NUTRITIONAL EVALUATION OF AERIAL TUBERS OF WILD AND DOMESTIC *DIOSCOREA BULBIFERA L.* (AIR POTATO OR AERIAL YAM) GROWN IN JIMMA ZONE, ETHIOPIA

LEMMA GUDETA GEMECHU

A THESIS SUBMITTED TO

THE DEPARTMENT OF APPLIED CHEMISTRY

SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER'S IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Lemma Gudeta have read and evaluated his thesis entitled "Nutritional Evaluation of Aerial Tubers of Wild and Domestic *Dioscorea bulbifera L*. (Air Potato or Arial Yam) Grown In Jimma Zone, Ethiopia" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

_____	_____	_____
Advisor	Signature	Date
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Internal Examiner	Signature	Date
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External Examiner	Signature	Date

DECLATION

I hereby declare that this MSc Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Lemma Gudeta Gemechu

Signature: _____

This MSc Thesis has been submitted for examination with my approval as thesis advisor.

Name: _____

Signature: _____

Date of submission: September

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Abbreviations

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ATSDR	Agency of Toxic Substances and Disease Registry
DCAP-OES	Direct Current Argon Plasma Optical Emission Spectrophotometer
ETAAS	Electro-thermal Atomic Absorption Spectrophotometer
FAAS	Flame Atomic Absorption Spectrophotometer
FAO	Food and Agriculture Organization
GFAA	Graphite Furnace Atomic Absorption
HGAAS	Hydride Gas Atomic Absorption Spectrophotometer
ICAP-OES	Inductively Coupled Argon Plasma Optical Emission Spectrophotometer
ICP-MS	Inductively Coupled Plasma Mass Spectrophotometer
WHO	World Health Organization
DDB	Domestic *Dioscorea*bulbifera
WDB	Wild *Dioscorea*bulbifera
MDL	Method Detection Limit
IDL	Instrument Detection Limit
DI	De-ionized water
ND	Not Detected
RAD	Recommended Dietary Allowance

Abstract

*Dioscorea bulbifera is an indigenous plant in Ethiopia. It is aerial tuber that has been used as a basic and traditional food in the south and southwestern parts of Ethiopia. However, its nutritional value has not been studied extensively.Therefore, this study aimed to evaluate the nutritional potential of two D.bulbifera L. varieties (wild and domestic) grow in Jimma Zone, Ethiopia. The standard procedures of AOAC (2003) were followed to analyze the proximate, nutrient compositions and minerals. The caloric value was calculated from crude protein, crude fat, and carbohydrate. While the results indicate that the domestic D.bulbifera contained carbohydrate (80.74%), crude fat (1.03%), crude protein (6.10%), crude fibre (3.03%) and Caloric value of 356.63 kca/100g while wild D.bulbifera contained higher content of crude fat (1.07%), crude protein (7.11%), crude fibre (6.20%) and lower content of carbohydrate (77.21%) and Caloric value (346.91kca/100g). Metals were analyzed by Flame Atomic Absorption Spectrophotometer (FAAS) and phosphorus was analyzed with Spectrophotometer . The average concentrations in(mg/kg) of the analyzed metals in domestic D.bulbifera were: Na (7.4), K(210.0), Mg(16.92), Ca(12.92), Fe(2.54), Zn(0.39), Cu(0.04), Mn(0.14) and P(2068.4)). In wild D.bulbifera higher level of Na(9.43), K(312.90), Mg(21.43), Ca(24.45), Zn(0.43),Cu(0.08), Mn(0.60), and lower level of Fe(1.78), P(1525.64) were obtained. One way ANOVA revealed significant variations in K, Mg, Ca, Fe Mn and P concentrations among the D.bulbifera varieties. It has been found that D.bulbifera contains lower levels of metals compare to other underground tubers and cereals food items exc*ept *maize. Both D.bulbifera varieties could be a better source of P, K, Ca, carbohydrate, energy, fiber and proximate. The wild D.bulbifera is was observed to be better in contents of K, Na, Mg, Ca, Zn, Mn, Cu, protein and fiber.*

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Key words: *Dioscorea bulbifera, Mineral Content, proximate, Evaluation, Mana Wered*

1. INTRODUCTION

1.1. Background

Roots and tubers refer to any growing plant that stores edible material in subterranean root, corm and tuber (Shajeela et al., 2011).They are the most important food crops in the tropics and subtropics (Behera et al., 2009). Cassava, sweet potato, potato and yam are grown in varied agroecologies and production systems contributing more than 240 million tons annually, covering around 23 million hectares. The aggregate value of yam, cassava, potato and sweet potato exceeds all other African staples, including cereal crops (NteranyaSanginga, IITA, 2015).

The reasons for encouraging these root and tuber crops for sustainable food production in Africa are:

(i) they are versatile staples to address food and nutrition security and produce more food per unit area of land, compared to many other crops

(ii) potato and sweet potato are short cycle crops and double cropping particularly the rain-fed system

(iii) yam and cassava are vital in the annual cycle of food availability due to theirbroaderagroecological adaptation, diverse maturity period and in ground storage capability,permitting flexibility in harvesting period for sustained food availability

(iv) they are also capable in efficiently converting natural resources into a more usable product

(v) they are a cheap but nutritionally rich staple food that contributes protein, vitamin C, vitamin A, zinc, and iron to meeting the dietary demands of the region's fast-growing towns and cities

(vi) they have high demand in local and national markets

(vii) they are far less susceptible to large-scale market shocks and price speculation experienced by more widely traded staples, such as grains, during international market

crises (NteranyaSanginga, IITA, 2015). Thus these crops have high potential towards household food security, local industries and natural resources base conservation.
The four crops have common and unique challenges related to quality of seed production, new variety adoption, losses due to insects and diseases, low productivity in poor soils, tolerance to stress associated with heat and drought, consumer preferences, and storage of harvested products (NteranyaSanginga, IITA, 2015).

The opportunities that these crops have in the future are: the resilience of the crop that continues to yield reliably high harvests under variable climatic conditions, providing better food and income opportunities with decreasing land holding sizes than mostothestaples in highland regions, the fast increasing demand from urban centers to pro vide better food and income opportunities and high starch content that can be transformed into many important products/derivatives chips, high quality flour starch, and ethanol (Nt eranyaSanginga, IITA, 2015).

Yam is a multispecies crop that belongs to the genus *Dioscorea* and family*Dioscoreacea*. It is found in Africa, India, Southeast Asia, Australia and tropical America with about 600 described species. It is the fourth most important tuber crop in economic terms), next to potatoes (Solanumtuberosum L.), cassava (Manihot esculenta Crantz), and sweet potatoes (Ipomoea batatas L.) (Mignouna et al., 2003). Out of the over 600 known yam species, only seven are mostly consumed. These include *Dioscorearotundata Poir* (White ya m), *Dioscoreacayenensis* (Yellow yam), *Dioscoreaalata* (Water yam), *Dioscoreabulbifer a* (Aerial yam), *Dioscoreaesculenta*, *Dioscoreapraehensalis* (Bush yam) and *Dioscoread umetorum* (Bitter yam) (Jayakody et al., 2007).

According to FAO statistics, 48.7 million tones of yams were produced on five million hectares in about 47 countries worldwide in 2005, and 97% of this was in sub-Saharan Africa West and Central Africa that accounts for 94% of world production.

Nigeria is the leading producer with 34 million tones followed by Côte d'Ivoire (5 million tonnes), Ghana (3.9 million), and Bénin (2.1 million tons) (FAO, 2010). Air potato (*Dioscoreabulbifera*) is a species of yam (*Dioscoreaceae*) family(Celestine,

2015). It is called air potato because it produces potato-like aerial bulbs in the leaf axils of the twining stems (Celestine, 2015).

Some varieties are edible and are cultivated as food crops in most hot humid tropical region of the world (Schultz, 1993).This yam species has two types of edible storage organs. One type is aerial tubers or bulbils formed in the leaf axils of the twining aerial stems while the second type is underground tubers formed beneath the ground (Celestine, 2015). Apart from providing basic food security and a source of income and diversity in diet, root and tuber crops also serve as additional source of protein, essential vitamins and minerals for the less affluent (Aregahegn et al., 2013).

In Ethiopia, especially in the south and southwestern parts, aerial yam has been a basic and traditional food crop for small-and medium-scale farmers (Tewodros, 2012). The country is only referred to as an isolated center of yam cultivation where a number of *Dioscorea* species are grown in complex cropping systems together with cereals and other root and tuber crops (Mulualem, 2006).

Main use of *D.bulbifera*is that the bulbils are normally cooked and eaten in a manner similar to other starchy root crops. The proximate and nutritional composition of the bulbils of D.bulbifera in terms of the dry weight has been reported by different studies. F or instance as reported by **(**Jacques Y.Achy et al., 2016) from Coted'Ivoire its proximate and nutritional compositions indicat ed as follows: moisture content (6.22%), fat (2.36%), protein (8.12 %), ash (3.44%), cellulose (0.91 %), carbohydrate (79.86%) and energy (37 3.16 kcal/100g). Again a report from India by (Subasini et al., 2013) indicates its proxim ate and nutritional composition as follws: total ash (4.7%), total Proteins (33.0 µg/100g), total Carbohydrate 685mg/100g and Crude fibre (3.8%).

D.bulbifera is also a good source of minerals such as iron, calcium and phosphorus (Abara, 2011).A report from India by (Subasini et al., 2013) indicates the mineral content of raw D.bulbifera as: Na(1.81mg/L), Ca(79.05mg/L), K(160.30mg/L), Li(0.55mg/L), Fe (0.474ppm), Cu(0.2635ppm), Mn(0.1220ppm), Ni(0.0990ppm), Zn(1.2825ppm), Co(0.1 780ppm) and Cr(0.4435ppm).

In Ethiopia also yams are hardly known to the scientific community (Mulualem, 2006). Even though their agronomic properties have been well documented, their food and industrial quality characteristics have not been studied extensively (Aregahegn et al., 2013).

Furthermore, most plant nutrients are dependent on the type of the plant species, soil type ,geographical location, and over all climatic conditions. Particularly mineral nutrients are more susceptible to these variables. Therefore, analyzing the mineral, proximate and nutritional composition of *D.bulbifera* is important to evaluate the effect of variation of th e above parameters in them. It also provides information about the nutrient quality characteristics of *D.bulbifera* in order to include it in daily meal and to use as raw materials for small-scale food industries.

1.2. Statement of the problem

Air potato *(*Dioscorea *bulbifera)* grows as wild in Jimma zone, Manna woreda. It is also c ultivated domestically. The aerial tubes of edible storage organs of the plant are consume d by the people. The people call it locally '**kontarie.** Boiling and roasting are the common methods of preparing the food for consumption. The tubers have bitter taste which disappears after proper boiling or roasting.

The people of this woreda also use it as medicinal plant; for instance, as a drug for kidney failure.The medicinal properties of *D.bulbifera* are well documented (Celestine, 2015). For example, as reported by (Al-Shehbaz et al., 1989) it is used in leprosy, asthma, cough, cold, tuberculosis, contraceptive, constipation, indigeton, abdominal pain. Root powder is used as component of local medicine for tuberculosis and maintains kidney function. In this area, the wild aerial yam has gotten very low attention by consumers.

Though, it is available, the people do not conserve and use it regularly as the domestic one as source of food and medicine. This might be due to lack of awareness about its potential as a source of nutrition and bad cultural practices.

Yet, there is no comparative study that shows the nutritional value differences and/ or similarities existing between the aerial tubers of wild and domestic air potato in the area.

It is known that the nutritional profiles of commodities are very important in encouraging a community to use the commodities as source of food (Tesfay et al., 2015). Investigating the nutritional value of the wild edible plants will be also important to conserve the wild edible plants in the home garden. This may lead to the formal domestication of the most nutritionally valuable wild edible plants. It also initiates the community to conserve nutrient full plants to minimize extinction of crucial wild edible plants (Baressa, 2016).

Therefore, it is important to have information that reveals the nutritional value of the aerial tubers of edible wild and domestic *Dioscorea bulbifera L.* (air potato or aerial yam) available in Jimma Zone, Manna woreda. So that, the information serve as the valuable knowledge to conserve and incorporate both the wild and domestic *Dioscorea bulbifera L* varieties regularly in our daily meal and to use them as raw materials in small-scale industries as others tuber crops.

1.3. Significance of the study

The world food crisis has been and will continue to be a major obstacle to humanity (Shajeela, et al., 2011). So that, there is an observed interest in search for alternative or additional food and feed ingredients is of paramount importance mainly for two reasons; one is the low production of oil seeds and grains and another is the stiff competition between man and the livestock industry for existing food and feed materials (Shajeela, et al., 2011).

Wild edible plants are neither cultivated nor domesticated, but are available from their wild natural habitat and used as sources of food (Lulekalet al., 2011).

The use of wild plant resource still continued in different by human beings (Baressa, 2016). The wild edible vegetables could be one of the alternative/ additional foods that give a solution for the food crises existing.

Scientific researchers also should support this by analyzing nutritive content of the food resources available which do not contradict with norms and values of the community in order to encourage the people to use any alternative food resources available.

Thus, this study is significant to reveal the nutritional quality of *D. bulbifera* as valuable knowledge in order to the society use the wild air potatoes regular alternative or additional source of food in alleviating the existing food crises rather than simply ignoring. It also significant to the scientific community to make base line on the little no data in the nutr-itional composition differences/similarities existing between wild and domestic air potato in this area. The study also provides adequate information to the community on the distribution of macro, micro, elements and proximate in ensuring the dietary safety of the individuals in the area. Lastly to initiate any interested body to conduct further detailed and comprehensive investigation on D.bulbifera.

1.4. Objectives of the study

1.4.1. General objective

The general objective of this study is to evaluate and compare the nutritional (chemical composition, proximate and minerals) constituents of the tubers of wild and domestic aerial potatoes available in Mana district of Kelaguda locality.

1.4.2. Specific Objectives

1. To evaluate the levels of Na, K, Mg, Ca, Fe, Mn, Cu, Zn & P concentration the raw aerial tubers of domestic and wild air potatoes (D.bulbifera) by AAS
2. To evaluate the moisture, volatile matter, ash, fixed carbon, protein, fat, fiber, carbohydrate & energy contents of these varieties.

