

**ADDIS ABABA UNIVERSITY**  
**COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES**  
**CENTER FOR FOOD SCIENCE AND NUTRITION**



**NUTRITIONAL QUALITY OF UNDERUTILIZED WILD EDIBLE FRUITS  
GROWN IN ETHIOPIA**

**BY**

**AYNACHEW TAFESSE**

A Thesis Submitted to College of Natural and Computational Sciences of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Food Science and Nutrition

**December, 2018**

**Addis Ababa**

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## DECLARATION

I the undersigned, declare that this is original work and has never been presented in this, in any other University as well as research centers. All source of materials used for this Thesis have been fully acknowledged.

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## **DEDICATION**

First and foremost, **this Thesis is dedicated to my big brother called Eyayu Tafesse**, for his endless love, support and encouragement to reach who I am now and he lives ever in my heart even if I lose him in unexpected way.

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## LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
AOAC	Association of Officials Analytical Chemistry
CAT	Catalase
CE	Catechin Equivalent
CP	<i>Carica Papaya</i>
CRD	Completely Randomized Design
DA	<i>Dovyalis Abyssinica</i>
Df	Degree of freedom
DNA	Deoxyribonucleic Acid
DPPH	2, 2-diphenyl-1-hydrazine
Dmb	Dry matter basis
DW	Dry Weight
ENH	Ethiopia National Herbarium
EPHI	Ethiopian Public Health Institute
g	gram
GAE	Gallic Acid Equivalent
GR	Glutathione Reductase
GT	Grand Total
FAO	Food and Agriculture Organization

FCR	Folin- Ciocalteu Reagent
GE	Gross Energy
GA	Gallic Acid
GP	Glutathione Peroxidase
Kg	Kilo-gram
LSD	Least Significance Difference
mg	Milligram
ml	Milliliter
MS	Mean Square
PE	Poly-Ethylene
ppm	parts per million
QE	Quercitin Equivalent
ROS	Reactive Oxygen Species
SE	Standard Error
SOD	Super Oxide Dismutase
SPSS	Statistical Product and Service Solutions
SS	Sum Square
Wb	Wait basis
WHO	World Health Organization
UWEFs	Underutilized Wild Edible Fruits
UV	Ultra-Violate

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This Research Thesis is only the beginning of my journey.

Thank you all

## ABSTRACT

*This study was carried out to evaluate nutritional composition, phytochemical constituents, anti-oxidant potentials, anti-nutritional factors, vitamin C content and to develop fruit leather products. Fruit pulps of Bedena/Balanites aegyptiaca (L) Del., Koshim/Dovyalis abyssinica (A.Rich.)Warb., Shola/Ficus mucoso Welw. Ficalho, Qoladi/Mimusops kummel Bruce A.DC., and Geba/Ziziphus spina-christi (L) Desf. sampled from North and East Shewa, Ethiopia were collected and characterized for their proximate composition, selected mineral contents, total soluble phenols, total flavonoids and total antioxidant capacity were done according to scientific standard procedure. Mineral contents were high, total soluble phenols ranged from 22.13±0.04 – 80.24±0.09 mg GAE/100g DW and total flavonoids were between 17.32±0.05 and 48.34±0.04 mg CE/100g DW. Total antioxidant capacity ranged from 0.07 – 11.60 mg QE/100g DW when measured in methanol extract using DPPH assay. The richness of these fruits in minerals and antioxidant compounds makes them considerable sources of nutrition and of potential impact on human health. Screening of anti-nutritional factors of five underutilized wild edible fruits were maximum oxalate content was found in Balanites aegyptiaca (18.59±0.31 mg/100g DW) and minimum value (5.04±0.11 mg/100g DW) was recorded in Mimusops kummel. While the phytate content ranged from 3.13±0.00 mg/100g DW (Mimusops kummel) to 3.45±0.10 mg/100g DW (Balanites aegyptiaca) among five edible fruits. Maximum value for condensed tannin content was exhibited in Ziziphus spina-christi (9.25±0.01 mg/100g DW) and lowest was recorded in Dovyalis abyssinica (2.26±0.05 mg/100g DW). The present results suggest that fruits contained lower amounts of all the anti-nutrients analyzed. Hence they may be recommended for consumption for human being. This study has shown that good quality fruit leathers and juices can be produced from the underutilized Ethiopian fruits including Dovyalis abyssinica and Carica Papaya using simple procedures suitable for small-scale commercial production including dehydration, osmotic dehydration, and mechanical juice extraction followed by hot water pasteurization.*

**Key words:** anti-nutrients, antioxidant activity, phytochemicals, minerals, wild edible fruits.

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1. Background

According to Abraham (2016) an underutilized wild edible fruit refers to species that are neither cultivated nor domesticated but available as wild natural habitat. These wild fruits are used as sources of food and can provide food nutrients that are essential for human body (Ramosweu, 1999). Most of these fruits are consumed in different forms. Mostly they are consumed as raw during rippling season. Recent study has proved that these wild fruits have wide use for dynamic sources of traditional medicine, nutrition, vitamins, minerals, building materials, fuel and transport materials. Therefore these underutilized wild edible fruits play an important role in the life of local as well as urban population (Gazali *et al.*, 1987; Abdelmuti, 1991; Ohiokpehai, 2003).

On the other hand wild edible fruits are getting increasing interest from researchers due to their medicinal properties, proteins and vitamin C content (Pardo-de-Santayana *et al.*, 2007). Wild edible fruits of Ethiopia are used as supplementary food because, they are seasonal or survival food sources in many cultural groups, and plays a role in source of income for rural population by selling it to the urban population (Ohiokpehai, 2003; Lulekal *et al.*, 2011). These underutilized wild edible fruits are collected traditionally in rural communities in developing countries to secure community food supply especially during times of food scarcity (Msuya *et al.*, 2010). That is why many tropical countries and rural people traditionally harvest wide ranges of fruits from the wild because of their taste, cultural uses, as food supplements or to tide over food shortage (Guinand and Dechassa, 2000; Kebu and Fassil, 2006; Kumar, 2012). Wild edible fruits consumption is still very common in rural area of Ethiopia, particularly by children and herdsman. Among of these fruits *Balanites aegyptiaca* (L) Del., *Dovyalis abyssinica* (A.Rich.) Warb., *Ficus mucoso* Welw.ex Ficalho, *Mimusops kummel* Bruce ex A.DC. and *Ziziphus spina-christi* (L) Desf., are common underutilized wild edible fruits consumed by children and

herdsman in Ethiopia. They are also used as income source for many rural community (Guinand and Dechassa, 2000; Getachew *et al.*, 2005).

Even if they are common as source of food (nutritional values, vitamins, minerals and phytochemicals) their contents are not studied well (Osman, 2004). Thus they are underutilized still now in different area of the country. There is limitation also on anti-nutrient factors information on these underutilized wild edible fruits. Therefore nutritional qualities (nutritional values, mineral contents, vitamins and phytochemical constituents) and anti-nutritional factors of these underutilized wild edible fruits was 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. Once physiological and biological alternation has occurred in human body, free radicals will be formed which leads to oxidative damage to biomolecules like lipids, proteins Deoxyribonucleic Acid (DNA) (Jayathilake *et al.*, 2016). Therefore uses of underutilized wild edible fruits and products are increasing nowadays due to their exerted beneficial properties such as antioxidant, anticancer, hypoglycemic and hypolipidemic activities. Because of the interaction between free radicals, antioxidant and co-factors is essential for keeping health, ageing and age related diseases (Limem *et al.*, 2016).

Therefore this study was conducted to reflect the massive unexploited nutritional capacity of these fruits especially those that have commercial value and mostly consumed. Nutritional value of food defines what a food is made of and its' impact on the body. Due to disease and weight control, it's particularly important to understand the nutritional value of food due to the impact on the body as it relates to cholesterol, fat, salt, and sugar intake. The food label is the primary tool enabling consumers to understand nutritional values in order to make informed decisions about consumption. In Ethiopia 233 species which cover 56 % of edible plant species have edibility report (Ermias *et al.*, 2011). Among these 51 % are fruits that are commonly used and have utilized edible parts and consumed in more than two community areas. Therefore these fruits have great contributions as sources of energy and micronutrients (vitamins and minerals).

According to Ayele (2014), underutilized wild edible plant products like fruits have been recognized as important source of micronutrients (minerals and vitamins), dietary fiber and phytochemicals that can support the health benefit of the consumers.

Table 1: General account of underutilized wild edible fruits selected for nutritional analysis

<b>Species</b>	<b>Local name</b>	<b>Family</b>	<b>Consumed as</b>	<b>Habitat</b>	<b>Where found</b>
<i>Balanites aegyptiaca</i> (L) Del.	Badeno/Bedena (Amharic)	Balanitaceae	Raw Fruits	Tree Shrub	Arid and semi-arid parts of Ethiopia
<i>Dovyalis abyssinica</i> (A. Rich.) Warb.	Koshim/Koshem Amharic/Oromo	Salicaceae	Raw Fruits	Shrub	Many parts of Ethiopia
<i>Ficus mucuso</i> Welw ex Ficalho	Shola/Odda Amharic/Oromo	Moraceae	Raw Fruits	Tree	Many parts of Ethiopia
<i>Mimusops kummel</i> Bruce ex A.DC.	Eshe/Qoladi Amharic/Oromo	Sapotaceae	Raw Fruits	Tree	Many parts of Ethiopia
<i>Ziziphus spina- christi</i> (L) Desf.	Geba/Qurqura Amharic/Oromo	Rhamnaceae	Raw Fruits and Dried	Tree shrub	Arid and semi-arid parts of Ethiopia

## 1.2. Statement of the problem

In Ethiopia and other African countries, there are hundreds of underutilized indigenous wild edible fruits gathered from the wild that contribute vital roles in the nutrition of the people mostly to rural population, but no attention is given to those fruits in our county. So there is a need to promote the utilization of these indigenous underutilized wild edible fruits. Growing and using wild edible fruits is an opportunity that has never been adequately prospected to alleviate malnutrition and ameliorate food nutrition.

On the other hand, hundreds of edibles including many fruits of wild/semi-wild origin are known to be periodically consumed by rural communities in Ethiopia. Ethiopia is one of the developing countries rich in endemic wild edible fruits. Among these, fruit of *Balanites aegyptiaca* (L) Del., (Beden/ Bedena), *Dovyalis abyssinica* (A.Rich.) Warb. (Koshim), *Ficus mucosa* Welw ex. Ficalho (Shola), *Mimusops kummel* Bruce A.DC. (Qoladi) and *Ziziphus spina-christi* (L) Desf., (*Geba/Qurqura*) are common wild edible fruits. They are not studied well of their phytochemicals composition, antioxidant capacities, nutrient composition and anti-nutritional factors of these fruits. This may be the reason why they are underutilized. In health perspective, chronic diseases like cancer, cardiovascular and diabetes are critical nowadays in Ethiopia as well as in the world. However no one gives values for these fruits unless there is food shortage and need of extra income. In general, the statement of the problem can be formulated as lack of scientific information on nutritive values and health benefits of these underutilized wild edible fruits has contributed for the absence of diversified use as food source in Ethiopia.

Despite the fact that wild fruits are widely consumed with no cultural inhibition and tend to be nutritious, there is lack of sufficient information on the nutritional factors of these wild fruits and disorders associated with these anti-nutrients. This study was therefore undertaken to assess the level of anti-nutrients, proximate composition, antioxidants, vitamin C content, phytochemicals and fruit leather product formulation in 5 wild fruits commonly consumed in Ethiopia.

Underutilized wild edible fruits are consumed in rural community of Ethiopia, but there is no information on their nutritional composition and phytochemical potentials for human health. This study was also undertaken to assess the level of nutritional, anti-nutritional factors and antioxidant activity of selected wild fruits commonly consumed in Ethiopia. As per our

knowledge there is no well-studied document for those of wild fruits regarding to nutrition and product formulation whether they can support during food scarcity periods or not. Overall a lack of scientific information on nutritive values and health benefits of underutilized wild edible fruits has contributed for the absence of diversified use as food source in Ethiopia.

### **1.3. Significance of the study**

This study was conducted to evaluate the nutritional composition, antioxidant activities, anti-nutritional factors and phytochemical constituents of underutilized wild edible fruits including fruit leather formulation. Once it was studied it is possible to announce the community to consume them for their nutrition requirement throughout their life. It will also contribute to the reduction of healthcare costs, which increase the wellness and healthy life style of the consumers. Responsible bodies will use the information from the study to intervene and create awareness of nutritional quality and can support risk mitigation practices along the consumer's chain. Policymakers and development organizations can use the outcome of the study to plan their policies and programs in the edible wild fruits sector for better cultivation and manageable way of these wild fruits farming in large scale. Because it is wild plant, it may have different chemicals that are secondary metabolites or they may have anti-nutritional factors. After knowing this it is possible to conduct product development which can prove for the full utilization of these fruits. It helps to know whether it fulfill vitamin C requirement for the consumers. Generally, this work will introduce fruit leather products that can improve nutritional status of the society.

### **1.4. Objectives of the study**

#### **1.4.1. General objectives**

The objective of this study was to investigate nutritional composition of the edible parts of underutilized wild edible fruits and product formulation of *Dovyalis abyssinica* (A.Rich.) Warb., (Koshim) collected from East Shewa Ethiopia to answer its palatability based on 9 hedonic scale sensory evaluation methods.

### **1.4.2. Specific objectives**

- The specific objectives were to determine nutritional composition, vitamin C content, anti-nutritional factors, phytochemical constituents (total phenol, total flavonoids) and anti-oxidant properties of 5 selected underutilized wild edible fruits.
- Fruit leather snack food product form edible part of fruits that is less palatable but more accessible *Dovyalis abyssinica* (Koshim) with cultivated and palatable fruit of *Carica Papaya* and evaluate its acceptability.

## CHAPTER TWO

### 2. LITERATURE REVIEW

#### 2.1. Origin and distribution of the selected underutilized wild edible fruit plants

##### 2.1.1. *Balanites aegyptiaca* (L) Delile (Family: Balanitiaceae)

According to Fayssa (2011) among of the underutilized wild edible plants in Ethiopia *B. aegyptiaca* is abundant in semiarid areas of Ethiopia and other parts of Africa (Sadiq, 2012). An important tree found in and all over Africa from arid and semi-arid regions to sub-humid savanna and in Asia. A small evergreen tree about 10 m, crown rounded in tangled mass of thorny branches and bark which is smooth and green, later changed to dark, cracked and corky. Thorns of the tree are long to 8 cm (which is soft at first, then woody). The leaves are distinctive pairs of grey-green leaflets and ovate. Flowers are fragrant, yellow-green clusters. Fruits are long to 5 cm, both ends round, yellow when ripe, a hard pointed seed within surrounded by yellow-brown bitter-sweet flesh, seed easily separated.

In Ethiopia it is common in the Dry and Moist Kolla agroclimatic zones of the Rift Valley in Shoa, Gamo Gofa, Sidamo, Tigray, Welo, Gojam, Ilubabor, Arsi and upland Harerge regions from (0–1,800 m) altitude and used for firewood, charcoal, timber (furniture), poles, utensils, tool handles, food (fruit), medicine (infusion from roots, emulsion from fruit, heated gum from the wood, fruits), fodder (leaves, young shoots, fruit), shade, mulch, windbreak, gum, ceremonial meetings, fencing (cut branches), oil (fruit), emulsion of fruit kills snails and fish (Asibey and Tayie, 1999).

Direct sowing of seeds at site is the way to propagate the species. The seed is large (4 x 2 cm) which is planted vertically with stem end down for better results. The germination of the seeds will take 1–4 weeks. It was estimated that about 1,000 seed are per kg. Treatment for germination: Soak seed for 24 hours in cold water, then change water and soak for another 24 hours. On the other way collect seeds that have passed through goats. Can easily be collected where livestock are kept overnight and germination would be successful from 50–70%. Dry and

insect-free seeds can be stored for up to one year after remove the seeds from its fruit. But as the seed is very susceptible to insect attack, storage is not recommended. Generally it is an important species for dry areas as it produces fruit even in very dry years. The wood is termite-resistant. Extracts of the fruit and bark can be used to kill the snail hosts of bilharzia. The free-swimming stages of both bilharzia and guinea worm are also killed if the extract is put into the infected water (Azene, 2007).



**a**



**b**



**c**

Figure 2.1 **a**, Tree of *Balanites aegyptiaca* **b**, Tree of *Balanites aegyptiaca* with its fruit **c**, Ripe and ready to eat underutilized wild edible fruits of *Balanites aegyptiaca* (L) Del.

### **2.1.2. *Dovyalis abyssinica* (A.Rich.) Warb. (Family Salicaceae)**

According to Azene's (2007), it is an indigenous plant shrub locally known as Koshim (Amharic), Ankakute, Dugo and Kurawa (Oromo), Ongolatz (Sumaligna) and Aihada (Tigrigna). This evergreen spiny shrub or tree has height to 10 m, crown rounded. Its bark is grey, spines to 4 cm long. Branchlets with very clear dotted breathing pores and leaves with shiny, dark green, oval, to 5 cm, tip blunt, edge unevenly rounded. This shrub's 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.

The ecology of this shrubby tree is found from Ethiopia, Somalia and South Africa to Malawi and Southern Asia (India and Sri Lanka) in upland rainforest, dry evergreen forest, on river banks and sometimes in more open woodland. In Ethiopia, usually found along river courses in humid lower highland forest and Juniperus and Podocarpus forest, of Moist and Wet Weyna Dega and Dega agroclimatic zones in most regions, at altitude of from 1,700- 3,000 m. Used for food (fruit), medicine (leaves), bee forage and live fence.

Seeds that are collected from the ripe fruits will be propagated Seedlings (sow in seedbed and prick out). It can be treated after collecting the fruits and soaked in water for 2–3 days. The water is then drained off and the fruits squeezed by hand to separate the seeds from the pulp. After washing, the seeds can be dried and stored for a short time. Use fresh seed for best results during storage and manage it by lopping and coppicing. The fruit is edible but acidic; excellent for jelly.

It is a shrub that belongs to family *Salicaceae*. *Dovyalis abyssinica* is native to and common in the forests of Eastern Africa countries like Ethiopia, Kenya Tanzania, Uganda and Mali (Hailu, 2011). It was described as a shrubs or trees which has height to 8m. The shrub usually has spiny branches with leaves having 2-7 mm long petioles and containing 9 x 4.5 cm blades. The male flowers are usually solitary or in 2-3 flowered fascicles with pedicels that are 5-13 mm long and sepals 4-5 or 5-6 mm long and colored pale green. The female flowers are also solitary with pedicels 4-12 mm long and sepals slightly enlarged in fruits. Fruits are round berries that are 2-4

cm in diameter and yellow in color, containing several small seeds that appear to be hairy (Vollesen, 2000). They are very juicy with an acidic flavor.

The ripe fruits of *D. abyssinica* which contain sweet-sour skin around the seeds are widely eaten raw. Mainly children and herdsman used it as supplementary diet. During food shortage seasons in most parts of Ethiopia, Kenya Uganda Mali and Tanzania it also used as supplementary food diet. Not only this but also the roots boiled in soup are consumed as a food and medicine in Kenya (Demele *et al.*, 2010). Traditionally it is used as treatment of such as hemorrhoids, ulcers, and swelling of throat, wound healing and toothbrush stick (Sileshi *et al.*, 2001).



Figure 2.2 **a**, *Dovyalis abyssinica* shrubs within fruits **b**, Ripe and ready to eat underutilized wild edible fruits of *Dovyalis abyssinica* and **c**, Peeled fruits of *Dovyalis abyssinica* for preparation of juice

### **2.1.3. *Ficus mucoso* Welw.ex Ficalho (Family: Moraceae)**

It is an indigenous plant family of *Moraceae*, is widespread African fig tree occurring in eastern Africa, extending east to Yemen and south to Angola and South Africa. In Ethiopia, it is found along river banks, in upland rain forest, mountain grassland or secondary scrub in moist and wet agro-climatic zones in nearly all regions, 1,400–2,500 m. Figs in heavy clusters on branches to 70 cm long from trunk or older wood, figs round, usually 2 cm across but can be larger, on stalks, orange-red, often hairy, soft and edible, and having many seeds and often insects too and it is used as food.



**a**



**b**



**c**



**d**



**e**

Figure 2.3 **a**, Unripened fruits of *Ficus mucuso* on the plant **b**, Trees of *Ficus mucuso* **c**, Branch of *Ficus mucuso* **d**, Well ripened fruits of *Ficus mucuso* and **e**, Sun dried fruits of *Ficus mucuso*

#### 2.1.4. *Mimusops kummel* Bruce A.DC. (Family: Sapotaceae)

It is an indigenous and widespread tree extending to East Africa and Eritrea, Sudan and West Africa in riverine vegetation and also in dry evergreen forest, in wooded grassland on rocky hills in dry areas. In Ethiopia, it occurs in drier mountain forest and humid highland forest. It performs well in Moist and Wet Weyna Dega agro-climatic zones in all regions, mainly along rivers and forest fringes, 1,000-2,500 m. The fruits used as food. The fruit is a hard drupe to 2 cm, pointed and orange yellow, contains one red-brown seed. About 2,500 fruits or seeds were expected per Kg of sample. It can be stored for some time but are susceptible for insect attack. Well ripe fruits are available from December to March and are sold in local market (Azene, 20007).



a



b



c

Figure 2.4 **a**, Tree branch and fruits of *Mimusops kumme* **b**, Well ripened fruits of *Mimusops kummel* **c**, Sun dried fruits of *Mimosops kummel*

### **2.1.5. *Ziziphus spina-christi* (L) Desf. (Family: Rhamnaceae)**

The genus *Ziziphus* belongs to the *Rhamnaceae* family which consists of about 100 species of evergreen trees and shrubs distributed throughout the tropical and subtropical regions of the world. According to (Feyssa, 2011) *Ziziphus spina-christi* is distributed in acacia bush lands in alluvial soils and along dry riverbeds, edges of cultivation and gardens. From these twelve species are cultivated (Hammer 2001). A thorny shrub becoming a tree 5 to 10 m, evergreen on wet sites but losing all its leaves in a long dry season. The tree lives a long time. It has bark which is grey-brown, when cut the edge is reddish, mature bark grooved and cracking. The paired spines are thumb pointer, the straight thorns long and thin. Small leaves, narrowly ovate, variable in length, 1–8 cm, shortly stalked, usually narrowed to the base where each side is similar, 3 clear veins from the base, the edge lightly toothed. The tree has small flowers (10–25 cm) in heads beside leaves, yellow-green, stalks and calyx hairy white.

A spiny shrub which grows in the Sahel from Senegal to the Sudan and Arabia, in wooded grasslands, on flooded river banks and at edges of cultivation. It prefers alluvial plains with deep soil. In Ethiopia, it occurs in Bereha and Dry and Moist Kolla agroclimatic zones in Afar plains, Diredawa, Hararge, Bale, Gamo Gofa, Shewa, Welo, and Tigray, 0–1,900 m. Used as firewood, charcoal, timber (spear shafts, roof beams), furniture, utensils, food (fruit), fodder (fruit, leaves), shade, live fence, fencing material/dry branches.

Fruits are round, 1–2 cm, woolly at first, ripening yellow to red, with edible flesh covering one hard stone that contains 2–3 seeds. The propagation is seedlings and cuttings. About 1,000–2,000 seed per kg will be counted. Remove the flesh, clean and dry for better storage. To treatment the seed the hard woody stones should be cracked with a hammer and soaked in warm but not hot water overnight. It develops an extremely deep taproot system. It can make an impenetrable thicket. The wood makes excellent firewood and charcoal. It coppices very well (Amina, 2007).

## **2.2. Mode of consumption**

Fruits of *Z. spina-christi* are used as food especially for people in northern, central and eastern Ethiopia, western and central Sudan, and other Saharan regions. Fruits are collected by women herdsman and children to sell on local markets. This provides an additional source of income for local people (Amina, 2007). They may use the income to buy important non-food items. Similarly, in Oman fruits are collected from wild and cultivated plants and sold on local markets (Gebauer *et al.*, 2007).

