CENTER FOR FOOD SCIENCE AND NUTRITION

Physicochemical, Antimicrobial, Antioxidant and Sensory Characterization of Belete-Gera Forest Honey, Jimma, Ethiopia

Thesis submitted to Natural and Computational Sciences,
Center for Food Science and Nutrition

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DECLARATION

I, the undersigned, declare that this is original work and has never been presented in this or any other university and that all the source materials used for this thesis have been duly acknowledged.

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The thesis has been submitted for examination with any approval as university Advisor.

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Addis Ababa University
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Physicochemical, Antioxidant, Antimicrobial and Sensory Characteristics of Belete-Gera Forest Honey, Jimma, Ethiopia.

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Thesis submitted to School of Graduate studies of Addis Ababa University in partial fulfilment of the Degree of masters of Science in Food Science and Nutrition

Approved by examining board		**Signature**	**Date**
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LIST OF ABRIVIATTIONS

a_w	Water activity
CHO	Carbohydrate
DPPH	2, 2 -Diphenyl 1-picryl hydrazyl assay
FRAP	Ferric Reducing Antioxidant Power
GI	Glycemic Index
HMF	Hydroxymethylfurfural
HPLC	High Pressure Liquid Chromatography
NHB	National Honey Board
ORAC	Oxygen Radical Absorbance Capacity
PCP	Polychlorinated biphenyls
ROS	Reactive Oxygen Species

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ABSTRACT

The study aimed to investigate the quality of honey based on botanical origin, physicochemical, antimicrobial, antioxidant and sensory properties collected from Belete -Gera forest, Jimma, Ethiopia. A total forty eight samples honey samples were collected from Jimma- Ethiopia and categorized into seven monofloral honey using melissopalynology. Sugars (fructose, glucose, sucrose, maltose) and hydroxylmethyfurfural were investigated using high performance liquid chromatography and moisture, pH, acidity, ash, a_w, conductivity and colour were analysed using harmonized method of the international honey commission. The antioxidant content (phenol, flavonoid) and antioxidant activity (2, 2 -Diphenyl 1-picryl hydrazyl assay, ferric reducing antioxidant power, Peroxide Radical scavenging activities) were analysed using UV-spectrophotometer. The antimicrobial properties were analysed using disc diffusion and sensory acceptability based on seven hedonic scale. The monoflora honey found in Belete-Gera were; Eucalyptus, Guizotia spp, Vicia faba, Trifolium, Vernonia, Hypericum and Sativa in which their total sugar content 64.67±6.18/100g (Eucalyptus), 63.8±5.74g/100g (Guizotia), 63.08±5.14g/100g (Viciafaba), 62.16±4.44g/100g (Tirifolium), 64.68±4.35g/100g (Vernonia), 63.45±5.19g/100g (Hypericum), 64.73±4.44g/100g (sativa), pH 4.10±0.01(Eucalyptus), 4.3±0.00 (Guizotia), 4.23±0.02 (Vicia faba), 4.25±0.00 (Trifolium), 4.09±0.03 (Vernonia), 3.70±0.01(Hypericum), 3.620±.00 (Sativa), moisture content 15.0±0.16 (Eucalyptus), 17.0±0.32 (Guizotia), 18.8±0.15 (Vicia faba), 15.8±0.32 (Tirifolum), 19.0±0.15 (Vernonia), 16.2±0.29 (Hypericum), 18±0.24% (sativa), electrical conductivity 0.50±0.04 (Eucalyptus), 0.20±0.01 (Guizotia), 0.2±0.00 (Viciafaba), 0.42±0.12 (Tirfolium), 0.31±0.07 (Vernonia), 0.37±0.05 (Hypericum), 0.26±0.04mS/cm (Sativa), HMF12.2±4.8 (Eucalyptus), 16.1±3.9 (Guizotia), 16.33±4.5 (Viciafaba), 21.95±0.87 (Trifolium), 18.23±1.72 (Vernonia), 12.25±4.94 (Hypericum), 19.20±4.20g/kg (Sativa), a_w 0.53±0.04 (Eucalyptus), 0.56±0.01 (Guizotia), 0.59±0.00(Vicia faba), 0.54±0.01(Trifolium), 0.60±0.00 (Vernonia), 0.55±0.00(Hypericum), 0.59±0.00%(Sativa), ash content 0.30±0.0 (Eucalyptus) , 0.1±0.02 (Guizitia), 0.30±0.0 (Vicia faba), 0.10±0.00 (Trifolium), 0.6±0.04 (Vernonia), 0.2±0.01, 0.1±0.00mg/kg (Sativa) respectively. Mean phenol content for Eucalyptus, Guizotia spp, Vicia faba, Trifolium, Vernonia, Hypericum and Sativa were: 615.6±5.50, 536.4±4.00, 453.1±3.12, 413.2±2.51, 425.0±2.00, 406.7±3.13, 61.95±1.47GAE/kg respectively; Flavonoids had 41.50±0.84, 24.22±0.73, 31.83±0.16, 11.09±0.11, 12.18±0.01, 25.62±0.08, 18.90±0.11mgQE/kg respectively; , DPPH had 47.40±1.13, 52.72±0.32, 42.27±1.13, 55.93±0.69, 37.29±0.11, 60.01±0.087, 72.76±5.09% respectively and peroxide radical scavenger had 63.6±2.82, 59.2±0.46, 86.3±11.03, 72.7±6.36, 76.2±7.21, 82.4±7.91, 65.0±1.13% respectively. The mean sensory acceptance for Eucalyptus and sativa taste was 5.44±0.95, 5.32±1.13 respectively; smell 5.54 ±0.95, 5.20±0.94, respectively; color 5.52±0.78, 5.86±0.83respectively and over all acceptability was 5.78±0.84, 5.58±0.99 respectively in a scale of seven. The results of honey indicated that, all the honey samples collected from Belete-Gera forest area satisfy the Codex, EU and Ethiopian standard. Eucalyptus honey had the best quality as compaiered from the rest and Honey is unique on its floral source of the nectar from which it was made and assessing the physicochemical and antioxidant property of honey from different area is necessary.

***Key words**: Antioxidant, Antimicrobial, Honey, Physicochemical*

1. INTRODUCTION

1.1. Background

Honey is the natural sweet substance produced by honey bees from nectar of plants or from secretions of living parts or excretions of plants sucking insects on the living parts of plants which bees collect, transform by combining with specific substances of their own deposit, dehydrate, store and leave in the honey comb to ripen and mature. Honey consists essentially of different sugars predominantly fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection. The color of honey varies from nearly colorless to dark brown. The consistency can be fluid, viscous or partially to entirely crystallised. The flavour and aroma vary which are derived from the plant origin (Codex, 2001).

Honey is the only available sweetener in which during ancient cultures had been used for nutritional and medical purpose. For long time in human history it has been used as an important carbohydrate source and the only largely available sweetener until industrial sugar production. The annual world honey production is 1.8 million tons, led by china with 27% of total and the consumption differs strongly from country to country. Ethiopia is currently ranked as the largest honey producer in Africa and third largest worldwide by producing 45,300t of honey in 2010 (FAO, 2012). The country ears an average of 35 million USD annually from the sale of honey (Gidey and Kibrom, 2010).

About 95% of the honey dry matter is composed of carbohydrate mainly fructose and glucose. 5-10% of the total carbohydrates are oligosaccharides in total about 25 different di- and tri-saccarides. Besides, honey contain also small amount of protein enzymes, amino acids, aroma compounds and polyphenols and possess antimicrobial and antioxidant effects (Bogdanov, et al, 2008). Honey possess valuable nourishing, healing and prophylactic properties. These properties can be interpreted by its physical and chemical composition (Gairola et al, 2013).

Honey has an appreciable antimicrobial and antioxidant activities (Alberto et al, 2016). Honey possess antimicrobial activity which deepened on its chemical component and geographical sources and are related to the amount of hydrogen peroxidises that is enzyme present in different types of honey and origin of nectar with which the bee is nourished (Mohsen et al., 2011). The antioxidant activity found in honey largely depends on polyphenols and flavonoids which are known to play major role to protect the body against the effects of free radicals (Tomasz et al, 2013).

The Properties and compositions of honey depend on its geographical floral origin, season, environmental factors and the techniques beekeepers used to handle the honey. It is one of the few non allergenic foods that the body easily assimilates and contains the macronutrients especially as energy provider of high energy carbohydrate food (80-85%), in the form of di and monosaccharaides. More than 22 sugars are found in honey; however fructose and glucose are the major constituents. Honey also contains more than 180 substances including amino acids, enzymes, protein, vitamins, minerals, organic acids and phenolic compounds (Sohaimy et al., 2015).

The physicochemical parameters of honey like pH, moisture, sugar composition and hydroxymethylfurfural (HMF) content, color, acidity and specific conductivity are the quality indicators that characterize different honey varieties (Boussaid et al., 2014). There are also other parameter like enzymes activity, hydroxymethylfurural and amino acids in honeys which are relatively low, but possibly used as a honey quality indicator (Belay, et al, 2017).

1.2. Statement of the problem

Ethiopia has a huge potential of honey production, this is most likely due to its diverse climate and vegetation resource. Honey is unique depending on its floral source of the nectar from which it is made. Assessment of the physicochemical and antioxidant property of honey from different area is necessary for resource exploitation. Accordingly, honey from Jimma area need further study which is believed to produce from diversified plant type. In addition, the area is also known by contributing a large amount of honey to the country. Belete-Gera forest

is one of the larger remaining tracts of forest left in the country. The honey quality in this biodiversity with in the forest however is not studied before, so it is essential to know the properties of honey from this potential source.

Honey consumption is growing significantly due to its high nutritional value and unique flavour. The price of natural honey is much higher than other sweeteners making it susceptible to adulteration with cheaper sweeteners primarily sucrose and the crystallization property of honey is affected by sugar and water contents (Bogdanov et al., 2008).

Sugar, moisture, HMF, conductivity, ash, which were analysed in this study used as quality standard by EU, Codex and Ethiopian standard agency. The viscosity which is the state of being thick and sticky due to internal friction is measure of resistance to gradual deformation by share stress. The viscosity property of honey can be used to categorize honey into Newtonian and non-Newtonian. The viscosity of honeys strongly depends on temperature. As temperature become lower the honey become thick and sluggish. Therefore, the viscosity of honey is affected greatly by both temperature and water contents (Eroglu, Belay et al., 2017).

Honey is a flavour rich product created by bees through an active search and collection of nectar from flowers. Depending on the visited flowers and on the climatic conditions which nectar produced honey may test quite differently. The botanical origin of honey is one of the main quality parameter and mostly its price often related to this floral origin. But there is a concern which is affecting honey market due to increasing for authenticity of original products. Recently customers have developed special interest for physicochemical and antioxidant characteristics of honey based on botanical and geographical origins. Thus, characterizations of Belete Gera forest honey possibly provide comprehensive information for science and market.

1.3. Research questions

The result of analysis were answered what the botanical origin, physicochemical properties, antioxidant, antimicrobial and sensorial acceptability of Belete Gera forest honey, Jimma, Ethiopia.

2. OBJECTIVES

2.1. General objective

The general objective of the study was to investigate the characteristics of honey based on botanical origin, physicochemical, antimicrobial, antioxidant and sensory properties of honey collected from Belete-Gera forest, Jimma, Ethiopia.

2.2. Specific objectives

The specific objective of the study was in order to:

1. Identify the botanical origin of honey.
2. Determine the physicochemical properties of honey.
3. Determine the antioxidant properties of honey.
4. Identify the antimicrobial properties of honey.
5. Determine the degree of sensorial acceptability of honey.

3. LITRATURE REVIEW

3.1. Botanical origin of honey

Botanical origin is determined based on the relative frequencies of pollen types of nectariferous species using harmonized methods of melissopalynology (Belay et al, 2016). Honey designated by the geographical or topographical region produced exclusively with in the area and according to floral or plant source it comes wholly or mainly from the particular source and the organoleptic, physicochemical and microscopic properties corresponding with the origin. Generally there are two types of honey those are blossom and honeydew honey. Due to different proportions of the possible sources, nectar and honeydew coming from greater variety of plant, no honey is completely the same as other one. Unifloral honeys are regarded as a more valuable class of honey and botanical denominations are widely considered (Stefan et al, 2014).

The major honey varieties according to the flower source are alfalfa which is white or light amber color, Avocado honey from the flowers of this plant tends darker in color, basswood honey derived from blossoms trees which is watery white color, Blueberry honey color range from light amber to amber color, buck wheat dark brown with strong flavour, clover honey its color is white water to extra light amber.

The properties of the most common unifloral honey species in the world are black locust (Acacia, Robinia pseudocacia) and linden are very similar all over the world and some types like Eucalyptus, thyme, orange blossom can vary considerably in taste and colour depending on the plant and country of origin. The appreciation of unifloral honey varies in the different parts of the world. Honey dew honey like fir and pine honey are especially appreciated in different parts of Europe.