2. REVIEW LITERATURE

2.1. Root and tuber crops

Tropical root and tuber crops including cassava, potato, sweet potato, yam and aroids are raw materials for small scale industries and consumed as staple foods especially in the les s developedcountries (Grusak MA and DD Pennas, 1999). They were critical components in the diet during the early evolution of humankind and were the most important food crops of very ancient origin in the tropics and subtropics (Ravi V et al., 1996).Root and tuber crops are found in a wide variety of production systems and do well under various levels of management from low to high input systems (Asha KI and MC Nair, 2002**)**. This is a distinctive feature, which makes them important for improving the productivity and richness of agro-systems (Aregahegn et al., 2013).Not only the domesticated ones, but also the wild edible vegetable plant species pays more attention towards extensive distribution and effortless availability will contribute new energy source, nutrition and food resources during natural calamities (Nandini et al.,2015*).*

2.2. Yams

Yams, the edible starchy tubers, are of cultural economic and nutritional importance in the tropical and subtropical regions of the world (Shajeela et al., 2011).Yam has been sug gest-ed to have nutritional superiority when compared with other tropical root crops. They are reported as good sources of essential dietary nutrients (Shanthakumarietal., 2008).

2.2.1. Uses of aerial yam tubers (D.bulbifera)

Throughout the plant's native range in three continents, several domesticated varieties, and in some areas, wild varieties, of *D.bulbifera* serve as food sources for local consumpt -ion and/or commercial distribution (Al-Shehbaz and Schubert 1989). The species has been in cultivation for several millennia in both Asia and Africa. Tubers of edible varietie s of *D.bulbifera*from Africa, Australia, Nepal, and Thailand are documented to have well text-ured flesh and a distinctly bitter taste in contrast to the softer flesh and sweeter taste of tubers produced by the varieties cultivated in much of Asia (Martin and FW, 1974).

In its wild state, it is extremely bitter. Under cultivation the plant loses its bitterness.Various preparation techniques are also used to lessen or fully eliminate bitterness. Techniqu es typically involve boiling/steaming and/or baking over coals after either cleaning (bulbils) or cleaning and peeling (tubers). In areas of the plant's native range, tubers of several of the toxic varieties of *D. bulbifera*are made palatable and can be used as a food source in emergency situations (i.e., periods of drought and or famine(Martin, and FW, 1974).

In addition to its food use, *Dioscorea bulbiferea L.* is a medicinal plant. Recent pharmacological findings also indicate that aerial tubers of *D.bulbifera* have long been used in many ways in folk medicines in all ranges (Martin and FW, 1974). Among many documented medicinal folk uses of the plant, some of the most well-known include: the use of bulbils for external treatment of sores and internal treatment of hemorrhoids (India); the use of a paste created from the tubers to treat swelling and as a cure for snakebite and scorpion stings (Africa, Central Asia); the use of the tuber for treatment of sore throat and struma (China); use of the tuber to remedy diabetes (Japan); use of the tuber for treatment of leprosy and tumors (northern regions of Bangladesh) (Gao et al., 2002).

Research conducted by (Gao et al., 2002) indicates the existence of anti-tumor promoting agents present in the tubers of *D. bulbifera*. Again research by (identified eight isolates present in tubers of *D.bulbifera* that exhibit antitumor promoting capabilities, all of which are furanonorditerpenes or glycosides: biosbulbin A-H and diosbulbinosides D and F. Inhibitory effects are promoted by several compounds characterized as flavonoids (Gao et al., 2002). Tubers or aerial bulbils of unpalatable varieties of *D.bulbifera* have been used to create poisons for various uses (Martin FW, 1974). Poisons are derived from alkaloids (i.e., dioscorine), saponins, sapogenins and/or tannins present in tubers of a given variety (Al-Shehbaz et al., 1989).

In various parts of Africa and on the island of Java, aerial tubers are used to make a fish poison (Al-Shehbazetal., 1989; Martin and FW, 974).The poison released by grated tubers placed in a stream acts to stun fish at fairly long distances (Al-Shehbaz et al., 1989).

2.2.2. Phytochemical compounds of air yam tubers (D.bulbifera).

Studies on preliminary phytochemical by qualitative and quantitative methods,physiochemical standards, elemental analysis can serve as a valuable source of information and provide suitable standards to determine the quality of this plant material in future investigations or applications (BarnadiDutta,2015).

Aerial bulbils contain alkaloids, hydrogen cyanide, saponins and flavonoids. It is already known that these toxic principles exhibit useful medicinal properties. So their presence in the yam species is a pointer to the medicinal value of the food material.

Table1 shows phytochemical compounds of *D.bulbifera* reported by (Celestine, 2015). The study revealed amounts of oxalate with the value (0.78 mg/100 g) whereas the amounts of HCN and tannin were lower than 1.48 mg/100 g.

Table1.Phytochemical profile of the *D.bulbifera L* .tubers (Aerial bulbils) that reported by (Celestine, 2015).

Phytochemical	Aerial bulbils
Phytate (mg/100 g	2.00±0.045
Oxalate (mg/100 g)	550.00±22.000
Tannins (mg/100 g)	37.41±6.235
Alkaloids (%)	3.10±0.100
Hydrogen cyanide (mg/100g)	43.69±1.211
Sapoins	++
Phenols	+
Flavonoids	++

2.2.3. Chemical nutrient composition of D.bulbifera

a) Crude fats

Yam tubers generally low and similar levels of fat contents which do not exceed 2% on dry weight basis and 0.3% in wet weight basis (Abara, 2011). However, the crude fat content of *D.bulbifera* was 0.55% as reported by (Celestine, 2015) from Nigeria.

b) Carbohydrates

Carbohydrates are the chief sources of energy by providing 70 – 80% of the calories in the human diet. The total carbohydrate determined by difference consists of sugars, dextrins, starches pectins, hemicelluloses, celluloses and lignin. The carbohydrate content of yams constitutes the major dry matter content of yam tubers (Abara, 2011).The carbohydrate content of *D.bulbifera* has been reported by the same person in 2003 was for instance 80.98%.

C) Crude Proteins

Proteins have a major role in the growth and maintenance of the human body and are, along with carbohydrates and lipids, the energy giving nutrients in the diet. In addition, proteins also pose a wide range of other functions in the body, such as enzymatic activity and transport of nutrients and other biochemical compounds across cellular membranes (Wu et al., 2014).Vegetables are generally high in carbohydrates and especially low in proteins. However, their nutritive value depends in part on the soil and climate in which they are grown (**A**kpabioet al., 2013).

Yam tubers generally low in levels of protein contents. On dry weight basis 6.35% and 6.18% protein content of *D.bulbifera* reported by (Abara, 2011) and (Celestine ,2015) respectively from Nigeria.

d) Crude Fiber

Dietary fiber is defined as lignin plus the polysaccharide components of plants which are indigestible by enzymes in the human gastrointestinal tract. On dry weight basis 2.89% and 7.97% fiber content of *D.bulbifera* reported by (Abara, 2011) and (Celestine, 2015) respectively from Nigeria.

Table 2 shows the chemical nutrient composition of the tubers of *D. bulbifera*. The result shows its contents of crude fats, crude fibre, ash, carbohydrate, energy and protein.

Table2. Chemical nutrient compositions profile of *D.bulbifera L.* tubers reported by (Celestine, 2015).

Proximate components and nutritional compositions	Aerial bulbils content
Crude Protein (%)	6.18±0.230
Crude fats (%)	3.30±0.400
Crude fibre (%)	7.97±0.751
Ash (%)	2.33±0.155
Moisture (dry weight) (%)	3.55±0.414
Moisture (fresh weight) (%)	66.57±1.276
Carbohydrate (%)	76.68±0.780
Energy value (KJ/100 g)	1530.61±26.336

2.2.4. Mineral element composition of air potato (D.bulbifera).

From the triple acid digested sample, sodium, potassium, calcium, magnesium, iron,copper, zinc and manganese can be using an atomic absorption spectrophotometer. Table 3 shows the levels of elements in tuber reported by (Celestine, 2015). Phosphorus is estimated colorimetrically (Shajeela1 et al., 2011).

Table3. Mineral profile of *D. bulbifera L.* tubers reported by (Celestine, 2015).

Mineral element (mg/100 g)	Aerial bulbils
K	316.72±0.431
Na	38.52±0.053
Ca	174.44±0.237
Mg	758.94±10.317
Mn	0.46±0.016 0.089
Fe	1.61±0.032
Zn	0.17±0.013
Cu	0.10±0.022
P	149.93±1.089
Cd	ND
Pb	ND
Cr	ND
Se	ND
Co	ND

Table4. Mineral composition of *D. Bulbifera L* reported from Nigeria by (Abara, 2011) and from Mexico by (Shanthakumari et al., 2008) in dry basis.

Mineral composition (mg/100g)		
	Report from Nigeria	Report from Mexico
Na	550.00± 6.36	63.38±0.01
K	440.00±3.54	1548.00±0.82
Ca	206.60± 4.24	228.15±0.08
Mg	139.00 ±2.83	440.17±0.10
P	150.00 ±0.74	120.50±0.06
Zn	1.52±0.16	1.30±0.01
Mn	4.00±0.18 (ppm)	10.60±0.03
Fe	5.90±0.28	3.90±0.12
Cu	5.00±0.45(ppm)	2.64±0.02 .

Root and tuber crops are also widely cultivated in southern Ethiopia, and support a considerable portion of the country's population as source of food. Prominent among these are: potato (Solanumtuberosum L.), sweet potato (Ipomoea batatas L.), enset (Enseteventricosum (Welw), Cheesman), godere (Colacasiaesculanta L.), yams (Dioscorea spp.), Ethiopian dinch (Coleus parviflorus), koteharrie (Diaspora bulbiferous), and anchote (Cocciniaabyssinica) (Aregahegn et al., 2013). In south and south-western parts, there are huge amounts aerial yam genotypes are distributed across diverse agro-geographical areas (Tewodros et al, 2013).

The food potential crops of root and tuber crops has not yet been fully exploited and utilized despite their significant contributions towards food security, income generation, provision of food energy and resource base conservation .According to FAO (2004) only 4568 tones of root and tuber crops were produced Ethiopia. Currently most root and tuber crops are grown as security crops against crop failures and/or to bridge the food deficit periods, as they are ready for harvest during "hunger months."

These crops are also, tolerant to termites, which might be attributed to their higher moisture content, and thrive well on very poor soils and under moisture stress conditions. They are a cheap but nutritionally rich staple food that contributes protein, vitamin C, vitamin A, zinc, and iron to meeting the dietary demands of the region's fast-growing towns and cities.For instance as reported by (Atlabachew et al., 2008) the minera l contentof Kocho from *Ensete (ventricosum)* is as follows in µg/g*:* Na(462-688), K(2,75 34,380), Ca(498–584), Mg(180–290), Fe(92.5135), Mn(8.5810.13) and Zn(3132.1). The mean concentration range (in µg/g) of each metal in *D.abyssinica* also reported by (Aregahegn et al., 2013) samples as: K (8,469–13,914), Na (133–405), Ca (172–448), Mg (180–354), Fe (28.3–144.5), Mn (12.0–14.5), Zn (12.3–44.5), Cu (7.26–17.6), Co (1.91–8.68), Cr (0.86–3.41) and Ni (2.43–5.31). *D.abyssinica* could be good sources of essential trace metals to the individuals, more particularly Fe and Zn.

The mineral and proximate content of tubers of anchote (Coccinia abyssinica (Lam.) were also reported by (Yenenesh Ayalew, 2016) as follows: 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, crude fat (0.24±0.05 - 0.75± 0.07%); crude fiber (3.63±0.04-6.96±0.24%) as well as total ash (4.63±0.31 - 6.83±0.02%), total amino acid content (45.12 to 62.89 g/100g); mineral contents in mg per 100 g dry weight: 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).

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

Nutrients (per 100g)	Potato		Sweet potatoes,	Cassava,	Yam,
,	White flesh and skin,	Red flesh and skin			
Proximate composition					
Energy (kcal)	69.0	70	86.0	160.0	118.0
Protein (g)	1.7	1.9	1.6	1.4	1.5
Total lipid (fat) (g)	0.1	0.1	0.1	0.3	0.2
Carbohydrate, by difference(g)	15.7	15.9	20.1	38.1	27.9
Fibre, total dietary (g)	2.4	1.7	3.0	1.8	4.1
Sugars, total (g)g	1.2	1.3	4.2	1.7	0.5
Minerals					
Calcium, Ca (mg)	9	10	30	16	17
Magnesium, Mg (mg)	21	22	25	21	21
Potassium, K (mg)	407	455	337	271	816
Phosphorus, P (mg)	62	61	47	27	55
Sodium, Na (mg)	16	18	55	14	9

Source (Anoma Chandrasekara and Thamilini Josheph Kumar, 2016)

Table 2 Nutritional composition (%) of some commonly consumed root & tuber crops from different sources.

Crops	Moisture	Dry matter	Total ash	Crude protein	Crude fat	Crude fibe	Total carb	Energy (Kcal/100g)
Solanum tuberosuma	78.90	21.90	1.45	4.74	0.71	0.77	14.20	NA
Ipomoea batatasa	70.57	29.43	0.97	3.13	0.79	0.90	24.54	NA
Manihot esculentaa	68.08	31.92	0.85	2.84	0.18	1.38	28.05	NA
Dioscorea								
Rotundataa	67.13	32.87	0.78	4.45	0.15	0.67	27.49	NA
Dioscorea alataa	77.40	22.60	1.05	2.51	0.14	0.42	18.90	NA
Xanthosoma								
Sagittifoliuma	82.27	7.73	1.03	5.47	0.20	1.28	11.03	NA
Colocasia esculentab	66.62	NA	4.09	6.40	0.78	1.83	86.58	378.93
Dioscorea bulbiferac	91.90	NA	NA	2.10	2.10	6.04	79.03	304.7
Coccinia abyssinicad	74.93	NA	2.19	3.25	0.19	1.73	19.44	82.12

Source: ((Yenenesh Ayalew, 2016); NA: data not available

2.2.5. Importance of measurement of chemical nutrient composition and pro ximate

2.2.5.1. Moisture content

Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food (Onwuka et al., 2005). High temperature and humidity may then activate the growth of bacteria and fungi which can affect the storage and shelf life of food. The moisture content of a feed affects its stability and overall quality. Therefore the level of moisture content is an important factor in both economy and storage (Thiex et al., 2003).

2.2.5.2. Volatile matter

Volatile matter in coal and coke is defined by the American Society for Testing Materials (ASTM, 1954) as "those products, exclusive of moisture, given off by a material as gas or vapor, determined by definite prescribed methods which may vary according to the nature of the material". It is one of the most important determinations in the proximate analysis.

2.2.5.3. Ash content

Ash in food contributes the residue remaining after all the moisture has been removed as well as the organic material (fat, protein, carbohydrates, vitamins, organic acid etc) have been incinerated at a temperature of about 500^{o}C).Ash content is generally taken to be a measure of the mineral content of the original food. Ash in feeds is useful for judging nutritional characteristics of the feed because ash has generally constantelement composition by feed material type as long as the feed does not contain earth and sand (Onwuka et al., 2005).