Fruits are consumed either fresh or dried and the sweet pulp of fruit is dried to produce fine flour. The flour is placed in small metal cups and cooked under steam. This process solidifies the flour to take the shape of the container. The dried pulp flour and water are also mixed with sesame and formed into small balls. The fruit pulp prepared in these two ways can be consumed either immediately or stored for future use. In Oman fruits including kernels are ground to produce an edible mealy substance which is either eaten raw or cooked in water, milk or butter milk (Miller and Morris, 1988).



Figure 2.5 **a**, Tress of *Ziziphus spina-christi* (L) Desf. **b**, Fruits of *Ziziphus spina-christi* (L) Desf. **c**, Sun dried fruits of *Ziziphus spina-christi* (L) Desf. **d**, Seeds isolated from fruits of *Ziziphus spina-christi* (L) Desf., fruits **e**, Leaves, spines, seeds and fruits of *Z. spina-christi* (L) Desf.

Fruit of *Balanites aegyptiaca* is consumed as raw, mixed with porridge, as juice by soaking the fruit in water. The oil from fruit seed is consumed with other staple foods which can increase milk of breastfeeding mothers. The fruits also fermented for alcoholic beverages.



Figure 2.6 Tree of *Balanites aegyptiaca*

## **2.3. Phytochemicals**

According to the WHO a medicinal plant is any plant which contains substances that can be used for therapeutic purposes, in one or more of its organs, and used as a precursor for chemopharmaceutical semi-synthesis. Such a plant will have its parts including fruits, leaves, roots, rhizomes, stems, barks, flowers, grains or seeds, employed in the control or treatment of a disease condition. Therefore they contain chemical components that are medically active. The composition may be Non-nutrient plant chemical compounds or bioactive components (phytochemicals or phyto-constituents). They are responsible for protecting the plant against microbial infections or infestations by pests (Liu, 2004; Nweze *et al.*, 2004; Doughari *et al.*, 2009).

The science of application of these indigenous or local medicinal cures including plants for treatment of diseases is currently called ethno pharmacology. Ethno pharmacology has been the mainstay of traditional medicines the entire world and currently is being integrated into mainstream medicine.

Accumulating evidence demonstrating that, absorption of dietary flavonoids in humans and significant contributions of phytochemicals such as flavones to the antioxidant capacity measured in fruits have a vital role in human health. Phytochemicals can be an important source of dietary antioxidants and may be responsible for the health benefits observed with increased consumption of fruits (Prior & Cao, 1999). Studies approved that a diet high in fruits and vegetables lowers risk for developing chronic diseases such as high blood pressure, heart disease, diabetes, cancer and stroke (Boeing *et al.*, 2012).

## **2.4. Some classes of phytochemicals**

### **2.4.1. Polyphenols**

Plant fruits that produce phenolic compounds include benzoic and cinnamic acid derivatives (phenolic acid), simple phenol, flavonoids, coumarins, stilbens, hydrolysable and condensed tannins, lignans and lignins, but the most important plant fruit secondary metabolites are phenols and flavonoids. They have distinctive biological activity as natural antibacterial more than synthetics ones. Generally, they are used as phytochemicals as well as phytoalexins, attractants

for pollinators, antioxidants and proactive agents against UV light (Gottlieb *et al.*, 2000; Hartmann, 2007; Jenke *et al.*, 2008).

Because of their perceived health-beneficial effects, polyphenols have become an intense focus of research interest. They occur in a variety of fruits, vegetables, nuts, seeds, flowers, bark, beverages, and in some manufactured food, as a component of the natural ingredients used. They have been reported to exhibit anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, anti-microbial, vasodilatory and analgesic effects (Wollgast and Anklam 2000; Abu-Bakar *et al.*, 2009, 2010). Interest in the research of polyphenols from different natural sources has grown because polyphenols can be utilized as antioxidants in the food industry. The beneficial effects of polyphenols on human health could be due to their free radical scavenger properties, which can block the harmful action of these molecules on cells (Akindahunsi and Salawu, 2005; Bobo-García *et al.*, 2015).

Several methods exist for the determination of phenolic compounds in foods. The most common methods include measurement of total polyphenols by Folin Ciocalteu Reagent (FCR) (Singleton and Rossi, 1965) and Prussian blue (Price *et al.*, 1978). The principle of the two methods relies on the reducing power of phenolic hydroxyl groups. The limitation of this method is interference of easily oxidizable non-phenolic compounds such as ascorbic acid. The other method is functional group methods which include the vanillin and HCl methods which allow the measurement of all flavonoids (Price *et al.*, 1978) and condensed tannins (Porter *et al.*, 1985) respectively.

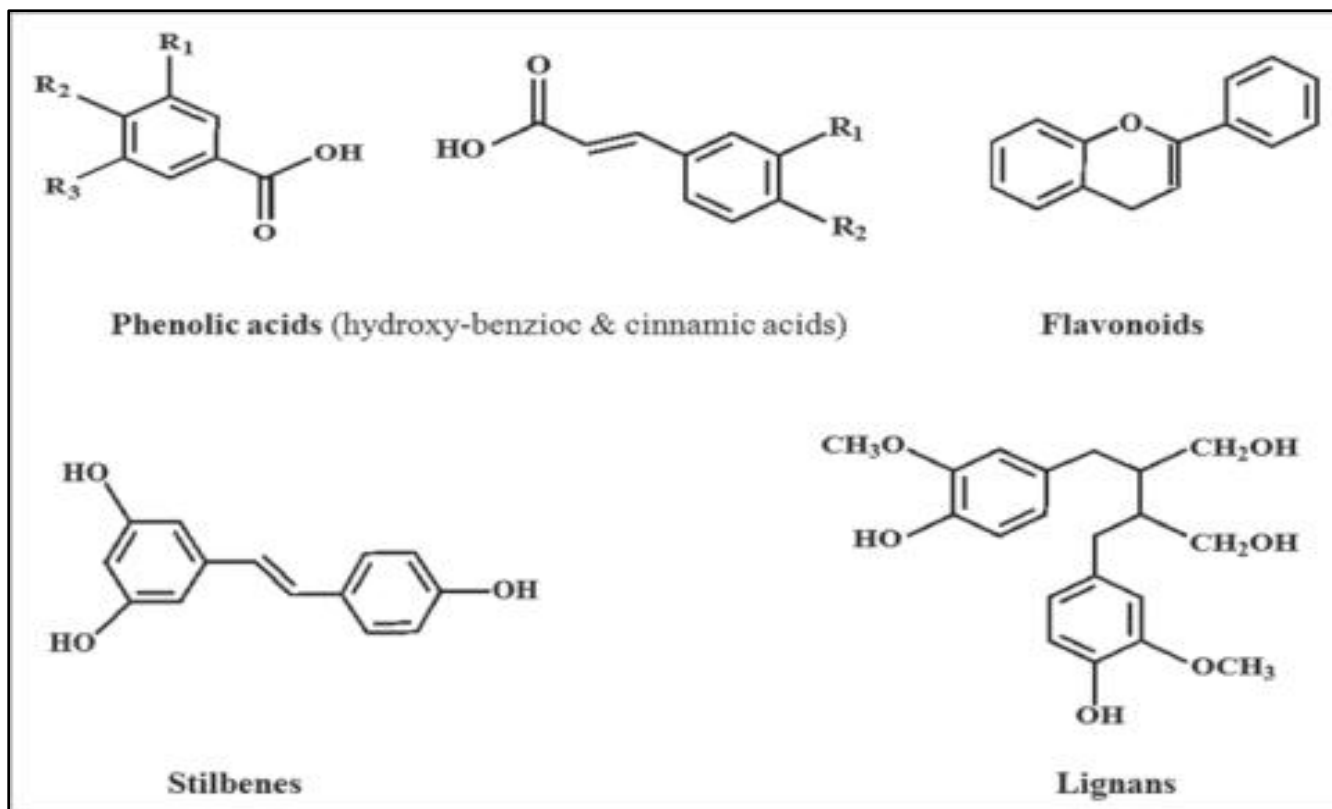
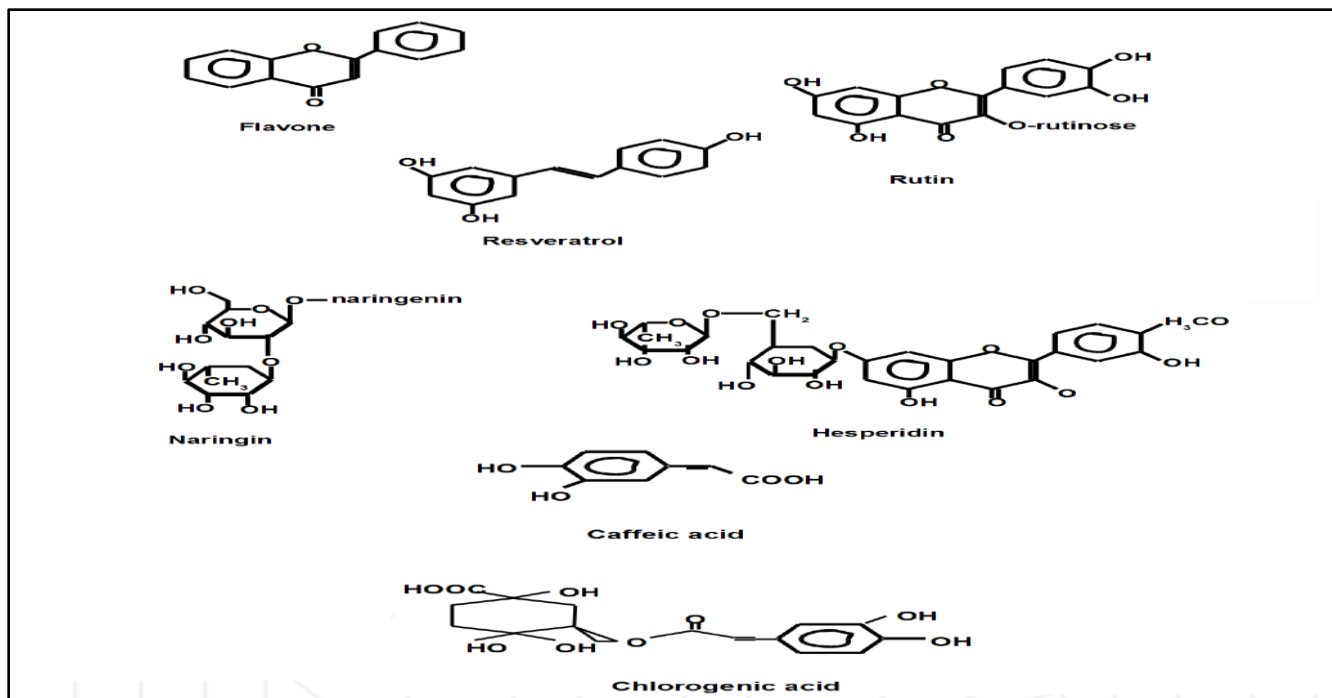


Figure 2.7 Chemical structures of the different classes of polyphenols (intechopen.com).

Polyphenols are classified on the basis of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. They are broadly divided into four classes; Phenolic acids, flavonoids, stilbenes and lignans. Phenolic acids are further divided into hydroxyl benzoic and hydroxyl cinnamic acids. Phenolic acids account for about a third of the polyphenol compounds in our diet and are found in all plant materials, but are particularly abundant in acidic-tasting fruits. Caffeic acid, Gallic acid, ferulic acid are some common phenolic acids. Flavonoids are most abundant polyphenols in human diet and share a common basic structure consisting of two aromatic rings, which are bound together by three carbon atoms that form an oxygenated heterocycle. Biogenetically, one ring usually arises from a molecule of resorcinol, and other ring is derived from the shikimate (C<sub>7</sub>H<sub>10</sub>O<sub>5</sub>) pathway. Stilbenes contain two phenyl moieties connected by a two carbon methylene bridge. Most stilbenes in plants act as antifungal phytoalexins, compounds that are synthesized only in response to infection or injury. The most extensively studied stilbene is resveratrol. Lignans are diphenolic compounds that contain a 2, 3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues.

#### **2.4.2. Flavonoids**

One hypothesis that has been advanced is that the protection against diseases, such as cancer and cardiovascular diseases can be attributed to a large class of antioxidant phytochemicals, termed flavonoids, mostly found in fruits and vegetables. The phytochemicals considered for the purposes of this review will, thus, refer primarily to the flavonoids found in fruits. Flavonoids are diphenylpropanes that commonly occur in plants. More than 4000 flavonoids have been identified. The common family members of flavonoids are flavones, iso-flavones, flavanones, anthocyanins, flavans, and proanthocyanidins. Flavonoids have long been recognized to possess anti-allergic, anti-inflammatory, anti-viral, anti-proliferative, and anti-carcinogenic activities. Flavonoids have been summarized in several reviews (Tiwari and Husain, 2017). There is no more evidence about possible synergistic or additive effects of flavonoids and the nutrient antioxidants, such as vitamins C or E. The view that flavonoids are irrelevant to human health or disease may need to be modified in view of the potentially health-promoting activities of the flavonoids that have recently been reported in experimental (Kumar *et al.*, 2014) and epidemiological studies.

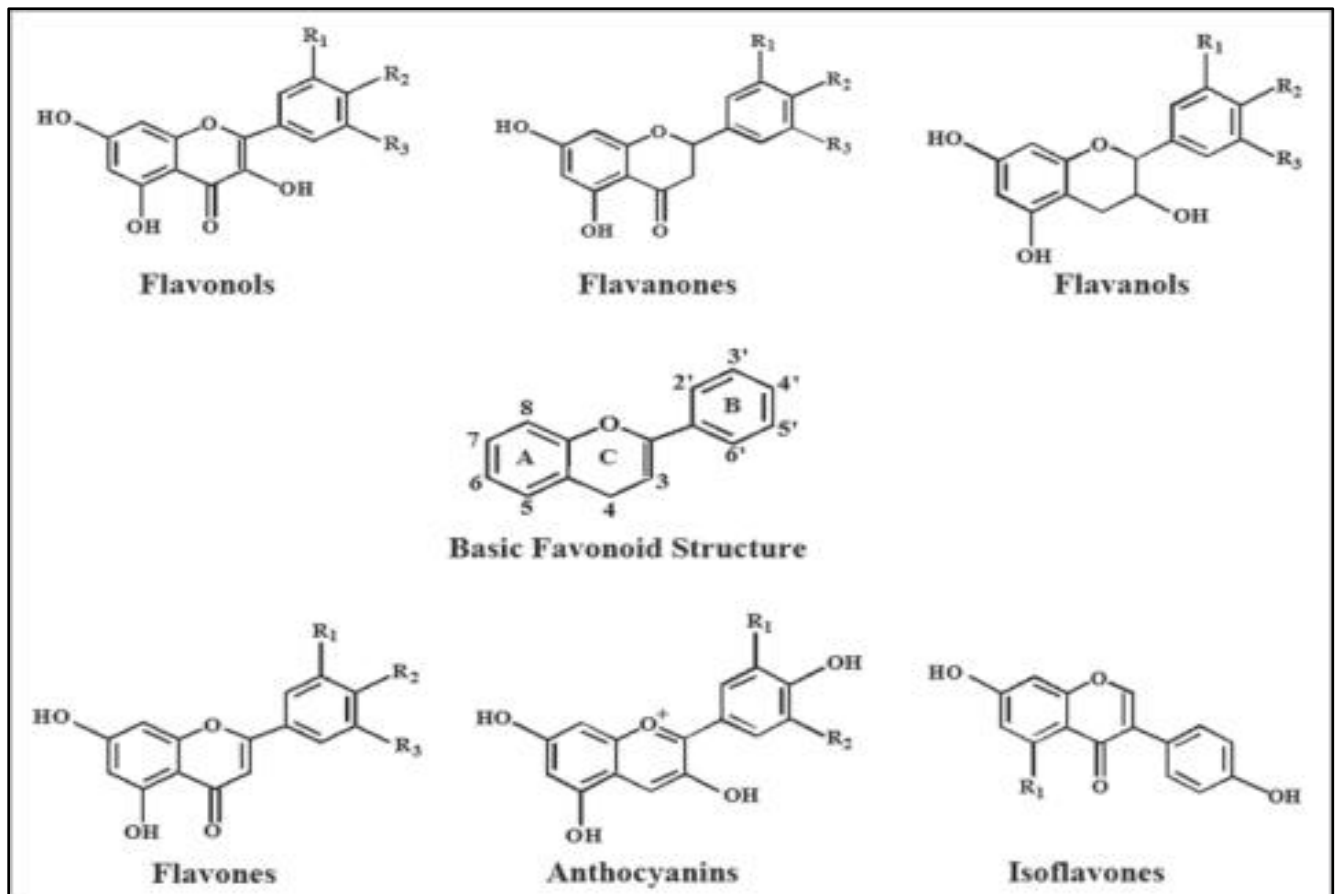
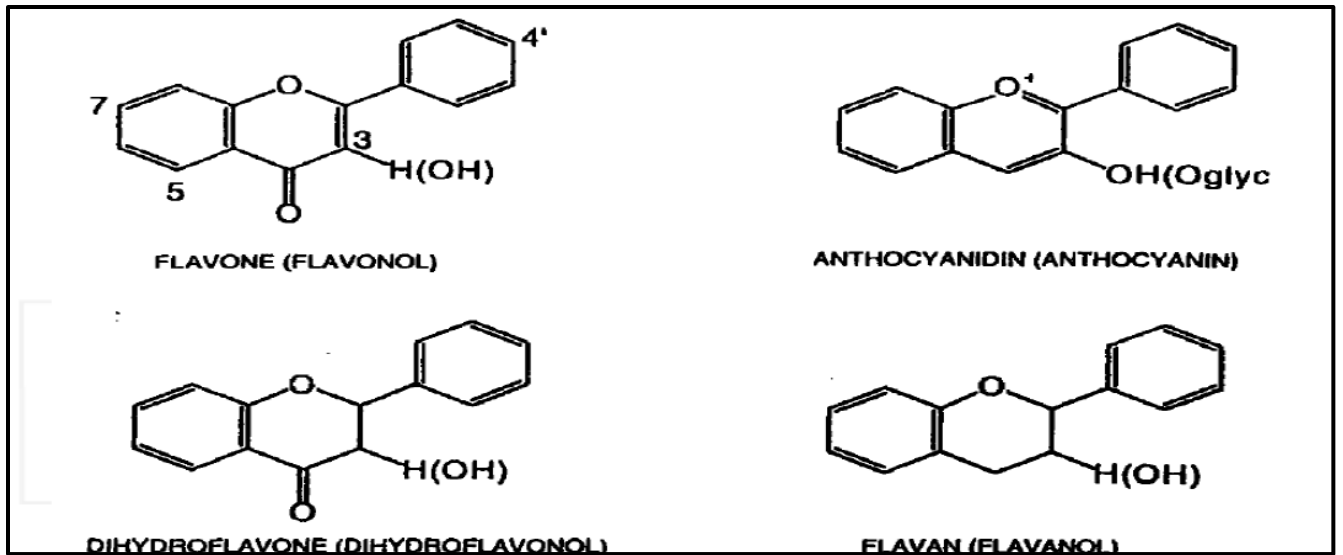


Figure 2.8 Chemical structures of sub-classes of flavonoids (intechopen.com).

Based on the variation in the type of heterocycle involved, flavonoids are divided into six major subclasses: flavonols, flavanones, flavanols, flavones, anthocyanins and isoflavones. Individual differences within each group arise from the variation in number and arrangement of the hydroxyl groups and their extent of alkylation and/or glycosylation. Flavonols (such as quercetin and kaempferol), have a 3-hydroxy pyran-4-one group on the C ring. Flavanones (such as naringenin and taxifolin), have an unsaturated carbon-carbon bond in the C ring. Flavanols (such as the catechins), lack both a 3-hydroxyl group and the 4-one structure in the C ring. Flavones (such as luteolin), lack a hydroxyl group in the 3-position on the C ring. Anthocyanins (such as cyanidin), are characterized by the presence of an oxonium ion on the C ring and are highly coloured as a consequence and in isoflavones (such as genistein), the B ring is attached to the C ring in the 3-position, rather than the 2-position as is the case with the other flavonoids.

## **2.5. Mechanism of action of phytochemical**

There are different mechanisms of action of phytochemicals that have been suggested from different scholars. They may inhibit microorganisms, interfere with some metabolic processes or may modify gene expression and signal transduction pathways (Kris-Etherton *et al.*, 2002; Egglar and Savinov, 2013; Surh, 2003). Phytochemicals may either be used as chemotherapeutic or chemo preventive agents with chemoprevention referring to the use of agents to inhibit, reverse, or retard tumor formation.

This indicates that chemo-preventive phytochemicals are applicable to cancer therapy. Since molecular mechanisms may be common to both chemoprevention and cancer therapy (D'Incalci *et al.*, 2005; Sarkar and Li, 2006). Plant extracts and essential oils may exhibit different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. To sum up the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents (Kang, 2011). Some specific modes of actions are antioxidant, anti-carcinogenesis, antimicrobial, anti-ulcer, anti-diabetic and anti-inflammatory.

### **2.5.1. Free radicals**

Free radicals are reactive chemical (oxygen) species having a single unpaired electron in an outer orbit. Which leads to unstable configuration can creates energy which is released through reactions with adjacent molecules, like proteins, lipids, carbohydrates, and nucleic acids (Valko *et al.*, 2006).

Oxygen –free radicals are major of free radicals that damage biological systems. They are more generally regarded as reactive oxygen species (ROS), which is by products formed in the cell of aerobic organisms, and can initiate autocatalytic reactions. So that molecules to which they react are themselves converted in to free radicals to propagate the chain of damage.

ROS are particularly active in the brain and neuronal tissue as the excitatory amino acids and neurotransmitters, whose metabolism is factory of ROS, which are unique to the brain and serve as sources of oxidative stress. ROS attack glial cells and neurons, which are post-mitotic cells and therefore, they are particularly sensitive to free radicals, leading to neuronal damage (Bahorun *et al.*, 2006).

Generally oxidative stress, can decrease sperm motility and reduce ability of sperm to fuse with an egg in men where us harm egg and follicular development, can interfere with corpus lutetium function and interfere with implementation of the egg.

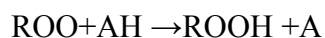
### **2.5.2. Free radicals and theory of aging**

Oxygen can be reduced by less than four electrons during normal metabolism to yield partially reduced reactive oxygen metabolites. Many of these reactive species (RS) are free radicals, i.e., they contain an odd number of electrons like of oxygen-derived free radicals include superoxide hydroperoxyl (HOO•), peroxy (ROO•), ( $O_2 -\bullet$ ), hydroxyl (OH•), and alkoxy (RO•) radicals. ROS that contain an even number of electrons, and thus are not free radicals, include hydrogen peroxide ( $H_2O_2$ ) and lipid hydroperoxide (ROOH). Other common RS produced in the body include nitric oxide (NO•) and peroxynitrite anion (ONOO•). Reactive species are able to initiate lipid peroxidation, a chain reaction, and oxidize other cellular components, such as DNA and proteins (Halliwell, 1997). To deal with RS, the body is equipped with an effective defense system, which includes: enzymes such as superoxide dismutase (SOD), catalase (CAT),

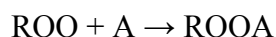
glutathione peroxidase (GP), and glutathione reductase (GR); high molecular-weight antioxidants such as albumin, ceruloplasmin, and ferritin. Low-molecular-weight antioxidants such as ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, glutathione, and uric acid are common. Oxidative stress is a state of imbalance between RS and antioxidants in favor of the former. The free radical or oxidative stress theory of aging states that, oxygen-derived free radicals or oxidative stress is the underlying cause of aging and age-related diseases such as cancer, cardiovascular disease, diabetes and neurodegenerative diseases (Yu, 1996).

### 2.5.3. Antioxidant activities

Chronic diseases are rapidly increasing worldwide and can cause economical, psychological and physical burden on an entire world. Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course. Physiological and biochemical alterations in the human body may result in overproduction of free radicals leading to oxidative damage to biomolecules (lipids, proteins, DNA). Use of medicinal plant based products (fruits) has increased recently because of their exerted beneficial properties such as antioxidant, anticancer, hypoglycemic and hypolipidemic activities (Jayathilake *et al.*, 2016). The primary antioxidants (AH) react with lipid peroxy radicals (ROO•) and convert them to more stable, antioxidant radicals (A•). The primary antioxidant is able to scavenge lipid radicals, e.g.:



Antioxidant radicals are stable due to delocalization of the unpaired electron around a phenol ring and cannot easily react with fatty acids. They are able to terminate radical chain process by reacting with radicals (Reische *et al.*, 2002). These free radical interceptors react with peroxy radicals (ROO•) to stop chain propagation; thus they inhibit the formation of peroxides.



