

HAWASSA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
CHEMISTRY DEPARTMENT



M.Sc. THESIS

By

ZEKARIAS ZERFU

**DETERMINATION OF SELECTED METALS AND NUTRITIONAL
COMPOSITION IN PAPAYA (*CARICA PAPAYA*) AT SAJA DISTRICT,
YEM SPECIAL WOREDA, SOUTHERN ETHIOPIA**

HAWASSA UNIVERSITY, ETHIOPIA

July, 2023

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IN PAPAYA (*CARICA PAPAYA*) FRUITS AT SAJA DISTRICT, YEM SPECIAL
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ZEKARIAS ZERFU

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Major Advisor: ENDALE TSEGAYE (PhD, Associate Professor)

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HAWASSA UNIVERSITY

HAWASSA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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We, the undersigned, members of the Board of Examiners of the final open defense by **Zekarias Zerfu** (ID. № ChemK/110/09) have read and evaluated his/her thesis entitled “**DETERMINATION OF SELECTED METALS AND NUTRITIONAL COMPOSITION IN PAPAYA (*CARICA PAPAYA*) FRUITS AT SAJA DISTRICT, YEM SPECIAL WOREDA, SOUTHERN ETHIOPIA**” and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree.

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DEDICATION

I dedicate this thesis to my family for nursing me with affection and love and for their dedicated partnership in the success of my life.

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By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I affirm that I have cited and referenced all sources used in this document. Every serious effort has been made to avoid any plagiarism in the preparation of this thesis. This thesis is submitted in partial fulfillment of the requirement for a degree from the School of Graduate Studies at Hawassa University. The thesis is deposited in Hawassa University Library and is made available to borrowers under the rules of the library. I solemnly declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. Brief quotations from this thesis may be used without special permission provided that accurate and complete acknowledgement of the source is made. Requests for permission for extended quotations from, or reproduction of, this thesis in whole or in part may be granted by the head of the School or Department or the Dean of the School of Graduate Studies when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author of the thesis.

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BIOGRAPHICAL SKETCH

The author was born on September 8, 1979 in Yem special woreada, Southern Ethiopia. He attended his primary education at Oya primary School and secondary education at FofaSecondary and preparatory School from 1997 to 2000. He received Bachelor degree in chemistry from Addis Ababa University in 2003. He was married and his major interests include creative writing and reading. Currently, he is working in saja Secondary& preparatory School.

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LISTS OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BITC	Benzyl is othiocyanate
CSA	Central Statistical Agency
CV	Coefficient of Variance
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agriculture Organization
IDL	Instrument Detection Limit
LSD	List Significant Difference
MDL	Method Detection Limit
SAS	Statistical Analysis System
SNNPRS	Southern Nations, Nationalities and Peoples Regional State
SPSS	Statistical Package for the Social Science
WHO	World Health Organization

ABSTRACT

Papaya is commonly known for its food and nutritional values throughout the world. The study was aimed to determine the level of selected metals as well as nutritional value of Papaya samples collected from six kebeles of Yem Special Woreda, SNNPRS. The optimized wet digestion method was used in the analysis. The results revealed that papaya has the ability to accumulate relatively higher amounts of Ca and Mg among the determined essential metals. The result of metal analysis revealed that papaya contains appreciable level of essential metals and the level of toxic heavy metals (Pb and Cd) were below maximum permissible value set by WHO/FAO indicating that the given fruit was safe to use The concentration ranges in dry weight basis in decreasing order were: Ca (7.13 -24.51) >Mg (9.76-10.87) > K (3.83 – 4.64) > Fe (1.18 – 5.76) >Mn (0.19-0.49) > Zn (0.14-0.45)>Pb(0.16-0.24) > Cu (0.14-0.16) >Cd (0.01) mg/kg. The proximate composition results indicated that the percentage of nutritional value in papaya ranges from (5.90 to 6.37), (70.56 to 72.42), (6.47 to 13.90), (2.08 to 2.85), (1.40 to 2.20), (6.21 to 11.45), (85.44 to 105.28); for ash, moisture, crude protein, crude fat, crude fiber, carbohydrates and total energy respectively. The efficiency of digestion methods was revealed that the excellent recoveries obtained which were found within the acceptable range for the analyzed metals. Regarding the proximate analysis, papaya was a good source of carbohydrate, fat, protein, and fiber and energy contents.

Keyword: Papaya, FAAS, Nutritional Value, Proximate composition

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Plants contain important chemicals used in traditional herbal drug dispensing for curative purposes. The usage of plants for curative and preventive remedies is an age-long activity. The advantage of medicinal plants was over orthodox or synthetic lies in the fact that it is cheap, common with low or no side effects on humans. Plants contain chemical ingredients of medicinal importance which has the capacity to change some bodily actions in the human body functions (Nissan et al, 2011). Medicinal plants have been naturally sought for thousands of years in the past ages, and have contributed immensely to the synthesis of a large number of modern-day drugs. They have found the usage as alternative remedies in the daily treatment of numerous sicknesses universally (Edoriet al, 2018). Papaya (*Carica papaya*) belongs to the order of plant kingdom known as Brassicales and the family of Caricaceae (Eno et al, 2000). The juice obtained from papaya has been applied to many domestic and medicinal uses such as softening of meat (during cooking), treatment of digestive problems (Akah et al, 2007). The chemical contents of pawpaw have also been found useful in increasing the immune system and dealing with cell tumour (Cordell, G. A. (2008).). The unripe fruit has been used to treat intestinal and other parasitic worms, skin diseases, urinary problems, and abnormalities (Anitha et al, 2018). The contributions of medicinal plants since ancient times have been indispensable sources of both preventive and curative traditional medicine (TM) preparations for human beings and livestock (Dery et al, 1999). Herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21 century. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China, and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians (Shad AK et al, 2008). Papaya (*Carica papaya*) is an important fruit in Ethiopia as it is a good source of vitamins, Dietary fiber and minerals and provides flavor, aroma and texture to the pleasure of eating. Fully ripened papaya fruits are usually eaten fresh as the enzymes in the fruit produce calm, Soothing feelings in the stomach. Papaya is known for its fine and natural laxative virtue which Aids digestion. Its fruits are rich in enzymes called papain (use as meat tenderizer) and /or the green

fruit is used by health and cosmetic industries amongst its many uses. It could be used as raw materials for soft drinks, jams and various preserves. The food canning industries could also use ripe fruits as filler in the manufacture of many products (Josef, 2008).

Different parts of *Carica papaya* are commonly used to treat a number of diseases; there is no any information about mineral contents in its different parts. The source of mineral nutrients for human being is plant materials consumed in the form of food or medicine. The human body requires both the metallic and the nonmetallic elements within certain permissible limits for growth. All minerals are important for human body because they serve necessary functions (Soetan et al, 2019 and Michael). Even though some minerals required in very small amount, deficiency of trace elements cause diseases, whereas their presence in excess may result in disturbing normal functioning of organs and central nervous system. Carica Papaya seed and leaves are widely used as traditional medicine in the world, having extensive commercial as well as social importance.

However, no literature report was found on comparative determination of the concentration of essential and non-essential metals in papaya fruits. Owing to this, the present study reported comparatively the concentration of Fe, Mn, Zn, Ca, K, Mg, Fe, Cu, Cd and Pb in papaya *nutritional composition in papaya fruit* and samples collected from Saja district using flame atomic absorption spectroscopy.

Food safety is vital for the survival of all living organisms involved in the food chain. In developing countries like Ethiopia, papaya fruits and other fruits contaminants with different metals. Lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) copper (Cu) and many other metals are potentially toxic to humans when they above biological limit and are widely dispersed in the environment (Morais et al, 2012). Therefore, aims of present study is to determine the selected metals (Ca, K, Mg, Fe, Co, Pd, Cu, Ni, Mn and nutritional compositions of papaya fruit in Saja districts as case study area in Yem Special woreda by using FAAS.

1.2 Statement of the problem

In Ethiopia the long history of using traditional medicinal plants for combating various ailments can be confirmed by referring to the medico-religious manuscripts in the country. In most developing countries like Ethiopia, the quest for rapid economic growth through industrialization and modern agriculture have resulted in a concomitant in flow of several contaminants (such as heavy metals and other important metals) into the environment. Although, metals occur as natural constituents of the earth crust, they are mostly considered persistent environmental contaminants since they cannot be degraded or destroyed. Hence, they can enter the body system through food, air, and water. However, the benefits of fruit consumption with some metals on human health are many, for instance consumption of *Carica papaya* effectively treats and improves all types of digestive and abdominal disorders. Specially, *Carica papaya* fruit is one of the commonest fruit in Saja districts and peoples of Yem Special Woreda traditionally used *Carica papaya* fruit to treat these diseases and others. The complex, largely uncharacterized, chemical composition of papaya, suggests the potential for uncharacterized effects on the health of humans or other organisms (Wilson et al. 2002).

In medicine heavy metal contents and other metals in fruits can be toxic when they exceed the recommended health levels or when they bio-accumulate in the body over a long period (Orisakwe et al., 2012). Documents related to the analysis of papaya in the study area was limited. This study, therefore aims at determining the selected metals in papaya fruit and its nutritional composition in Saja district as case study area.

1.3. Objectives of Study

1.3.1. General Objective of the study

To determine selected metals and some nutritional composition in papaya fruit in Saja by using Flame Atomic Absorption Spectroscopy (FAAS).

1.3.2. Specific objectives of the study

The specific objective of the present study is:

1. To determine the level of selected metals (Ca, Mg, K, Zn, Cu Mn and, Fe,) in papaya.
2. To identify some nutritional compositions in *Carica Papaya* fruits.
3. To find out nonessential metals lead (Pb) and Cadmium (Cd) in *Carica Papaya* fruits.

4. To compare the concentrations of selected metals with literature values.

1.4 Significance of the study

The result of this study can use in many ways. For instance, it may use as guideline or baseline for:

- ✓ Those who are working in public sector.
- ✓ Preliminary document for further research in the area.
- ✓ Other neighbor districts to know essential metals in *Carica Papaya* fruit.

Moreover, the findings of this research would be used to provide metal composition of *Carica papaya* fruit, these were very useful tools in public health safety, given their potential for determining metal amounts and that could then be addressed by public health authority and will be used by higher study institution as reference material.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Overview of carica papaya

Papaya, (*Carica Papaya*), also called pawpaw, succulent fruit of a large plant of the family Caricacea (Anjum et al; 2013). Though its origin is rather obscure, the papaya may represent the fusion of two or more species of *Carica* native to Mexico and Central America. Today it is cultivated throughout the tropical world and into the warmest parts of the subtropics. The papaya fruit is slightly sweet, with an agreeable musky tang, which is more pronounced in some varieties and in some climates than in others. It is a popular breakfast fruit in many countries and is also used in salads, pies, sherbets, juices, and confections. The unripe fruit can be cooked like squash. The papaya plant is considered a tree, though its palm like trunk, up to 8 metres (26 feet) tall, is not as woody as the designation generally implies (Anjum et al; 2013).



