^{a-d}
240407-1	73.99±0.45^{c-i}	26.01±0.45^{a-g}	73.04±0.73^{a-d}	26.96±0.73^{bcd}
240407-B	76.39±0.45^{a-h}	23.61±0.45^{b-i}	72.13±0.42^{a-e}	27.28±0.41^{a-d}
240407-G	75.52±4.40^{a-h}	24.48±4.40^{b-i}	72.30±0.09^{a-e}	27.70±0.09^{a-d}
KICHI	75.18±0.74^{b-h}	24.82±0.74^{b-h}	69.57±4.05^{ef}	30.43±4.05^{a}
KICHI-1	79.09±0.86^{ab}	20.91±0.86^{hi}	72.83±1.47^{a-d}	27.17±1.47^{a-d}
KUWE	75.59±1.37^{a-h}	24.41±1.37^{b-i}	72.78±0.00^{a-e}	27.22±0.00^{a-d}
Mean	75.90	24.10	72.34	27.38

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$)

Annex E- Proximate composition of Anchote tubers for 44 accessions (dry basis)

Accessions	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total ash (%)	Utilizable carbohydrate (%)	Gross energy (kcal100g-1)
90801	6.15±0.00^{u}	6.03±0.39^{qr}	0.58±0.06^{a-i}	5.22±0.34^{f-k}	5.63±0.01^{f-l}	82.73±0.06^{de}	361.12±1.95^{g-k}
90802	7.87±0.08^{jk}	6.78±0.27^{p}	0.56±0.05^{a-j}	5.22±0.12^{f-k}	5.52±0.15^{g-l}	81.75±0.50^{fgh}	359.84±1.34^{h-l}
90802-1	8.12±0.01^{i}	6.80±0.27^{p}	0.69±0.02^{abc}	4.44±0.05^{n-s}	5.52±0.50^{g-l}	82.54±0.84^{def}	362.82±2.07^{d-h}
207984	8.62±0.02^{g}	7.03±0.26^{p}	0.39±0.03^{i-m}	3.95±0.13st	4.76±0.04^{qr}	83.80±0.18^{abc}	366.71±0.11^{abc}
223085	6.46±0.09^{tu}	11.80±0.15^{c}	0.43±0.01^{h-m}	4.89±0.37^{j-n}	5.28±0.03^{h-p}	77.59±0.26^{r}	362.00±1.53^{f-i}
223086	7.51±0.03^{lm}	13.35±0.00^{ab}	0.30±0.04^{lm}	5.40±0.26^{e-i}	5.70±0.05^{d-i}	75.26±0.27^{s}	357.07±1.41^{lm}
223087-1	6.01±0.03^{v}	13.25±0.12^{b}	0.50±0.06^{b-l}	5.22±0.19^{f-k}	5.11±0.00^{k-r}	75.93±0.36^{s}	361.66±0.47^{g-j}
223088	7.50±0.02^{lm}	8.64±0.01^{hij}	0.36±0.32^{j-m}	5.79±0.08^{de}	5.63±0.10^{e-k}	79.73±0.50^{lo}	356.14±0.89^{mn}
223090	5.36±0.03^{x}	6.26±0.26^{q}	0.47±0.20^{d-l}	5.04±0.00^{g-m}	4.90±0.15^{n-r}	83.35±0.62^{bcd}	363.40±0.41^{d-g}
223090-1	6.61±0.04^{s}	10.82±0.27^{de}	0.51±0.06^{b-k}	4.66±0.13^{k-q}	5.42±0.04^{g-m}	78.70±0.38^{pq}	362.37±1.01^{e-i}
223092	7.85±0.00^{k}	10.45±0.13^{e}	0.68±0.01^{abc}	4.78±0.01^{k-p}	5.41±0.04^{g-n}	78.67±0.16^{pq}	362.27±0.04^{e-i}
223092-1	6.45±0.18t	8.76±0.02^{h}	0.67±0.08^{a-d}	4.96±0.07^{g-n}	6.30±0.01^{bc}	79.32±0.02^{nop}	358.30±0.71^{j-m}
223093	8.44±0.17^{h}	10.90±0.12^{d}	0.32±0.00^{klm}	4.77±0.08^{k-p}	5.35±0.01^{g-o}	78.66±0.19^{pq}	361.22±0.25^{g-k}
223094	5.64±0.02^{w}	7.94±0.00^{lmn}	0.45±0.00^{f-l}	3.95±0.06st	4.94±0.06^{m-r}	82.95±0.00^{cd}	367.21±0.01^{ab}
NJ	7.32±0.08^{nop}	10.70±0.26^{de}	0.43±0.03^{g-m}	6.42±0.00^{bc}	6.83±0.02^{a}	75.62±0.25^{s}	350.17±0.25^{p}
223096	8.15±0.05^{i}	6.13±0.14^{qr}	0.50±0.03^{b-l}	4.06±0.14st	4.89±0.02^{n-r}	84.38±0.05^{a}	366.67±0.48^{abc}
223097	7.10±0.04^{q}	13.72±0.10^{a}	0.58±0.01^{a-i}	6.04±0.10^{cd}	5.75±0.01^{d-h}	73.89±0.22^{t}	355.73±0.50^{mn}
223097-1	9.64±0.01^{d}	9.57±0.00^{fg}	0.66±0.30^{a-f}	5.48±0.10^{e-g}	5.31±0.02^{h-p}	78.95±0.18^{opq}	359.29±1.98^{i-l}
223099	8.68±0.04^{g}	9.40±0.14^{g}	0.49±0.11^{b-l}	5.08±0.09^{g-l}	5.17±0.02^{j-q}	79.84±0.04^{lo}	361.35±0.27^{e-j}
223100	6.97±0.01^{q}	9.81±0.13^{f}	0.64±0.06^{a-g}	4.84±0.12^{j-o}	4.86±0.05^{o-r}	80.05±0.01^{k-n}	365.15±0.97^{b-f}
223101	7.99±0.02^{j}	8.38±0.13^{h-k}	0.67±0.01^{a-e}	4.29±0.19^{p-s}	5.07±0.07^{h-r}	81.62±0.29^{ghi}	366.06±0.58^{a-d}
223104	5.60±0.01^{w}	9.23±0.52^{g}	0.57±0.08^{a-j}	5.44±0.01^{e-h}	5.44±0.01^{g-m}	79.59±0.47^{mno}	359.85±0.51^{h-l}
223105	7.23±0.12^{p}	6.75±0.25^{p}	0.47±0.00^{d-l}	4.45±0.22^{n-s}	5.08±0.04^{k-r}	83.26±0.03^{bcd}	364.43±1.10^{b-g}
223105-1	7.29±0.03^{op}	8.71±0.14^{hi}	0.46±0.01^{e-l}	4.36±0.01^{o-s}	5.20±0.03^{i-q}	81.27±0.10^{hij}	364.24±0.20^{b-g}
223109-1	10.04±0.02^{b}	7.59±0.14^{no}	0.52±0.02^{b-k}	5.77±0.14^{de}	6.05±0.09^{b-e}	79.90±0.07^{k-n}	355.30±0.12^{mn}
223110	7.44±0.01^{lmn}	5.82±0.00^{r}	0.52±0.01^{b-k}	4.45±0.01^{n-s}	4.83±0.04^{pqr}	84.41±0.04^{a}	365.48±0.24^{a-e}
223112	7.00±0.09^{q}	8.22±0.01^{kl}	0.53±0.00^{b-k}	6.57±0.90^{ab}	6.42±0.00^{ab}	78.24±0.92^{qr}	350.72±3.65^{op}
223112-1	9.97±0.05^{bc}	7.02±0.13^{p}	0.75±0.07^{a}	5.33±0.73^{e-j}	5.28±0.62^{h-p}	81.37±1.29^{hij}	360.00±5.07^{h-l}
223113	7.33±0.11^{nop}	7.86±0.27^{lo}	0.59±0.02^{a-i}	4.93±0.10^{j-n}	5.37±0.31^{g-o}	81.23±0.71^{hij}	361.75±1.55^{ghi}
229702	6.16±0.01^{u}	8.29±0.12^{jkl}	0.54±0.00^{a-j}	4.52±0.05^{m-r}	5.33±0.30^{h-p}	81.62±0.38^{ghi}	363.60±1.02^{e-g}
229702-1	6.02±0.02^{v}	9.17±0.13^{g}	0.61±0.03^{a-h}	4.87±0.00^{j-o}	5.30±0.30^{h-p}	80.06±0.14^{k-n}	362.76±1.38^{e-h}
220563	7.55±0.07^{l}	7.51±0.26^{o}	0.50±0.08^{b-l}	5.10±0.08^{g-l}	5.16±0.00^{k-q}	81.72±0.25^{fgh}	361.43±0.74^{e-j}
220563-1	9.86±0.01^{c}	6.23±0.12^{q}	0.70±0.03^{ab}	6.96±0.24^{a}	6.37±0.31^{b}	79.33±0.24^{nop}	349.14±0.10^{p}
DIGGA	10.20±0.01^{a}	6.81±0.13^{p}	0.67±0.12^{a-e}	4.89±0.04^{j-n}	4.63±0.31^{r}	82.57±0.34^{def}	363.45±0.77^{e-g}
DIGGA-1	6.43±0.03^{t}	8.39±0.00^{h-k}	0.59±0.03^{a-i}	5.13±0.02^{g-l}	5.11±0.60^{k-r}	81.12±0.59^{hij}	362.01±2.60^{f-i}
DIGGA-2	8.43±0.02^{h}	6.34±0.14^{q}	0.50±0.03^{b-l}	4.81±0.06^{j-o}	5.86±0.31^{c-g}	82.48±0.36^{d-g}	359.60±1.16^{h-l}
230565	9.35±0.00^{e}	9.46±0.13^{fg}	0.66±0.04^{a-e}	5.64±0.09^{def}	5.69±0.00^{d-j}	78.55±0.26^{pq}	357.57±0.17^{lm}
230566	7.23±0.05^{p}	6.37±0.00^{q}	0.24±0.05^{m}	4.23±0.14^{qrs}	5.36±0.30^{g-o}	83.90±0.10^{ab}	362.62±0.83^{e-i}
240407-1	6.76±0.01^{r}	6.83±0.40^{p}	0.35±0.01^{j-m}	3.63±0.04^{t}	4.70±0.00^{qr}	84.51±0.43^{a}	368.48±0.24^{a}
240407-B	7.41±0.03^{mno}	8.08±0.00^{klm}	0.33±0.03^{klm}	5.05±0.01^{g-m}	6.02±0.00^{b-f}	80.50±0.02^{j-m}	357.98±0.32^{klm}
240407-G	10.18±0.05^{a}	8.26±0.00^{kl}	0.48±0.05^{c-l}	5.03±0.01^{g-m}	5.06±0.31^{k-r}	80.80±0.29^{ijk}	361.17±1.56^{g-k}
KICHI	6.07±0.06^{uv}	7.79±0.01^{mno}	0.59±0.10^{a-i}	5.00±0.08^{g-m}	4.88±0.30^{o-r}	81.91±0.11^{e-h}	364.06±1.36^{b-g}
KICHI-1	9.21±0.09^{f}	8.12±0.12^{klm}	0.69±0.00^{abc}	6.42±0.03^{bc}	6.12±0.00^{bcd}	78.61±0.13^{pq}	353.30±0.04^{no}
KUWE	6.34±0.03^{t}	8.46±0.13^{h-k}	0.60±0.02^{a-i}	4.92±0.04^{j-n}	5.53±0.00^{g-l}	80.57±0.07^{jkl}	361.47±0.03^{e-j}
Mean	7.58	8.50	0.53	5.05	6.83	80.52	360.84

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$)

Annex F- Proximate composition of Anchote leaves for 44 accessions (dry basis)

