SALALE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF CHEMISTRY

INVESTIGATION OF THE LEVEL OF VITAMIN C IN TEN WILD EDIBLE PLANTS CONSUMED IN NORTH SHOA ZONE, OROMIA REGION, ETHIOPIA

By: CHALCHISA ABEBE SORI

ADVISOR: GIRMA REGASSA (PhD.)

FICHE, ETHIOPIA

NOVEMBER, 2022

INVESTIGATION OF THE LEVEL OF VITAMIN C IN TEN WILD EDIBLE PLANTS CONSUMED IN NORTH SHOA ZONE, OROMIA REGION, ETHIOPIA

By: CHALCHISA ABEBE SORI

ADVISOR: GIRMA REGASSA (PhD.)

A MASTER THESIS SUBMITTED TO SALALE UNIVERSITY, SCHOOL OF GRADUATE STUDIES, COLLEGE OF NATURAL SCIENCES, DEPARTMENT OF CHEMISTRY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MSc.) IN ANALYTICAL CHEMISTRY.

SALALE UNIVERSITY

FICHE, ETHIOPIA

NOVEMBER, 2022

APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Chalchisa Abebe Sori have read and evaluated his/her thesis entitle "Investigation of the Level of Vitamin C in Ten Wild Edible Plants Consumed in North Shoa Zone, Oromia Region, 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 (MSc.) in Analytical Chemistry

______________________________ __________ ______________

Name of major Advisor/supervisor Signature Date

______________________________ __________ ______________

Chairperson Signature Date

______________________________ __________ ______________

Internal Examiner Signature Date

______________________________ __________ ______________

External Examiner Signature Date

DECLARATION

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: Chalchisa Abebe Sori

Signature: ________________

This MSc dissertation has been submitted for examination with my approval as thesis advisor/ dissertation Supervisor.

Name: Girma Regassa (PhD.)

Signature: _________________

Date of submission**:** ___________

SUPERVISOR'S/ADVISOR'S APPROVAL SHEET

To **Chemistry department**

Subject: **Thesis submission**

This is to certify that the thesis entitled "Investigation of the Level of Vitamin C in Ten Wild Edible Plants Consumed in North Shoa Zone, Oromia Region, Ethiopia submitted in partial fulfillment of the requirements for the degree of Master's degree in Analytical Chemistry, the Graduate program of the department of Chemistry and has been carried out by Chalchisa Abebe Sori Id. No – RM0102/13, under my/our supervision. Therefore, I/we recommend that the student has fulfilled the requirements and hence hereby he/she can submit the thesis to the department.

______________________________ _________ ______________

Name of major Advisor/supervisor Signature Date

______________________________ _________ ______________

Name of Co-Advisor Signature Date

DEDICATION

First and foremost, this Thesis is dedicated to my wife Chaltu Bayisa Tura, for her endless love, support and encouragement to reach who I am now and to my son Firaol and newborn daughter Sena for everlasting and true love they had for me.

LIST OF FIGURES

FIGURES **PAGE**

LIST OF TABLES

TABLE **PAGE**

LIST OF ABBREVIATIONS

AA	Ascorbic Acid
DHA	Dehydroascorbic Acid
FAO	Food and Agricultural Organization
FW	Fresh Weight
MPA	Meta Phosphoric Acid
RDA	Recommended Daily Allowance
SPE	Solid Phase Extraction
USDA	U.S. Department of Agriculture
WEPs	Wild Edible Plants
WSVs	Water Soluble Vitamins

ACKNOWLEDGMENT

I owe the deepest gratitude to my adviser Dr. Girma Regassa for his constructive professional assistance from the beginning to the completion of this thesis work. His support and encouragement from the beginning up to the completion of this study was kindly appreciated.

I also proceed my long list of thanks for the research laboratory team of Ethiopian Health and Nutrition Research Institute from the head of the center to laboratory personal for their politeness and hospitality. I am very thankful to Mr. Tadesse Melaku, Mr. Nahom Haile, the technical staffs of the research center especially, Mr. Asrat Yehulashet, for their collaboration and providing all the necessary laboratory facilities needed for the analysis.

Another long list of gratitude goes to Fiche College of Teachers Education for their assistance specially by covering stationary and copying service.

I am also grateful to my family for their assistance and other graduate students whose discussions and friendship were socially valuable in the study.

Finally I would like to thank almighty God for adding this day in my life.

ABSTRACT

Vitamin C also known as ascorbic acid is the main constituents of fruits and green leafy vegetables. It is an essential antioxidant. Presence of this organic acid plays an important role in human health. This study therefore intended to determine the level of vitamin C in selected wild edible plants such as *Balanites aegyptiaca*, *Carrisa spinarium*, *Dovyalis abyssinica*, *Ficus mucuso welw*, *Opuntia ficus indica, Rubus apetalus, Syzygium giuneens sp.*, *Thymus shimperi, Utrica simensis and Zzizphus spina-chriti* which are commonly consumed around Fiche town. Acid hydrolysis method of sample preparation was used for plant sample preparation. 6% trichloro acetic acid solution was used as extraction solvent. In this method bromine water was used to oxidize ascorbic acid to dehydroascorbic acid. After coupling with 2, 4-dinitrophenyl hydrazine at 37 ^{O}C temperature for three hours, the solution was treated with 85% H_2SO_4 to produce a red color complex. Double beam UV-VIS spectrophotometer method was utilized for investigation of the level of vitamin C in wild edible plants and the absorbance was measured at 515 nm. Based on this method, it was investigated that the content of vitamin C was 0.175 ± 1.90 mg/gm to 1.612 ± 0.06 mg/g in investigated plants with percent relative standard deviation of 0.06 to 2.85% values *(Utrica simensis* 1.612 ± 0.06, *Thymus shimperi* 1.386 ± 1.44, *Syzygium guineense sp*. 1.274 ± 0.78, *Doviyalis abyssinica* 1.130 ± 0.84, *Carissa spinarum* 0.722 ± 1.38, Ziziphus spina-christi 0.531 ± 1.88, Balanites aegyptiaca 0.402 ± 1.99, *Rubus apetulas* 0.350 ± 2.85 and $Opuntia\ ficus\ indica$ 0.175 ± 1.14). Validation experiments revealed good percentage recovery of 98.14, good linearity with R^2 -values 0.997 within the established concentration range. The LOD of the method was 0.05 mg/g whereas the LOQ was 0.15 mg/g. This study showed that the vitamin C contents of fruits and leaves sample varied with fruit and leaf type, and the vitamin C contents of leaves such as *Utrica simensis* and *Thymus shimperi* were higher than some domesticated green leafy vegetables such as cabbage and mustard spinach, moreover, the vitamin C contents of fruits such as *Syzygium guineense sp.*, *Dovyalis abyssinica and carissa spinarum* were higher than some domesticated citrus fruits like lemon and orange. The levels of vitamin C in these wild edible plants were comparable with that of literature value. Therefore, it is recommended that considerable attention should be given to wild edible plants to supplement food insecurity.

Key word: edible wild plants, fruits, leaves, vitamin C, UV-VIS spectrophotometry.

CONTENTS

CHAPTER PAGE

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

Vitamins are fundamental components of the diet which are required in minimum amounts in the body constantly to maintain regular prosperity and diverse physiological limits in the human body. They are extensively circling in ordinary sustenance sources and can be successfully familiarized in the weight control plans which satisfy each day's needs. Vitamins are a group of organic compounds and can be classified into two groups depending on their solubility: fat-soluble vitamins and water-soluble vitamins. The former incorporates lipid soluble nutrients A, D, E, and K and various carotenoids. The latter is made up of water soluble vitamins C and eight B-vitamins, specifically thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and cyanocobalamin (B12) (Fatima, Z. *et al*. 2019). Vitamins play a very important role in maintaining health (Chimezie, A. *et al.* 2008), contributing to a healthy immune system and providing all the nutrients essential for good health. They have complex biochemistry and play an essential role in human nutrition and health.

Water soluble vitamins (WSVs) are well known for their special role in energy metabolism as well as in the maintenance of healthy muscles, skin, eyes, hair, and liver. They act as precursors of coenzyme and enzyme cofactors in different metabolic reactions occurring in the body for example; lipids, carbohydrates and protein metabolism. Their deficiency or excess in the human body can cause several metabolic and other health problems such as beriberi, anemia, neurological diseases, oral lesions, and pellagra. Vitamin C plays a beneficial role in some health-promoting effects like biosynthesis of collagen, carnitine and hormones, immune response, iron absorption and its inadequate intake can cause fatal disease scurvy. Some WSVs also exhibit antioxidant activity especially vitamin C is a well-known food additive because of this property (Fatima, Z. *et al*. 2019).

Vitamin C also known as ascorbic acid (AA) is an organic compound having the formula $C_6H_8O_6$. It is a valuable food component because of its antioxidant and therapeutic properties. As a potent antioxidant, it has the capacity to eliminate several different free radicals (Desai, A.P. and Desai, S. 2019).

It also has important biological and metabolic functions, particularly with respect to its role in the biosynthesis of connective tissue. Vitamin C aids the metabolism of tyrosine, folic acid, and tryptophan.

Vitamin C lowers low density lipoprotein (LDL) which is bad cholesterol and triglyceride levels in the blood, which are both risk factors for heart disease. It is outright vital for cognitive development of infants during pregnancy, and there are higher dietary requirements of it during pregnancy and lactation which have to be met wholly through dietary intake of vitamin C and any deficiency may result in cognitive impairment to the child (Desai, A.P. and Desai, S. 2019). Vitamin C is also useful as a nutritious food additive, a reducing agent, a stabilizer, and a color stabilizer. As a result, figuring out what this molecule is crucial for pharmaceutical and biological purposes. Scurvy, anemia, and numerous infections and mental illnesses are all caused by a lack of vitamin C in the body. The Food and Drug Administration (FDA) recommendations for vitamin C intake are 75–90 mg daily for adult females and males, respectively (Hagos, M. *et al*. 2022).

Plants rich in fruits, vegetables, whole grains, and legumes, provide an abundance of vitamins and minerals to meet one's nutritional needs. The therapeutic potential of the vegetables is largely dependent on the presence of vital vitamins as well as micronutrients. Even though vitamin is required in a small amount per day in health, it plays a vital role in our health. The consumption of leafy vegetables and fruits rich in vitamin C are reported to reduce the risk of attack of various acute and chronic diseases (Seal, *et al*. 2017a). Plant-derived vitamin C is of great interest because of its impact on human health. Vitamin C is essential for metabolism because of its redox chemistry and role as enzymatic cofactors, not only in animals but also in plants. It has a strong antioxidant potential. Humans, unlike most animals, are unable to synthesize vitamin C, thus it must obtained through the diet. The best sources of vitamin C are fruits and vegetables. Our bodies need vitamin C to make a substance collagen which is important for the health and repair of our skin, bones, teeth and cartilage. Vitamin C was first isolated in 1928. In 1932 it was proved to be the agent, which prevents scurvy, a diseased condition which occurs due to deficiency of vitamin C in the body (Desai, S. 2019).

Different wild edible plants (WEPs) have played a significant role in different geographical regions of the world throughout human history (Sekeroglu, N. *et al*. 2006).

Wild vegetables and fruits contribute to people's food security and health in many rural areas of the world. According to Dirres A.D. (2016) wild edible plant refers to species that are neither cultivated nor domesticated but available as wild natural habitat. These wild edible plants are used as sources of food and can provide food nutrients that are essential for human body (Ramosweu, P. 1999). Most of these plants are consumed in different forms. Mostly they are consumed as raw during ripping season. Therefore these underutilized wild edible plants play an important role in the life of local as well as urban population (El-Gazali, G. E. *et al*. 1987; Abdelmuti, O.M.S. 1991; Ohiokpehai, 2003). Since wild plants contain vitamins, minerals and trace elements, they complement staple foods towards a balanced diet even under normal living conditions (Dogan, Y. *et al*. 2013). These plants also provide some useful products like medicine, fibre, fodder, dyes etc (Kayang, H. 2007). The study of wild edible plants is important to identify the potential sources which could be utilized as alternative food.

Recent study has proved that these wild edible plants have wide use for dynamic sources of traditional medicine, nutrition, vitamins, minerals, building materials, fuel and transport materials. Wild edible plants have always been an essential and widespread food source for food-insecure families living in poverty in developing countries. Many wild edible plants are rich in nutrients and vitamin C, which make them particularly valuable in terms of a balanced diet in communities that have shortage of food. Even if they are common as source of food (nutritional values, vitamins, minerals and phytochemicals) their contents are not studied well (Osman, M.A. 2004). Thus they are underutilized still now in different area of the country. Therefore nutritional qualities (vitamin C content) of these underutilized wild edible plants were investigated and summarized. Recent studies show that chronic diseases are increasing in the world rapidly. Hence diet and nutrition are factors affecting the promotion and maintenance of health wellness in the entire life time.

Food security concerns and the remarkable nutritional content of wild edible plants demanded the investigation of these plants for food contents. Distinctive instrumental techniques have been utilized for the determination of Vitamin C including direct titration, fluorometric methods, chromatographic methods, and spectrophotometric methods (Norfun, *et al*. 2016). Vitamin C has strong UV absorption, which is the basis of spectrophotometric methods for the quantification of vitamin C (Elgailani, I.E. 2017).

Low consumption of vitamin C in diet is one of the major factors which lead to deficiency of vitamins and other minerals. Vitamin C cannot be synthesized by animals and must be provided from plants or foods. Considering the inadequate concentration in the diet and the potential of wild edible plants as a source of vitamin C, the researcher was interested in investigating the content of vitamin C in ten wild edible plants that are commonly consumed in North Shoa zone of Oromia Region.

1.2. Statement of the Problems

Vitamins play a very important role in maintaining health (Chimezie, A. *et al*. 2008), contributing to a healthy immune system and providing all the nutrients essential for good health. Diets that do not contain adequate amounts of vitamins often result in vitamin deficiency-related diseases, including blindness and mental retardation, depending on the particular vitamin that is lacking. For example, insufficient vitamin C intake causes scurvy, which is characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility (Sharaa, I.E. and Mussa, S.B. 2019). Vitamin C is regarded as one of the most suitable dietetic antioxidant agents since it is naturally available in great quantities in vegetable foods (Moyer, R. A. and Sellappan, S. 2002).

In Ethiopia and other African countries, there are a number of underutilized indigenous wild edible fruits and leaves gathered from the wild that play vital roles in the nutrition of the people, mostly to rural population, but no attention is given to those edible plants in our country. Hence, there is a need to promote the utilization of these indigenous underutilized wild edible plants. Growing and using wild edible fruits and leaves is an opportunity that has never been adequately prospected to alleviate malnutrition and promote food nutrition (Aynachew, T. 2018).

On the other hand, a lot of edibles plants, including many fruits of wild/semi-wild origin and leaves are known to be periodically consumed by rural communities in Ethiopia. Ethiopia is one of the developing countries rich in endemic wild edible plants. Common wild edible fruits include *Balanites aegyptiaca* (L) (Baddannoo), *Dovyalis abyssinica* (Koshommii), *Ficus* *mucuso welw* (harbuu), *Mimusops kummel bruce* (Qolaadi) *and Ziziphus spina-christi (qoqorii/qurquraa). Utrica simensis* (Doobbii) is common edible wild leaf. Low-income people depend on edible wild plants both for cash and survival, and can be exploited. Many WEPs in Ethiopia were reported as emergency, supplementary or seasonal food sources to prevent food insecurity in households of Ethiopian cultural groups. For example, the invasive *Opuntia ficus-indica* (Cactaceae), was found to be widely exploited for its fruit in many parts of northern Ethiopia, playing a significant role in food source diversification (Addis, G. *et al.* 2009).

They are not well studied for their phytochemicals composition, antioxidant capacities, and nutrient composition of these wild edible plants. This may be the reason why they are underutilized. Many WEPs are mentioned for their rich sources of AA, natural antioxidants, carotenoids and folic acid. Generally, the information available from the nutritional analysis of WEPs shows their potential contribution to dietetic diversity and food security (Lulekal, E. *et al*. 2011). Lack of scientific information on nutritive values and health benefits of these underutilized wild edible plants has contributed to the absence of diversified use as a food source in Ethiopia.