Figure 1: Papaya tree (*Carica papaya*)

The plant is crowned by deeply lobed leaves, sometimes 60 cm (2 feet) across, borne on hollow petioles (leaf stalks) 60 cm long. Normally, the species is dioecious, male and female flowers being produced on separate plants, but hermaphroditic forms are known, and numerous irregularities in the distribution of the sexes are common. Male flowers are borne in clusters on stalks 90 cm long; the flowers are funnel-shaped, about 2.5 cm (0.1 inch) long, and whitish, with 10 stamens in the throat. The female flowers are considerably larger, on very short stalks, and often solitary in the leaf axils; they have five fleshy petals that are united toward the base and a large cylindrical or globose superior ovary that is crowned by five fan-shaped sessile stigmas. The fruit is commonly spherical to cylindrical in form, is 75 to 500 mm (3 to 20 inches) or even more in length, and sometimes weighs as much as 9 to 11.5 kg (20 to 25.5 pounds). The very juicy flesh is deep yellow or orange to salmon-coloured. Along the walls of the large central cavity are attached the numerous round, wrinkled black seeds.

2.2 Medicinal properties of *Carica papaya* plant.

The different parts of the *Carica papaya* plant proved to have medicinal value including leaves, seeds, latex and fruit. *C. Papaya* has a wide variety of medicinal properties including anticancer, antiviral, anti-inflammatory, antimicrobial, antidiabetic, antihypertensive, wound healing activity, free radical scavenging activity and increase in thrombocyte count, etc. The phytochemical constituents of *Carica papaya* and its medicinal properties are presented as follows.

2.2.1 Phytochemical Compounds and antioxidants present in *Carica papaya*

Phytochemical analysis of *Carica papaya* leaf extracts revealed the presence of alkaloids, glycosides, flavanoids, saponins, tannins, phenols and steroids (Gill, 1992; Owoyelet al, 2008). The stems, leaves and fruits of papaya contain plenty of latex. The latex of *Carica papaya* is a rich source of four cysteine end peptidases namely papain, chymopapain, glycyloendopeptidase and caricain a papaya endopeptidase II (Azarkan et al, 2003).

2.2.2 Antioxidants and free radical scavenging activity

The leaves, seeds and juice of papaya show free radical scavenging and antioxidants activity. The antioxidant activity of various fractions (ethanol, petroleum ether, ethyl acetate, n-butanoland aqueous extract) from seeds of *C. papaya* was evaluated and showed that ethyl

acetate and n-butanol fractions demonstrated antioxidant and free radical scavenging activity than other fractions (Zhou K et al, 2011). Papaya juice is an efficient scavenger of highly reactive hydroxyl radicals ($\cdot\text{OH}$) (Webman et al, 1989), which significantly decreased the lipid peroxidation levels and increased the antioxidant activity in rats (Mehdipour et al, 2006). The leaf extract of *C.papaya* evidenced significant antioxidant and free radical scavenging potential (Okoko and Ere, 2012).

2.2.3 Anticancer activity

An in vitro study using extract of the papaya seed homogenate was highly effective in inhibition of super oxide generation and inducing apoptosis in acute promyelocytic leukemia cell line HL-60 and the activity was mainly contributed by benzyl isothiocyanate [BITC] (Nakamura Y et al, 2007). The aqueous extract of *Carica papaya* exposed to onion bulbs disturbed the mitotic cell division of *Allium cepa* by affecting the spindle formation and hence shows its cytotoxic effect (Akinboro and Bakare, 2007). The aqueous extract of papaya flesh (0.01% - 4% v/v) treated with breast cancer cell line MCF7 revealed significant inhibition of cell proliferation (García et al, 2009).

2.2.4 Treatment for dengue fever

A case report from Pakistan Ahmad N et al (2011), documented that aqueous extract of *C.papaya* leaves administered to a patient affected with dengue fever twice daily for 5 consecutive days exhibited elevated platelet count from $55 \times 10^3/\text{ul}$ to $168 \times 10^3/\text{ul}$. Another study in murine model also evidenced increase in platelet and RBC count without any acute toxicity after oral administration of *C.papaya* extract (Dharmarathna et al, 2013).

2.2.5 Antidiabetic activity

Aqueous extract of unripe papaya fruit significantly inhibited the key enzymes amylase and glycosidase involved in type 2 diabetes and also inhibited the lipid peroxidation in rat pancreatic cells studied in vitro (Oboh, 2013).

2.3 Metals in Papaya

Metals in medicine are used in organic systems for diagnostic and treatment purposes. Inorganic elements are also essential for organic life as cofactors in enzymes called metalloproteinase.

When metals are scarce or high quantities, equilibrium is set out of balance and must be returned to its natural state via interventional and natural methods. Metals can be toxic in high quantities. Either ingestion or faulty metabolic pathways can lead to metal poisoning. Sources of toxic metals include cadmium from tobacco, arsenic from agriculture and mercury from volcanoes and forest fires. Nature, in the form of trees and plants, is able to trap many toxins and can bring abnormally high levels back into equilibrium. Toxic metal poisoning is usually treated with some type of chelating agent. Heavy metal poisoning, e.g., Hg, Cd, Pb, are particularly pernicious. Carica papaya tissues and supporting soil had significantly higher levels of pH, EC and heavy metals in the sampled plots than the control plot. The heavy metal concentrations in Carica papaya and soil occurred in the decreasing order of Pb>Cu>Hg>Zn>Cd. The fruit of C. papaya had the highest mean concentrations of Pb (51.4±14.1 mg/kg) and Zn (26.4±1.9 mg/kg), while the leaf had the highest mean concentration of Hg (32.0±2.3 mg/kg). The pH, Cu and Zn in the supporting soil were significantly correlated with the levels in the C. papaya tissues (Eludoyin and Ogbe, 2017). According to Edori, O. (2019), Leaves and peels of papaya bought from open market within Port Harcourt metropolis were examined for metals and qualitative phytochemical compositions. The metals examined in the leaves and peels of the papaya were manganese (Mn), potassium (K), sodium (Na), zinc (Zn), magnesium (Mg), calcium (Ca), iron (Fe), arsenic (As), nickel (Ni), copper (Cu), lead (Pb), and selenium (Se). Their concentrations were in the order of Fe > Mn > Zn > Mg > K > Cu > Ca > Ni > Na > Pb > Ar = Se in the leaves, while those of the peels were in the order of Fe > Mn > Zn > Mg > Cu > Ca > K > Ni = Na > Pb > Ar = Se. The results of heavy metals in leaves and peels were not significantly different from each other, but were slightly higher in the peels than the leaves (Eludoyin and Ogbe, 2017).

2.4 Importance of Carica Papaya

The nutrients found in papaya are thought to have a range of health benefits. They may help protect against a number of health conditions. According to the World Health Organization (WHO) report, almost 80% of people in marginal communities use only medicinal plants for the treatment of various diseases. Papaya plant produces natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumors and pesticidal properties (Ayoola and Adeyeye, 2010). The tea, prepared with the green papaya leaf (Figure 2 (c)), promotes digestion and aids in treatment of ailments such as chronic indigestion,

overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2005) . The efficacy of treatments with *Carica papaya* is dependent on the quantity of the different compounds in the preparation. The quantity of the compounds, as previously indicated, differs in the fruit, latex, leafs, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree ((Ayoola and Adeyeye, 2010) and (Afolabi, 2011)) .

Papaya leafs are also believed to be several health benefits including removing intestinal parasites, as aid in correcting digestion problems and can potentially reduce inflammation in several parts of the body (Ayoola and Adeyeye, 2010). The papaya seeds (Figure 2(b)) are very pungent and peppery, making them almost unpalatable. Papaya seeds have antibacterial properties and are effective against *E.coli*, *Salmonella* and *Staphylococcus* infections; protect the kidneys from toxin-induced kidney failure; eliminate intestinal parasites and cure for piles and typhoid fever (Timothy , 2011). It is also reported that seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for impotence (Elizabeth, 1994). Papaya is considered one of the most important fruits because it is a rich source of antioxidant nutrients (e.g., carotenes, vitamin C, and flavonoids), the B vitamins (e.g., folate and pantothenic acid), minerals (e.g., potassium, magnesium etc), and fiber.

In addition, papaya is a source of the digestive enzyme papain, which is used as an industrial ingredient in brewing, meat tenderizing, pharmaceuticals, beauty products, and cosmetics (FAOSTAT, 2019).

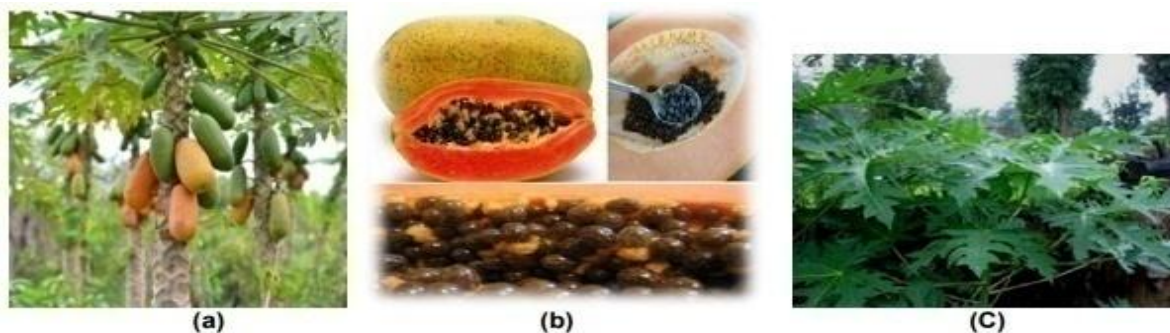


Figure 2: *Carica papaya*; plant (a), (b) Seeds, (c) leaf in Odo-shakiso, Ethiopia Source: (Menegsha Tigist et al, 2014).

Different parts of *Carica papaya* is very important to cure a number of diseases and hence used by most of individuals in a regular basis in the country particularly in Guji Zone. For example *Carica papaya* leaves are best known to treat malaria. To treat malaria disease the leaves are boiled for a while and the extracted solution is inhaled from three to five days in an average. Powder of papaya seeds are extensively used to cure typhoid fever and to eliminate intestinal parasites (as dewormers). In addition young females used papaya seeds for illegal abortion. Even though different parts of *Carica papaya* are commonly used to treat a number of diseases, there is no any information about mineral contents in its different parts. The source of mineral nutrients for human being is plant materials consumed in the form of food or medicine. The human body requires both the metallic and the nonmetallic elements within certain permissible limits for growth.