Accessions	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total Ash (%)	Utilizable carbohydrate (%)	Gross energy (kcal100g-1)
90801	8.78±0.01^{dg}	18.84±0.80^{ghi}	3.34±0.08^{ijp}	10.26±0.16^{ops}	12.16±0.04^{dh}	55.40±0.92^{jk}	327.02±1.20^{el}
90802	9.09±0.01^{c}	16.30±0.39^{lm}	3.64±0.09^{gn}	12.73±0.29^{ad}	12.02±0.18^{ei}	54.07±1.32^{kn}	319.18±0.88^{pt}
90802-1	8.74±0.03^{di}	19.27±0.65^{ghi}	2.77±0.01^{qr}	10.98±0.18^{kp}	11.37±0.00^{mno}	54.20±1.52^{klm}	324.45±0.80^{lo}
207984	8.40±0.18^{lo}	15.82±0.64^{m}	3.47±0.01^{ip}	11.84±0.82^{dk}	11.95±0.01^{ej}	56.92±1.45^{gij}	322.17±3.16^{ms}
223085	8.50±0.05^{hm}	19.32±0.26^{ghi}	3.62±0.03^{gn}	13.05±0.08^{a}	11.99±0.01^{ei}	52.02±0.35^{n}	317.89±0.10^{tu}
223086	8.89±0.15^{cf}	18.06±0.25^{hk}	3.71±0.05^{el}	12.29±0.32^{af}	11.77±0.04^{hm}	54.16±0.00^{klm}	327.71±1.06^{ek}
223087-1	8.51±0.02^{hm}	30.68±0.71^{bc}	3.71±0.07^{el}	10.73±0.05^{kq}	12.00±0.13^{ei}	42.89±0.82^{t}	316.49±0.14^{tu}
223088	8.75±0.08^{di}	29.61±0.04^{c}	3.06±0.01^{pq}	12.90±0.03^{abc}	11.81±0.08^{gl}	42.63±0.02^{tu}	318.41±4.51st
223090	8.50±0.01^{hm}	23.62±0.39^{f}	3.50±0.08^{ip}	12.00±1.22^{di}	12.77±0.01^{b}	47.35±0.64^{pq}	318.51±1.04st
223090-1	7.75±0.13st	34.58±0.29^{a}	3.72±0.00^{el}	11.98±0.27^{di}	12.46±0.09^{bcd}	38.15±0.30^{w}	325.40±3.51^{ho}
223092	7.96±0.07^{rs}	16.69±0.66^{klm}	3.26±0.08^{mnp}	11.43±0.74^{fm}	12.48±0.01^{bcd}	55.25±1.26^{jkl}	321.29±0.62^{ns}
223092-1	8.29±0.26^{mnp}	18.09±0.77^{hk}	4.15±0.13^{be}	12.51±0.27^{ad}	11.81±0.08^{gl}	53.87±0.93^{kn}	318.88±0.97^{qt}
223093	8.50±0.24^{hm}	18.08±0.16^{hk}	3.28±0.18^{kp}	12.38±0.17^{ad}	12.01±0.12^{ei}	54.07±0.07^{kn}	329.46±1.29^{bi}
223094	7.89±0.08^{rs}	26.64±0.72^{de}	3.12±0.15^{opq}	10.32±0.73^{ns}	11.22±0.60^{op}	48.71±0.71^{op}	326.40±1.72^{fm}
NJ	8.85±0.08^{cf}	25.71±0.05^{e}	3.73±0.06^{ej}	12.32±0.45^{ae}	10.74±0.04^{q}	47.50±0.60^{pq}	321.01±2.97^{os}
223096	9.32±0.04^{b}	16.23±0.01^{lm}	3.99±0.39^{eh}	13.03±0.23^{a}	11.70±0.02^{in}	55.05±0.12^{jkl}	329.80±3.41^{bh}
223097	8.92±0.10^{cde}	30.15±0.34^{bc}	3.30±0.01^{jip}	9.84±0.28^{ns}	11.76±0.60^{hm}	44.94±1.22^{rs}	323.20±0.50^{kq}
223097-1	8.40±0.23^{lo}	13.27±0.03^{no}	3.56±0.03^{ho}	12.27±0.03^{af}	11.37±0.15^{mno}	59.53±0.21^{def}	333.61±3.53^{ab}
223099	8.70±0.09^{ej}	10.46±0.28^{q}	4.12±0.06^{bf}	9.84±0.73^{ns}	11.91±0.08^{ek}	63.66±0.46^{a}	321.03±1.27^{os}
223100	8.96±0.08^{cd}	8.96±0.01^{r}	3.73±0.01^{ek}	12.40±0.21^{ad}	12.00±0.10^{ei}	62.91±0.28^{ab}	332.90±0.26^{abc}
223101	8.77±0.01^{dg}	16.83±1.20^{jkm}	3.88±0.05^{di}	9.60±0.00^{s}	12.03±0.01^{ei}	57.67±1.24^{fgh}	325.26±0.55^{ho}
223104	8.40±0.01^{lo}	18.07±0.67^{hk}	3.69±0.09^{fm}	11.14±0.22^{io}	12.17±0.04^{dh}	54.94±1.02^{jkl}	330.44±0.19^{bf}
223105	8.26±0.09^{mnp}	17.80±0.25^{ijl}	4.29±0.10^{ad}	11.18±0.00^{hn}	11.57±0.09^{io}	55.16±0.42^{jkl}	325.60±1.58^{gn}
223105-1	8.50±0.06^{hm}	26.04±0.18^{de}	3.34±0.08^{ip}	10.16±0.32^{ps}	12.63±0.04^{bc}	47.82±0.38^{pq}	334.84±0.41^{a}
223109-1	8.22±0.03^{nop}	35.42±0.05^{a}	3.32±0.11^{jip}	7.89±0.03^{t}	12.55±0.00^{bcd}	40.82±0.19^{uv}	329.15±0.46^{ei}
223110	8.66±0.02^{fk}	25.27±0.27^{e}	3.38±0.07^{jip}	9.61±0.00^{s}	12.33±0.02^{cde}	49.41±0.23^{o}	329.44±0.56^{bi}
223112	8.58±0.10^{gl}	20.27±0.02^{g}	4.44±0.03^{ab}	10.95±0.02^{kp}	12.25±0.08^{cf}	52.08±0.1^{lmn}	329.39±0.27^{bi}
223112-1	8.75±0.02^{dh}	19.67±0.67^{gh}	4.23±0.01^{bcd}	11.16±0.03^{in}	11.76±0.05^{hm}	53.19±0.76^{klmn}	332.34±3.44^{ad}
223113	7.59±0.01^{t}	13.71±0.26^{n}	4.68±0.84^{a}	10.56±0.42^{mqr}	12.21±0.23^{dg}	58.83±1.29^{efg}	325.22±0.54^{io}
229702	8.44±0.01^{kn}	18.38±0.41^{hij}	3.74±0.02^{ej}	12.04±0.10^{eh}	11.32±0.01^{nop}	54.52±0.50^{kl}	322.33±0.14^{ms}
229702-1	8.22±0.02^{nop}	18.45±0.66^{hij}	3.56±0.03^{ho}	12.42±0.00^{ad}	11.50±0.07^{ko}	54.08±0.55^{kn}	322.10±6.19^{ms}
220563	8.58±0.11^{gl}	16.30±3.21^{lm}	4.37±0.21^{abc}	13.00±0.26^{ab}	10.99±0.06^{pq}	55.35±3.32^{jkl}	326.32±2.36^{gm}
220563-1	8.06±0.29^{pqr}	11.81±0.42^{opq}	3.99±0.07^{eh}	10.89±0.09^{kp}	11.65±0.14^{jm}	61.66±0.76^{bc}	330.09±0.71^{bg}
DIGGA	8.57±0.05^{gl}	11.38±0.00^{pq}	3.96±0.10^{eh}	11.43±0.13^{fm}	12.79±0.00^{b}	60.44±0.02^{cde}	322.91±1.00^{kr}
DIGGA-1	8.11±0.07^{pqr}	34.00±0.19^{a}	3.14±0.06^{opq}	11.05±0.08^{io}	11.98±0.19^{ej}	39.83±0.52^{vw}	323.59±0.72^{jp}
DIGGA-2	8.76±0.02^{dg}	29.13±0.54^{c}	2.44±0.27^{r}	11.88±0.52^{dj}	12.02±0.27^{ei}	44.53±1.06st	316.58±4.48^{tu}
230565	8.45±0.04^{jn}	16.95±0.26^{jkm}	3.25±0.00^{nop}	11.33±0.38^{gm}	11.78±0.30^{hl}	56.70±0.18^{hij}	323.80±0.33^{io}
230566	8.95±0.10^{cd}	11.52±0.39^{pq}	4.01±0.09^{eg}	10.67±0.53^{kr}	12.33±0.21^{cde}	61.49±0.03^{bcd}	328.13±0.83^{dj}
240407-1	8.46±0.11^{jn}	30.38±0.01^{bc}	3.27±0.16^{kp}	10.71±0.00^{kr}	12.04±0.22^{ei}	43.61±0.03st	325.55±1.63^{ho}
240407-B	9.41±0.03^{b}	14.06±0.68^{n}	4.45±0.02^{ab}	12.13±0.04^{bg}	12.02±0.03^{ei}	57.34±0.66^{ghi}	325.09±0.14^{io}
240407-G	8.37±0.10^{lo}	12.69±0.01^{nop}	2.76±0.01^{qr}	11.36±0.38^{gm}	13.59±0.02^{a}	59.60±0.35^{def}	314.00±1.35^{u}
KICHI	8.19±0.07^{opq}	31.21±0.28^{b}	3.33±0.01^{jip}	9.91±0.30^{ns}	11.47±0.02^{lo}	44.08±0.59st	331.15±1.09^{ae}
KICHI-1	9.79±0.02^{a}	27.26±1.58^{d}	3.60±0.38^{gn}	10.66±0.11^{kr}	11.91±0.14^{fk}	46.56±1.72^{qr}	327.65±2.92^{ek}
KUWE	9.03±0.05^{dc}	13.16±0.53^{no}	3.97±0.33^{eh}	11.49±0.04^{el}	11.89±0.08^{fk}	59.50±0.16^{def}	326.32±1.46^{fm}
Mean	8.56	20.50	3.63	11.42	11.96	52.56	325.05

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$)

Annex G- Anti-nutritional content (mg/100g dry basis) of Anchote tuber and leaf part for 44 accessions