2.2.5.4. Fixed carbon

Fixed carbon is a calculated value of the difference between 100 and the sum of the moisture, ash, and volatile matter where all values are on the same moisture reference base.

2.2.5.5. Crude protein

Crude protein is defined as the value obtained by quantitating nitrogen in a sample by the Kjeldahl method(in which nitrogen compounds in the sample is degraded by sulfuric acid to become ammonia, sodiumhydroxide is added, steam distillation is conducted under the alkaline conditions, distilled ammonia is absorbed in acid and measured by titration) and multiplying the result by the factor 6.25 (6.38 for milk products) (Wu et al.,2014).

Proteins have a major role in the growth and maintenance of the human body and are, along with carbohydrates and lipids, the energy giving nutrients in the diet.

In addition, proteins also pose a wide range of other functions in the body, such as enzymatic activity and transport of nutrients and other biochemical compounds across cellular membranes. Being such important constituents of human diet it is crucial to know the protein content in foods and thus it is important to have reliable analytical methods(Wolfe and R.R,2006).

2.2.5.6. Crude fat

Crude fat refers to the sum of triglycerides, phospholipids, wax ester, sterols and minor a mount of non-fatty materials. Fat is very good sources of energy and aids in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes). Crude fat can be determined by intermittent soxhlet extraction apparatus. Ether extract contains, in addition to fat, oil-soluble dyes (such as chlorophyll and

carotenoids), wax, freefatty acids, lecithin, cholesterin, and phospholipids, etc.(Pamela et al., 2005).

When feeds are stored for a long period, a phenomenon is observed in that moisture does not change while ether extracts decreases gradually. This is because unsaturated fatty acids contained in feeds are oxidatively polymerized absorbing oxygen in the air and becomes insoluble in ether (Onwuka et al., 2005).

2.2.5.7. Crude Fiber content

Crude fiber is primarily measured to comprehend indigestible parts in feeds, and is consisted mainly of a part of lignin, pentosan, chitin, etc., in addition to cellulose. These compounds are collectively called as fiber. Crude fiber is commonly used to estimate the quality of foods of plant origin on the premise that it constitutes their least digestible fraction.It is important for the removal of waste from the body thereby preventing constipation and many health disorders.

Consumption of vegetable fiber has been shown reduce the cholesterol level, risk of coronary heart diseases, colon and blood cancers and hypertension, enhance glucose tolerance and increase insulin sensitivity.

Common food sources of fiber are fruits, vegetables and whole grains (Kaur, 2016).There is a close relationship between the crude fiber content and the nutrition value of the feed, andgenerally the higher the crude fiber content is, the lower the nutrition value is (Kaur, 2016).

2.2.5.8. Carbohydrate

Carbohydrate content a food is estimated by subtracting the values obtained for fat and protein from organic matter. The percentage of organic matter was calculated by subtracting the percentage of ash from one hundred (100) (Onwuka et al., 2005).

2.2.5.9. Energy calculation

The percent calories in selected samples are calculated by multiplying the percentage of crude protein and carbohydrate by 4 and crude fat by 9. The values are then converted to calories per100gm of the sample (Onwuka et al., 2005).

2.2.6. Importance and classification of minerals in food

Although the minerals composed of only a few percent of the body of animals and green plants, they take a disproportionately large part in the body metabolism for both plants and animals (Andersonet al., 2002).

Numerous investigators have shown that the normal physical activities are not possible in the absence of minerals (Anderson et al., 2002; Galston and A.W, 1968). Of the mineral elements: P, Ca, K, S, Na and Cl are sometimes called macronutrients, not because they a re more important but because they are necessary in somewhat greater amounts than othe rs (Anderson et al., 2002; Galston and A.W, 1968).The first five essentials to both plants and animals, and the last two to animals alone (Andersonet al., 2002; Galston and A.W, 1968).

Based upon the amount that is required in human nutrition, metallic elements are classified as:

(1) Bulk mineral elements or macro metals; Ca, Mg, K and Na (Recommended Dietary AllowanceRDA > 200 mg/day).
(2) The Recommended Dietary Allowance (RDA) of the non- metallic element P is > 200 mg/day except for infants,(Santos et al., 2004).
(3) The most important micronutrients (RDA < 200 mg/day) are Cr, Cu, Fe, Mn, Mo, Se, V and Zn. They are used in the enzymatic systems and can be harmful when their injection rate istoo high (Santos et al., 2004).

The various mineral elements are generally being imbibed into the plants from the soil, water and atmosphere. The level of mineral elements in plant varies depending upon the environmental factors and the type of plant itself. Among plant types growing in the same environment, fungi lichen and mosses accumulate more metals than the others. Moreover, the concentration of elements also varies with the age of the plant (Subasini et al., 2014).

The delimitation of the present study was to evaluate and compare the (mineral and proximate) nutritional values ofraw aerial tubers of domestic and wild aerial yam that grow in Oromia regional State, Jimma zone, Manna woreda on dry basis. The metallic

elements of interested in this study were K, Na, Ca, Mg, Fe, Zn, Cu and Mn and the non-metallic element interested was P. In the following section general roles played by these elements of in animals are presented.

a) Potassium

Potassium is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. Deficiency symptoms include weakness, anorexia, nausea, drowsiness and irrational behavior (Santos et al., 2004).

b) Sodium

Sodium is an essential nutrient involved in fluid and electrolyte balance and is required for normal cellular function. It is an essential mineral or micronutrient which along with potassium helps to regulate the body's fluid balance.

Dietary deficiency of sodium is not common due its widespread occurrence in foods. Sodium is present in foods as a normal constituent at a low level.

It is also added to foods as NaCl (table salt) while processing, cooking and immediately prior to consumption. However, excess sodium intake is linked with high blood pressure and heart disease.

The current recommendation is to consume less than 2,400 milligrams of sodium a day. This is about 1 teaspoon of table salt per day.

It includes all salt and sodium consumed, including sodium used in cooking and at the table salt (Anderson et al, 2002).

c) Calcium

Calcium is the outstanding single constituent of bones and teeth. It is the most abundant mineral in the body with RDA for adults 1200 milligrams (Santos et al., 2004). It occurs in the bone in the ratio of 3:1 mole $Ca_3(PO_4)_2$ and $CaCO_3$, respectively. Very small fraction of calcium can also serve in heart, muscles, and nerve systems. Ca and PO_4^{3-} are properly utilized in the body by vitamin D (Anderson et al., 2002).The best natural sources are sea vegetables, low-fat yogurt, skim milk, beans, seeds, nuts, green

vegetables, etc. Intakes over 2000 milligrams per day may lead to hypocalcaemia, induce constipation, and inhibit the intestinal absorption of iron, zinc, and other essential minerals (Galston and A.W,1968; Banerji et al., 2005).

d) Magnesium

Magnesium is the essential composition of bone and teeth with RDA for adults of 350 milligrams. It plays important role in metabolism of phosphorus, starch and sugars. Many biochemical and physiological processes require magnesium. It is necessary for vitamin C and calcium metabolism.It keeps teeth healthy, brings relief from indigestion and can aid in fighting depression. More than300 enzymes are known to be activated by magnesium minerals (Galston and A.W, 1968; Banerji et al., 2005).

e) Iron

Iron is a constituent of hemoglobin. Body iron content is regulated by the amount absorbed. The absorption is influenced by body stores and by the amount and type of iron in ingested foods. The RDA for adults is 15 milligrams (Banerji et al., 2005). It is a vital component of many enzymes; it can promote resistance to disease and prevent fatigue.

A deficiency can cause anemia, resulting in impaired concentration, reduced physical performance and work capacity, and decreased immune function. Ascorbic acid is necessary for the proper assimilation of iron (Banerji et al., 2005).

f) Zinc

Zinc function in essential enzyme systems in animals (Banerji et al., 2005). The RDA is 15 milligrams per day for men and 12 milligrams per day for women. Recent research suggests that men have a higher need for zinc than do women. Thus, it is appropriate the RDA is sex-specific for zinc. Zinc is an essential trace element that must be supplied in the diet of human beings so that growth and health can be maintained. It is necessary for protein synthesis and the metabolism of vitamin A; it helps the healing process of internal and external wounds, decreases cholesterol deposits and promotes mental awareness. A deficiency can cause loss of appetite, growth retardation and immunological

abnormalities (Banerji et al., 2005).

g) Copper (Cu)

Copper in living organisms, including humans, forms an essential component of many enzymes (cuproenzymes) and proteins. The biochemical role for copper is primarily catalytic, with many copper metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen, for example cytochrome-C-oxidase and superoxide dismutase. Studies have also shown that copper is required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases (Wardlaw and Insel et al., 1996).

h) Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function. While a deficiency of this mineral is uncommon, it is often lost in processed foods.

A deficiency of manganese may affect brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. Grains and cereal products are the best food sources of manganese, while animal products are the poorest. Toxicity from manganese is uncommon (Minaleshewa etal., 2007).

Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent (ATSDR, 2001).

i) Phosphorus

Phosphorus is the second most abundant essential mineral in the human body after calcium. It not only plays a role in numerous biologic processes, including energy metabolism and bone mineralization, but also provides the structural framework for DNA

and RNA. It is synthesized through various biochemical pathways such as glycolysisn and beta oxidation. Approximately 80% to 90% of phosphorus is present in the bones and teeth in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The remainder is present in extracellular fluid (ECF), soft tissues and erythrocytes. Serum and plasma contain only a small fraction of total body phosphorus in the form of inorganic phosphate, lipid phosphorus, and phosphoric ester phosphorus. Thus, changes in serum phosphate levels do not necessarily reflect the body's total store of phosphorus.

A normal diet provides approximately 20 mg/kg/day of phosphorus of which 16mg/kg per day is absorbed in the small intestine e(predominantly in the jejunum) by both para-cellular and intracellular processes. Deficiency of calcium, phosphorous and vitamin D leads to the classic bone symptoms associated with rickets, such as bowlegs, knock knees, curvature of the spine and pelvic and thoracic deformities (Raina et al., 2012).

2.2.7. Methods of nutrition values analysis

Chemical composition of a substance constitutes are the different classes of nutrients present in the samples such as carbohydrates, protein, fat, crude fibre, ash and moistu-re as well as caloric value calculated from values of carbohydrate, fat and protein.

2.2.7.1. Proximate analysis

The moisture, volatile matter, and ash results are typically among the primary parameters used for assessing the quality of a solid material.

a) Ash

Crude ash is quantified by international standards ISO 5984 (2002) animal feeding stuff. By this method crude ash is defined as the weight of a sample measured after heating at 550-600 °C for 2 hours to incinerate in a crucible. Another method is ASTM standard method. This method is heating the sample in the furnace at a temperature of 550°C for 4h and weighed after cooling in a dessicator to obtain the weight of ash.

b) Moisture

Determination of Moisture

Methods which have commonly been used for this determination may be subdivided according as to whether their approach is physical or chemical.

Physical methods include:

(a) Oven-drying, in which the weighed material is heated to a suitable temperature and the loss in weight determined

(b) Determination of loss in weight of the sample after drying in vacuo at room or elevated temperature with or without the use of desiccants

(c) Direct water determination by measuring after distillation with an immiscible liquid

(d) Other methods depending on the existence of a constant relation between water content and physical properties, such as electrical conductivity and detectable alteration of any significance other than the reversible loss of moisture.

Loss on drying (Analytical Standard of Feed): This is a method in which an analysis sample is heated with a temperature-controlled dryer and the loss is quantitated as moisture (loss on drying).

Moisture assay methods by international standards ISO 6496 (1996) animal feeding stuffs: This method is the determination of moisture and other volatile matter content.

An oven temperature of 104-105 °C is desirable for the satisfactory determination of moisture in air potato (Wikins et al., 1987).

c) Volatile matter

As ASTM standard method, it is termed as the weight loss due to heating of 1gm of biomass at $925^{o}C \pm 5^{o}C$ in furnace for 7 minutes.

2.2.7.2. Nutrient composition analysis methods

a) Carbohydrate

Carbohydrate can be determined y different methods.

Total carbohydrate:

By difference: 100 - (weight in grams [crude protein + crude fat + moisture + ash + crude fiber] in 100 g of food)

By direct analysis: weight in grams - (mono + disaccharides + oligosaccharides + polysaccharides, including fibre)

Available carbohydrate:

By difference: 100 - (weight in grams [crude protein +crude fat + moisture + ash + crude fibre] in 100 g of food).

By direct analysis: weight in grams - (mono + disaccharides + oligosaccharides + polysaccharides, excluding fibre)

Determination of available carbohydrate by difference is considered acceptable for purposes of energy evaluation for most foods (FAO, 2010).

b) Crude protein

For many years, the protein content of foods has been determined on the basis of total nitrogen content, while the Kjeldahl (or similar) method has been almost universally applied to determine nitrogen content (AOAC, 2003).Nitrogen content is then multiplied by a factor to arrive at protein content. This approach is based on two assumptions: that dietary carbohydrates and fats do not contain nitrogen, and that nearly all of the nitrogen in the diet is present as amino acids in proteins.

On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content (Wikins et al., 1987).

Total nitrogen can be determinate by of the Conventional Kjeldahl analysis with modifications, Kjeltec analysis with block digestion and semiautomated distillation, and t he Hach methods.When conventional Kjeldahl analysis with modifications, Kjeltec ana-lysis with block digesti-on and semiautomated distillation, and the Hach method for determining nitrogen (N) were compared using a wide range of samples. Twenty different sample types were ground and mixed. Each sample type was divided into 5 subsamples which were analyzed for N by each of the 3 methods. In each sample type, differences ($P<0.05$) were detected among the 3 N determination methods in 5 of the 20 N sources analyzed. The mean N content over all 20 samples was higher with Kjeldahl analysis ($P<0.05$) than with Kjeltec, while Hach analysis produced intermediate results.

Thus, the conventional Kjeldahl analysis with modifications is the best of the three (Wikins et al., 1987).

c) Crude Fibre

Crude fiber is primarily measured to comprehend indigestible parts in feeds, and is consisted mainly of a part of lignin, pentosan, chitin, etc., in addition to cellulose. For crude fibre, numerous methods may be in use, but only a few have reached the status of an international standard.

The criteria for evaluating fiber methods are the recovery of indigestible plant residues and the analytical performance of the methods and their official status.

There are different methods for crude fiber determination. For instance, ISO 5498(AOAC 962.09) describes a general method and ISO 6541 a method for some food products.