The honey bee species *Apis mellifera* which is spread all around the world its honey is the most widely collected and marketed around the world however there honey made by other species *Apis Cerana* in china, *A.dorsata*, and *A.flora* in tropical Asia (Stefan et al, 2015).

3.2. Physicochemical properties of honey

The quality of honey is mainly determined by its sensorial, chemical, physical and microbial characteristics (White et al, 2013). The physical properties of honey vary depending on water content, the type of flora used to produce the honey, temperature and the proportion of the specific sugars it contains. Fresh honey is a supersaturated liquid containing more sugar than the water can typically dissolve at ambient temperatures. At room temperature honey is super cooled liquid in which the glucose will precipitate into solid granules. This forms a semisolid solution of precipitated glucose crystals in a solution of fructose and other ingredients (White et al, 2013). Non osmotic and enzymatic activity, antimicrobial properties, microbial quality, color and chemical composition of honey are influenced by heat and storage (Susana et al, 2014).

The melting point of crystallized honey is between 40^{o}C and 50^{O}C depends on its composition. Below this temperature honey can be either in meta stable state meaning that it will not crystallize until a seed crystal is added. The rate of crystallization is affected by many factors but the primary factor is the ratio the main sugars fructose to glucose. Honeys that are supersaturated with very high percentage of glucose such as brassica honey crystallize almost immediately after harvesting while honeys with low percentage of glucose such as chestnut or tupelo honey do not crystallize. Crystallization is also affected by water content because a high percentage of water inhibits crystallization as high dextrin content. Temperature also affects the crystallization with the fastest rate occurring between 13^{o}C and 17^{o}C (White et al, 2013).

The reason for testing honey for quality control purposes is to verify the authenticity of the product and to reveal the possible presence of artificial components or adulterants as well as to address processing and market needs. This requires not only determining the moisture and mineral content (ash) but also the levels of HMF, acidity and sugars (Meda et al, 2005).

The physical and chemical feature of honey has low susceptibility have low susceptibility to the proliferation of microorganisms (low PH, low moisture content, oxidation reduction

potential, antimicrobial constituents. Nevertheless external factors such as environmental conditions and handling and storage can have a negative effect on the final quality. Reduced number of microorganisms under normal moisture conditions would not interfere with the quality of honey. The moisture content influences the flavor, preservation, viscosity, specific weight, crystallization and palatability contributes to the development of fermenting microorganism (Ananias et al 2011).

3.2.1. Sugars in honey

Carbohydrates (CHO) are the main constituents of honey comprising about 95 % of the honey dry weight. The main sugars are monosaccharides fructose and glucose. Additionally about 25 different oligosaccharides are present. The principal oligosaccharides in blossom honey are sucrose, maltose, trehalose and turanose as well as some nutritionally relevant one such as panose1-ketose, 6-ketose and palatinose. Compared to blossom honey honeydew honey contains higher amount of the oligosaccharides melezitose and raffinose (Bogdanovetal, 2015).

The largest portion of the dry matter in honey consists of sugar and this concentrated solution of several sugars results in the characteristics physical properties of high viscosity, stickiness, high density, granulation tendencies, tendency to absorb moisture from air and immunity from some types of spoilage (White et al, 2013).

A study showed that the mean total sugar content of honey ranged from 72.4 to 79.7g/100g of which monosaccharide sugars were dominant among those the fructose content was found higher than glucose (Belay et al, 2017).

A 100 gram serving honey provides 304 kilocalories having 82% carbohydrate and its glycemic index (GI) ranges from 31 to 78 depending on the variety and the specific composition, color aroma and flavor of honey and the flowers foraged by bees that produced the honey. A study conducted at several United States regions shows that honey typically

contains fructose 38.2%, Glucose 31.3%, maltose 7.1%, sucrose 1.3%, water 17.2% and higher sugar 1.5% (Khan et al, 2015).

Honey is highly concentrated aqueous solution of glucose and fructose along with small amount of different sugars and it is composed of different types of sugars monosaccarides, disaccharides and oligosaccharides which constitute about 95% of total dry weight of honey (Shafiq et al, 2014).

Sugars govern honey property and are related to degree of maturity and botanical origin of honey. The maximum concentration of fructose (43.1± 0.4 g/100g) in *Becium glandiflourm* and the maximum glucose content was found in *Leucas abyssinica* (37.2±0.4g/100g) and minimum in *Becium grandiflorum* (29±3g/100g) and the mean total sugar content of honey ranged from 72.4 to79.7g/100g honey, the fructose content was found to be higher than that of glucose. Sucrose is an essential sugar for honey quality detection and screening (Belay et al, 2017).

Botanical origin of honey is related to sugar composition because the carbohydrates are more than 95% of honey solids. Reducing sugars invert sugars mainly fructose and glucose have been found to be the major constituent of honey. A high concentration of sucrose most of the time means an early harvest of honey because sucrose has not been fully transformed to glucose and fructose by the action of invertase and the mean percentage of sucrose (3.62+0.13%) of all honey samples (Derebasi et al, 2014).

Sugars are present in many foods varying from naturally occurring products such as honey formulate foods including jellies and gels as well as confectionary. Honey is composed mainly of sugars which the monosaccharide fructose and glucose are the most abundant approximately 70-80% of the honey solids further more small quantities of disaccharides maltose and sucrose are also present (Costa et al, 2015).

3.2.2. Moisture content

Moisture content of honey is a limiting factor in determination of its quality, stability and spoilage resistance against yeast fermentation. Lower moisture (<20%), elongates honey shelf life and protects honey from attack by microorganisms which would be a feature of large majority of commercial honey products (Sohaimy S. et al 2015) and contributes to its ability to resist fermentation and granulation during storage. An increase in moisture content is indicative of adulteration and low moisture content protects honey from the attack by microorganisms (Adenekan et al, 2012). The moisture content of honey depend on various factors such as harvesting season, source plant's floral type and the moisture content of the original plant nectar (Serajul et al., 2014, Tadesse et al, 2015). Moisture is largely influenced by geographical position from where the nectar and pollen producing plant and the bee colony, degree of maturity, botanical origin of honey and harvesting techniques (Belay et al, 2017).

3.2.3. pH

Honey contains a number of acids which include amino acids (0.05-0.1) and organic acids 0.57% and the average pH of honey is 3.9 with a typical range of (3.4 to 6.1). A pH level between 3.2 and 4.5 and the natural acidity of the honey inhibit the growth of microorganism as the optimal pH for most organisms is between 7.2 and 7.4. Free acidity is an important parameter related to the deterioration of honey and it is characterized by the presence of organic acids in equilibrium with lactone and internal esters and some inorganic ions such as phosphates, sulphates and chlorides and the presence of different organic acids , geographical origins and harvest seasons can affect the honey 's acidity (Roseaneet al, 2015).

3.2.4. Hydroxymethylfurfural

The compound 5-hydroxymethyl-2 furalde-hyde (HMF) is resulted from the decomposition of monosaccharides or the maillard reaction, when honey is heated or stored for a long time as well as the heat treatment temperature and storage time increases (Laleh et al, 2013). HMF is

produced at pH 5 or lower and HMF occurs naturally in honey especially in warm climates (White et al, 2013).

HMF content is presented as indicative of honey determination. HMF content varied between 0 and 4.12mg/kg which can be considered fresh honey. Other factors that influence the level of 5-HMF are the sugar profile presence of organic acid, pH, moisture content and floral source. Therefore the 5-HMF content gives only an indication of overheating or inadequate storage conditions. The HMF formation increases as a result of bad storage and heating. It is an excellent indicator of honey freshness and proper storage (Dubero et al, 2015). A high 5-HMF content in honey can also indicate falsification by adding invert syrup because 5-HMF can be produced by heating sugars in the presence of an acid to the inversion of sucrose (Roseane et al, 2015). The presence of 5-HMF is an aldehyde that is often used for the assessment of honey quality is considered an important physic-chemical parameter to determine the status of honey samples.

HMF is often used as an indicator for the quality of honey and is generated by the decomposition of fructose in acidic conditions. It occurs naturally over time in most honey. In addition to this high levels of HMF may be result due to inadequate storage and adulteration with sugar or severe heat treatment. Even though HMF is not thought to be a harmful substance, food standards in many countries regulate the levels of HMF in honey (Keppy et al, 2009).

3.2.5. Electrical conductivity

Electrical conductivity of honey is used in routine honey quality control and purity. The conductivity is a good criterion of the botanical origin which depends on the ash acid content of honey and the higher their content results high conductivity (Bogdanov et al, 1999).

It gives an indication regarding its origin, floral or honey dew the source of nectar and also can detect whether bees have been artificially fed with sugars. Honey contains acids and mineral salts, organic acids, proteins and polyols, compounds which chemically are called

ionisable in a solution form and have the property to conduct electric current. The electrical conductivity of honey is defined as that of 20% (w/v) weight in solution at $20^0C \pm 0.5$ where the 20% refers to the anhydrous honey and is expressed in mill Siemens per centi meter (Desissa et al, 2014).

Electrical conductivity values with solutions of 20-25% dry matter and proposed taking measurements with solution of 20% dry mater is standard in many countries (Sancho et al., 2013).Electrical conductivity is a good criterion of the botanical origin of honey (Bogdanov et al, 1999).

3.2.6. Specific rotation

The main carbohydrates of honey are fructose, glucose, sucrose and maltose. Therefore low content of sucrose and high content of glucose and fructose in honey are parameters of characterization of honey quality. Several content of honey contain high content of maltose and each carbohydrate has specific angle of rotation of polarized light (specific rotation) and it depends on rotation as well as content of carbohydrates in honey (Fredijs et al, 2015).

Honeys and honey dews have the property of rotating the polarization plane of polarized light and this property depend s largely on the sugars of honey their type and relative proportion. The importance of this method is that appears to be valid to separate floral honeys from honeydew honeys.

The floral honeys normally present higher content in fructose and honeydew honeys present lower content of fructose but higher in melezitose, glucose and erlose. Therefore Each carbohydrate has a specific angle of rotation of polarized light (specificrotation)Fructose[α] D20=-92,4°,Glucose[α]D20=+52,7°, Melezitose[α]D20=+88,2°, Erlose [α]D20=+121,8° (Serrano et al, 2010).

According to their origin, honeys are divided into honeydew honeys and nectar honeys. Honeydew honeys are produced by honey bees from execration from plant sucking insects,

nectar honeys are made up of the nectar or secretion of plants. Both kinds of honeys are distinguished by optical rotation as honeydew honeys show positive rotations while in nectar honeys the optical rotation is negative. This is caused by opposed optical rotation of fructose and glucose/sucrose. Optical rotation in honey is there for determined by the composition and used in quality control to determine the contamination of honey dew honeys with blossom honeys (Anton, 2016).

Optical rotation is a parameter that is a relation to determination of botanical origin of honey groups blossom, honeydew and compound honeys. The specific rotation calculated from angular rotation, ray circuit length and grams of taken dry mater (Pridal et al, 2002).
Honey has the property of rotating the plane of polarization of polarized light. Sugars which are the major components of honey are chiral compounds and like almost all naturally occurring carbohydrates are the D molecules. Each sugar at a given concentration affects the rotation by an amount that that is characteristics of the sugar. The optical rotation of a mixture depends on the relative proportions of the sugars present. Measure the optical rotation of honey is a supersaturated solution of mostly D-fructose and D-glucose which a small amount of sucrose (Esso, 2001).

3.2.7. Water activity

The water activity of the honey samples were in the range of 0.481 to 0.542 and the water activity of honey is a key factor concerned in spoilage by fermentation and is a major factor preventing or limiting microbial growth and in several cases aw is the primary parameter responsible for stability, modulating microbial response and determining type of microorganisms encountered. The knowledge of water activity of honey is needed to predict moisture exchange with the environment since water activity is the driving force behind water transfer from /to honey. The water activity in honey results mainly from the concentration in water of honey of monosaccharide's fructose and glucose and to a lesser extent to some disaccharides such as sucrose and maltose (Chirife et al, 2016).

The moisture content of honey is highly important for its shelf life if there is less water activity creates non satisfactory condition for the activity of several microorganisms that depend on higher amount of free water for growth and survival (Lorena, et al, 2012).

The stability of honey primarily affects the availability of water which is characterized by water activity. The water activity of honey during storage may vary and may occur many physical and chemical changes. Storing of honey in an atmosphere at too high humidity causes the water vapor adsorption from the environment which increases the honey water activity (Aleksandra et al, 2014).

3.2.8. Viscosity

Like most viscose liquids honey is thick and sluggish with decreasing temperature at 20^0C and appearance of honey feel solid but it can continues to flow at very low rates (Hemant et al, 2013). The viscosity of honey is affected by temperature, moisture content and flora source and viscosity changes as temperature, moisture content and floral source change and viscosity of honey decreases rapidly as its temperature rises.