Compared to domesticated plant food sources, wild plant foods tend to be overlooked. However, there is substantial evidence that indicates the importance of WEPs in terms of the global food basket. Many WEPs are mentioned for their rich sources of vitamin C, natural antioxidants, carotenoids, and folic acid. Generally, the information available from the nutritional analysis of WEPs shows their potential contribution to dietetic diversity and food security (Lulekal, E. *et al.* 2011). Even though ethno botanical studies explain the existence of these food sources, the level of nutrients and vitamin C in these WEPs is not highly researched yet. In other words, in most parts of Ethiopia, there is a problem with vitamin malnutrition in many communities, especially in poor rural communities (Balemie, K. and Kebebew, F. 2006). This controversial issue therefore motivates the researcher to study the vitamin C content of selected wild edible plants commonly consumed around Fiche town.

1.3. Objectives of the Study

1.3.1. General Objective

The general objective of this research is to study the contents of vitamin C in commonly consumed WEPs around Fitche town.

1.3.2. Specific Objectives

- To assess the vitamin C content of selected wild edible plants
- To isolate vitamin C from plant matrices by using preferable extraction methods
- To determine the vitamin C content of selected wild edible plants
- To compare vitamin C content of current work with commonly consumed domestic fruits and leaves.

1.4. Significance of the Study

The study may help to promote the utilization of WEPs, which are sources of food and nutrients that are essential for the human body. It may also help to explain the role of WEPs in the efforts to achieve food security and aid to explain their potential for food supplementation. It is used in proper utilization of large accessible biodiversity in our environment. Furthermore, it may be used as a source of information for those who are in need.

1.5. Scope of the Study

Analysis of the content of vitamin C in WEPs is necessary to promote its consumption and nutritional value, especially for communities who live in rural areas in developing countries like Ethiopia. However, because of the shortage of time and budget for this research, the study was limited to investigation of ascorbic acid (vitamin C) in ten WEPs consumed around Saka Sole.

1.6. Limitation of the Study

During the journey for sample collection, the researcher lacked access to transportation, a per diem for each day, and seasonal variation of sample fruits. After all these, the major challenge was the scarcity of laboratory instruments for sample preparation and analysis, which resulted in the cost and time-consuming nature of finishing thesis work within the scheduled time.

1.7. Thesis Outlines

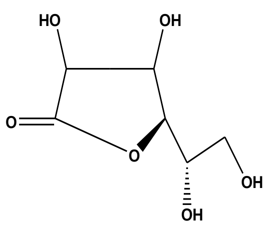
The first chapter introduces the project (what it's all about, what analytical techniques were used, as well as the gap and what influenced the choice of this investigation). The second chapter gives a detailed background on vitamins, wild edible plants, sample pretreatment, and methods of determination of vitamins from WEPs. The third chapter discussed research methodology and materials used for research and chapter four gives a detailed discussion of results, while the conclusion and recommendations were described in chapter five. The last section is the reference section.

CHAPTER TWO

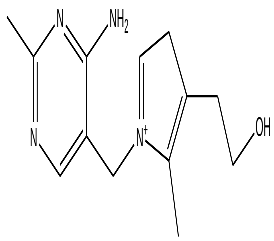
2. REVIEW OF RELATED LITERATURE

2.1. Vitamins

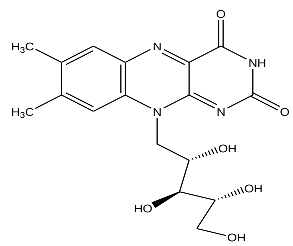
Vitamins are a group of compound, required in small quantity for controlling metabolism and body functioning. Vitamins are two types, fat soluble (A, D, E, K) and water soluble (B and C). Different vitamins are necessary for the body to maintain normal health, as reported by the US National Institute of Health. In recent years, the essential roles of vitamins in human health have received extensive attention. For people who are at risk of vitamin deficiencies, vitamin supplementation is regarded as an effective treatment (Zhang, Y. *et al*. 2018).



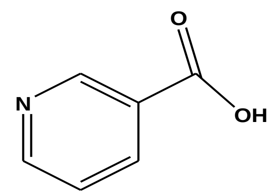
Vitamin C



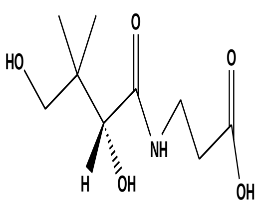
Vitamin B1



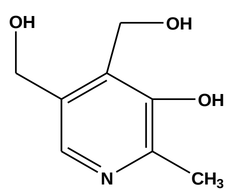
Vitamin B2



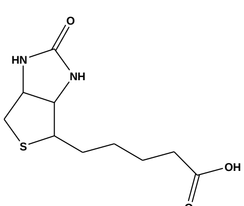
Vitamin B3



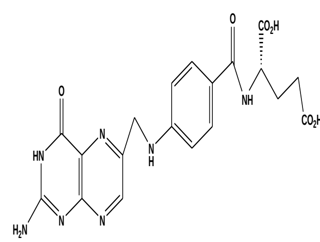
Vitamin B5



Vitamin B6



Vitamin B7



Vitamin 9

Figure 1 Structure of water soluble vitamins (Tyskiewicz, K. *et al*. 2018).

2.1.1. Solubility and Stability of Water Soluble Vitamins

The water-soluble vitamins tend to have one or more polar or ionizable groups (carboxyl, keto, hydroxyl, amino, or phosphate). Vitamin C, thiamin (B1), riboflavin (B2), and biotin (B7) are poorly stable to oxidation. They must be protected from heat, oxygen, metal ions (particularly Fe^{2+} and Cu^{2+}), polyunsaturated lipids undergoing peroxidation, and ultraviolet light. Some vitamins can undergo degradation by reacting to factors in sample storage and/or preparation, for example; ascorbic acid reacts with plant ascorbic acid oxidase (Ball, G.F. 2005).

Table 1 Solubility and stability of water soluble vitamins (Thermo Fisher Scientific**,** 2017)

No	Name of vitamins	Solubility	Stability
1	Thiamine (vitamin B1)	Soluble in water; slightly soluble in ethanol; insoluble in ether and benzene.	Stable in acidic solution, unstable in light or when heated.
2	Riboflavin (vitamin B2)	Soluble in basic aqueous solution; slightly soluble in water and ethanol; insoluble in chloroform and ether.	Unstable in light, and heating; slightly unstable in basic solution.
3	Nicotinamide/niacin (vitamin B3)	Soluble in water, ethanol, and glycerol.	Stable in acidic and basic solutions; stable when exposed to air.
4	Nicotinic acid (vitamin B3)	Soluble in water.	Stable in acidic and basic solutions; stable when exposed to air.
5	Pantothenic acid (vitamin B5)	Soluble in water, ethanol, alkali carbonate hydroxide solution and alkali solution; insoluble in ether.	Unstable in acidic and basic solutions; unstable when heated; calcium salt is stable.
6	Pyridoxine/pyridoxal hydrochloride (vitamin B6)	Soluble in water, ethanol, methanol, and acetone; insoluble in ether and chloroform.	Stable in acid solution; unstable in alkali solution.

Table 1 (Continued)

7	Folic acid (vitamin B9)	Soluble in alkali solution; slightly soluble in methanol; insoluble in water and ethanol.	Stable when exposed to air; unstable when exposed to light.
8	Ascorbic acid (vitamin C)	Soluble in water; slightly soluble in ethanol; insoluble in ether.	Unstable when exposed to air.
9	Cyanocobalamine (vitamin B12)	Soluble in water and ethanol; insoluble in ether, acetone, and chloroform.	Unstable in alkali and strong acid solutions.

2.2. Ascorbic Acid

Vitamin C also called L-Ascorbic acid, has molecular weight 176.12 g/mol, 300 g/L at 20 ^{o}C solubility in water, less soluble in alcohol (20 g/L at 20 °C) and insoluble in chloroform, ether and benzene (Velisek, J., Cejpek, K. 2007). The structure of ascorbic acid is as follow.

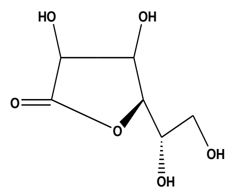


Figure 2 Structure of Ascorbic acid

L-ascorbic acid is a white to very pale yellow crystalline powder with a pleasant sharp acidic taste. Almost odorless (NTP, 1992)**.** L-ascorbic acid or Vitamin C has many roles in our body and has been linked to impressive health benefits. It is also called ascorbic acid. It is water soluble and found in different fruits and vegetables, including strawberries, kiwi fruit, bell peppers, kale, broccoli, spinach, lemon and as well in underutilized wild edible plants. The recommended daily intake for vitamin C is 90 mg for adult men and 75 mg for adult women. Children require around 15–45 mg daily and adolescents 65–75 mg, while infants (12 months or less) require 40–50 mg daily (Whitney, E.N. and Rolfes, S.R. 2015).

2.2.1. Significance of Vitamin C

Vitamins are a class of nutrients that are essentially required by the body for its various biochemical and physiological processes. Popular literature suggests vitamins as a class of nutrients that are essentially required by the body for its various biochemical and physiological processes. Vitamin C helps in metabolism of tyrosine, folic acid and tryptophan (Iqbal, K. *et al*. 2004). It regulates blood cholesterol level and helps in the synthesis of the ascorbic acid carnitine and catecholamine that regulate nervous system (Elgailan, I.E. *et al*. 2017). Ascorbic acid is an antioxidant that protects the body from the harmful effects of free radicals and pollutants. According to Grosso, G. *et al*. (2013), vitamin C is of paramount importance in the formation and maintenance of the collagen and as a powerful antioxidant (Elgailan, I.E. *et al*. 2017), protecting the body against oxidative stress, and present in the human diet as a vital vitamin. Previous studies have shown that vitamin C is essential for the natural synthesis of dopamine in the human body. On the other hand, large doses of ascorbic acid proved to reduce the risk of kidney stone formation in women. The deficiency of vitamin C compound in the body result in causing scurvy, anaemia and various infection and mental disorders. However, Pisoschi, *et al*. (2014) argued that vitamin C if excessively consumed can cause gastric irritation, and some of its metabolites like oxalic acid, causes renal problems. Furthermore, it may result in the inhibition of natural processes occurring in food and can contribute to taste/aroma deterioration in food and beverages if in excess.

2.3. Wild Edible Plants

Wild edible plants are those plants with edible parts that grow naturally on farm land and on fallow or uncultivated land (Dansi, A. 2008 and Duguma, H.T. 2020). Different wild edible plants have played a significant role in different geographical regions of the world throughout human history (Sekeroglu, N. *et al.* 2006). They may have remarkable nutrient values and can be an important source of vitamins, fibers, minerals, and fatty acids; they may also show important medicinal properties. Wild edible plants have always been an essential and widespread food source for food-insecure families living in poverty in developing countries. They are also important for many communities in rural villages and even those in urban areas, especially among the poor and marginalized. Wild edible plants are of crucial importance in all parts of the world in supporting the global food basket (Chakravarty, S. *et al.* 2016). According to Lulekal E. *et al.* (2011) about one billion people in the world use wild foods (mostly from plants) on a daily basis. Although many wild edible plants are used as a food supplement or as a means of survival during drought and famine, the importance of wild edible plants has been overlooked by the majority of the rural population (Yves, G. and Dechassa, L. 2000).

Ethiopia is known as the biodiversity hotspot and centre of origin and diversification for a significant number of food plants and their wild relatives. The wide range of climatic and edaphic conditions permitted the growth of a variety of wild edible plants (Addis, G. *et al*. 2005). Ethiopia is one of the developing countries which depend on wild edible plants to fulfill nutritional needs in addition to domesticated cultivars, especially in poor rural communities during periods of food scarcity. In most parts of Ethiopia, wild edible plants are a recovering part of the feeding habits of many communities. Lulekal *et al.* (Chakravarty, S. *et al.* 2016) noted that about 413 kinds of WEPs are consumed in Ethiopia. Thus, many rural people of Ethiopia usually feed on wild edible plants for survival during food shortages. The findings of the study conducted in Yayo forest show that indigenous fruits, vegetables, and tubers can make a positive contribution to food and nutrition security, as they are well adapted to local environmental conditions. Increasing the production of these underutilized food plants in farmer's home gardens and promoting their consumption could contribute to a more sustainable approach to prevent protein/energy malnutrition and micronutrient deficiencies (Duguma, H. T. 2020). Wild vegetables can constitute important local commodities fetching high prices on local and regional markets and as such contribute to local cash income. Research has shown that many of the wild edible plants have been found to be rich sources of one or more of the nutritionally important substances, such as proteins, carbohydrates, vitamins, and minerals. Besides the dietary substances, some of them also contain considerable amounts of a variety of health-promoting compounds, such as phenolic compounds (Seifu, T. *et al*. 2017).

2.4. Wild Edible Plants and Their Role in Combating Food Insecurity

Wild edible plants are with one or more parts that can be used for food if gathered at the appropriate stage of growth, and properly prepared. Plants have been the source of food materials from the dawn of human civilization (Shaheen, S. *et al.* 2017). For instance, about 300 million people obtain part or their entire livelihood and food from wild, forests in the world (DMP, 1982). Over 70% of the wild edible plants are consumed when food scarcity is high and at times of starvation (Teklehaymanot, T. and Giday, M. 2010). On the other hand Addis, G. *et al.* (2005) stated that wild plants in Ethiopia are used as source of food both at times of plenty and of food shortage. Despite agricultural the fact that societies primarily rely on crop plants, the tradition of eating wild plants has not completely disappeared, their nutritional role and health benefits being reported in many surveys worldwide. Thus, wild edible plants still play an important role in human nutrition especially in the time of starvation (Mavengahama, S. *et al*. 2013). Globally, an estimated 1.02 billion people are undernourished (FAO, 2009). Wild food plants are of high nutritional content such as protein, vitamin B2, and vitamin C, which used as alternatives to conventional vegetables in the human diet (Mengistu, F. and Hager, H. 2008). According to many sources, the amount of vitamins, minerals and other nutrients in wild food is on the average greater in cultivated foods (Hinnawi N.S. 2010). Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases (Arnold, 1995 cited in Khanal, 2006). According to FAO (2010), more than 35% of Ethiopian people are food insecure. The country's ever increasing population along with recurrent drought, war and poor agricultural practices with low productivity, have pulled the country into a vicious circle of food insecurity. In addition, over dependence on a limited number of food sources and poor efforts to diversify dietary sources aggravate the country's food insecurity problem. Many WEPs in Ethiopia were reported as emergency, supplementary or seasonal food sources to prevent food insecurity in households of Ethiopian cultural groups. However, consumption of wild edible plant is more common in food insecure areas than in other areas in the country (Teklehaymanot, T. and Giday, M. 2010).

Many WEPs are mentioned for their rich source of vitamin C. According to many of the research done, fruits are a valuable source of anticancer and anti-mutagenic substances, or substances which help protect against cardiac disorders. Those diseases often result from oxidation changes due to free radicals that damage lipids, proteins, and nucleic acids. Vitamins C contained in fruit have antioxidant features and can play an important role in the prevention of many diseases (Jablonska-Rys, E. *et al*. 2009).

Despite the wide availability and utilization of WEPs in Ethiopia, information on food contents and nutritional values of Ethiopian plants is limited. Hence, there is still a need for nutritional analysis and domestication of WEPs to assist in the nationwide effort to combat food insecurity and ensure dietetic diversity. For example, invasive *Opuntia ficus indica* (Cactaceae), was found to be widely exploited for its fruit in many parts of northern Ethiopia, playing a significant role in food source diversification (Addis, G. *et al*. 2009). The fruits of this plant are also sold in many local markets in the Tigray and many part of Ethiopia along with other cultivated food sources such as potato, carrot, bean and maize. Hence it is important that policy and decision makers consider all available ethno botanical information on Ethiopian WEPs so as to develop regional and national plans for the conservation, management and sustainable utilization of the country's underused wealth of WEPs (Lulekal, E. *et al*. 2011).