Accession Number	Tannin		Phytate	
	Tuber	Leaf	Tuber	Leaf
90801	182.16±10.22^{e}	264.93±3.31^{i}	174.43±0.74^{c-f}	252.91±2.65^{no}
90802	223.19±13.87^{d}	214.11± 3.55^{kl}	215.03±13.52^{b}	270.12±5.51^{h-k}
90802-1	76.44±7.48^{nop}	325.12±3.34^{cd}	147.16±5.85^{h-k}	226.86±1.74st
207984	87.82±4.37^{mn}	267.42±7.41^{i}	151.19±8.10^{g-j}	282.29±1.67^{cde}
223085	107.76±2.25^{jkl}	221.46±3.32^{jk}	315.57±1.56^{a}	257.14±1.83^{mn}
223086	142.96±5.30^{gh}	271.53±3.83^{i}	325.03±8.46^{a}	265.98±3.89^{i-l}
223087-1	329.92±4.39^{a}	179.42±6.93^{mn}	138.54±10.28^{i-l}	287.18±2.43^{bcd}
223088	81.01±0.71^{mno}	189.29±3.30^{mn}	195.19±17.20^{c}	248.20±4.63^{o}
223090	312.24±0.96^{b}	268.28±6.84^{i}	142.56±13.95^{h-l}	248.26±2.15^{o}
223090-1	174.58±6.23^{ef}	189.70±6.57^{mn}	127.14±8.99^{k-n}	262.38±0.53^{klm}
223092	270.38±7.57^{c}	226.58±3.64^{j}	143.72±14.86^{h-l}	252.81±0.71^{no}
223092-1	158.05±8.71^{fg}	268.01±10.83^{i}	117.70±12.14^{mno}	223.66±1.76^{tu}
223093	113.92±8.42^{jk}	182.17±3.00^{mn}	179.12±8.94^{cde}	217.49±1.35^{uv}
223094	213.32±6.20^{d}	204.42±6.54^{l}	143.45±10.61^{h-l}	239.14±4.39^{pq}
NJ	119.29±10.59^{ij}	385.63+6.27^{a}	113.76±10.43^{no}	236.18±4.14^{qr}
223096	158.52±4.85^{fg}	290.73±6.72^{g}	163.22±7.00^{e-h}	213.42±3.26^{v}
223097	121.34±15.58^{ij}	270.47±7.06^{i}	185.53±9.26^{cd}	211.11±3.93^{v}
223097-1	132.97±6.09^{hi}	308.51±2.65^{ef}	106.58±0.20^{op}	235.82±1.32^{qr}
223099	110.75±8.11^{jk}	336.26±7.05^{bc}	88.31±2.88^{pq}	191.50±0.37^{x}
223100	105.89±2.88^{jkl}	286.23±3.30^{gh}	136.94±10.56^{i-m}	258.80±3.19^{lmn}
223101	50.11±4.18^{r}	343.51±6.80^{b}	78.72±5.85^{qr}	183.25±6.27^{y}
223104	177.18±4.13^{e}	318.03±3.29^{de}	123.46±10.64^{l-o}	232.03±2.11^{qrs}
223105	139.62±12.14^{h}	298.11±3.63^{fg}	64.04±2.93^{rs}	262.97±1.82^{klm}
223105-1	87.65±2.74^{mn}	286.53±7.01^{gh}	79.90±0.98^{qr}	203.09±1.82^{w}
223109-1	55.51±9.84^{qr}	95.17±3.38^{s}	62.09±14.76^{rs}	229.98±3.78^{rst}
223110	26.65±2.53st	216.15±3.30^{jkl}	82.57±6.22^{qr}	246.77±4.60^{o}
223112	144.97±4.81^{gh}	176.66±3.14^{n}	180.75±15.16^{cde}	279.52±0.28^{efg}
223112-1	27.81±4.44^{s}	210.48±13.11^{kl}	169.04±14.06^{d-g}	272.07±3.41^{g-j}
223113	24.70±6.31st	113.57±6.67^{r}	44.29±5.58st	218.42±1.93^{uv}
229702	68.37±12.90^{opq}	182.14±6.57^{mn}	36.39±0.39^{tu}	280.36±1.17^{def}
229702-1	57.66±12.87^{qr}	161.21±6.61^{o}	37.73±2.58^{tu}	273.35±2.95^{f-i}
220563	46.56±4.14^{r}	276.96±3.04^{ih}	138.67±7.53^{i-l}	265.45±5.28^{jkl}
220563-1	9.83±5.74^{t}	188.50±7.30^{mn}	79.51±1.44^{qr}	266.90±3.48^{ijk}
DIGGA	14.33±0.56st	149.36±3.36^{op}	29.49±14.59^{tu}	245.76±3.61^{op}
DIGGA-1	77.04±2.00^{nop}	127.18± 6.75^{q}	20.38±0.95^{u}	277.11±4.16^{e-h}
DIGGA-2	52.37±10.07^{qr}	135.86±3.36^{q}	194.26±7.93^{c}	295.98±4.41^{a}
230565	56.33±13.28^{qr}	147.82±3.37^{p}	193.23±12.70^{c}	293.31±5.68^{ab}
230566	97.83±12.07^{klm}	114.39±3.53^{r}	45.60±9.30st	247.79±4.28^{o}
240407-1	135.32±4. 85^{hi}	54.11±6.84^{t}	44.42±11.07st	246.86±2.48^{o}
240407-B	167.61±7.66^{ef}	207.98± 3.32^{l}	174.26±1.95^{c-f}	238.08±4.07^{q}
240407-G	20.24±3.35st	158.59±6.62^{op}	150.63±2.32^{g-j}	275.11±6.16^{e-h}
KICHI	60.47±1.76^{pqr}	133.63±0.08^{q}	131.03±6.60^{j-n}	289.25±2.87^{abc}
KICHI-1	17.19±6.98st	127.74±3.37^{q}	142.68±4.56^{h-l}	276.84±1.33^{e-h}
KUWE	91.18±1.29^{lmn}	153.26±3.62^{op}	154.91±2.62^{f-i}	231.91±4.32^{qrs}
Mean	112.02	216.53	131.10	250.30

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex H- Major mineral elements concentration (mg/100g dry basis) of Anchote tubers for 44 accessions

Accession	Na	P	K	Ca	Mg
90801	62.04±0.25^{k-q}	34.18±0.60^{d-g}	74.64±1.81^{d}	234.61±0.21^{m}	30.85±0.55^{ij}
90802	66.31±1.25^{i-n}	39.70±3.30^{b}	53.64±0.09^{jkl}	83.53±1.87^{z}	20.96±0.39^{opq}
90802-1	55.32±0.07^{pq}	29.17±0.24^{jk}	28.83±3.01^{qr}	118.18±1.02^{v}	17.56±1.51^{qrs}
207984	64.07±0.86^{j-p}	28.76±1.11^{jkl}	65.75±0.29^{ef}	80.64±0.66^{z}	17.07±0.59^{q-t}
223085	55.77±1.11^{opq}	31.96±0.05^{f-j}	14.61±1.96^{t}	105.50±1.11^{w}	25.82±5.23^{lmn}
223086	68.18±7.39 $^{h-m}$	33.03±1.37^{e-i}	34.72±1.55^{op}	316.67±2.85^{d}	24.00±4.08^{mno}
223087-1	85.64±12.91^{bc}	17.51±0.06^{r}	13.63±1.70^{t}	124.47±0.57^{u}	13.26±0.63^{t-w}
223088	61.58±2.94^{l-q}	38.44±2.29^{b}	43.08±0.26^{m}	343.01±1.10^{b}	36.64±1.01^{efg}
223090	42.78±0.51^{r}	29.52±0.69^{ij}	30.09±1.82^{qr}	235.09±0.15^{m}	29.18±2.13^{i-l}
223090-1	53.05±0.92^{q}	33.97±0.21^{d-h}	37.21±0.47^{no}	249.65±2.10^{k}	26.51±0.65^{k-n}
223092	68.83±0.70^{h-m}	37.79±1.89^{bc}	62.89±0.38^{efg}	99.27±0.10^{x}	19.64±1.03^{pqr}
223092-1	79.61±2.69^{c-f}	19.44±2.08^{qr}	80.59±3.43^{c}	305.36±5.47^{f}	30.75±0.85^{ij}
223093	77.69±2.00^{c-g}	24.40±0.64^{mno}	95.28±3.65^{a}	84.14±5.92^{z}	12.39±0.48^{uvw}
223094	73.54±0.18^{d-g}	19.78±1.20^{pqr}	54.02±5.19^{jkl}	234.06±5.44^{m}	28.84±1.27^{i-l}
NJ	80.20±1.71^{cde}	23.78±2.12^{mno}	87.41±1.18^{b}	310.84±1.31^{ef}	59.36±4.24^{a}
223096	65.00±0.02^{i-o}	34.54±0.70^{c-f}	62.55±0.26^{efg}	118.04±4.92^{v}	22.34±0.39^{nop}
223097	63.55±0.60^{j-p}	30.35±0.57^{hij}	52.70±0.69^{kl}	310.87±3.16^{ef}	30.33±2.37^{ijk}
223097-1	65.84±0.23^{i-n}	31.73±1.27^{f-j}	60.48±0.12^{ghi}	91.31±3.16^{y}	20.00±0.43^{o-r}
223099	66.03±0.40^{i-n}	31.32±0.03^{f-j}	66.65±0.96^{e}	123.78±0.27^{u}	16.02±0.36^{r-u}
223100	70.67±0.25^{g-l}	21.64±3.65^{opq}	61.33±0.75^{fgh}	280.97±0.16^{gh}	41.37±1.36^{de}
223101	59.47±0.36^{m-q}	36.46±0.37^{b-e}	72.86±1.76^{d}	223.09±2.39^{n}	16.18±2.10^{r-u}
223104	57.92±6.29^{n-q}	45.74±4.42^{a}	19.64±1.32^{s}	135.07±0.16^{t}	13.52±0.11^{s-v}
223105	71.07±0.44^{f-k}	23.80±2.00^{mno}	61.13±0.12^{ghi}	306.53±1.14^{f}	38.33±1.43^{efg}
223105-1	55.49±4.37^{pq}	37.49±0.89^{bcd}	55.95±2.82^{jk}	152.94±1.52^{s}	25.09±3.30^{lmn}
223109-1	62.62±1.19^{k-p}	21.00±1.62^{o-r}	74.40±0.63^{d}	315.03±1.00^{de}	9.33±0.04^{w}
223110	78.00±7.51^{c-f}	24.07±0.67^{mno}	56.77±0.02^{ijk}	270.38±4.18^{i}	45.60±1.82^{bc}
223112	63.29±7.68^{j-p}	48.64±1.22^{a}	19.02±2.24^{s}	163.10±1.26^{r}	11.03±1.02^{vw}
223112-1	98.78±12.96^{a}	30.99±0.03^{f-j}	39.11±1.05^{n}	251.90±0.09^{jk}	32.65±1.03^{hi}
223113	81.33±0.56^{cd}	21.47±2.43^{opq}	57.27±0.51^{hij}	269.03±4.77^{i}	39.21±0.82^{ef}
229702	63.90±0.07^{j-p}	25.90±1.20^{klm}	61.04±0.13^{ghi}	251.33±0.25^{jk}	43.67±0.62^{cd}
229702-1	60.55±0.13^{m-q}	24.17±1.20^{mno}	61.97±1.71^{fg}	270.16±4.11^{i}	40.55±2.47^{def}
220563	43.17±2.15^{r}	37.17±1.34^{bcd}	29.47±4.87^{qr}	207.41±0.85^{p}	25.69±3.64^{lmn}
220563-1	68.49±1.69^{h-m}	38.30±1.71^{b}	43.51±1.16^{m}	372.16±1.39^{a}	38.47±0.24^{efg}
DIGGA	72.26±3.25^{e-j}	39.12±0.72^{b}	31.24±0.03^{pq}	317.89±4.54^{d}	28.02±0.37^{j-m}
DIGGA-1	66.22±0.32^{i-n}	23.20±2.71^{m-p}	61.72±1.12^{fg}	241.55±2.59^{l}	47.99±1.76^{b}
DIGGA-2	95.88±2.58^{a}	33.98±0.36^{d-h}	43.80±3.45^{m}	284.79±0.95^{g}	25.94±0.00^{lmn}
230565	75.63±4.88^{d-g}	29.42±0.98^{ij}	31.65±0.17^{pq}	167.31±1.09^{r}	20.38±0.60^{opq}
230566	65.08±1.05^{i-o}	23.59±1.21^{mno}	56.02±0.23^{jk}	277.06±3.23^{h}	39.77±0.33^{def}
240407-1	60.09±1.17^{m-q}	14.09±1.81^{s}	49.95±0.00^{l}	226.45±1.87^{n}	40.50±0.20^{def}
240407-B	63.89±0.88^{j-p}	22.13±1.52^{n-q}	76.63±0.17^{d}	217.70±4.48^{o}	39.25±0.70^{ef}
240407-G	62.62±1.89^{k-p}	30.61±0.02^{g-j}	31.05±2.27^{pq}	325.09±0.86^{c}	26.08±1.80^{lmn}
KICHI	63.94±0.21^{j-p}	17.78±0.06^{r}	60.77±0.83^{ghi}	256.76±1.39^{j}	34.83±1.50^{gh}
KICHI-1	91.03±0.33^{ab}	32.04±0.70^{f-j}	26.02±4.01^{r}	188.98±0.44^{q}	18.47±2.97^{pqr}
KUWE	58.50±1.46^{n-q}	25.63±0.21^{lmn}	63.12±0.32^{efg}	278.44±3.49^{h}	42.58±0.76^{cde}
Mean	67.38	29.5	51.46	223.19	28.77

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex I- Minor mineral elements concentration (mg/100g dry basis) of Anchote tubers for 44 accessions