ISO 12099 gives guide lines for the application of NIR spectrometry and underlines the importance of using validated standard methods for the calibration and validation of NIR methods.

Different methods for the determination of fibre have been evaluated by analysing results reported with the proficiency testing scheme of the Association of American Feed Control Officials. No significant difference between methods. This indicates that the most common methods currently in use are robust and reliable (FAO, 2010).

d) Crude fat

Most fat in the diet is in the form of triglyceride (three fatty acids esterified to a glycerol molecule backbone). There are also non-glyceride components such as sterols, e.g. cholesterol.

While there is considerable interest in the roles that these non-glyceride components may play in metabolism, they are not important sources of energy in the diet (FAO, 2010).There are accepted AOAC gravimetric methods for crude fat, which includes phospholipids and wax esters, as well as minor amounts of non-fatty material (AOAC, 2003). Total fat can be expressed as triglyceride equivalents determined as the sum of individual fatty acids and expressed as triglycerides (FAO, 2010). This method is

satisfactory for the determination of fat in a wide variety of foods. A gravimetric method is acceptable for energy evaluation purposes (AOAC, 2003).

2.2.7.3. Analysis of metals

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Several spectrometry techniques have been used for macro and trace elements determination in plants or biological materials.

Flame Atomic Absorption Spectrophotometer (FAAS), hydride graphite atomic absorption spectrophotometer (HGAAS) or electrotermal atomization atomic a bsorption spectrophotometer (ETAAS),graphite furnace atomic absorption (GFAA), Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICAP-OES), direct current argon plasma optical emission spectroscopy (DCAP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are most commonly used for the determination of metals in plant materials because of their inherent selectivity, sensitivity, precision and accuracy(Jacobus et al., 2007).

2.2.8. Sample decomposition

The various techniques for elemental determination do not all requires the same degree of sample matrix break down.

The choice of a digestion technique should take into account the objective of the final determination and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory (Ebdonet al, 1998). The purposes of sample decomposition are (Quevauvilleret al, 1995).

- Converting all the species in which a given element is present in such a way that it becomes present in one defined form
- Eliminating interfering substances from the matrix
- Obtaining the element in a homogeneous and easily accessible matrix. The different decomposition methods could be classified in to three categories.

a) Wet digestion

The majority of wet digestion methods involve different combinations of five acids (HNO_3, H_2SO4, $HClO_4$, HCl, and HF) and H_2O_2. The choice of an individual acid or combinations of acids depends upon the nature of the matrix to be composed.

The procedure could take place either in open system or closed system (bomb decomposing with conventional or microwave heating) (Santos et al, 2004).

b) Dry ashing

Dry ashing is usually performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. After decomposition the residue is dissolved in acid and transferred to a volumetric flask prior to analysis.

Typical ashing temperatures are 450 to 550 °C. Magnesium nitrate is commonly used as an ashing aid. Dry ashing is also conducted at 50-100°C under reduced pressure in an oxygen plasma discharge.

Keeping its advantage and simplicity, it has some limitation.

These are:

- Losses due to retention to the ashing container and volatilization.
- Contamination from the muffle furnace and sample container
- Physical loss of 'low density' ashes when the muffle furnace door is opened.
- Formation of toxic gases in poorly ventilated area(Santos et al, 2004).

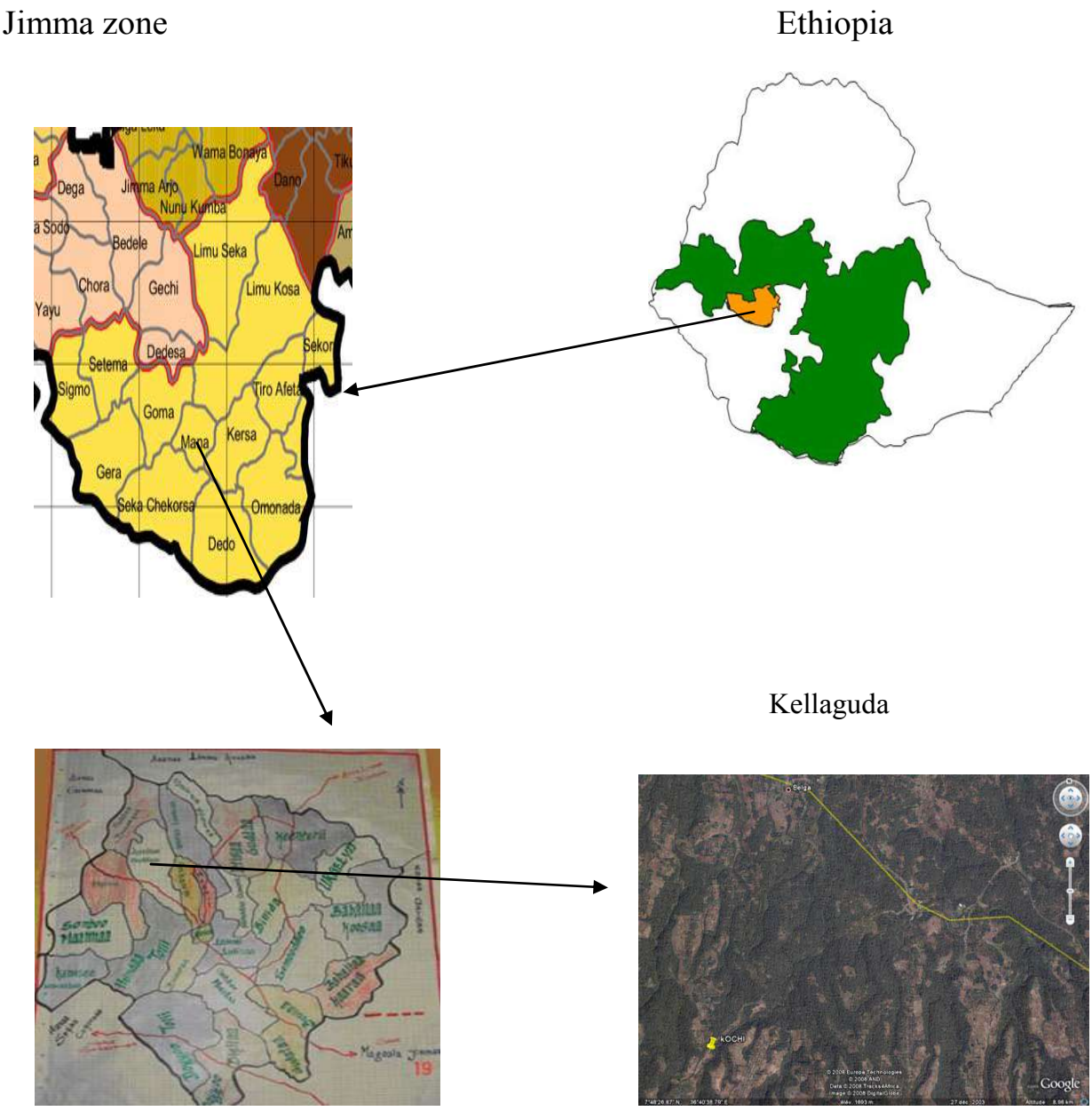
c) Fusion

Fusion is a powerful technique especially both for organic matrices and those with a high silica and alumina content. Since solid and aggressive fusion reagents are difficult to purify, fusion cannot be recommended as ultra-trace analysis. A second disadvantage is that the method is carried out in contact with ambient air. Risks of volatilizations are large (Negash et al, 2002).

3. MATERIALS AND METHOD

3.1. Description of the study area

Mana is one of the Jimma zone woredas in the Oromia Region of Ethiopia It is located at 368 km southwest of Addis Ababa and 20 km west of Jimma town. The district constitutes 12% highland, 65% intermediate highland and 23% lowland with altitude ranges between 1470–2610maltitude.The mean minimum and maximum temperatures are 13.0^0C and 24.8^0C, respectively (ARDO, 2008). Based on long term (15 years) weather data obtained from the nearby JARC meteorological station, the average annual rainfall is 1523 mm. Distric Nitosols and Orthic Acrisols are the dominant soil types with slightly acidic PH, which is suitable for production of coffee, cereals and vegetables(ORG, 2003).According to Wikipedia the landscape of Mana includes mountains, high forests and plain divided by valleys. A survey of the land in this woreda shows that 89.1% is arable or cultivable (86.1% was under annual crops), 2.7% pasture, 2.8% forest, and the remaining 5.4% is considered swampy, degraded or otherwise unusable. Coffee is import ant cash crop for this woreda; over 5,000 hectares are planted. Khat is also another import ant cash crop for this woreda**.** Kelaguda is one of the localities in Mana district where *D.b ulbifera* is commonly cultivated and consumed. According to data collected from Kellaguda administration office (2007) it is situated between 7°46.5 and 7°51.5 in North while 36°40 and 36°42 in East.



Mana wereda

Figure1. Map of the study area (adopted from Google map)

3.2. Apparatus, chemicals, reagents and standard solutions

3.2.1. Apparatus

A hand slicer was used to peel and chop the samples into pieces. Analytical balance (OHAUS Switzerland) was used to weigh the air potato samples, and air dry oven (Contherm 260M) was used to remove the moisture content of air potato samples in the preparation of their powder form for metal analysis. Grinder was used to crush air potato samples in to powder and to pass through 1.00mm sieve. Muffle furnace (SX-2.5-12 model) was used for ashing of air potato samples.750mL Erlenmeyer flask with a reflux condenser and laboratory hot plate, California Modified Buchner Funnel were used for de termination of crude fiber. BUCHI Distillation Unit K-355appratu, BUCHI Digest Auto mat K-438 block digestion apparatus were used for determination of crude protein.Thimb le, glass dish and Soxhlet apparatus for determination of crude fat. Borosilicate beakers (250mL) have been used for the digestion of samples, volumetric flasks 50mL and 100mL (PYREX, UK) were used in holding the prepared sample solutions. Measuring cylinders and pipettes were used during measuring of different volumes of chemicals and reagents. Flame atomic absorption spectrophotometers (Varian spectraAA-20plus models) equippe d with deuterium arc background correctors were used for analysis of the analyte metals using air acetylene flame and UV-VIS Spectrophotometer (DR200/USA model was used for determination of phosphorus.

3.2.2 Chemicals, reagents and standard solutions

Chemicals and reagents used in the analysis were all analytical grade. Working standard solutions of Na, K, Mg, Ca, Fe, Zn, Mn, Cu and P were used for the calibration of instruments and spiking experiments. Double ionized water was used for dilution and preparation of reagents and standards. Concentrated nitric acid (HNO_3) 68%, 30% H_2O_2 and hydrochloric acid (HCl) 35.4% were used for the digestion of the samples.

Concentrated sulphuric acid, sodium hydroxide solution 40% w/w, digestion mixture of Potassium sulphate (K_2SO_4) and copper sulphate ($CuSO_4$), dissolved 4% of boric acid and methyl red were used for determination of crude protein.

Sodium hydroxide solution (0.312N), sulfuric acid solution (0.255 N), prepared ceramic fiber (Cat. No. 1740 M), 95% ethyl alcohol and Dow Corning Antifoam A Emulsion diluted (1+4) with water were used for determination of crude fiber. Petroleum ether was used for the determination of crude fat.

3.3. Cleaning of apparatus

Apparatus such as volumetric flasks, measuring cylinders, beakers, porcelain crucibles, pipettes and polyethylene bottles were soaked with acid, washed with detergents and tap water, rinsed with de-ionized water, dried in oven and then kept in dust free place until analysis of metal was performed. Prior to each use apparatus was soaked and rinsed in de-ionized water.

3.4. Sample collection and pretreatments

Representative samples of the tubers of both wild and domestic *D.bulbifera* were collected from Manna District, Jimma Zone of Ethiopia. The tubers of domestic D.bulbifera were collected from the farm area of three sites *Afeta, Bosoka* and *Kujo,* while that of the wild tuber was collected from forests located in these sites. . From each sampling site, about ten matured plants were randomly collected. Sample collection was restricted only to this particular locality and the harvests of 2017/18 year, in order to reduce the effects of soil type, temperature, topography and other factors on mineral composition of these varieties. Three tubers were taken from each plant and brought to the laboratory of Chemistry Department of Adama Science and Technology University, Ethiopia.

In the laboratory, the samples were washed first with tap water, then by a detergent solution and finally de-ionized water to remove soil particles and dust. Following this the samples were peeled and chopped into slices using a hand slicer.

The slices were then dried in air, powdered with a grinder and passed through a 1.0 mm sieve to remove large debris.

The powdered samples were homogenized according to their varieties (mixed 100 g each) bagged in sealed transparent polythene bottles which were properly labeled and stored in the cold room (4-10°C) for further analysis.

3.5. Proximate Analysis

Ash, volatile matter, fixed carbon and moisture content of the biomass are key parameters determined for estimating the quality of *D.bulbifera*. They were determined following different standard procedure: Each parameter was determined in triplicates.

3.5.1. Determination of Moisture Content

The procedure for the determination of moisture content of the three air potato varieties (wild and domestic) was as outlined by the Association of Official Analytical Chemists (AOAC, 2003). This involves drying of 2.0g air potato samples to a constant weight for 4 hours at 105^0C and calculating moisture content as the loss in weight of the dried samples as follows:

$$MC\% = \frac{W_2 - W_3}{W_2 - W_1}\ x\ 100 \quad (1)$$

Where,

MC= moisture content

W_1 = weight of dry crucible

W_2= weight of moist sample + weight of dry crucible

W_3= weight of dry (sample + crucible)

W_2- W_3= moisture mass

W_2-W_1= initial mass

3.5.2 Determination of Percentage volatile matter (VM %)

To determine VM%, the oven dried sample of each air pota

to varieties were incinerated in (SX-2.5-12 model) muffle furnace at temperature of 950℃ for seven minutes as outlined in ASTM D 3172-73: VM% was calculated as follows:

$$VM\% = \frac{W_2 - W_3}{W_2 - W_1} x\ 100 \quad (2)$$

Where,

VC = Volatile content

W_1 = weight of dry crucible

W_2 = weight of dry (sample + crucible)

W_3 = weight of charred sample

W_2-W_3 = volatile mass

W_2-W_1 = initial mass = mass of dry sample

3.5.3. Determination of percent Ash Content

Total ash content of the samples was determined as described by (AOAC, 2003) and using (SX-2.5-12 model) furnace. All samples after the determination of volatile content of air potato varieties, the charred content was weighed and placed in muffle furnace at 550^{o}C for 4 hours until white ash formed.