2.5. Wild Edible Plants Under Investigation

The study concerned with ten potent commonly consumed wild edible plants. Here under were the details of wild edible plants under investigation.

Table 2 Wild edible plants under investigation

S.No	Afaan Oromoo	Family	Plant scientific name	Habitat	Parts used	Modes of consumption
1	Adaamii	*Cactaceae*	*Opuntia ficus indica(L)*	Shrub	fruit	Ripe fruits are eaten raw
2	*Agamsa*	*Apocynaceae*	*Carissa spinarum*	Shrub	fruit	Ripe fleshy fruit eaten raw
3	Baddannoo	*Balanitaceae*	*Balanites aegyptica*	Tree	fruit	Ripe eaten raw
4	Doobbii	*Utricaceae*	*Utrica simensis*	Herb	Leaf	Cocked
5	*Goosuu*	*Myrtaceae*	*Syzygium guineens sp.*	Tree	fruit	Ripe fruits eaten fresh & raw
6	Goraa	*Rosaceae*	*Rubus apetalus*	Shrub	fruit	Ripe fruits eaten raw
7	*Harbuu*	*Moraceae*	*Ficus mucuso welw*.	Tree	fruit	Ripe fruits are eaten raw
8	*Koshommii*	*Salicaceae*	*Dovyalis abyssinica*	Shrub	fruit	Ripe fleshy fruit eaten raw
9	Qoqorii	*Rhamneceae*	*Ziziphus spina-christi*	Tree	fruit	Ripe fruits eaten raw
10	Xoosinyii	*Lamiaceae*	*Thymus shimperi*	Herb	Leaf	Cocked

1. Dovyalis abyssinica **(Koshommii-Afaan Oromoo)**

According to Bekele,T. A. and Tengnas, B. (2007) it is an indigenous plant shrub locally known as Koshommii (Afaan Oromoo). This evergreen spiny shrub or tree has height to 10 m, crown rounded. Its bark is grey, spines to 4 cm long. Branch lets with very clear dotted breathing pores and leaves with shiny, dark green, oval, to 5 cm, tip blunt, edge unevenly rounded.

This shrubs flowers are green sepals, females single but male flowers in clusters with many stamens that has fruit which is round berry about 2 cm across, surrounded by the calyx, green and hairy at first then smooth orange-yellow, with edible sweet sour around the seeds. Micronutrient analysis demonstrates the fruit to be an appreciable source of minerals such as potassium, phosphorus and sodium as well as vitamins such as thiamine, riboflavin and niacin. Other micronutrients reported in moderate amounts include; copper, iron and calcium (Waweru, D.M. *et al* 2022).

Dovyalis Ab. shrubs with fruits

ripe and unripe fruit

ripe fruit of *Dovyalis abyssinica*.

Figure 3 *Dovyalis abyssinica* plant.

2. Carissa spinarum **(Agamsa – Afaan Oromo.)**

It is a spiny shrub or small tree growing up to 5 m found in the study site. It grows in dry and midland climatic zones. Bark is grey, smooth with straight woody spines to 5 cm, often in pairs, rarely branching and milky latex. Leaves are opposite, leathery, shiny dark green to 5 cm, tip pointed, base rounded, stalk very short. Flowers are fragrant, in pink-white terminal clusters, lobes overlap to the right. Flowering begins in March and fruit ripen during late autumn to summer. Studies showed that this wild fruits have higher values of carbohydrate, fiber, fat, protein, minerals such as Fe, Ca, K, P, Mn and Zn and different vitamins (Siyum, Z.H. and Miresa, T.A. 2021).



Ripe fruit of *Carrisa spinarum.* Unripe fruit of *Carrisa spinarum* *Carrisa spinarum fruit*

Figure 4 *Carrisa spinarum* plant.

3. Syzygium guineenses **(Goosuu-Afaan Oromoo)**

The species of *Syzygium* is a tree growing to 12 m in favorable conditions, but remain at 5 to 8 m producing edible berries. Berries are deliciously eaten by monkeys, apes, birds or other wild animals including humans. Shepherds collect, eat and take home to share the trophy with family members and friends in the seasons. Flower in February and give blue black ripen berry fruit in April and May which eaten fresh and propagated from seeds. Those grown near river bank can give ripen fruit even in dry season. This wild edible fruit contain high concentrations of Ca, Mg, Fe, K and P (Saka, J.K. and Msonthi, J.D. 1994). *Syzygium guineense* is included among the African plant species that are active against malaria. Its bark is used in traditional medicine to treat gastro-intestinal upsets and diarrhea (Amusan, O.O. *et al*. 2002).

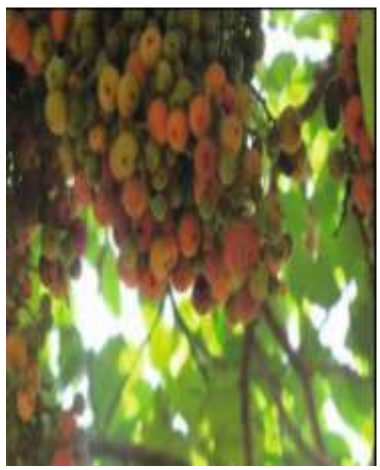


Tree of *Syzvgium guineense* *Syzygium guineense* with fruit *Ripe fruit of syzygium guineens*

Figure 5 *Syzvgium guineense* tree with fruit.

***4. Ficus mucuso welw* (*Harbuu-Afaan Oromoo*)**

Ficus mucuso welw also known as *Ficus capensis* thunb (harbuu Afaan Oromoo) is a tree of variable height (4 to 9 m). It is an indigenous plant family of Moraceae, is widespread in Ethiopia. It has spherical crown, often low-branched leaves and widespread in tropical Africa and South Africa of variable species. *Ficus capensis* also known as the Cape Fig and is a native of tropical Africa and the Cape Islands. The plant is a deciduous tree with spreading roots and branches and broad green leaves. *Ficus capensis* produces fleshy fruits all year round in a single or branched raceme along the trunk and the main braches (Pawlos, Z. *et al*. 2021). Furthermore, *Ficus mucuso welw* fruits might be used as a good source of vitamin A, essential minerals such as Ca, Mg, K and Fe for good health. The fruit helps to maintain acid alkali balance for the human body, used as blood purifier and also in the treatment of skin infection (Modi, S.K. *et al*. 2013).


Tree of *Ficus mucuso w***.** *Ripe Ficus mucuso w***.** edible part of *Ficus mucuso w*.

Figure 6 The species of *Ficus mucuso welw*.

5. Opuntia ficus indica (Adaamii-Afaan Oromoo.)

Opuntia ficus-indica is a large, trunk-forming, segmented cactus that may grow to 5 to 7 m with a crown of over 3 mm diameter and a trunk diameter of 1 m large pads are green to blue-green, bearing few spines up to 2.5 cm or may be spineless. Typically grow with flat, rounded cladodes also called platyclades containing large, smooth, fixed spine. This plant survives the drought due to its succulent leaves. This fruit is a good natural reservoir of sugar, minerals such as Mg, Ca and K whose nutritive components and antioxidants such as vitamin C can be used as food supplements (Chiteva, R. and Wairagu, N. 2013).

Opuntia ficus-indica plant *Ripe fruit of Opuntia ficus-indica*

Figure 7 The species of *Opuntia ficus indica.*

6. Rubus apetalus (Goraa-Afaan Oromoo)

A shrub in the Rosacea family, 1 to 3 m tall, with branches hanging down and sickle-shaped thorns. Leaves are green, odd-pinnate; flowers are made up of five petals, without fragrance glands on the bottom. Stems are smooth with 1 to 3 pink flowers 5 cm in diameter. Flowers have a large amount of pollen and are therefore attractive to bees. Rosehip has small, dry, one-seeded fruits (hips) red to orange in colour, which may range from dark purple to black in some species. Blooming period is in June, ripening from late summer to autumn. Study showed that the specie *Rubus apetulas* fruit contain high amount of minerals such as Ca, Fe, Zn, vitamin A and hold up to 10 times more beta-carotene than mango (*Mangifera indica*) (Aragaw, H.S. *et al*. 2021).

Figure 8 *Rubus apetalus* shrub with fruit.

***7. Ziziphus spina-christi* (Qoqorii- Afaan Oromoo)**

Ziziphus spina-christi is a plant species that has edible fruits and a number of other beneficial applications that include the use of leaves as fodder. It is a spiny shrub or small tree (Figure 9). It is known to be drought tolerant and very resistant to heat. It is evergreen but can drop some of its leaves during very dry seasons. The tree can reach a size of 5 to 10 m and a trunk diameter of up to 45 cm. The bark is whitish brown or pale grey and is deeply fissured. The crown is rounded or umbelliform with dense branches that spread widely and have a tendency to weep at the ends. Spines are light brown in colour and paired, with one of each pair being up to 8 mm long, straight and directed forward while the other is shorter and slightly curved. Fruits are collected by women and children and sold on local markets. Fruits are consumed either fresh or dried. Analysis of bioactivities and chemical composition of *Ziziphus spina-christi* indicated that it contain carbohydrate, protein, starch and minerals indicating their nutritional value for both human and animals (Mariod, A.A. 2019). *Ziziphus* plants have been widely used in traditional medicine for curing many diseases including diabetes, tuberculosis and anaemia.

Ziziphis spina-christi tree

Ziziphis spina-christi fruit

Figure 9 *Ziziphis spina-christi* tree and fruit.

***8. Balanites aegyptiaca* (Baddannoo-Afaan Oromoo)**

***B**alanites aegyptiaca* also called desert date belongs to the family of Balanitaceae and species of the genus *Balanites aegyptiaca* is spiny shrub alternately tree, generally conveyed on dry area regions about Africa and South Asia. It is widespread in most arid, semi-arid to sub-humid tropical Savannas in Africa.

The *Balanites aegyptiaca* tree reaches 10 m in height with a generally narrow form. The branches have long, straight green spines arranged in spirals. The dark green compound leaves grow out of the base of the spines and are made up of two leaflets which are variable in size and shape. The fluted trunk has grayish-brown, ragged bark with yellow-green patches where it is shed. Analysis of chemical composition of *Balanites aegyptiaca* indicated that it contain protein, sugar, minerals and vitamins indicating their nutritional value for human (Mariod, A.A. 2019). *Balanites aegyptiaca* fruit also used as medicinal plant which decreased the blood sugar content and could be considered as a high potential anti-diabetic product.

Figure 10 *Balanites aegyptiaca* tree and its fruit.

9. Utrica simensis* (Doobbii- Afaan Oromoo*)

Urtica simensis is a dark green perennial wild plant that has been used for food in the past, especially during droughts. It has a lot of promise and can help with food security by meeting human nutritional needs. *Urtica simensis* is well known for its stinging hairs that grow beneath the stems and on the lower leaf surface. The plant is completely covered in stinging hairs. Herbal nettles are one meter tall, dioecious, erect, and non-branched. The leaves are opposite and simple, with united stipules and interpetiolar lengths of 0.5 to 1 cm. The leaves are slightly cordate at the base, broad and sharp at the apex, and serrated at the border. It is a dark green perennial plant whose leaf is used as food by various ethnic groups in Ethiopia (Tsegaye, W. *et al.* 2008). It is common in North Shoa zone of Oromia. Leaves are cooked with water and eaten with traditional flatbread made out of teff flour. The leaf of this plant is also used for the treatment of various diseases. For example, the leaves are used for the treatment of gastritis. The leaves of *Urtica simensis* contain a significant amount of dietary and health important biochemicals.

In particular, the leaves are found to be rich in carbohydrates, minerals, moderate fat (essential fatty acids) (Bayba, K.B. *et al*. 2020).


Figure 11 *Utrica simensis* plant.

***10.Thymus shimperi* (Xoosinyii- Afaan Oromoo)**

Thymus schimperi, known by the local language name Tosegn is indigenous into northern Ethiopia and Oromia, which is a small perennial herb shrub of very high altitude areas. The herb which is wild, though not yet cultivated, is a useful condiment. It is also reportedly used in the control of gonorrhea. Furthermore, when it is added to boiling water and drunk, it helps against cough and liver disease. As a spice, *Thymus shimperi* is dried, ground and mixed with other spices that are used in the preparation of pepper (ripen, dried and ground pepper) and “Shirro” (powder form of roasted and ground legume grains). In some provinces, it is used to flavor tea or drunk alone as tea (Kassegn, H.H. and Mekelle, E.P. 2016). *Thymus* leaves offer significant levels of quality phytonutrients profile. 100 gm. of fresh leaves provides (% of RDA), 38% of dietary fiber, 27% of vitamin B6 (pyridoxine), 266% of vitamin C, 158% of vitamin A, 218% of iron, 40% of calcium, 40% of magnesium, and 75% of manganese but zero cholesterol (USDA National Nutrient data base).

Figure 12 *Thymus shimperi* herb.

2.6. Nutritional Content of Wild Edible Plants

Plant-derived vitamins are of great interest because of their impact on human health. They are essential for metabolism because of their redox chemistry and role as enzymatic cofactors (Asensi-Fabado, M.A. and Munne Bosch, S. 2010). Many local people in the world were living near the forest use the wild edible plants as food resources. These are free and easily collected by the local people as a good source of nutrients and vitamins (Suwardi, A.B. *et al.* 2018). Research has shown that many of the wild edible plants have been found to be rich sources of one or more of the nutritionally important substances, such as proteins, carbohydrates, vitamins, and minerals. Besides the dietary substances, some of them also contain considerable amounts of a variety of health promoting compounds, such as phenolic compounds. WEPs have high nutritional contents such as protein and vitamin C, which are used as alternatives to conventional vegetables in the human diet (Duguma, H.T. 2020). They are an overlooked source of vitamins and minerals that could help combat malnutrition. More than a thousand wild growing edible plants have been identified that could supply vitamins from the B group – thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5) and folate (B9), vitamin C and fat soluble vitamins (Cantwell Jones, A. *et al*. 2022).

Leaves, stems, fruits, flowers, tubers, barks, seeds, roots, and so on, of lots of wild edible plants are still consumed for their dietary value in many communities around the globe. Some of these wild edible plants are used as primary food sources while others are used as secondary condiments in dishes prepared from domesticated cultivars (Tyrona Lockett and Louis E

Grivetti, C. 2000). These plants play an important role as a source of energy and micronutrients (Afolayan A.J. and Jimoh, F.O. 2009). In addition, Becker (1983) reported the presence of vitamins A, B2, and C in wild edible plants of Senegal. Research on six wild edible plants from Spain also confirmed the occurrence of lipids, fatty acids and carotenes in the leaves of these six investigated wild plants (Guill-Guerrero and Rodriguez-Garcia, I. 1999). Protein content in a proportion that is comparable to the amount in domesticated plants was also reported from a nutritional study of wild edible plants in South Africa (Afolayan A.J. and Jimoh, F.O. 2009). A study on the dietary value of eight wild edibles in Iran and India also showed the presence of sodium, calcium, potassium, iron, zinc, protein, and fat in a ratio comparable to that found in cultivated plants (Aberoumand, 2009). Many wild leafy vegetables are also mentioned for their rich source of vitamin C, natural antioxidants, carotenoids and folic acid (Luczaj, 2010).

2.7. Determination of Vitamins in Wild Edible Plants

2.7.1. Sample Preparation of Plant Materials for Chemical Analysis

Plant material is a very complex matrix composed of a great variety of organic compounds. Sample preparation, even for chromatographic techniques of high separation power, is a difficult process composed of many operations. The basic operations include prewashing, grinding which is aimed at obtaining a homogenous, dividing a sample in such a way that representative subsamples are obtained, centrifuging, drying the extract, solvent evaporation, and extract cleanup (Romanik, G. *et al*. 2007). Before sample preparation starts, the sampled plants fruit and leaf must be pre-processed. At this stage, the plants must be washed with tap and deionized water, then dried to remove moisture for preservation, preventing bacteria activity and restricting fungal growth. To increase the surface area of the dried plant materials for improved contact with the solvent during the subsequent extraction process, the dried plant must first be ground. An increased surface area of the dried plant materials will improve the performance of the extraction process. The particle size of the milled plants is one of the factors that can affect the extraction yield (Mohammed Azmin, S.N. *et al.* 2016). A crucial step of sample preparation is selective isolation and enrichment requiring very sophisticated operations when trace components in inherently complex plant matrices are to be determined (Zygmunt, B. and Namiesnik, J. 2003).