Accessions	Fe	Cu	Zn	Mn	B
90801	3.03±0.13^{f-j}	0.62±0.00^{o-r}	0.71±0.00^{de}	1.27±0.01^{mn}	2.34±0.04^{x}
90802	6.03±0.25^{b}	0.97±0.03^{e}	0.36±0.01^{jk}	1.64±0.02^{cd}	2.80±0.04^{s}
90802-1	2.42±0.32^{f-j}	0.47±0.00^{t}	0.51±0.02^{e-j}	1.04±0.01^{x}	4.15±0.08^{j}
207984	3.77±0.01^{d-g}	1.07±0.00^{d}	0.45±0.03^{g-k}	1.04±0.01^{wx}	4.74±0.03^{g}
223085	1.64±0.07^{j}	0.65±0.00^{l-o}	0.35±0.02^{k}	1.66±0.03^{c}	3.70±0.08^{k}
223086	2.71±0.31^{f-j}	0.59±0.00^{qr}	0.53±0.03^{e-k}	1.68±0.04^{c}	4.95±0.18^{f}
223087-1	1.58±0.00^{j}	0.72±0.00^{ij}	0.35±0.02^{k}	1.48±0.03^{f}	3.69±0.02^{kl}
223088	3.23±0.18^{e-j}	0.69±0.00^{j-m}	0.66±0.02^{d-h}	1.33±0.00^{ijk}	3.05±0.06^{q}
223090	3.02±0.18^{f-j}	0.70±0.00^{ijk}	1.39±0.19^{c}	0.97±0.01^{z}	2.19±0.03^{yz}
223090-1	2.41±0.07^{f-j}	0.50±0.00st	0.69±0.01^{d-g}	1.11±0.01^{t-w}	2.73±0.08^{t}
223092	18.65±4.15^{a}	0.86±0.03^{g}	0.49±0.02^{e-k}	1.25±0.00^{nop}	2.93±0.05^{qr}
223092-1	3.13±0.23^{f-j}	0.59±0.01^{pqr}	0.49±0.03^{e-k}	1.02±0.02^{xy}	3.07±0.08^{q}
223093	3.77±0.17^{d-g}	1.60±0.01^{a}	0.44±0.00^{h-k}	1.08±0.00^{u-x}	2.73±0.03^{t}
223094	4.81±0.45^{b-e}	0.60±0.00^{pqr}	0.32±0.37^{k}	1.01±0.01^{y}	2.66±0.08^{tuv}
NJ	4.86±0.23^{b-e}	0.58±0.00^{r}	0.61±0.00^{e-i}	1.85±0.02^{a}	2.62±0.06^{vw}
223096	5.05±0.26^{bcd}	1.18±0.02^{d}	0.37±0.01^{ijk}	1.21±0.00^{rst}	3.03±0.06^{q}
223097	4.08±0.01^{c-f}	0.68±0.00^{j-m}	0.69±0.01^{def}	1.67±0.01^{c}	2.83±0.08^{s}
223097-1	3.79±0.23^{d-g}	0.91±0.04^{f}	0.40±0.00^{ijk}	1.27±0.02^{mno}	2.81±0.07^{s}
223099	3.52±0.08^{d-h}	1.08±0.03^{d}	0.46±0.02^{g-k}	1.17±0.01^{rst}	2.83±0.09^{s}
223100	5.50±0.42^{bc}	0.61±0.00^{o-r}	0.47±0.00^{f-k}	1.28±0.01^{lm}	2.14±0.06^{z}
223101	3.14±0.12^{f-j}	0.63±0.03^{n-q}	0.52±0.02^{e-k}	1.08±0.01^{vwx}	3.71±0.07^{k}
223104	1.61±0.00^{j}	0.51±0.00^{s}	0.69±0.06^{def}	1.39±0.01^{gh}	3.72±0.02^{k}
223105	3.39±0.02^{e-h}	0.73±0.00^{hi}	0.50±0.00^{e-k}	1.24±0.00^{pq}	3.30±0.03^{p}
223105-1	1.65±0.00^{j}	0.64±0.00^{m-p}	0.47±0.05^{f-k}	1.16±0.01^{stu}	3.56±0.09^{no}
223109-1	3.34±0.30^{e-i}	1.21±0.03^{c}	0.43±0.03^{h-k}	1.72±0.02^{b}	4.67±0.09^{h}
223110	3.80±0.15^{d-g}	0.70±0.00^{i-l}	0.44±0.02^{h-k}	0.99±0.01^{yz}	3.61±0.06^{mn}
223112	1.72±0.15^{ij}	0.61±0.00^{o-r}	2.79±0.35^{b}	1.37±0.01^{ghi}	3.60±0.08^{mn}
223112-1	5.61±0.06^{bc}	0.99±0.04^{e}	0.60±0.00^{e-j}	1.19±0.02^{rst}	7.04±0.04^{a}
223113	3.07±0.13^{f-j}	0.53±0.02^{s}	0.53±0.02^{e-k}	1.29±0.01^{lm}	5.88±0.12^{b}
229702	3.47±0.06^{d-h}	0.60±0.01^{pqr}	0.54±0.01^{e-k}	1.23±0.01^{rs}	5.52±0.18^{d}
229702-1	2.78±0.06^{f-j}	0.68±0.00^{j-m}	0.55±0.01^{e-k}	1.40±0.01^{gh}	5.47±0.03^{de}
220563	5.66±1.03^{b}	0.53±0.00^{s}	0.35±0.04^{k}	1.25±0.02^{opq}	5.51±0.09^{d}
220563-1	2.93±0.08^{f-j}	0.76±0.05^{h}	0.37±0.02^{jk}	1.36±0.00^{hij}	5.67±0.05^{c}
DIGGA	3.65±0.32^{d-h}	0.68±0.05^{j-m}	0.44±0.01^{h-k}	1.23±0.02^{qr}	5.86±0.05^{b}
DIGGA-1	3.66±0.00^{d-h}	0.50±0.00st	0.55±0.00^{e-k}	1.32±0.01^{jk}	4.26±0.05^{i}
DIGGA-2	2.86±0.09^{f-j}	0.58±0.04^{r}	0.49±0.00^{e-k}	1.13±0.00^{s-v}	3.64±0.06^{lm}
230565	3.33±0.48^{e-i}	1.29±0.02^{b}	0.47±0.02^{f-k}	1.14±0.01^{s-v}	3.54±0.08^{no}
230566	2.13±0.17^{g-j}	0.70±0.03^{i-l}	0.50±0.01^{e-k}	1.12±0.00^{s-v}	3.05±0.09^{q}
240407-1	2.44±0.11^{f-j}	0.67±0.00^{k-n}	0.43±0.02^{h-k}	1.11±0.01^{s-v}	2.99±0.04^{qr}
240407-B	3.41±0.08^{e-h}	0.58±0.00^{r}	0.43±0.01^{h-k}	1.46±0.03^{g}	2.58±0.07^{w}
240407-G	3.38±0.03^{e-h}	0.67±0.00^{k-n}	3.41±0.33^{a}	1.37±0.02^{hi}	2.51±0.0^{w}
KICHI	3.07±0.45^{f-j}	0.62±0.00^{o-r}	0.44±0.00^{h-k}	1.15±0.01^{stu}	2.72±0.06^{tu}
KICHI-1	2.07±0.18^{hij}	0.50±0.00st	0.86±0.02^{d}	1.57±0.01^{e}	2.23±0.04^{yz}
KUWE	1.59±0.24^{j}	0.63±0.00^{o-r}	0.53±0.00^{e-k}	1.32±0.00^{kl}	2.68±0.06^{tuv}
Mean	3.65	0.73	0.64	1.29	3.62

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex J- Major mineral elements concentration (mg/100g dry basis) of Anchote leaves for 44 accessions

Accessions	Na	P	K	Ca	Mg
90801	44.16±1.93^{pq}	62.22±2.77^{j-n}	114.99±2.50^{s}	110.34±2.64^{rs}	37.13±0.83^{mn}
90802	58.62±2.14^{g-j}	63.16±0.22^{h-m}	114.83±0.02^{s}	138.54±9.36^{j-o}	46.66±2.82^{ghi}
90802-1	43.12±0.00^{q}	67.55±0.86^{d-j}	157.78±0.62^{cd}	125.15±1.56^{o-r}	49.68±0.37^{efg}
207984	51.13±0.52^{k-o}	75.21±1.56^{abc}	163.95±0.84^{b}	132.63±5.44^{l-q}	49.79±0.03^{efg}
223085	53.26±0.88^{j-n}	60.28±0.00^{k-o}	125.86±0.51^{opq}	98.39±2.07st	44.56±0.25^{g-j}
223086	55.41±0.49^{i-m}	77.75±5.85^{ab}	123.11±3.07^{qr}	123.80±0.02^{o-r}	52.32±1.03^{def}
223087-1	92.24±14.12^{a}	72.46±4.45^{b-f}	149.30±1.10^{ghi}	172.15±9.84^{fg}	48.46±0.27^{e-h}
223088	59.21±1.05^{g-j}	57.72±0.92^{l-q}	130.44±1.17^{no}	152.14±7.88^{h-k}	70.65±7.47^{a}
223090	56.01±0.47^{i-m}	74.37±1.63^{a-d}	124.56±1.61^{pq}	119.10±6.25^{qr}	44.94±2.33^{g-j}
223090-1	69.99±1.26^{de}	54.22±1.71^{opq}	128.02±0.87^{opq}	96.76±2.61st	35.92±1.38^{mn}
223092	51.61±0.55^{k-o}	60.26±0.40^{k-o}	128.94±0.79^{op}	94.49±2.26^{tu}	40.24±2.90^{j-m}
223092-1	65.10±2.23^{d-g}	58.76±2.36^{l-p}	146.55±0.13^{ij}	128.58±3.65^{m-q}	30.63±0.11^{o}
223093	60.96±0.82^{ghi}	58.33±0.00^{l-p}	155.95±2.10^{c-f}	101.84±3.30st	31.84±0.09^{no}
223094	53.26±2.14^{j-n}	71.88±0.58^{b-f}	108.05±1.67^{rs}	143.12±7.54^{i-m}	44.71±3.28^{g-j}
NJ	63.43±2.05^{fgh}	67.99±4.03^{d-j}	152.57±2.23^{c-h}	124.96±0.57^{o-r}	32.00±0.04^{no}
223096	56.63±1.50^{i-l}	67.54±0.59 $^{d-j}$	108.50±3.39^{t}	137.33±0.84^{k-p}	46.47±0.03^{ghi}
223097	67.68±0.36^{def}	69.68±5.82^{c-h}	157.88±1.01^{c}	180.93±0.51^{ef}	43.07±0.73^{h-k}
223097-1	47.84±0.58^{n-q}	67.36±2.48^{e-j}	122.59±1.73^{qr}	100.09±5.21st	39.07±2.15^{klm}
223099	47.22±0.14^{n-q}	74.09±0.61^{a-e}	156.76±0.74^{cde}	186.10±3.52^{c-f}	53.83±0.17^{cde}
223100	49.72±4.30^{m-p}	57.42±0.77^{m-q}	130.69±1.34^{no}	147.33±1.93^{i-l}	52.21±0.49^{def}
223101	79.18±0.14^{c}	75.18±3.51^{abc}	175.43±0.44^{a}	189.68±14.20^{b-e}	55.87±0.31^{bcd}
223104	50.70±1.86^{k-p}	73.43±5.79^{a-e}	157.54±0.20^{cd}	226.93±1.00^{a}	53.48±0.88^{cde}
223105	64.58±0.98^{d-g}	71.78±8.60^{b-f}	157.19±1.60^{cde}	155.32±4.70^{hi}	39.81±0.67^{j-m}
223105-1	30.45±1.96^{r}	57.90±1.66^{l-q}	148.47±1.09^{hi}	175.97±6.60^{efg}	37.79±0.06^{lm}
223109-1	86.29±3.75^{b}	73.87±0.52^{a-e}	127.00±0.54^{opq}	64.10±1.12^{v}	48.47±8.56^{e-h}
223110	63.67±4.76^{efg}	70.07±5.59^{c-g}	167.97±0.90^{b}	165.49±8.69^{gh}	57.47±1.92^{bc}
223112	49.64±2.75^{m-p}	63.13±5.62^{h-m}	151.77±0.01^{d-i}	196.53±6.00^{bcd}	46.93±0.05^{f-i}
223112-1	50.26±3.12^{l-p}	79.15±1.57^{a}	178.91±3.94^{a}	156.26±4.82^{hi}	47.06±0.61^{f-i}
223113	30.64±1.24^{r}	62.81±0.58^{i-m}	151.33±1.04^{e-i}	187.26±2.52^{b-f}	47.83±0.84^{f-i}
229702	32.17±1.34^{r}	58.77±0.00^{l-p}	150.57±0.41^{f-i}	200.69±4.12^{bc}	45.92±0.72^{ghi}
229702-1	70.13±0.44^{d}	65.99±1.56^{f-k}	154.08±0.71^{c-h}	183.13±2.36^{def}	36.12±0.49^{mn}
220563	52.78±2.57^{j-n}	69.01±1.04^{c-i}	154.78±0.18^{c-g}	218.55±5.61^{a}	47.06±0.72^{f-i}
220563-1	33.36±0.21^{r}	66.28±0.40^{f-k}	154.59±1.38^{c-g}	226.95±4.09^{a}	46.04±0.35^{ghi}
DIGGA	45.23±2.81^{opq}	53.65±0.03^{opq}	131.27±8.84^{mno}	81.44±14.29^{u}	32.37±3.00^{no}
DIGGA-1	47.56±1.05^{n-q}	42.46±0.12^{r}	140.25±3.51^{kl}	125.36±17.16^{o-r}	42.74±3.24^{i-l}
DIGGA-2	53.65±3.14^{j-n}	63.65±3.14^{g-m}	153.83±0.19^{c-h}	201.85±4.99^{b}	49.95±0.79^{efg}
230565	63.81±0.71^{d-g}	58.63±2.85^{l-p}	142.68±5.41^{jk}	153.15±12.41^{hij}	29.72±0.29^{o}
230566	49.32±0.39^{m-q}	53.15±0.59^{pq}	107.72±1.50^{t}	127.34±0.57^{n-q}	36.03±0.06^{mn}
240407-1	50.99±1.27^{k-o}	64.42±0.31^{g-l}	124.46±2.82^{pq}	122.88±6.59^{o-r}	45.73±1.15^{ghi}
240407-B	57.20±0.39^{h-k}	70.00±0.34^{c-g}	136.36±2.50^{lm}	124.71±9.17^{o-r}	60.17±4.30^{b}
240407-G	51.77±0.40^{k-o}	51.30±0.03^{q}	118.31±2.26^{rs}	151.64±7.64^{h-k}	48.52±1.58^{e-h}
KICHI	52.74±1.65^{j-n}	56.86±1.29^{m-q}	117.68±3.94^{rs}	142.04±14.09^{i-n}	46.87±3.22^{f-i}
KICHI-1	32.00±0.74^{r}	69.32±2.02^{c-i}	135.37±6.79l^{mn}	190.52±0.14^{b-e}	48.90±0.08^{efg}
KUWE	51.29±0.64^{k-o}	55.43±1.73^{n-q}	113.17±0.19st	121.50±1.82^{pqr}	36.79±0.33^{mn}
Mean	54.46	64.63	139.82	147.8	45.04