The resulting ash removed from the furnace; cooled in desiccators and weighed. The ash content was determined as follows:

$$\text{Ash\%} = \frac{(Ash\ mass)}{(initial\ mass)}\ x\ 100 \qquad (3)$$

Where,

Initial mass= mass of dry sample

3.5.4. Determination of Percentage fixed carbon

The percentage of sample mass that remained after removal of volatile matter and ash content was used to determine the fixed carbon content and the percentage fixed carbon (FC %) was computed by subtracting the sum of VM (%) and AC(%) from 100 as shown in the Equation below:

$$\text{Fixed Carbon} = 100 - ([\text{Moisture}] + [\text{Volatile}] + [\text{Ash}]) \qquad (4)$$

3.5.5. Determination of crude protein

The crude protein content in the sample was determined by Kjeldahl method using (BUCHI Digest Automat K-438) block-digestion apparatus at Jije Labo Glass PLC, Addis Ababa. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture.

The mixture was then made alkaline. Ammonium sulphate thus formed, released ammonia which was collected in 4% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor(6.25) and the amount of protein was calculated (5).

Digestion Procedure:

First1.0 g of dried samples was taken in digestion flask. 12 ml of concentrated H_2SO_4 and 7.8 g of digestion mixture i.e. K_2SO_4: $CuSO_4$ (7: 0.8) were added to it. Then, 3ml of hydrogen peroxide was added to prevent foaming. Then after, the flask was swirled in order to mix the contents thoroughly and the heat side shields were attached to the tube rack. Secondly the exhaust manifold was placed tightly on the tubes and the water aspirator was turned on completely. Next, the rack of tubes was placed in the pre-heated (420°C) digestion block. After 10 min, the water aspirator was turned down until the acid fumes were just contained within the exhaust hood. Digestion was carried out for an additional 50 min; thirdly the digestor was turned off when the digestion mixture bec-ome clear (blue green in color). The rack of tubes with the exhaust still in place was removed and put in the stand to cool for 20 min.

When fuming was stopped, the manifold was removed and the aspirator was shut off and the side shields were removed.The digest was cooled and transferred to 100ml volumetric flask and volume was made up to mark by the addition of distilled water.

Distillation and titration: The digest was transferred to the distillation unit. Distillation of the digest was performed in distillation apparatus (**BUCHI Distillation Unit K-355)**.Ten milliliters of digest was introduced in the distillation tube then 10 ml of 0.5 N NaOH was gradually added through the same way. A conical flask containing 25 ml of 4% the boric acid solution was placed under the outlet of the condenser in such a way that the delivery tube is below the surface of the excess boric acid solution. The distillation unit was adjusted to dispense 50 ml of sodium hydroxide solution.

Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in a conical flask containing 25 ml boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color appears due to NH_4OH.

The distillate was then titrated against standard 0.1 N HCl solutions till the appearance of pink color. A blank was also run through all steps as above. Percent crude protein content of the sample was calculated by using the following formula:

% Crude Protein = 6.25* x %N (*. Correction factor) (5)

$$\%N = \frac{(Sv-Bv) \times N \times 0.014 \times Vd \times 100}{\text{Wt. of the sample} \times V} \quad (6)$$

Where

Sv = ml of sample titration reading

Bv = ml of blank titration reading

N = Normality of HCl

Vd = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

3.5.6. Determination of crude fat

Dry extraction method for fat determination was implied (AOAC, 2003). Fats were determined by intermittent soxhlet extraction apparatus. Crude fat was determined by ether extract method using Soxhlet apparatus at Jije Labo Glass PLC, Addis Ababa.

Procedure: Approximately 2 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater were turned on to start extraction.

After 4-6 siphoning at condensation rate of 5-6/second for 4hrs the ether was evaporated and the beaker was disconnected before last siphoning. The extract was transferred into clean glass dish with ether washing and the ether was evaporated on water bath.

Then the dish was placed in an oven at 105^{o}C for 2hrs and cooled it in desiccators. The percent crude fat was determined by using the following formula:

$$\%\text{Crude Fat} = \frac{\text{Wt. of ether extract} \times 100}{\text{Wt.of sample}} \quad (7)$$

3.5.7. Determination of crude fiber

Principle: Crude fiber was determined gravimetrically (ISO5498 (AOAC962.09) after chemical digestion and solubilization of other materials present at Jije Labo Glass PLC, Addis Ababa. The fiber residue weight is then corrected for ash content after ignition.

Procedure: Accurately about 2g(W_0) of sample was weighed and transferred to a 9 cm hard filter paper supported on supported on a filter cone in a 60 ° funnel. Then it was extracted with three 25mL portions of ether and vacuum was applied until sample was dried. Then the extracted sample was transferred quantitatively by brushing into a 75mL Erlenmeyer flask fitted with a reflux condenser. 20mL of well-mixed ceramic fiber suspension (containing about 1.5 g of fiber - dry weight), 200mL of boiling 1.25% sulfuric acid solution, and 1 drop of diluted antifoam were added.

The Erlenmeyer flasks with a reflux condenser was placed on a hot plate and boiled exactly for 30 minutes, rotating flask periodically to keep solids from adhering to sides. The flask was then removed and contents were filtered through California Buchner funnel precoated with about 0.75g of ceramic fiber - dry weight. Then after, the flask was rinsed with 75mL of boiling water, and washed through funnel. This was repeated with three 50mL portions of water, and sucked dry. Fiber mat with residue was returned to flask by blowing back through funnel. 200mL of boiling 1.25% sodium hydroxide solution was added, returned to heater and boil exactly 30 minutes. The flask was removed and the sample was filtered as before.

The sample was then washed with 25 mL of boiling 1.25% sulfuric acid solution, three 50mL portions of water, and 25mL of alcohol. Mat and residue were removed, and transferred to ashing dish.

Fiber mat and residue were dried at 130^{o}C for 2 hours and cooled in a desiccator and weighed (W_1).

Then, they were ignited at 600^{o}C to constant weight 30 minutes and cooled in desiccator and weighed (W_2).

Calculation was done by using the formula:

$$\%\text{Crude Fiber} = \frac{(W_1 - W_2) \times 100}{W_0} \quad (8)$$

Where

W_0= mass sample

W_1= mass of oven dried fiber mat and residue

W_2 = mass of furnace ignited fiber mat and residue

3.5.8. Carbohydrate

Total carbohydrate was calculated by the difference between 100 and the sum of moisture, ash, crude fat, crude protein and crude fibre as outlined in (AOAC, 2003) by the formula:

% CHO = 100 - (% moisture+ %protein + % ash+ %fat + % Crude fiber) (9)

3.5.9. Energy calculation

The caloric value of the sample was calculated using "Atwater factor" by multiplying the value of the crude protein, lipid and carbohydrate by 4, 9, 4 respectively and taking the sum of the as outline in (AOAC, 2003).The values were then converted to calories per 100gm of the sample.

3.6. Preparation of the Samples for Metals Analysis

3.6.1. Sample Digestion

A 1.0g of each powdered *D. bulbifera* variety was weighed and transferred in to a clean 250ml borosilicate flasks labeled according to the sample type, with 3glass beads. Cautiously, 10mL 50% $HNO_3$was added to the flasks and covered with ribbed watch glass and heated to 95^oC. Then the solution was refluxed for 15 minutes and cooled. 5mL conc.HNO_3 was added to the solution and refluxed for another 30 minutes at 95^oC.

The resulting solution was again cooled. After that, 5mL conc. HNO_3 was added and it was refluxed for another 30 minutes at 95^oC until evaporation to about 5mL. After cooling, 2mL DI water and 3mL 30% H_2O_2were added.

Then the flask was covered with watch glass and heated slowly (to avoid losses by excessive reaction) to initiate peroxide reaction.

Heating the flasks was continued until effervescence subsides. The beaker was cooled and 7mL 30% H_2O_2 (1mL at a time) was added while heating. The resulting solution was cooled; 5mL conc. HCl, and 10mL DI water were added. Covering with watch glass the solution was refluxed for an additional 15 minutes without boiling. The digest was cooled, diluted with 20mL of distilled water and transferred into a 50mL volumetric flask while filtering with Whatman® filter paper No. 541. Lastly the sample was diluted to the mark of 50mL standard volumetric flask with DI water.
The diluted solution was transferred to polyethylene bottle and stored in refrigerator until analysis. All samples were prepared identically in triplicates.Six blank solutions for both type of samples were prepared to check for background contamination following the same procedure of sample preparation.

3.6.2. Determinations of the Minerals

a) Determinations of the Metals

Intermediate standard solutions containing 100 mg/kg were prepared in 1000mL volumetric flask from the atomic absorption spectrophotometer standard stock solutions that contained 1000 mg/kg. Four working standards for each metal of interest were prepared from these intermediate standard solutions and used for calibration and spiking purposes. Then, Na, K, Mg, Ca, Fe, Zn, Cu, and Mn were analyzed by flame atomic absorption spectrophotometer (Varian spectraAA.20plus) model equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the alignment of parameters (burner, lamp current, slit width and wavelength) were optimized for maximum signal intensity of the instrument.

For each element, respective hollow cathode lamp was used, and the solution was successively aspirated into the flame. Three replicate determinations were carried out

for each sample at the laboratory of Oromia Water Works Design and Supervision En -terprise, Addis Ababa.The elements were determined by absorption/concentration mode and then the instrument readout was recorded for each solution. The same analytical procedure was employed for the determination of elements in the six digested blank solutions.

Table5. Instrumental operating conditions for the determination of metals in *D.bulbifera* samples

Element	Wave length (nm)	Lamp current (mA)	Slit width (nm)	Instrument detection limit (mg/kg)	Flame type
Na	589.0	5	0.5	0.006	Air acetylene
K	766.5	5	1.0	0.006	"
Mg	285.2	4	0.5	0.006	"
Ca	422.7	10	0.5	0.005	"
Fe	248.3	5	0.2	0.006	"
Mn	279.5	5	0.2	0.006	"
Cu	324.8	4	0.5	0.007	"
Zn	213.9	5	5.0	0.002	"

b) Determination of phosphorus – Photometric method

Phosphorus in the sample was determined at the laboratory of Ethiopia Construction Design and Supervision Corporation, Addis Ababa by Uv-VIS spectrophotometer (DR200/USA model) according to AOAC 965.17 procedure as described below:

Molybdovanadate reagent preparation

40 g ammonium molybdate $4H_2O$ was dissolved in 400mL hot DI and water cooled. 2 g ammonium metavanadate was dissolved in 250mL hot DI water and cooled; 250 mL 70%

$HClO_4$ was added. Gradually molybdate solution was added to vanadate solution with stirring, and diluted to 2 litre.

Phosphorus standard solutions preparation:

(i) Stock solution -2 mg/mL. 8.788 g KH_2PO_4 was dissolved in DI H_2O and diluted to 1L

(ii).Working solution – 0.1 mg/mL. 50 mL stock solution was diluted to 1L.

Preparation of standard curve:

i) Aliquots of working standard solution containing 0.5, 0.8, 1.0 and 1.5mg P were transfe rred to100mL volumetric flasks). Standard Curve was prepared by plotting mg P against per cent T on semi log paper.

ii) Determination using U-VIS spectrophotometer: 2g sample was ashed in 150mL beaker about 4 h at 600ºC. Then it was cooled, 40mL HCl (1+3) and several drops HNO_3 were added and brought to boiling point. Then after, it was cooled, transferred to 200mL volumetric flask and diluted to volume with DI H_2O. Aliquot containing 0.5-1.5 mg P was filtered and placed in 100mL volumetric flask. 20mL molybdovanadate reagent was added and diluted to volume with DI H_2O, mixed well and stood 10 min. Then percent T was read at 400nm against 0.5 mg standard set at 100% T; mg of P standard determined was from curve.

Calculation: P (%) = mg P in aliquot / (g sample in aliquot x 10)

Recover test of metal analysis

In the absence of certified reference material for the *D. bulbifera* samples, the validity of the optimized procedure was checked by adding known concentration of each metal in 1.0g sample from the stock solution. Spiking process was preformed. The resulting spiked samples were digested, diluted and analyzed for the metals of interest. Levels of Na, K, Mg, Fe, Mn, Ca, Zn and Cu metals in the spiked samples were also analyzed at Addis Ababa University. The spiking procedure was as below:

For domestic *D.bulbifera*: 10.00 µg of Na, 10.00 µg of Ca, 10.00 µg of Mg and 5.00µg of Fe were spiked at once in to one beaker containing 1.00 g of sample and the remaining metals: 10.00 µg of K, 0.04 µg of Cu, 3.4µg of Zn and 0.14 µg of Mn, were spiked at once in to another beaker containing 1.00g of sample.

For wild *D. bulbifera:* 10.00 µg of Na, 10.00 µg of Ca, 10.00 µg of Mg and 5.00µg of Fe were spiked at once in to one beaker containing 1.00 g of sample and the remaining metals: 10.00 µg of K, 0.04 µg of Cu, 3.4µg of Zn and 0.14 µg of Mn, were spiked at once in to another beaker containing 1.00g of sample and the same digestion system was performed. The spiking levels were chosen based on the levels that will double the sample concentration. Spiking process was performed in two categories to minimize the effect that might be arise due the concentration of one metal suppress the concentration of other metal.

Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer. The recovery test for each sample was performed in triplicates.

$$R\% = \frac{([\text{Concentration in Spiked Sample}]-[\text{Cononcentration in Unspiked Sample}])}{[\text{Total standard added}]} \text{ x } 100 \qquad (10)$$

3.7. Method detection limit

Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero, and is determined from analysis of a sample in a given matrix containing the analyte (Theodore et al., 2003).

The limit of detection is most commonly defined as the amount of analyte that gives a signal equal to three times the standard deviation of a blank. In this study, after digestion of six blank solutions six readings were taken for each blank and the standard deviations of these were calculated.

The calculated value in such away represent the measured minimum concentration of the analytes and the values reported with 99 % confidence that the analyte concentration greater than zero. The method detection limit of each element was

obtained by multiplying the standard deviation of the reagent blank by three(Jeffrey et al., 996).

$MDL = 3XSD_{blank}$ (11)

SD_{blank} - is the standard deviation of the blank readings

3.8. Data Analysis

The organized data were analyzed using SPSS software and Microsoft Excel 2007. The calibration curve of each element from its respective standards used was analyzed by Microsoft Excel 2007. Mean comparisons, variations in levels of minerals of interest and standard deviation of the triplicate measurement for each mineral of interest in each *D.bulbifera* varieties were analyzed using SPSS software. Descriptive data have generated and presented as means ± standard deviation for the results of proximate analysis and concentration of minerals in each sample. The mean variations in data among two D.*bulbifera* varieties were analyzed using T-test. The variation in mean values resulted are considered as significant if the calculated p-values <0.05 at 95% confidence interval.