A basic knowledge of plant structure is necessary before collecting samples. When drawing samples of fresh fruits, sampling should be carried out in such a way that the samples represent the lot. Sampling should be done in a clean area to minimize the risk of contamination. Samples should be taken randomly, i.e., minimum of three plants from various locations (top, middle and bottom). The sample should not be overfilled the sample container or permit punctures by wire closure. Sample containers must be securely sealed after filling so that they cannot leak or become contaminated during normal handling. The packaging of the samples should be done directly at the site of sampling to avoid the possibility of contamination. Samples must be well packaged to maintain their integrity. Shipping containers and packaging material should be an insulated container of sufficient capacity. Appropriate packing materials such as paper bag, or any other suitable material should be needed to secure samples within the shipping container. Plastic containers filled with refrigerant or well frozen ice packs in plastic bags should be used to keep sample units cool. Dry ice is needed to keep frozen samples in a frozen state (ISO 874: 1980: Fresh Friuts and Vegetables Sampling). In the case of sampling leaf, the most recent mature leaf (MRML) should be sampled because it is the first fully expanded leaf below the growing point. It is neither dull from age nor shiny green from immaturity.

2.7.2. Sample Pretreatment Methods

Due to their chemical instability, particular attention should be given to sample pretreatment procedures while analyzing WSVs. There are different factors involved in the degradation of vitamin C and their different bioactive forms (Farah, H.S. *et al*. 2020). Light, either in the form of sunlight or white fluorescent light, can have an effect on the stability of vitamin C, when exposed to light it has been found to lose vitamin C. Vitamin C or ascorbic acid is relatively stable in dry air, but is unstable in the presence of moisture. Ascorbic acid is readily oxidized in aqueous solutions, first forming dehydroascorbic acid, which is then further and rapidly oxidized. The rate of ascorbic acid degradation in aqueous solutions is pH dependent (Moura, T. *et al.* 1994). If the ascorbic acid sits in a very acidic environment there will be a lot of protons around it. This could slow down the reaction. It has been found that the reaction takes place fastest at a pH of 4 (The nutrition handbook for food processors). At a pH higher (thus more alkaline) or lower (thus more acidic), the reaction will proceed more slowly. Oxygen is known to be a very good oxidizer. When left open to the air, which contains oxygen, ascorbic acid is prone to oxidation. Hence, if no oxygen is present, less oxidation of ascorbic acid will take place (Jutkus, R.A. *et al*. 2015). Vitamin C losses can occur during the frozen storage of foods, and work has shown that oxidation of ascorbic acid is faster in ice than in the liquid water (Herbig, A.L. and Renard, C.M. 2017). Hence, proper care must be given during storage of plant sample for vitamin C analysis. Trace heavy metals such as Cu, Fe and Zn ions act as catalysts to the degradation of ascorbic acid. They don't actually get used during the reaction, but can serve as temporary storage place for electrons (Fatima, Z. *et al*. 2019).

As a precaution, all sample and standard preparation should be carried out under subdued lights, using amber glass wares and maintaining optimum pH and temperature range during the entire cycle of analysis for more precise and accurate results. Furthermore, the utilization of appropriate stabilizers/antioxidants such as TCEP (tris(2-carboxyethyl)phosphine) is also recommended for some vitamins such as vitamin C in order to prevent its oxidation. A complex and well-optimized method is often required to extract vitamins from natural complex matrices, like biological fluids and foods of plant and animal origin, because vitamins frequently interact with other compounds such as protein, carbohydrates and phosphate groups (Fatima, Z. *et al*. 2019).

2.7.2.1. Sample Preparation Methods for Analysis of Vitamins in Wild Edible Plants

Sample preparation requires precise handling and manipulation of samples from the time they enter the lab until the vial is placed on the tray. The development of modern sample preparation techniques has significant advantages over conventional methods, in terms of reduction in organic solvent consumption and in minimizing sample degradation (Kothari, V. *et al*. 2012). They also result in the elimination of undesirable and insoluble components from the extract. The modern methods includes microwave assisted extraction (MAE), ultrasonication assisted extraction (UAE), supercritical fluid extraction (SFE), solid phase micro extraction (SPME) (Teng, H. *et al.* 2009) etc.

Furthermore, elimination of additional sample clean-up and concentration steps before analysis, improvement in extraction efficiency, and selectivity are also the benefits of modern processes (Kothari, V. *et al.* 2010). The selection of method to isolate active components with best yield and highest purity from natural sources is mainly dependent on the nature of compounds and raw material which is going to be processed (Kothari, V. *et al.* 2009). In recent years, the most reported applications of dispersive liquid-liquid micro extraction (DLLME) have focused on more complex matrices such as wine, fruit and juice, honey, milk, wastewater, as well as pharmaceutical and biological samples (Saraji, M. and Boroujeni, M.K. 2014).

2.7.2.2. Sample Preparation Method for Analysis of Vitamin C in Plants

Due to its chemical instability, particular attention should be given to sample pretreatment procedures while analyzing vitamin C. As a precaution, all sample and standard preparation should be carried out under subdued lights, using amber glass wares and maintaining optimum PH and temperature range during the entire cycle of analysis for more precise and accurate results. Furthermore, the utilization of appropriate stabilizers or antioxidants are also recommended for some vitamins such as vitamin C (Fatima, Z. *et al*. 2019).

Acid hydrolysis method can be used to extract vitamin C from fruit sample. Acid hydrolysis has been used to release bonded vitamins into their free form and for reduction of number of possible vitamers. The most commonly used acids for acid hydrolysis includes hydrochloric acid (HCl), per chloric acid (PCA), metaphosphoric acid (MPA) and sulphuric acid. 5% MPA has been widely used for extraction of vitamin C with high recovery because MPA precipitates protein and also ensure the stability of vitamin C as compared to other acids (Chebrolu, K.K. 2012). Acid hydrolysis is a comparatively simpler and time saving approach for the extraction of vitamin C and gives better quantitative results (Fatima, Z. *et al*. 2019).

2.7.2.3. Sample Preparation Methods for Analysis of Vitamin C in Wild Edible Plants

Several techniques would be used in sample preparation for WSVs in WEPs, ranging from classical procedures like weighing, dilution, and filtration, to relatively new technologies of sample preparation which is quick, easy, cheap, effective, rugged, and safe. Solid phase extraction (SPE) based methods are frequently used as clean-up and pre-concentration steps before determination of WSVs (Chamkouri, N. 2015). SPE can be an advantageous approach because of its ability of modification according to studied compounds. New SPEs have been fabricated and employed for vitamin C analysis recently, by using different adsorbents based on well-defined chemical and physical characteristics (nature of the matrix, porosity, surface area, selectivity, durability, and reproducibility) (Chamkouri, N. *et al.* 2015).

2.7.3. Analytical Techniques Used for Analysis of Vitamin C in Wild Edible Plants.

The development of rapid, simple, and inexpensive analytical methods is the areas of growing interest, in environmental and industrial fields. Many analytical methods can be used for the quantitative determination of vitamin C content in fruit and leaf sample, such as: spectrophotometry, biological methods, electrochemical methods (voltametry, fluorometry, and potentiometry) and chromatographic method (Khan, M.R. *et al*. 2006). Vitamin C is usually analyzed by classical techniques such as volumetric methods - titration), potassium iodates or bromate (Matei, N. *et al*. 2004). Spectrophotometry is one of the most frequently used simple methods, because vitamin C is able to absorb UV ray (Fadhel, D.H. 2012). Direct ultraviolet (UV) spectrophotometry can provide a fast, simple and reliable method for the determination of vitamin C. UV Spectrophotometry is mostly used to determine vitamin C because it is simple method, and Vitamin C is able to absorb UV rays. Since Ascorbic acid is able to absorb UV rays (Elgailani, 2017), the method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables. Table below shows the application of UV-VIS. Spectrophotometry for the determination of ascorbic acid from plant matrices.

Table 3 Quantification of Vitamin C in five fruits using UV Spectrophotometer *(*Desai, A.*P*. 2019, Mohammed, Q. Y. *et al. 2009)*

Sr.No	Sample	Biological Name	Amount of Vitamin C (mg/g)	Sample preparation methods	Method used
1	Star gooseberries	*Phyllantus acidus*	0.0829	Acid hdrolysis method using MPA.	UV-VIS spechtrophotometry
2	Mulberry	*Morus Nigra*	0.418		
3	Karanda	*Carissa carandas*	0.4468		
4	Black currant	*Ribes nigrum*	4.4683		
5	Grape	*Vitis vinifera*	0.0558		

MPA – Meta Phosphoric Acid

Generally, recently, different methods for determination of vitamins in various types of samples have been proposed. The key roles of these methods are played by sample preparation techniques, and the main efforts in this field have been focused on the optimization of the preparation, extraction and clean-up steps and on the enhancement of the environmental safety of these procedures. The method with the most promise in achieving these goals is micro extraction methods. The main advantages of this approach is its good compatibility with high throughput multi residue analytical procedures and it's relatively low cost. Therefore, these techniques are expected to have the most pronounced development in the future. The currently proposed analytical approaches for the detection of vitamin C in this study was mainly based on UV-VIS spectrophotometry.

CHAPTER THREE

3. METHODS AND MATERIALS

3.1. Description of Study Area

Sample was collected from the nearby forest near Fiche town, in particular, a place called Saka Sole, one of the district in Wadesa Amba kebele which is 15 km from Fiche town, the capital of the North Shoa zone. Wadesa Amba is found in Girar Jarso Woreda, one of the districts of North Showa Zone, Oromia National Regional State. This district has Latitude: 9.81 North, Longitude: 38.79 East and Altitude: 1,866.00m/6,122.05F. The Woreda lies along the highway to Amhara National Regional State in the Northwestern direction at a distance of 112 km from Addis Ababa. It shares border with Amhara Region in the North, Yaya Gullalle Woreda in the East, and Debre Libanos Woreda in the South and Degem Woreda in the West. The total area of the Woreda is about 3,321.25 hectares. The altitude of the Woreda ranges from 2,738 to 2,782 meters above sea level. The woreda is located between the longitudes of 9°45′18" and 9°48′42"N and the latitudes of 38°48′28-38°42′54"E. According to Fiche Station meteorological data the average rainfall amount of the Woreda is about 1,200 mm, and maximum and minimum rainfall is about 1,115 mm and 651 mm, respectively. Temperature of the woreda ranges from a minimum of 11.5 °C to a maximum of 35 °C according to Girar Jarso Woreda Agricultural Office. Growth forms of WEPs comprise trees, shrubs, herbs and climbers.

Figure 13 Map of Ethiopia, Oromia, North Shoa, Gerar Jarso woreda and study kebele.

3.2. Research Methodology

Samples were collected from different plants depending on the availability and maturity of edible parts. For each fruit and leaf samples, a mixed and composite sampling strategy was applied. The sampled plants fruit and leaf was pre-processed. At this stage, the samples were washed with tap and deionized water then put in the refrigerator until analysis.

3.3. Chemicals and Reagents

All standards, reagents and solvents were of analytical grade. Analytical reagent-grade sulfuric acid (H_2SO_4) 98% (Ranbaxy, India), meta phosphoric acid (M-H_3PO_4) 65.5% (Ranbaxy, India), 2,4-DNPH ($C_6H_6N_4O_4$) (Merck, India), thiourea (CH_4N_2S) (Merck, India), saturated Bromine solution (Merck, India), trichloroacetic acid (TCA) ($C_2HCl_3O_2$) (Ranbaxy, India) and ascorbic acid standard 99.9% ($C_6H_8O_6$ (Merck, India)), deionized water (conductivity 0.055 µS).

3.4. Apparatus and Instruments

The equipment used to conduct this study were: Double beam UV-Visible Spectrophotometer (UV-1800, Shimadzu, Japan), Freeze drier **(**1220 Volt/50 Hz**,** upright teflon coated collector**),** adjustable micropipette 100-500 µl, desiccators, water bath (1.5 KW, 335 x 405 x 100 mm, thermostatic) & ice bath (model 796, Orion, T -80 to 300 °C, 470 mm immersion), analytical balance (Mettler Toledo, model-AG240, Switzerland), centrifuge (EBA 20 Hettich, Tuttlingen, Germany) , micropipette tips 100-500 µl, pipette 5 ml & 10 ml, volumetric flask 50 ml, 100 ml (borosilicate), spatula, conical flask, filter paper, funnel, test tube, mortar & pestle, and vortex mixer (Heidolph Unimax, England).

3.5. Sample Collection, Preservation and Handling

Saka Sole was selected based on its wide distribution of wild edible plants and is one of the dense forest sites of the woreda, where the local community used this forest both for wild food and income generation. Depending on the availability of wild edible plant samples, a representative number of fruits and leaves were collected from Saka Sole forest.

Ascorbic acid is relatively stable in dry air, but is unstable in the presence of moisture. In fresh foods, the L-ascorbic acid form normally predominates but processing, storage and cooking increase the proportions of the dehydroacsorbic acid (DHA) form. Due to its chemical instability, particular attention should be given to sample pretreatment procedures while analyzing vitamin C. This begins from sample collection and handling in vitamin C analysis from plants (Fatima, Z. *et al*. 2019). Samples were taken randomly, i.e., minimum of three plants from various locations (top, middle and bottom). After collection, the samples were selected for uniformity of size, ripeness/maturity, and absence of defects. Then washed with tap water followed by de-ionized water. Paper bag in an ice box was used for holding and shipping sample. The samples were then kept in deep freeze until extraction for analysis. Maintaining optimum pH and temperature range during the entire cycle of analysis is also recommended for more precise and accurate results.

Laboratory analysis requires less than one milliliter of sample. However, a good sample contains enough samples to represent the area sampled. Therefore, the larger the area is, the larger the sample size needs to be. Sample size also varies with crop types. The studied wild edible plants were selected based on wider distribution in selected area, availability of edible part during collection time and use during normal times, food scarcity and famine periods.

Depending on the availability of plant sample, representative amount of fruits were collected from Saka Sole forest. The sampling protocol was taken into consideration to include the condition of the sample and its environment, the biological condition of the plant and the sampled plant part (healthiness of sampled plants). The collected samples were washed with tape water and rinsed three times with deionized water to make them free of extraneous substances, including soil and dust particles, and foliar spray residues that may influence analytical results. All of the samples were sealed in dried paper bag and transported to the laboratory using ice box where further sample pre-treatments were made. Accordingly, after cutting into small, all the samples were grinded by mortar and pestle then extracted for determination of ascorbic acid content.

3.6. Preparation of Standard Solution

The stock standard solutions of vitamin C was prepared by dissolving 100 mg ascorbic acid standard in 5% metaphosphoric acid in 100ml volumetric flask to obtain 1000 mg/L of stock solution and the volume was made up to the mark with 5% metaphosphoric acid. Working standard of 100 mg/L was prepared from stock standard solution. From this working standard, serial dilutions were made to obtain 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40 1.60, 1.80 and 2.0 mg/L of ascorbic acid solution.