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex K- Minor mineral elements concentration (mg/100g dry basis) of Anchote leaves for 44 accessions

Accessions	Fe	Cu	Zn	Mn	B
90801	2.98±0.47^{q-t}	0.80±0.08^{no}	4.36±0.02^{b}	1.25±0.04^{ef}	10.75±0.05^{a*}
90802	7.12±1.12^{ij}	0.68±0.08^{p-s}	4.18±0.02^{c}	1.20±0.02^{fg}	9.60±0.17^{b}
90802-1	3.78±0.00^{m-s}	1.47±0.00^{d}	0.73±0.02^{o-s}	0.26±0.02^{v}	6.65±0.12^{d}
207984	2.36±0.06st	0.99±0.00^{kl}	3.16±0.00^{h}	1.68±0.08^{d}	5.42±0.08^{i}
223085	11.56±0.02^{ef}	0.56±0.00^{tu}	3.02±0.13^{i}	0.87±0.02^{mno}	6.38±0.17^{de}
223086	6.70±0.22^{jk}	0.61±0.04st	3.93±0.03^{d}	1.13±0.03^{ghi}	5.18±0.09^{i}
223087-1	5.34±0.48^{klm}	2.18±0.00^{a}	2.19±0.00^{m}	0.87±0.11^{mno}	7.64±0.16^{c}
223088	10.51±0.11^{fg}	0.76±0.02^{op}	3.49±0.01^{g}	1.00±0.01^{jkl}	4.83±0.06^{op}
223090	4.83±0.40^{l-p}	0.72±0.04^{o-r}	4.08±0.01^{c}	1.18±0.07^{fgh}	5.47±0.06^{i}
223090-1	44.27±1.11^{a}	1.08±0.01^{h-k}	0.68±0.00^{p-t}	ND	3.49±0.10^{a}
223092	5.31±0.59^{klm}	0.69±0.07^{p-s}	3.69±0.01^{ef}	1.07±0.02^{hij}	3.84±0.09^{y}
223092-1	8.21±0.25^{hi}	1.12±0.03^{ghi}	0.70±0.00^{p-t}	ND	4.98±0.01^{mn}
223093	16.15±0.10^{bc}	1.00±0.14^{jkl}	0.70±0.02^{p-t}	0.12±0.01^{vw}	4.25±0.09^{uv}
223094	11.44±2.58^{ef}	0.56±0.16^{tu}	3.63±0.01^{ef}	1.05±0.05^{ijk}	4.49±0.04^{r}
NJ	17.02±0.60^{b}	2.10±0.06^{ab}	0.85±0.01^{o}	0.40±0.01^{u}	4.40±0.06^{s}
223096	4.14±0.03^{l-r}	0.81±0.01^{no}	3.56±0.05^{fg}	1.02±0.04^{jkl}	4.33±0.05^{t}
223097	3.45±0.16^{q-t}	0.65±0.00^{qrst}	0.63±0.01^{rst}	2.06±0.12^{b}	4.97±0.09^{mn}
223097-1	6.54±0.02^{jk}	0.49±0.01^{u}	2.74±0.07^{j}	0.79±0.02^{opq}	3.64±0.05^{z}
223099	4.52±0.01^{l-q}	1.03±0.00^{i-l}	0.72±0.04^{o-s}	2.44±0.08^{a}	4.87±0.06^{no}
223100	12.25±0.07^{de}	0.64±0.08^{rst}	2.54±0.08^{k}	0.73±0.04^{pqr}	3.36±0.07^{b}
223101	9.31±1.24^{gh}	2.06±0.00^{b}	0.77±0.02^{o-r}	1.62±0.07^{d}	5.96±0.06^{f}
223104	8.74±1.69^{h}	0.95±0.00^{lm}	0.81±0.04^{op}	0.25±0.01^{v}	5.12±0.07^{lm}
223105	8.51±0.32^{hi}	1.10±0.00^{hij}	0.72±0.01^{o-s}	0.81±0.03^{nop}	4.24±0.06^{uv}
223105-1	3.21±0.08^{q-t}	0.95±0.00^{lm}	0.48±0.04^{u}	0.45±0.02^{u}	5.34±0.17^{j}
223109-1	45.18±0.15^{a}	1.17±0.00^{fgh}	0.82±0.03^{op}	1.89±0.18^{c}	4.42±0.06^{rs}
223110	2.85±0.23^{rst}	1.33±0.00^{e}	0.73±0.06^{o-s}	0.80±0.04^{nop}	5.65±0.03^{h}
223112	3.03±0.40^{qrst}	1.21±0.00^{f}	0.65±0.01^{q-t}	0.94±0.06^{klm}	5.08±0.06^{lm}
223112-1	3.69±0.33^{n-s}	1.49±0.00^{d}	0.79±0.00^{opq}	0.72±0.04^{pqr}	5.26±0.08^{k}
223113	1.85±0.01^{t}	1.22±0.00^{fg}	0.56±0.01^{tu}	0.69±0.02^{pqrs}	4.69±0.09^{q}
229702	3.55±0.14^{o-s}	1.69±0.00^{fg}	0.62±0.00^{rst}	0.63±0.03^{rs}	3.95±0.10^{wx}
229702-1	13.48±0.62^{d}	0.86±0.05^{mn}	0.69±0.02^{p-t}	0.20±0.02^{vw}	6.32±0.09^{e}
220563	3.24±0.29^{q-t}	1.10±0.00^{ijk}	0.70±0.00^{p-t}	0.59±0.01st	5.04±0.05^{lm}
220563-1	2.37±0.13st	1.32±0.00^{e}	0.69±0.01^{p-t}	0.72±0.03^{pqr}	2.64±0.04^{d}
DIGGA	5.45±0.38^{kl}	0.47±0.03^{u}	1.58±0.02^{n}	0.91±0.03^{lmn}	5.86±0.22^{g}
DIGGA-1	8.50±0.16^{hi}	0.56±0.00^{tu}	2.34±0.15^{l}	0.68±0.03^{qrs}	4.84±0.09^{op}
DIGGA-2	2.85±0.35^{rst}	1.00±0.00^{jkl}	0.69±0.02^{p-t}	1.72±0.08^{d}	4.96±0.08^{mn}
230565	14.99±0.08^{c}	1.00±0.03^{jkl}	0.65±0.01^{q-t}	0.50±0.01^{tu}	4.51±0.16^{r}
230566	5.17±0.01^{k-n}	0.64±0.01^{rst}	3.74±0.07^{e}	1.08±0.05^{hij}	4.01±0.07^{w}
240407-1	11.13±0.59^{ef}	0.56±0.02^{tu}	2.65±0.03^{jk}	0.76±0.01^{opq}	4.90±0.16^{n}
240407-B	13.29±1.87^{d}	0.50±0.04^{u}	2.60±0.05^{k}	0.71±0.02^{pqr}	4.20±0.11^{uv}
240407-G	5.11±0.33^{k-o}	0.77±0.00^{op}	4.68±0.31^{a}	1.35±0.00^{e}	4.29±0.08^{tu}
KICHI	6.42±0.57^{jk}	0.66±0.04^{q-t}	3.71±0.04^{e}	1.07±0.00^{hij}	3.81±0.08^{y}
KICHI-1	2.20±0.09st	1.06±0.00^{ijk}	0.61±0.02^{stu}	0.67±0.03^{qrs}	3.91±0.08^{x}
KUWE	2.98±0.04^{q-t}	0.75±0.05^{opq}	4.09±0.02^{c}	1.18±0.06^{fgh}	3.27±0.09^{c}
Mean	8.54	0.99	1.95	0.95	5.02

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex L- Concentrations of toxic heavy metals in tubers of 44 Anchote accessions (dry basis)