Viscosity of different honeys at different share rates correlates with other physicochemical and sensory property. As temperature increases viscosity falls due to less molecular friction and reduced hydrodynamic forces. The rheological behavior of honey and the influence of temperature need to investigate for proper handling, packaging and processing issues. An increase in temperature produces a clear decrease in the value of viscosity which has dramatic decrease in the low range of temperature (Gomez et al, 2009).

The surveys of rheological properties play an important role in food industry. The viscosity value of honey depends on water content. The higher water content in the honey means lower viscosity and higher fluidity. Most honeys show Newtonian behavior. The honey with the highest value of activation energy display the highest viscosity value (Travnicek et al, 2017).

The temperature effect on viscosity follow an Arrhenius type relationship and honey varieties exhibited Newtonian behavior. This show dependence of viscosity on increasing temperature applied (Mossel et al, 2000).

3.2.9. Colour

Color is the first attribute of honey and is very important for commercialization and it is an important parameter in the quality, acceptance and presence of consumers (Susana et al, 2015). The color of honey is classified into seven which includes water white, extra white, white, extra light amber, light amber, amber and dark amber (NHB, 2008).

Honey also contains the colors orange, yellow and green. The rosemary honeys the green color which is negative value while mint honey had highest redness followed by eucalyptus honey. The color of honey depends on its ash content, the temperature at which the honey remains in the hive and storage time (Roseane, et al, 2015).

The color in liquid honey varies from clear and colorless (like water) to dark amber or black. The various honey colors are basically all shades of yellow and amber. Color varies with botanical origin, age and storage conditions but clarity depends on the amount of suspended particles such as pollen. Crystallized honey turns lighter in color because the glucose crystals are white (Esteraf-Oskouei et al 2013).

3.2.10. Optical density

The density of honey depends on temperature and honey density decrease with increasing temperature. The optical density of a medium is not the same as its physical density. The physical density of honey refers to mass volume ratio. It related to the honey sluggish tendency to maintain the absorbed energy of an electromagnetic wave in the form of vibrating electrons before reemitting it as a new electromagnetic disturbance. The more optical dense that honey is, the slower that a wave will move through the material (Owayes et al, 2005).

Honey density expressed as specific gravity is greater than water density by about 50% and it also depends on the water content of honey. Because of the variation in density, it is sometimes possible to observe distinct satisfaction of honey in large storage tanks. The high water content (less dense) honey settles above the denser honey. Such inconvenient separation can be avoided by more through mixing (Bogdanov et al, 2009).

Measurement of the optical density of honey with a colorimeter using the complete light spectrum offers a precise, reproducible method for classifying honey according to its color. The method can be used for honeys from most floral sources and provides a practical systemic basis for blending honey to any required color (Townsend et al, 2015).

3.3. Antimicrobial properties of honey

The antimicrobial capacity of honey has been attributed mainly to the presence of hydrogen peroxide which is produced from glucose oxidase and phenolic compounds found naturally in honey used at low concentration induce wound healing (Sara et al, 2015). The main actors of antibacterial potency of honey are hydrogen peroxide and gluconic acid which originated from dissolution of sugar by honey's glucose oxidase and the action of honey linked with osmolarity which create unfavourable to bacteria growth and proliferation. The acidic pH of honey comprised between 4.31 and 6.02 play a role in microbial control. Additional honey components such as aromatic acids or phenolic compounds contribute to the overall antimicrobial activity (Aggad et al, 2014).

Honey has the potential to be used as an antibacterial agent to prevent and control infection with *S.aureus* and the use of natural products to enhance wound healing is common practice in many part of the world and the honey which consists of supersaturated solution of sugars and has a low pH together with honey's high osmolarity and presence of hydrogen peroxide, reduces bacterial growth at the wound site. When used as wound dressing honey provides an ideal environment for the rapid tissue repair, regeneration and re-modeling that are essential for growth the wound bed. The *S.aureus* is the most frequently isolated wound pathogen and

it is becoming increasingly resistant to antibiotics. Honey is effective in eradicating antibiotic resistant *S.aureus*(Nagi et al, 2009).

Honey has higher antimicrobial activity against *colusridumperfergens* and there are many factors in honey that effect on the growth of *C.perfringens*. the *colustridumperferingens* is not tolerant of low water activity (a_w) reported values for tolerance between 0.93 and 0.97 and honey is a supersaturated sugar solution with 0.56-0.62 aw which explains the inhibitory activity against *C.perfringes* and the *C.perfringens* initiate growth most readily at neutral pH although excellent growth occurs between pH 6 and 7 and the growth of C. perfirenges is severely limited at pH <5.0 and pH .8.3 and the pH of honey is between 3.2 and 4.5. Different honeys have varying and diverse effects on the growth of bacteria each organism has unique response profile to different honeys and the antimicrobial effects are due to combination of several factors in honey (Oinaale et al, 2015).

Honey exhibits a level of antibacterial activity which generally increased with increasingly concentrations and the degree of antibacterial activity varied according to the type of bacteria and type of honey. The manuka honey inhibits bacterial species and acts on both gram positive and gram negative bacteria. As compared the antibacterial activity of organic honey and Manuka honey the most inhibition was obtained in manuka honey against *Escherichia Coli* and *Staphlococcusepidermidis* with a peak inhibitory against staphylococcus epidermis at 30% concentration (Swapna et al, 2013).

Honey has powerful antimicrobial activity against dermatological relevant microbes. These finding are particularly promising in current times when the problem of antimicrobial drug resistance is considered a global crisis. Honey can reverse antimicrobial resistance and reduced microbial pathogenicity and no honey resistant microbial strains have emerged to date due to the multi factorial nature of the antimicrobial properties of honey. As honey from diverse floral origins have been shown to have antimicrobial activity against a range of skin relevant microbes and there are countless varieties of honey being produced worldwide and some may have superior antimicrobial activities (Paulin et al, 2015).

Antimicrobial activity of honey sample against S.aureus in different concentrations (25, 50, 75, 100%of honey samples were checked for their antimicrobial properties. The minimum inhibitory concentration of the honey samples were determined using broth dilution technique and the sample of the honey showed inhibitory effects in vitro at 50, 75 and 100% concentration and the minimum inhibitory was 12.5mg/ml and the study showed that honey like antibiotics has antimicrobial effect which can be used as alternate therapy against bacteria and have antimicrobial action against a broad spectrum of bacteria both gram positive and gram negative bacteria (Taha et al, 2009).

In recent years a marked increase in antibiotic resistance by certain pathogenic bacteria has been seen and this increase is due to the overuse and misuse of antibiotics and has resulted in several high resistant strain of staphylococcus aureus frequently causing nosocomial infections and alternative methods of traditional antibiotics should be explored like honey against staphylococcus aureus and honey was found to have inhibitory effect on bacteria growth and comparable in strength with to the antibiotic compares and potential for application as viable methods of bacterial control particularly as first line treatment for mild infection or as preventive treatment (Monica et al, 2014).

Honey can be effectively used as antimicrobial and antioxidant agent to overcome the problem of bacterial infections and multi drug resistant microbial strains as to enable & enhance the market revenue throughout the world and honey a cheap antibacterial agent can be used to manage chronic wound types such as burns, leg ulcer or surgical wounds which infect with drug resistant staphylococcus bacteria (Azeem et al, 2014).

The production of new antibiotics substances requires enormous budgetary costs. The possible alternative use of natural substances should reconsider. Among those the hive products such as honey which is not toxic very efficient antimicrobial with a broad spectrum of action. The use of honey in wound management has become widely available. This renewed interest is mainly due to the growing clinical problems of antibiotic resistant bacteria and the combined difficulties in managing chronic wound types that may become infected for example with methicillin resistant staphylococcus aureus (Aggad et al, 2014).

Honey has been known to possess antimicrobial property as well as wound healing activity. The healing property of honey is due to the fact that it offers antibacterial activity maintains a most wound conditions and is high viscosity helps to provide a protective barrier to prevent infection. The antimicrobial activity in most honeys is due to the enzymatic production of hydrogen peroxide manuka honey displays significant antimicrobial (antibacterial effect) which related to the low PH level of honey and its high sugar content (high osmolarity) that hinder the growth of microbes (Manisha et al, 2011).

3.4. Antioxidant properties of honey

Honey represents a natural product that does not carry side effect which can harmful to health. Among the compounds found in honey vitamin C, phenol compounds, catalase, peroxides, glucose oxydase, enzymes have antioxidant properties. Honey also contains flavonoids and carotinoids and the high level of these indicators to ensure a high level of antioxidants in honey. The amount, type of antioxidant compounds depends largely upon the floral source and variety of the honey. In general darker honeys have higher antioxidant content than lighter honey (Khalil et al, 2014).

Honey serves as a source of natural antioxidant which plays an important role in food preservation and human health by combating damage caused by oxidising agents. The antioxidants present in honey include both enzymatic and non enzymatic substances (Isabel C.et al2014). The amount and type of antioxidants largely depends on the floral source or honey variety and a correlation between antioxidant activities with total phenolic content (Linkon et al, 2015).

The antioxidant activity of natural honeys depends on their chemical composition such as phenolics, flavonoids, enzymes, organic acids amino acids, millard reaction products ascorbic acids carotenoids as well as their origins. Phenolics or polyphenols are one of the most important classes of compounds found in honey the total concentration of phenols in honey is highly depends on its plant source (Ibrahim et al, 2012).

Honey have beneficial to health due to its antioxidant properties and is very complex mixture containing a number of ingredients involved in oxidant or antioxidant physiological processes including hydrogen peroxide, nitrite, nitrate, glucose oxidase, chlorine, iodine, catalase, flavonoides, phenolic acids and maillard reaction products (Moussaet al 2012).

Honey is recognized as having different biological properties including antioxidant effect; phenolic acid and flavonoids are the main antioxidant and phenolic content of honey are responsible for free radical scavenging and antioxidant activity. The total flavonoid and phenolic content generally determine using a spectrophotometer assay based on the formation of alimunium chloride complex which applies for quantification of flavonoids in honey (Silvoetal, 2015).

Honey is recognized as having different biological properties including antioxidant effect, phenolic acid flavonoids are the main anti oxiodants and the phenolic content of honey using folin-ciocaletu method and the phenolics are responsible for free radical scavenging and antioxidant activity. The total flavonoid contents are generally determined using a spectrophotometer assay based on the formation of aluminum chloride complex which applies for quantification of flavonols in honey (Silvo et al, 2015).

4. MATERIALS AND METHODS

4.1. Study area

The Belete-Gera forest is 150, 000ha in size which is found in Jimma zone of Oromia regional state consists of two disjoint forests namely Gera forest and Belete forest and it is 430km away from Addis Ababa the capital of the country (Merga et al 2013). Belete-Gera forest has a tropical rainforest climate with long annual wet season from March to October and temperature between 20 -25^{o}C. The forest is found in Jimma, Ethiopia which is recognized as key biodiversity area. Belete Gera forest has a geographical coordinates Afalo ($7^{0}38^{0}13'16'$E and quacho ($7^{0}46^{0}20'7''$E) (Beenhouwer et al, 2015).

Figure 1: Location of Belete Gera forest, Jimma-Ethiopia.

4.2. Honey Sampling

The honey samples were collected based on floral calendar as stated by Belay et al. (2017). Six potential areas namely Achuwa, Shebe, Gera, Waskala, Beleteand Deri were purposely selected based the honey potential and 48 honey samples were randomly collected from traditional hives at farm gate. The collected honey samples immediately strained and pour in a food grade jars and stored at a temperature of less than 4^{o}C (Boulanouar et al, 2017).

Figure 2: Sample Collection process

4.3. Floral Origin Determination

The floral source of the honey were determine based on the relative frequency of the pollen types of nectariferous species using harmonized method of melissopalynology. The analysis were carried out to determine major honey source by centrifuging 10g sample of honey dissolved in 20ml of distilled water. The solution was centrifugate for 10min; and another 20ml of distilled water was added to completely dissolve centrifuged for 5min. The residue was allowed to dry in microscope slides by spreading with micro spatula. After applying glycerine jells; sample was examined through microscope and the plant source was identified using reference slides (Nuru, 2004, Ohe, 2004, Belay et al, 2017).

Melissopalynological analysis of the pollen type of honey could be placed into different class according to their percentage of dominance, predominant pollen (>45%), Secondary pollen (15% to 45%), important minor pollen (3% to 15%) and minor pollen (<3%). Pollen catalogue could be used to identify the botanical affinity of the pollen types (Taniamarie et al., 2013).The total number of pollen is calculated by using dilution factor and number of pollen in the sample (Rosdi et al., 2016).