3.7. Methods of Sample Preparation and Ascorbic Acid Determination

3.7.1. Sample Preparation Procedures

Following the standard sample preparation techniques, ascorbic acid was extracted from wild edible fruits and leaves by the modification of commonly used method developed by Desai, A.P. and coworkers (2019) which was developed for the determination of ascorbic acid content in aqueous extracts of fruit. The edible wild plants named koshommii *(dovyalsis abyssinica),* harbuu *(Ficus mucuso welw),* goosuu *(Syzygium guineense sp.*, hagamsa *(carrisa spinarium),* adaamii *(Opuntia ficus-indica),*goraa (*Rubus apetalus),* qoqorii *(Ziziphus spina-christi),* baddannoo *(Balanites aegyptiaca),* doobbii *(Utrica simensis) and* xoosinyii *(Thymus shimperi)* were collected from forest in the vicinity of Fiche town area and the laboratory sample was prepared according to the nature of edible part of the sampled plant. The steps in sample preparation/extraction for analysis were; 5 g blended sample was put in mortar and pestle, then 100 ml of 6% trichloro acetic acid solution was added and mixed together or ground manually with pestle for 3 minutes at room temperature. After mixing for 3 minutes, the mixture was transferred into conical flask and homogenized by vortexing for 30 seconds and then centrifuged for 2 minutes at 2,000 rpm. The sample solution was filtered with whatman No. 4 filter paper into another conical flask. The sedimented solids were removed by filtration. 2 ml of saturated bromine solution was added to a conical flask containing sample solution to oxidize the ascorbic acid to dehydroascorbic acid & was aerated. 10 ml of aliquot was taken and mixed with 10 ml of 2% thiourea to remove the excess bromine and thus the clear solution was obtained, and 4ml was pipetted into each of three test tubes. 1 ml of 2% 2, 4-DNPH was added to all test tubes to produces an osazone which on treatment with H_2SO_4 forms reddish colored solution (2,4-DNPH act as dye in this method). For the completion of the reaction, all test tubes were put in water bath (thermostatic) at 37 ℃ for 3 hour and cooled in an ice bath for 5 minutes, then 5 ml 85% H_2SO_4 solution was added slowly while the tubes were in an ice bath; as a result, colored solutions were obtained. Blank was prepared from the sample by a series of extraction of the residue until vitamin C free solution was produced. All test tubes were stood at room temperature for 30 minutes. Finally, the absorbance of the standards, blank, test samples and spiked samples were read at 515 nm. Triplicate sample preparation and triplicate reading were done for each sample. The spectroscopic analysis was carried out at Nutrition Laboratory of Ethiopian Health and Nutrition Research Institute (EHNRI).

3.7.2. Analytical Method

UV- Visible spectrophotometer (Double beam) (model-1800, Shimadzu, Japan) having matched quartz cells of light path 1cm with software: UV probe Version of software: 2.42 was used. The calibration curve was established by running the previously prepared standard solutions. The sample solutions of fruits and leaves were run on UV-VIS spectrophotometry and the absorbance of each plant sample were recorded. Triplicate readings were carried out on each sample. The same analytical procedure was employed in the determination of ascorbic acid in each of ten plant sample. The content was calculated using the calibration curve by plotting absorbance against concentration of the respective standard sample. The results were reported as means ± % RSD (percentage relative standard deviation) for ten plant samples.

Figure 14 Double beam UV-VIS Spectrophotometry used in Analysis

3.8. Method of Data Analysis

The data were analyzed quantitatively. The analysis of plant samples were carried out thrice for each sample, and the mean and percentage relative standard deviation were calculated. All calculations were done with Microsoft excels.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Evaluation of Analytical Figures of Merit

Validation of analytical methods is an essential component in analytical method development. Therefore, in this study, each analytical method that are intended to be developed, would verified whether it fits for the intended purpose or not before applying on to the real samples. Validation is the formal and systematic way to demonstrate the suitability of a developed method for testing the analyte to provide useful analytical data within defined limits (Hagos, M. *et al*. 2022).Typical analytical performance characteristics which would be considered in method validation include accuracy, precision (repeatability and reproducibility), detection limits, quantification limits, and linearity range of the system. The most common validation parameters for the analytical methods were discussed here under.

4.1.1. Linearity of Calibration Curve

The linearity of an analytical method refers to the ability to obtain results either directly, or after mathematical transformation proportional to the concentration of the analyte in the sample within a given range (Shabir, G.A. 2003; Chandran, S. and Singh, R.S. 2007). Linearity is established by measuring the instrument response of a sufficient number (at least five) of standard solutions in the expected range of the analyte. It is estimated by the equation of the regression line ($y = ax + b$) by plotting concentrations (x) versus the response (y) (Caldas, S.S. *et al.* 2009). Typically, the correlation coefficient is used to express the acceptability of the linearity of the regression line (Chandran and Singh, 2007; ICH, 1994). In this research, calibration curves for the determination of ascorbic acid in the prepared plant samples were constructed by plotting the absorbance as a function of the corresponding concentrations as shown in Figure 15. There was observed a proportional increase in absorbance at the respective λmax with increasing concentration of each ascorbic acid standard solutions.

Table 4 Concentration versus Absorbance of ascorbic acid standard (n = 9)

C (mg/g)	0.20	0.40	0.60	0.80	1.0	1.20	1.40	1.60	1.80	2.0
A	0.2225	0.411	0.58	0.7275	0.907	1.0645	1.3145	1.5001	1.69	1.877

C = Concentration   A = Absorbance

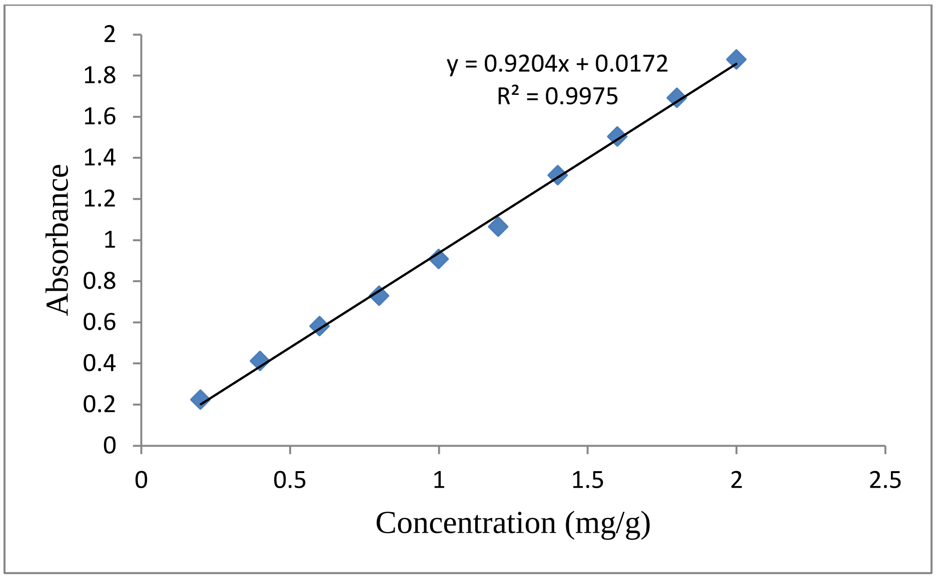


Figure 15 Calibration curve for the determination of ascorbic acid (vitamin C).

The linearity of the method was tested from 0.2 to 2.0 mg/g, and it exhibited good linearity with a regression of y = 0.9204x + 0.0172 and a regression coefficient R^2 = 0.997 (Figure 15). The linear relationship of absorbance with the concentration of ascorbic acid was shown in Figure 15.

Repeatability

The precision of an analytical method is defined as the degree of an agreement among individual tests obtained when the method is applied to multiple sampling of a homogenous sample. It is usually expressed in terms of standard deviation (SD) or relative standard deviation (% RSD) for replicate measurements of the standard at low, mid and high concentrations. In this study, the precision of the method was tested by calculating percent relative standard deviation for triplicate measurements. The % RSD of the method repeatability was found to be 0.06 to 2.85 which were summarized in table 5.

4.1.2. Determination of Method Detection Limits and Quantification Limits

Detection limit is the lowest concentration level that can be determined to be statistically different from an analyte blank or the minimum concentration that can be detected by the analytical method with a given confidence limit. There are numerous ways of determining detection limits of a given measurement. According to EPA (Environmental Protection Agency) of America it is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analytical concentration is greater than zero. The LOQ is the least amount of analyte that can be quantified accurately and precisely. The standard deviation and slope were calculated using the linearity of calibration curve (Hagos, M. *et al.* 2022). In this work, LOD and LOQ were calculated using the formula LOD = $3.3 \times \sigma / S$ and LOQ = $10 \times \sigma / S$ based on the SD of the response and slope where S is the slope of the calibration curve and σ is the standard deviation of the response which were summarized in Table 5.

Table 5 Analytical parameters of the proposed UV-VIS method such as wavelength, regression equation, R^2, LOD, LOQ, RSD, and recovery (n =9)

Wavelength (nm)	Regression equation	R^2	LOD (mg/g)	LOQ (mg/g)	% RSD	% R
515	y= 0.9204x + 0.0172	0.997	0.05	0.15	0.06-2.85	98.14 $\pm$ 1.49

4.1.3. Study of Recovery (Procedure of Spiking)

Recovery has been defined by the IUPAC as the proportion of the analyte quantity, present or added to the analytical portion of materials tested, which is extracted and presented for measurement (Thompson, *et al*. 1999). It is important to obtain high recoveries (close to 100%) with good precision. Most often, recoveries of organic compounds are acceptable in the range of 70 – 110% (Linsinger, T.P. 2008). In this work; using the standard addition method, the accuracy of the UV-VIS spectrometric method was assessed. A known concentration of ascorbic acid standard solution was added to the fruit samples. The spiked samples were subjected to the same sample preparation and analyzed three times in order to achieve an average recovery. The following formula was used to compute the percent recovery:

$$\% \text{ R} = \frac{\text{CS}-\text{C}}{\text{CA}} \text{ X } 100$$

Where CS is the concentration of the spiked sample, C means the concentration of the unspiked sample, and CA the concentration of the spiked ascorbic acid (Hagos, M. *et al*. 2022). To confirm the efficiency of analytical method used, spiking experiments in which known volume and concentration of standard solutions, were used. 0.07 mg/g of AA standard solution were added to the samples. This means that 3.5 ml AA standard solution was spiked to each sample. Then samples were extracted and prepared by the adopted procedures for all plant samples discussed under 3.7. As used for unspiked samples triplicate spiked samples were prepared and triplicate readings were recorded and the results revealed good percent recovery (% R) of 94.28 to 102.85 with % RSD ranging between 0.02 to 2.05. The results were given in Table 6.

Table 6 Analytical results obtained for validation of the method procedure after spiking with standard solutions (n = 9, triplicate sample preparation and triplicate reading).

Fruit sample	Amount added (mg/g)	Amount before addition(mg/g)	Amount found(mg/g)	(%)Recovery
Balanites aegyptiaca	0.07	0.390 ± 1.99	0.458 ± 0.41	97. ± 2.05
Carissa spinarum	0.07	0.702 ± 1.38	0.770 ± 0.23	97.14 ± 1.49
Doviyalis abyssinica	0.07	1.15 ± 0.84	1.217 ± 0.13	95.71 ± 1.23
Ficus mucuso welw	0.07	—	—	—
Opuntia ficus indica	0.07	0.165 ± 1.14	0.232 ± 1.11	95.71 ± 0.02
Rubus apetulas	0.07	0.351 ± 2.85	0.417 ± 0.35	94.28 ± 1.56
Syzygium g. sp.	0.07	1.272 ± 0.78	1.343 ± 0.30	101.42 ± 1.73
Thymus shimperi	0.07	1.390 ± 1.44	1.461 ± 0.14	101.42 ± 0.25
Utrica simensis	0.07	1.621 ± 0.06	1.693 ± 0.21	102.85 ± 1.52
Ziziphus spina-christi	0.07	0.491 ± 1.88	0.560 ± 0.02	98.57 ± 1.25

4.2. Determination of Ascorbic Acid in the Plant Samples by Spectrophotometer

Vitamin C is the most important vitamin in fruits and vegetables. It is widely distributed in nature, mostly in plant products such as fruits and green vegetables. Wild edible plants play a critical role in ensuring food and livelihood security for countless families and communities around the world (Khan, F.A. 2017). Many wild leafy vegetables are also mentioned for their rich source of vitamin C, a natural antioxidants (Luczaj, L. 2010). The role of vitamin C in our body demands its determination in different sources like wild edible plants. Based on this, the current research work clearly indicated that concentration of vitamin C varies with the type of edible plant analyzed. The amount of vitamin C (ascorbic acid) in ten wild edible fruits and leaves ranged from 0.175 ± 1.14 mg/g to 1.612 ± 0.06 mg/g. The quantity of vitamin C was found highest in the leaf of *Utrica simensis* (1.612 ± 0.06 mg/g). The leaf of *Thymus shimperi* contained the second highest amount of vitamin C (1.386 ± 1.44 mg/g) and followed by the fruit of *Syzygium guineense* sp. (1.274 ± 0.78 mg/g), *Doviyalis abyssinica* (1.130 $\pm$ 0.84), *Carissa spinarum* (0.722 $\pm$ 1.38) respectively. Moderate amount were detected in *Ziziphus spina-christi* (0.531 $\pm$ 1.88), *Balanites aegyptiaca* (0.402 $\pm$ 1.99) and appreciable amount were detected in *Opuntia ficus-indica* (0.175 $\pm$ 1.14). Out of analyzed edible fruits, the vitamin C content of *Ficus mucuso welw* was not quantified and reported since its vitamin C content was under instrument detection limit. As indicated in table 7, the concentration pattern of vitamin C in plants under investigation follow: *Utrica simensis* $>$ $Thymus\ shimperi$ $>$ *Syzygium guineense sp.* $>$ *Dovyalsis abyssinica* $>$ *Carrisa spinarum* $>$ *ziziphus spina-christi* $>$ *Balanites aegyptiaca* $>$ *Rubus apetalus* $>$ *Opuntia ficus-indica.* Therefore, these wild edible fruits and leaves under investigation could be considered good sources of vitamin C except *Ficus mucuso welw* which was not quantified. Therefore, the level of vitamin C in these wild edible plants could be able to satisfy the recommended daily allowances which are 90 mg for adult men and 75 mg for adult women and 15-45 mg for children.

Table 7 Amount of vitamin C in ten wild edible plants samples by spectrophotometric method (n = 9, triplicate sample preparation and triplicate reading were done).

S. No.	Wild edible Fruits	Mean A ± % RSD	Mean C ± % RSD (mg/g)
1	*Balanites aegyptiaca*	0.3872 ± 0.01	0.402 ± 1.99
2	*Carissa spinirum*	0.6817 ± 0.14	0.722 ± 1.38
3	*Doviyalis abyssinica*	1.057 ± 0.21	1.130 ± 0.84
4	*Ficus mucuso welw*	ND	ND
5	*Opuntia ficus indica*	0.1782 ± 1.21	0.175 ± 1.14
6	*Rubus apetulas*	0.3393 ± 0.45	0.350 ± 2.85
7	*Syzygium guineense sp.*	1.1897 ± 1.01	1.274 ± 0.78
8	*Thymus shimperi*	1.2928 ± 1.05	1.386 ± 1.44
9	*Utrica simensis*	1.5008 ± 0.78	1.612 ± 0.06
10	*Ziziphus spina-christi*	0.5059 ± 0.15	0.531 ± 1.88

A = Absorbance C = Concentration ND = Not Detected

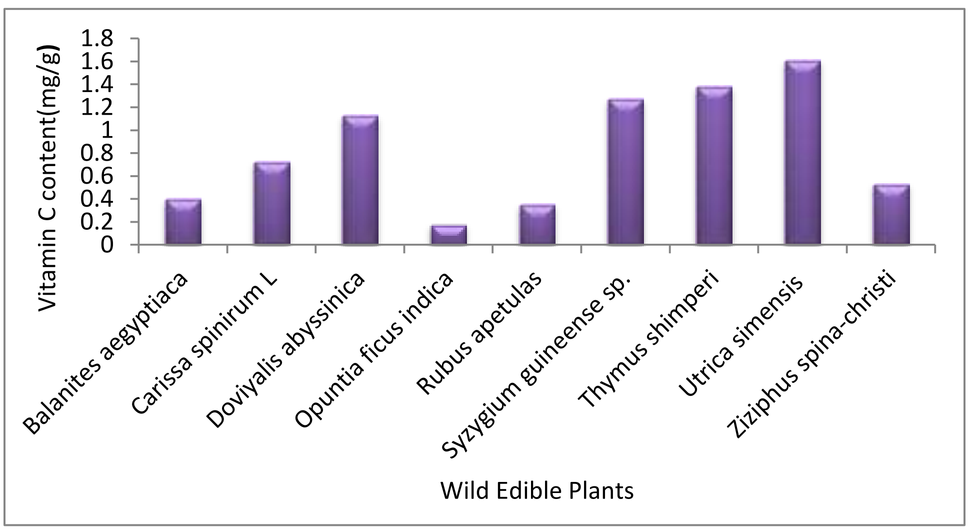


Figure 16 Ascorbic acid content in ten fresh wild edible plants by using UV Spectrophotometer.