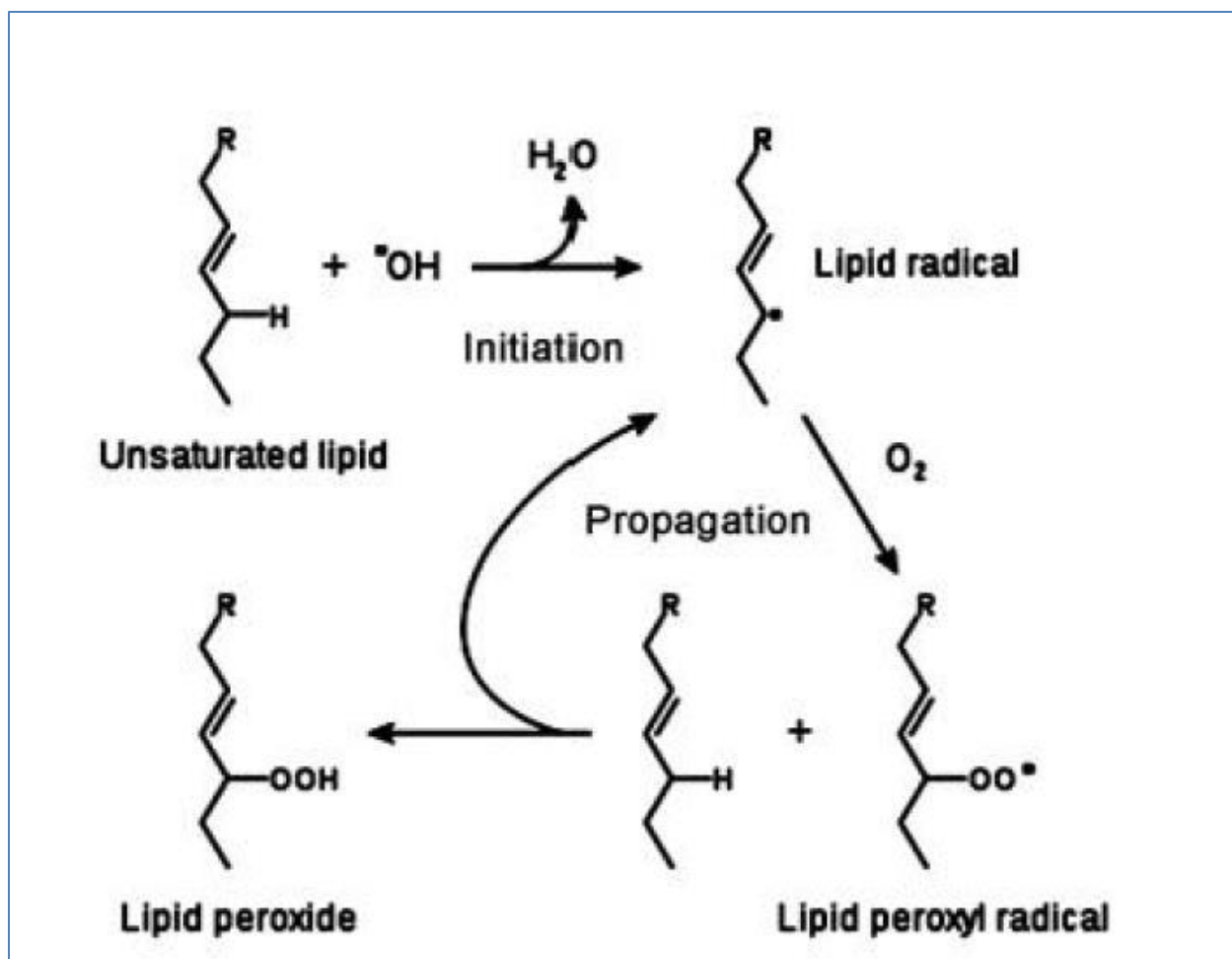


Figure 2.9 Chemical reaction of free radical formation (Gad and Sayd, 2015).

The most effective antioxidants interrupt the free radical chain reaction and usually contain aromatic rings capable of donating  $\text{H}\cdot$  to the free radical formed during lipid oxidation. The formed antioxidant radical is stabilized by delocalization of the unpaired electron around the phenol ring to form a stable resonance hybrid.

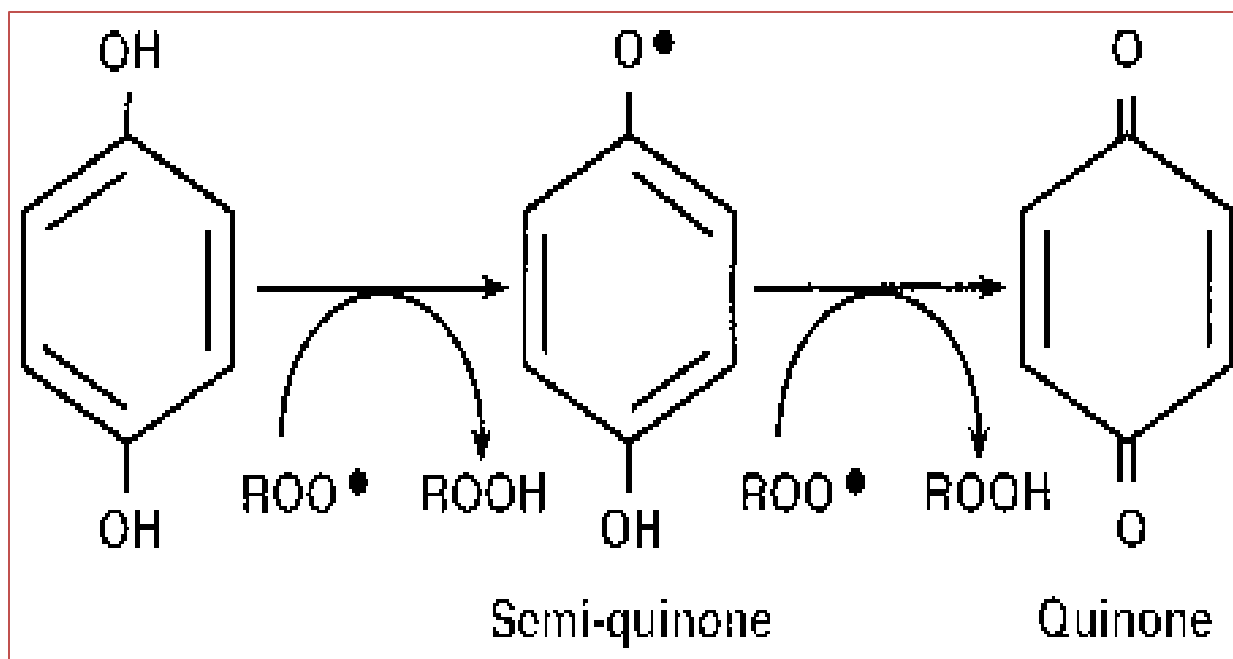


Figure 2.10 Two-step hydrogen donations by a phenolic antioxidant to lipid radicals (PNG image).

## 2.6. Anti-nutritional factors of wild edible fruits

Anti-nutritional factors are substances that can affect the availability of nutrients required by the human as well as animal body. The anti-nutritional factors interfere with metabolic process so that growth and bioavailability of nutrients are negatively influenced (Binita and Khetarpaul, 1997; Umaru *et al.*, 2007; Sisay 2017 unpublished).

## **2.7. Fruit leather**

Fruit leathers are dried sheets of fruit pulp which have a soft, rubbery texture and a sweet taste. They can be made from most fruits, although mango, apricot, banana and tamarind leathers are amongst the most popular. Leathers can also be made from a mixture of fruits. It is easy to eat and convenient to pack (Raab, 1999). Fruit leathers are eaten as snack foods. They are also used as ingredients in the manufacture of cookies, cakes and ice cream. The preservation of fruit leathers depends on their low moisture content (15-25%), the natural acidity of the fruit and the high sugar content. When properly dried and packaged, fruit leathers have a shelf life of up to 9 months (FAO).

## **2.8. Food drying method and dehydration**

One of the oldest methods to preserve food is drying. Drying involves heat and mass transfer that may cause changes in product quantity and quality. Among of them physical changes includes shrinkage, puffing and crystallization. These may cause desirable and undesirable biochemical, physical and chemical changes or reactions which leads in color, texture, taste, odor and other properties (Maskan, 2001).

Among of food drying technique dehydration is common. This method is dipping perishable fruit slice or juice in sugar solution prior to hot-air drying. This method is preferable for most perishable fruits that is wasted during post-harvest process and for those of underutilized wild edible fruits of less palatable but more accessible (Cheman and Taufic, 1995; Charles, 2015).

## **2.9. Consumer preference acceptance sensory evaluation test**

Functional food and nutrition-modified (health enhancing) foods have grown rapidly in the last decades. Due to socio-economic changes, like increase in life expectancy, the need of high health care costs and better quality of life, create more demand of the consumer for these products (Valls *et al.*, 2013). Consumers have their own characteristics which depend on age, gender, diet-health awareness, life style and psychological factors. For this matter sensory evaluation is must after new product development to determine which mixing ration or formulation is accepted based on 9-consumer hedonic scale.

## 2.10. Color of food products

There are three main color spaces used to define color: the red, green, and blue (RGB) color space, the cyan, magenta, yellow, black (CMYK) color space, and the  $L^*a^*b^*$  color space. Among them, the  $L^*a^*b^*$  model has the largest range encompassing all colors in the RGB (Adobe Systems, 2002). RGB color space is composed of three colorimetric components namely, Red, Green, and Blue, each of which varies in the range 0-255. Every pixel in RGB images has certain values of red, green, and blue components.

The  $L^*a^*b^*$  color space is an international standard for color measurement developed by the Commission Internationale d'Eclairage (CIE) in 1976. The  $L^*a^*b^*$  color consists of a luminance or lightness component ( $L^*$  value, ranging from 0 to 100), along with two chromatic components (ranging from -120 to +120):  $a^*$  component (from green to red) and the  $b^*$  component (from blue to yellow) as shown in Figure 2.24. The  $L^*a^*b^*$  color is device independent, providing consistent color regardless of the input or output device such as digital camera, scanner, monitor, and printer. The  $L^*a^*b^*$  values are often used in food research studies.

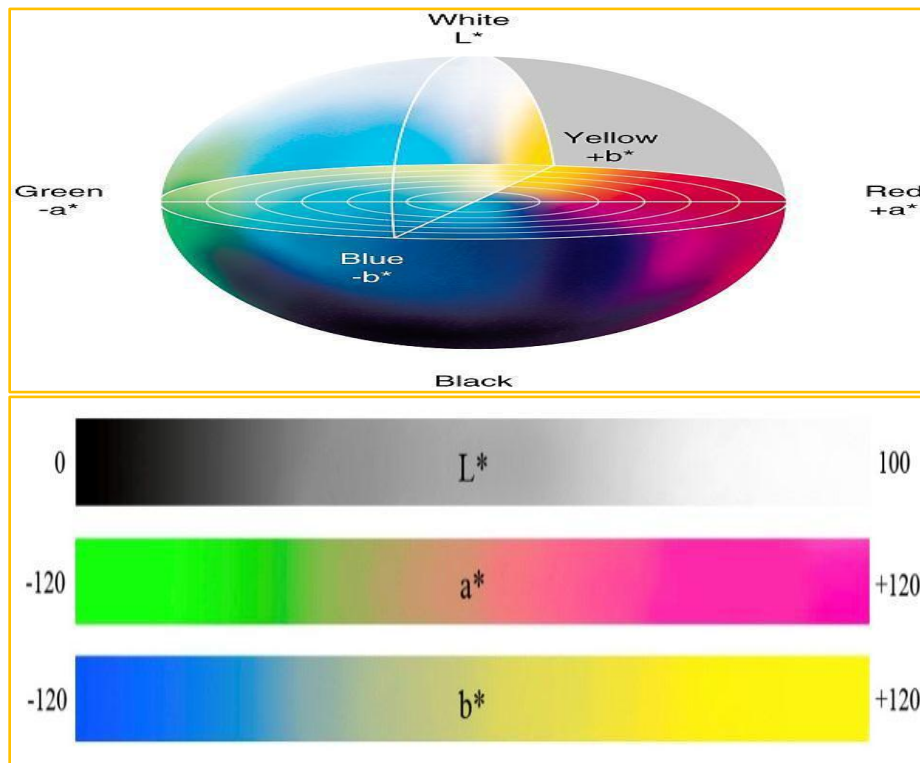


Figure 2.11 Laboratory Space Color

## CHAPTER THREE

### 3. MATERIAL AND METHODS

#### 3.1. Materials

Aluminum foil, poly ethylene bag, baking papers, fruit juice and sample containers are purchased from Shoa supermarket and local juice houses. Reagents such as NaOH (99%), HCl (37%), H<sub>2</sub>SO<sub>4</sub> (98%), Ferric chloride hexahydrate (99%), AlCl<sub>3</sub> (99%), methanol (99.5%), ethanol absolute (99.8%), KMnO<sub>4</sub> (99.5%), Vanillin (≥99%), Boric acid (99.5%), KI (98.5%), are products of Central Drug House (CDH), Labo-Chemie, Sigma Aldrich and Uni-Chem and analytical grade whereas, standards such as D-Catechin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Quercetin and Phytic acid sodium salt hydrate are purchased from Sigma Aldrich Switzerland, which is HPLC grade solid standards. Test tubes, blenders, different laboratory equipment are purchased from Wise team, Google and Neway chemicals and laboratory equipment suppliers. Sample preparation was done at Addis Ababa University College of Natural and Computational Sciences, Center for Food Science and Nutrition Laboratory. The proximate composition, anti-nutritional factors analysis, antioxidant activities and phytochemicals were carried out at the center using Kjeldhal, Muffle furnace, spectrophotometer (Lambda 950, UK) and drying oven (OV/125/SS/F/DIG/A, Genlab Limited) whereas minerals were analyzed in Arba Minch University Department of Chemistry laboratory using AAS (PerkinElmer AA 800, Canada) and fiber and vitamin C were analyzed at the Ethiopian Public Health Institute (EPHI).

#### 3.2. Sampling site

Samples are collected from where they are available as they are seasonal fruits. *Balanites aegyptica* (L) Del., *Mimusops kummel* Bruce A.DC. and *Z. spina-christi* (L) Desf. Were collected from North Shewa Ethiopia, Dera wereda district whereas *Ficus mucuso* Welw .ex Ficalho was collected from Debre Birhan area. *Dovyalis abyssinica* (A.Rich.) Warb., was collected from East shewa-Debre zeith town. These fruits were identified by experts of Taxonomy and Plant Biology of Addis Ababa University and confirmed by Ethiopia National Herbarium Department (ENH).

### **3.3. Sample collection**

The 5 fruit samples were collected from North and East Shewa in different zones. 2 kg of mature or well ripped fruit samples were purchased from different sellers in local market (*Balanites aegyptica*, *Mimusops kummel* and *Ziziphus spina-christi*) from north Shoa Ethiopia specifically from Dera Wereda (Latitude: 10<sup>0</sup>09'60.00"N and Longitude: 38<sup>0</sup>39'59.99"E). 5 kg of *Dovyalis abyssinica* (A.Rich) Warb., fruit was collected from shrubs found in East Shewa Ethiopia Debre Zeith area (Lat: 8<sup>0</sup>45'8.75" and Log: 38<sup>0</sup>59'40") and 2.5 kg of *Ficus mucoso* Welw.ex Ficalho fruit from Debre Brihan from trees (Lat: 9<sup>0</sup>40'19"N and Log: 39<sup>0</sup>32'2"E) (CSA, 2007). All samples are altitudinal and ripe on its own trees or shrubs. After collecting the sample it was packed with poly ethylene bag. Then it was transported to laboratory using public transport to the research center and put it inside the refrigerator below 8 °C.

#### **3.3.1. Sample preparation for analysis**

The samples collected from different locations and that was stored in refrigerator are inspected visually, sorted manually and washed using potable water before further processing. Then fruits were subjected to sun light drying. Then edible part of the fruit was separated from seeds with pestle and mortar and manually. After this each sample was dried in oven for reduction of moisture and to make it is easy for universal disintegrator (Model; FW100, China). Then the samples were grounded to powder. The composite was sieved by using 0.425 mm sized sieve and packed in Poly Ethylene (PE) bags. Then the samples were placed at ambient temperature for analysis at research center inside sample storage.

### **3.4. Product Development of Fruit Leather**

#### **3.4.1. Materials**

Locally grown ripe Koshim *Dovyalis abyssinica* fruit was collected from Debrezeith town whereas *Carica Papaya* was purchased from fruit shop Addis Ababa. Prior to processing, fruits were stored at ambient temperature.

### 3.5. Determination of proximate composition

Nutrient contents were analyzed on dry matter basis including moisture, carbohydrate, ash, crude fat, crude fiber and crude protein using the recommended methods of the Association of Officials Analytical Chemists.

#### 3.5.1. Moisture content

Moisture content was determined according to AOAC, (2007). Briefly the moisture dish was dried at 130 °C for one hour and placed in a desiccator for 20 min and weighted (M1). Then 5 gram of well milled and homogenized samples were transferred in the moisture dishes (M2) and was dried in an oven at 105 °C for 3 hours and until constant weight was obtained (M3). The measurement was made in triplicates. The percentage weight loss was determined as follows:

$$\text{Moisture content (dmb \%)} = \left( \frac{M1-M3}{M2-M1} \right) * 100 \dots\dots\dots\text{eq (1)}$$

Dry matter of samples was obtained by subtracting moisture content (%) from 100.

$$\text{Moisture content (wb \%)} = \frac{((Md/100+Md) x 100)}{1} \dots\dots\dots\text{eq (2)}$$

Where: dmb - dry matter basis

Wb – wait basis

Md – Moisture content in dry matter basis

#### 3.5.2. Crude protein

Crude protein content of each fruit sample will be determined by Kjeldahl method. The steps are sample digestion, neutralization, distillation and trapping of ammonia and titration with standard sulfuric acid.

Crude protein content of samples was determined according to the AOAC standard method 979.09 (2000). Briefly, homogeneously powdered 500 mg will be placed in Kjeldahl flask in duplicate. Then to the flask 6 mL of Concentrated sulfuric acid was added. After an hour 3.5 mL of H<sub>2</sub>O<sub>2</sub> was added. The 3 g of catalyst (potassium sulfate: copper sulfate, 1:15) was added and

heated at 370 °C using Kjeldhal digester until the mixture turns light clear. The mixture was distilled using distillation apparatus. So the auto distiller adjusted to add 30 mL distilled water and 40 ml of NaOH (35%). After collection of distillate for 8 min in 250 mL Erlenmeyer flask that contain 25 mL of boric acid (2%) with 5 drops of methyl red indicator. Then titration was carried out with 0.1 N hydrochloric acids to a reddish color (end point of titration) and determination was carried out as follows.

$$\text{Nitrogen (\%)} = \frac{(\text{Corrected acid volume}) \times (\text{NHCl}) \times (14\text{gN}) \times 100}{\text{gram of sample}} \dots \text{eq (2)}$$

$$\text{Protein (\%)} = 6.25 \times \% \text{ nitrogen} \dots \text{eq (3)}$$

### 3.5.3. Crude fat

Crude fat contents (which include triglycerides, phospholipids, sterols and related compounds) of the samples were extracted by the continuous extraction (Soxhlet) method (AOAC, 2000) with slight modification. Extraction cylinder was washed with hot water to remove any impurities then it was put in to the oven for an hour at a temperature of 110 °C. Then the cylinders were put in to desiccator and weighted them as W1. Put them again in desiccator. An extraction thimble was covered with a layer of fat free cotton. Then 2 g of sample was weighted (W2) in to an extraction thimble and covered with absorbent cotton. Then 50 mL of solvent (petroleum ether boiling point of 40-60 °C) was added to pre weighted round bottom flask and was set to Soxhlet apparatus. The samples were suggested to extraction with solvent for 5 hr. After that the flask was dried in an oven a 110 0C for 1 hour and was cooled to room temperature. Determination was made in duplicates. Crude fat was calculated as follows.

$$\text{Crude fat content (\%)} = \frac{\text{Extracted fat (g)} \times 100}{\text{sample weighed (g)}} \dots \text{eq (4)}$$

### 3.5.4.Total ash content

Ash content of each samples were analyzed according to AOAC method (2000). A Porcelain crucible was cleaned and dried in a muffle furnace for 30 min at 550°C. Then crucible was cool in a desiccator (with granular silica gel) for about 30 minutes or more at room temperature and weighed (M1). 2.5g of dry powder of *Balanities egyptica* (L) Del, *Dovyalis abyssinica* (A.Rich) warp, *Ficus mucuso* Welw.ex Ficalho, *Mimusops Kummel* Bruce A.DC. and *Ziziphus*

*spina christi* (L) Desf samples were weighted (M2) to an accuracy of 4 decimal places in the dish. Then samples were charred on a hot plate under a fume –hood by increasing the temperature slowly until smiking ceases. Then each samples were ashed in muffle furnace at 550<sup>0</sup>C for 5 hour. Then it was removed from furnace and cooled to room temperature and weighted again(M3). The ash conent will be determined using he following equation

$$\text{Total Ash(\%)} = (M3-M1/M2-M1) \times 100 \dots \text{eq (5)}$$

### 3.5.5. Crude fiber

Crude fiber analysis was conducted using the method of AOAC(2000). About 1.6 g weighted sample was transferred in to a 600 ml beaker and about 200 ml 1.5 % sulfuric acid was added and boiled fro 30 min. Recording took place by placing a watch glass over the mouth of the beaker. After 30 min heating by gently keeping the level constant with distilled water, 20 ml Of 28% KOH was added and boiled gently again for another 30 min. Subsequently, washing was conducted with 1 % sulfuric acid and KOH solution. After, filtering it was then dried in an electric oven for 2 hours. Further more, it was cooled at room temperature for 30 min in a desicators and weighted, then trasferred the crucibles to muffle furnace for 30 min ashing at 550<sup>0</sup>C. Finally, it was cooled again in desicators and reweighted. The crude fiber content was determined by using the formula:-

$$\% \text{ Crude fiber} = (w2 - w3/w1) \times 100 \dots \text{eq (6)}$$

Where W1 = crucible weight after drying

W2 = Crucible weight after ash

W3 = Dry weight

### 3.5.6. Utilizable carbohydrate determination

The utilizable carbohydrate was conducted by difference with the exclusion of crude fiber.

$$\text{Utilizable carbohydrate (\%)} = 100 - (\text{crude fat} + \text{crude fiber} + \text{crude protein} + \text{ash}) \dots \text{eq (7)}$$

### 3.5.7. Total energy in kilocalories

The gross energy (GE) content in each sample was determined mathematically using the formula  
Gross energy (Kcal) = (9 x crude fat) + (4 x crude protein) + (4 x utilizable carbohydrate).....eq (8)

### 3.6. Minerals analysis

Iron and Zinc standard solution was prepared by dissolving 1.0g of Fe and Zn in 14 mL of deionized water. Then 7 mL of HNO<sub>3</sub> was added to each 1L volumetric flask. Then it was diluted with deionized water to the mark, whereas Ca standard was prepared from 1000ppm calcium (Ca) stock solution.