4.4. Physicochemical properties

4.4.1. Sugars

The sugar in honey was identified based on AOAC (1990) 969.38 and International Honey Commission (IHC, 2009). The sugar content of honey sample was determined by HPLC with RI-detection.

The sugar content (fructose, sucrose, glucose and maltose) in the honey samples were identified based on their retention times and the flow rate was 1.3ml $min^{-}1$, mobile phase acetonitrile/water (80:20v/v), column and detection temperature $30^{o}C$ and the sample volume of 10µl dissolved in 40ml water and transferred qualitatively to the flask and filled to the mark with water fructose (2gm), glucose (1.5gm), sucrose (0.25gm) maltose (0.15gm).

Equipments used include 25ml pipette, sample vials, calibrated flasks 100ml volume, ultrasonic bath, syringe, membrane filter, high performance liquid chromatography and analytical stainless steel column and the reagents used were methanol for HPLC, acetonitrile for HPLC, distilled water, standard substances fructose, glucose (Bogdanov, 2009, Shobham et al, 2017).

4.4.2. Moisture

Moisture was determined using AOAC (1989) and Harmonised method of IHC (2009). Honey moisture is the quality criterion that determines the capacity of honey to remain stable and

resist spoilage by yeast fermentation. The moisture of content of the honey was determined using Abbe refractometer based on ICH.

The digital refractometer was calibrated with distilled water, in which the refractive index of the water at 20^{o}C was found to be 1.3330 as standard. After the refractometer had cleaned and dried the honey samples were measured in triplicate and mean value was taken (Bogdanov et al, 2009).

4.4.3. Ash

Ash is determined based on AOAC (1999) and ICH (2009). The ash content of honey which is the residue was obtained by heating ash dish in electrical furnace at ashing temperature, and subsequently cool in desiccators to room temperature and was weighted (Bogdanov et al, 1999).

10g of sample was taken and heated to about 350^{0}C.After preliminary ashing, the dish was incinerated at high temperature (550^{0}C) in a burning muffle furnace for 5hrs. Finally, proportion of ash was calculated. The Equipment used was ash dish, hot plate, infra-red heater for preliminary evaporation, electrical furnace, desiccators, analytical balance and Olive oil was used as a reagent.

The ash content was calculated using the following equation

$$WA = \frac{(M1 - m2) * 100}{M0}$$

Where

W_A=Weight of Ash

M_1=weight of crucible +ash

M_2= weight of crucible

M_0=weight of sample

4.4.4. pH

The pH and acidity was determined based on AOAC (1989) and ICH (2009). 10g of honey were dissolved in 75ml distilled water in a 250ml beaker. The solutions were titrated with 0.1M sodium hydroxide solution at pH 8.30 after calibration with standard buffer solutions of pH 4, 7 and 10; the pH was measured using pH Meter (AB150 fisher scientific pH meter).

4.4.4. Acidity

Representative 10g sample was dissolved in 75ml of carbon dioxide free water in a 250 ml beaker, stir with magnetic stirrer and was titrated with 0.1M NaOH to pH 8.30. The reading was obtained with 120 sec. of starting titration and complete titration was completed within 2minutes (Bogdanov et al, 2009). The acidity of the honey was expressed using the following association:

Milliequvalent /kg honey =ml of 0.1M NaOH*10

4.4.5. Electrical conductivity

Conductivity was determined based on AOAC (1989) and ICH (2009). 20g dry mater of honey was dissolved in 100m1 distilled water and conductivity was measured using a conductivity meter. The electrical conductivity result of the honey solution was calculated using the formula:

$$SH = \mathrm{K.G}$$

Where

S_H = Electrical conductivity of the honey solution in $mS.cm^{-1}$

K= Cell constant

G=Conductance in mS.

4.4.6. Hydroxymethylfurfural

The hydroxymethylfurfural (HMF) was determined based AOAC (1989) and International Honey Commission (2009), by clear, filtered aqueous honey solution using reverse phase HPLC equipped with UV detection and result was expressed in milligrams per kilogram (Bogdanov, Keppy et al, 2009).

The absorbance of a clarified aqueous honey solution was measured against the reference solution of the same honey in which the 284 nm chromophore of HMF was destroyed by biosulfate. The HMF content of honey was then calculated using the following equation:

$$\text{HMF}\left(\frac{\text{mg}}{100\text{g}}\text{of honey}\right) = \frac{(\text{A284}-\text{A336})*}{W} Factor$$

Where:

W= Weight of sample in grams

Factor = 126*100*1000*100= 74.87

16830*1000

126= the molecular weight of honey

16830= the molecular absorptive of HMF

HMF= Hydroxymethylfurfural

Mg= milligram

A284= Absorbance at 284

A336= Absorbance at 336

4.4.7. Viscosity

The viscosity of honey was determined based on Belay et al (2017), with some modification at over a range of shear rates (2.58-258.1 s^{-1}), at 25, 30, 35, 40 and 45 0C. The flow behaviour of honey was described by fitting experimental data of shear stress and share rate in Newtonian fluid. Viscosity dependence of temperature was evaluated using Arrhenus model:

$$n = no * Exp * (Ea/RT)$$

Where

n = viscosity (Pa s)

no =rate constant (Pa S^n)

Ea= activation energy (kJ/mol)

T = absolute temperature (K)

R= gas constant (kJ/mol/K)

4.4.8. Color

The colour intensity of honey sample was measured according to the Pfund classification using photometer and colour grades which were expressed in millimetre grade by comparing analytical grade glycerol standard (Belay et al., 2013). Accordingly, the pfund grade was categorized as water white, extra white, white, extra light amber, light amber, amber and light amber that range from 8 to 114mm pfund.

4.4.9. Optical density

The optical density of honey sample was assessed based on Owayes (2005) with some modification. 1g of honey diluting with 9ml of distilled water and centrifuged for 10min at 3000g and the absorbance of the filtrate supernatant was measured using spectrophotometer at 530nm against distilled water as a blank (Owayes et al 2005).

4.4.10. Specific rotation

The specific rotation of honey was determined based on AOAC (1989) and ICH (2009).The angular rotation of a clear, filtered aqueous solution of honey sample was measured by a polarimeter in which its value related to the carbohydrate composition with the specific rotation $[\alpha]_{20}D$**.**

12g of honey was dissolved in distilled water and 10ml of Crazze I solution was added thoroughly mixed for 30 second and 10ml of Crarez II solution added and mixed again for 30

seconds and make up to volume in 100ml volumetric flask with distilled water. The next day, solution was filtered, rinsed and filled in a clean 2-dm polarimeter tube, placed the tube in polarimeter and the angular rotation [α] was read at 20^{o}c.

The specific optical rotation $[\alpha]_{20}D$ is the angle of rotation polarized light at the wave length of sodium D line (λ=589.3nm) at 20^{O}Cof aqueous solution of 1dm depth and containing 1g per ml. The specific rotation of honey calculated using the following formula.

$$[\alpha]20_{D} = \frac{\alpha * 100}{L * M}$$

Where:

α = Angular rotation found

L = length in decimetres of the polarimeter tube

M = grams of dry matter taken

4.5. Antimicrobial properties of honey

The disc diffusion test was used to measure the minimum inhibitory concentration and minimum bacterial concentration. Aseptically plates were swabbed with Staphylococcus aureus which was obtained from Ethiopian biodiversity institute, by dipping sterile swabs into inoculums for antibiotic disk diffusion. Sterile paper discs were dipped in different dilution of honey placed on swab plates of S.aureus in specific dilution and were put on agar plate and the agar plates were incubated at 37^{O}C for 48 hour. The agar plates were observed and measured inhibition zone from each paper disk in mm and checked the test organism grows on the disc and safely measured the test organism (Motior et al, 2010).

Samples of staphylococcus aureus was incubated for 48hours at 37^{0}C and the antibiotic comparison tests were done using antibiotic infused disks with tetracycline (30µg) and the disks were placed onto inoculated Muller Hinton agar plates, in which subsequent incubated

for 48hours at 37^0C, after incubation the zones of inhibition were measured (Monica et al, 2014).


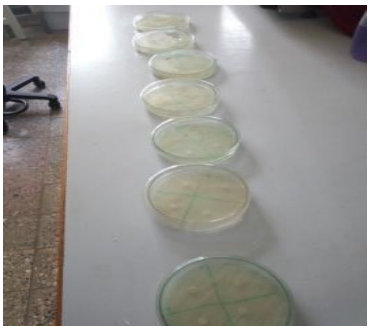

Figure 3: Analyzing Antimicrobial Properties of Honey.

4.6. Antioxidant properties of honeys

The antioxidant properties of honey was determined based on antioxidant content (phenol and flavonoids) and antioxidant activity (DPPH, FRAP, POS). 2g honey sample was extracted by stirring with 25 ml of methanol and 25 ml of distilled water and placed at 25^0C for 60 minute maceration using temperature shaker incubator and filtered through Whatman 4 paper and residue was extracted in the same way with two additional 25 ml portion of methanol. Combined Methanol extracts were evaporated at 40^oC to dry and dissolve in methanol at concentration of 50mg/ml and store at 4^0C for further uses.

4.6.1. Antioxidant contents

4.6.1.1. Polyphenols

Polyphenol content of honey was determined based on Asli et al (2010). First the standard solution was prepared in five different concentrations which were 0, 25, 50, 75 and 100mg/L, and the stock gallic acid solution was made by dissolving 25mg of gallic acid in 100ml of 70% methanol. The standard working solution 0, 1, 2, 3,4ml of gallic acid solution (250mg/L) was pipetted in a10ml volumetric flask and subsequently diluted to volume with 70% methanol. To make the standard curve, 1ml of each of the standard solution was pipetted into

a separate test tube then 5ml of aqueous dilution of folin-ciocalteu reagent was added and mixed well using a vortex mixer for about 1 min. After 5min 4ml of a 75g/L Na_2CO_3 solution was added and the mixture was mixed thoroughly for another 1min, and incubated for 15min (Asli et al, 2010).

To determine phenolic content of honey Folin-ciocalteu method were used by diluting 5gm of honey sample to 50ml distilled water. 0.5ml of solution were mixed with 2.5ml of 0.2N Folin-Ciocalteu reagent for 5min and 2ml of 75g/l sodium carbonate, after incubation the absorbance of the mixture were measured at 765nm against a methanol blank. Gallic acid was used as standard to produce the calibration curve.

The sample was prepared by dissolving five grams of each honey samples in 50ml of 70% methanol and one ml from each methanolic honey solution was transferred to test tubes and measured by the procedure used for constructing the standard curve. Blank sample was prepared for each honey samples by pipetting 20ml of honey extract into 100ml glass beaker and the honey extract was shaken by mechanical shaker at 320rpm for 20min and the mixture was filtered through whatman filter pape 4 and 1ml of filtrate was taken and tested. The absorbance of standards, samples solution and reference were read in UV-spectrophotometer at 765nm against zero absorbance of blank. The following formula was used to calculate the total phenolic content:

Total phenolic conten (mg GAE/Kg honey)=

$$\frac{\text{GAE (mg/L)*Total volume of methanol extract (ml)*sample weight (g)*}10^{-3}\text{(kg/g)}}{10^{-3}\text{(L/ml)*Dilution factor}}$$

Where

GAE = Gallic acid equivalence

Kg = Kilogram

Mg = milligram

L = Litre

Ml = Millilitre

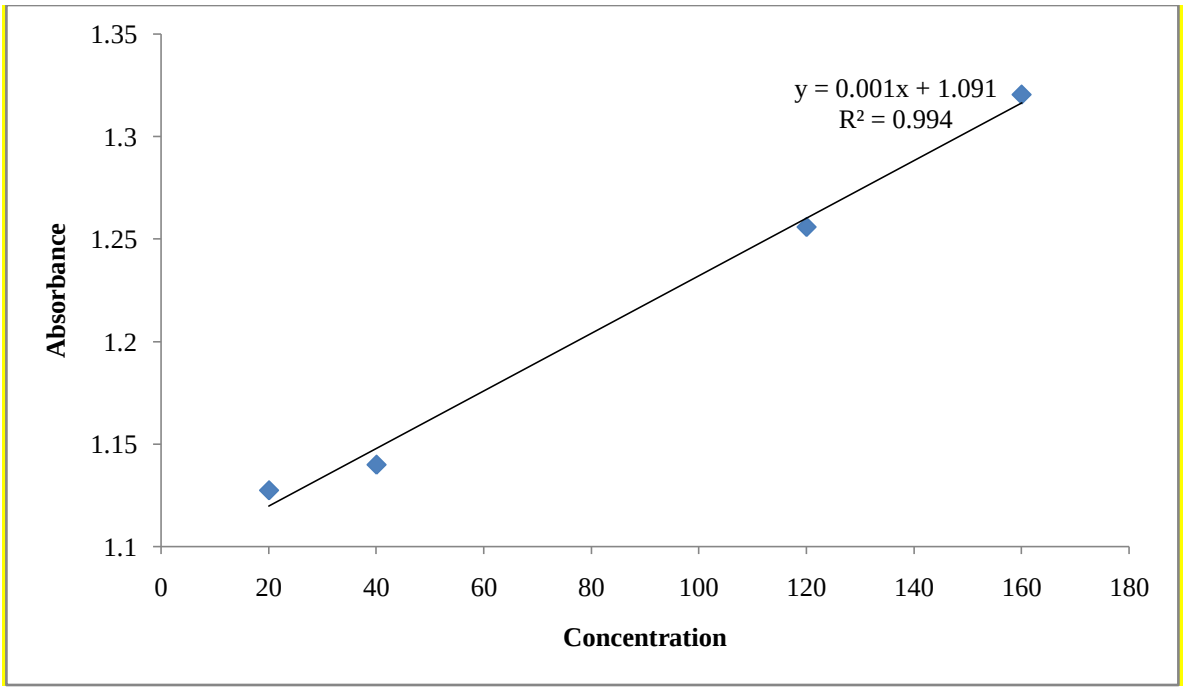

Figure 4 Standard curve for phenolic content of Belete-Gera honey Jimma-Ethiopia.