4.3. Comparison of Observed Ascorbic Acid Concentration with Literature Values

Determination of ascorbic acid in WEPs has received considerable attention due to its important role in maintaining health (Chimezie, A. *et al*. 2008) contributing to a healthy immune system and providing all the nutrients essential for good health. Researches have reported the concentration of ascorbic acid in wild edible plants.

There are attempts of determining vitamin C in wild edible plants however in the current study vitamin C was determined in ten plants including fruits and vegetables with relatively cost effective instrument UV-VIS. There was also no published work in the determination of vitamin C in edible plants found in North Shoa. The results reported by different researchers were given in table 8.

Table 8 Comparison of *Dovyalis abyssinica* and *Opuntia ficus indica* concentration of present study with literature report (mg/g) (Literature values were not expressed with %RSD).

Wild fruits	Present study	Ascorbic acid concentration (mg/g)	Reference	Country	Method used
Doviyalis abyssinica	1.130 ± 0.84	1.204	Cavalcante, I. and Martins, A. (2005)	Brazil	Titration
		1.09	Waweru, D.M. et al. (2022)	Kenya	UV-VIS
		1.405	Almeida, et al. (2004)	Brazil	Titration
		1.129	Aynachew, T.(2018)	Ethiopia	UV-VIS
		1.43	Rotili, *et al*. (2018)	Brazil	UV-VIS
Opuntia ficus indica	0.175 ± 1.14	0.0517	Chiteva, R. and Wairagu, N. (2013).	Kenya	Titration
		0.137	El Samahy, *et al*. (2006)	Egypt	Minolta Color Reader CR-10

Table 8 indicated that the vitamin C content of *Doviyalis abyssinica* in present study was found in the range of literature report. It's vitamin C content was above the minimum which was reported by Waweru, D.M. *et al.* (2022) but below the maximum which was reported by Rotili, *et al*. (2018). The vitamin C content of *Opuntia ficus indica* in present study was also found near literature report. It is known that the nutritional composition of these fruits varies with respect to age, postharvest handling, season and temperature condition (Chiteva, R. and Wairagu, N. 2013).

Table 9 Comparison of *Carrisa spinarum, Syzygium guineense* and *Rubus apetulas* concentration of present study with literature report (mg/g)

Wild fruits	Present study	Ascorbicacid concentration (mg/g)	Reference	Country	Method used
Carissa spinarum	0.722 ± 1.38	0.176	Siyum, Z.H. and Miresa, T.A. (2021)	Ethiopia	Titration
		2.565	Aragaw, H.S. *et al.* (2021)	Ethiopia	Iodometric Titration
Syzygium guineense sp.	1.274 ± 0.78	0.750	Singh, D.B. *et al.* (2001)	India	volumetrically by standard methods (A.O.A.C. 1975)
		3.307	Aragaw, H.S. *et al.* (2021)	Ethiopia	Iodometric Titration
		0.119	Stadlmayr,B. *et al.* (2013)	Kenya	Literature Review
Rubus apetulas	0.35 ± 2.85	2.942	Aragaw, H.S. *et al.* (2021)	Ethiopia	Iodometric Titration
		0.320	Probst, Y. (2015).	Australia	Literature Review

This table shows that the level of ascorbic acid in *Carissa spinarum* in present study was found in the range of literature report. Its vitamin C content was above the minimum which was reported by Siyum, Z.H. and Miresa, T.A. (2021) but below the maximum which was reported by Aragaw, H.S. *et al*. (2021). The level of ascorbic acid in *Syzygium guineense sp.* in present study was also found in the range of literature report. It's vitamin C content was above the minimum which was reported by Stadlmayr, B. *et al.* (2013) but, below the maximum which was reported by Aragaw, H.S. *et al*. (2021). The table also shows that the level of ascorbic acid in *Rubus apetalus* in present study was found with in the range of literature report that was above the minimum which was reported by Probst, Y. (2015) and below the maximum which was reported by Aragaw, H.S. *et al*. (2021).

Table 10 Comparison of *Balanites aegyptica* and *Ziziphus spina-christi* concentration of present study with literature report (mg/g)

Wild fruits	Present study	Ascorbic acid concentration (mg/g)	Reference	Country	Method used
Ziziphus spina-christi	0.531 ± 1.88	0.0864	Ahmed and Sati. (2018)	Saudi Arabia	RP-HPLC
		0.014	Singh, *et al*. (2012)	Oman	Titration
		0.030	Berry-Koch, *et al*. (1990)	Atlanta	Literature Review
		1.60	Orwa, *et al*. (2009)	Kenya	Titration
Balanites aegyptiaca	0.402 ± 1.99	0.0966	Mariod, A.A. (2019)	Sudan	Literature Review
		0.0687	Sagna, *M.B. et al.* (2014)	Senegal	RP-HPLC
		0.5060	Debela, H.F. and Zemede, A. (2015)	Ethiopia	Redox Titration
		0.310	Stadlmayr, B. *et al.* (2013)	Kenya	Literature Review

Table 11 Comparison of *Utrica simensis* and *Thymus shimperi* concentration of present study with literature report (mg/g)

Wild fruits	Present study	Ascorbic acid concentration (mg/g)	Reference	Country	Method used
Utrica simensis	1.612 ± 0.06	1.58–3.36	Babya,B.K. *et al.* (2020)	Ethiopia	UV-VIS
		2.28-2.42	Seifu T. *et al.* (2017)	Ethiopia	UV-VIS
Thymus shimperi	1.386 ± 1.44	1.601	USDA National Nutrient data base	USA	Standard Reference
		1.601	Dauqan, E.M. and Abdullah, A. (2017	Malaysia	Literature Review

Data presented in Table 10 clearly indicated that concentration of vitamin C in *Ziziphus spina-christi* in present study was found in the range of literature report. It's vitamin C content was above the minimum which was reported by Singh, *et al*. (2012) but, below the maximum which was reported by Orwa, *et al*. (2009). The concentration of vitamin C in *Balanites aegyptica* in present study was also found in the range of literature report. It's vitamin C content was above the minimum which was reported by Sagna, *M.B. et al. (*2014) but, below the maximum which was reported by Debela, H.F. and Zemede, A. (2015). Table 11 indicated that the vitamin C concentration in the present study in *Utrica simensis* was found in the range of literature report. It's vitamin C content was above the minimum and below the maximum which was reported by Babya, B.K. *et al*. (2020). The vitamin C content of *Thymus shimperi* was found to be near literature values but is high enough to satisfy RDA. Therefore, from the results given in Table 10 and 11, it could be concluded that the concentration of vitamin C in the present study was found within the range of literature values and were enough to satisfy RDA.

There are considerable differences in the values of ascorbic acid obtained in the present study with those reported by several other studies for some fruit and leaf samples. All such differences in the contents of ascorbic acid in the present study and previous studies might be as a result of variations in maturity stage and regional varieties of fruits. Different techniques of measuring and squeezing process may also affect the ascorbic acid content of fruits extract. The amount of ascorbic acid could even vary between the different fruit samples of the same species (Mahdavi, R. *et al*. 2010; Bekele, D. and Geleta, G. 2015). Factors including climate; temperature; and various physical conditions such as light can also affect the concentration of ascorbic acid in fruits. The amount of ascorbic acid content in fruits can also be affected by the type, duration of storage and handling. Therefore, it is necessary that fruit sample should be stored at cool temperature and not to stay for a long time in order for its ascorbic acid contents not to decrease (Shrestha, N. *et.al*. 2016).

4.4. Comparison of Observed Ascorbic Acid Concentration with Domesticated Citrus Fruits

Fruits such as orange and lemon contain high amount of vitamin C. It is widely known that the best sources of vitamin C are citrus fruits and their juices (Khan, M.R. *et al*. 2006). Vitamin C is vital for the proper function of the immune system, making it a nutrient to turn to for the prevention of recurrent ear infections, colds, and flu. Orange and citrus fruits are supposedly the kings of Vitamin C**,** as they are known to have the vitamin in high quantities. Oranges for example contain 0.32 to 0.78 mg/g of vitamin C which is near recommended daily allowance for human. Consumption of citrus fruits or their products is found to alleviate from several diseases. Citrus fruits and juices are rich sources of bioactive compounds. Citrus fruits provide about 51% of vitamin C. Citrus are the main fruit grown and consumed in the world. Nowadays, world production is about 108 million tons. Orange is the first by production quantities. According to European Agriculture, Forestry and Fishery Statistics of 2017, 6.3 million tons were produced annually. Moreover, production of citrus fruits has expanded rapidly (Marti, N. *et al*. 2009). These were the reason why these two citrus fruits were selected for comparison. Table 12 shows vitamin C content of orange (*Citrus simensis*) and lemon (*Citrus limon*) in selected countries (Marti, N. *et al*. 2009).

Table 12 Comparison of observed Ascorbic acid content with domesticated citrus fruits

Fruits	Condition	Total vitamin C.(mg/g FW)	References	Country	Method used
Lemon	Fresh	0.531	Mohammed, Q.Y. *et al.* (2009)	Iraq	UV-VIS spectrophotometry
		0.358	Khan, M.R. *et al.* (2006)	Bangladesh	
		0.415	Vahid, B. (2012)	Iran	
Orange	Fresh	0.433	Mohammed, Q.Y. *et al.* (2009)	Iraq	
		0.50	Acevedo, D. *et al.* (2018)	Iran	
		0.535	Vahid, B. (2012)	Iran	

As observed from the table above, different researchers at different time and country reported their findings of ascorbic acid contents in citrus fruits. The concentration of ascorbic acid in *Utrica simensis*, *Thymus shimperi*, *Syzygium guineense*, *Doviyalis abyssinica* and *Carissa spinarum* in present study were found to be higher than these domesticated fruits. This indicates that these wild fruits and leaves are rich in ascorbic acid and the level of ascorbic acid in these wild edible plants could be able to satisfy the recommended daily allowance of vitamin C even more than the domesticated fruits like lemon and orange.

However, contents of ascorbic acid in *Opuntia ficus indica* in present study was identified to be lower than the value in above citrus fruits.

Table 13 Comparison of observed Ascorbic acid content with domesticated fruits by country (mg/g) (Khan, M.R. *et al*. (2006), Mohammed, Q.Y. *et al*. (2009), Mussa, S.B. and Sharaa, I.E. (2014)

Fruits	Condition	Pakistan	USA	Libya	Iraq	Bangladesh	India	Method used
Lemon	Fresh	0.287	0.530	ND	0.531	0.3583	0.564	UV-VIS spectrophotometer
Orange	Fresh	0.321	0.532	0.3245	0.433	NR	NR	

Another literature report indicated that it was found that the average concentration of ascorbic acid in lemon; bitter orange; grapefruit and sweet orange were about 0.348 mg/g , 0.298 mg/g, 0.398 mg/g, and 0.251 mg/g, respectively (Shrestha, N. *et al.* 2016). This shows that the concentration of ascorbic acid in *Utrica simensis*, *Thymus shimperi*, *Syzygium guineense*, *Doviyalis abyssinica*, *Carissa spinirum* in present study were found to be higher than these domesticated fruits. In addition, these edible wild plants had a higher content of vitamin C than pineapple (0.61 mg/g of FW), carambola (0.35 mg/g of FW), papaya (0.46 mg/g of FW), mango (0.53 mg/g of FW), and passion fruit (0.30 mg/g of FW) (Cavalcante, I.H. and Martins, A.B. 2005). Thus, supplementations of these wild fruits obviously mitigate vitamin C malnutrition which may occur because of lack of these domestic fruits. This may advantageous nutritionally as well as economically.

4.5. Comparison of Observed Ascorbic Acid Content in *Utrica simensis* and *Thymus shimperi* Leaf with Cabbage and Mustard Spinach; Commonly Used Domesticated Leafy Vegetables.

Cabbage botanically known as *Brassica oleracea* is a leafy vegetable that is usually green in color. It is a member of the cruciferous family of vegetables. It is a low-calorie food that is rich in vitamin C, a vitamin that is necessary by the human body. It also contains phytochemicals, such as fiber and carotenoids, which may have health-promoting properties. While all leafy greens are rich in vitamin C, mustard spinach is the most abundant.

In addition to vitamin C, mustard spinach is also regarded as an excellent source of many other nutrients, primarily K, Ca, vitamin A, and Mn. Vitamin C is known to be a powerful antioxidant that protects the immune system of human (Singh, R. 2019).

Table 14 Comparison of Observed Ascorbic Acid Content in *Utrica simensis* and *Thymus shimperi* Leaf With Cabbage and Mustard Spinach; Commonly Used Domesticated Leafy Vegetables.

Vegetables	Total vitamin C (mg/g)	References	Country	Method used
Cabbage *(Brassica oleracea)*	0.83	Puupponen-Pimia, R. *et al*. (2003)	Finland	RP-HPLC
	1.07	Sikora, E. *et al*. (2008)	Poland	Tillman's Method
	0.703	Branion, H.D. *et al*. (1948)	America	Titration
	0.55	Singh, R. (2019)	South Africa	Titration
	3.686-8.298	Alemu, T. *et al*. (2022)	Ethiopia	Voltammetric method
	0.381	Hailemariam, G.A. and Wudineh, T.A. (2020)	Ethiopia	UV-VIS
Mustard Spinach (*Brassica rapa*)	1.30	Musa, N. *et al*. (2012)	Malaysia	Titration
	0.62	Toledo, M.E. *et al*. (2003)	Japan	2,4-DNPH Method.
	0.53	Singh, J. *et al*. (2007)	India	RP-HPLC

This table shows that vitamin C content of leaf of *Utrica simensis* and *Thymus shimperi* were found to be almost higher than these commonly used domesticated green leafy vegetables except that the value that was reported by Alemu, T. *et al*. (2022). Depending on the finding of this study, it is advisable that diversification and use of these wild leafy vegetables instead of mentioned domesticated green leafy vegetables is economically as well as nutritionally helpful since these wild leafy vegetables are easily available within local community. In general, the concentrations of ascorbic acid observed were more or less comparable with the reported literature values. The level of vitamin C in these wild edible plants could be able to satisfy the recommended daily allowances of vitamin C. It was almost high enough to provide vitamin C, which is known to be a powerful antioxidant that protects the immune system of human.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Vitamin C also known as ascorbic acid is an organic compound that is a valuable food component because of its antioxidant and therapeutic properties. This study showed that the underutilized wild edible plants studied are good sources of vitamin C, which is comparable with or sometimes higher than domesticated fruits. Spectrophotometric method was used to quantify the content of vitamin C in edible plant samples. The concentration of ascorbic acid varies from one plant to another plant and the average concentration of ascorbic acid was about 1.612 ± 0.06 mg/g for *Utrica simensis* and is about 0.175 ± 1.14 mg/g for *Opuntia ficus indica* fruit. It was found that the average concentration of ascorbic acid in *Thymus shimperi, Syzygium guineense sp., Carissa spinarum, Doviyalis abyssinica, Ziziphus spina-christi, Balanites aegyptiaca, Rubus apetalus* plants were about 1.386 ± 1.44, 1.274 ± 0.78, 0.722 ± 1.38, 1.130 ± 0.84, 0.531 ± 1.88, 0.402 ± 1.99, 0.350 ± 2.85 mg/g respectively. Hence the average concentration of ascorbic acid determined by spectrophotometric methods were found to be maximum in *Utrica simensis* whereas minimum in *Ficus mucuso welw* among ten wild edible fruits and leaves collected from Saka Sole forest. The concentrations of most of wild edible fruits/leaves detected were found to be high in vitamin C content and almost were included within the range of literature value. This study also showed that the concentration of ascorbic acid in *Syzygium guineense sp., Doviyalis abyssinica, Carissa spinarum* in present study were found to be higher than the concentration of ascorbic acid in domesticated citrus fruits such as lemon; orange; grapefruit and the concentration of ascorbic acid in *Utrica simensis* and *Thymus shimperi* leaves were found to be higher than commonly eaten green leafy vegetables such as cabbage and mustard spinach when compared. Hence, these wild edible plants under investigation might be considered as good sources of vitamin C. Therefore, the level of vitamin C in these wild edible fruits and leaves could be able to satisfy the recommended daily allowances which are 90 mg for adult men and 75 mg for adult women and 15-45 mg for children. This indicates that, these underutilized wild edible fruits and leaves can be used to mitigate micronutrient malnutrition and improving food security. Furthermore, these wild edible plants might be used instead of domesticated plants mentioned above since they have higher amount of vitamin C and easily accessible in the community. Hence, this study could aid in promoting the application of the studied wild edible plants, to be economically exploited for nutraceutical applications and included into food formulations for the benefit of human health.