All minerals are important for human body because they serve necessary functions ((Soetanet al., 2010) and (Michael et al., 2009)). Even though some minerals required in very small amount, deficiency of trace elements cause diseases, whereas their presence in excess may result in disturbing normal functioning of organs and central nervous system. For example in human adults, peripheral nerve damage has been observed at 40 to 60 $\mu\text{g}/\text{dl}$ lead contamination and anemia at 80 $\mu\text{g}/\text{dl}$ (Peterso et al., 2007). High levels of lead exposure can result in stillbirth or miscarriage. It is one of the most toxic nonessential and mobile metallic elements found in soils and it affects animals and plant adversely (Chaturvedi. 2004). Intake of cadmium-contaminated food causes acute gastrointestinal effects, such as vomiting and diarrhea (Godt et al., 2006). Inhalation exposures to high levels of cadmium damage the respiratory system (bronchial and pulmonary irritation), headache, chest pains, muscular weakness, cancer and death (Peterso et al., 2007).

Plants can easily be contaminated by heavy metals in the course of cultivation or later during the processing stage. The content of heavy metals is one of the criteria for the use of plant material as food or traditional medicines. Hence determination of mineral compositions in food and medicinal plant is essential for understanding their nutritive importance and health risk. Accordingly, control of heavy metals in medicinal plants and their products should be made such to ensure safety and efficacy of herbal products (Endashaw, 2007). *Carica Papaya* seeds and

leaves are widely used as traditional medicine in the world, having extensive commercial as well as social importance. However, no literature report was found on comparative determination of the concentration of essential and non-essential metals in papaya seeds, leaves. Owing to this, the present study reported comparatively the concentration of K, Mg, Ca, Mn, Cu, Zn, Co, Ni, Cr, Cd and Pb in papaya seed, leaves and supporting soil samples collected from OdoShakiso district using flame atomic absorption spectroscopy. Some additional benefits of carica papaya as follows:

2.4.1 Age-related macular degeneration

Zeaxanthin, an antioxidant found in papaya, filters out harmful blue light rays. It is thought to play a protective role in eye health, and it may ward off macular degeneration. However, a higher intake of all fruits has been shown to decrease the risk of and progression of age-related macular degeneration (Megan Ware RDN LD, 2019).

2.4.2 Asthma prevention

The risk of developing asthma is lower in people who consume a high amount of certain nutrients. One of these nutrients is beta-carotene, contained in foods like papaya, apricots, broccoli, cantaloupe, pumpkin, and carrots (Megan Ware RDN LD, 2019).

2.4.3 Cancer

Consuming the antioxidant beta-carotene, found in papayas, may reduce cancer risk. Among younger men, diets rich in beta-carotene may play a protective role against prostate cancer, according to a study published in the journal Cancer Epidemiology and Prevention Biomarkers (Megan Ware RDN LD, 2019).

2.4.4 Bone health

Low intakes of vitamin K have been associated with a higher risk of bone fracture. Adequate vitamin K consumption is important for good health, as it improves calcium absorption and may reduce urinary excretion of calcium, meaning there is more calcium in the body to strengthen and rebuild bones (Megan Ware RDN LD, 2019).

2.4.5 Diabetes

Studies have shown that people with type 1 diabetes who consume high-fiber diets have lower blood glucose levels, and people with type 2 diabetes may have improved blood sugar, lipid, and insulin levels. One small papaya provides about 3 grams of fiber, which is equivalent to just 17 grams of carbohydrates (Megan Ware RDN LD, 2019).

2.4.6 Digestion

Papayas contain an enzyme called papain that aids digestion; in fact, it can be used as a meat tenderizer. Papaya is also high in fiber and water content, both of which help to prevent constipation and promote regularity and a healthy digestive tract (Megan Ware RDN LD, 2019).

2.4.7 Heart disease

The fiber, potassium, and vitamin content in papaya all help to ward off heart disease. An increase in potassium intake along with a decrease in sodium intake is the most important dietary change that a person can make to reduce their risk of cardiovascular disease (Megan Ware RDN LD, 2019).

2.4.8 Inflammation

Choline is a very important and versatile nutrient found in papayas that aids our bodies in sleep, muscle movement, learning, and memory. Choline also helps to maintain the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat, and reduces chronic inflammation (Megan Ware RDN LD, 2019).

2.4.9 Skin and healing

When used topically, mashed papaya appears to be beneficial for promoting wound healing and preventing infection of burned areas. Researchers believe that the proteolytic enzymes chymopapain and papain in papaya are responsible for their beneficial effects. Ointments

containing the papain enzyme have also been used to treat decubitus ulcers (bedsores) (Megan Ware RDN LD, 2019).

2.5 Severe Side Effects of Eating Too Much Papaya

The use of papaya leaf, fruit and root extracts as traditional medicines (Akah et al. 1997; Eno et al. 2000) and the complex, largely uncharacterised, chemical composition of papaya latex, suggests the potential for uncharacterised effects on the health of humans or other organisms. A compound present in crushed papaya seed that is believed to have activity against helminthic intestinal parasites, benzyl isothiocyanate (BITC), has been shown to have an effect on vascular contraction using a canine carotid artery in vitro model (Wilson et al. 2002). Other studies have suggested possible purgative effects of root extracts (Akah et al. 1997) and antihypertensive activity of fruit extracts (Eno et al. 2000). The presence of cyanogenic compounds in papaya has also been reported (Seigler et al. 2002). Some problems related to eating too much Papaya as follows:

2.5.1 May Cause Miscarriage

This stems from the fact that papaya can often cause latex allergy in individuals. This can be potentially dangerous to the mother as well as the unborn child. Other studies also speak of the enzyme papain in papaya, which suppresses progesterone (a sex hormone) that is needed to prepare the uterus for conception. Papain may also damage certain membranes in the woman's body that are essential for fetal development. Further research also tells us that the papain in papaya can poison the foetus and cause birth defects in the new-born. Papaya may not also be safe during breastfeeding. There is some speculation in this regard, and research is not very clear. Hence, please consult your doctor and avoid papaya during this period(Ravi Teja Tadimalla, 2019).

2.5.2 Can Lead to Digestive Issues

The very same papain that can calm your stomach can also lead to digestive distress if taken in excess. Papaya is rich in fiber, and too much of it can lead to digestive upset. Also, the skin of the fruit contains latex – which can irritate the stomach and cause pain. The papain in the fruit works well in breaking down fiber. But if the fiber is not digested well, you will have to suffer

from stomach gas. Papaya can also cause diarrhea in certain individuals – the fiber in the fruit can bind with the stools and cause diarrhea, leaving you terribly dehydrated. The abundant fiber in the fruit can also cause constipation. To avoid this, ensure you drink plenty of water (Ravi Teja Tadimalla, 2019).

2.5.3 Papaya Can Cause Respiratory Disorders

The papain in the fruit is also a potential allergen. Individuals consuming a lot of papayas end up ingesting excessive papain – and this can lead to respiratory allergies. Some of these include asthma, wheezing, nasal congestion, and, in severe cases, even breathing difficulties. In fact, this can be a problem for pregnant women as well. Respiratory allergies can also affect the foetus, causing further complications (Ravi Teja Tadimalla, 2019).

2.5.4 May Lead to Kidney Stones

Papaya is replete with vitamin C, and research shows that excess of vitamin C (more than 2,000 mg per day for adults, and more than 1,200 mg per day for children) can lead to toxicity symptoms, including kidney stones. Vitamin C, once metabolized in the body, produces oxalates that can turn into stones (Ravi Teja Tadimalla, 2019).

2.5.5 Can Be Unsafe For Infants

While we need more studies to corroborate this fact, some sources state that papaya may be unsafe for infants below one year of age. The excess fiber in the fruit can be the culprit. This can lead to loose stools in the infant (diarrhea) and even constipation (due to excess fiber). Please check with the baby's pediatrician before giving it any fruit (Ravi Teja Tadimalla, 2019).

2.5.6 Nutritional quality of papaya (Carica Papaya)

The nutritional qualities and medicinal value of papaya are closely related. The papaya can be considered as a nutrient dense food, as it provides many more nutrients on per calorie basis as compared to other foods. The whole papaya fruit is an excellent source of dietary fiber and therefore can also help in preventing the constipation. The fiber content of

papaya can help in lowering the high blood cholesterol levels. Papaya is rich in vitamins C and A. One serving of papaya can provide about 100% daily requirement for vitamin C and 30% of vitamin A. It also contributes to small quantities of vitamin E, K, thiamine, riboflavin, niacin, pyridoxine and folate. Folic acid is needed for the conversion of homocysteine to cysteine and methionine. The increased level of homocysteine in blood is considered a significant risk factor for a heart attack or stroke as it can directly damage the wall of blood vessels (Antoniades et al., 2009, Seo et al., 2010). Papaya could be a candid source to reverse the homocysteine mediated cardiovascular diseases since it has profuse amount of folic acid. The nutrients contained in papaya can also help to prevent the oxidation of cholesterol. The oxidized cholesterol sticks to the internal lining of blood vessels, forming dangerous plaques that can eventually cause heart attacks or strokes. Data from various studies indicate that dietary vitamin E and C may exert some effect in preventing the oxidation of cholesterol because of their suggested association with paraoxonase, an enzyme that inhibits the oxidation of LDL and HDL cholesterol (Jarvik et al., 2002, Schürks, et al., 2010, Gaby, 2010).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area

Saja is a city administration in the yem special woreda of Ethiopian southern Nations, Nationalities, and Peoples Region (SNNPR). The exact location of Yem people; the people of Yem are found in the south western Ethiopia. Though they wide spread over the whole area

Of Jimma zone, Yem people are found in Yem special woreda revelry consistent socio-cultural life. With reference to the geographical positioning system, Yem special woreda is located $7^{\circ} 30'N$ to $8^{\circ} 27'N$ latitude and $37^{\circ} 20'E$ to $41^{\circ} 55'E$ longitude. The special woreda

Covers a total area of 724.55 square km. The special woreda is bounded by Gurage and Hadya across the Gibe river in the east and Oromia in the North, south and west. Many Yem people scattered from the special woreda and lived in various areas. Especially into Jimma zone. and intr-mingle their culture with Oromo people