Minerals content was determined according to the method of Association of Official Analytical Chemists (AOAC, 2000). About 2.5 g of powder sample was charred to remove organic matter. Then ash in muffle furnace at 550 °C for 5 hours for the determination of the total ash content. Three drops of 1M HNO<sub>3</sub> acid and few drops of deionized water added to the sample in each of the crucibles. The ash was digested by using 3N and 6N hydrochloric acid. The digested sample was filtered into the sample bottles each using the Whatman filter paper (42 mm) prior to analysis after filtration made up the volume to 50 mL with deionized water. For calcium determination 2.5 mL of 10 % LaCl<sub>3</sub> was added. The Ca, Fe and Zn content in the sample was determined by Flame Atomic Absorption Spectrophotometer (PerkinElmer AA 800, Canada) using air acetylene flame and calcium cathode lamp at 422.7 nm. Iron content was determined at 248.3 nm using Iron cathode lamp whereas Zinc content was measured at 213.9 nm using Zinc cathode lamp. Using AAS (PerkinElmer AA 800, Canada) calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100 g for all of the above minerals (Appendix 7.1). Then reading was taken from the graph which depicted the metal concentrations that correspond to the absorption or emission value of the sample and the blank. The metal content were calculated by using the formula

$$\text{Metal content (mg/100g)} = \frac{(A-B)}{10W} \times V \dots\dots\dots\text{eq (9)}$$

Where A = Concentration of sample solution in ppm

B = Concentration of blank solution from curve in ppm, W = Weight of sample and

V = Volume of extract

### **3.7. Determination of antioxidant activities**

#### **3.7.1. Sample extraction**

Samples were extracted based on the procedure Siddhuraju and Becker ( 2003). The powdered fruit samples were homogenized and weighted in 5g was by using top load balance which was calibrated early. Then the samples were transferred to 250ml conical flask. After this 25 ml of methanol was added at room temperature. Then it was placed in the incubator shaker (ZHWHY-103B, China) for 24 hours at 150 rpm. Then the sample was filtered by using whatmman filter no. 1 filter paper. Then 25 ml of methanol was added again to the filtered sample and extracted as described above. Then these sample and methanol mixture was evaporated at 40 °C by using rotary evaporator and redissolved again with methano solution in concentration of 50mg/ml.

#### **3.7.2. Free radical scavenging activity by DPPH**

DPPH scavenging activity of the fruits methanolic extrac was measured according to the method of Latta and Eskin, (1980). IC50 values of the extracts and concentration of the extracts necessary to decrease the iniatial concentration of DPPH by 50% were calculated. The hydrogen atom or electrons donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple colored methanol solution of DPPH. The effect of methanolic extracts on DPPH radical were estimated according to Kirby and Schmidt (2004). Briefly, 4ml of 0.004% solution of DPPH radical solution in methanol was mixed with 1 ml of various concentrations (2-12 mg/ml) of the extracts in methanol with a vortex mixer. The samples were incubated for 30 min in the dark at room temperature. Then scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm using a UV-Vis spectrophotometer (PerkinElmer, Lambda 950, UK). Inhibition of free radical DPPH in percent (I%) was calculated in the following way.

$$I \% = \frac{Ac - As}{Ac} \times 100 \dots\dots\dots \text{eq (10)}$$

Where;

- Ac = is the absorbance of the control reaction (containing all reagents except the test compound).
- As = sample is the absorbance of the test compound and
- %I = Percent of inhibition

The concentration of scavenging activities at IC50 was calculated using the %I from the absorbance of control (Quercetin) and absorbance sample solution.

### 3.7.3. Total Polyphenols

Total phenol content was determined by the method described by Singleton and Rossi, (1965) with some modification. Aliquots (75µL) of each fruit extract were taken in to test tube made up to the volume of 1 mL with methanol. Then 1 mL of Folin-Ciocalteu reagent (1:9 with deionized water) were added consecutively in each test tube and blank. After vortexing the reaction it was incubated for 1-8 minutes at room temperature. After 3 min, 1 mL of saturated sodium carbonate solution (20%) was added and the mixture was adjusted to 10 mL with distilled water. Then the reaction was kept for 90 min in dark and then transferred to 1cm cuvette and absorbance was measured at 725 nm UV-VIS spectrophotometer (PerkinElmer, Lambda 950, UK). For determination of total phenol content gallic acid was used to construct the standard curve. The results were mean ± standard error and expressed as mg of gallic acid equivalent per gram of extracts (GAEs/g DW). Molar mass of Gallic acid (C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>), 170.12 g/mol. 0.05g gallic acid was dissolved in 1 mL of methanol and then diluted to 10 mL with water (5g/L) stock. Then 0.1, 0.2, 0.5, 1 mL was transferred and dissolved to 10 mL with water to create standards with 50, 100, 250 and 500 mg/L (ppm) concentrations respectively. The total phenolic content in extract was calculated using the formula:

$$\text{Total phenol content (TPC)} = \frac{GAEC \times V}{W} \left( mg \frac{GAE}{100g} DM \right) \dots\dots\dots \text{eq (11)}$$

Where: GAEC = is concentration of gallic acid equivalent (mg/mL) from curve

V = is total volume of the extract (mL) and

W = is sample weight (g)

### 3.7.4. Total Flavonoids

The flavonoids content was determined by aluminium trichloride method using catechin as reference compound (Zhishen *et al.*, 1999). A volume of 50µL sample extract was mixed with 950µL of methanol and 1ml of (2%) aluminium trichloride. The final volume of the solution was adjusted to 2000 µL with distilled water. After 10 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm UV-VIS spectrophotometer (PerkinElmer, Lambda 950, UK) . The total flavonoids content was expressed as (mg CEs/100g) DM.

## 3.8. Determination of anti-nutritional factors

### 3.8.1. Oxalate

Total oxalate content of the fruits extract was determined by permanganate reduction using 0.1N KMnO<sub>4</sub> solutions (AOAC, 2005) which was modified by Tilahun, (2017); Day and Underwood, (1986). One g of sample was weighed from each sample type and transferred to 100 mL conical flask. 75 mL of 3M of sulfuric acid was added and stirred carefully intermittently with a magnetic stirrer for an hour. The slurry was filtered using whatman No.1 filter paper. 25 mL of sample extract (filtrate) was collected and titration was carried out against with hot (80-90 °C) 0.1N of KMnO<sub>4</sub> solution until a faint pink colour appeared that persisted for at least 30s. The concentration of oxalate in each sample could be obtained from the calculation.

1 mL of 0.1N of permanganate = 0.006303 g of oxalate.....eq (12)

### 3.8.2. Condensed tannins

Tannins were determined using the method (Iqbar, 2011) with slight modification. One gram of each underutilized wild edible fruit powder was weighed in a screw capped test tube and 10 mL of 1% HCl in methanol was added to each test tube containing the samples. Then the tubes were put in to mechanical shaker for 24 hours at room temperature. After 24 hours of shaking, the tubes were centrifuged using (DYNAC II centrifuge, Clay Adams division of Becton and Dickinson Company, USA) for 5 min. One ml of the clear supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared from equal volume of 8% concentrated HCl in methanol and 4% of vanillin in methanol). In other test tube the mixture was allowed to stand for 20 min to

complete the reaction. D-catechin was used as a standard for condensed tannin determination. A 40 mg of D-catechin was weighted and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. About 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of stock solution was taken in test tube and the volume of each test tube was adjusted to 1 ml with 1% HCl in methanol. About 5 mL of vanillin HCl reagent was added in to each test tube. After 20 min the absorbance was measured at 500 nm by UV-VIS spectrophotometer (PerkinElmer, Lambda 950, USA). The concentration of tannin was calculated by using D-Catechin standard curve and results were expressed as of D-Catechin equivalent in mg/100g dry weight bases. The standard curve was shown in appendix 1.

$$\text{Tannin (mg/100g)} = \frac{[(A_s - A_b) - \text{intercept}]}{S * D * W} \dots\dots\dots \text{eq (13)}$$

Where:  $A_s$  = sample absorbance

$A_b$  = blank absorbance

$D$  = density of solvent (0.791g/mL)

$W$  = weighted of sample in gram and  $S$  = slope from calibration curve

### 3.8.3. Phytate

The phytate content was determined according to the method described and latter modified by Latta and Eskin, (1988); Vaintraub and Lapteva, (1988). A series of standard solution was prepared containing 5-40 ppm phytic acid in 0.2N HCl solution. 3 mL of each standard and 3 mL 0.2N HCl (blank) was pipetted to 15 mL of centrifuge tubes. Then 2 mL of the wade reagent was added to each test tube and mixed on vortex mixer (K-GEMMY, MODEL: VM-300P, Taiwan) for 5 seconds. Then the solution was centrifuged at 3000 rpm/10 min and supernatant was collected. Then it was measured at 500 nm by making zero the spectrophotometer using deionized water.

0.5g of dried sample was extracted with 10 mL of 0.2N HCl for an hour at an ambient temperature and centrifuged at 3000 rpm/30 min. The clear supernatant was used for phytate estimation. Then 2 mL of wade reagent (0.03% solution of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  containing 0.3% sulfosalicylic acid in water) was added to 3 mL of supernatant sample solution and homogenized and centrifuged the solution at 3000 rpm/10 minutes. Then the absorbance was measured at 500 nm using UV-Vis spectrophotometer (PerkinElmer, Lambda 950, UK). The phytate concentration was determined from the difference between the absorbance of the blank ( 3 mL of 0.2N HCl and

2 mL of waste reagent) and from assayed sample. Then amount of phytic acid was calculated using phytic acid standard curve and result was expressed as phytic acid in  $\mu\text{g/g}$  of fresh weight.

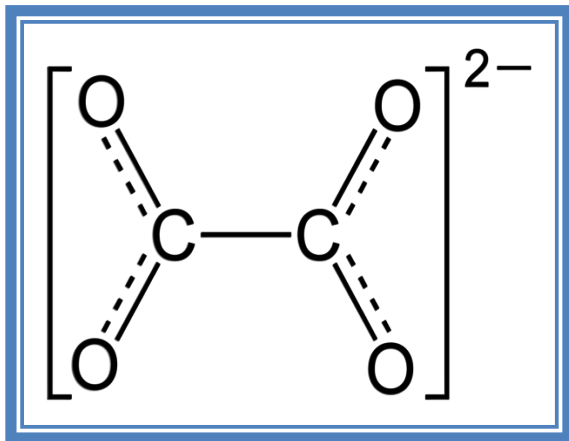
$$\text{Phytic acid in } \mu\text{g}/100\text{g} = \frac{[(Ab-As)-Intercept]*10}{Slope*w*3} \dots\dots\dots\text{eq (14)}$$

Where:  $A_s$  is sample absorbance

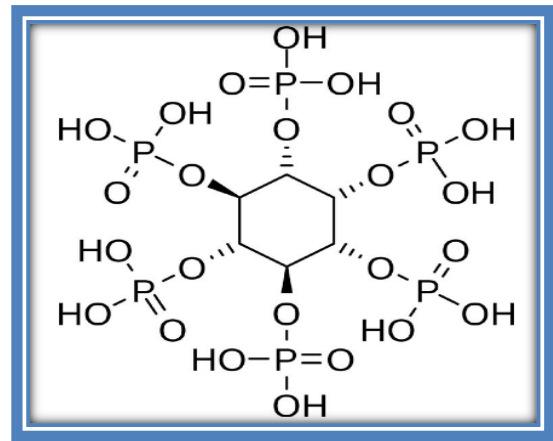
$A_b$  is blank absorbance

$W$  is weight of sample and

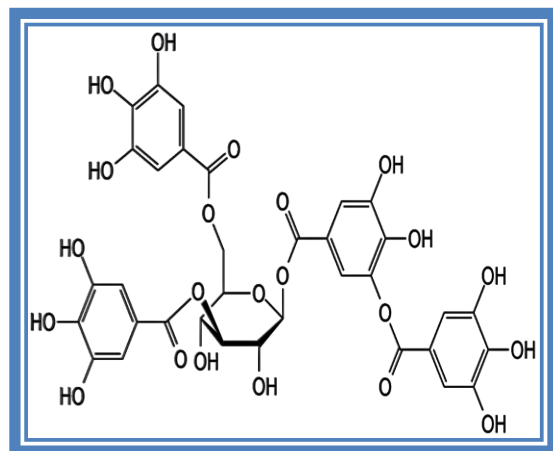
$Int$ - is intercept obtained from calibration curve



Oxalate ion



Six-sided phytic acid molecule with a phosphorus atom in each arm



Tannic acid structure



Tannic acid powder

Figure 3.1 Chemical structure of common antinutritional factors found in the food (PNG Image)

### 3.9. Determination of Vitamic C (Ascorbic Acid) Content by Tiration

Ascorbic acid was estimated following titration method developed by Harris and Ray, (1935). This method is to determine vitamin C concentration in a solution by a redox titration using iodine. As iodine is added during the titration, the ascorbic acid is oxidized to dehydroascorbic acid, while the iodine is reduced to iodine ions.

Ascorbic acid + I<sub>2</sub> → 2 I<sup>-</sup> + dihydroascorbic acid

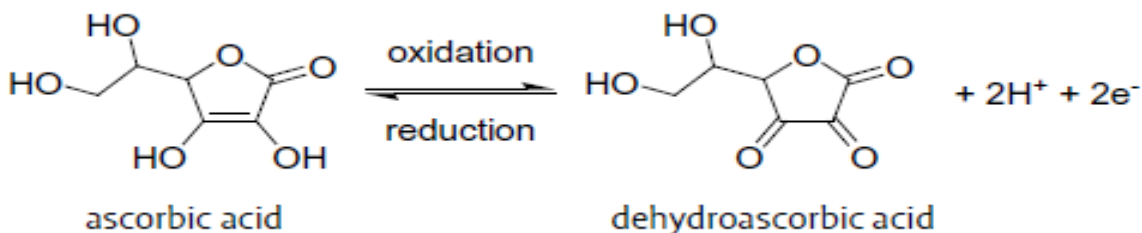
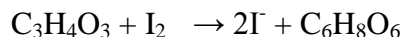


Figure 3.2 Oxidation reduction reaction of ascorbic acid with iodine (outreach.canterbury.ac.nz).

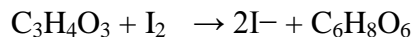
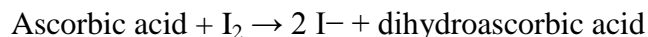
Due to this reaction the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid has been oxidized. The excess iodine is free to react with the starch indicator, forming the blue black starch-iodine complex which is end point of the titration.

**Sample extraction:** Weight 10 g of each sample powder and transfer to 250 mL of beaker. Then add 50 mL of hot distilled water and stir for an hour. After blending, strain the pulp powder through cheesecloth, washing it with a few 10 mL portion of distilled water. Finally make the extract solution up to 100 mL in a volumetric flask.

**Titration:** 20 mL of aliquot of the sample solution was pipetted in to 250 mL conical flask. Then 150 mL of distilled water was added followed by 1 mL of starch indicator solution. Then titration was carried out with 0.005M iodine solution until permanent trace of dark blue-black color developed which is end point of titration.

### Calculation:

First the average volume of iodine solution that was consumed during titration was calculated by taking final volume when end point is observed. Then a mole of iodine and ascorbic acid that was reacted was determined according to the equation given below.



### 3.9.1. Juice Preparation (PA) from *Carica Papaya* (CA) and *Dovyalis abyssinica*

*Dovyalis abyssinica* and *Carica Papaya* (CP) fruits were manually sorted for apparent damage, washed and sanitised. The fruit pulp and seeds were placed on a plastic sieve and pressure was applied with a metal spoon, until juice was completely separated from seeds. The fruit was pulped with a fruit finisher (home blender) using 0.15 cm and 0.08 cm finisher sieves consecutively. To obtain clarified juice, the process and system described by (Vaillant *et al.*, 2005). Extracted juices from *DA* which has an attractive deep yellow color but rather harsh flavor and orange color juice of *CP* were packaged in kettle and followed by hot water pasturization (63 °C for 30 minutes) and stored at 20 °C for 3 days.

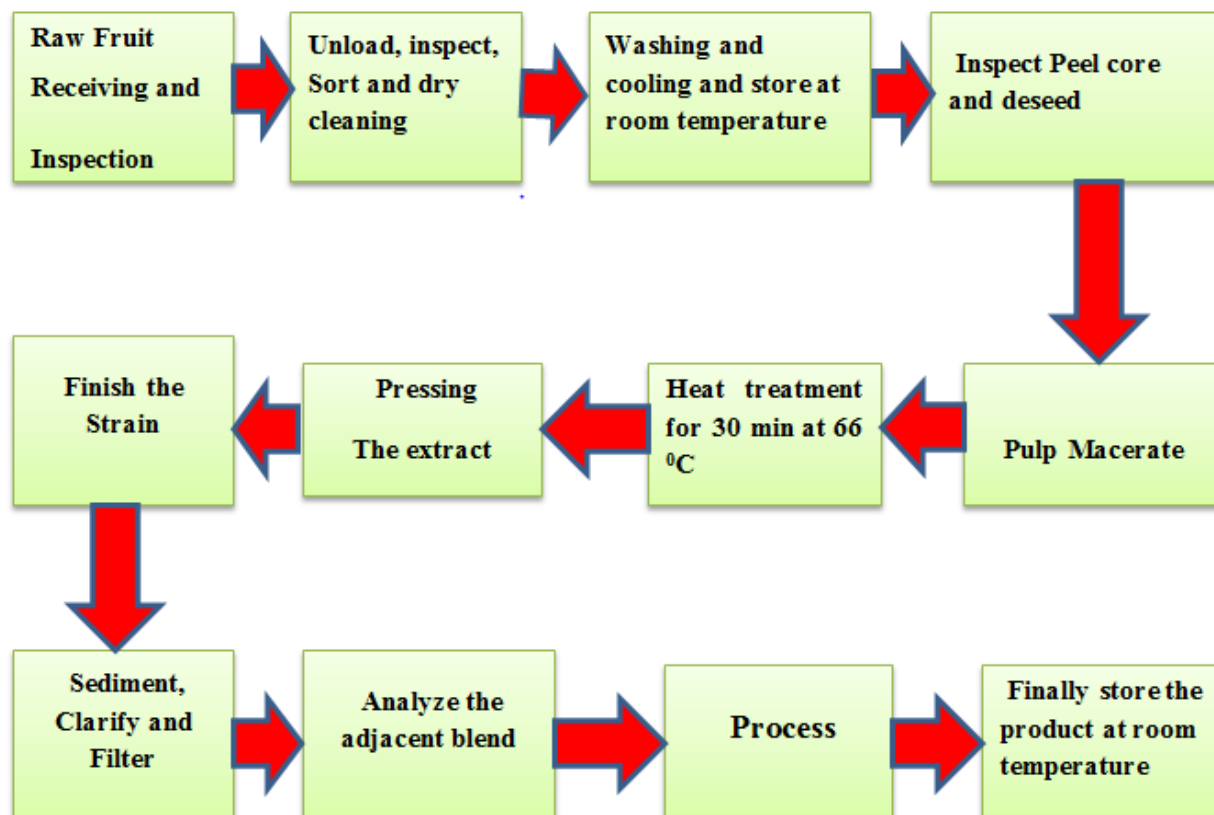


Figure 3.3 Process flow chart for production of safe and healthier fruit pulp juice

### 3.9.2. Determination of Degree Brix, pH and Titrable Acidity (TA) Value

To determine degree brix of the raw *DA* fruit juice, Refractometer (ATAGO PAL-1, Japan) was rinsed with distilled water until the brix of distilled water becomes 0.00 during calibration. The fruit juice was added to the instrument and measured. Degree brix is important to develop products like juice and jam. For this work only *Dovyalis abyssinica* was our focus from 5 selected wild fruits to develop fruit leather product mixing with *Carica Papaya*.

**pH** of a fruit juice was determined by pH meter (Micro 600, Singapor). According to manufacturer instruction 100 mL of fruit juice was transferred to 150 mL of dried beaker. Then 1/3 of pH meter electrode was immersed to the beaker containing juice. Then the reading was taken after the value was constant in three trials.

**Titrateable acidity (TA)** of juice was conducted according to juice manufacturing procedure. 10 mL of raw *Dovyalis abyssinica* fruit juice was measured and transferred to 100 mL erlenmeyer

flask. Then 5 drops of phenolphthalein indicator was added to the beaker. After that it was titrated against with 0.1N of NaOH solution until the deep yellow solution was changed to slightly pink color. The value of TA was calculated as follows.

$$TA = \frac{\Delta V * 0.064}{Sg \text{ of } ^\circ B} \dots\dots\dots \text{eq (15)}$$

Where:  $\Delta V$  is change in titrant (0.1N NaOH) solution.

$Sg$  is specific gravity of juice degree brix and

$^\circ B$  is degree brix of fruit juice

### **3.9.3. Fruit Leather Product Development From *Carica Papaya* and *Dovyalis abyssinica***

Dehydration method is applied with slight modification (Chemmanur and Taufik, 1995). First fruit pulp juice was prepared as described above. After pasteurizing it at 63 °C for 30 min the juice was stored for three days at ambient temperature. Then followed by hot-air drying in oven according to method of Falade and Aworh, (2004; 2005) at 60 °C for 8 hours.

## **3.10. Color Analysis**

### **3.11. Image Capturing**

The 3 fruit leathers were taken and imaged before and after baking. To avoid light reflection in the space and preventing from fluctuation in imaging, dark place was prepared and a leather sample was placed in the dark room for imaging. The images were captured by a digital camera (Y530-U051, China) which was connected to computer via USB port. The camera was fixed parallel to and at a distance of 30 cm from leather samples.

#### **3.11.1. Image Processing**

500\*500 pixel (Area = 250000 pixels) pieces are cut from the captured images and saved under BMP format. The captured image was in RGB color space, we have to convert it to L\*a\*b\* color spaces by using ImageJ1.46r software. By aid of the ImageJ package referred to as “Color-Space-Converter”.

### **3.11.2. Analyzing Color**

The converted L\*a\*b\* images were analyzed using ImageJ 1.4 software and the mean value and standard deviation of color intensity in the image pixels was obtained and saved in Microsoft Excel 2010.

### **3.11.3. Sensory Evaluation**

The final product were evaluated by 15 semi-trained panellists, center for 18 to 40 years of age (staff and students of the Universities of Addis Ababa, Food Science and Nutriion Center, Institute of Technology). The panelists were selected for participation on the basis of their knowladge on sensory analysis. Randomized, stored (25<sup>0</sup>C) samples of product were served in clear, and odorless plate. These were marked with three digit random numbers. Bottled water was provided for rinsing of mouth during the testing. One times used new plasic spitting cup was provided for storage of the wase from their mouth. Evaluations took place in the mornings between 9:00 and 11:00 a.m. and afternoon from 03:00 pm to 05:00 pm were conducted at room temperature (20 <sup>0</sup>C) under fully light sensory evaluation room. The fruit leather were evaluated for appearance (color and texture), taste and overall acceptability according to the 9-hedonic scale (like extremely, like very much, like moderately, like slightly, niether like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely) using procedures of Dias *et al.*, (2007) and Whasley *et al.*, (2010).

### **3.12. Statistical Analysis**

Compleely Randemized Design (CRD) was used for the experitmet. The statistical analysis of the sensory evaluation was conduced by one way of ANOVA using statstical t-test and Friedman's test (P<0.05). The line graph relating to proximate, phytochemical constituents, vitamins and anti-nutritional factors were conducted using SPSS version 20.0. and Minitab V17. Differences between media were determined by using least significance difference (LSD). The color of the fruit leather was done with ImageJ 1.4r version 0.0.0. Microsoft office exel 2010 was used for physico-chemical anaysis.

## CHAPTER FOUR

### 4. RESULTS AND DISCUSSION

In this section proximate composition, phytochemical constituents, antinutritional factors, minerals, vitamins C, Physico-chemical properties of fruits and sensory evaluation of developed products are discussed. The tables and graphs shows the nutritonal quality, antinutritional factors and sensory evaluation of each underutilized wild edible fruits that are analyzed. All analysis were performed in dry matter basis (dmb).

#### 4.1. Proximate composition of underutilized wild edible fruits

From the result obtained the underutilized wild edible fruit species which are more consumed by local communities have appreciable amount of food nutrients and energy which can be used as daily requirement as well as a food supplement. These fruits have an importance for supplement, source of nutrition and vitamins. Maybe local communities that have not access for cultivated fruits ought to for their needs.