4.6.1.2. Flavonoids

The total flavonoid content of honey was estimated by aluminum chloride ($AlCl_3$) to quantify the total flavonoid content; Quercetin was used as the reference which was expressed as quercetin equivalent (QE).

A standard curve of known concentration of quercetin was generated by preparing and testing concentrations of quercetin standard solution. Stock quercetin solution was prepared by dissolving 25mg of querctin in 100ml of 80% ethanol then the standard solutions were made up by pipeting stock solution(250mg/l) into 10ml volumetric flask and adjusting the volume with 80% ethanol by using test tubes 1ml of each standard solution was reacted with 3ml of 95% ethanol (Asli et al, 2010).

0.2ml of 10% aqueous dilution of $AlCl_3$ reagent 0.2ml of potassium acetate and 5ml of distilled water and the mixture was mixed thoroughly by vortex mixer for about 30 second

and allowed to stand at room temperature for 30 min and absorbance was measured using UV-spectrophotometer at 415nm.

Dowd method was used to determine the flavonoid content by mixing 5ml of 2% aluminum trichloride in methanol with the same volume of honey solution and absorption reading at 415nm were measured in UV-visible spectrophotometer after 10 minute against a blank sample consisting of 5ml honey solution without aluminum trichloride and total flavonoid were determined with Quercetin standard.

The samples were prepared by dissolving five gram of each honey samples in 25ml of 80% methanol one ml each honey solution was transferred to a test tube and similar procedure was used for constructing the standard curve applied to 1ml of each standard solution and a sample blank was similarly prepared for each honey sample but the same amount 10% $AlCl_3$ solution (0.2ml) was replaced by distilled water and the absorbance reading were taken by UV-spectrophotometer at 415 and the following formula was used to calculate the final total flavonoid content.

Total flavonoid content (mgQE/Kg honey) =

$$\frac{\text{QE (mg/L)*Total volume of Ethanol extract *Sample weight(g)*}10^{-3}\text{(kg/g)}}{\text{Ml*}10^{-3(}\text{L/ml)*dilution factor}}$$

Where

QE= Quercetin equivalent

Mg=milligram

ML= millilitre

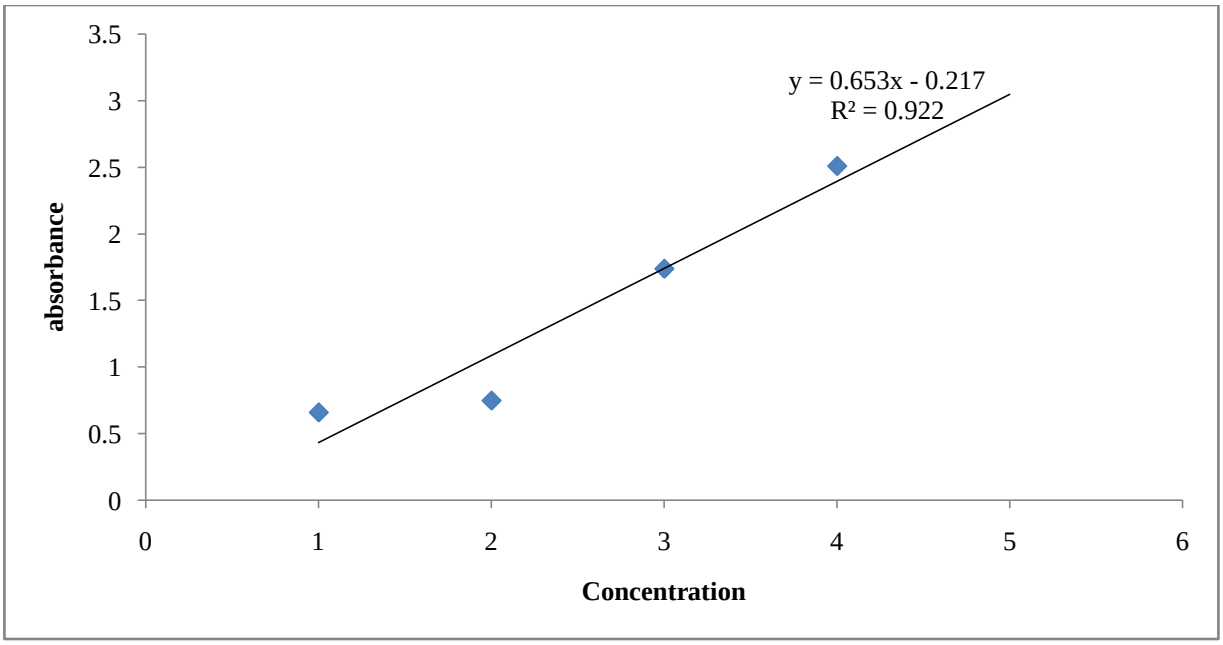

Figure 5: Standard curve for flavonoid content of Gera honey Jimma-Ethiopia.

4.6.2. Antioxidant activity

4.6.2.1. Radical Scavenger activity

The radical scavenging activity of honey extract was determined using assay according to Chang et al (2001). The decrease in the absorption of DPPH solution after the dilution of an antioxidant was measured at 517nm. Ascorbic acid 10mg/ml was used as reference.

2, 2Diphenyl1-PicrylHydrazyl is a stable in powder form free radical with red color which changed to yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. Reagent was prepared by dissolving: 0.1mMDPPH solution in 4mg of DPPH in 100ml of methanol.

Working procedure: Honey radical scavenging activity was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) dissolving honey sample with methanol and mix 0.75 samples with

1.5 ml of DPPH and absorbance were measured at 517 nm and ascorbic acid was used as control.

The scavenging activity of honey samples for the radical 2, 2-diphenyl -1-picryl hydrazyl (DPPH) with different concentrations of ascorbic acid (positive control) and honey sample 2.5, 5, 7.5, 10mg /ml were dissolved in methanol and 1.5ml of each sample was mixed with 3ml of DPPH in methanol (0.02mg/ml), with methanol serving as blank sample. The mixture was vigorously shaken and allowed to stand at room temperature for 15min in a dark room and the absorbance was read at 517nm using UV-Visible spectrophotometer and the degree of stable DPPH decolourization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the sample (Venugopal et al, 2010).

Percentage radical scavenging was calculated using the following formula

$$\%RSA = \frac{\text{Abs control} - \text{abs sample}}{\text{Abs control}} * 100$$

Where

RSA= Radical Scavenger activity

Table: RSA Absorbance of honey samples

S.No	Sample	Absorbance of sample	Absorbance	Result (%)	DPPH radical
1.	Eucalyptus honey	0.4246	517nm	47.40	0.80723
2.	Guizotia-spp	0.3816		52.72	
3.	Vicia faba	0.4660		42.27	
4.	Trifolium	0.3557		55.93	
5.	Vernonia	0.5062		37.29	
6.	Hypericum	0.3228		60.01	
7.	Sativa	0.2198		72.76	

4.6.2.2. Ferric reducing antioxidant power (FRAP) assay

FRAP was determined based on Bolanos (2014). FRAP assay 75µl of sample (0,125, 250, 500, 1000µm) was added to 1.5ml micro-centrifuge tubes followed by 1425µl of working

FRAP solution and the mixture were incubated in the dark for 30miutes at 37^{o}C and absorbance reading were recorded at 700nm using spectrophotometer (Bolanos et al, 2014).

The antioxidant power of honey was determined by 1ml of ethanolic honey extract (10%v/v) mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferri-cyanide after incubation 2.5ml of trichloroacetic acid was mixed and the mixture had centrifuged at 3000rpm for 10min. Finally 2.5 supernatant was mixed with equal amount of water and 0.5 ml of 0.1% $FeCl_{3.}$ Absorbance was measured at 700nm using spectrophotometer and ascorbic acid had used as a reference standard. Ferric reducing antioxidant power was calculated using the following formula:

$$\text{FRAP value} = \frac{C * A_{700}\text{Test}}{A7_{00}Fe^{2+}}$$

Where

C=Concentration

A=Absorbance (µm)

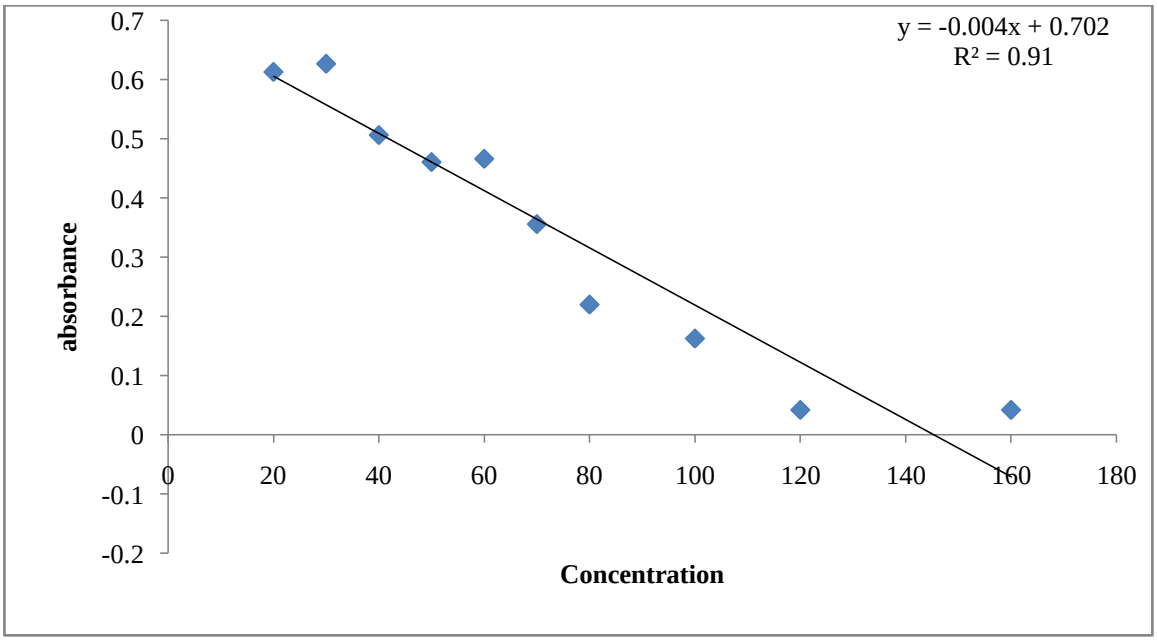

Figure 6: DPPH Radical Scavenging activity with ascorbic acid of Belete-Gera honey Jimma Ethiopia

4.6.2.3. Peroxide Radical scavenging activity

The peroxide radical scavenging activity of honey sample was determined by honey solution 8ml in deionised water mixing with1.2 ml H_2O_2 phosphate buffer after incubating for 110 min absorbance were measured at 230nm against phosphate buffer as blank. H_2O_2 phosphate was used as negative control and ascorbic acid was used as positive control and percent inhibition were calculated as

Percent inhibition = (Abs of –ve control- Abs of sample/Abs of –ve control)*100

Where

Abs= Absorbance

Table: Peroxide absorbance of honey samples

S.No	Sample	Absorbance of samples	Absorbance	Result(% inhibition)	Blank
1.	Eucalyptus honey	0.3012	230nm	63.6	0.8276
2.	Guizotia-spp	0.3376		59.2	
3.	Vicia faba	0.1133		86.3	
4.	Trifolium	0.2259		72.7	
5.	Vernonia	0.1969		76.2	
6.	Hypericum	0.1456		82.4	
7.	Sativa	0.2896		65.0	

4.6.3. Sensory evaluation

The honey samples were evaluated for sensory acceptability based on the seven point hedonic scale rated from dislike extremely (1) to like extremely(7) for taste, flavour, colour and over all acceptability of honey samples. A 40g of honey sample were put into sampling plate for each assessor and spoon, cup and water for rinsing were served.