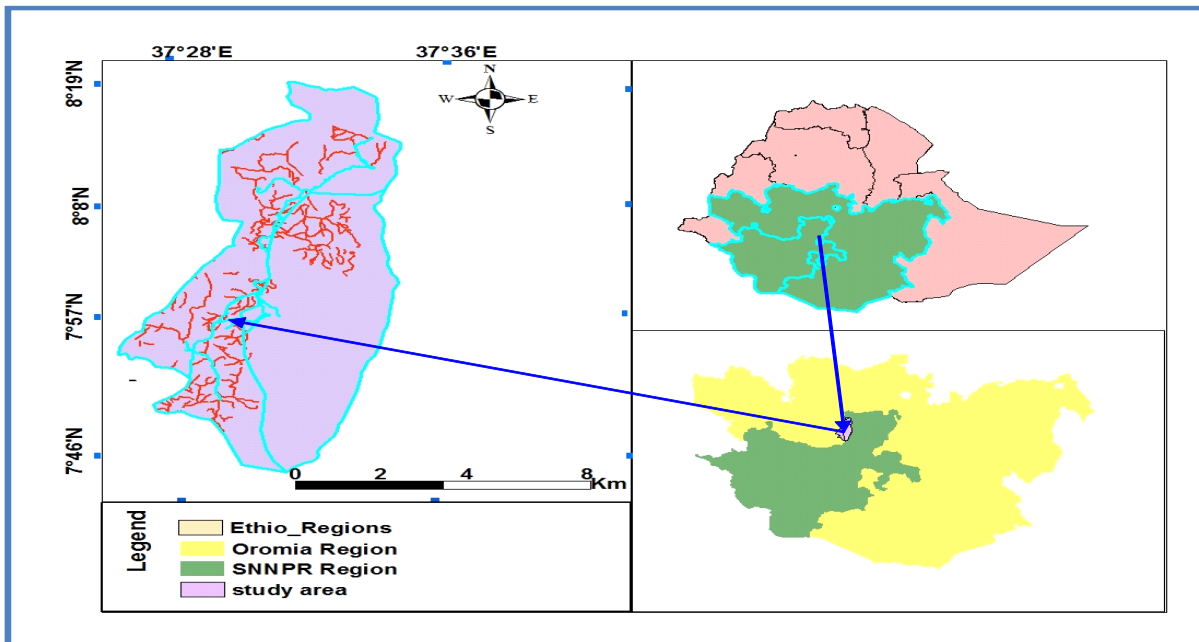


Figure 3: Map of area

The Yem people used staple food of Enset and the recent (2005) national population and housing census result reported that Yem has the population size 160,447 and at Saja town only 10200 inhabitants existed in 2020. The major economic activity of the Yem people is agriculture

.The capital of Yem Special Woreda is Saja located at 239Km away from Addis Ababa and 527 Km from Hawassa. The surface configuration of Yem special woreda comprises mountain ranges, gorges and deep valleys. The highest mountain peak is Bor Amor (Ama) which culminates about 2939 meter above sea level and the lowest point is 1000 meter below sea level. And Saja town surround by mountain and demarcate by River to west by Gibe, north east zabe and the south Simini river. saja town located in the lower part of Yem topography . The climate of SAJA town is hot relatively than the rest part of yem special woreda like Deri, Fofa and Toba.

3.2 Instruments and Equipment

“Kjeldahl digestion apparatus, Flame atomic absorption spectroscopy (FAAS), volumetric flasks, pestle, oven, mortar, knife, aluminum foil, polyethylene bag filter paper

3.3 Chemicals and solvents

69-72% HNO₃, 35% HCl, 70% HClO₄, H₂O₂, Stock standard solutions containing 1000 mg/Kg of the metals Ca, K, Mg, Fe, Cd ,Mn,Cu,Zn, and Pd, distilled water, de-ionized water and tap water(Mebrahtu ,2016).

3.4 Sample collections

Mature and clean green papaya (*Carica papaya* L.) fruits were collected from six Kebeles namely, Asha, Lafton, Sajakenama, Ashri, Sewuda and Tigri kebeles. To draw a representative sample from each sampling Kebele, from each of three subsamples were taken about some distance away from each other and put in clean polyethylene plastic bags by systematic name. They were brought to the laboratory for further pre-treatment (**Mebrahtu, 2016**).

3.4.1 Preparation of sample

The fruit sample was washed with distilled water and the fruit was cut in to small pieces. It was dried at 80-100°C in electric oven and ground using pestle and mortar. The powdered materials were stored in electric oven till they acquired constant weight (*Chauhanet al., 2015*).

3.4.2 Optimization of digestion procedure

To select an optimum procedure for digestion parameters like digestion time, reagent volume, volume ratio of reagents and digestion temperature was optimized by varying one parameter a time and the others constant (Chauhan *et al.*, 2015).

3.4.3 Digestion of Plant Sample

Digestion occurs in two methods wet and dry methods.

Wet digestion

Acid digestion involves the utilization of mineral or oxidizing acids and an external heat source to decompose the sample matrix in open or closed vessels. The choice of the acid or the combination of the acids depends upon the nature of the matrix to be decomposed. Wet digestion is performed by using concentrated acids including nitric acid, perchloric acid, hydrogen peroxide and mixture of acids in open or closed vessels. Incomplete digestion of the matrix is a common problem. In such a case the mixture of H_2SO_4 , $HClO_4$ or HNO_3 is one of the best means to dissolve organic components. For plant species including varying amounts of silicates, hydrogen fluoride is used for complete digestion

Dry ashing

Combustion (Dry ashing) or oxidation is usually performed by placing the sample in an open vessel to remove the organic portion of the sample by thermal decomposition, normally in the presence of an ashing aid, using a muffle furnace. Typical ashing temperatures are 450 to 550°C at atmospheric pressure for 1 to 6 h. The analyte is then extracted from the ash with a mineral acid. The degree of volatilization loss is a limiting factor and depends on the applied temperature, the form in which the analyte is present in the sample and the chemical environment in the ashing stage. The most important advantages of the ashing are the ability to decompose large sample sizes and dissolving the resulting ash in a small volume of acid, and the need for little or no reagents

2g fruit powder was weighted into separate digestive flasks and was digested with 35% mL HNO₃ or 69% HClO₄. The flasks were heated in digestive apparatus around 80-100°C in hood. After heated for an hour, the content of flasks were digested by adding 10 mL HNO₃ followed by 2 mL of H₂O₂. Heating was continued until the volume of the content was reduced to semi dried mass via increment of H₂O₂. The contents of flasks were cooled by diluting around 5 mL HNO₃. It was filtered through Whatman # 42 paper into volumetric flasks and marked as stock sample solution for the determination of metals by FAAS.

3.5 Instrumental calibration

Calibration metal standard solutions were prepared for each of metals from an standard solution containing 10mg/L from Atomic Absorption spectroscopy stock that contain 100ml.

3.6 Determination of Metals

The concentration of Ca, k, Mg, Fe, Pb, Ni, Mn, Cu, Co and Cr were analyzed with FAAS. The parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Hollow cathode lamps for each metal operated at the manufacturers recommended conditions were used at its respective primary line source. The acetylene and air flow rates were managed to ensure suitable flame conditions. Finally, the absorbance of each standard was measured and then absorbance versus concentration curve was plotted. Once the FAAS was activated, the fruit sample solution was aspirated into FAAS via sample introduction valve.

3.7 Method validation and method performance

To validate the analytical method, parameters such as precision, accuracy, in terms of recovery test, method of detection limit, limit of quantitation, analysis of laboratory sample, matrix spike and matrix spike duplicate was carried out.

$$\%Recovery = \frac{\text{Amount of analyte recovered}}{\text{Amount added}} \times 100 \dots \dots \dots \text{equation 1}$$

3.8 Proximate Analysis

3.8.1 Moisture content

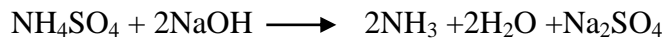
The dishes used for the moisture determination were dried at 130⁰C for 1 hr in drying oven and placed in desiccators for about 30 min. The mass of each dishes was measured (M_1) and about 5 g of the sample was weighed in to each of the dishes (M_2). The sample was then mixed thoroughly and dried at 100⁰C for 6 hr. After drying is completed, the mass was measured (M_3). The moisture content was calculated from the equation (AOAC, 2000).

$$\text{Moisture (\% W/W)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100 \dots\dots\dots \text{equation 2}$$

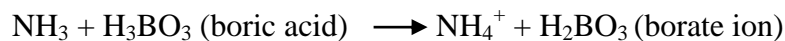
Where; M_1 =mass of the dish, M_2 =mass of the dish and the sample before drying and
 M_3 =mass of the dish and the sample after drying.

3.8.2 Crude protein analysis (Kjeldahl Method)

About 0.5 g of the papayas ample was weighed by Adventurer analytical balance and added to the digestion flask. Then 6 ml of acid mixture (concentrated orthophosphoric acid and concentrated sulphuric acid) and 3.5 ml of 30% hydrogen peroxide solution was added in to the digestion flask step by step. The tubes wereshaked until the violet reaction disappeared. About 3g of the catalyst mixture made of 0.5 g of selenium and 100 g of potassium sulphate was added in to the digestion flask. The solution was then digested at 370⁰C for 1 hr by Tecator digester. After digestion completed, the content in the flask was diluted by water and 40% sodium hydroxide was added to neutralize the acid and to make the solution slightly alkaline.



The ammonia was then distilled into receiving flask that consisted solution of excess 4% boric acid solution for reaction with ammonia. The borate ion was formed as the result of the reaction of the boric acid and the ammonia; and this was titrated with standard acid (0.1N sulphuric acid solution).



The nitrogen content was calculated from the equation (AOAC, 2000)

$$\text{Nitrogen (\% w/w)} = \frac{V_2 - V_1 \times 14}{W} \times 100 \dots\dots\dots \text{equation 3}$$

Where V_2 = volume (ml) standard H_2SO_4 solution used in the titration of the blank,

V_1 = volume (ml) standard H_2SO_4 solution used in the titration of the sample,

W = sample weight and 14 is the molecular weight of nitrogen.

The protein content was calculated from the equation (A.O.A.C, 2000)

$$\text{Protein Content (\% W/W)} = 6.25 \times \%N \dots\dots\dots \text{equation 4}$$

3.8.3 Ash

Crucibles for the analysis were cleaned by drying at $120^\circ C$ in at drying oven and ignited at $550^\circ C$ in furnace for 3 hr. Then the crucibles were removed from furnace and cooled in desiccators .The mass of each of the crucibles were measured by Adventurer analytical balance (M_1) and about 2.5 g of the sample was weighed in to each crucible (M_2). The crucibles were dried at $120^\circ C$ for one hour on a Wag tech hot plate. The crucibles were then placed in a furnace at about $550^\circ C$ for 1 hr. The crucibles were then removed from the furnace and will be cooled. A 5 drop of distilled water was then added to each of the crucible and placed in the furnace at $550^\circ C$ for 30 min. Crucibles were again removed from the furnace, allowed to cool and 5 drops of distilled water and nitric acid was added to each. Then the crucibles onceagain were inserted in to the furnace until they became free from carbon and the residue appears grayish white. Then they were removed from the furnace and placed in desiccators. Finally the mass of each dish and sample after dried was weighed as (M_3).

The total ash was calculated from the equation (A.O.A.C, 2000).

$$\text{Ash (\% W/W)} = \frac{M_3 - M_1}{M_2 - M_1} \times 100 \dots\dots\dots \text{equation 5}$$

Where; M_1 =mass of the dried dish, M_2 =mass of the dish and the sample and M_3 =mass of dish and sample after dried

3.8.4 Crude fiber Content

Fiber is edible part of plant that not easily digested in small intestine but fermented in the large intestine of human by bacterial enzymatic activities that can partially degrade them (the micro flora), to short-chain fatty acids and several gases such as carbon dioxide, hydrogen and methane, are an important direct source of energy for the colonic mucosa (Opera, 2003).Fiber is physiological and nutritional concept relating to those carbohydrate components of foods (Opera, 2003). It includes cellulose, hemicelluloses, polysaccharides, oligosaccharide of plant substances (Puwastienet *al.*, 2011). Foods rich in fiber have also the capacity of binding bile acids, metabolites of cholesterol, which plays an important role in the digestion and absorption of lipids in the small intestine (Rodriguez *et al.*, 2006).Fruits, vegetables, seeds, and whole grains supply most of the fiber in the human diet.