5.2. Suggestions for Further Work (Recommendation)

Vitamin C which is used as food supplements are available in many WEPs in many natural habitats. Due to the resource limitation only vitamin C was considered in this study hence, it will be essential to focus on the determination of the remaining water soluble vitamins spectrophometrically. Due to its chemical instability, particular attention should be given to sample pretreatment procedures while analyzing vitamin C. The following recommendation should be forwarded by the researcher.

- The study analyzed only ten wild edible plants samples, other wild edible plants available within the study area can also be investigated by other researchers to make valid generalization about ascorbic acid found in plants grown in the area.
- Bioactive compounds and additional nutritional contents of wild edible plants need further investigation.
- These underutilized wild edible plants are essential for human health. Therefore, these plants have to be diversified.
- It will be very important to recognize the nutritional values of wild edible plants and set plans for conservation, management and sustainable utilization.
- It should be worked on the indigenous knowledge of the people which is transferring only by oral and visual along with its documentations and keeping it from loss.
- The researcher suggests that nutrition policies have to promote the utilization of wild edible plants as one pillar of food and nutrition security.

6. REFERENCES

Abdelmuti, O.M. (1991). Biochemical and Nutritional Evaluation of Famine Foods of the Sudan. *Faculty of Agriculture. Khartoum, Sudan, University of Khartoum, Doctoral dissertation in Biochemistry and Nutrition, Sudan*.

Aberoumand, A. and Deokule, S.S. (2009). Studies on Nutritional Values of Some Wild Edible Plants from Iran and India. *Pakistan Journal of Nutrition*, *8*(1), pp.26-31.

Acevedo, D. Montero, P. and Martelo, R. (2018). Determination of Quality in Orange Juices Marketed in the City of Cartagena de Indias-Colombia. *International Journal of Engineering and Technology*, *10*(1), pp.336-339.

Addis, G. Asfaw Z. and Woldu Z. (2009). Underutilized Edible Plants as a Means of Food Source Diversification in Ethiopia. In *Issues and challenges in food security: Proceedings of a national workshop organized by the Biological Society of Ethiopia. Addis Ababa University, Addis Ababa, Ethiopia*. pp.58-78.

Addis, G. Urga, K. and Dikasso, D. (2005). Ethnobotanical Study of Edible Wild Plants in Some Selected Districts of Ethiopia. *Human Ecology*, *33*(1), pp.83-118.

Afolayan, A.J. and Jimoh, F.O. (2009). Nutritional Quality of Some Wild Leafy Vegetables in South Africa. *International journal of food sciences and nutrition*, *60*(5), pp.424-431.

Ahmed, F.A. and Sati, N.M. (2018). Chemotaxonomic Study and Botanical Overview of Some Ziziphus spp. *Sudan. International Research Journal of Biological Sciences*, *7*(5), pp.32-39.

Alemu, T., Zelalem, B. and Amare, N. (2022). Voltammetric Determination of Ascorbic Acid Content in Cabbage Using Anthraquinone Modified Carbon Paste Electrode. *Journal of Chemistry*, volume *2022*.p.9.

Almeida, E.J. (2004). Clonagem e Fenologia de Dovyalis sp. (Doctoral Dissertation). Jaboticabal. Universidade Estadual Paulista. Wild Fruits: Composition, Nutritional Value and Products. P.60

Amusan, O.O., Dlamini, P.S. Msonthi, J.D. and Makhubu, L.P., (2002). Some Herbal Remedies from Manzini Region of Swaziland. *Journal of Ethnopharmacology*, *79*(1), pp.109-112.

Anthony, C., Dweck (2005). A Review of Zizyphus Spina-Christi. FLS FRSC FRSH–Technical Editor, Personal care.

Aragaw, H.S., Nohr, D. and Callo-Concha, D. (2021). Nutritional Potential of Underutilized Edible Plant Species in Coffee Agroforestry Systems of Yayu, Southwestern Ethiopia. *Agroforestry Systems*, *95*(6), pp.1047-1059.

Asensi-Fabado, M.A. and Munne-Bosch, S. (2010). Vitamins in Plants: Occurrence, Biosynthesis and Antioxidant Function. *Trends in plant science*, *15*(10), pp.582-592.

Aynachew T. (2018). Nutritional Quality of Underutilized Wild Edible Fruits Grown in Ethiopia, p.4, Addis Ababa University(unpublished).

Balemie, K. and Kebebew, F. (2006). Ethnobotanical Study of Wild Edible Plants in Derashe and Kucha Districts, South Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *2*(1), pp.1-9.

Ball, G.F. (2005). *Vitamins in Foods: Analysis, Bioavailability, and Stability*. CRC press. New York. p.4.

Bayba, K.B., Dubale, A.A., Mehari, B. and Atlabachew, M. (2020). Chemical Composition of Urtica Simensis Grown in Different Regions of Ethiopia. *Journal of Chemistry*, pp. 2-8.

Becker, B. (1983). The Contribution of Wild Plants to Human Nutrition in the Ferlo (Northern Senegal). *Agroforestry systems*, *1*(3), pp.257-267.

Bekele D., Geleta G. (2015). Iodometric Determination of the Ascorbic Acid (vitamin C) Content of Some Fruits Consumed in Jimma Town Community in Ethiopia. *Research Journal of Chemical Science.* 5(1): 60-63.

Bekele-Tesemma, A. and Tengnäs, B. (2007). *Useful Trees and Shrubs of Ethiopia: identification, propagation, and management for 17 agroclimatic zones* (p. 552). Nirobi: RELMA in ICRAF Project, World Agroforestry Centre, Eastern Africa Region.

Berry-Koch, A., Moench, R., Hakewill, P. and Dualeh, M. (1990). Alleviation of Nutritional Deficiency Diseases in Refugees. *Food and Nutrition Bulletin*, *12*(2), pp.1-7.

Branion, H.D., Roberts, J.S., Cameeon, C.R. and McCready, A.M. (1948). The Ascorbic Acid Content of Cabbage. *Journal of the American Dietetic Association*, *24(2)*, pp.101-104.

Caldas, S.S., Demoliner, A. and Primel, E.G. (2009). Validation of a Method Using Solid Phase Extraction and Liquid Chromatography for the Determination of Pesticide Residues in Groundwaters. *Journal of the Brazilian Chemical Society*, *20*, pp.125-132.

Cantwell-Jones, A., Ball, J., Collar, D., Diazgranados, M., Douglas, R., Forest, F., Hawkins, J., Howes, M.J. Ulian, T., Vaitla, B. and Pironon, S. (2022). Global Plant Diversity as a Reservoir of Micronutrients for Humanity. *Nature Plants*, *8*(3), pp.225-232.

Cavalcante, I.H. and Martins, A.B. (2005). Physical and Chemical Characterization of Dovyalis Fruits. *International journal of fruit science*, *5*(4), pp.39-46.

Chakravarty, S., Bhutia, K.D., Suresh, C.P., Shukla, G. and Pala, N.A. (2016). A review on Diversity, Conservation and Nutrition of Wild Edible Fruits. *Journal of Applied and Natural Science*, *8*(4), pp.2346-2353.

Chamkouri, N., Khodadoust, S. and Ghalavandi, F. (2015). Solid-Phase Extraction Coupled With HPLC-DAD for Determination of B Vitamin Concentrations in Halophytes. *Journal of chromatographic science*, *53*(10), pp.1720-1724.

Chandra, R., Kumar, S., Singh, S., Sharma, K.D., Alam, M.D.N. and Verma, D. (2013). Quantitative Assay Evaluation of Vitamin C From Formulated Tablets: Application On RP-HPLC and UV-Spectrophotometry. *International Journal Of Analytical, Pharmaceutical and Biomedical Sciences*, *2(3)*, pp. 19-23

Chandran, S. and Singh, R.S.P. (2007). Comparison of Various International Guidelines for Analytical Method Validation. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *62*(1), pp.4-14.

Chebrolu, K.K., Jayaprakasha, G.K., Yoo, K.S., Jifon, J.L. and Patil, B.S. (2012). An Improved Sample Preparation Method for Quantification of Ascorbic Acid and Dehydroascorbic Acid by HPLC. *LWT, Food Science. and Technology*.*47*(2), pp.443-449.

Chimezie, A., Ibukun, A., Teddy, E. and Francis, O. (2008). HPLC Analysis of Nicotinamide, Pyridoxine, Riboflavin and Thiamin in Some Selected Food Products in Nigeria. *African Journal of Pharmacy and Pharmacology*, *2*(2), pp.029-036.

Chiteva, R. and Wairagu, N. (2013). Chemical and Nutritional Content of Opuntia ficus-indica (L.). *African Journal of Biotechnology*, *12*(21).pp.3309-3312.

Dansi, A., Adjatin, A., Adoukonou-Sagbadja, H., Falade, V., Yedomonhan, H., Odou, D. and Dossou, B. (2008). Traditional Leafy Vegetables and Their Use in the Benin Republic. *Genetic Resources and Crop Evolution*, *55*(8), pp.1239-1256.

Dauqan, E.M. and Abdullah, A., (2017). Medicinal and Functional Values of Thyme (Thymus vulgaris L.) herb. *Journal of applied biology and biotechnology*, *5*(2), pp.0-2.

Debela, H.F. and Zemede, A. (2015). Nutritional Contents of Balanites aegyptiaca and its Contribution to Human Diet. *African Journal of Food Science*, *9*(5), pp.346-350.

Desai, A.P. and Desai, S. (2019). UV Spectroscopic Method for Determination of Vitamin C (ascorbic acid) Content in Different Fruits in South Gujarat Region. *International Journal of Environmental Sciences & Natural Resources*, *21*(2), pp.41-44.

Dirres, A.D. (2016). Collection and Identification of Wild Fruits Tree/Shrub Species in Amhara Region, Ethiopia. *Journal of Natural Sciences Research*, *6*(11), pp.26-31.

Dogan, Y., Ugulu, İ. and Durkan, N. (2013). Wild Edible Plants Sold in the Local Markets of Izmir, Turkey. 45: pp. 177-184.

Duguma, H.T. (2020). Wild Edible Plant Nutritional Contribution and Consumer Perception in Ethiopia. *International Journal of Food Science*, pp. 1-16

Elgailani, I.E., Elkareem, M.A., Noh, E., Adam, O. and Alghamdi, A. (2017). Comparison of Two Methods for the Determination of Vitamin C (Ascorbic Acid) in Some Fruits. *American Journal of Chem*istry, *2*(1), pp.1-7.

El-Ghazali, G.E. (1987). Medicinal Plants of the Sudan: Medicinal Plants of the Eastern Nuba Mountains. *National Council for Research.* Khartoum, pp.8-31

El-Samahy, S.K., Abd El-Hady, E.A., Habiba, R.A. and Moussa, T.E. (2006). Chemical and Rheological Characteristics of Orange-Yellow Cactus-Pear Pulp from Egypt. *Journal of the Professional Association for Cactus Development*, *8*, pp.39-51.

Fadhel, D.H. (2012). Spectrophotometric Determination of Ascorbic Acid in Aqueous Solutions. *Al-Nahrain Journal of Science, 15*(3), pp.88-94.

Farah, H.S., Alhmoud, J.F., Al-Othman, A., Alqaisi, K.M., Atoom, A.M., Shadid, K., Shakya, A. and AlQaisi, T. (2020). Effect of pH, Temperature and Metal Salts in Different

Storage Conditions on the Stability of Vitamin C Content of Yellow Bell Pepper Extracted in Aqueous Media. *Systematic Reviews in Pharmacy*, *11*(9), pp.661-667.

Fatima, Z., Jin, X., Zou, Y., Kaw, H.Y., Quinto, M. and Li, D. (2019). Recent trends in Analytical Methods for Water-Soluble Vitamins. *Journal of Chromatography A*, *1606*, pp. 1-72.

Grosso, G., Bei, R., Mistretta, A., Marventano, S., Calabrese, G., Masuelli, L., Giganti, M.G., Modesti, A., Galvano, F. and Gazzolo, D. (2013). Effects of Vitamin C on Health: a Review of Evidence. *Front Biosci (Landmark Ed)*, *18*(3), pp.1017-1029.

Guil-Guerrero, J.L. and Rodríguez-García, I. (1999). Lipids Classes, Fatty Acids and Carotenes of the Leaves of Six Edible Wild Plants. *European Food Research and Technology*, *209*(5), pp.313-316.

Hagos, M., Redi-Abshiro, M., Chandravanshi, B.S. and Yaya, E.E. (2022). New Analytical Methods for the Determination of Ascorbic Acid Content in Aqueous Extracts of Flesh, Peel and Seeds of Pumpkin (Cucurbita maxima). *Bulletin of the Chemical Society of Ethiopia*, *36*(2), pp.277-290.

Hailemariam, G.A. and Wudineh, T.A. (2020). Effect of Cooking Methods on Ascorbic Acid Destruction of Green Leafy Vegetables. *Journal of Food Quality*, volume *2020*,p.5

Herbig, A.L. and Renard, C.M. (2017). Factors that Impact the Stability of Vitamin C at Intermediate Temperatures in a Food Matrix. *Food Chemistry*, *220*, pp.444-451.

Hinnawi, N.S.A. (2010). *An ethnobotanical Study of Wild Edible Plants in the Northern West Bank* (Doctoral dissertation).

ICH (1994). Validation of Analytical Procedures: Text and methodology. *Report Number: Q2(R1).* [Online] Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf [Accessed 21st February 2022].

Iqbal, K., Khan, A. and Khattak, M.M.A.K. (2004). Biological Significance of Ascorbic Acid (Vitamin C) in Human Health-a Review. *Pakistan Journal of Nutrition*, *3*(1), pp.5-13

Jablonska-Rys, E., Zalewska-Korona, M. and Kalbarczyk, J. (2009). Antioxidant Capacity, Ascorbic Acid and Phenolics Content in Wild Edible Fruits. *Journal of Fruit and Ornamental Plant Research*, *17*(2), pp.115-120.

Jutkus, R.A., Li, N., Taylor, L.S. and Mauer, L.J. (2015). Effect of Temperature and Initial Moisture Content on the Chemical Stability and Color Change of Various Forms of Vitamin C. *International Journal of Food Properties*, *18*(4), pp.862-879.

Kassegn, H.H. and Mekelle, E.P. (2016). Determination Proximate Composition of the Wild Abyssinian Thyme Herb (Thyme Schimperi L.) Grown in High Lands of Southern Tigray, North Ethiopia. *Food Science and Quality Management*, *50*, pp.1-3.

Kayang H. (2007). Tribal knowledge on Wild Edible Plants of Meghalaya, Northeast India. *Indian Journal of Traditional Knowledge*. 6(1), pp. 177-181

Khan, F.A., Bhat, S.A. and Narayan, S., (2017). Wild Edible Plants as a Food Resources: Traditional Knowledge. *Division of post-harvest technology shere_Kashmir university of Agricultural sciences and technology of Kashmir*. *India*. pp. 1-19

Khan, M.R., Rahman, M.M., Islam, M.S. and Begum, S.A. (2006). A Simple UV-Spectrophotometric Method for the Determination of Vitamin C Content in Various Fruits and Vegetables at Sylhet Area in Bangladesh. *Journal of Biological Science*, *6*(2), pp.388-392.