4.7. Statistical Analaysis

The statistical analysis was conducted with SPSS version 20. Results were expressed as the mean ±sd and at $p \leq 0.05$ were considered to be significant.

5. RESULT AND DISCUSSION

5.1. Floral Origin Determination

The results of pollen dominance of floral origin honey samples of melissopalynology finding indicated that seven monofloral honeyswere found in Belete Gera forest. The monofloral honeys were: *Guizotiaspp., Eucalyptus spp., Hypericum spp., Trifolium spp., Viciafaba, Vernonia spp., Satvia ssp.*,(Table 1).

The level of dominance ranged from 45% (*Viciafaba*) to 88.9% (*Sativa*). The honey samples obtained from Belete-Gera showed that the presence of variety of pollen grains. The highest pollen grain was found in Sativa and the eucalyptus (81.8%). This is in line with a study done in Romania, which showed that about 72% of honey samples were mono floral and about 28% were multifloral (Claudia et al, 2016). The identification and quantification of the pollens in honey sample is one of the best ways to determine the range of nectar type of honey. Melissopalynological also help in identifying the geographical origin (Singh et al, 2016).

Table 1. Floral oirigin of Belete gera honey Jimma-Ethiopia (n=48).

Honey sample	Source plants (%)						
	Eucalyptus spp	Guizotia spp	Vicia faba	Trifolium	Vernonia spp	Hypericum spp	Sativa spp
A1	81.8	12	0	0	0	6.2	0
A2	11.4	13	68	0	0	0	7.6
A3	8.7	12.3		76	3	0	0
A4	10	4	79	0	0	1	6
A5	0	0	13.1	0	0	86.9	0
A6	2	0	0	8	58	0	32
A7	72	12	0	0	0	15.1	0
A8	11.4	70	12.6	6	0	0	0
B9	18.5	15.3		64	0	2.2	0
B10	10	3	3	0	79	0	5
B11	5.9	0	14.1	0	0	80	0
B12	22.5	6.4	0	5	49.5	0	16
B13	42.5	8.1	0	8	0	4	37.4
B14	11.4	9.6	68	11	0	0	0
B15	8.7	15.3	0	46.5	29.5	0	0
B16	23	0	45	0	28	0	4
D17	0	0	14.1	0	0	85.9	0
D18	12.2	6	9	7	14.8	0	51
D19	81.8	9	0	3	0	6.2	0
D20	9	68	11	0	10	0	2
D21	10.7	12		70	0	0	7.3
D22	10	7.3	8	0	5.7	0	69
D23	47	0	10.1	0	0	31.9	11
D24	33	11	0	49	5	0	2

Honey sample	Source plants (%)						
	Eucalyptus	Guizotia spp	Vicia faba	Trifolium	Vernonia	Hypericum	Sativa
G25	75	13.8	0	3	0	8.2	0
G26	11.4	11.6	0	8	68	1	0
G27	15.5	15.3		69	0	0	0
G28	9	5	80	0	0	0	6
G29	0	0	11.1	0	0	72	18.9
G30	54	21	0	11	14	0	0
G31	81.8	12	0	0	0	6.2	0
G32	12	17	6	0	62	3	0
S33	9.1	0	0	0	2	0	88.9
S34	9	83	4	0	0	0	4
S35	4	0	12.1	0	0	83.9	0
S36	0	3	0	6	80	0	11
S37	79	12	0	0	0	7	2
S38	14	11	67	2	0	0	6
S39	10.7	15.3		70		4	0
S40	8	83	4	0	0	0	5
W41	0	0	11.1	0	0	85.9	0
W42	0	18	0	14		0	68
W43	81.8	12	0	0	0	6.2	0
W44	10	7	72	11	0	0	0
W45	10.1	15.3		0	74.6	0	0
W46	10	4	0	79	0	0	7
W47	13	0	11	0	0	76	0
W48	10	0	0	9	23	0	58

5.2. Sugar Contents

The sugar content of the honey was stated in (Table 2) the sum of Fructose and glucose was found to be a dominant sugars, ranged from 60.76to 63.32g/100g with a mean of 61.80g/100g in *Sativa* and *vernonia* honeys, respectively. This was in line with the codex and EU standard which is ≥60g/100g (Codex, 2001).

This finding was lower than the (Belay et al, 2017) finding which was maximum concentration of fructose (43.1±0.4g/100g) was found in Acacia honey and minimum 35±4g/100g) was *Becium grandiflorum* and the maximum glucose content found in *Leucas abyssinca* (Belay et al, 2017).

Table 2: Mean Sugar content of Belete Gera honey Jimma-Ethiopia.

Honey Types	**Sugar profiles (g/100g)**			
	Fructose	Glucose	Sucrose	Maltose
Eucalyptus spp	32.020±0.77ab	30.82±4.53a	1.30±0.57b	0.53±0.30a
Guizotia-spp	30.8±1.02b	31.18±3.96a	1.12±0.31b	0.70±0.45a
Vicia faba	31.56±1.70b	29.52±2.70a	1.380±0.54b	0.69±0.20a
Trifolium	32.32±1.29ab	28.45±1.92a	0.880±0.58b	0.51±0.65a
Vernonia	34.86±1.22a	28.46±1.57a	0.840±1.16b	0.52±0.40a
Hypericum	31.90±1.56ab	29.98±2.29a	0.950±0.45b	0.63±0.89a
Sativa	33.71±1.61ab	27.05±1.74a	3.500±0.38a	0.47±0.08a

Means for monofloral honey with different letters are signifiantly different.

Another study showed that sugar content of samples ranged from 71g/100kg of honey to 80g/100kg of honey (Shobham et al., 2017). A study conducted in United States regions shows that honey typically contains fructose 38.2%, Glucose 31.3%, maltose 7.1%, sucrose 1.3%, (Khan et al, 2015).This variation was happened due to the difference in botanical origin and geographical location.

The sum of the fructose and glucose is very close to the sum of all reducing sugars as fructose and glucose represents mostly more than 90% of all reducing sugars. The fructose/glucose ratio and the sucrose concentrations are good criteria for differentiating between unifloral honeys (Bogdanov et al, 1999).

The sucrose content of the honey samples ranged from 0.84 t0 3.50g/100gm and it was within the codex standard (<5g/100gm).Sucrose is an essential sugar for honey quality and screening. The contribution of sucrose to total sugar in honey can be increased, if honey is harvested before ripening. The ripening process in the combs, the sucrose level is reduced by the action of the enzyme invertase (Bogdanov, 1999, Belay, 2017).
Sugar is the major component that governs honey property and its content is firmly related to the degree of maturity and botanical origin of honey.

The maximum concentration of fructose (34.86$\pm$0.86g/100g) was found in Vernonia and the minimum Guizotia spp (30.8$\pm$0.72g/100g). A significant diference $p<0.05$ was observed between Vernonia and other monofloral honeys in fructose content. Glucose was the second most aboundent in Guizotia honey.

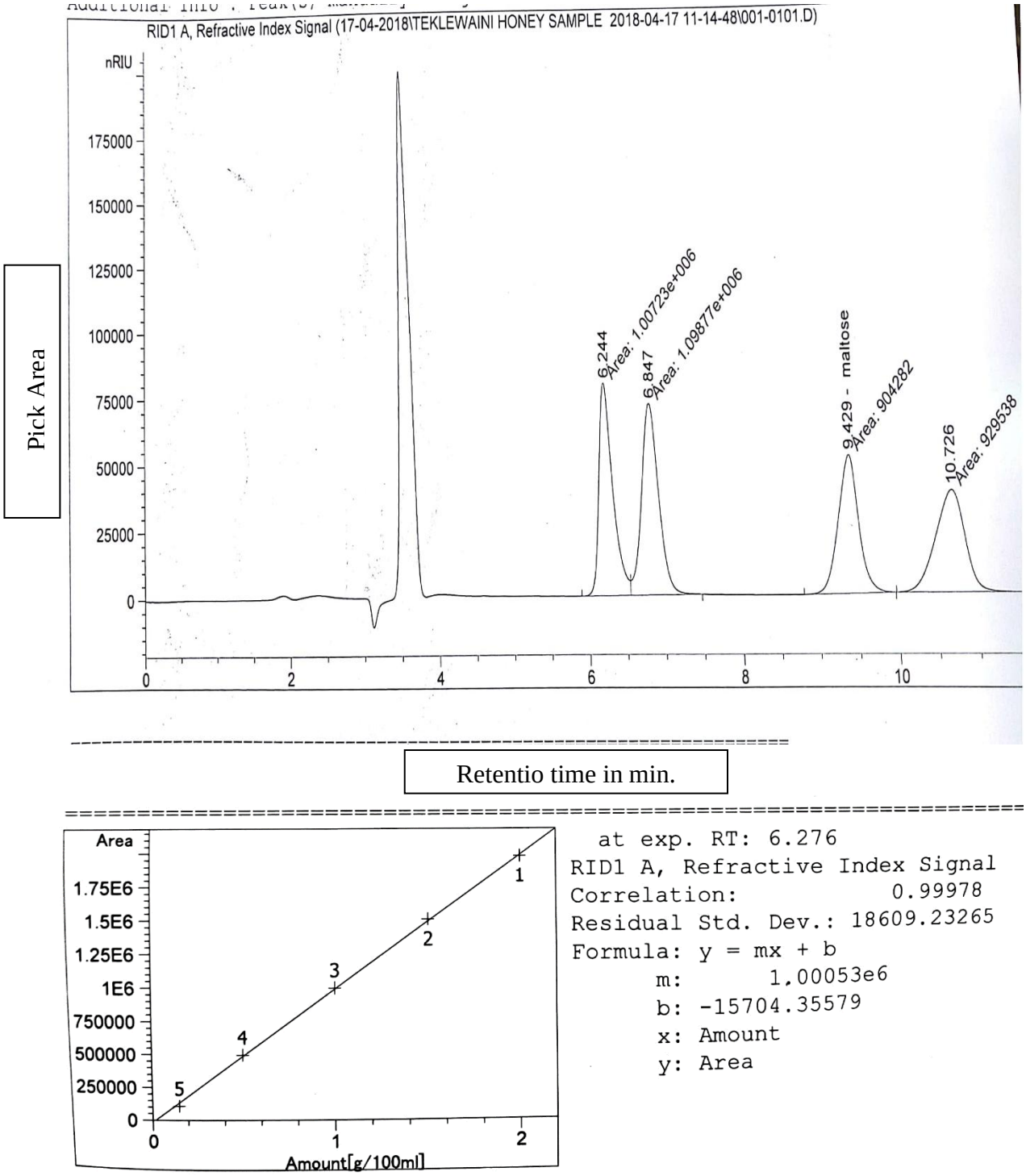

Figure 7: The retention time of sugar analysis by HPLC result of Belete-Gera Honey.

Table 3: Mean ±sd physicochemical properties of Belete Gera honey, Jimma- Ethiopia (n=48).

Honey type	Refractive index	Moisture content	Aw	pH	Ash	HMF	Optical density	EC	Specific rotation	Free acidity
Eucalyptus	1.4992	15.0	0.53	4.1	0.30	12.2	0.5203	0.50	-12.9	40.0
	±0.004b	±0.16e	±0.04c	±0.01c	±0.01b	±4.8ba	±0.00a	±0.04a	±0.90bc	±1.30a
Guizotia-spp	1.4940	17.0	0.56	4.3	0.10	16.1	0.3792	0.20	-12.7	26.0
	±0.002d	±0.32c	±0.01bac	±0.00a	±0.02d	±3.9ba	±0.001c	±0.01c	±0.73bc	±1.23d
Vicia faba	1.4895	18.8	0.59	4.23	0.30	16.3	0.3792	0.20	-14.5	23.0
	±0.001a	±0.15ba	±0.0ab	±0.02b	±0.01b	±4.5ba	±0.00c	±0.00c	±0.84ba	±0.59e
Trifolium	1.4970	15.8	0.54	4.25	0.10	21.95	0.3791	0.42	-11.3	26.0
	±0.002bc	±0.32d	±0.01c	±0.00b	±0.00d	±0.87a	±0.00c	±0.12ba	±0.84ba	±1.17d
Vernonia	1.4891	19.0	0.60	4.09	0.6	18.23	0.4498	0.31	-16.0	32.0
	±0.004cd	±0.15a	±0.00a	±0.04c	±0.04a	±1.72ba	±0.002b	±0.09bc	±0.67dc	±0.60b
Hypericum	1.4960	16.2	0.55	3.70	0.2	12.25	0.4497	0.37	-10.2	24.0
	±0.005bc	±0.29d	±0.0bc	±0.01d	±0.01c	±4.94b	±0.002b	±0.05ba	±0.91a	0±0.35ed
Satvia	1.4905	18.4	0.59	3.62	0.1	19.20	0.3792	0.26	-12.9	29.0
	±0.004cbd	±0.24b	±0.0ba	±0.00e	±0.00d	±5.94ba	±0.001c	±0.04bc	±0.60d	±0.43e

Means for monofloral honey with different letters are significantly different.