Total fiber content was calculated from the equation (AOAC, 2000).

$$\text{Total crude fiber} = \frac{M_1 - M_2}{M_3} \times 100 \dots\dots\dots 6$$

Where; M_1 is dried sample, M_2 is dried ashes sample and M_3 is the weight of sample.

3.4.5 Determination of crude fat

Determination of fat content was performed by soxhlet extraction. The solvent used for extraction fat was diethyl ether. 2g of sample was added in extractor thimble (w_1).A clean, dried round bottom extraction flask containing a few granules of boiling chips were weighed (W_2). The extraction thimble and flask was fitted on the extractor unit and 160 ml of diethyl ether was poured into the flask using a tube connected on the top of the extraction unit. Condenser was connected to the Soxhlet extractor and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction is carried out for 5 hr. The solvent is recovered and the oil dried in an oven set at 70 °C for 1 hr. The round bottom flask and oil was cooled in a desiccator and then weighted (W_3) (AOAC, 2000).The extract is calculated as:

$$\% \text{ Crude fat} = \frac{(w_3 - w_2)}{w_1} \times 100 \dots\dots\dots \text{equation 7}$$

3.8.6 Determination of Crude Carbohydrate Content

The total carbohydrate content was determined by difference from other nutrients, according to (Egan *et al.*, 1981).

$$\text{Carbohydrate} = 100 - (\%M + \%P + \%F + \%Fb + \%A) \dots \dots \dots \text{equation 8}$$

Where: C = Carbohydrate content, M = Moisture content, P= Protein, F = Fat content, Fb = Fiber content and A =Ash content.

3.8.7 Determination of Energy Value

The energy value of the samples was determined by multiplying the protein content by 4, Carbohydrate content by 4 and fat content by 9 (Livesey, 1995).

$$\text{ED (energy density)} = 9 \times \text{crude fat (\%)} + 4 \times \text{carbohydrates (\%)} + 4 \times \text{proteins (\%)} \dots \dots \dots \text{equation 8}$$

However, the metabolizable energy (ME) content of the chicken experimental diets was determined according to (Wiseman, 1987).

3.9. Statistical Analyses

The data derived obtained from laboratory was subjected to statistical analysis including mean, standard deviation, and ANOVA. (The means were compared to determine whether they were significantly different using SAS software (SAS Institute, 2002).

Three replicate determinations were carried out for each sample. When P values ($P < 0.05$) was found significant, the means of each parameter was compared using the least significant differences (LSD) procedures of the appropriate software.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Optimization of Digestion Procedure

Table 1: Different conditions of digestion procedure tested for one gram papaya sample

Trial No	Reagent used	Volum e ratio	Temperature(⁰ C)	Digestion time(min)	Observation
1	HNO ₃ :HClO ₄	3:1	120	10	Pale yellow solution
2	HNO ₃ :HClO ₄	3:1	150	25	Pale yellow solution
3	HNO ₃ :HClO ₄	3:1	150	40	Slight Pale yellow sol.
4	HNO ₃ :HClO ₄	3:1	160	65	Slight pale yellow sol.
5	HNO ₃ :HClO ₄	2:2*	90*	80*	Clear colorless sol.
6	HNO ₃ :HClO ₄	3:1	200	95	Colorless solution
7	HNO ₃ :HClO ₄	3:1	210	115	Colorless
8	HNO ₃ :HClO ₄	3:1	240	120	Cloudy suspension

The optimal digestion procedure chosen was the one that require 2hrs for complete digestion of onegram of papaya flour with 2ml (70%) HNO₃ and 2ml of 70% HClO₄ (Table 1).

*Optimum condition.

4.2. Instrument Calibration

Calibration curves were prepared to determine the concentration of the metals in the sample solution. A series of working standard solutions were prepared from the 10 mg/L intermediate standard solutions of their respective metals. Wavelengths, concentration of the intermediate standards, working standard solutions and the correlation coefficients of the calibration for each of the metals are presented in Table 2.

Table 2: Concentration of Working Standards and Correlation Coefficients of the Calibration Curves

No	Metal	Wavelength (nm)	Concentration of intermediate standard (mg/L)	Concentration of working standards (mg/L)	Correlation coefficient of calibration curves
1	K	766.5	10	1.0, 3.0, 5.0, 7.0, 10	0.998
2	Ca	422.7	10	1.0, 3.0, 5.0, 7.0, 9	0.999
3	Mg	285.2	10	1.0, 3.0, 5.0, 7.0, 10	0.996
4	Cu	324.8	10	0.1, 0.5, 1,1.5, 2.0	0.998
5	Zn	213.9	10	0.1, 0.5, 1,1.5, 2.0	0.997
6	Mn	279.5	10	0.5, 1.5, 3.0, 5, 6.5	0.998
7	Fe	248.3	10	1.0, 2.5, 5, 8, 10	0.999
8	Cd	228.9	10	0.1, 0.5, 1,1.5, 2.0	0.999
9	Pb	283.2	10	0.1, 0.5, 1,1.5, 2.0	0.996

4.2. Analytical Method Detection Limits

The detection limit for the methods was calculated by multiplying the standard deviation of

Table 3:Instrument and method detection limits of metals in Papaya

No.	Metal	Instrument detection limit (mg/L)	Method detection limit for papaya (mg/L)
1	K	0.010	0.04
2	Ca	0.010	0.05
3	Mg	0.001	0.005
4	Cu	0.020	0.062
5	Zn	0.005	0.022
6	Mn	0.0010	0.002
7	Fe	0.030	0.034
8	Cd	0.005	0.01
9	Pb	0.100	0.12

Seven blank signals each determined in triplicate by three. The calculated MDL are given in table 3 above.

4.3. Evaluation of Analytical Method

As shown in Table 4 below, the percentage recovery for papaya samples lie in the range 90-104%, which are within the acceptable range for metals (Hight, 1998). This confirms that the method is of good precision and accuracy.

Table 4: Recovery test for the optimized procedure of papaya sample.

Metal	Conc. in sample (mg/L)	Amount added (mg/L)	Conc. in spiked sample (mg/L)	Amount recovered (mg/L)	Recovery (%)
K	4.00	5	9.20	5.2	104
Ca	15.00	5	19.50	4.5	90
Mg	10.50	5	16.00	5.5	94
Mn	0.50	2	2.40	1.9	98
Fe	3.50	5	8.30	4.8	96
Zn	0.40	2	2.5	2.1	102
Cu	0.14	2	2.10	1.96	99.2
Cd	0.01	2	2.00	1.99	99.8
Pb	0.20	2	2.20	2	100

4.4. Distribution of Metals in Papaya Samples

The levels of metals in papaya samples determined were expressed per dry weight as shown in Table 5. Generally, the observed trend from table 5 below was Zn>Pb>Cu > Cd. Based on this, the distribution pattern of specific metals in papaya was discussed in detail as follows:

The levels of Ni ranged from 0.14to 0.45mg/kg in papaya. The highest value observed in Ashri and the lowest value observed in Laften of papaya fruit. Analysis of variance (ANOVA) showed that the mean concentration of nickel was significantly different in all sampling sites. Among the sample sites, level of Zn in papaya fruit in increasing order;Laften<Tigri<Asha<Kenama<Sewuda<Ashri. This difference may be due to difference in

climate, soil nature, fertilizer and soon. The permissible limit set by FAO/WHO (1984) for edible plants is 27.4 mg/kg which is higher than that found in the present study.

Table 5: Concentration (mean \pm SD, n= 3 in mg/kg dry wt) of metals in papaya samples

Sites	Zn	Cd	Cu	Pb
Asha	0.22 ^d \pm 0.01	0.01 ^a \pm 0.00	0.14 ^c \pm 0.00	0.16 ^a \pm 0.05
Laften	0.14 ^f \pm 0.00	0.01 ^a \pm 0.00	0.14 ^c \pm 0.0	0.17 ^a \pm 0.06
Kenama	0.30 ^c \pm 0.01	0.01 ^a \pm 0.00	0.14 ^c \pm 0.00	0.20 ^a \pm 0.07
Ashri	0.45 ^a \pm 0.010	0.01 ^a \pm 0.00	0.16 ^a \pm 0.00	0.22 ^a \pm 0.07
Sewda	0.38 ^b \pm 0.00	0.01 ^a \pm 0.00	0.14 ^c \pm 0.00	0.21 ^a \pm 0.08
Tigeri	0.16 ^e \pm 0.00	0.01 ^a \pm 0.00	0.15 ^b \pm 0.00	0.24 ^a \pm 0.09
LSD	0.01	0.002	0.005	0.12
CV	1.951	0.06	1.941	4.29

NB:-Means with the same letter in the same column are not significantly different.

As shown in table 5 above, the concentration of Cd in all sites was 0.01 mg/kg. One way analysis of variance showed that there was no significant difference in the level of Cd in papaya among sample sites. The permissible limit for Cd set by FAO/WHO (1984) in edible plants was 0.2 mg/kg. However, for medicinal plants the permissible limit for Cd set by WHO (2005), China and Thailand was 0.3 mg/kg in finished herbal products. The level of cadmium in papaya was below these standards implying that there was no Cd contamination.

As shown in Table 5, the levels of copper ranged from 0.14 to 0.16mg/kg in papaya. The highest value observed in Ashri and the lowest value observed in Asha. Analysis of variance showed that the mean concentration of copper in Ashri was significantly higher than other sites. But the difference in Asha, Laften, Saja Kenama, and Sewuda was not significant. The level of copper obtained in this study was higher than the level reported by (Okonet *al.*, 2017). And the value was lower than the concentration reported by (Belstian and Belete, 2018). This difference may be due to different agricultural practice and soil nature. The concentration of lead was ranged from 0.16

to 0.22 mg/kg. One way analysis of variance showed that the concentration of lead was not significantly different among sampling sites. The maximum permissible limit of Pb in fruits and vegetables set by WHO/FAO was 0.30 mg/kg. The level of Pb obtained in this study was lower than the maximum permissible value revealing that there was no Pb contamination (WHO/FAO, 2012).