##### 4.1.1. Moisture content

Moisture content is amount of water present in a given food sample either as wet basis or as dry matter basis. This parameter has to be determining in a food; because it has great contribution for fruits perishability and shelf life of foods. The moisture content of 5 wild fruits in wait basis is 5.26% for *Balanites aegyptica* (L) Del, 11.11% for *Dovyalis abyssinica* (A.Rich) Warb, 3.00% for *Ficus mucuso* Ficalho, 3.85% for *Mimusops kummel* Bruce A.DC and 5.39% for *Ziziphus spina-christi* (L) Desf. The moisture content of each underutilized wild edible fruits in dry matter basis was ranged from  $3.10 \pm 0.10$  to  $12.50 \pm 0.00\%$  (Table 2). The maximum moisture content was  $12.50 \pm 0.00\%$  for *Dovyalis abyssinica*, whereas the minimum moisture content was observed for *Ficus mucuso* value (3.10%). The moisture content of other fruits is between these figures. Moisture content of these wild fruits is highly comparable with wild edible fruits of doum (*Hyphaene thebaica* L. Mart.) 7.5, baobab (*Adansonia digitata* L.) 4.23, tamarind (*Tamarindus indica* L.) 12.82 and jujube (*Ziziphus spina-christi* L. Willd.) 10.53% Salih, and Yahia (2015).

### 4.1.2. Crude protein

The protein content of fruits is presented in (Table 2). The content was ranged from  $3.15\pm 0.00$  to  $6.52\pm 0.62\%$ . From the collected and analyzed fruits protein content the maximum is for *Ziziphus spina-christi* and minimum value is from *Mimusops Kummel* which is  $3.15\pm 0.00\%$ . The other is between these values. Protein content of *Balanites aegyptiaca* and *Ziziphus spina-christi* is 4.90% and 6.52%, which is higher and different from Debela's finding value of 1.4 and 2.13%, respectively (Debela *et al.*, 2011). But protein content of *Ziziphus spina-christi* is comparable with Amina's finding which 4.8g/100g of dried sample (Amina *et al.*, 2007). These may be due to area of sample collected which may vary in soil fertility, ripening and maturity index of sample and other factors. Generally the fruits have qualified as a source of protein for the consumers in any age group.

Most of these fresh fruits are good sources of proteins and rich in carbohydrate but low in fat and fiber. Among them *Ficus mucoso Ficalho* has more energy 391.63 Kcal/100g whereas *Balanites aegyptiaca* has less amount of energy 349.13 Kcal/100g. These are due to the fat and protein content of *Ficus mucoso*, which is more than others fruits. Especially the energy that is calculated from carbohydrate gets high value. That means they contain carbohydrate more than 80 % from proximate analysis observed. Generally, these fruits are good sources of protein, carbohydrate and vitamins but less in fat and fiber as compared to others. Therefore, it is possible to conclude that herdsman, and children are more advantageous from these underutilized wild edible plant fruits for their nutritional requirement as they are living in rural community and consumed more.

### 4.1.3. Crude fat

The crude fat content of these fruit samples are reported in (Table 2). The result showed that the fat content of these samples were ranged in 0.05% to 3.75%. The maximum value was recorded for *Mimusops Kummel* which is 3.75%, whereas the fruit that is low in fat content is *Balanites aegyptiaca* which shows 0.05% formerly reported 2.5% in ether extract. It may be vary due to sampling location and extraction chemical used. From different literature fat content of fruits were not significant ( $p\geq 0.005$ ) except avocado values 77% fat by calories making them even

higher in fat than most animal foods. This indicates that fruits have low fat content when compared with other food source. *Ziziphus spina-christi* contains 1% which is comparable with Amina's finding 0.9g/100g of dried sample (Amina *et al.*, 2007).

#### **4.1.4. Total ash**

The value of ash in analyzed wild edible fruit samples was reported on (Table 2), which ranges from 3.40 to 12.75% but 12.50% in dry base matter according to Getachew's finding (2011). From the result observation the minimum ash content was for *Mimusops kummel* 3.40% but higher in fat and maximum value was registered for *Balanites aegyptiaca* which is  $12.75 \pm 5.63\%$ , but low in fat (0.05%). The more ash content in food results from inorganic materials that are essential or toxic (intensely or chronically) for consumers.

#### **4.1.5. Crude fiber**

The crude fiber content of these underutilized wild edible fruits was between values of 0.03 to 0.34% (Table 2). As to the other fruit fiber content, these analyzed fruits have good source of fiber content. The minimum fiber content is for *Balanites aegyptiaca* (L) Del. 0.03% and maximum value is 0.34% for *Dovyalis abyssinica* (A.Rich.) Warb. in dry matter bases.

#### **4.1.6. Utilizable Carbohydrate**

From the result obtained these underutilized wild edible fruits are nutritionally rich. They are good source of carbohydrate which ranges from 82.27 to 92.62% (Table 2). As of before the minimum value (82.27%) is for *Balanites aegyptiaca* and maximum value (92.62%) is for *Mimusops kummel*. These fruits were consumed by children and herdsman in all over Ethiopia. The content of carbohydrate of *Ziziphus spina-christi* (86.2%) is comparable with Amina's finding which reported as (80.6%) and Ethiopian food composition Table which is reported as 78.2% of edible portion of fruit. To sum up, one hundred gram of *Z.spina-christi* dried fruit pulp contains 382 kcal, 86.82 g of carbohydrate,  $6.52 \pm 0.62$  g of protein and  $1.00 \pm 0.70$  g of fat.

Table 2 : Proximate composition of the analyzed fruits pulp in dry matter basis (dmb)

Analysis of nutrients (%)	Fruit Samples				
	<i>Balanites aegyptiaca</i>	<i>Dovyalis abyssinica</i>	<i>Ficus mucoso</i>	<i>Mimusops kummel</i>	<i>Ziziphus spina- christi</i>
Moisture	5.55 <sup>c</sup> ±0.24	12.50 <sup>e</sup> ±0.00	3.10 <sup>a</sup> ±0.10	4.00 <sup>b</sup> ±0.00	5.70 <sup>d</sup> ±0.10
Crude Fat	0.05 <sup>a</sup> ±0.00	1.00 <sup>c</sup> ±0.00	3.75 <sup>d</sup> ±0.25	0.75 <sup>b</sup> ±0.25	1.00 <sup>c</sup> ±0.70
Crude Protein	4.90 <sup>c</sup> ±0.49	4.74 <sup>b</sup> ±1.24	6.13 <sup>d</sup> ±0.24	3.15 <sup>a</sup> ±0.00	6.52 <sup>e</sup> ±0.62
Total Ash	12.75 <sup>e</sup> ±0.63	10.20 <sup>d</sup> ±0.85	6.60 <sup>c</sup> ±0.00	3.40 <sup>a</sup> ±0.28	5.60 <sup>b</sup> ±0.00
Crude Fiber	0.03 <sup>a</sup> ±0.00	0.34 <sup>c</sup> ±0.00	0.18 <sup>b</sup> ±0.00	0.08 <sup>a</sup> ±0.00	0.06 <sup>a</sup> ±0.00
Carbohydrate	82.27 <sup>a</sup>	83.72 <sup>c</sup>	83.34 <sup>b</sup>	92.62 <sup>e</sup>	86.82 <sup>d</sup>
G. Energy (Kcal/100g)	349.13 <sup>a</sup>	362.84 <sup>b</sup>	391.63 <sup>e</sup>	389.83 <sup>d</sup>	382.36 <sup>c</sup>

All the given values are means of three determinations ± standard error (SE), means in the same raw with different letters are significantly different ( $P < 0.05$ ) and letters a, b, c, d and e are superscripts given to show the significance difference between means. G- Gross

## 4.2. Minerals Content

It is well known that over exposure or deficeincy to various elements has noticeable effects on human health. The effec of an element is detemined by several characterstics like absorption, metabolisim and degree of interaction with physiological process. Three essential minerals were analyzed on these five underutilized wild edible plant fruits. Wild edible plant are deep roots, the micronutrients in studied wild fruits were appreciably high. Therefore these plant fruits are potential source of minerals for those of community living in rural area as well as for those of consumers that purchase and used from market (Abbaspour and Kelishadi, 2014).

### 4.2.1. Calcium

From the result obtained on Table 3 calcium content is ranged from 60.31±0.06 mg for *Balanites aegyptiaca* to 168.21±0.23 mg/100g DW for *Ziziphus spina-christi* which is highly comparable with Salih's result (173.0 mg/100g DW), but slightly far from Amina's finding (140

mg/100g DW). For *Dovyalis abyssica* fruit  $108.09 \pm 0.05$  mg/100g DW,  $154.23 \pm 0.03$  mg/100g DW for *Ficus mucoso* (Joshi, 2014). As of the most abundant mineral in our body, calcium is essential for our body's overall nutrition and health. It used as disease prevention and absorption of other nutrients. Calcium is required daily from 1000 -1200 mg for optimal nutrition and health of healthy men and women whose age is greater than 31 years. Health care professionals recommend that consuming any supplement of these minerals at least two hours before or after you eat calcium-rich foods. Therefore, fruits *Ziziphus spina-christi* is rich in Calcium whereas fruits of *Balanites aegyptiaca* is less when it compares with the other. From scientific fact there is no evidence for *Mimusops Kummel* to compare its calcium content with the literatures. Generally, they may be good source of Calcium (Amina 2007; Salih *et al.*, 2015).

#### **4.2.2. Iron**

The iron content of the samples was less than calcium content of fruits in 100g sample in dry matter basis. The result was ranged from  $0.13 \pm 0.03$  (*Z.spina-christi*) to  $0.78 \pm 0.04$  mg/100g (*Dovyalis abyssinica*) that is listed below on (Table 3). Iron is an essential element in living organisms in a wide variety of metabolic activities including O<sub>2</sub> transport, deoxyribonucleic acid (DNA) synthesis and electron transport. Therefore, using of these fruits may have benefit for human health due to their iron that the fruits contains. Iron content value of these wild fruits is comparable with wild edible fruits of doum (*Hyphaene thebaica* L. Mart.) 13.4, baobab (*Adansonia digitata* L.) 11.0, tamarind (*Tamarindus indica* L.) 6.1 and far from jujube (*Ziziphus spina-christi* L. Willd.) 61.7 mg/kg Salih and Yahia (2015).

#### **4.2.3. Zinc**

It is essential element for human and found in cells throughout the body (Bhatnagar and Taneja, 2001). It is needed for the body defensive or immune system to properly work. It also plays a great role in cell division, cell growth, wound healing and breakdown of carbohydrates (Roohani *et al.*, 2013). It used for a sense of smell and taste. Generally, it is used to improve athletic performance and strength, support male and female reproductive health and fertility, prevent cancer and boost immune function, improve cardiovascular health, become more sensitive to insulin and prevent diabetes, getting the super antioxidant effect of zinc, prevent Alzheimer's and promote brain health, improve sleep, cognition and energy levels and finally

elevate mood and avoid depression (Roohani *et al.*, 2013). The result of zinc content from 100g dry matter sample is ranged from 0.04±0.06 mg (*Z. spina-christi*) to 0.80±0.02 mg/100g (*Dovyalis abyssinica*) as shown in (Table 3). Zinc content of these wild fruits is comparable with and some are higher than wild edible fruits of Doum (*Hyphaene thebaica* L. Mart.) 2.1, Baobab (*Adansonia digitata* L.) 2.9, Tamarind (*Tamarindus indica* L.) 1.9 and far from Jujube (*Ziziphus spina-christi* L. Willd.) 3.3 mg/kg Salih and Yahia (2015). Daily recommendation of Zinc for adults (Females and males) is 8 to 11 mg/day. Therefore, from the analyzed wild edible fruits these three minerals can support for Recommended Dietary Allowance (RDA) and Adequate Intake (AI) for human being.

Table 3: Minerals content of fruits in dry matter basis (dmb, mg/100g)

Fruit samples	Analysis of minerals		
	Ca	Fe	Zn
<i>Balanites aegyptiaca</i> (L) Del	60.31 <sup>a</sup> ±0.06	0.24 <sup>b</sup> ±0.02	0.22 <sup>b</sup> ±0.01
<i>Dovyalis abyssinica</i> (A.Rich.) Warb.	108.09 <sup>c</sup> ±0.05	0.78 <sup>c</sup> ±0.04	0.80 <sup>e</sup> ±0.02
<i>Ficus mucoso</i> Welw ex. Ficalho	154.23 <sup>d</sup> ±0.03	0.68 <sup>d</sup> ±0.01	0.52 <sup>c</sup> ±0.03
<i>Mimusops kummel</i> Bruce A.DC.	74.61 <sup>b</sup> ±0.02	0.48 <sup>c</sup> ±0.07	0.65 <sup>d</sup> ±0.10
<i>Ziziphus spina-christi</i> (L) Desf.	168.21 <sup>e</sup> ±0.23	0.13 <sup>a</sup> ±0.03	0.04 <sup>a</sup> ±0.06

All the given values are means of three determinations ± standard error (SE), means in the same column with different letters are significantly different (P < 0.05) and letters a, b, c, d and e are superscripts given to show the significance difference between means.

### 4.3. Vitamin C Content of the Fruits

Vitamin is very important nutrient in our life. Among of them vitamin C is essential. It can be produced by the body. Nevertheless, it has many roles in your 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 fruits. The recommended daily intake for vitamin C is 90 mg for adult men and 75 mg for adult women (Whitney and Rolfes, 11<sup>th</sup> Ed.).

Generally, vitamin C has the following impressive health importance for human. It is strong antioxidant that may reduce the risk of chronic diseases. It may help to battle high blood

pressure, fights heart disease risk factors; potentially lowering heart disease risk, could reduce blood uric acid level and help to prevent gout attacks, helps to prevent iron deficiencies by improving iron absorption, can boost immunity by helping white blood cells function better, to protect our memory and thinking as we age (Ryan Raman, 2018). Therefore, from the study ascorbic acid value was ranged from  $2.32 \pm 0.03$  to  $112.90 \pm 0.01$  mg/100g of dry sample. For *Z. spina-christi* fruit level of ascorbic acid is ( $29.43 \pm 0.02$  mg/100g) comparable with Amina's finding (30.04 mg/100g) (Duke 1985; Berry-Koch *et al.*, 1990; Amina 2008). In other study, ascorbic acid value of *Ziziphus spina-christi* is (98 mg/100g) of dry sample which is high compared with orange (50 mg), grape (30 mg) and strawberry (59 mg) reported by Eromosele *et al.*, (1991). The variation may be due to the level of ascorbic acid that depends on the stage of maturity and ripeness of the fruits. Vitamin C content of *Balanites aegyptiaca* was  $2.32 \pm 0.03$  mg/100g which is comparable with 1.26 mg/100g (Sadiq *et al.*, 2012). Ascorbic acid level of *Dovyalis abyssinica* is  $112.90 \pm 0.01$  mg/100g much with Martin's finding (120.30 mg/100mg) but far from Rotili's finding 143.43 mg/100mg (Martins, 2005; Rotili *et al.*, 2018). *Ficus mucuso* has  $3.00 \pm 0.4$  mg/100g of dry matter. The fruit is rich in ascorbic acid and comparable with Joshi's review 3.3 mg/100g (Joshi *et al.*, 2014). Ascorbic acid level of *Mimusops Kummel* is  $30.34 \pm 0.11$  mg/100g. The result is higher than that of Ajay Kumar (2012) work 25.22 mg/100g.

Table 4: Ascorbic acid content of underutilized wild edible fruits in dry matter basis (dmb)

Fruit Samples	Vitamin C/ Ascorbic Acid Content (mg/100g)
<i>Balanites aegyptiaca</i> (L) Del	$2.32^a \pm 0.03$
<i>Dovyalis abyssinica</i> (A.Rich.) Warb.	$112.90^e \pm 0.01$
<i>Ficus mucuso</i> Welw ex. Ficalho	$3.00^b \pm 0.4$
<i>Mimusops kummel</i> Bruce A.DC.	$30.34^d \pm 0.11$
<i>Ziziphus spina-christi</i> (L) Desf.	$29.43^c \pm 0.02$

All the given values are means of three determinations  $\pm$  standard error (SE), means in the same column with different letters are significantly different ( $P < 0.05$ ) and letters a, b, c, d and e are superscripts given to show the significance difference between means.

#### **4.4. Antioxidant activities of underutilized wild edible fruits**

There is a claim and research based document in other counties on fruits *Ziziphus spina-christi* used as medicine. The fruit is eaten in Ethiopia and Sudan by rural communities, herdsman and children as source of food, to treat malaria, diarrhea as antispasmodic. Not only this but also there is a claim that the fruit is used for hypoglycemic, anti-inflammatory, antioxidant, antimicrobial, hypotensive, antitumor, liver protective agent like immune system stimulant (Robinson, 2006; Said, 2006 ). There are a many claims on analyzed fruits in this study from different rural community and dry land area peoples. Therefore the antioxidant activity of these fruits was explained as follows which is reported in (Table 4).

Oxidative stress can be generated by free radicals or reactive oxygen species such as superoxide (O<sub>2</sub>), peroxy (OOH, ROO) and hydroxyl (OH). Buildup of these radicals may result oxidative damage in human body as well as in animals. The result of damage can introduce degenerative diseases like coronary heart diseases, cancer, ageing, inflammation and neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's, and Multiple Sclerosis). Therefore antioxidant is needed to prevent these disorders. Hence underutilized wild fruits plays giant role to protect and reduced amount of free radical formed in our body in everyday life, if we consumed them as per required level. They are nutritionally qualified as equal as those fruits which are available in our home easily. Therefore underutilized wild edible fruits are the source of antioxidant for rural community, herdsman and children in their life (Rahate *et al.*, 2013; Rebaya *et al.*, 2015).

##### **4.4.1. Free radical scavenging activity by DPPH**

The result of DPPH value at IC<sub>50</sub> is shown in (Figure 4.1) and reported on (Table 5). It ranges from 0.07 to 11.60 mg/100ml of methanol extract samples. As the value of IC<sub>50</sub> decreases the scavenging activities of the sample increases; whereas the value of IC<sub>50</sub> increases the result is vice versa. Therefore, from underutilized wild edible fruits grown in Ethiopia and analyzed in these work, Koshim (*Dovyalis abyssinica*) has the same scavenging activity with ascorbic acid standard as well for Shola (*Ficus mucuso*) and Qoladi (*Mimusops kummel*). Bedeno (*Balanites aegyptiaca*) and Geba (*Ziziphus spina-christi*) have less scavenging capacity when it compared with the ascorbic acid standard. In general they have an ability to scavenge free radicals from our

body in an efficient manner. The scavenging activity is arranged as follows from higher to lower. *Dovyalis abyssinica* (A.Rich) warb. (11.6 mg) > *Mimusops Kummel* Bruce A.DC. (0.07 mg) > *Ficus mucuso* Welw ex. Ficalho (6.88 mg) > *Ziziphus spina-christi* (L) Desf. (5.21mg) > *Balanites aegyptiacea* (L) Del. (4.29mg/100ml) sample (Figure 4.1).

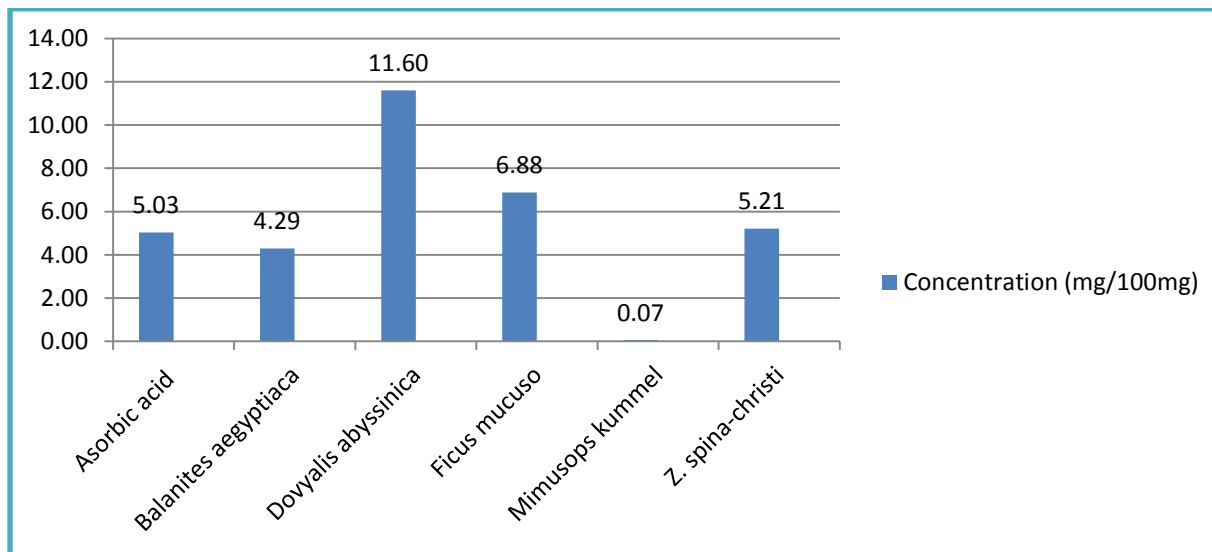


Figure 4.1 DPPH free radical scavenging activity, concentration of extracts and ascorbic acid standard at IC50 in a form of bar graph.

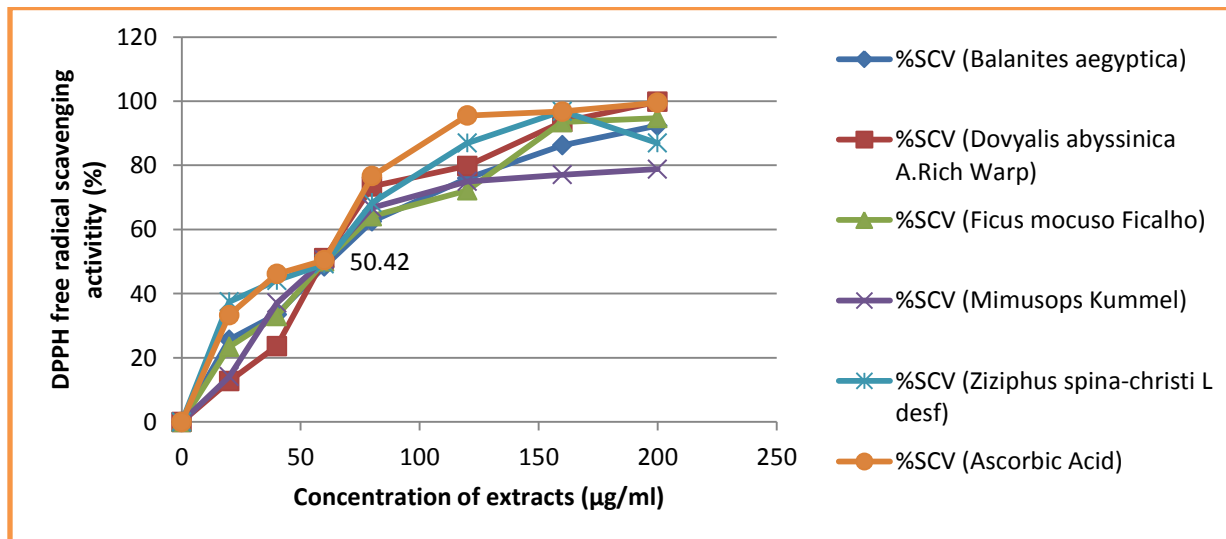


Figure 4.2 DPPH free radical scavenging activity of concentration of extracts and ascorbic acid

From the graph it possible to say that no need of more consumption for *Dovyalis abyssinica* and *Ziziphus spina –Christi* fruits for the sleek of scavenging activities. Overall these fruits have good scavenging activities at IC50.

#### **4.4.2. Total phenol**

Total phenol was determined by using Gallic acid standard and expressed as (mg GAE/100g dmb). The maximum value of total phenol was obtained from fruits of *Dovyalis abyssinica* which is  $80.24 \pm 0.09$  mg GAE/100g DW of sample. According to Hailu's study the 80% extract of the fruits of *Dovyalis abyssinica*, proves to support healing of wounds as the traditional claims as evidenced by an increase in wound contraction, hydroxyproline content and tensile strength and a decrease in epithelization period compared with control results (Winslow *et al.*, 1998; Ramawat *et al.*, 2008; Ahlem *et al.*, 2014). It also possesses a significant anti-inflammatory effect as shown by a significant decrease in edema compared with control results supporting the wound healing effect. The lowest value of total phenol was  $22.13 \pm 0.04$  mg GAE/100g of dmb of *Ficus mucuso* sample (Table 5).