The water activity had significant correlation at $p<0.05$ with refractive index, electrical conductivity, specific rotation. The electrical conductivity also had correlation with moisture, optical density and water activity.

5.3. Physicochemical properties

5.3.1. Moisture Content

The moisture content of the honey samples was stated in (Table 3) which ranged from 15.8 to 19.8% with an average of 17.17% which was in the range of codex standard $\leq$20%. This result was in line with other studies in which the water content in honeys ranged from 14% to 18.6% (Helena et al, 2007).

Another study done in Sudan shows moisture content of analysed honey ranged from 17.2% to 20.40% (Salah et al, 2015).The moisture content has important role in the shelf life of honey; the lower the moisture content the longer will be the shelf life of honey because it prevent the fermentation by osmo-tolerant yeast.

A study in Burkinafaso showed that the moisture content of honey varied from 15.01$\pm$0.01 to 25.1$\pm$0.0 which mainly concluded that the premature extraction of these honey samples can lead to a greater risk for fermentation (Meda et al, 2005).

Another study in Brazil showed that the moisture content present in honey influenced the flavour, density, crystallization, solubility and conservation. The moisture content of Brazilian honey is 18.8% (Costa et al, 2015).

The moisture content is the composition criteria which has a part of the standard has to be fulfilled in world honey trade and honey having a high water content is more likely to fermented. In routine honey control carried out by the international honey commission during 1989-1997 in more than 3000 honey samples 91-95% of all honeys had water content of less than 20g/100g (Bogdanov et al, 1999).

The refractive index of honey samples in the current study were ranged from 1.4891 to 1.4992 which was in line with the international honey commission standard which is 1.0 to 2.5.

5.3.2. Water activity

The water activity of honey samples were ranged from 0.53 to 0.60, with average of 0.56 (Table 3), a_w can be calculated from moisture content based on the following equation:

$$a_w = 0.262 + 0.0179 * \text{Moisture}.$$

The water activity in honey results mainly from the concentration in the water of honey of monosaccharides fructose and glucose and some disaccharides such as sucrose and maltose (Chirife et al, 2006). There is no more microbial growth under 0.6aw (Novasina et al, 2008). The water activity in honey varied from 0.479 to 0.557(Helena, et al 2007).

The water activity of honey is below 0.6 which is enough to inhibit the growth of osmotolerant yeast present in honey. Water activity of honey depends on the concentration of soluble compounds and thus substances which are present in very small amount like oligosaccharides, nitrogenous compounds, acids, flavour and mineral have little effect on lowering of water activity. Therefore water activity mainly depends upon the concentration of fructose and glucose in the water of honey to lesser extent on some disaccharide's like sucrose and maltose (Shafiq et al, 2014).

5.3.3. Ash

The ash content of honey sample stated in Table 3, which ranged from 0.10 to 0.60gm with average 0.24gm/100gm. The ash content of the sample were within the accepted range which is $\leq$0.6gm/100gm of range (Codex alimentarious, 2001).

This study was in line with (Derebasi et al, 2014) ash content of honey samples were ranged from 0.00 to0.52%.

Ash content is a quality criterion used for determination of the botanical flora and geographical origin of honey samples. Harvesting process, beaking techniques and the material collected by the bees foraging on the flora can affect the ash content of honeys.

5.3.4. Free Acidity

The free acidity of the honey samples range from 23.0 to 40.0 with average 28.57 (Table 3). The free acidity of honey according to European Union standards $\leq$ 40meq/kg and according to codex alematarious $\leq$50 meq/kg. The free acidity value was low which indicate honey freshness and the free acidity of honey ranged from 9.2meq/kg to 41.4meq/kg (Shobham et al, 2017).

The free acidity is an important quality criteria and honey fermentation causes an increase in acidity value. The standard fixed a maximum 40milli-equivalents/kg which has increased to 50milliequivalents/kg in the codex as there are honeys which have a higher natural acidity (Bodanov et al, 1999). A study done in Burkinafaso showed that the free acidity varied from 20.3$\pm$0.4 to 60.8$\pm$0.4meq per kg (Meda et al, 2005).

5.3.5. Electrical conductivity

The electrical conductivity of the honey samples were ranged from 0.20 to 0.5mS/cm which were measured according to the harmonized method of the European honey commission and expressed in mS/cm. Electrical conductivity was measured at 20^0c using conductivity meter and all measurements were preformed in triplicate.

Electrical conductivity value of honey samples varied within the range 0.41 to 0.79mS/cm (Mohammed et al, 2013). A study done in Argentina showed the average values obtained for electrical conductivity of honey (0.33mS/cm and pH (4.17) indicate that the analysed honey s came mainly from nectar (Pia et al, 2010). Another study done in India showed that the electrical conductivity values ranged from 0.45 mS/cm to 0.46mS/cm (Shobham et al, 2017).

5.3.6. Specific rotation

The specific rotation of the honey range from -16 to -10.25 which was similar with the research done in Lativa which was from -16 to -8 and the specific rotation was determined by

polarimetry with the angular rotation of clear filtered aqueous solution which the value is related sugar composition and the specific rotation is one of the physical attribute of honey classification (Fredijs et al, 2008).

The overall value for specific rotation is a result of the values of the different honey sugars and the measurement of specific rotation used in EU countries was found negative values of specific rotation (Bogdanov et al, 1999).

5.3.7. Hydroxymethylfurfural

The HMF content of the honey samples found in Table 3 ranged from 3.2mg/kg to 37mg/kg with an average result of 16.60mg/kg and were within the Codex $\leq$80mg/kg and $\leq$40mg/kg ICH standards respectively. HMF is the quality index which is a good model to estimate shelf life of honey (Biagio et al, 2009). HMF activity is an international parameter used for the control of the limit for thermal treatment to honey (Chua et al, 2014).

Several factors such as temperature, time of heating during processing, storage conditions, aging of honey products and sources of floral were found to influence the HMF content in honey (Mohamed et al, 2013). The HMF level of honey ranged from 2.0$\pm$2 to 41.9$\pm$0.1 mg/kg (Meda et al, 2005).

5.3.8. Viscosity

The viscosity of honey affected by temperature and floral sources and as the temperature rise from 25^{o}c to 45^{o}c the viscosity of the honey samples decreased and showed Newtonian flow.

Table 4: The Viscosity of Belete Gera honey samples at different temperature (25-45) Jimma-Ethiopia (n=48).

S.No	Monofloral honeys	Viscosity Pa s at different temperature				
		25°c	**30°c**	**35°c**	**40°c**	**45°c**
1.	Eucalyptus spp	8.21	6.80	5.42	3.09	2.94
2.	Guizotia-spp	4.23	3.07	2.01	1.38	1.02
3.	Viciafaba	8.20	7.14	5.72	4.03	3.91
4.	Trifolium	11.36	10.35	3.72	3.53	3.51
5.	Vernonia	12.83	11.74	4.08	3.84	3.06
6.	Hypericum	6.21	5.24	3.06	2.16	2.00
7.	Sativa	6.58	4.29	3.01	2.08	1.73

The temperature dependent of viscosity of monofloral honey was assessed by applying the Arrhenius type model. The fitting of the logarithmic of viscosity in Pa s versus the reciprocal temperature data was examined at a temperature range of 298.15-318.15K and projected by Arrhenius model. The monofloral honeys GuizotiaSpp, Sativa, Euclaptus, Viciafaba and Hypericum showed Newtonian behaviour. The highest viscosity was observed at 25°C, while at a temperature 45°C become smaller and the finding was in line with (Belay et al, 2017).

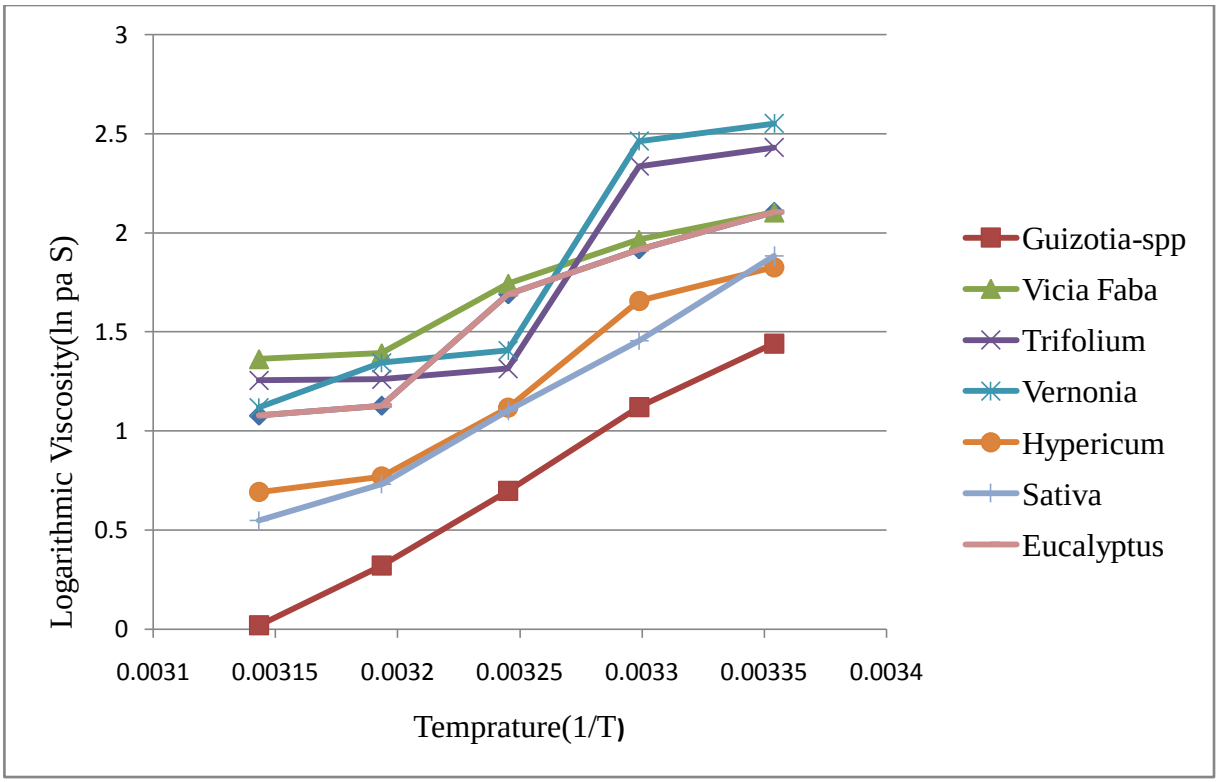

Figure 8: The Arrhenius logarithmic value of viscosity (in Pa s) versus the reciprocal value of temperature(1/T) of Belete-Gera honey Jimma-Ethiopia.

5.3.9.Colour

The colour of honey sample was measured by Pfund and the result ranged from extra light amber Viciafaba (35mm) to amber Hypericum (87mm) (Table 5).

The result honey color of this study was in more lighter than as compared with a study done in India showed that the color of honey ranged from amber to dark amber and the color of honey depend on various parameters such as phenolic compounds and pollen content in the honey as well as its origin and floral varieties (Shobham et al, 2017).

Another study in Nigeria showed that the colour of honey ranged from light amber to dark amber (Adenekan et al, 2010).

Table 5. Color of Belete-Gera honey Jimma-Ethiopia (n=48).

S.No	Honey Type	Colour	Pfund
1.	Eucalyptus spp	Light Amber	52
2.	Guizotia-spp	Amber	87
3.	Viciafaba	Extra light Amber	35
4.	Trifolium	Amber	87
5.	Vernonia	Light amber	52
6.	Hypericum	Amber	87
7.	Sativa	Light amber	52

5.3.10. Optical Density

Optical density (OD) of the honey samples were from 0.3792 to 0.5203 (Table 3), and the finding was in line with the study of (Owayes et al 2005). One gram of honey was diluted with 9ml of distilled water and centrifuged for 10min at 3000g the absorbance of the filtrate supernatant was measured at 530nm against distilled water as a blank using a spectrophotometer

5.3.11.pH

The pH value of the honey ranged from 3.62 to 4.30 with average of 4.04 which is within the range according to European union honey pH standards should in the range 3.6-4.6 and according to codex alematarious 3.6-4.6. The result of this study was in line with study done in Sudan showed that the pH values of analyzed honey sample ranged from 3.98 to 4.61 which was from Blue Nile and Darfur respectively (Salah et al, 2015).

Another study done in Romania showed that the pH values ranged from 3.61 to 4.72(Claudia et al, 2016). Another study showed that honey pH values varied from 3.5$\pm$0.1 to 4.7 $\pm$0.1 (Meda et al, 2005).