4. RESULTS AND DISCUSSION

4.1. Proximate and nutritional composition of *D.bulbifera* varieties

a) Moisture Content

The results of moisture content analysisof the two air potato varieties were given in table 6. Domestic *D.bulbifera* contained high moisture content of 6.73%, while moistu re content of wild *D.bulbifera* is 5.83% (Table 6). These values were higher than value s (4.58%) and (3.55%) reported by (Polycarp, 2012) and(Celestine, 2015), respectivel y.However, the values were lower than values (9.20%) reported by (Abara, 2011) for the same species. In terms of natural product stability, moisture content higherthan15 % tends to promote microbial contamination and chemical degradation as it provides a medium for many reactions to occur (Polycarp, 2012). The higher moisture content of domestic *D.bulbifera* also indicated that,it dead to spoilage due to its susceptibilitytom icrobialattackmoreeasilythanthewild*D.bilbifera.*

b) Volatile Matter Content

The results of Volatile Matter content analysis of the two air potato varieties were give -n in table 6. Domestic D.*bulbifera* contains higher volatile matter content (77.20 %) than *D.bulbifera* (76.28%). This indicates that, the domestic D.*bulbifera* contains more products exclusive of moisture that is given off as gas or vapor due to high temperatur e (ASTM, 1954).

c) Ash Content

The results of ash content analysis of the two air potato varieties were given in table 6. The ash content was 2.37% for domestic *D.bulbifera* and 2.58% for wild *D.bul bifera*. The ash content of both varieties is in the line with the value 2.86% that report- ed by (Shanta-kuwri et al., 2008) for the same species.

The ash content is generally recognized as a measure of quality for the assessment of the functional properties of foods. The higher ash content of wild *D.bulifera* indicates higher minerals content (Onwuka et al., 2005). It indicates wild *D.bulifera* has better mineral absorption ability.

d) Crude protein

The crude protein content was 6.10% for domestic *D.bulbifera* and 7.1% for wild *D.b ulbifer*a as shown in (Table 6). Thus the domestic *D.bulbifera contained* higher level o f protein than the wild one. The obtained values of protein are higher than 3.40% repor ted by (Subhash et al., 2012) but lie in the line with the l value of 6.35% that reported by (Abara, 2011) for the same plant. The protein content of yams varies considerably among species and between cultivars due to climate, maturity at harvest and length of storage time. These factors may be responsible for the differences observed in the crude protein content in this work and other works (Abara, 2011). According to (Pam-ela *et al.,* 2005), proteins from plant sources have lower quality but their combination with many other sources of protein such as animal protein may result in adequate nut ritional value.

e) Crude fibre

The crude fibre obtained for domestic *D.bulbifera*was 3.03% and 6.20% for wild *D.bulbifera* as shown in (Table 6). The value obtained for domestic *D.bulbifera* is comparable to 3.8% the values that reported for the same plant by (Subasini et al., 2013). The value obtained for wild *D.bulbifera* is comparable to 7.97% which was reported by (Celestine, 2015) for the same plant. Crude fibre in food or plant is an indication of the level of non-digestible carbohydrate and lignin Thus, the lower fiber content of domestic *D.bulbifera* is good in aiding absorption of glucose and fat and the higher level fiber content of domestic *D.bulbifera* is good in enhancing digestibility. However, its presence in high level can cause intestinal irritation, lower digestibility and decreased nutrient usage (Oladiji et al., 2005).

The differences observed in the crude fiber content in this work and other works might attributed to cultivars, climate and maturity at harvest differences (Abara, 2011).

f) Crude fat

The crude fat content obtained was1.03% for domestic *D.bulbifera* and 1.07% for wild *D.bulbifera* as shown in (Table 6). The obtained values of crude fat are comparable to 1.20% that reported by (Subhash and Sarla, 2012) but lower than 3.30% reported by

(Celestine, 2015) but higher than 0.55% reported by (Polycarp, 2012). Fat provides very good sources of energy and aids in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes (Jones *et al.,* 1985, Pamela *et al.,* 2005).

g) Carbohydrate

The results of carbohydrate content analysis of the two air potato varieties were given in table 6. Domestic *D.bulbifera* contained carbohydrate content 80.74% while the wild *D.bulbifera* carbohydrate content is 77.21%. These values are comparable to literature values 76.68% reported by (Celestine, 2015) and 82.50% by (Abara, 2011). The carbohydrate content of yam differs due to age of tuber, species and cultivar differences (Abara, 2011). The plant is a high source of carbohydrate when compared with the Recommended Dietary Allowance (RDA) of 130g (Pamela *et al.,* 2005). High percentage of carbohydrates shows that bulbils are energy food that can contribute to food security in developing countries (FAO, 2001). They were low in fat and protein contents as compared to carbohydrate content.

h) Caloric value

The caloric value of domestic *D.bulbifera* was 356.63kcal/100g and that of wild *D.bulbifera* was 346.91kcal/100g. An average person requires 2000-3000 kcal per day (Jones *et al.,* 1985). The plant can contribute to the caloric requirement of the body. With this energy value, both (*Dioscorea bulbifera*) varieties could be used as energy in the flour porridge for infants and children (Butte, 1996).

Table6.Average proximate and nutritional composition of domestic and wild *D.bulbifera* varieties (Mean ± SD)

Proximate components	DDB	WDB
Moisture (dry weight) (%)	6.73±0.10	5.83 ± 0.018
Volatile matter (%)	77.20 ± 0.42	76.28 ± 0.71
Ash (%)	2.37 ± 0.15	2.58 ± 0.11
Fixed carbon%	13.70 ± 0.05	15..31 ± 0.51
Crude Protein (%)	6.10 ±0.01	7.11± 0.11
Crude fats (%)	1.03± 0.03	1.07± 0.01
Crude fibre **(%)**	3.03± 0.02	6.20± 0.10
Carbohydrate (%)	80.74±0.34	77.21±0.25
Energy value kca/100 g)	356.63±0.27	346.91±0.56

Where DDB refers to domestic *D.bulbifera* and WDB refers to wild *D.bulbifera*

4.2. Results of Method Validation for metal analysis

Instrument Calibration

The qualities of results obtained for essential and toxic metals analysis can be affected bythe calibration and standard solution preparations procedures.

The instrument was calibrated using four series of working standards. Working standa rd solutions of each metal were prepared freshly by diluting the intermediated standard solutions (Table 7). The calibr-ation graph of each of metal of interest is shown in Appendix 2.

Table 7: Series of working standards and correlation coefficients of the calibration curves for the determination of metals in the barley varieties using FAAS

Metal	Concentration of Standards (ppm)	Absorbance	Regression Equation	Correlation Coefficient (R)
Na	5, 10, 15, 20	987748.90, 1845524.56, 2766395.52 ,3639355.02	Y=17751x+90834	0.999
K	5, 7.5, 10, 15	1528.88, 2217.12, 3062.18, 4568.64	Y= 306.5x-30.05	0.999
Mg	0.25, 0. 5,0.75,1	0.1878, 0.3755, 0.7292	Y=1.115 + 0.001	0.996
Ca	0.25, 0.5,1.0,2.0	0.2423, 0.4442, 0.8091	Y=0.018x-0.00	0.999
Fe	5, 10, 15, 20	0.13511, 0.27160, 0.40635, 0.54056	Y= 0.027x+0.000	0.999
Mn	2.5, 5, 10,20	0.062, 0.127, 0.239,0.462	Y=0.022x + 0.009	0.999
Cu	0.5, 1, 1.5, 2	0.072,0.143,0.214, 0.285	Y=0.142x + 0.001	0.999
Zn	0.25, 0.5, 0.75, 1.0,	0.02105, 0.04079, 0.06346, 0.08486	0.085x-0.001	0.999

Method Detection Limits (MDL)

Six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Na, K, Mg, Ca, Fe, Zn, Cu & Mn) using flame atomic absorption spectrophotometers (Table 8). The standard deviation for each element was calculated based on equation (11) from six blank measurements for the determination of method detection limit (MDL) by SPSS Statistics 20.

Table 8: Method detection limit for metals analysis in DDB & WDB samples (n=6)

Metal	MDL(mg/kg)
Na	1.98
K	0.36
Mg	1.30
Ca	0.89
Fe	1.03
Mn	0.54
Zn	0.30
Cu	0.21

Recovery test (R %)

The optimum digestion procedure chosen was the one that requires 2hrs and 15 minute for complete digestion of 1.0 g of raw tubers of both domestic and wild D.bulbifera with 15mL HNO_3 (68 %), 10 mL H_2O_2 (30 %) and 5mL 35.4 % HCl depending upon: clarity of digests, minimal digestion time, and minimum reagent volume consumption, absence of undigested D.bulbifera samples, simplicity and low heating temperature. The recoveries of the detected metals (Ca, Mg, K, Na, Fe, Mn, Zn and Cu) in the spiked samples for raw domestic *D.bulbifera* tubers lies within the range 84.12 – 110.11% and for raw wild *D.bulbifera* tubers 82 – 115.2 % as shown in (Table 9) and (Table 10) respectively. They are within the accepted range 80-120 %.

In general, good recoveries were obtained for metals which were detected in the present study. Thus, the optimized procedure has good accuracy and precision.

The correlation coefficients (R-values) ranges from 0.996 – 0.999 from the calibration curves (Appendix 2). Thus, the obtained calibration curves were fairly linear, which assured that linearity of instrumental response for individual analyte.

Table 9: Recovery test for domestic D.bulbifera sample

Sample	Metal	Con in unspiked Sample (mg/kg)	Amount added (mg/kg)	Con in Spiked sample (mg/kg)	Recovery (%)
Domestic D.bulbifera (DDB)	Na	7.34	10	16.99	96.46%
	K	215.33	10	225.55	102.15%
	Mg	17.53	10	26.4	88.7%
	Ca	12.51	10	21.80	98.29%
	Fe	2.34	5	7.66	106.3%
	Zn	2.86	3.5	5.80	84.12%

Table 10: Recovery Test for wild D.bulbifera

Sample	Metal	Con in un spiked Sample (mg/kg)	Amount added (mg/kg)	Con in Spiked sample (mg/kg)	Recovery (%)
Wild D.bulbifera (WDB)	Na	14.75	10	22.95	82%
	K	313.06	10	322.38	93.23%
	Mg	19.0	10	30.52	115.2%
	Ca	25.54	10	34.69	91.5%
	Fe	4.55	5	10.15	112%
	Zn	1.46	3.5	4.93	99.1%
	Mn	BDL	-	-	-
	Cu	**BDL**	**-**	**-**	**-**

4.4. Levels of metal contents in the analyzed D.bulbifera samples

Potassium (K)

The concentration of potassium in the *D.bulbifera* varieties under this study was found to be 312.90 mg/kg for wild D.bulbifera and 210.03mg/kg for domestic *D.bulbifera*as shown in (Table 11). Statistical T-test ($P<0.05$) revealed that there is significant variation of potassium concentration between the *D.bulbifera* varieties. The detected levels of K in both tuber varieties were higher than 160.38mg/kg reported by (Subasini et al., 2013) but lower than 3167.7mg/kg report for the same species by (Cel estine, 2015).

From the analyses carried out, it was quite evident that potassium is one of the second elements next to phosphorus. The estimated minimum requirement by adolescents and adults are 2.0 g/day. However, many nutritionists now recommend higher dose of 3.5g to reduce the risk of high blood pressure (FNB, 2004). The concentration of potassium analyzed in the varieties is safe to use these varieties as a source of potassium.

The differences observed might be due to environment and maturity at harvest factors menti-oned earlier.

Magnesium (Mg)

In the present study the mean values of magnesium was 16.92mg/kg for domestic *D.bulbifera* and 21.43mg/kg for wild *D.bulbifera* as shown in table11. Statistical T-test ($P<0.05$) revealed that there is insignificant variation of magnesium concentration between the *D.bulbifera* varieties. The values are lower than 835mg/kg that was reported by (Polycarp, 2012).The use of these varieties in diet is safe as the food and nutrition board of the institute of medicine (FNB, 1997) recommended up to 400 mg magnesium per day. Therefore, these D.bulbifera varieties can be consumed safely as source of Mg.

Sodium (Na)

The level of sodium content of was 7.42mg/kg for domestic *D.bulbifera* and 9.43mg/kg for wild *D.bulbifera*as shown in table 11. Statistical T-test ($P>0.05$) revealed that there is insignificant variation of sodium concentration between the *D.bulbifera* varieties. The

detected levels of Na in both tuber types were higher than 1.81mg/kg that reported by (Subasini et al., 2013) but lower than 385.2mg/kg that reported for the same species in India by (Celestine, 2015). The nutritional requirement for sodium is about 1100 – 3300 mg (FNB, 1989), however, much sodium is added to food in sodium chloride form and the contribution of unprocessed food source is only about 10 to 15%. Thus, the analyzed level of sodium could be safe to consume these *D.bulbifera* varieties as good source of diet. Because of the reciprocal effects of Na and K authorities have argued that a diet high in potassium and low in sodium (low urinary Na and K ratio) favours lower blood pressure. Increasing dietary potassium as the chloride salt has shown to decrease blood pressure in some hypertensive individuals. It is also possible that a low Na and high K diet would decrease the development of cardiovascular disease (Celestine, 2015).

Calcium (Ca)

As shown in table 11 the Ca concentration was found to be 12.92 mg/kg for domestic *D.bulbifera* and 24.45mg/kg for wild *D.bulbifera*. Calcium was the forth abundant mineral found in wild *D.bulbifera* and the third abundant mineral found in wild *D.bulbifera*.Statistical T-test ($P<0.05$) revealed significant variation of calcium concentration between the *D.bulbifera* varieties. The detected levels of Ca in both tuber types were lower than values report for the same species in India by (Subasini et al., 2013) which is 79.05mg/kg. The concentration of Ca differs between caltivars due to amount of water available, time of planting, composition of soil upon it was grown, experimental method of analysis and cultivar differences (Abara, 2011).

Iron (Fe)

Iron was found with its higher mean concentration in domestic *D.bulbifera* (2.54mg/kg) and lower mean
concentration (1.78 mg/kg) in wild *D.bulbifera* (Table13).

T-test shows that there was significant difference ($p<0.05$) in levels of Fe between *D.bulbifera* varieties. The detected levels of Fe in both tuber types were lower than

39.0mg/kg the values report for the same species in India by (Shanthakumari et al., 2008) but higher than 0.4740mg/kg that reported by (Subasini et al., 2013) for the same species. The differences observed between the result of the study and from the other works might be due to composition of soil upon it was grown, experimental method of analysis and cultivar differences (Abara , 2011).