Accessions	Cd (ng/g DM)	As (ng/g DM)	Pb (ng/g DM)
90801	0.74±0.17 $^{i-l}$	1.59±0.11 ^{c}	4.80±0.62 ^{lm}
90802	1.52±0.13 ^{ef}	1.87±0.08 ^{a}	9.07±0.22 ^{g}
90802-1	2.24±0.09 ^{c}	0.93±0.04 ^{hi}	23.82±0.82 ^{d}
207984	1.36±0.16 ^{f}	0.79±0.10 ^{jk}	12.07±0.60 ^{f}
223085	6.26±0.39 ^{a}	1.55±0.08 ^{c}	26.02±1.04 ^{c}
223086	2.68±0.21 ^{b}	1.70±0.07 ^{b}	30.26±1.35 ^{b}
223087-1	1.22±0.16 ^{g}	0.61±0.03 ^{mno}	5.66±0.61 ^{k}
223088	1.66±0.09 ^{de}	0.68±0.08 ^{lm}	14.25±0.49 ^{e}
223090	0.90±0.04 ^{hi}	0.51±0.03 $^{o-r}$	5.75±0.40 ^{jk}
223090-1	0.56±0.03 $^{l-s}$	0.67±0.10 ^{mn}	4.68±0.23 ^{m}
223092	0.79±0.07 $^{h-k}$	0.87±0.06 ^{ij}	5.77±0.38 ^{jk}
223092-1	0.16±0.10 ^{vw}	0.78±0.10 ^{jkl}	5.98±0.30 ^{j}
223093	0.64±0.47 $^{j-p}$	0.79±0.04 ^{j}	8.77±1.59 ^{h}
223094	0.84±0.18 ^{hij}	0.49±0.06 ^{pqr}	3.91±0.90 ^{no}
NJ	1.78±0.74 ^{d}	1.46±0.09 ^{d}	62.60±2.24 ^{a}
223096	0.32±0.06 ^{uv}	0.77±0.07 ^{jkl}	6.61±0.42 ^{i}
223097	0.64±0.08 $^{k-p}$	1.35±0.07 ^{e}	2.65±0.09 ^{t}
223097-1	0.07±0.23 ^{w}	0.57±0.27 $^{n-q}$	2.98±0.60 ^{rs}
223099	0.52±0.03 $^{m-t}$	1.04±0.07 ^{g}	1.49±0.13 ^{x}
223100	0.63±0.04 $^{k-p}$	1.56±0.15 ^{c}	5.95±0.25 ^{j}
223101	0.38±0.07 ^{stu}	0.66±0.05 ^{mn}	1.87±0.20 ^{vw}
223104	0.60±0.06 $^{l-q}$	1.22±0.09 ^{f}	4.10±0.07 ^{n}
223105	0.69±0.13 $^{j-m}$	0.69±0.18 ^{lmk}	2.29±0.49 ^{u}
223105-1	0.51±0.09 $^{m-u}$	0.93±0.07 ^{hi}	2.49±0.35 ^{tu}
223109-1	0.40±0.02 $^{q-u}$	0.82±0.11 ^{j}	3.13±0.20 ^{r}
223110	0.33±0. 06 ^{uvt}	0.68±0.14 ^{lm}	2.89±0.15 ^{s}
223112	0.67±0.14 $^{j-n}$	0.16±0.21 ^{t}	3.72±0.95 ^{op}
223112-1	0.47±0.04 $^{n-u}$	1.00±0.06 ^{gh}	3.56±0.10 ^{pq}
223113	0.47±0.06 $^{n-u}$	0.45±0.05 ^{r}	1.65±0.07 ^{wx}
229702	0.97±0.16 ^{h}	0.48±0.08 ^{qr}	0.24±0.30 ^{b}
229702-1	0.40±0.10 $^{q-u}$	0.56±0.06 ^{opq}	0.82±0.32 ^{z}
220563	0.46±0.05 $^{o-u}$	0.59±0.04 $^{m-p}$	2.32±0.17 ^{u}
220563-1	0.65±0.05 $^{j-o}$	0.82±0.04 ^{j}	3.20±0.12 ^{r}
DIGGA	0.60±0.07 $^{k-p}$	0.91±0.09 ^{hi}	1.11±0.09 ^{y}
DIGGA-1	0.58±0.06 $^{l-r}$	1.21±0.04 ^{f}	0.09±0.04 ^{b}
DIGGA-2	0.49±0.11 $^{m-u}$	0.27±0.06 ^{s}	1.91±0.06 ^{v}
230565	0.50±0.07 $^{m-u}$	0.80±0.03 ^{j}	2.61±0.12 ^{t}
230566	0.51±0.05 $^{m-u}$	0.61±0.05 ^{mno}	4.83±0.19 ^{lm}
240407-1	0.64±0.09 $^{k-p}$	0.61±0.07 ^{mno}	2.60±0.19 ^{t}
240407-B	0.46±0.07 $^{p-u}$	0.48±0.08 ^{qr}	0.55±0.11 ^{a}
240407-G	0.38±0.05 $^{r-u}$	0.93±0.16 ^{hi}	3.82±0.18 ^{o}
KICHI	0.35±0.06 ^{tu}	0.44±0.06 ^{r}	8.99±0.31 ^{g}
KICHI-1	0.51±0.10 $^{m-u}$	0.19±0.44 st	4.94±2.73 ^{l}
KUWE	0.57±0.06 $^{l-s}$	0.59±0.08 $^{m-p}$	3.45±0.14 ^{q}

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex M- Concentrations of toxic heavy metals in leaves of 44 Anchote accessions (dry basis)

Accessions	Cd (ng/g DM)	As (ng/g DM)	Pb (ng/g DM)
90801	1.06±0.05 $^{g-l}$	2.89±0.16 $^{f-i}$	13.13±0.42 ^{i}
90802	0.79±0.07 $^{j-m}$	2.45±0.30 $^{i-o}$	9.79±0.88 ^{pqr}
90802-1	0.94±0.06 $^{j-m}$	2.85±0.20 $^{f-j}$	10.40±0.28 ^{nop}
207984	0.96±0.10 $^{i-m}$	2.64±0.15 $^{g-l}$	10.55±0.86 ^{mno}
223085	0.90±0.11 $^{j-m}$	2.51±0.14 $^{h-n}$	11.17±0.19 ^{klm}
223086	1.40±0.06 ^{fgh}	2.34±0.12 $^{k-p}$	15.03±0.87 ^{h}
223087-1	1.07±0.09 $^{g-l}$	1.99±0.14 $^{o-t}$	10.97±0.51 $^{k-n}$
223088	0.83±0.09 $^{j-m}$	1.68±0.16 st	10.08±0.41 ^{opq}
223090	1.01±0.19 $^{h-m}$	2.99±0.43 ^{fg}	13.68±1.43 ^{i}
223090-1	0.96±0.20 $^{i-m}$	6.90±1.14 ^{a}	16.26±2.53 ^{g}
223092	1.60±0.19 ^{def}	2.06±0.44 $^{n-t}$	8.07±1.62 ^{u}
223092-1	1.04±0.08 $^{g-m}$	4.78±0.10 ^{b}	14.67±0.58 ^{h}
223093	1.01±0.10 $^{h-m}$	2.78±0.16 $^{f-k}$	12.33±0.13 ^{j}
223094	1.02±0.08 $^{h-m}$	2.57±0.19 $^{g-m}$	13.32±0.46 ^{i}
NJ	1.11±0.14 $^{g-k}$	1.82±0.11 $^{q-t}$	13.19±0.26 ^{i}
223096	0.73±0.07 $^{j-m}$	1.78±0.13 ^{rst}	10.44±0.56 $^{m-p}$
223097	0.77±0.16 $^{j-m}$	2.02±0.56 $^{o-t}$	10.53±2.58 ^{mno}
223097-1	1.13±0.04 $^{g-j}$	2.16±0.06 $^{m-r}$	10.71±0.09 $^{l-o}$
223099	0.90±0.10 $^{j-m}$	3.51±0.04 ^{de}	9.57±0.15 ^{qrs}
223100	1.44±0.06 ^{efg}	3.12±0.15 ^{ef}	18.65±0.76 ^{d}
223101	0.91±0.05 $^{j-m}$	3.09±0.24 ^{ef}	9.21±0.35 ^{rst}
223104	1.07±0.22 $^{g-l}$	2.63±0.49 $^{g-l}$	8.67±1.44 ^{tu}
223105	1.73±0.34 ^{def}	3.62±0.75 ^{d}	16.98±3.09 ^{f}
223105-1	0.85±0.09 $^{j-m}$	2.94±0.12 ^{fgh}	8.14±0.21 ^{u}
223109-1	0.96±0.05 $^{i-m}$	3.14±0.11 ^{ef}	9.08±0.31 st
223110	0.76±0.10 $^{j-m}$	1.71±0.13 ^{rst}	5.68±0.18 ^{x}
223112	1.08±0.13 $^{g-k}$	2.10±0.36 $^{m-s}$	11.36±1.30 ^{kl}
223112-1	0.72±0.08 $^{j-n}$	2.56±0.25 $^{g-m}$	5.43±0.30 ^{x}
223113	0.33±0.15 ^{n}	0.49±0.63 ^{u}	7.11±1.41 ^{v}
229702	0.65±0.14 ^{lmn}	1.74±0.36 ^{rst}	4.07±0.90 ^{y}
229702-1	1.82±0.09 ^{de}	1.90±0.11 $^{p-t}$	6.39±0.44 ^{w}
220563	0.63±0.07 ^{mn}	2.02±0.10 $^{o-t}$	6.53±0.49 ^{vw}
220563-1	0.75±0.16 $^{j-m}$	2.55±0.50 $^{g-m}$	10.17±1.81 ^{opq}
DIGGA	0.68±0.15 $^{k-n}$	2.57±0.19 $^{g-m}$	7.97±0.27 ^{u}
DIGGA-1	0.69±0.06 $^{k-n}$	4.55±0.20 ^{b}	4.23±0.22 ^{y}
DIGGA-2	1.91±0.08 ^{d}	2.16±0.10 $^{m-r}$	17.10±1.02 ^{f}
230565	1.37±0.04 $^{f-i}$	2.70±0.05 $^{f-l}$	12.20±0.63 ^{j}
230566	7.04±0.27 ^{a}	2.39±0.15 $^{j-o}$	95.41±3.86 ^{a}
240407-1	1.85±0.10 ^{d}	2.14±0.15 $^{m-s}$	11.49±0.44 ^{k}
240407-B	3.16±0.15 ^{b}	2.29±0.16 $^{l-q}$	27.17±1.06 ^{c}
240407-G	1.73±0.12 ^{def}	4.06±0.30 ^{c}	17.80±1.26 ^{e}
KICHI	2.70±0.18 ^{c}	2.51±0.02 $^{h-n}$	28.96±0.79 ^{b}
KICHI-1	1.56±0.11 ^{def}	1.62±0.10 ^{t}	10.80±0.18 $^{k-o}$
KUWE	1.09±0.09 $^{g-k}$	2.03±0.21 $^{o-t}$	10.99±0.52 $^{k-n}$

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex N- Molar ratios of phytate to calcium, iron, zinc and phytate x calcium: zinc in tubers of 44 Anchote accessions

Accessions	Phytate: Calcium	Phytate: Iron	Phytate: Zinc	Phytate x Calcium/Zinc
90801	0.05±0.00^{jk}	4.88±0.23^{fgh}	24.23±0.22^{ghi}	141.87±1.42^{i}
90802	0.16±0.01^{b}	3.01±0.06^{l-p}	58.70±2.05^{b}	122.40±7.01^{ijk}
90802-1	0.08±0.00^{f}	5.21±0.88^{fg}	28.80±2.47^{efg}	84.89±6.56^{lm}
207984	0.11±0.01^{d}	3.40±0.17^{j-n}	33.16±0.27^{e}	66.73±0.00^{m-p}
223085	0.18±0.00^{a}	16.32±0.62^{a}	89.54±5.65^{a}	235.79±17.36^{cd}
223086	0.06±0.00^{h}	10.21±1.43^{b}	60.91±2.33^{b}	481.36±22.77^{a}
223087-1	0.07±0.00^{gh}	7.42±0.54^{d}	39.39±0.50^{cd}	122.34±2.10^{ijk}
223088	0.03±0.00^{m-q}	5.13±0.73^{fg}	29.12±1.81^{ef}	249.23±14.73^{c}
223090	0.04±0.00^{l-o}	3.99±0.15^{h-l}	10.16±0.42^{mno}	59.62±2.40^{m-q}
223090-1	0.03±0.00^{opq}	4.47±0.45^{ghi}	18.23±1.57^{jk}	113.52±8.82^{jk}
223092	0.09±0.01^{e}	0.66±0.08^{uv}	28.85±1.87^{efg}	71.45±4.56^{l-o}
223092-1	0.02±0.00^{rst}	3.18±0.09 $^{k-o}$	23.72±0.97^{hi}	180.78±10.61^{fgh}
223093	0.13±0.00^{c}	4.02±0.02^{h-l}	40.54±1.59^{cd}	85.23±9.34^{lm}
223094	0.04±0.00^{l-o}	2.54±0.42^{n-r}	24.94±1.09^{f-i}	145.55±3.01^{i}
NJ	0.02±0.00st	1.99±0.27^{p-s}	18.38±1.69^{jk}	142.56±12.47^{i}
223096	0.08±0.00^{e}	2.74±0.03^{m-q}	43.91±0.78^{c}	129.37±7.69^{ij}
223097	0.04±0.00^{m-p}	3.85±0.20^{i-l}	26.53±1.58^{fgh}	205.74±10.17^{ef}
223097-1	0.07±0.00^{fg}	2.39±0.15^{n-r}	26.29±0.13^{fgh}	59.90±1.78^{m-q}
223099	0.04±0.00^{jkl}	2.12±0.02^{p-s}	19.03±0.10^{jk}	58.76±0.17^{m-q}
223100	0.03±0.00^{pqr}	2.11±0.00^{p-s}	28.69±1.97^{efg}	201.14±13.93^{efg}
223101	0.02±0.00^{t}	2.12±0.08^{p-s}	15.08±0.45^{kl}	83.93±1.59^{lm}
223104	0.06±0.00^{i}	6.47±0.56^{e}	17.67±0.06^{jk}	59.56±0.13^{m-q}
223105	0.01±0.00^{uvw}	1.60±0.06^{r-u}	12.75±0.62^{lmn}	97.52±5.12^{kl}
223105-1	0.03±0.00^{opq}	4.09±0.04^{h-k}	17.02±1.54^{jkl}	64.99±6.53^{m-p}
223109-1	0.01±0.00^{u-x}	1.60±0.52^{r-u}	14.58±4.46^{klm}	114.64±35.40^{jk}
223110	0.02±0.00^{tu}	1.84±0.21^{q-t}	18.61±2.05^{jk}	125.46±11.87^{ij}
223112	0.07±0.01^{gh}	8.92±0.04^{c}	6.42±0.26^{opq}	26.14±0.84^{r}
223112-1	0.04±0.00^{k-n}	2.55±0.19^{n-r}	27.94±2.43^{fgh}	175.58±15.18^{hg}
223113	0.01±0.00^{vwx}	1.22±0.10^{s-v}	8.21±0.74^{opq}	55.15±5.93^{n-q}
229702	0.01±0.00^{vwx}	0.89±0.03^{tuv}	6.63±0.19^{opq}	41.59±1.17^{pqr}
229702-1	0.01±0.00^{vwx}	1.15±0.10^{s-v}	6.74±0.38^{opq}	45.44±1.85^{o-r}
220563	0.04±0.00^{k-n}	2.12±0.50^{p-s}	39.10±6.30^{d}	202.26±31.77^{efg}
220563-1	0.01±0.00^{uv}	2.30±0.10^{o-r}	21.09±0.66^{ij}	195.85±6.87^{e-h}
DIGGA	0.01±0.00^{wx}	0.70±0.40^{uv}	6.67±3.37^{opq}	53.08±27.52^{n-q}
DIGGA-1	0.01±0.00^{x}	0.47±0.02^{v}	3.69±0.16^{q}	22.22±0.75^{r}
DIGGA-2	0.04±0.00^{klm}	5.76±0.41^{ef}	39.36±1.85^{cd}	279.69±12.24^{b}
230565	0.07±0.01^{fg}	4.98±1.04^{fgh}	40.90±1.14^{cd}	170.73±3.63^{h}
230566	0.01±0.00^{vwx}	1.80±0.23^{q-t}	8.98±1.67^{nop}	62.18±12.24^{m-q}
240407-1	0.01±0.00^{u-x}	1.55±0.46$^{r=u}$	10.23±3.04^{mno}	57.75±16.68^{m-q}
240407-B	0.05±0.00^{j}	4.33±0.15^{ghj}	40.24±1.83^{cd}	218.45±5.43^{de}
240407-G	0.03±0.00^{qrs}	3.77±0.03$^{i=l}$	4.39±0.49^{pq}	35.65±4.04^{qr}
KICHI	0.03±0.00^{opq}	3.64±0.35^{i-m}	29.34±1.48^{ef}	188.00±10.48^{fgh}
KICHI-1	0.05±0.00^{jk}	5.86±0.34^{ef}	16.37±0.05^{kl}	77.19±0.06^{lmn}
KUWE	0.03±0.00^{n-q}	8.35±1.10^{c}	28.78±0.49^{efg}	199.99±5.89^{efg}
Mean	0.05	3.81	27.79	142.20