The medicinal aspect of *Balanites aegyptiaca* (Desert date) fruit is a multipurpose tree as of others. Nursing mothers and consumers used it within porridge and oil from the seed is used to treat headache that can improve lactation (Cregan *et al.*, 2002). This can improve human health if it is consumed in any style that people prefer like as raw, within porridge or as juice after soaking it in water for long time which can improve stomach pain.

#### **4.4.3. Total flavonoids**

Total flavonoid content of these fruits was determined by using Catechin standard. The result is expressed in mg of CE/100g of dry matter basis sample. The highest value is  $48.34 \pm 0.04$  mg CE/100g dmb for fruit of *Ziziphus spina-christi*. The lower flavonoid content is obtained from fruits of *Mimusops kummel* ( $17.32 \pm 0.05$  mg CE/100g) of dmb sample. Flavonoid content of the fruits was arranged from highest to the lowest under (Table 5) shown below.

Table 5: Total phenolic content (TPC), Total Flavonoids Content (TFC) and antioxidant activity of the fruit extracts in dry matter basis

Fruit Samples	Total phenol content (mg GAE/100g)	Total flavonoid content (mg CE/100g)	DPPH (IC50) (mg/100mg)
<i>Balanites aegyptiaca</i> (L) Del.	47.84 <sup>d</sup> ±0.05	21.54 <sup>b</sup> ±0.03	4.29 <sup>b</sup>
<i>Dovyalis abyssinica</i> (A.Rich.) Warb.	80.24 <sup>e</sup> ±0.09	29.08 <sup>c</sup> ±0.02	11.60 <sup>e</sup>
<i>Ficus mucuso</i> Welw ex. Ficalho	22.13 <sup>a</sup> ±0.04	38.10 <sup>d</sup> ±0.03	6.88 <sup>d</sup>
<i>Mimusops kummel</i> Bruce A.DC.	46.74 <sup>c</sup> ±0.05	17.32 <sup>a</sup> ±0.05	0.07 <sup>a</sup>
<i>Ziziphus spina-christi</i> (L) Desf.	35.84 <sup>b</sup> ±0.07	48.34 <sup>e</sup> ±0.04	5.21 <sup>c</sup>

All the given values are means of three determinations ± standard error (SE), means in the same column with different letters are significantly different (P < 0.05) and letters a, b, c, d and e are superscripts given to show the significance difference between means.

Flavonoid content of UWE Fruits of *Z. spina-christi* (48.34±0.04 mg/100g) > *Ficus mucuso* (38.10±0.03 mg/100g) > *Dovyalis abyssinica* (29.08±0.02 mg/100g) > *Balanites aegyptiaca* (21.54±0.03 mg/100g) > *Mmisops Kummel* (17.32±0.05 mg/100g of CE in dry matter basis. `

#### 4.5. Anti-nutritional factors of underutilized wild edible fruits

Anti-nutritional factors of the analyzed fruits are reported as follows. Oxalate, phytate and tannin content of edible portion of the fruits were analyzed. The parameters may not be much in fruits but to put their anti-nutrition profile it is important to do the analysis ( Bhabdari, 2006).

##### 4.5.1. Oxalate content

The maximum oxalate content in a column was reported for *Balanites aegyptiaca* (18.59±0.31 mg/100g) followed by *Dovyalis abyssinica* (13.25±0.14 mg/100g) and the minimum value of oxalate content in the column is (5.04±0.11 mg/100g) of dry matter basis represent *Mimusops Kummel* fruit (Table 6). Oxalate is derivatives of oxalic acid and its slats occur as end product of metabolism in a number of plant tissues. When these plants are eaten; they may have an adverse health effect in human body, because oxalate can bind with calcium, magnesium and other minerals. In the other way calcium oxalate is insoluble and it will precipitate with soft tissue; the

lost calcium by itself can cause osteoporosis of bone, teeth's and impairment of blood clothing. Process (Badifu and Okeke, 1992).

Oxalic acid is normal end product of human body metabolism; hence extra consumption of oxalate may introduce stone formation in urinary tract/kidney stone during excretion of acid through urine. Cooking and soaking of foods rich in oxalate can reduce the oxalate content to acceptable level by leaching. The mean daily intake of oxalate in developed countries is 70-150 mg/100g of dry matter basis. When oxalate content of these analyzed wild fruits compares with daily intake it is too low. To sum up, the content of oxalate in the analyzed fruits are not in a toxic level rather it is in very low amount compared with the standard set for this factors which make the fruits safe from anti-nutritional perspective (Noonan *et al.*, 1999).

#### **4.5.2. Phytate content**

During the past few years, courtesy has been given to phytic acid (PA) as anti-nutritional factor in the diet of humans; due to inability to utilize phytate. Minerals that are binned with PA are not bioavailable which can leads to deficiencies in human population whose staples are wheat, rice and maize seeds in their nutrition. Vitamin A, Fe and Zn are the main micronutrient malnutrition in the entire world (WHO, 2002). Negatively charged Phosphate in PA can binds strongly with those of metallic cations like Ca, K, Fe, Mg, Mn, and Zn making them insoluble and thus unavailable as nutritional factors (Lisbeth *et al.*, 2008). From the study phytate content is maximum in a column for *Balanites aegyptiaca*  $3.45 \pm 0.10$  mg/100g and the minimum value of phytate content in the column is  $3.13 \pm 0.00$  mg/100g of dry matter basis represent *Mimusops Kummel* fruit. Generally phytate content of analyzed fruits are less when it compares with phytate rich seeds such as unpolished rice, maize bread and toasted sesame of values were 12.7-21.6, 4.3-8.2 and 39.3-57.2 mg/g on dry matter basis, respectively (Kumar, *et al.*, 2010).

#### **4.5.3. Condensed Tannin content**

The tannin content of analyzed underutilized wild edible fruits is reported in (Table 6). From the result observation the maximum tannin content for *Ziziphus spina-christi* is  $9.25 \pm 0.01$  mg/100g. The minimum content also isolated which is  $2.26 \pm 0.05$  mg/100g represent *Dovyalis abyssinica* fruit. Tannin content of *Balanites aegyptiaca* and *Ziziphus spina-christi* listed below in the table

is comparable with Umaru's finding (2006)  $7.40 \pm 0.14$  and  $5.28 \pm 0.09\%$ , respectively. Astringent taste that can impose palatability is caused by tannin found in fruits (Umaru *et al.*, 2006). Not only this but also it has a negative effect on growth and reduction of food intake. Tannin by nature can affect utilization of protein binding with endogenous, exogenous proteins and enzymes of digestive tract. As the level of tannin content of underutilized wild edible fruits grown in Ethiopia and analyzed in this work is low, that can make them safe for consumption without any restriction and further processing (Table 6).

Table 6: Level of anti-nutritional factors in underutilized wild edible fruits in (mg/100g) of dry matter basis

Fruit samples	Oxalate content	Phytate content	Tannin content
<i>Balanites aegyptiaca</i> (L) Del.	$18.59^d \pm 0.31$	$3.45^{bc} \pm 0.10$	$6.12^c \pm 0.02$
<i>Dovyalis abyssinica</i> (A.Rich.) Warb.	$13.25^c \pm 0.14$	$3.40^b \pm 0.01$	$2.26^a \pm 0.05$
<i>Ficus mucoso</i> Welw ex. Ficalho	$6.30^b \pm 0.00$	$3.37^b \pm 0.01$	$3.84^b \pm 0.01$
<i>Mimusops Kummel</i> Bruce A.DC.	$5.04^a \pm 0.11$	$3.13^a \pm 0.00$	$7.71^d \pm 0.00$
<i>Ziziphus spina-christi</i> (L) Desf.	$6.30^b \pm 0.00$	$3.36^b \pm 0.00$	$9.25^e \pm 0.01$

All the given values are means of three determinations  $\pm$  standard error (SE), means in the same column with different letters are significantly different ( $P < 0.05$ ) and letters a, b, c, d and e are superscripts given to show the significance difference between means.

#### 4.6. Color parameters

Color measurement instruments can show us a slight difference between two samples even if the samples seem the same for a person. If the color of a sample does not adequate enough for the standard, customer satisfaction may be compromised and rework and cost will increase. Therefore, it is important to produce products that have the exactly same color. This indicates that production process has to be controlled in each batch or continuous process according to the system we used. To identify color difference using  $L^*a^*b^*$  or  $L^*C^*H^*$  Coordinates is applicable. Recently color measurements have been used as quality parameters and indicator of color in food industries (Yohannes, 2014). Dehydration is the phenomenon of removal of water from lower concentration of solute to higher concentration. Dehydration found wide application in the preservation of food-materials since it lowers the water activity of fruits and vegetables. It

is preferred over other methods due to their color, aroma, nutritional constituents and flavor compound retention value (Tiwari, 2005).



Figure 4.3 **a**, Fruit leather product developed from *Carica Papaya* **b**, *Dovyalis abyssinica* and **c**, from mixture of two fruits, respectively

Color parameters were analyzed as in (Table 7). The L value ranged from 82.46 (672, Fruit leather of *Carica Papaya*) to 133.88 (521, Fruit leather of *Dovyalis abyssinica*). The result shows that lightness of sample 672 is in range whereas the other samples were out of lightness range. Then a\* value is ranged from 82.46 (762) to 150.77 (521). The value of b\* is ranged from 75.30 (672) to 113.49 (521), which is within range. From the Table it is possible to say that fruit leather 672 is more acceptable than sample 654 (Fruit leather from *Carica Papaya* and *Dovyalis abyssinica*) and 521. Therefore *Carica papaya* and the mixture with *Dovyalis abyssinica* were more acceptable than *Dovyalis abyssinica* fruit leather. Mixing of *Dovyalis abyssinica* with other colorful fruit is advisable to get colorful product and palatable leather fruits.

Table 7: Analysis of color parameter for developed fruit leather product

Product code	L*	a*	b*
672	82.46 <sup>a</sup>	90.879 <sup>a</sup>	75.30 <sup>a</sup>
654	125.90 <sup>b</sup>	141.03 <sup>b</sup>	108.33 <sup>b</sup>
521	133.88 <sup>c</sup>	150.77 <sup>c</sup>	113.49 <sup>c</sup>

All the given values are means of three determinations  $\pm$  standard error (SE), means in the same column with different letters are significantly different ( $P < 0.05$ ) and letters a, b and c are superscripts given to show the significance difference between means.

Product code 672 =100% *Papaya* fruit leather, 654 = 100% *Dovyalis abyssinica* (A. Rich.) warb fruit leather, 521 = 50% of *Dovyalis abyssinica* and 50% of *Papaya* fruit leather.

#### 4.7. Production Yield

*Dovyalis abyssinica* fruit has a good production yield. For the analysis well ripped fresh fruits were weighted, peeled and deseed. Then the juice was weighted. The result is shown in (Table 8). Therefore *Dovyalis abyssinica* is very juicy fruits in terms of production 75.55% is extractable juice. The total content of soluble solids in the juice is 14.40%. Hence, the volume of the fruit may be more benefit able in aspect of economic for further production.

Table 8: Yield analysis and evaluation of *Dovyalis abyssinica* fruit juice

Fruit sample	Weight of fruits (g)	Weight of juice (g)	Weight of waste (g)	Juice yield (%)
<i>Dovyalis abyssinica</i>	224.98	172.23	52.75	76.55

The physical and chemical analysis shows that the fruit juice was very acidic and cannot deteriorate easily. The pH and TA values indicate that the less pH values the higher titratable acidity value. From the result it is possible to say that degree brix and TA value is directly proportional whereas pH is indirectly proportional for both of degree brix and TA values. The concentration of free protons in a juice or wine ranges from 0.1 to 1 mg/L, whereas TA values might be 4 to 8 g/L (Kumar *et al.*, 2014). Therefore TA value of the sample was within the range

of the standard. Therefore this fruit can support to protect fruits that are easily perishable like *Avocado*, *Papaya* and *Banana* for long shelf life (Table 9).

Table 9: Physical and chemical analysis of raw fruit juice of *Dovyalia abyssinica* (A.Rich.) Warb.

<sup>0</sup> Brix	PH	TA	Taste	Odor	Appearance
14.40±0.08	2.48±0.01	5.35±0.00	Acidic	Pungent	Deep yellow

All the given values are means of three determinations ± standard error (SE)

Yield output is important for product development. From the result obtained 39.60g for *Dovyaliss abyssinica*, 37.65g for *Carica Papaya* and 36.15g (50% *DA* and 50% *CP*) per 300g of fruit leather was recorded from 300 g of fruit juice added to heat drying before processing.

Table 10: Estimation of yield for developed leather product

Product sample code	Yield in g/300g
Fruit leather of <i>Carica Papaya</i> (672)	37.65 <sup>b</sup> ±0.05
Fruit leather of <i>Carica Papaya</i> and <i>Dovyalis abyssinica</i> , 1:1 ratio (654)	36.15 <sup>a</sup> ±0.04
Fruit leather of <i>Dovyalis abyssinica</i> (521)	39.60 <sup>c</sup> ±0.08

All the given values are means of three determinations ± standard error (SE), means in the same column with different letters are significantly different (P < 0.05) and letters a, b and c are superscripts given to show the significance difference between means.

## 4.8. Sensory Evaluation

Among of 15 different panelists participated for sensory evaluation of new product that was formulated; different perceptions was observed and summarized as follows. For sample 672 all evaluator was interested and it is their preference (Table 11). Even if fruit leather is new for them they impressed in the texture and strength it has as it is new product. Fruit leather has a tendency to attract and good sense of edibility as a snack food especially for children. It is the method to increase fruits consumption in other form which promotes good health for any one consuming it. The process is easy and fast. It can save fruits that are easily perishable and wasted in different

area. Therefore this information may help our community one step to consume fruits in a form of fruit leathers in addition of their stable food staff. The moisture content of the product is less than that of raw dried sample which increase the shelf life of products. As we know fruits are easily perishable unless it was conserved properly in anywhere it is.

Dehydration allows for the development of a variety of new shelf-stable food products from perishable fruits thus reducing post-harvest fruit losses and ensuring that seasonal fruit products are available throughout the year. Osmotic pre-treatment of fruits, by dipping them in sugar solutions, prior to hot-air drying produces relatively low-cost intermediate moisture fruit products with good color, flavor and texture characteristics, and shelf stability at tropical ambient temperatures. Fruits dried with infused sugar are sometimes referred to as candies due to the large amount of sugar added to the fruit. They are also referred to as leathers because the infused sugar imparts a leathery texture to the dried product as opposed to the woody texture of non - osmosed dried fruit products. Fruit leathers/candies are moist enough to be eaten without re-hydration, yet shelf-stable (Cheman & Taufik, 1995).

Laboratory studies on the production of fruit leathers from *C.Papaya* revealed that the best products. From the standpoint of consumer acceptance, cost and energy savings, ripe *C. Papaya* fruit pulps by hot-air drying at 60 °C were preferred (Falade & Aworh, 2004 & 2005). Similar studies with *Dovyalis abyssinica* indicated that it has good response for chewiness, texture, color, but low score in taste and aroma (Falade, 2002). Although hot- air dried *Dovyalis abyssinica* and *C. Papaya* fruit juice received consistently better scores for color, taste, chewiness and overall acceptability relative to controls that did not receive osmotic pre-treatment but *Dovyalis abyssinica* was generally less preferred than *C. Papaya* (Falade, 2002; Falade & Aworh, 2005). Different scholars proved that Osmotic pre-treatment and drying inhibited microbial growth; osmo-dried products from these fruits were free of pathogenic microorganisms including intestinal pathogens (Salmonella and Shigella) and Staphylococcus (Falade, 2002; Falade and Aworh, 2004, 2005).

#### **4.8.1. Difference teste**

It one of the most popular sensory testing methods. It is often the first sensory method used in newly developed sensory programs (Costell, 2002). Difference testing is a way to determine if a

sensory difference actually exists between samples. The degree or nature of the difference cannot be quantified. However, Descriptive tests are generally needed to truly define differences. There are four types of difference tests which can be used to answer some practical questions. The most common for use in the wine and food industry are the triangle difference test and the duo-trio difference test (Zoecklein *et al.*, 1999 and 2005).

These are questions raised during difference test method of sensory evaluation of a give products. Which product do you prefer, Which product do you like, How well do you like this product and How often would you use this product. In this study it is to answer the attributes based on the paneslist participated but no asked the questions of the methds. From the sensory evaluation 66.67% of panelists said that there is a difrence between three samples on overall appreance whereas 33.33% said no overall difference between the samples. 73.33% is differ in color intesity whereas 26.67% is the same. The same as above parammeter taste of the sample is completly differ from one another. There is similarity of score for texture (mouth feel) and color which is 73.33% said that the sample is the same whereas 33.33% is differ Table 11 (Babalola, 2008).

Table 11: Panelists response from samples evaluation of difference in overall appearance, color, taste, texture (mouth feel) between them

Attributes	Score	
	Different	The same
Overall appearance	10	5
Color	11	4
Taste	15	0
Texture (mouth feel)	11	4

#### 4.8.2. Ranking Preference test

Using the Friedman's test, the formula to test whether there are differences based on the  $\chi^2$  statistics;  $\chi^2 = 3.133$  and from Friedman's table  $\chi^2$  critical values for  $\alpha$  0.05 and degree of

freedom N-1 is 2.726. From the Friedman's test, the  $\chi^2$  value is greater than the table value of 2.762. Therefore, the preference rank differs significantly at 5% level interval.

Table 12: Showed ranking of the three fruit leather products from most preferred to least preferred by scoring a number from 1 to 3

Panelists	Sample code		
	672	654	521
1	2	2	2
2	1	2	3
3	1	2	3
4	3	1	2
5	1	2	3
6	1	2	3
7	2	1	3
8	1	2	3
9	1	2	3
10	1	2	3
11	1	2	3
12	1	2	3
13	1	2	3
14	1	2	3
15	1	3	2
<b>Rank sums</b>	<b>19<sup>a</sup></b>	<b>29<sup>b</sup></b>	<b>42<sup>c</sup></b>
<b>Treatment mean</b>	<b>1.27<sup>a</sup>±0.59</b>	<b>1.93<sup>b</sup>±0.46</b>	<b>2.80<sup>c</sup>±0.41</b>

All the given values are means of central tendency  $\pm$  standard deviation (SD), means in the same row with different letters are significantly different ( $P < 0.05$ ) and letters a, b and c are superscripts given to show the significance difference between means.

From the panelists sensory evaluation there is a significance difference between three samples. The LSRD value is 6.82 ( $p \geq 0.05$ ) as shown in (Table 13). Since rank numbers that haven't the

same superscript are significantly different. Therefore product 672 is significantly preferred over product 654 and 521. Product 654 is preferred than 521 but less preferred than that of 672 at 5% level interval.

Table 13: Least significant ranked difference for samples coded in random number

Sample	672	654	521
Rank sum	19 <sup>a</sup>	29 <sup>b</sup>	42 <sup>c</sup>

All the given values are means of central tendency  $\pm$  standard deviation (SD), means in the same row with different letters are significantly different ( $P < 0.05$ ) and letters a, b and c are superscripts given to show the significance difference between means.

672 = Fruit leather product of *Carica Papaya*, 654 = Fruit leather product of *Carica Papaya* and *Dovyalis abyssinica* in equal ratio, 521 = Fruit leather product of *Dovyalis abyssinica* fruit pulp.

### 4.8.3. The 9-point hedonic scale test

For measuring product liking and preference, the nine-point hedonic scale is probably the most useful sensory method. A hedonic scale includes a series of verbal statements that convey a level of like or dislike. The method occupies a unique role for sensory evaluation. Since product development has been used extensively with a wide variety of products and with considerable success. Smiling faces with more child-friendly terminology or just pictures of facial expressions are common approaches with children (Aworh, 2015).

Table14: Comparative evaluation of fruit leathers made from *Carica Papaya*, *Dovyalis abyssinica* and their mixture without additional component

Panelists	Fruit Leathers			Panelist Total
	672	654	521	
1	8	7	6	21
2	9	8	7	24
3	9	8	7	24
4	8	7	4	19
5	9	2	1	12
6	9	5	5	19
7	9	2	1	12
8	9	7	3	19
9	8	6	6	20
10	8	6	6	20
11	9	7	7	23
12	8	6	2	18
13	8	7	7	22
14	8	8	8	24
15	9	8	8	25
Treatment total	$\Sigma x = 128^c$	$\Sigma x = 94^b$	$\Sigma x = 78^a$	GT = 302
Treatment mean	$8.53^c$	$6.27^b$	$5.20^a$	
	$\Sigma x^2 = 1096^c$	$\Sigma x^2 = 642^b$	$\Sigma x^2 = 488^a$	$\Sigma x^2 = 2226$

All the given values are means of central tendency  $\pm$  standard deviation (SD), means in the same row with different letters are significantly different ( $P < 0.05$ ) and letters a, b and c are superscripts given to show the significance difference between means.

From the ANOVA analysis there is a significant difference between fruit leather samples on their overall acceptability, since the calculated F ratio is 12.91 is greater than that of tabulated F value 3.34 at 5% level of significance (Table 15). This indicates that there is a likely occurring chance to be different even if temperature and time is blocked or remain the same in all products. From

panelist observation there is a significance difference between panelist, since the calculated F critical (2.27) is as large as F tabulated (2.04) at 5% level of significance of product overall acceptability from the likely chance of probability.

Table 15: ANOVA table for 9-hedonic scale sensory evaluation for overall acceptability

Score of variation	df	SS	MS	F ratio	
				Calculated	Table ( $p < 0.05$ )
Panelists(P)	14	73.90	5.28	2.27 <sup>b</sup>	2.04 <sup>a</sup>
Treatment(TR)	2	60.18	30.09	12.91 <sup>b</sup>	3.34 <sup>a</sup>
Error(E)	28	65.15	2.33		
Total(T)	44	199.25	4.53		

All the given values are means statistical and calculated. Means in the same row with different letters are significantly different ( $P < 0.05$ ) and letter a and b is superscripts given to show the significance difference between means.

## CHAPTER FIVE

### 5. CONCLUSION AND RECOMMENDATIONS

#### 5.1. CONCLUSION

These underutilized wild edible fruits are good sources of carbohydrate, protein, fiber and total energy. Therefore, they will help for daily requirements. They also important for proper growth of children and can meet the daily need of human nutrition. These wild fruits are low in fat and total ash content.

The investigated underutilized wild fruit pulps possess high phenolic compounds and antioxidant capacity and good amount of minerals. It is possible to conclude that the analyzed pulps seem to have a high nutritional value and bioactive potential. Among them *Dovyalis abyssinica* (A.Rich.) Warb is high in vitamin C content and antioxidant activity than the other analyzed wild fruits.

The anti-nutritional factors of five underutilized wild fruits commonly consumed in Ethiopia showed that all the wild fruits contained oxalate, phytate, and tannin. However, values obtained for these fruits are lower than the established toxic level. Hence they can be consumed without any restriction. However, consumption in large amounts of fruits with higher levels of anti-nutrients should be avoided. The consumption of these vegetables was therefore encouraged.

From study it is evident that these underutilized wild edible plant fruits possess high nutritional value. These fruits may use to various cuisines and flavor, garnish, or complement for other foods. Most of this traditional knowledge only survives in the memory of the elderly and is now in danger of vanishing. This paper attempts to compile and disseminate that knowledge in order to help maintain cultural traditions and facilitate research into food history and new food sources.

This study showed that the underutilized wild edible fruits studied are good sources of nutrients including minerals such as Ca, Fe and Zn, which is comparable with or sometimes higher than conventional wild edible fruits. Therefore, most of these underutilized wild edibles can be used to mitigate macronutrient malnutrition and improving food security.