In humans, iron is an essential component of hundreds of proteins and enzymes. Iron is very important in the formation of haemoglobin in red blood cells and deficiency of iron leads to anemia. The recommended daily requirement of iron for man is 6 – 40mg/kg (Bolt et al., 1978). The two varieties contain the element (Fe) with level below the maximum tolerable limit, thus can be consumed safely as important source of it.

Zinc (Zn)

From the present results (Table 11) it can be seen that, the mean level of Zn was 0.39mg/kg for domestic D.bulbifera (DDB) and 0.43 mg/kg for wild D.bulbifera (WD B). T-test revealed that there insignificant differene ($p>0.05$) in levels of Zn between t he two samples. These values are comparable to literature value1.7mg/kg that is report ed by (Celestine , 2015) and 1.2825mg/kg reported (Subasini et al., 2013). The low lev el of Zn in D.bilbifera is associated to the presence of phytic acid which will form inso luble complex (Abara, 2011).

The concentration of Zn obtained from this study is below the maximum tolerable limit (FAO/WHO, 2001) guidelines (99.4 mg/kg).Thus, D.bulbifera varieties can be consumed safely as important source of it. Zinc containing organic compounds is employed as astringent and antifungal agents. It aids wound healing and metabolism of nucleic acid and insulin. Zinc in excess causes anemia and if deficient in the body can lead to dermatitis (FNB, 2004).

Phosphorus (P)

As shown in (Table 11) the level of phosphorus was 2068.40mg/kg for domestic *D.bulbifera* DDB and 1525.64mg/kg for wild *D.bulbifera* WDB. Phosphorus levels that

were analyzed in the two D.bulbifera varieties were higher when compared to 1449.3mg/kgt he value reported for the same species by (Celestine, 2015) and659.8mg/kg that was reported by (Libra et al., 2011) (Table, 13).

D.bulbifera and wild *D.bulbifera* varieties showed significant variation ($P < 0.05$). Food and Nutrition Board, Institute of Medicine, National Academies gave Dietary Reference Intakes (DRIs) for phosphorus as 4000mg/d of tolerable upper intake level**s** for adults .A normal diet provides approximately 20mg/kg/day of phosphorus. Thus, *D.bulbifera* varieties can be consumed safely and it can be considered as good source of phosphorus). The phosphorus content of the (*Dioscorea bulbifera*) varieties is an indication that the food products of this plant will help in the formation of teeth and bones in children and their proper development.

Table 11: Result of average concentrations of macro and micro elements and proximate in D. bulbifera samples and statistical p-values (Average con. as mean ± Std, n=3)

a. Proximate profile of the two types of *D. bulbifera* tubers

	DDB	WDB	T-test	
Proximate components	Mean ± Std	Mean ± Std	$P_{0.05}$	significance
Moisture (dry weight) (%)	6.73±0.10	5.83 ± 0.018	0.001	significant
Volatile matter (%)	77.20 ± 0.42	76.28 ± 0.71	0.142	insignificant
Ash (%)	2.37 ± 0.15	2.58 ± 0.11	0.015	significant
Fixed carbon%	13.70 ± 0.05	15..31 ± 0.51	0.010	significant
Crude Protein (%)	6.10 ±0.01	7.11± 0.11	0.003	significant
Crude fats (%)	1.03± 0.03	1.07± 0.01	0.567	insignificant
Crude fibre **(%)**	3.03± 0.02	6.20± 0.10	0.001	significant
Carbohydrate (%)	80.74±0.34	77.21±0.25	0.002	significant
Energy value kca/100 g)	356.63±0.27	346.91±0.56	0.001	significant

b. Mineral profile of the two types of *D. bulbifera* tubers

	DDB (mg/kg(	WDB (mg/kg)	T-test	
Minerals	Mean ± Std	Mean ± Std	$P_{0.05}$	Significance
Na	7.42 ±1.2	9.43± 0.71	0.175	Insignificant
K	210.03± 0.32	312.90 ± 11.32	0.004	Significant
Mg	16.92± 0.94	21.43 ± 0.60	0.002	Significant
Ca	12.92 ± 3.54	24.45 ± 3.95	0.02	Significant
Fe	2.54 ± 0.18	1.78 ± 0.26	0.018	Significant
Zn	0.39 ± 0.06	0.43± 0.15	0.314	Insignificant
Cu	BDL	-	-	-
Mn	BDL	-	-	-
P	2068.40 ± 28.92	1525.64 ±18.94	0.001	Significant

Where DDB refers to domestic *D.bulbifera* and WDB refers to wild *D.bulbifera*

Table 12: Comparison of proximate in *D.bulbifera*with literature values of the same plant

Proximate	Present study	Subasini et al	Celestine	Shathakumari et al
Crude protein (%)	6.10 -7.11	4.7	6.18	15.75
Carbohydrate (%)	77.21-80.74		76.68	-
Crude fat (%)	1.03-1.07	-	3.30	8.13
Crude fiber (%)	3.03-6.20	3.8	7.97	3.92
Ash (%)	2.37-2.58	-	2.33	2.86
Energy(kca/100g)	346.91-356.63	-	1530.61	-

Table 13: Comparison of metal concentration (mg/kg) in *D.bulbifera*with literature values of the same plant

Element	Present study (mg/kg)	Subasini et al (mg/kg)	Celestine (mg/kg)	Shajeelaet al (mg/kg)
Na	7.42-9.43	1.81	385.2	782.4
Ca	12.92- 24.45	79.05	1744.4	3381.5
Mg	16.92 -21.43	-	7589.4	3962.0
K	210.03-312.90	160.38	3167.7	15543.6
Zn	0.39 – 0.43	1.2825	1.7	14.8
Fe	1.78-2.54	0.4740	16.1	192.0
P	1525.63-2068.40	-	1449.3	1544.2

4.5. Comparison of levels of minerals in *D. bulbifera* with other tuber plants and Cereals

Results of the present study are compared with other varieties of dioscorea species grown in other parts of the world and other tuber staple foods enset (*Ensete ventricosum*) food products kocho which is locally used in order to evaluate the potential of *D. bulbifera* to supplement mineral nutrients. As it can be seen from (Table, 15) concentration ranges of the studied metals of *D.bulbifera* tubers are lower with significant differences.

This variation is not only due to species variability but also variations in agricultural practices and other parameters listed so far.

Let alone different crops, even for a particular species, the concentration level generally decreases in the order root >stem > leaves > fruit > seed when the source of the mineral element is only the soil (U. (Subasinietal., 2015). As shown in (Table,14) when the metal levels of D.bulbifera are compared with that of cereal like barley, maize, wheat and sorghum reported by (Wodaje, 2015), the studied tuber cultivars accumulated similar concentration of Fe and Zn with maize but contains lower levels of these metals than barley, wheat and sorghum.

Table14: Comparison of the analyzed concentration of metals in D.bulbiferasamples with literature values of other underground tubers pants (mg/kg)

Tuber plant		Macro metals				Reference(s)
		Na	K	Mg	Ca	
D.bulbifera	*Analyzed*	7.42-9.43	210.03-312.90	16.92 -21.43	12.92- 24.45	This study
Kocho from *Ensete ventricosum*	Reported	462–688	2,753–4,380	180–290	498–584	Atlabachew et al., 2008
S.tuberosum	Reported	17.1–377	4,890–5,989	221–2,450	50.6–87.3	Reveroetal., 2003
D.abyssinica	Reported	133–405	8,469–13,914	180–354	172–448	Aregahegn et al., 2013
M. esculenta	Reported	46.9–63.7	2,600–3,900	700–1,300	700–900	Adeniji et al., 2007

Tuber plant		Micro metals				Reference(s)
		Fe	Mn	Zn	Cu	
D.bulbifera	Analyzed	1.78-2.54	BDL	0.39 – 0.43	BDL	This study
S.tuberosum	Reported	7.19–10.4	1.43-1.77	2.18–4.58	0.54-1.72	Reveroetal., 2003
M. esculenta	Reported	117.3-184.2	24.5–32.3	5.6–8.5	4.6-10.9	Adeniji et al., 2007
Kocho from *Ensete ventricosum*	Reported	92.5–135	8.58–10.13	31–32.1	3.4-4.3	Atlabachew et al., 2008
D.abyssinica	Reported	28.3-144.7	12.0-14.5	12.3-44.5	7.26-17.6	Aregahegn et al., 2013

Table15: Comparison of the analyzed concentration of major metals in D.bulbifera samples with literature values of other cereals (mg/kg)

Metal	D.bulbifera	Barley	Wheat	Maize	Sorghum	Reference
	This study	Reported	Reported	Reported	Reported	
Fe	1.78-2.54	31.85	10.64	0.40	36.45	(Wodaje, 2015)
Zn	0.39 – 0.43	3.85	8.54	0.66	5.40	(Wodaje, 2015)
Cu	BDL	0.15	1.72	0.13	1.25	"
Mn	BDL	1.67	7.67	0.42	2.34	"

The absence of the toxic heavy metals in tuber indicates the safety of the food resource with respect to heavy metal toxicity, perhaps the plant is not a high accumulator of the elements or the soil load is minimal. The Na/K ratio (0.12 for tuber) is <1.0, an indication that the food resource has the potential to prevent hypertension. Their Ca/P ratio was found to 1.16.These value is higher than 1.0 suggesting that the food has the potential to promote calcium absorption in the intestine.

5. CONCLUSION AND RECOMMENDATION

The present study tried to determine contents of the basic chemical nutrients including crude fats, ash, crude fibre, carbohydrate, calorific value, crude protein and moisture and the concentration of minerals(Na, K, Mg, Ca, Mn, Cu, Zn, Fe and P) in raw domestic and wild D.bulbifera, collected from **Kelaguda** locality of Mana woreda.

This study T-test significant test method confirmed the two types of tubers of *D. bulbifera* are not differ significantly in their contents of the basic chemical nutrients crude fats and moisture content but differ significantly in their contents of fiber, protein, ash, carbohydrate and energy value . The contents of carbohydrate and fibre observed in the tubers of this yam species are remarkably high, indicating its high potential to provide dietary energy and lower the risks of several human health disorders.

T-test significant test method confirmed significant differences in the level of the majority of the minerals (K, Mg, Ca, Fe and P) between the two varieties. The wild *D.bulbifera* contains higher amount of the interested minerals than domestic D.bulbifera sample. Moreover, K and P are the most accumulated minerals in both D.bulbifera varieties

Overall, the results of this research work gives a general conclusion that, the two *D.bulbefera* varieties grown in Mana wereda Kelaguda locality can be consumed as good sources of proximate, P and K elements. Since the mineral contents of wild *D.bulbifera* is higher than the domestic one, it is recommended to incorporate it to meal as regularly as the domestic one rather than having low attention to it. However, further investigation is recommended with more sampling areas especially on great P and K contents variation between the two *D.bulbifera* varieties.

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7. APPENDICES

Appedisx1. Figures of apparatuses, instruments used and photos of activities during the study

a) Soxhlet apparatus

b) BUCHI Digest Automat K-438 block-digestionapparatus

c) BUCHI Distillation Unit 355apparatu

Figure2. Apparatuses for the determinations of crude fat and protein

a) Domestic *D.bulbiferab)*

Wild *D.bulbifera*

C) While digesting on Hot plate withd) While digesting on Hot Plate with DH_2O_2 and HNO_3 and HCl

e) Final solution for metal analysis

Figure3. Photos that show each stage of the D.bulbifera samples in the processes of experimentation for the intended metal analysis

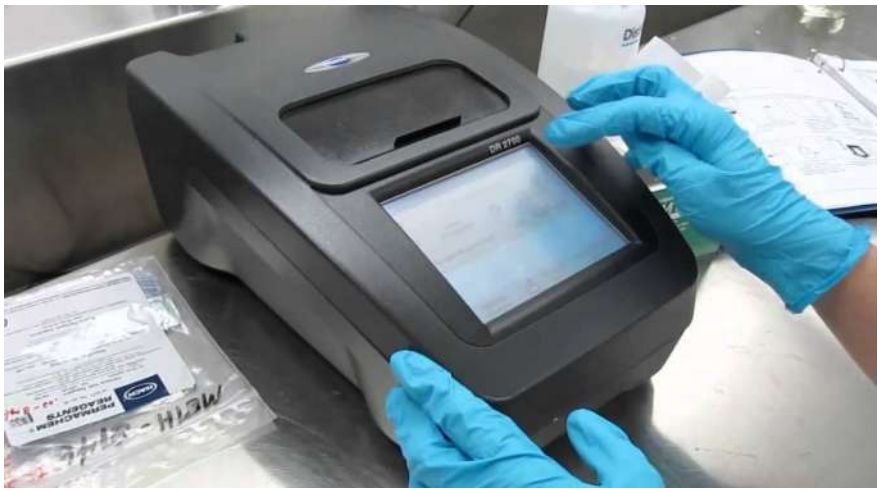


Figure 4.Spectrophotometer (DR200 /USA model)

Figure5. Flame Atomic Absorption Spectrophotometer (varian spectraAA-20plus) model

Appendix2: Data of triplicate measurements of concentrations of metals and proxim ate in the two D.bulbifera samples.

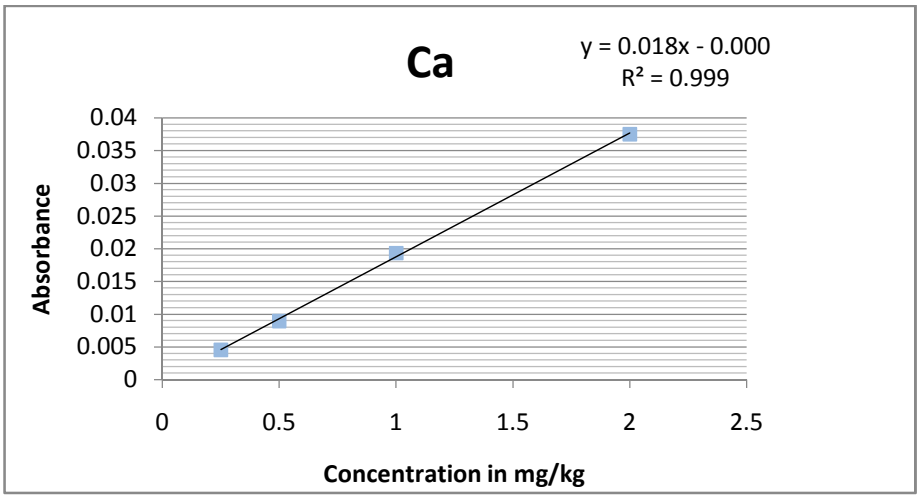
a) Data of triplicate measurements of concentrations of minerals in the two D.bulbifera samples by FAAS instruments in mg/kg. samples by FAAS instruments in mg/kg.