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex O- Molar ratios of phytate to calcium, iron, zinc and phytate x calcium: zinc in leaves of 44 Anchote accessions

Accessions	Phytate: Calcium	Phytate: Iron	Phytate: Zinc	Phytate x Calcium/Zinc
90801	0.14±0.00^{ef}	7.26±1.07^{ed}	5.75±0.09^{q}	15.83±0.12^{s}
90802	0.12±0.01^{g-j}	3.26±0.58^{k-n}	6.40±0.09^{pq}	22.12±1.18^{qrs}
90802-1	0.11±0.00^{i-m}	5.08±0.04^{gh}	30.82±0.89^{g}	96.26±3.99^{kl}
207984	0.13±0.00^{e-h}	10.13±0.33^{ab}	8.86±0.05^{mno}	29.31±1.38^{pqr}
223085	0.16±0.00^{cd}	1.88±0.02^{p-s}	8.45±0.30^{m-p}	20.73±0.29^{qrs}
223086	0.13±0.00^{e-h}	3.36±0.16^{k-n}	6.71±0.15^{opq}	20.73±0.46^{qrs}
223087-1	0.10±0.00^{l-o}	4.57±0.45^{ghi}	12.98±0.11^{k}	55.76±3.66^{n}
223088	0.10±0.01^{l-o}	2.00±0.06^{p-s}	7.04±0.12^{n-q}	26.70±0.93^{p-s}
223090	0.13±0.01^{e-i}	4.36±0.40^{hij}	6.03±0.03^{q}	17.91±0.85^{rs}
223090-1	0.16±0.00^{c}	0.50±0.01^{t}	38.01±0.23^{cd}	91.77±3.03^{kl}
223092	0.16±0.00^{c}	4.05±0.46^{ijk}	6.79±0.04^{opq}	16.00±0.30^{rs}
223092-1	0.11±0.00^{j-n}	2.31±0.09^{o-r}	31.66±0.33^{fg}	101.57±1.82^{jk}
223093	0.13±0.00^{e-h}	1.14±0.01st	30.72±1.18^{g}	78.11±5.53^{m}
223094	0.10±0.01^{k-o}	1.82±0.44^{p-s}	6.52±0.11^{pq}	23.28±0.84^{qrs}
NJ	0.11±0.00^{h-l}	1.17±0.06st	27.51±0.73^{h}	85.76±1.90^{lm}
223096	0.09±0.00^{mno}	4.36±0.04^{hij}	5.94±0.01^{q}	20.36±0.15^{qrs}
223097	0.07±0.00^{qrs}	5.18±0.33^{gh}	33.15±0.07^{ef}	149.66±0.73^{f}
223097-1	0.14±0.01^{de}	3.05±0.01^{l-o}	8.52±0.27^{m-p}	21.26±0.43^{qrs}
223099	0.06±0.00^{s}	3.58±0.00^{j-m}	26.27±1.26^{h}	121.93±3.52^{hi}
223100	0.11±0.00^{j-n}	1.79±0.03^{qrs}	10.11±0.20^{lm}	37.18±1.21^{op}
223101	0.06±0.00^{s}	1.68±0.28^{qrs}	23.44±0.24^{i}	110.98±9.43^{ij}
223104	0.06±0.00^{s}	2.29±0.42^{o-r}	28.40±1.17^{h}	160.80±5.93^{e}
223105	0.10±0.00^{k-o}	2.62±0.12^{n-q}	36.34±1.00^{d}	140.78±0.37^{fg}
223105-1	0.07±0.00^{rs}	5.35±0.08^{g}	42.37±3.60^{b}	185.73±8.82^{c}
223109-1	0.22±0.01^{a}	0.43±0.01^{t}	27.88±1.51^{h}	44.56±1.64^{o}
223110	0.09±0.00^{nop}	7.36±0.72^{ed}	33.61±3.31^{ef}	139.14±20.93^{fg}
223112	0.09±0.00^{o-r}	7.88±1.04^{d}	42.38±0.81^{b}	207.76±2.40^{b}
223112-1	0.11±0.00^{j-n}	6.26±0.64^{f}	33.92±0.62^{e}	132.28±6.51^{gh}
223113	0.07±0.00^{qrs}	9.99±0.05^{ab}	38.82±0.32^{c}	181.36±0.95^{cd}
229702	0.08±0.00^{o-r}	6.69±0.29^{ef}	44.56±0.05^{a}	223.13±4.84^{a}
229702-1	0.09±0.00^{nop}	1.72±0.06^{qrs}	39.23±0.84^{c}	179.26±6.14^{cd}
220563	0.07±0.00^{p-s}	6.96±0.48^{ef}	37.74±0.75^{cd}	205.85±9.37^{b}
220563-1	0.07±0.00^{qrs}	9.53±0.39^{bc}	38.31±0.03^{cd}	216.93±3.73^{ab}
DIGGA	0.19±0.04^{b}	3.83±0.32^{i-l}	15.38±0.02^{j}	31.25±5.45^{pq}
DIGGA-1	0.14±0.02^{efg}	2.76±0.01^{m-p}	11.75±0.95^{kl}	36.97±8.00^{op}
DIGGA-2	0.09±0.00^{npq}	8.85±1.22^{c}	42.37±1.58^{b}	213.50±13.23^{ab}
230565	0.12±0.01^{h-l}	1.66±0.04^{qrs}	44.91±1.45^{a}	171.37±8.37^{de}
230566	0.12±0.00^{gkl}	4.05±0.06^{ijk}	6.56±0.01^{pq}	20.83±0.11^{qrs}
240407-1	0.12±0.01^{f-j}	1.88±0.08^{p-s}	9.23±0.19^{mn}	28.27±0.93^{p-s}
240407-B	0.12±0.01^{h-l}	1.53±0.19^{rs}	9.08±0.01^{mn}	28.26±2.10^{p-s}
240407-G	0.11±0.01^{i-m}	4.56±0.20^{ghi}	5.84±0.26^{q}	22.10±2.08^{qrs}
KICHI	0.12±0.01^{f-j}	3.82±0.30^{i-l}	7.72±0.00^{n-q}	27.35±2.72^{p-s}
KICHI-1	0.09±0.00^{n-r}	10.64±0.40^{a}	45.05±0.94^{a}	214.17±4.64^{ab}
KUWE	0.12±0.00^{h-l}	6.58±0.20^{ef}	5.62±0.14^{q}	17.03±0.16^{rs}
Mean	0.11	4.31	22.47	90.72

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p < 0.05$).

Annex P- Concentration of phytate phosphorus, non-phytate phosphorus and phosphorus as phytate in tubers of 44 Anchote accessions

Accessions	Phytate phosphorus (mg/100g)	Non-Phytate phosphorus (mg/100g)	Phosphorus as phytate (%)
90801	48.84±0.21^{c-f}	14.66±0.81^{e-i}	1.43±0.03^{fgh}
90802	60.21±3.78^{b}	20.51±7.08^{b-f}	1.53±0.22^{e-h}
90802-1	41.21±1.64^{h-k}	12.03±1.39^{ghi}	1.41±0.04^{fgh}
207984	42.33±2.27^{g-j}	13.57±1.16^{f-i}	1.47±0.02^{fgh}
223085	88.36±0.44^{a}	56.40±0.49^{a}	2.76±0.02^{a}
223086	91.01±2.37^{a}	57.98±3.74^{a}	2.76±0.19^{a}
223087-1	38.79±2.88^{i-l}	21.28±2.82^{b-f}	2.21±0.16^{b}
223088	54.65±4.81^{c}	16.21±7.10^{e-h}	1.43±0.21^{fgh}
223090	39.92±3.91^{h-l}	10.40±3.22^{hi}	1.35±0.10^{ghi}
223090-1	35.60±2.52^{k-n}	1.63±2.31^{jkl}	1.05±0.07^{jkl}
223092	40.24±4.16^{h-l}	2.45±6.05^{jk}	1.07±0.16^{i-l}
223092-1	32.96±3.40^{mno}	13.52±1.32^{f-i}	1.70±0.01^{def}
223093	50.15±2.50^{cde}	25.75±3.15^{bc}	2.06±0.16^{bc}
223094	40.17±2.97^{h-l}	20.39±4.17^{b-f}	2.04±0.27^{bc}
NJ	31.85±2.92^{no}	8.07±0.80^{ij}	1.34±0.00^{g-j}
223096	45.70±1.96^{e-h}	11.16±2.66^{ghi}	1.32±0.08^{g-j}
223097	51.95±2.59^{cd}	21.60±2.02^{b-e}	1.71±0.05^{def}
223097-1	29.84±0.06^{op}	-1.89±1.33^{k-n}	0.94±0.04^{l}
223099	24.73±0.81^{pq}	-6.59±0.84^{m-q}	0.79±0.03^{lmn}
223100	38.34±2.96^{i-m}	16.70±6.60^{e-h}	1.81±0.44^{cde}
223101	22.04±1.64^{qr}	-14.41±1.27^{rs}	0.60±0.04^{mno}
223104	34.57±2.98^{l-o}	-11.17±7.40^{p-s}	0.76±0.14^{lmn}
223105	17.93±0.82^{rs}	-5.87±2.82^{l-p}	0.76±0.10^{lmn}
223105-1	22.37±0.27^{qr}	-15.12±1.16^{rs}	0.60±0.02^{mno}
223109-1	17.39±4.13^{rs}	-3.61±5.75^{k-o}	0.84±0.26^{lmn}
223110	23.12±1.74^{qr}	-0.95±1.07^{klm}	0.96±0.05^{kl}
223112	50.61±4.25^{cde}	1.97±3.02^{jk}	1.04±0.06^{jkl}
223112-1	47.33±3.94^{d-g}	16.34±3.91^{e-h}	1.53±0.13^{e-h}
223113	12.40±1.56st	-9.06±0.87^{n-r}	0.58±0.01^{mno}
229702	10.19±0.11^{tu}	-15.71±1.31^{rs}	0.39±0.02^{op}
229702-1	10.56±0.72^{tu}	-13.61±1.92^{qrs}	0.44±0.05^{op}
220563	38.83±2.11^{i-l}	1.66±0.76^{jkl}	1.04±0.02^{jkl}
220563-1	22.26±0.40^{qr}	-16.03±1.31^{rs}	0.58±0.02^{mno}
DIGGA	8.26±4.09^{tu}	-30.86±3.36^{t}	0.21±0.10^{p}
DIGGA-1	5.71±0.27^{u}	-17.50±2.98^{s}	0.25±0.04^{p}
DIGGA-2	54.39±2.22^{c}	20.41±2.58^{b-f}	1.60±0.08^{d-g}
230565	54.10±3.56^{c}	24.69±4.54^{bcd}	1.84±0.18^{cd}
230566	12.77±2.60st	-10.82±3.82^{o-s}	0.54±0.14^{no}
240407-1	12.44±3.10st	-1.65±1.29^{k-n}	0.88±0.11^{lm}
240407-B	48.79±0.55^{c-f}	26.66±2.06^{b}	2.21±0.18^{b}
240407-G	42.18±0.65^{g-j}	11.57±0.68^{ghi}	1.38±0.02^{gh}
KICHI	36.69±1.85^{j-n}	18.91±1.91^{c-g}	2.06±0.11^{bc}
KICHI-1	39.95±1.28^{h-l}	7.91±0.58^{ij}	1.25±0.01^{h-k}
KUWE	43.38±0.73^{f-i}	17.75±0.52^{c-g}	1.69±0.01^{def}
Mean	36.71	7.21	1.28