## 5.2. RECOMMENDATIONS

- ❖ These underutilized wild edible fruits are essential for human health. Therefore, the fruit plants have to be diversified. Product development on these fruits can help to promote their nutritional quality. Not only this but also it is important to develop products with other food stuffs in different formulation for better use of the fruits wasted on underground and to increase the palatability of the fruits.
- ❖ Biological studies are needed to explore the advantageous effect of these wild fruits for human health.
- ❖ These underutilized wild edible fruits have their own seeds which will be source of oil and other source of nutrition. Therefore, it is important to put their nutritional quality and nutrition profile including their seeds.
- ❖ These plants should be explored further for overcoming macronutrient, vitamin and phytochemicals malnutrition, particularly in the developing world.
- ❖ Application of modern processing methods along with incorporation of traditional knowledge will definitely provide a substantial base for the commercial exploitation of these plants for developing new foods or for bio-fortification, as well as for use in the pharmaceutical industry.
- ❖ Application of modern biotechnological methods might provide sufficient support to develop transgenic plants with less anti-nutrients or toxicological factors in the underutilized wild edible plants.
- ❖ Still, a wide gap in our knowledge exists with regard to exploring the actual gene pool, in evaluating beneficial secondary metabolites, phytochemicals, and other nutritional features in these underutilized wild edible plant resources.

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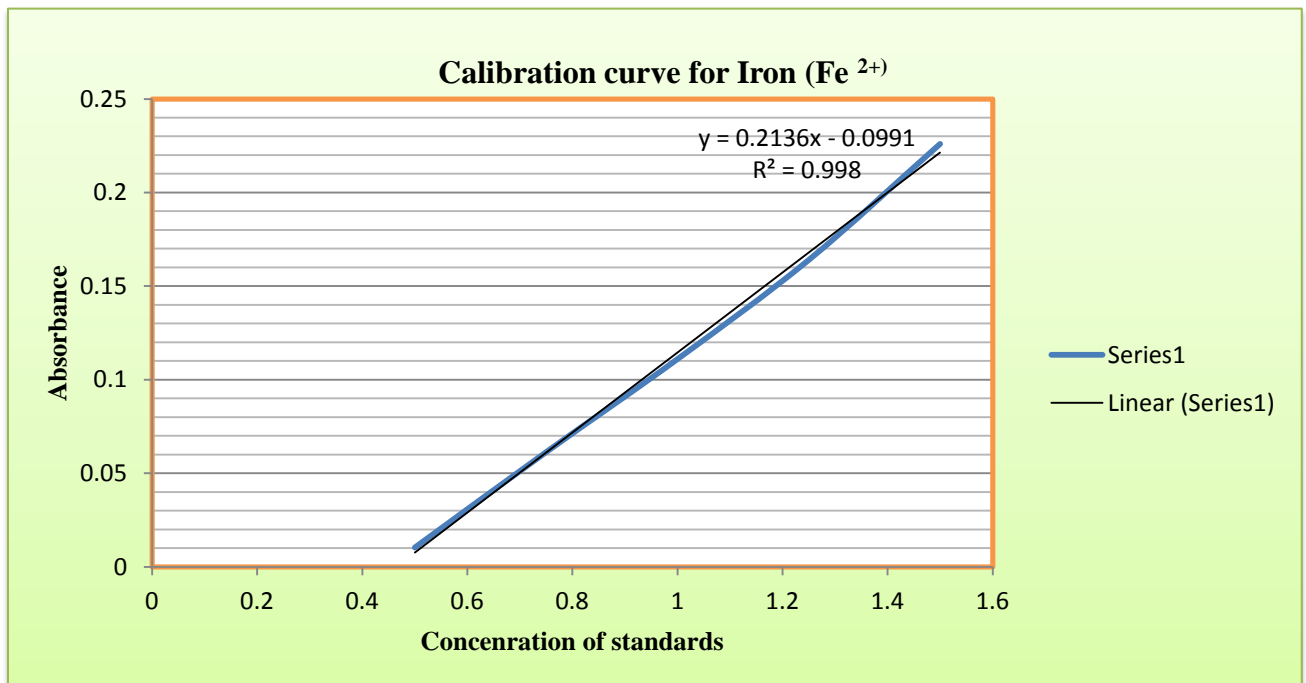
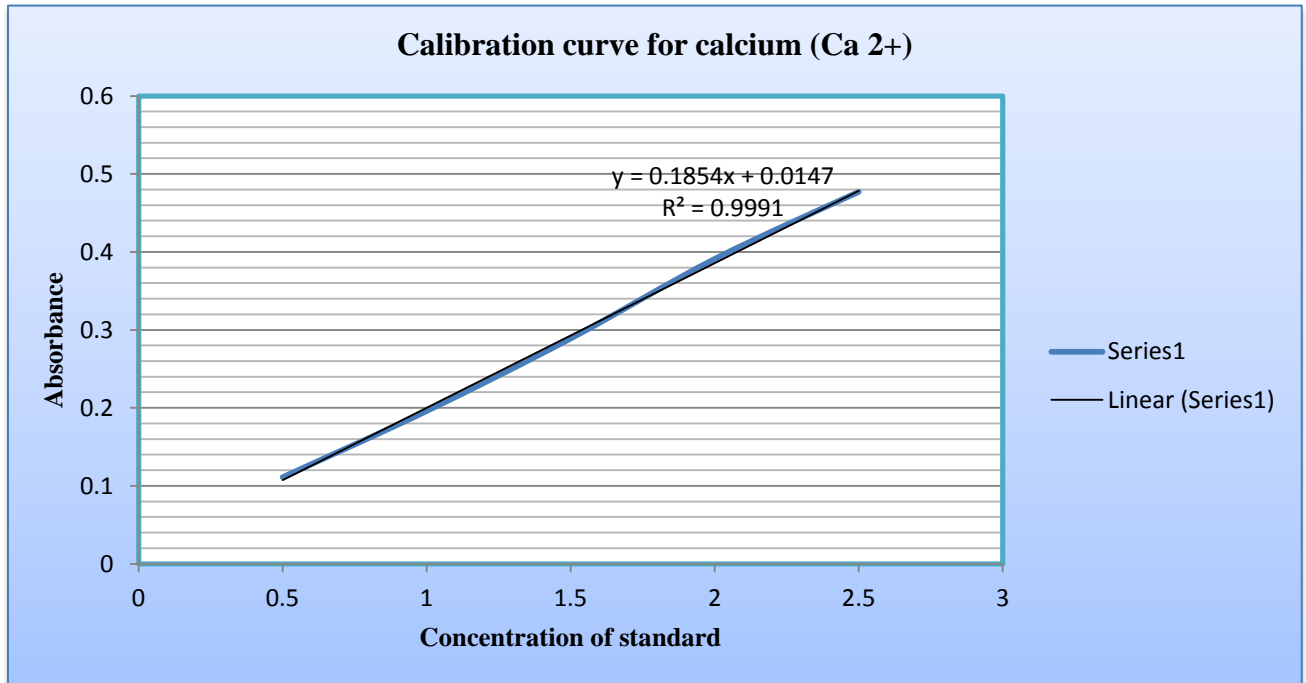
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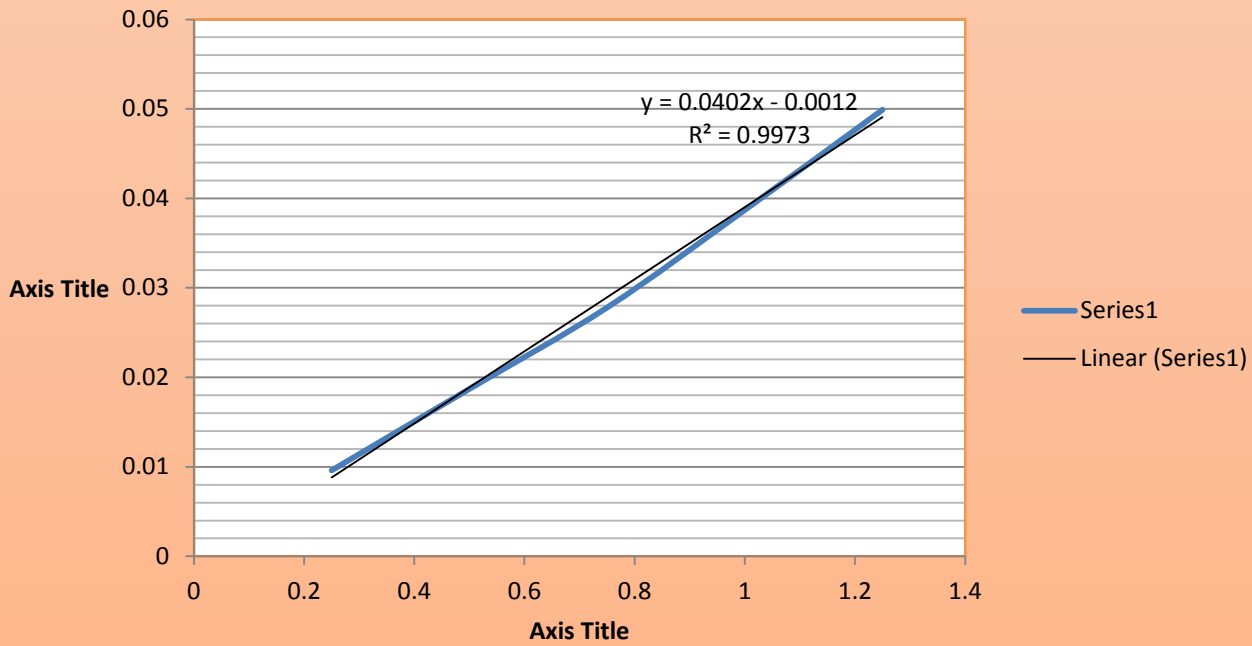
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## 7. APPENDIXES

### 7.1. Calibration curve for mineral analysis

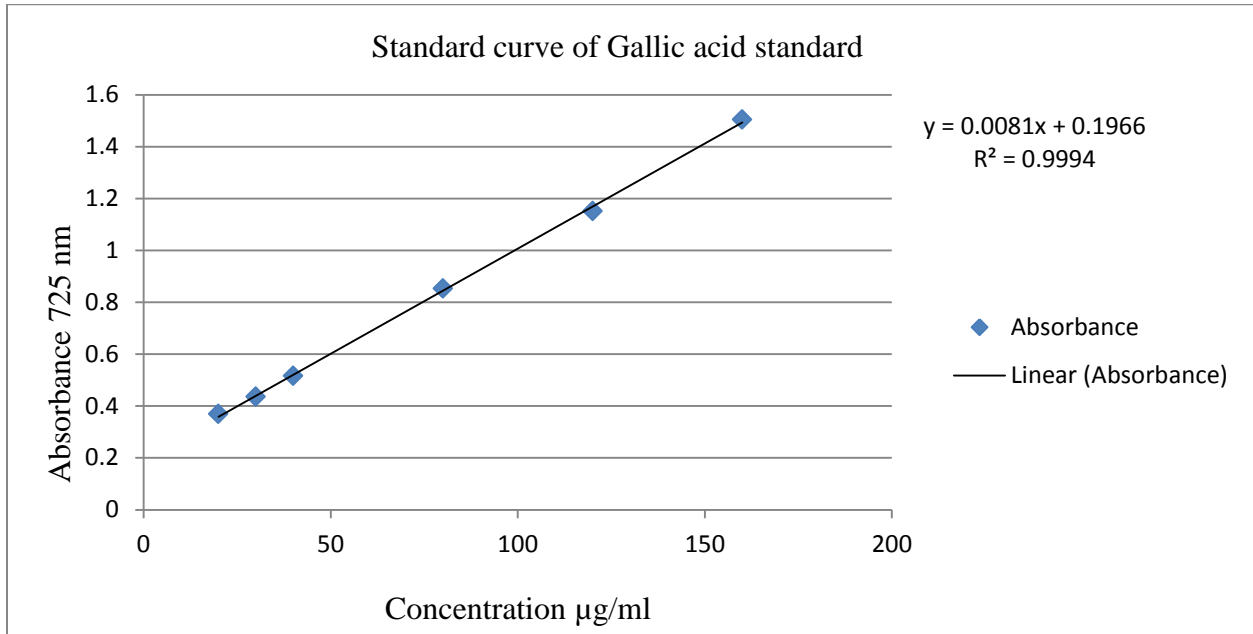


Calibration curve for Zinc ( $Zn^{2+}$ )

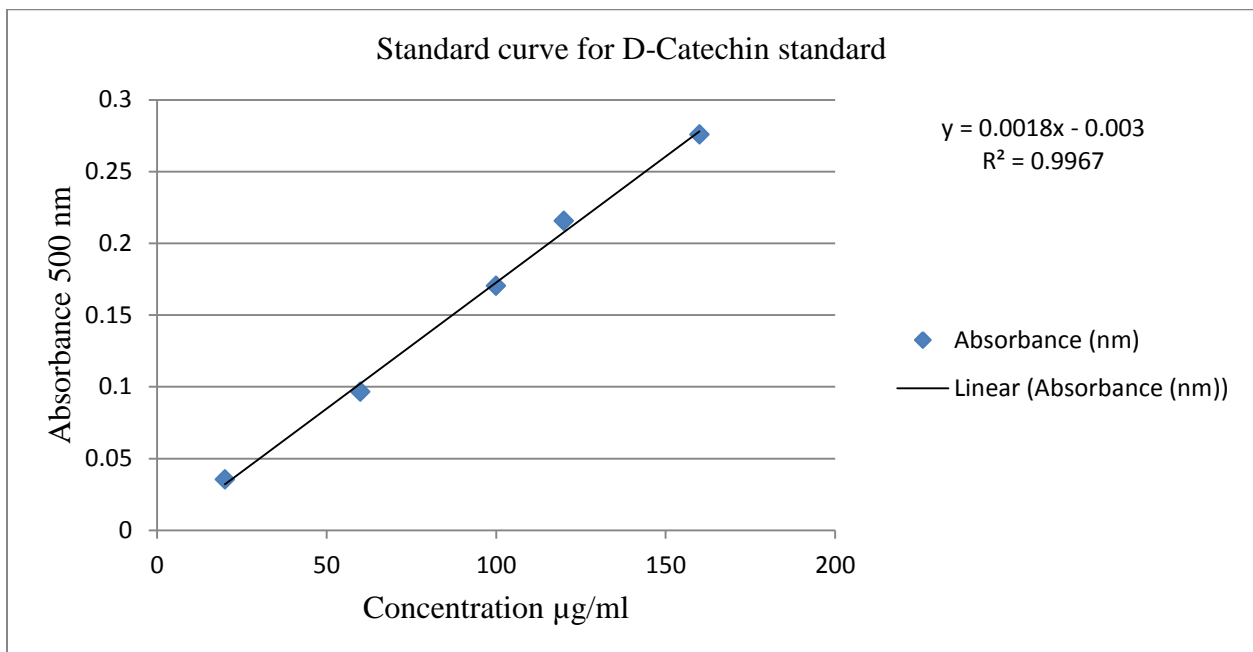


## 7.2. Standard curve of concentration verses absorbance

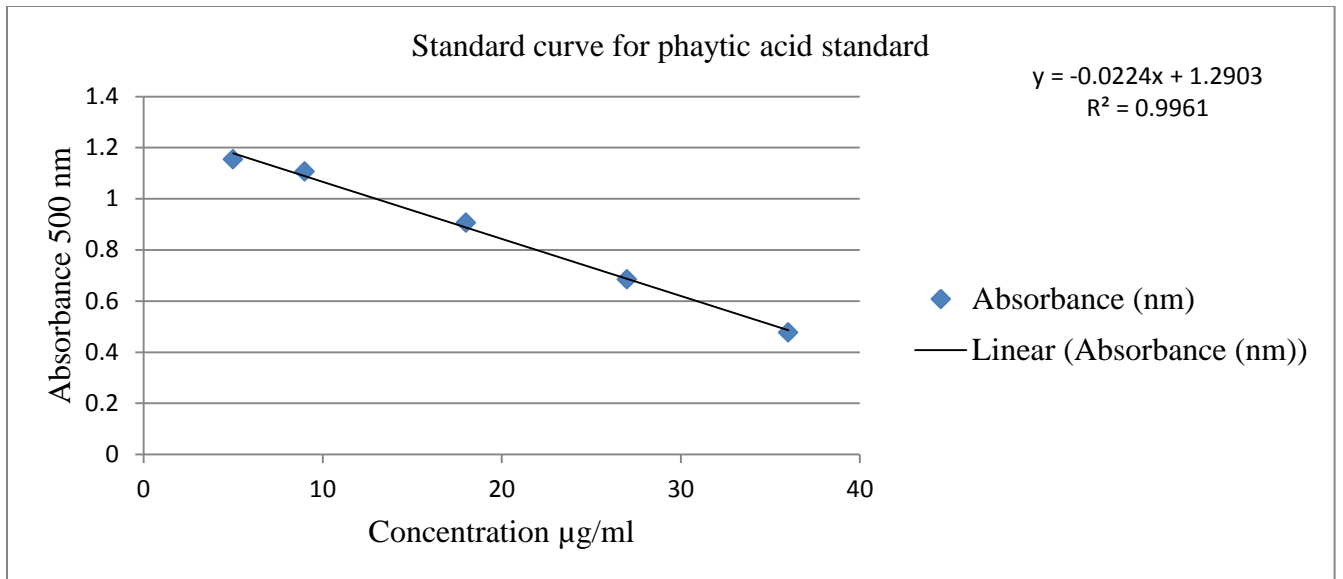
### 7.2.1. Standard curve for phenol analysis



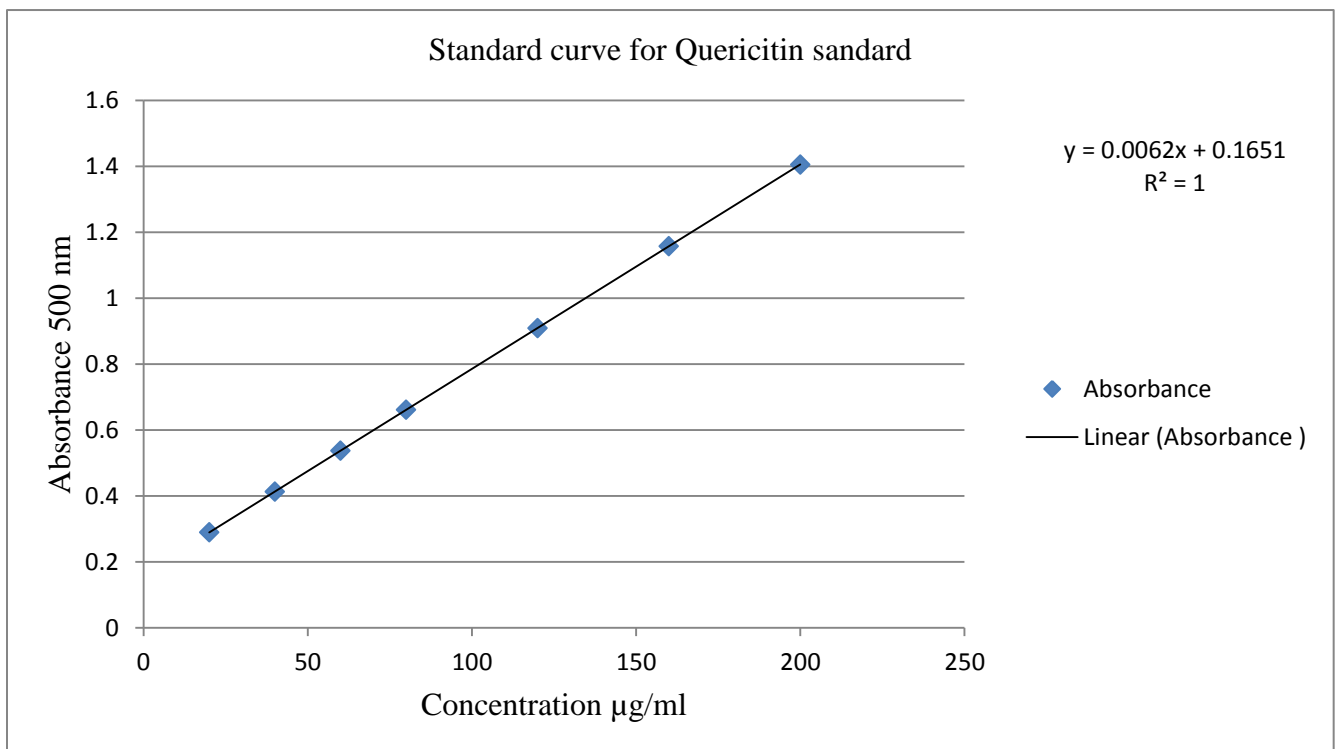
### 7.2.2. Standard curve for tannin analysis



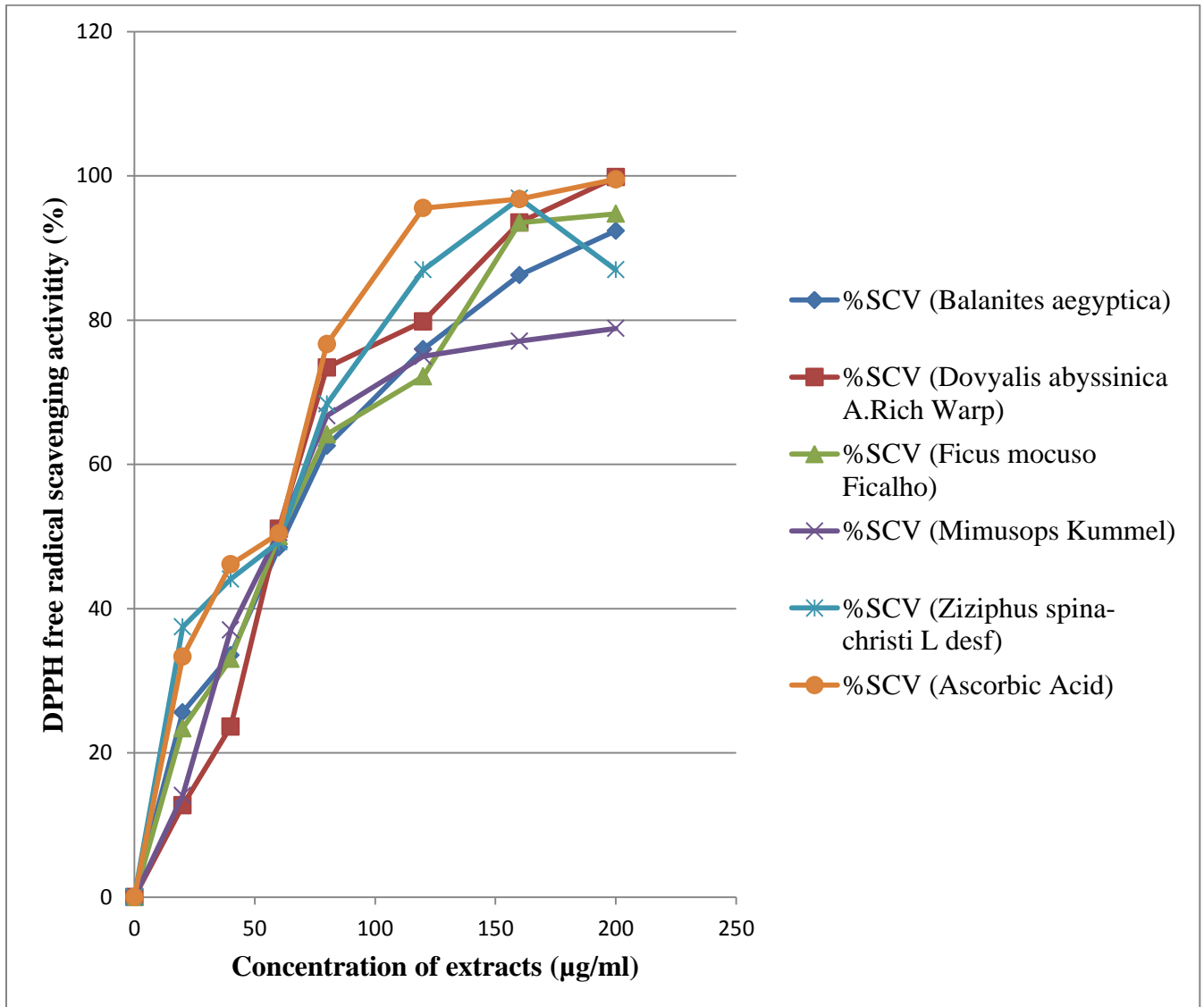
### 7.2.3. Standard curve for determination of phytic acid