5.3.12. Antimicrobial properties of honey

The values of zone of inhibition using disc diffusion for all monofloral honey were stated in Table 6. The zone of inhibition ranged from 12-19mm.Honey exhibits antimicrobial activities against S.aureus bacteria and this study was aimed to compare the effect of seven types of honey.

The inhibitory effect of honey on bacterial growth showed Sativa honey(19mm inhibition) had highest antibacterial growth effect to S.aureus and the efficacy of different types of honey against S.aureus was depend on the types of those honeys.

This study was in line with (Selvamohan et al, 2016) which showed maximum zone of inhibition 18mm and minimum 6mm inhibition against S.aureus. Another study indicated that honey sample shown marked inhibition of growth of S.aureus the maximum inhibition zone was shown at concentration of 100% as 20mm which reduce to 10mm at 75% concentration (Taha et al, 2009). The antibacterial activity of raw honey compared with popular antibiotics showed that the zone of inhibition ranged from 6.94mm to 37.94mm (Chauhan et al, 2010).

Table 6: Antimicrobial properties of Belete Gera Honey Jimma-Ethiopia (n=48).

S.No	Honey types	Inhibition zone Result	Tetracycline control
1.	Eucalyptus honey	17mm	26mm
2	Guizotia-spp	10mm	
3.	Viciafaba	14mm	
4.	Trifolium	15mm	
5.	Vernonia	11mm	
6.	Hypericum	16mm	
7.	Sativa	19mm	

5.3.13. Antioxidant property

The phenol, flavonoid, DPPH, FRAP and peroxide scavenge value of honey sample is stated in Table 7.

Table 7: Mean ±sd for phenol, flavonioid, peroxide scavenging and ferric reducing properties of Belete-Gera Honey Jimma-Ethiopia, (n=48).

S.No	Honey Type	DPPH Free radical scavenger(%)	Phenolic content(GAE/kg)	Flavonoid Content (mgQE/kg)	Peroxide radical scavenger(%)	FRAP Values (%)
1.	Eucalyptus honey	47.40±1.13	615.6±5.50	41.50±0.84	63.6±2.82	16.10±11.17
2.	Guizotia-spp	52.72±0.32	536.4±4.00	24.22±0.73	59.2±0.46	23.30±13.43
3.	Vicia faba	42.27±1.13	453.1±3.12	31.83±0.16	86.3±11.03	20.46±8.62
4.	Trifolium	55.93±0.69	413.2±2.51	11.09±0.11	72.7±6.36	15.42±10.26
5.	Vernonia	37.29±0.11	425.0±2.00	12.18±0.01	76.2±7.21	62.82±16.99
6.	Hypericum	60.01±0.87	406.7±3.13	25.62±0.08	82.4±7.91	46.30±19.96
7.	Sativa	72.76±5.09	61.95±1.47	18.90±0.11	65.0±1.13	67.82±18.13

5.3.14. Phenol content

The total phenolic content of the honey samples ranged from 406.7 to 615.6mg GAE/kg honey with mean 415.90±3.10mg GAE/kg (Table 7), which was done by the modified folin ciocateu method which was sensitive to phenol and polyphenol entities. A study in Malaysia

honey showed that the total phenolic compound range from 316.67 to 561.67mg GAE/kg honey (Wen et al, 2014).

Another study in Argentina showed that the total phenolic content ranged from 40.3 to 193.0mg GAE/100g and Eucalyptus honeys exhibited significantly higher content and free-radical scavenging activity (Maria et al, 2014).
A study done Turkey showed that the total phenolic content of honey varied from 35.36 to 365.94 mg GAE/kg with a mean of 155.55mg GAE/kg using a standard curve of gallic acid (Asli et al, 2010). The total phenolic content ranged from 250 to 548 mg galic acid per kg of honey (Silvio et al, 2015).

5.3.15. Flavonoids

The total flavonoid content of honey sample varied from 11.09 to41.50mgQE/kg and average 23.55mg QE/kg, with the lowest and highest observed in sample Trifolium and eucalyptus honeys respectively Table 7, and in this study we used a spectrophotometer quantification of flavonoids with aluminum chloride.

This result was in line with the study done in Turkey which showed that the total flavonoid content of honey samples was found ranged from 4.80 to 54.78mg of QE/kg with a mean of 22.80mg of QE/kg (Asli et al, 2010). The total flavonoids result ranged from 9 to 48.6mg of quercetine per kg of honey (Silvio et al, 2015).

5.3.16. Free radical scavenging activity

The free radical scavenging activity of the honey samples resulted from 37.29 to 72.76% with an average of 52.60% discoloration of the DPPH Table 7. The IC50 value which is the concentration of antioxidant (honey concentration) that causes 50% inhibition of DPPH was calculated from the equation of the curve for each individual samples.

The antioxidant properties of each honey sample was investigated by evaluating the free radical scavenging activity of DPPH radical and the extracted honey 0.5ml was mixed with

methanolic solution 2.7ml containing DPPH radical 0.024mg/ml and the mixture was shaken and left to stand for 15min in the dark in order for the absorbance to stabilize and the redaction of the DPPH radical was determined by measuring the absorbance of the mixture at 517nm finally the radical scavenging activity was calculated as the percentage of DPPH discoloration using the following equation.

$\%RSA = ({}^{A}DPPH\text{-}As/{}^{A}DPPH)*100$

5.3.17. Peroxide Radical scavenging activities

All Belete-Gera honey samples have good peroxide scavenging activity in which most of them exhibited from 59.2 to 86.3 % with an average of 72.7% Table 7, peroxide inhibition which was higher as compared with study done in Malaysia with total inhibition ranged from 20.95 to 76.99% (Wen et al, 2014).

5.3.18. Ferric reducing antioxidant power

The ferric reducing antioxidant power of honey samples ranged from 15.42 to 67.825% an average 30. (Table 7), this was in line with the study in Italy with value 18.83 to 79.5% and in Slovenian study (Saric et al, 2012).

5.3.19. Sensory analysis

Two groups of samples were set up one Eucalyptus honey and the other Sativa honey, the samples were tested by a panel of 50 assessors who were asked to evaluate the degree of acceptability by giving a score from 1 to 7 (7-extermely liked and 1 extremely disliked) about taste, smell, colour and general appearance of the samples.

Table 8: Sensory quality attribute of Belete-Gera honey Jimma-Ethiopia.

Honey Types	Parameters	Total panellist (N)	Mean±sd
Eucalyptus honey	Taste		5.44±0.951
	Smell	50	5.54±0.952
	Colour		5.52±0.788
	General acceptance		5.78±0.840
Sativa Honey	Taste	50	5.32±1.13
	Smell		5.20±0.947
	Colour		5.86±0.833
	General acceptance		5.58±0.991

The main factors on mean acceptability of Gera forest honey with respect to taste, smell, and colour and over all acceptability are presented in Table 8. The score of the taste acceptance values ranged from 5.32±1.13 to 5.44±0.95 with mean value 5.38±1.04. There were no significant difference ($p>0.05$) in the taste acceptance among Eucalyptus and Sativa honey samples and all the taste score of the honey were between like and extremely like thus the taste of honey samples were acceptable by the panellists.

The score of smell acceptance ranged from 5.20±0.94 to 5.54±0.95 with a mean 5.37±0.945. There was no significant difference ($p>0.05$) in smell acceptance among Eucalyptus and sativa honey and the entire smell test were between liked and extremely liked. Therefore the smell of Gera forest honey was acceptable by panellists.

The score of the colour acceptance test ranged from 5.52±0.78 to 5.86±0.83 and the mean value of colour acceptance was 5.69±0.80, there was no significant ($p>0.05$) difference in colour among Eucalyptus and Sativa honey samples and all the colour scores of honey samples were in between like and extremely like based on the scale indicated the colour of Gera honey was acceptable to the panellists.

The score of general acceptance ranged from 5.58±0.99 to 5.78±0.84 in a scale of seven with a mean of 5.68±0.915. Generally the acceptance level of Belete-gera honey was between liked and extremely like and all the scores of acceptability were above liked which indicate high level of acceptability.

The Sensory profile of the honey samples evaluated for their various attributes (Taste, Smell, Colour and General appearance). There is statistical difference between the honey samples and this can be explained by the fact that honey characteristics are different by several factors including botanical source and climatic conditions. The sensory analysis showed higher intensity of sweetness and smell for eucalyptus honey than the sativa honey and sensory methodology in combination with physicochemical and melisnological analysis improves the assignation of floral origin of honey.

The color, aroma and flavour are major sensory characteristics of honey which are mainly determined by the botanical origin of honey as well as by processing and storage conditions (Nadezda et al, 2014).

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The physical properties of honey are related with the chemical composition of honey and it is dependent on the botanical and geographical origin. The physical properties could be used to predict the antioxidant activity in honey and pH, color, electrical conductivity determine the quality of honey.

The results obtained in this study confirmed that honey's composition depends on a great extent on its botanical origin thus floral origin is also influence most honey's health promoting properties and the most appropriate analytical method which to evaluate the antioxidant capacity by determining the total phenolic compounds in a sample with combination of the radical scavenging activity.

Honey is rich in phenolic and flavonoids which exhibit a wide range of biological effects and acts as natural antioxidant. The analysis of polyphenols had been regarded as very promising way of studying floral and geographical origins of honey. The analytical procedure to determine individual phenolic compounds involves extraction from matrix, analytical separation and quantification.

Finally as a conclusion the quality of honey mostly depend on its chemical composition and floral origin. This study showed that the honey samples contained phenolic compounds, flavonoids and radical scavenging activities. The obtained results of honey analyse indicate the high quality of honey produced in Jimma forest and the quality of honey corresponds to Codex, EU and Ethiopian standards.

6.2 Recommendation

Based on this research the following points are recommended

1. Honey is unique on its floral source of the nectar from which it was made; accordingly assessment of the physicochemical and antioxidant property of honey from other area is necessary.
2. Quality honey is produced in Belete Gera forest; Jimma, Ethiopia so entering to international market is required.

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8. APPENDIXES

Appendix I. Sensory analysis questioner

Dear panelist,

Use the number scale below to mark which number that you like or dislike about honey samples

7. Extremely liked

6. Liked a lot

5. Liked

4. Nether liked nor disliked

3. Disliked

2. Much disliked

1. Extremely disliked

----------General appearance

----------Taste

----------Smell

----------Color

Appendix II. ANOVA for sugars

ANOVA for the effect of Fructose

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	22.76184286	3.79364048	2.10	0.1770
Error	7	12.64270000	1.80610000		
Corrected Total	13	35.40454286			

ANOVA for the effect of Glucose

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	30.84920000	5.14153333	0.62	0.7105
Error	7	57.89420000	8.27060000		
Corrected Total	13	88.74340000			

ANOVA for the effect of Sucrose

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	10.56234286	1.76039048	4.50	0.0346

Error	7	2.73720000	0.39102857		
Corrected Total	13	13.29954286			

ANOVA for the effect of Maltose

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.13657143	0.02276190	0.09	0.9950
Error	7	1.71320000	0.24474286		
Corrected Total	13	1.84977143			

Appendix III. ANOVA for physicochemical

ANOVA for RI

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.27787122	0.04631187	3104.61	<.0001
Error	7	0.00010442	0.00001492		
Corrected Total	13	0.27797564			

ANOVA for moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F

Model	6	30.14857143	5.02476190	80.90	<.0001
Error	7	0.43480000	0.06211429		
Corrected Total	13	30.58337143			

ANOVA for a_w

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.00914286	0.00152381	3.56	0.0607
Error	7	0.00300000	0.00042857		
Corrected Total	13	0.01214286			

ANOVA for Acidity

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	415.4285714	69.2380952	86.18	<.0001
Error	7	5.6242000	0.8034571		
Corrected Total	13	421.0527714			

ANOVA for pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.88360000	0.14726667	343.62	<.0001
Error	7	0.00300000	0.00042857		

Corrected Total	13	0.88660000			

ANOVA for Ash

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.39428571	0.06571429	143.75	<.0001
Error	7	0.00320000	0.00045714		
Corrected Total	13	0.39748571			

ANOVA for HMF

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	162.1829714	27.0304952	1.54	0.2911
Error	7	122.8056000	17.5436571		
Corrected Total	13	284.9885714			

ANOVA for optical density

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.03699040	0.00616507	2157.77	<.0001
Error	7	0.00002000	0.00000286		
Corrected Total	13	0.03701040			

ANOVA of EC

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	0.15468571	0.02578095	5.47	0.0210
Error	7	0.03300000	0.00471429		
Corrected Total	13	0.18768571			

ANOVA for SR

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	6	48.31428571	8.05238095	12.59	0.0019
Error	7	4.47560000	0.63937143		
Corrected Total	13	52.78988571			