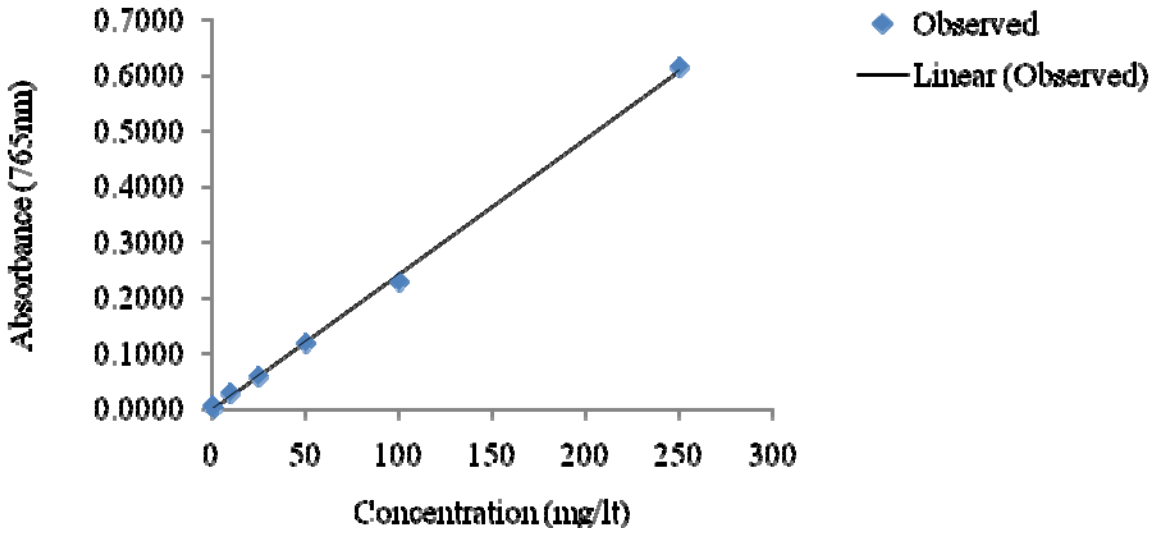
Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex Q- Concentration of phytate phosphorus, non-phytate phosphorus and phosphorus as phytate in leaves of 44 Anchote accessions

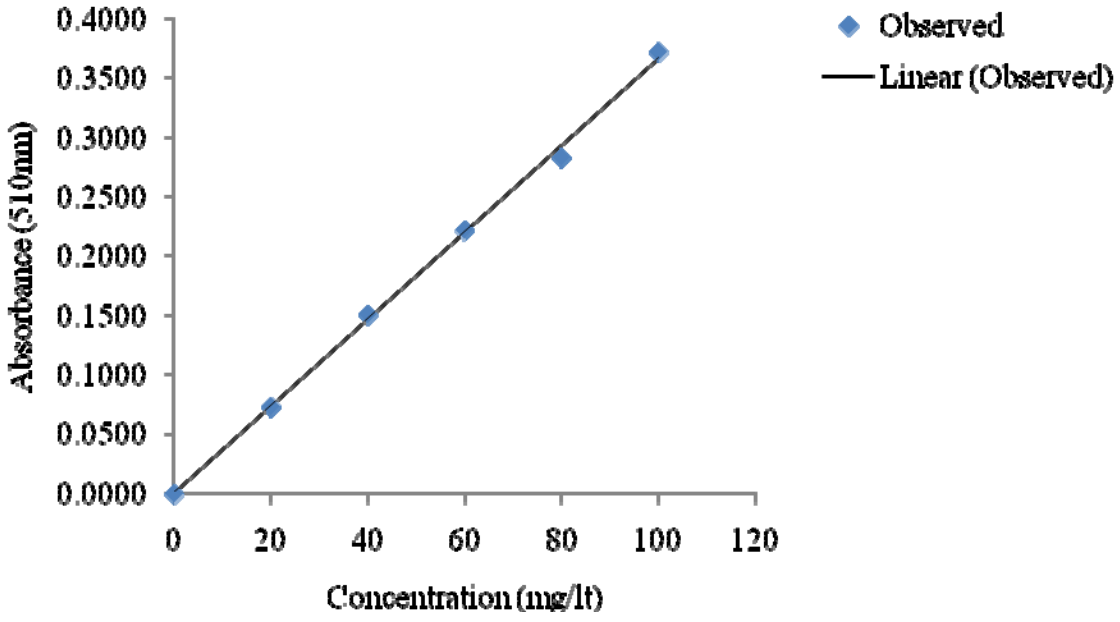
Accessions	Phytate phosphorus (mg/100g)	Non- Phytate phosphorus (mg/100g)	Phosphorus as phytate (%)
90801	61.22±2.77^{j-n}	9.60±3.51^{ei}	1.16±0.06^{h-k}
90802	63.16±0.22^{h-m}	12.47±1.33^{efg}	1.20±0.02^{f-i}
90802-1	67.55±0.86^{d-j}	-4.03±1.35^{m-r}	0.94±0.02^{o-s}
207984	75.21±1.56^{abc}	3.83±2.03^{i-l}	1.05±0.03^{lmn}
223085	60.28±0.00^{k-o}	11.71±0.51^{e-h}	1.19±0.01^{f-i}
223086	77.75±5.85^{ab}	-3.28±6.94^{m-q}	0.96±0.09^{n-r}
223087-1	72.46±4.45^{b-f}	7.95±5.13 $^{g-j}$	1.11±0.08^{i-l}
223088	57.72±0.92^{l-q}	11.77±2.22^{e-h}	1.20±0.04^{f-i}
223090	74.37±1.63^{a-d}	-4.85±1.03 $^{n-r}$	0.93±0.01^{p-s}
223090-1	54.22±1.71^{opq}	19.25±1.56 ^{cd}	1.36±0.04^{cd}
223092	60.26±0.40^{k-o}	10.52±0.20^{e-i}	1.17±0.00^{g-j}
223092-1	58.76±2.36^{l-p}	3.87±2.85^{ikl}	1.07±0.05^{klm}
223093	58.33±0.00^{l-p}	2.57±0.38^{j-m}	1.04±0.01^{l-o}
223094	71.88±0.58^{b-f}	-4.92±0.65^{n-r}	0.93±0.01^{p-s}
NJ	67.99±4.03^{d-j}	-1.85±2.87^{l-p}	0.97±0.04^{m-r}
223096	67.54±0.59^{d-j}	-7.78±1.50^{o-r}	0.88±0.02^{qrs}
223097	69.68±5.82^{c-h}	-10.57±4.72^{r}	0.85±0.06^{s}
223097-1	67.36±2.48^{e-j}	-1.33±2.11^{k-o}	0.98±0.03^{m-q}
223099	74.09±0.61^{a-e}	-20.47±0.72^{s}	0.72±0.01^{t}
223100	57.42±0.77^{m-q}	15.04±1.66^{def}	1.26±0.03^{d-g}
223101	75.18±3.51^{abc}	-23.87±1.75^{s}	0.68±0.01^{t}
223104	73.43±5.79^{a-e}	-8.46±5.20^{pqr}	0.89±0.06^{qrs}
223105	71.78±8.60^{b-f}	1.85±9.11^{j-n}	1.03±0.13^{l-p}
223105-1	57.90±1.66 $^{l-q}$	-1.03±1.15^{k-o}	0.98±0.02^{m-q}
223109-1	73.87±0.52^{a-e}	-9.48±1.58^{qr}	0.87±0.02^{rs}
223110	70.07±5.59^{c-g}	-0.97±4.31^{k-o}	0.99±0.06^{m-q}
223112	63.13±5.62^{h-m}	15.13±5.70^{def}	1.24±0.11^{e-h}
223112-1	79.15±1.57^{a}	-2.97±2.52^{m-q}	0.96±0.03^{n-r}
223113	62.81±0.58^{i-m}	-1.66±0.04^{l-o}	0.97±0.00^{m-r}
229702	58.77±0.00^{l-p}	19.73±0.33^{bcd}	1.34±0.01^{cde}
229702-1	65.99±1.56^{f-k}	10.55±2.39^{e-i}	1.16±0.04^{h-k}
220563	69.01±1.04^{c-i}	5.32±0.43^{h-k}	1.08±0.01^{j-m}
220563-1	66.28±0.40^{f-k}	8.45±0.58^{f-j}	1.13±0.01^{i-l}
DIGGA	53.65±0.03^{opq}	15.16±1.04^{def}	1.28±0.02^{def}
DIGGA-1	42.46±0.12^{r}	35.13±1.04^{a}	1.83±0.02^{a}
DIGGA-2	63.65±3.14^{g-m}	19.23±1.90^{cd}	1.30±0.04^{de}
230565	58.63±2.85^{l-p}	23.50±1.26^{bc}	1.40±0.04^{c}
230566	53.15±0.59^{pq}	16.23±1.78^{de}	1.31±0.04^{de}
240407-1	64.42±0.31^{g-l}	4.70±0.39^{ijkl}	1.07±0.01^{j-m}
240407-B	70.00±0.34^{c-g}	-3.34±1.48^{mnopq}	0.95±0.02^{n-s}
240407-G	51.30±0.03^{q}	25.73±1.69^{b}	1.50±0.03^{b}
KICHI	56.86±1.29^{m-q}	24.13±2.09^{bc}	1.42±0.05^{bc}
KICHI-1	69.32±2.02^{c-i}	8.20±1.65^{ghij}	1.12±0.03^{i-l}
KUWE	55.43±1.73^{n-q}	9.50±0.52^{efghi}	1.17±0.01^{g-j}
Mean	64.63	5.46	1.11

Values are expressed as means ± standard deviations (SD); Means followed by different superscript letters in the same column are significantly different ($p< 0.05$).

Annex R- Standard curve for the determination of total phenols concentration (R2= 0.9989)



Annex S- Standard curve for the determination of total flavonoids concentration (R2= 0.9984)



DECLARATION

I, the undersigned, declare that the thesis is my original work, has not been presented for degrees in any other university and all sources of material used for the thesis have been duly acknowledged.

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Date: __11/10/2017__