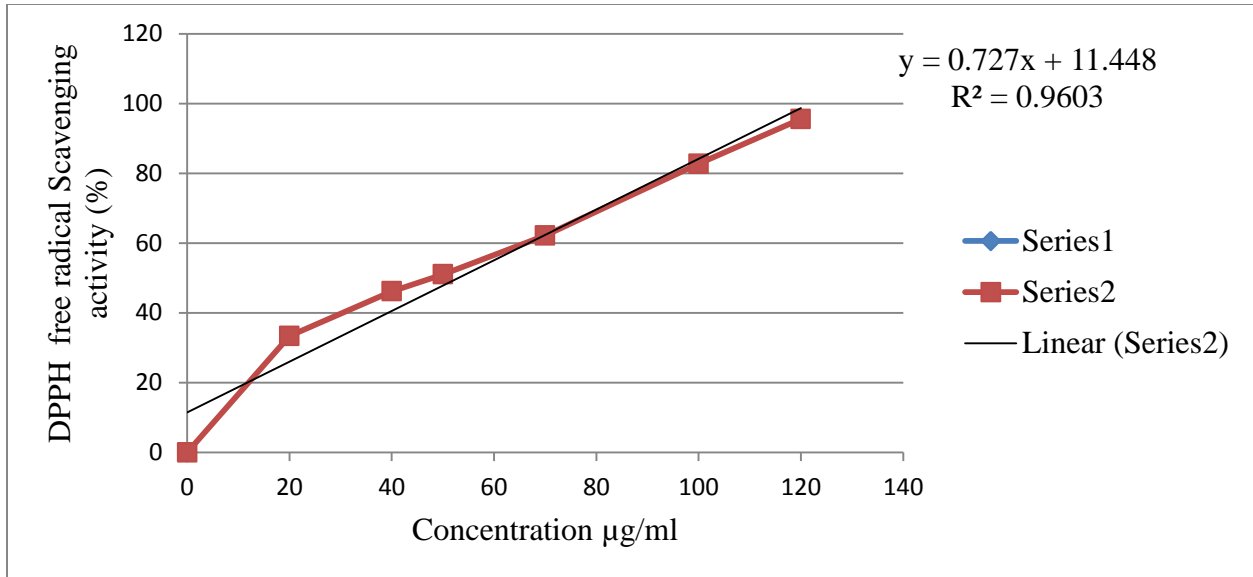
### 7.2.4. Standard curve for Total flavonoids



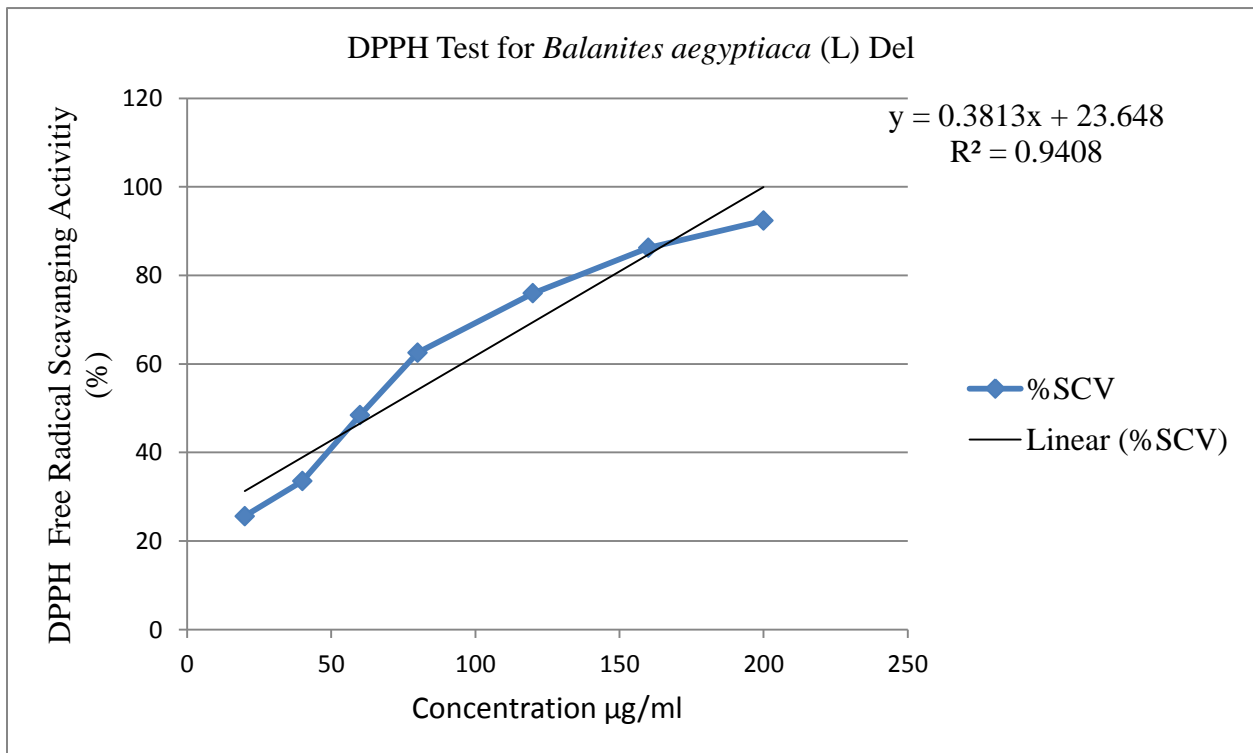
### 7.3. DPPH free radical scavenging activity of concentration of extracts and Ascorbic acid



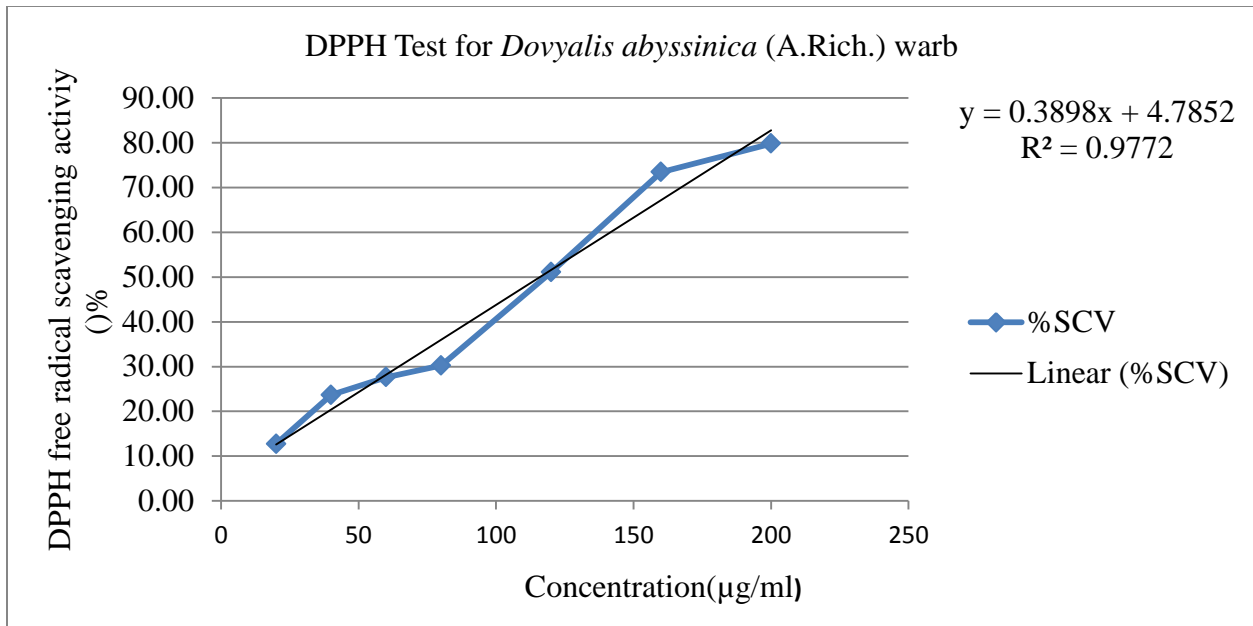
7.3.1. DPPH test for Ascorbic acid standard



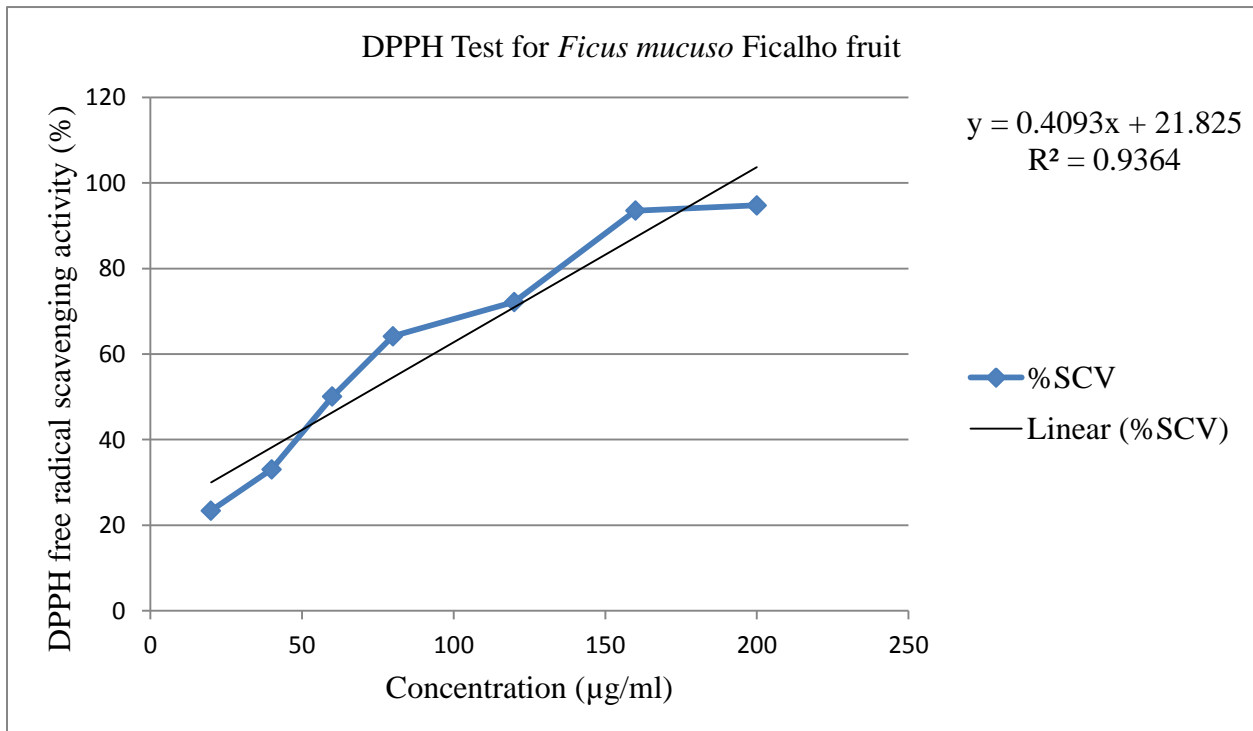
7.3.2. DPPH test for *Balanites aegyptiaca* (L) Del. fruit



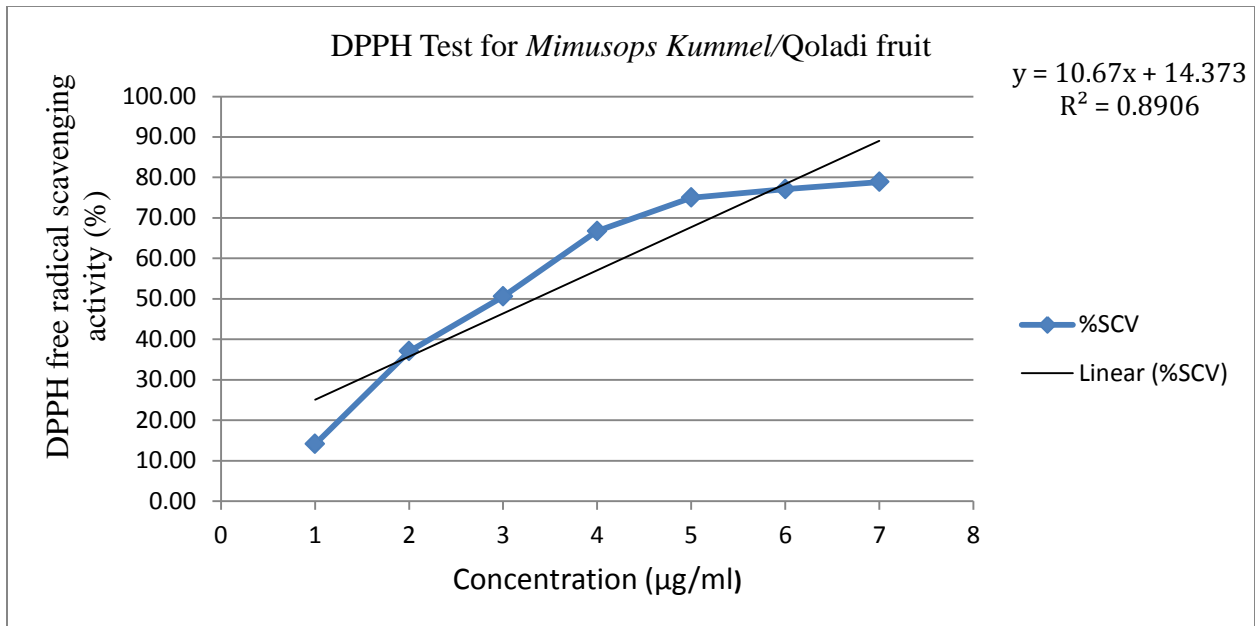
7.3.3. DPPH test for *Dovyalis abyssinica* (A.Rich.) warb. fruit



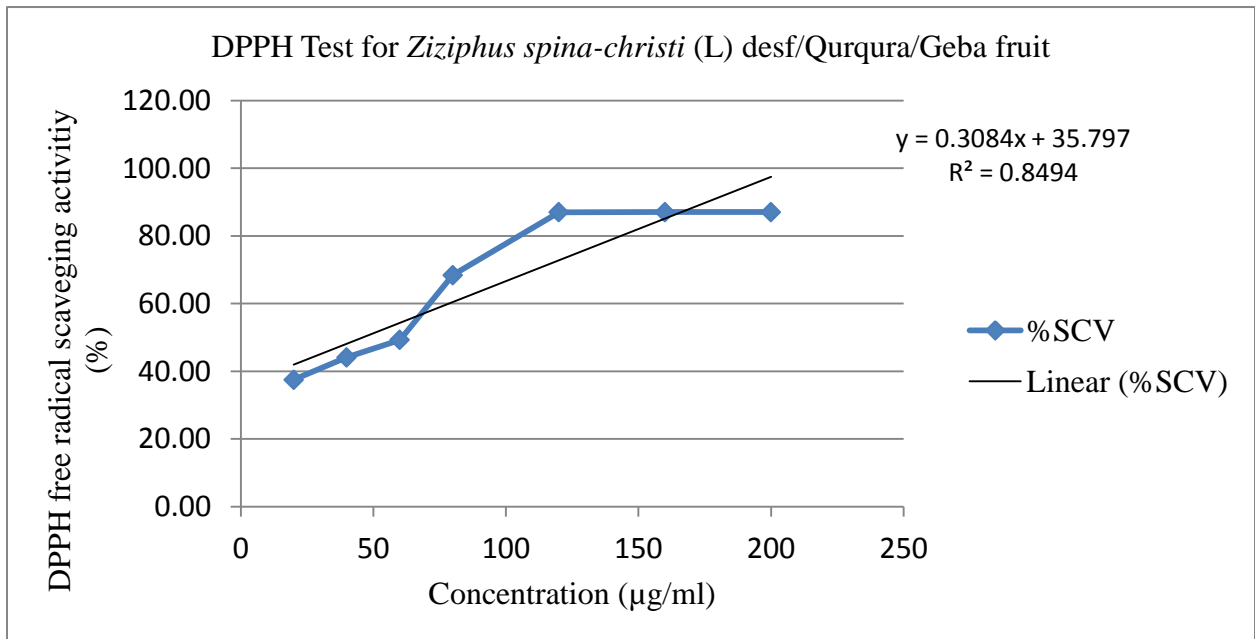
7.3.4. DPPH test for *Ficus mucuso* Welw.ex Ficalho fruit



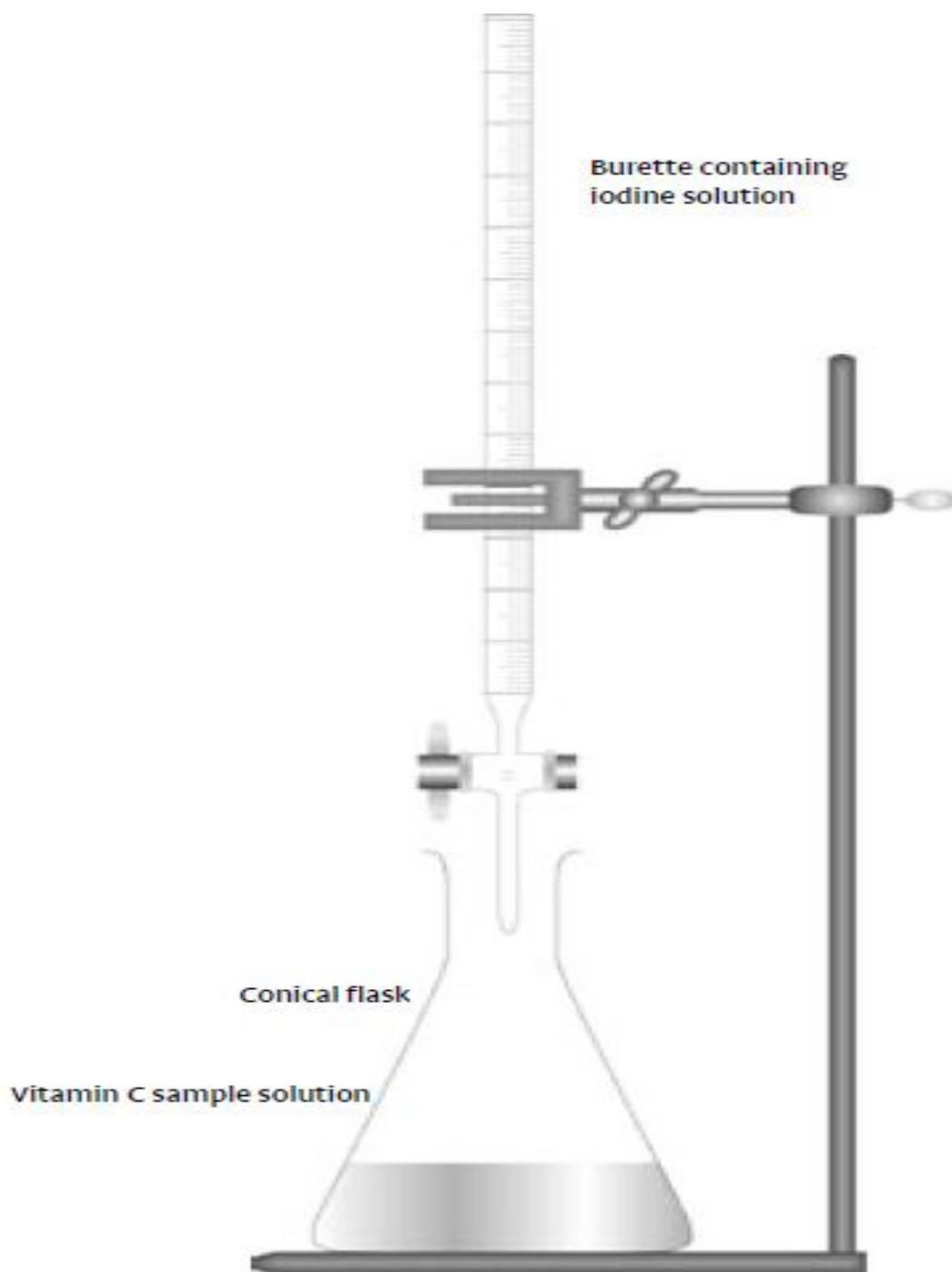
7.3.5. DPPH test for *Mimusops kummel* (Qoladi) fruit



7.3.6. DPPH test for *Ziziphus spina-christi* (L) desf/Geba/Qurqura fruit



7.3.7. Set up for determination of Vitamin C content by titration



## 7.4. INFORMED CONSENT FOR SENSORY EVALUTION

### WELLCOME AND THANK YOU FOR PARTICIPATING

**Consumer preference acceptance test for leather fruit product developed from *Doviyalis abyssinica* (A Rich.) warp pulp juice with *Carica Papaya* (*Caricaceae*) and white sugar.** Dear consumer you are invited to participate in a study entitled "**Nutritional quality of underutilised wild edible fruits grown in Ethiopia**". The overall objectives of the study is to asses the nutritional quality of the underutilized wild edible fruits, anti-nutrituonal components, phytochemical constituents and increase the palatablity of the fruits by developing fruit leather products. The product will be evaluated by 9- hedonic scale sensory evaluation methods. You will be oriented about the test instructions to identify name and classify a range of sample attributes (color, taste, texture and overall appreance). You will be asked to taste and spit samples and to rate the samples for intesity of each attributes. If you have a prior expreance of any allergic reactions to leather fruits products you should not participate in this sensory evaluation.

Don't foerget to rinse your mouth with water between samples, and wait for 30 seconds before you taste the next.

#### 7.4.1. Difference test format

Date \_\_\_/\_\_\_/\_\_\_ time \_\_\_\_\_ product \_\_\_\_\_

Dear panelist here are samples for evaluation. Are samples differ or the same in overall appearance, color, taste, texture (mouth feel).

Attributes	The same	Different
Overall appreance		
Color		
Taste		
Texture (mouth feel)		

## 7.4.2. Preference Ranking test format

Preference ranking test	
Fruit Leather	
Name _____ Session code _____ Date _____	
Please rinse your mouth with water before starting, before each sample and any time you need to.	
In front of you there are 3 samples. Beginning with the sample on the left, taste each one. After you taste all samples, you may re-taste as often as if you need. Rank the samples from the most preferred (=1) to least preferred (=3).	
Sample	Rank (1 to 3), Ties are not allowed.
672	_____
654	_____
521	_____
<b>Thank you for your participation.</b>	

$$\chi^2 = \frac{12}{(k)(J)(J+1)} \times \sum T^2 - 3(k)(J+1)$$

Where k=number of panelists, J=number of products and T<sub>j</sub>=rank sums with degrees of freedom for  $\chi^2=(k-1)$

When it has been determined that  $\chi^2$  is significant, a comparison of rank is done, it is referred to as the “least significant ranked difference”(LSRD) given by the formula:

$$\text{LSRD} = t \times \sqrt{\frac{N \times k(k-1)}{6}}$$

Where k=the number of samples, N=the number of panelists,  
t=the critical t-value at df=N-1 and  $\alpha =0.05$

### 7.4.3. A 9-point hedonic scale rating format

Dear participant, as a representative of the consuming population, quantify the degree of liking or disliking the products one by one separately. Please taste each of the coded samples in the set sequence presented from top to bottom. Use the appropriate scale to show your attitude by checking at the point best describe your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us. Please put ‘√’ sign in the box that best describes your overall opinion of the sample.

Don't forget to rinse your mouth with water between samples, and wait for 30 seconds before you taste the next.

Score	Sensory perception	Sensory quality attribution				
		Appearance	Color intensity	Taste	Texture mouth feel	Overall acceptability
9	Like extremely					
8	Like very much					
7	Like moderately					
6	Like slightly					
5	Neither like nor dislike					
4	Dislike slightly					
3	Dislike moderately					
2	Dislike very much					
1	Dislike extremely					

Additional comment if any

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Thank you

Every panellist tested and evaluated overall acceptability for each formulation by using a paper based scorecard, which presented a linear, structured scale with ends and middle anchored “I really dislike it”, “I neither like nor dislike it” and “I like it very much”. Each formulation was coded with random three-digit numbers. Before evaluating a given set of samples, the panellists were instructed to taste and evaluate from left to right, and to rinse their mouths twice with water between samples to minimise residual flavour effects. They were also told to score each sample before moving to the next one.