Domestic D.bulbifera (DDB)									
	Na	K	Mg	Ca	Fe	Cu	Zn	Mn	P
1	8.35	210.40	17.96	15.95	2.62	BDL	0.46	BDL	2036.85
2	7.85	209.90	16.66	13.78	2.66	-	0.35	-	2074.71
3	6.07	209.80	16.13	9.02	2.33	-	0.35	-	2093.65
mean	7.42	210.03	16.92	12.92	2.54	-	0.39	-	2068.40
std	1.20	0.32	0.94	3.54	0.18	-	0.06	-	28.92
Wild D.bulbifera(WDB)									
1	9.89	317.50	20.76	28.72	1.97	-	0.32	-	1506.70
2	9.80	300.50	21.92	20.92	1..49	-	0.38	-	1544.57
3	8.61	321..20	21.62	23.20	1.89	-	0.60	-	1525.64
mean	9.43	312.90	21.43	24.45	1.78	-	0.43	-	1525.64
std	0.71	11.32	0.60	3.95	0.26	-	0.15	-	18.94

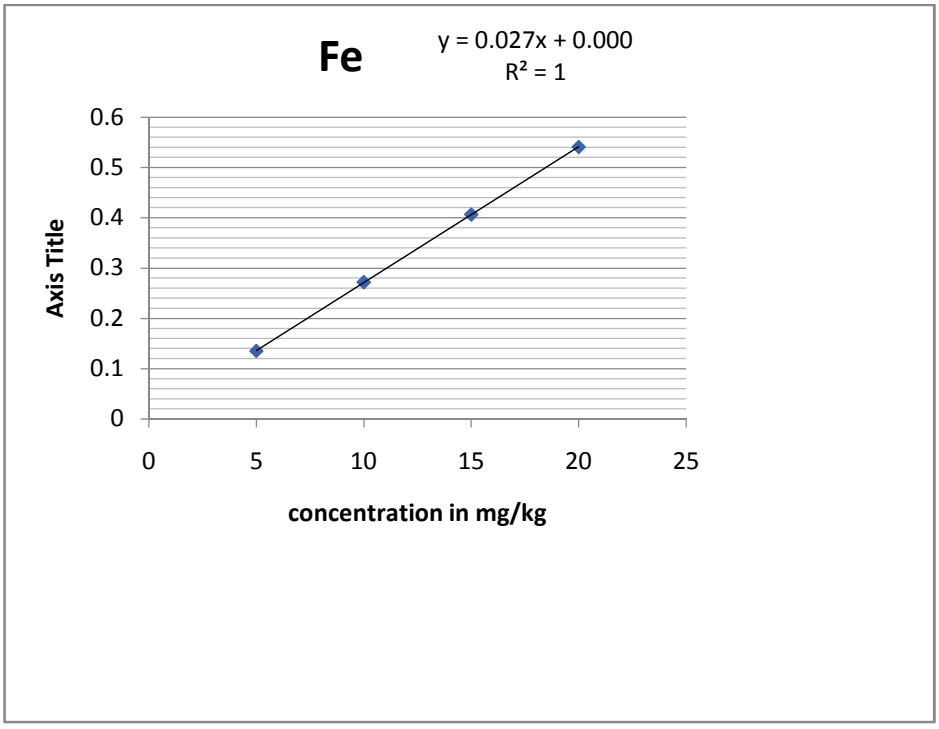
b) Data of triplicate measurements of proximate in two the D.bulbifera samples in percentage

	Domestic D.bulbifera (DDB)			Wild D.bulbifera(WDB)		
	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)
1	6.72	1.02	3.02	7.22	1.00	6.35
2	6.05	1.05	3.07	7.01	1.05	6.15
3	6.13	1.01	3.00	7.10	1.16	6.10
Mean	6.10	1.03	3.03	7.11	1.07	6.20
std	0.23	0.013	0.027	0.073	0.06	0.01

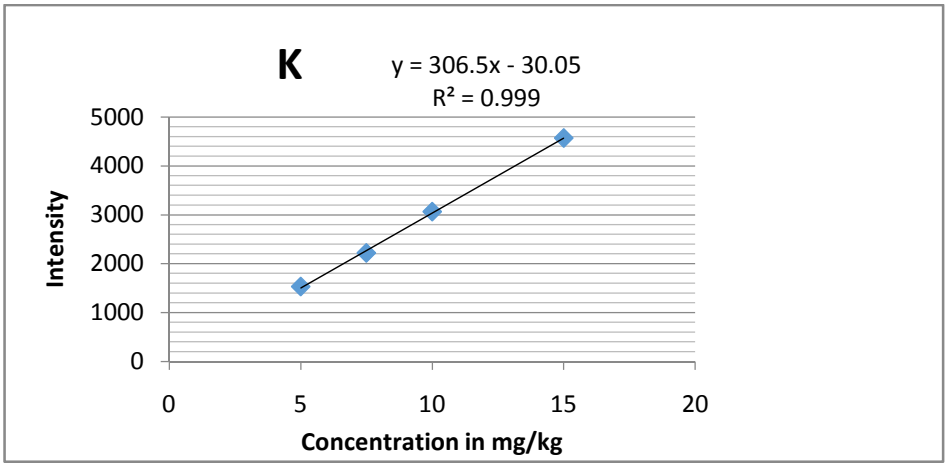
Appendix 3Figure (a-h): The calibration graphs of ten metals of interest analyzed from barley varieties using Microsoft Excel 2007



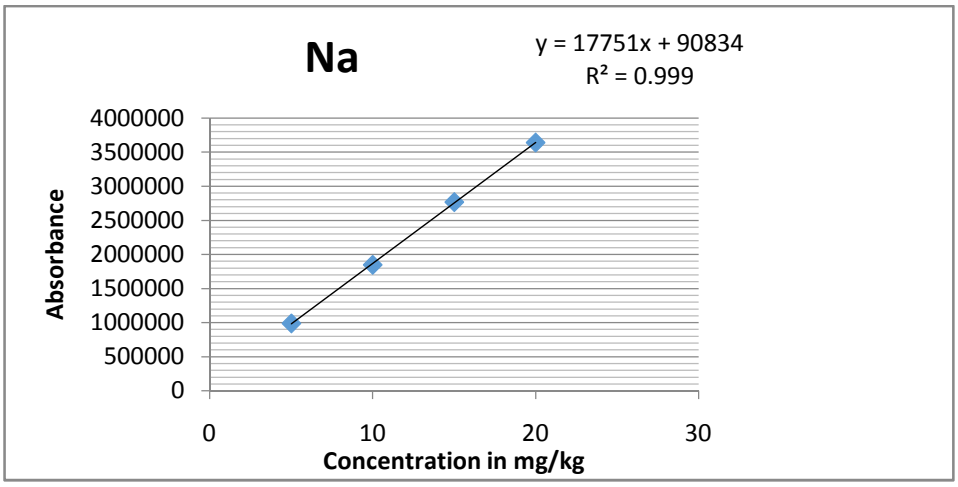
a) Calibration graph of calcium standards



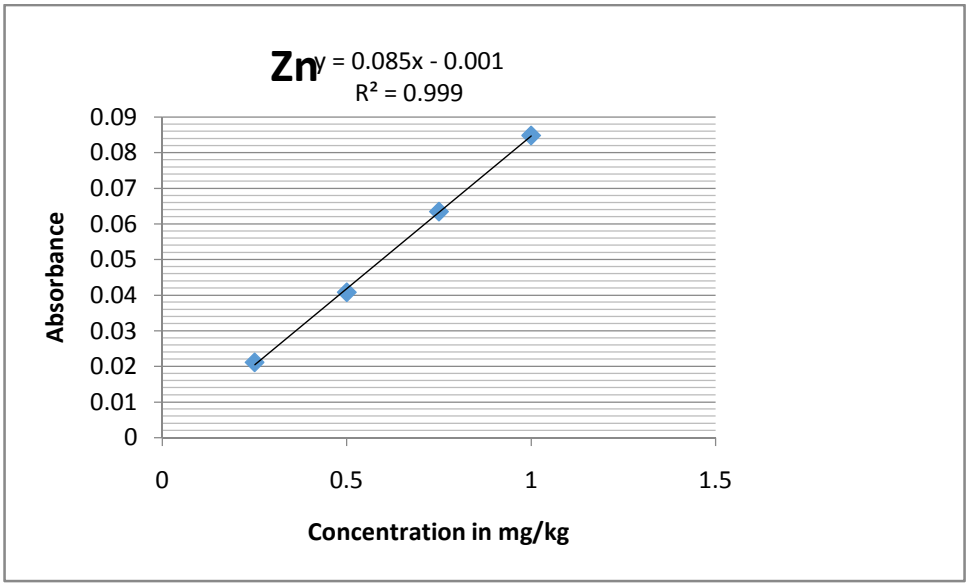
b) Calibration graph of iron standards



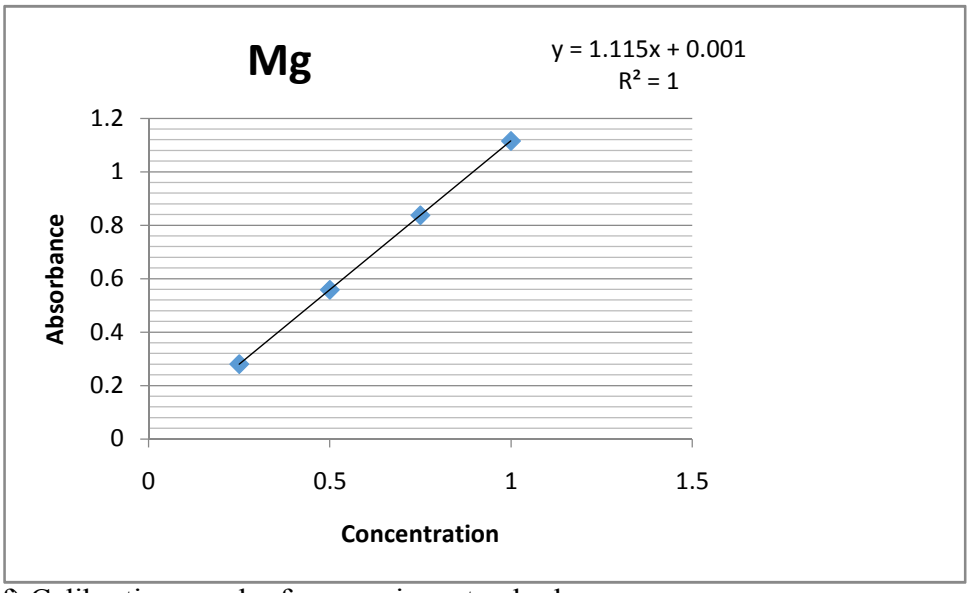
c) Calibration graph of potassium standards



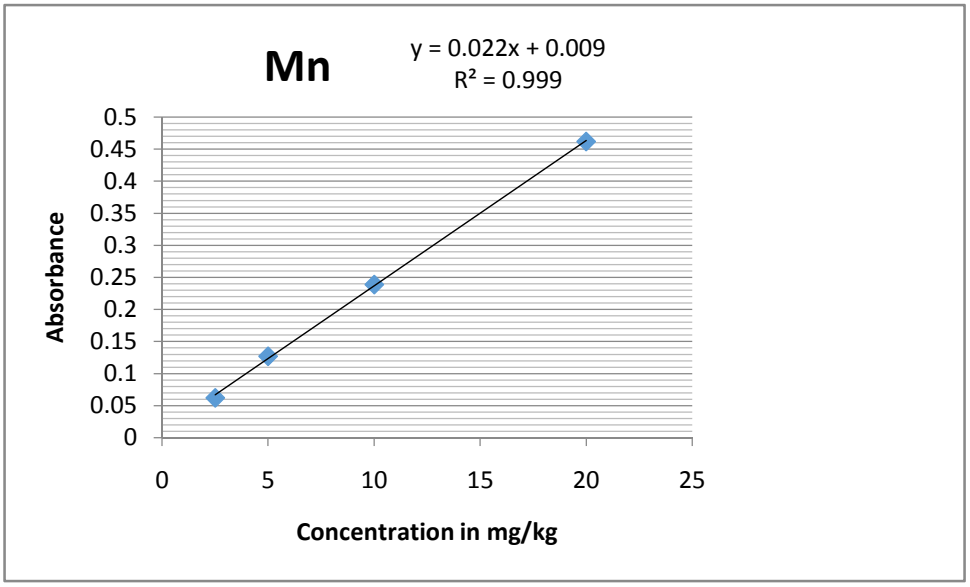
d) Calibration graph of sodium standards



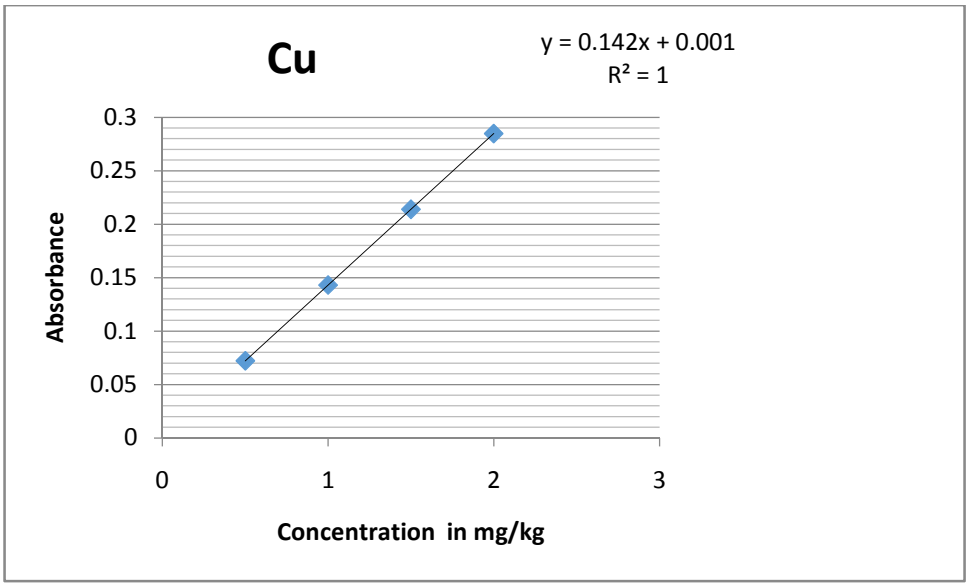
e) Calibration graph of zinc standards



f) Calibration graph of magnesium standards



g) Calibration graph of manganese standards



h) Calibration graph of copper standards