Kothari, V., Gupta, A. and Naraniwal, M. (2012). Comparative Study of Various Methods for Extraction of Antioxidant and Antibacterial Compounds from Plant Seeds. *Journal of Natural Remedies*, *12*(2), pp.162-173.

Kothari, V., Pathan, S. and Seshadri, S. (2010). Antioxidant Activity of M. zapota and C. limon Seeds. *Journal of . Natural. Remedies*, *10*(2), pp.175-180.

Kothari, V., Punjabi, A. and Gupta, S. (2009). Optimization of Microwave-Assisted Extraction of Annona squamosa seeds. *The Icfai University Journal of Life Sciences*, *3*(1), pp.55-60.

Linsinger, T.P. (2008). Use of Recovery and Bias Information in Analytical Chemistry and Estimation of its Uncertainty Contribution. *TRAC-Trends in Analytical Chemistry*, *27*(10), pp.916-923.

Luczaj, L. (2010). Changes in the Utilization of Wild Green Vegetables in Poland Since the 19th Century: a Comparison of Four Ethnobotanical Surveys. *Journal of ethnopharmacology*, *128*(2), pp.395-404.

Lulekal, E., Asfaw, Z., Kelbessa, E. and Van Damme, P. (2011). Wild Edible Plants in Ethiopia: a Review on Their Potential to Combat Food Insecurity. *Afrika focus*, *24*(2), pp.71-122.

Mahdavi, R., Nikniaz, Z., Rafraf, M. and Jouyban, A. (2010). Determination and Comparison of Total Polyphenol and Vitamin C Contents of Natural Fresh and Commercial Fruit Juices. *Pakistan Journal of Nutrition*, *9*(10), pp.968-972.

Mariod, A.A. ed. (2019). *Wild fruits: Composition, Nutritional Value and Products*. Springer International Publishing.

Marti, N., Mena, P., Canovas, J.A., Micol, V. and Saura, D. (2009). Vitamin C and the Role of Citrus Juices as Functional Food. *Natural product communications*, *4*(5), pp.677-700.

Matei, N., Magearu, V., Birghila, S. and Dobrina, S. (2004). The Determination of Vitamin C From Sweet Cherries and Cherries. *Revista de Chimie*, *55*(5), pp.294-296.

Mavengahama, S., McLachlan, M. and De Clercq, W. (2013). The Role of Wild Vegetable Species in Household Food Security in Maize Based Subsistence Cropping Systems. *Food Security*, *5*(2), pp.227-233.

Mengistu, F. and Hager, H. (2008). Wild Edible Fruit Species Cultural Domain, Informant Species Competence and Preference in Three Districts of Amhara Region, Ethiopia. *Ethnobotany Research and Applications*, *6*, pp.487-502.

Modi, S.K., Prasad, B.D. and Basavaraj, M. (2013). The Influence of Moisture Content and Density on Thermal Conductivity of Ficus Carica Linnaus (Fig Fruit) by Transient Line Heat Source Method. *International Journal of Engineering and Innovative Technology*, *3*(6), pp.177-180.

Mohammad Azmin, S.N., Abdul Manan, Z., Wan Alwi, S.R., Chua, L.S., Mustaffa, A.A. and Yunus, N.A. (2016). Herbal Processing and Extraction Technologies. *Separation & Purification Reviews*, *45*(4), pp.305-320.

Mohammed, Q.Y., Hamad, W.M. and Mohammed, E.K. (2009). Spectrophotometric Determination of Total Vitamin C in Some Fruits and Vegetables at Koya Area-Kurdistan Region/Iraq. *Journal of Kirkuk University-Scientific Studies*, *4(2)*, pp. 46-54.

Moura, T., Gaudy, D., Jacob, M. and Cassanas, G. (1994). PH Influence on the Stability of Ascorbic Acid Spray-Drying solutions. *Pharmaceutica Acta Helvetiae*, *69*(2), pp.77-80.

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B. and Wrolstad, R.E. (2002). Anthocyanins, Phenolics, and Antioxidant Capacity in Diverse Small Fruits: Vaccinium, Rubus, and Ribes. *Journal of agricultural and food chemistry*, *50*(3), pp.519-525.

Musa, N., Zaharudin, N., Misni, F. and Kabeb, S.M. (2012). *Determination of Vitamin C and Minerals from Spinach (Amaranthus Viridis) Chips for Nutrients Facts* (Doctoral dissertation, UMP).

Mussa, S.B. and Sharaa, I.E. (2014). Analysis of Vitamin C (Ascorbic Acid) Contents Packed Fruit Juice by UV-Spectrophotometry and Redox Titration Methods. *IOSR Journal of Applied Physics*, *6*(5), pp.46-52.

Norfun, P., Arqueropanyo, O., Liawruangrath, S. and Ounnunkad, K. (2016). Electrochemical Flow Injection Determination of Ascorbic Acid in Fruit Samples Employing a Graphene-Polyaniline Electrode. *International Journal of Chemical Engineering and Applications*, *7*(2), p.142.

Ohiokpehai, O (2003). and Ramosweu, P. (1998). The Role of Agricultural Processing and Nutrition in Improving Food Security at Household Level. In *Proceeding of the National Post Harvest Conference held October* (pp. 6-8).

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. (2009). Agroforestree Database: a Tree Reference and Selection Guide. Version 4. *Agroforestree Database: a tree reference and selection guide. Version 4.*

Osman, M.A. (2004). Chemical and Nutrient Analysis of Baobab (Adansonia digitata) Fruit and Seed Protein Solubility. *Plant foods for human nutrition*, *59*(1), pp.29-33.

Pawlos, Z., Chandravanshi, B.S., Yohannes, W. and Embiale, A. (2021). Levels of Selected Metals in *Ficus sur forssk* Fruit and soil of the Plant Grown in Different Parts of Ethiopia. *SINET: Ethiopian Journal of Science, 44*(1), pp.1-12.

Pisoschi, A.M., Pop, A., Serban, A.I. and Fafaneata, C. (2014). Electrochemical Methods for Ascorbic Acid Determination. *Electrochimica Acta*, *121,* pp.443-460.

Probst, Y. (2015). A Review of the Nutrient Composition of Selected Rubus Berries. *Nutrition & Food Science*, *45*(2), pp.242-254.

Puupponen-Pimia, R., Hakkinen, S. T., Aarni, M., Suortti, T., Lampi, A. M., Eurola, M., Piironen, V., Nuutila, A. M. and Oksman-Caldentey, K. M. (2003). Blanching and Longterm Freezing Affect Various Bioactive Compounds of Vegetables in Different Ways. *Journal of the Science of Food and Agriculture* 83(14): 1389-1402.

Romanik, G., Gilgenast, E., Przyjazny, A. and Kaminski, M. (2007). Techniques of Preparing Plant Material for Chromatographic Separation and Analysis. *Journal of biochemical and biophysical methods*, *70*(2), pp.253-261.

Rotili, M.C., Villa, F., Braga, G.C., França, D.L., Rosanelli, S., Laureth, J.C.U. and Silva, D.F.D. (2018). Bioactive Compounds, Antioxidant and Physic-Chemical Characteristics of the Dovyalis Fruit. *Acta Scientiarum. Agronomy*, *40*, pp. 2-8.

Sagna, M.B., Diallo, A., Sarr, P.S., Ndiaye, O., Goffner, D. and Guisse, A. (2014). Biochemical Composition and Nutritional Value of *Balanites aegyptiaca* (L.) Del Fruit Pulps from Northern Ferlo in Senegal. *African Journal of Biotechnology*, *13*(2).pp.337-342

Saka, J.K. and Msonthi, J.D. (1994). Nutritional Value of Edible Fruits of Indigenous Wild Trees in Malawi. *Forest ecology and Management*, *64*(2-3), pp.245-248.

Saraji, M. and Boroujeni, M.K. (2014). Recent Developments in Dispersive Liquid–Liquid Microextraction. *Analytical and bioanalytical chemistry*, *406*(8), pp.2027-2066

Seal, T., Chaudhuri, K. and Pillai, B. (2016). Identification and Quantification Flavonoids in Three Wild Edible Plants, *Houttuynia cordata*, *Solanum gilo and Solanum kurzii* of

North-Eastern Region in India, Using High Performance Liquid Chromatography with Diode Array Detection. *Journal of Chemical and Pharmaceutical Research*, *8*(8), pp.859-867.

Seifu, T., Mehari, B., Atlabachew, M. and Chandravanshi, B. (2017). Polyphenolic Content and Antioxidant Activity of Leaves of *Urtica simensis* Grown in Ethiopia. *Latin American Applied Research*, *47*(1), pp.35-40.

Sekeroglu, N., Ozkutlu, F., Deveci, M., Dede, O. and Yilmaz, N. (2006). Evaluation of Some Wild Plants Aspect of Their Nutritional Values Used as Vegetable in Eastern Black Sea Region of Turkey. *Asian Journal of Plant Sciences*, 5(2), pp.185-189

Sellappan, S., Akoh, C.C. and Krewer, G. (2002). Phenolic Compounds and Antioxidant Capacity of Georgia-Grown Blueberries and Blackberries. *Journal of agricultural and food chemistry*, *50*(8), pp.2432-2438.

Shabir, G.A. (2003). Validation of High-Performance Liquid Chromatography Methods for Pharmaceutical Analysis: Understanding the Differences and Similarities Between Validation Requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of chromatography A*, *987*(1-2), pp.57-66.

Sharaa, I.E. and Mussa, S.B. (2019). Determination of Vitamin C (Ascorbic Acid) Contents in Vegetable Samples by UV-Spectrophotometry and Redox Titration Methods and Estimation the Effect of Time, Cooking and Frozen on Ascorbic Acid Contents. *International Journal of Progressive Sciences and Technologies*, *15*(2), pp.281-293.

Shrestha, N., Shrestha, S. and Bhattarai, A. (2016). Determination of Ascorbic Acid in Different Citrus Fruits of Kathmandu Valley. *Journal of Medical and Biological Science Research*, *2*(1), pp.9-14.

Sikora, E., Cieslik, E., Leszczynska, T., Filipiak-Florkiewicz, A. and Pisulewski, P.M. (2008). The Antioxidant Activity of Selected Cruciferous Vegetables Subjected to Aquathermal Processing. *Food Chemistry*, *107*(1), pp.55-59.

Singh, D.B., Attri, B.L., Sharma, T.V.R.S. and Sreekumar, P.V. (2001). Nutrient Composition of Some Wild Edible Fruits of Andaman and Nicobar Islands. *Journal of Applied Horticulture*, *3*(1), pp.60-62.

Singh, J., Upadhyay, A.K., Prasad, K., Bahadur, A. and Rai, M. (2007). Variability of Carotenes, Vitamin C, E and Phenolics in Brassica Vegetables. *Journal of Food Composition and Analysis*, *20*(2), pp.106-112.

Singh, R. (2019). The Ascorbic Acid Content of Cabbage (Brassica oleracea). *Bulletin of Pure and Applied Sciences.* 38(2), P.82-84.

Singh, V., Guizani, N., Essa, M.M., Rahman, M.S. and Selvaraju, S. (2012). In Vitro Antioxidant Activities of *Ziziphus spina-christi* Fruits (red date) Grown in Oman. *Biotechnology*, *11*(4), pp.209-216.

Siyum, Z.H. and Meresa, T.A. (2021). Physicochemical Properties and Nutritional Values of Carissa Spinarum L. Agam fruit. *International Journal of Fruit Science*, *21*(1), pp.826-834.

Snyder, L.R., Kirkland, J.J. and Dolan, J.W. (2011). *Introduction to Modern Liquid Chromatography*, John Wiley & Sons.

Stadlmayr, B., Charrondiere, U.R., Eisenwagen, S., Jamnadass, R. and Kehlenbeck, K. (2013). Nutrient Composition of Selected Indigenous Fruits from Sub-Saharan Africa. *Journal of the Science of Food and Agriculture*, *93*(11), pp.2627-2636.

Suwardi, A.B., Indriaty, N.Z. and Navia, Z.I. (2018). Nutritional Evaluation of Some Wild Edible Tuberous Plants as an Alternative Foods. *Innovare Journal of Food Science*, *6*(2), pp.9-12.

Teklehaymanot, T. and Giday, M. (2010). Ethnobotanical Study of Wild Edible Plants of Kara and Kwego Semi-Pastoralist People in Lower Omo River Valley, Debub Omo Zone, SNNPR, Ethiopia. *Journal of ethnobiology and ethnomedicine*, *6*(1), pp.1-8.

Teng, H., Ghafoor, K. and Choi, Y.H. (2009). Optimization of Microwave-Assisted Extraction of Active Components from Chinese Quince Using Response Surface

Methodology. *Journal of the Korean Society for Applied Biological Chemistry*, *52*(6), pp.694-701.

Thompson, M., Ellison, S.L., Fajgelj, A., Willetts, P. and Wood, R. (1999). Harmonized Guidelines for the Use of Recovery Information in Analytical Measurement. *Pure and applied chemistry*, *71*(2), pp.337-348.

Toledo, M.E.A., Ueda, Y., Imahori, Y. and Ayaki, M., (2003). L-Ascorbic Acid Metabolism in Spinach (Spinacia oleracea L.) During Postharvest Storage in Light and Dark. *Postharvest biology and technology*, *28*(1), pp.47-57.

Tsegaye, W., Urga, K. and Asres, K. (2008). Antidiabetic Activity of Samma (Urtica simensis hochst. Ex. A. Rich.) in Streptozotocin-Induced Diabetic Mice. *Ethiopian Pharmaceutical Journal*, *27*, pp.75-82.

Tyrone Lockett, Louis E. Grivetti, C. (2000). Food-Related Behaviors During Drought: a Study of Rural Fulani, Northeastern Nigeria. *International Journal of Food Sciences and Nutrition*, *51*(2), pp.91-107.

Tyskiewicz, K., Debczak, A., Gieysztor, R., Szymczak, T. and Roj, E. (2018). Determination of Fat-and Water-Soluble Vitamins by Supercritical Fluid Chromatography: a review. *Journal of Separation Science*, *41*(1), pp.336-350.

Vahid, B. (2012). Titrimetric Determination of Ascorbic Acid Contents in Plant Samples by 2, 6-Dichlorophenolindophenol Method. *Journal of the Chemical Society of Pakistan*, *34*(6), pp.1510-1512.

Velisek, J. and Cejpek, K. (2007). Biosynthesis of Food Constituents: Vitamins. 2. Water-soluble Vitamins: Part 1-a Review. *Czech journal of food sciences*, *25*(2), p.49.

Waweru, D.M., Arimi, J.M., Marete, E., Jacquier, J.C. and Harbourne, N. (2022). Chemical and Antioxidant Characterization of *Dovyalis affra and Dovyalis abyssinica* Fruits in Kenya. *Heliyon*, p.e11064. *Journal of Pre Proof*. pp. 1-17.

Whitney, E.N. and Rolfes, S.R. (2015). *Understanding Nutrition*. Cengage Learning.

Yves, G. and Dechassa, L. (2000). Wild-Food Plants in Southern Ethiopia: Reflection on the Role of "Famine-Food" at a Time of Drought. *UNDP, UN-Emergencies Unit for Ethiopia*.pp. 1-17.

Zhang, Y., Zhou, W.E., Yan, J.Q., Liu, M., Zhou, Y., Shen, X., Ma, Y.L., Feng, X.S., Yang, J. and Li, G.H. (2018). A Review of the Extraction and Determination Methods of Thirteen Essential Vitamins to the Human Body: An update from 2010. *Molecules*, *23*(6), pp. 2-25

Zygmunt, B. and Namiesnik, J. (2003). Preparation of Samples of Plant Material for Chromatographic Analysis. *Journal of chromatographic science*, *41*(3), pp.109-116.