Table 6: Concentration (mean \pm SD, n= 3 in mg/kg dry wt) of metals in papaya sample

Site	K	Ca	Mg	Fe	Mn
Asha	3.83 ^b \pm 0.12	6.14 ^e \pm 0.12	10.33 ^c \pm 0.06	1.85 ^{cd} \pm 0.12	0.24 ^d \pm 0.01
Laften	3.85 ^b \pm 0.06	7.13 ^c \pm 0.17	9.76 ^d \pm 0.05	1.18 ^e \pm 0.12	0.20 ^e \pm 0.01
Kenama	4.64 ^a \pm 0.06	6.88 ^d \pm 0.06	10.54 ^b \pm 0.05	4.79 ^b \pm 0.23	0.32 ^c \pm 0.01
Ashri	3.93 ^b \pm 0.06	24.51 ^a \pm 0.17	10.87 ^a \pm 0.06	5.76 ^a \pm 0.35	0.49 ^a \pm 0.01
Sewuda	4.58 ^a \pm 0.06	21.23 ^b \pm 0.17	10.51 ^b \pm 0.06	2.30 ^c \pm 0.40	0.42 ^b \pm 0.02
Tigri	3.91 ^b \pm 0.05	7.23 ^c \pm 0.12	10.18 ^c \pm 0.17	1.77 ^d \pm 0.17	0.19 ^e \pm 0.01
LSD	0.13	0.25	0.17	0.46	0.02
CV	1.72	1.16	0.85	8.71	3.79

NB:-Means with the same letter in the same column are not significantly different.

As shown in above Table 6, papaya contains higher amount of Ca (7.13-24.51mg/kg), followed by Mg (9.76-10.87 mg/kg) and K (3.83 – 4.64mg/kg). The higher level of K, Ca and Mg in the papaya was probably due to the fact that they are highly mobile in the plant tissue (Ayenew *al.*, 2014; Minaleshewa, 2010; Sailus, 2014). The other probable reason for higher concentration of K, Mg and Ca is due to the present of fertilized soil with manure and organic residues which is rich with these metals (Weeraratnaet *al.*, 1977). The concentration of Fe (1.18 – 5.76 mg/kg) in papaya was higher among determined trace metals. Higher levels in the papaya may be attributed to the availability of this metal in relatively acidic soils of the farmland (Ishibashi *et al.*, 2004).

The levels of potassium ranged from 3.83mg/kg to 4.64mg/kg in papaya. The highest value observed in Sajakenama and the lowest value observed in Asha in papaya fruit. Potassium was the third most accumulated metal in papaya. This higher concentration of K in papaya is may be the result of its higher concentration in the soil. Weeraratna et al. (1977) reported that addition of K fertilizers to the soils rapidly increase concentration of available K that could be attributed to mineralization of the organic matter, the solubilization effect of rain water and due to increased chemical and biological fixation of potassium in the presence of fertilizers. The concentration of K found in this study was lower than the value reported by (Adesuyi and Ipinmoroti, 2011). This difference may be due to difference in climate, soil fertility, fertilizer. Calcium was the most accumulated metal in papaya. The levels of calcium ranged from 6.14 mg/kg to 24.51 mg/kg. The highest value observed in Ashri and the lowest value observed in Asha. Analysis of variance showed that the mean concentration of calcium was significantly different among all sampling sites except Laften and Tigri. Among the sample sites, level of calcium in papaya fruit in Asha < Kenama < Laften = Tigri < Sewuda < Ashri.

This difference is based on climate change, soil fertility and so on. Magnesium was the second most accumulated metal in papaya next to calcium. The levels of magnesium ranged from 9.76mg/kg to 10.87 mg/kg in papaya. The highest value observed in Ashri and the lowest value observed in Laften. Analysis of variance showed that the mean concentration of magnesium in Ashri was significantly higher than the level of magnesium in other sampling sites. Comparable level (11.37 mg/kg) of magnesium was reported from Nigeria (Adesuyi and Ipinmoroti, 2011). The levels of iron ranged from 1.77mg/kg to 5.76mg/kg in papaya.

The highest value observed in Ashri and the lowest value observed in Laften. Analysis of variance showed that the mean concentration of iron in Ashri was significantly higher than the level of iron in other sampling sites. This might be due to geographical and geological differences of the soil and different agricultural practices including the use of different agro-chemicals. Comparable level (3.87 mg/kg) of iron was reported from Nigeria (Adesuyi and Ipinmoroti, 2011). The maximum permissible limit of iron set by WHO for edible fruits and vegetables was 15 mg/kg (WHO/FAO, 2012). The value obtained in this study was lower than this permissible limit indicating that there was no Fe contamination in the given fruit.

The levels of manganese ranged from 0.19mg/kg to 0.49mg/kg in papaya. Analysis of variance showed that the mean concentration of iron in Ashriwas significantly higher than other sampling sites. Higher amount of Mn was reported by (Adesuyi and Ipinmoroti, 2011). This might be due to geographical and geological differences of the soil and different agricultural practices including the use of different agro-chemicals.

4.5. Comparison of Metal Levels of papaya with Literature Values

The comparative study of the metal concentration of papaya determined in this study and reported values of other researchers are presented in Table 7. The level metals in papaya showed good agreement with different literature values reported by different researchers. But some metals showed variation with the reported values, and this variation was attributed to difference in soil nature, different farming system including the use of agrochemicals, different method of analysis, different geographical and climatic conditions. The concentration of Mg in papaya found in this study was comparable with the value reported by (Adesuyi and Ipinmoroti, 2011). The concentration of Fe in papaya found in this study was in range with the value reported by (Okon et al., 2017).

Table 7:-.Comparison of determined metals concentration with reported values in the \papaya.

Metal	Country	Conc.(mg/kg)	Reference
K	Nigeria	17-47.6	Adesuyi and Ipinmoroti, 2011
	Saja, Southern Ethiopia	3.83-4.64	Present Study
Ca	Nigeria	25.2-41.4	Adesuyi and Ipinmoroti, 2011
	Saja, Southern Ethiopia	6.14-24.51	Present Study
Mg	Nigeria	5.27-28.13	Adesuyi and Ipinmoroti, 2011
	saja Zone, Southern Ethiopia	9.76-10.87	Present Study
Mn	Kenya	14.25	Kipkemboi, 2009
	saja, Southern Ethiopia	0.19-0.49	Present study
Fe	Kenya	136.3	Kipkemboi, 2009
	Nigeria	2.8	Okon et al., 2017
	Saja, Southern Ethiopia	1.18– 5.76	Present Study
Cu	Kenya	3.61	Kipkemboi, 2009
	Nigeria	0.5-1.9	Adesuyi and Ipinmoroti, 2011
	Saja, Southern Ethiopia	0.14-0.16	Present study
Zn	Nigeria	0.7	Okon et al., 2017
	Saja, Southern Ethiopia	0.14-0.45	Present study
Cd			
	Saja, Southern Ethiopia	0.01	Present study
Pb	Nigeria	0.001	Okon et al., 2017
	Saja, Southern Ethiopia	0.16-0.24	Present study

4.6. Analysis of Proximate Composition in Papaya

As shown in Table 8 below, the average protein contents ranged from 6.47 to 13.90%. The highest amount of crude protein was found in the Asha kebele, while the lowest was found in the Tigir kebele. The protein contents showed a significant variation among sampling sites.

This may be due to the condition differences environmental conditions, agricultural practices and fertilizer application (Sharma et al., 1990). The level of crud protein obtained in this study was in agreement with the value (12.49%) reported from Nigeria (Williams, 2017).

Table 8: Proximate Composition in Papaya (mean±SD)

Sample sites	% CP	%MO	%Ash	%Fiber
Asha	11.45c±0.58	71.83b±0.23	6.37c±0.88	1.80b±0.10
Laften	6.81e±0.12	72.42a±0.29	6.83b±0.56	1.40c±0.28
Kenam	8.27d±0.23	68.99e±0.10	7.43a±0.34	1.50c±0.12
Ashri	13.90a±0.11	70.56d±0.28	4.93e±0.06	1.90b±0.00
Sewuda	12.83b±0.23	71.22c±0.50	5.90d±0.01	2.20a±0.23
Tigri	6.47e±0.29	72.39a±1.0	7.10ba±0.35	1.87b±0.11
LSD	0.54	0.44	0.42	0.30
CV	3.034	0.34	3.65	9.37

The moisture contents varied from 68.99 to 72.42% in various locations. The highest amount of moisture was found in the Laften kebele, while the lowest was found in the Saja kenama. Analysis of variance showed that the mean concentration of Moisture of Laften and Tigri was not significantly different. Among the sample sites the concentration level of moisture in papaya fruit can be arranged as Kenama<Ashri<Sewuda<Tigri<Laften. Williams (2017) reported lower moisture content (26.07) than the present study. But higher content of moisture (80.13%) was reported by (Belsti and Belete, 2018). These differences may be due to difference in climate and soil nature. Ash contents ranged from 4.93 to 7.43%.

The highest amount of ash was found in the Saja Kenama while the lowest was found in Ashri kebele. Analysis of variance showed that the mean concentrations of ash of Laften, Kenama and Tigri were not significantly different. The percentage of ash gives ideas about the content of minerals of food and that indicates total mineral residue left after incineration of organic matter. Total ash content directly proportional with inorganic element content. Hence the samples with high percentage of ash contents were expected to have high concentrations of various mineral elements (Puwastien *et al.*, 2011). The content of fiber ranged from 1.40 to 2.20%. The highest amount of fiber was found in the Sewuda kebele, while the lowest was found in the Laften kebele. Analysis of variance showed that the mean concentration of fiber in Sewuda was significantly higher than other sample sites. But its content in Laften, Kenama and Tigri was not significantly different. Comparable level of fiber (1.86) was reported by (Williams, 2017). Diets with a high content of fiber have a positive effect on health that decreased prevalence of several types of cancer. It also decreases the absorption of Cholesterol from the gut, delaying the digestion and conversion of starch to simple sugars.

As indicated in Table 9 below, the crude fat ranged from 2.08 to 2.85%. The highest amount of crude fat was obtained in Asha and the lowest value was found in Kenama. The analysis of variance showed that the content of crude fat obtained in Asha was significantly higher than other sample sites. Slightly lower content of crude fat (1.11) was reported by (Williams, 2017). The variation in crude fat contents may be due to the difference in environmental conditions, agricultural practices and fertilizer application, seed storage conditions.

Table 9: Proximate composition of fat, carbohydrates and energy (kcal)

Sample sites	%Fat	total %CH	Energy (kcal)
Asha	2.85a±0.06	6.30d±0.17	95.11c±5.70
Laften	2.11cd±0.11	10.40b±0.17	88.47d±4.04
Kenama	2.08d±0.07	11.45a±0.15	98.06b±5.88
Ashri	2.71b±0.12	6.29d±0.16	105.28a±2.89
Sewuda	2.15cd±0.05	6.21d±0.20	94.89c±1.15
Tigri	1.87b±0.11	9.97c±0.06	85.44e±1.15
LSD	0.13	0.27	0.72
CV	3.08	1.79	0.43

As shown in the table 9 above, total carbohydrate content ranged from 6.21 in Sewuda to 11.45% in Kenama. One way analysis of variance showed that the content of total carbohydrate in Sewuda was significantly higher than other sample sites. Higher level of total carbohydrate was reported from Nigeria (Williams, 2017). The variation in contents may be due to the difference in environmental conditions, agricultural practices and fertilizer application, seed storage conditions.

The mean energy content of papaya ranged from 85.44 to 105.28Kcal/g. The highest content of energy was found in Ashri and the lowest content was found in Tigri. One way analysis of variance showed that the energy content in papaya was significantly different among all sample sites.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

Based on the results of the study the following conclusions were made: The efficiency of digestion methods was revealed that the excellent recoveries obtained which were found within the acceptable range for the analyzed metals. A one gram of papaya sample was digested using a mixture of 2 ml HNO₃ and 2 ml HClO₄ at 90 °C for 80 minutes. The result of metal analysis revealed that papaya has the ability to accumulate relatively higher amounts of Ca and Mg among the determined essential metals. The concentration ranges in dry weight basis in decreasing order were: Ca (7.13 -24.51) >Mg (9.76-10.87) > K (3.83 – 4.64) > Fe (1.18 – 5.76) >Mn (0.19-0.49) > Zn(0.14-0.45)>Pb (0.16-0.24) > Cu (0.14-0.16) >Cd (0.01) mg/kg. The proximate composition results indicated that the percentage of nutritional value in papaya ranges from (5.90 to 6.37), (70.56 to 72.42), (6.47 to 13.90), (2.08 to 2.85), (1.40 to 2.20), (6.21 to 11.45), (85.44 to 105.28); for ash, moistures, crude protein, crude fat, crudes fiber, carbohydrates and total energy respectively. The result of metal analysis revealed that papaya contains appreciable level of essentials metals and the level of toxic heavy metals were below maximum permissible value set by WHO/FAO indicating that the given fruit was safe to use. Regarding the proximate analysis, papaya was a good source of carbohydrate, fat, protein, and fiber and energy contents.

5.2 Recommendation

It is recommended that papaya, due to high nutritional value and acceptable levels of selected trace metals could be used in daily human diet. Awareness of people was needed to expand papaya production in the study area and other parts of the country. Further studies should be carried out on other heavy metals, phytochemicals and anti-nutritional value and anti-oxidants of the papaya.

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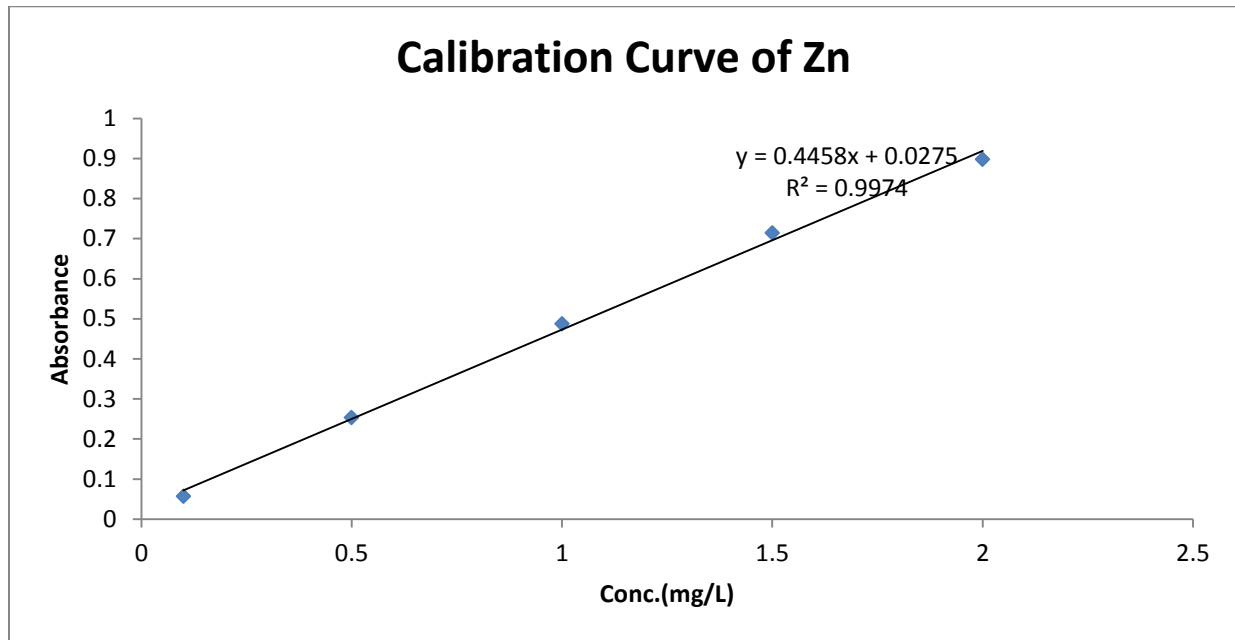
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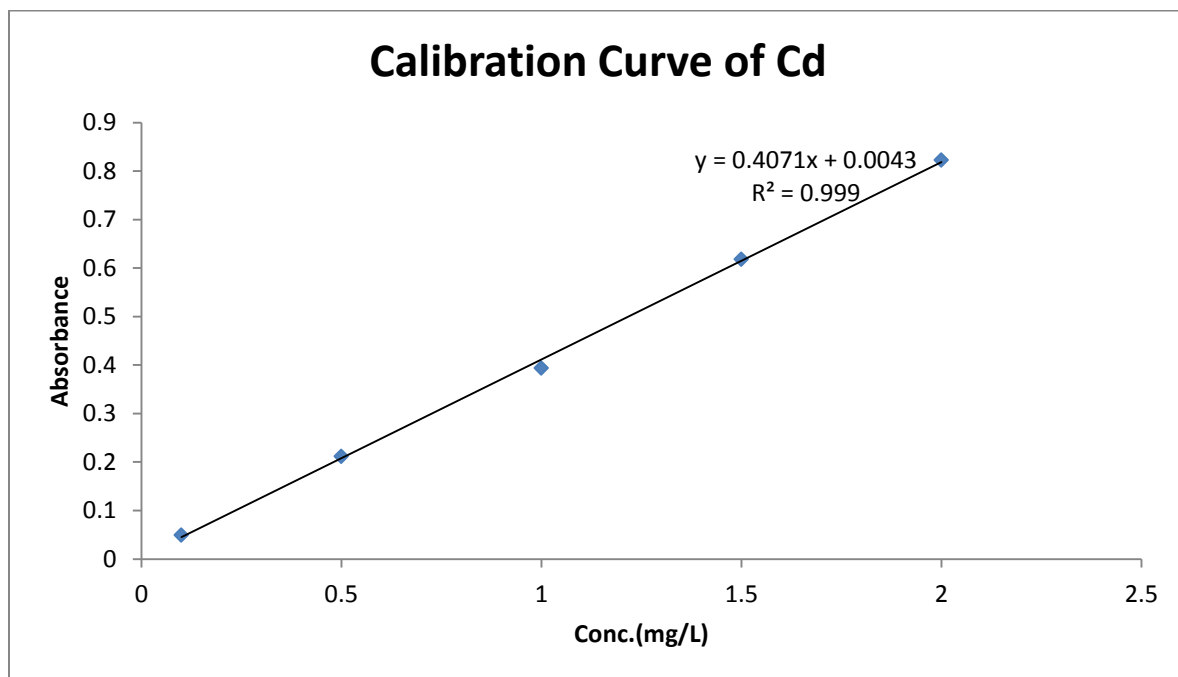
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APPENDIXES

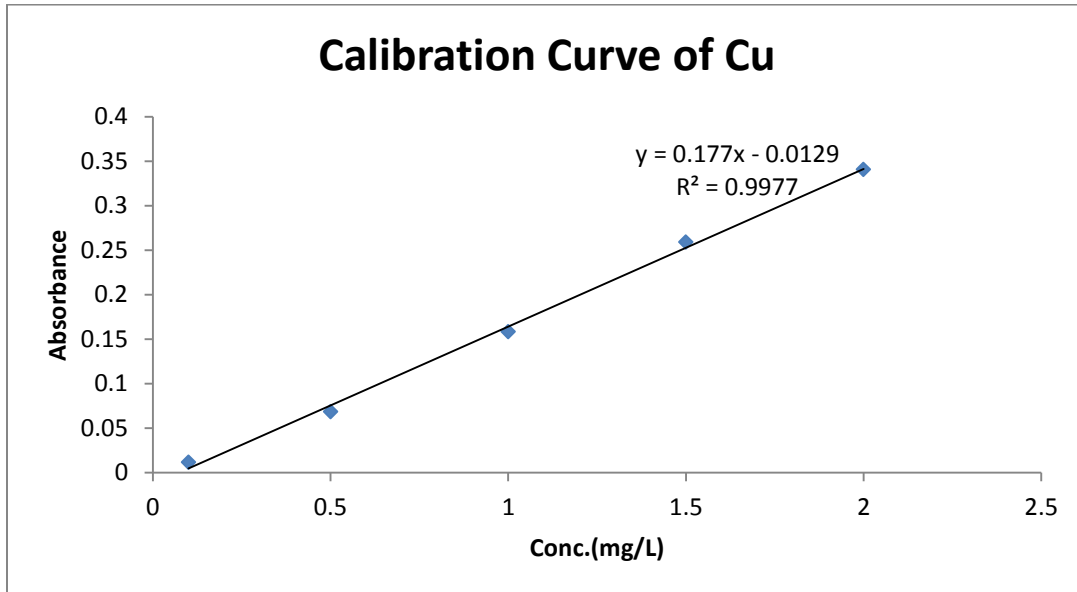
Appendix 1: Calibration curve for Ni



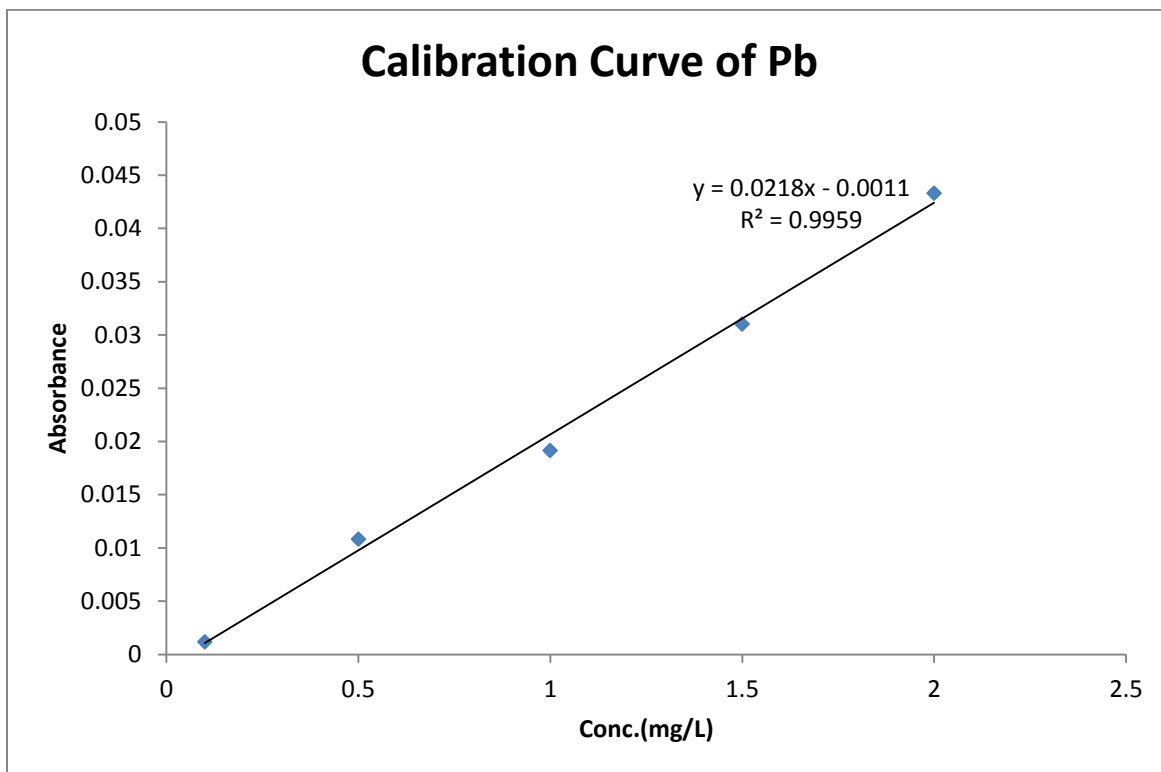
Appendix 2: Calibration curve for Cd



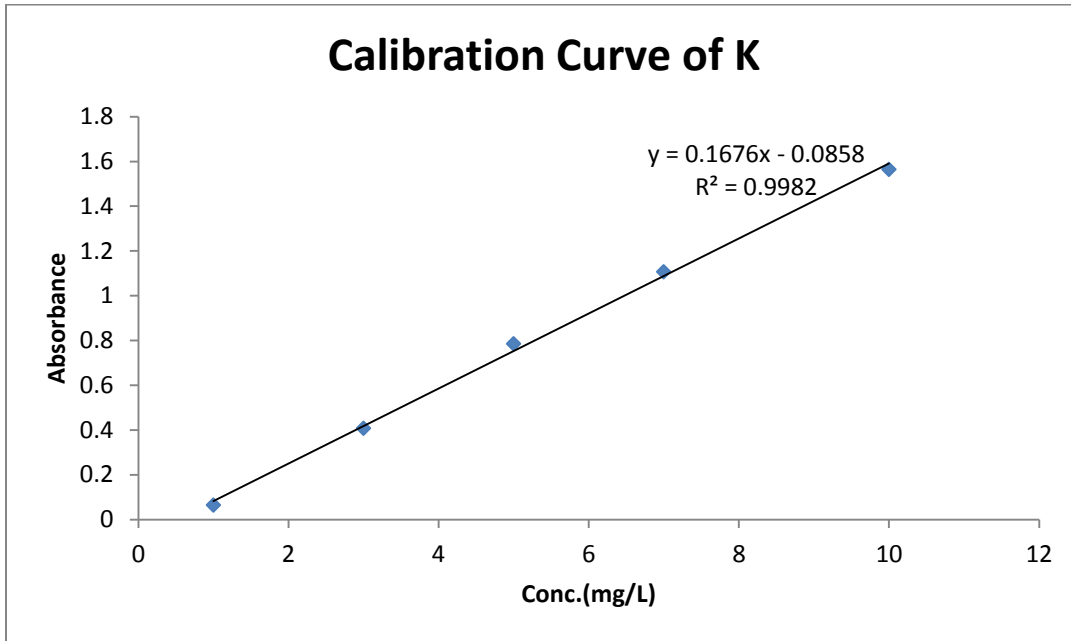
Appendix 3: Calibration curve for Cu



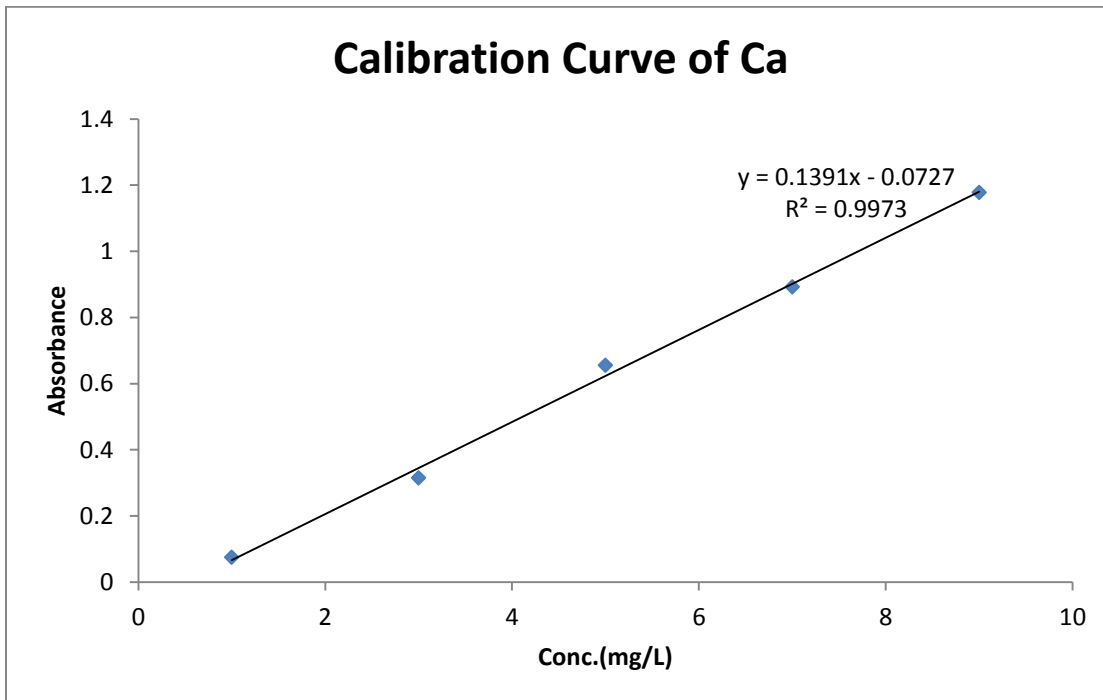
Appendix 4: Calibration curve for Pb



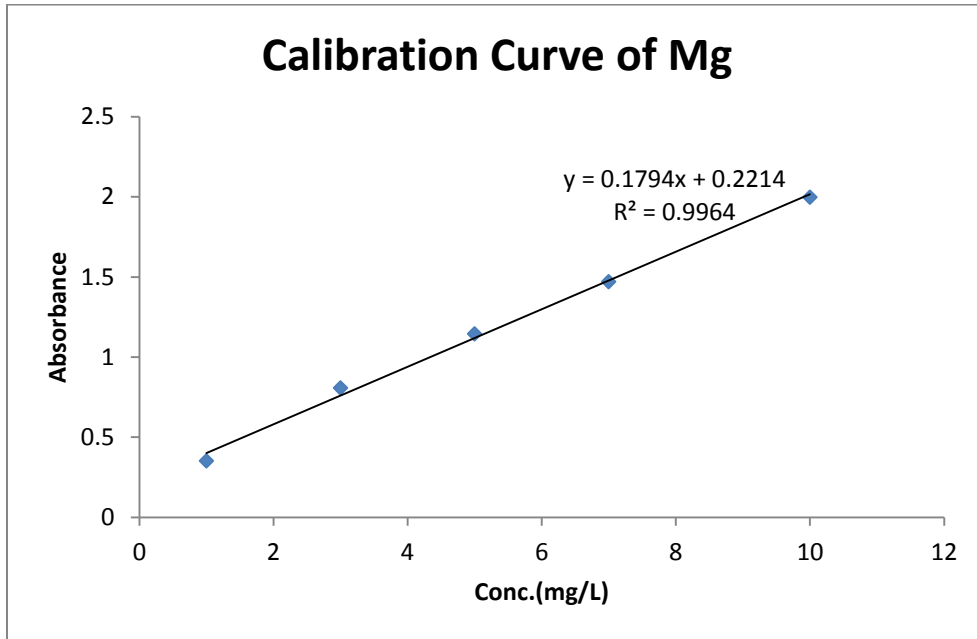
Appendix 5: Calibration curve for K



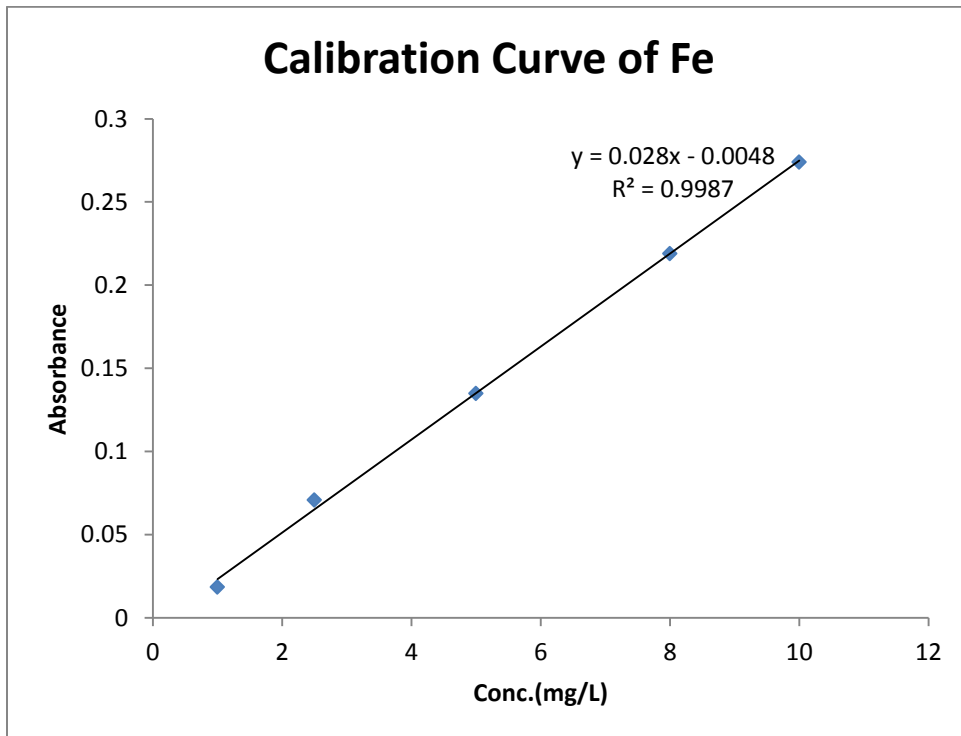
Appendix 6: Calibration curve for Ca



Appendix 7: Calibration curve for Mg



Appendix 8: Calibration curve for Fe



Appendix 9: Calibration curve for Mn

