Physicochemical and Antioxidant Properties of Honey and Pollen from West Shoa, Ethiopia

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GRADUATE PROGRAMMES

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Abstract

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Adds Ababa University, 2018

Honey is the sweet substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. Pollen is the male reproductive cells of flowers. The objectives of the study were to investigate the botanical origin, physicochemical and antioxidant proprieties of West Shoa honey and pollen of Ethiopia.The physicochemical properties of those honeys were determined using moisture content, electrical conductivity, $_{P}$H, ash, free acidity, Hydroxymehylfurfural, sugar, total protein content and fat for different types of honeys and the proximate composition of pollen was analyzed. The antioxidant content and antioxidant activities of the honey and pollen were evaluated. The botanical origin found were Trifolium *species, Guizotia scraba, Eucalyptus globulus and Multiflora* honey. *Plantago lanceolata* and *Eucalyptus globulus* were used pollen characterization. The highest moisture content were seen in *Trifolium* spp honey (16.54 ± 1.68%) while that of multiflora honey had lowest moisture content (15.65±0.93%). The highest total phenolic content recorded in multiflora honey (196.54±6.47 mg of GAE/100g) and the highest ferric reducing antioxidant power recorded in *Guizotia scabra* honey (70.76±4.79). The highest radical scavenging activity content recorded in *Plantago lanceolata* pollen (70.71±0.39%).Accordingly it is possible to state that West Shoa honey and pollen are good quality honey and pollen in relation to the physicochemical and a high antioxidant source of food.

Keywords: Antioxidant, Botanical origin, Honey, Physicochemical, pollen

DECLARATION

This thesis is my original work except where due reference has been made in the acknowledgments. This work has not been submitted for a degree in any other University.

Signature……

Date…….

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

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Place and date of submission: Center for Food Science and Nutrition

Addis Ababa University

June, 2018

DEDICATION

This thesis is dedicated to my lovely mother Denbale Tafesse for all scarifies she made in her life.

Abbreviations and Acronyms

AOAC Association of Official Analytical Chemists

ARP anti- radical power

CA Codex Alimentarius

CSA Central Statistical Agency

DPPH 2,2-diphenyl-1-picrylhydrazyl

EU European Union

FRAP Ferric reducing antioxidant power

FAO Food and Agriculture Organization

HPLC High performance liquid chromatography

IHC Harmonized methods of the International Honey Commission

QSAE Quality and Standards Authority of Ethiopia

TAP antioxidant power

WHO World Health Organization

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List of Figures

Figure Page

List of Tables

Tables page

1. INTRODUCTION

1.1. Background

Honey has been used as a food and medicine since the ancient times. Honey is the sweet matter made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees (Belay *et al.*¸ 2013). It contains about 181 substances (Alvarez-Suarez*etal.*, 2010; Abdulrhman*etal.*,2011; Ibrahimetal.,2012).

The compositions of honey are mainly the monosaccharide sugars. Next to sugars, moisture is the 2nd most abundant component of honey. The water content of honey ranges between 15 to 20% (average 17.2%) Abdulrhman *et al.* (2011). Glucose and fructose account for about 85% of the honey solids. In addition, various disaccharide sugars (sucrose, maltose isomaltose and turanose), acids, proteins and minerals have also a part in the solid component of honey (White, 1975; White, 1980).Honey also contain phenolic compounds, which act as natural antioxidants and plays potential role in contributing to human health (Khalil &Sulaiman, 2010).

According to Codex Alimentarius (2001), physicochemical propriety of honey has a role in describing the quality property of honey. These physicochemical properties are: moisture content, reducing sugars, sucrose content, total acidity, ash content and hydroxymethylfurfural (HMF). These quality parameters are used to characterize individual honey types (Feás *et al.*,2010). According to Belay *et al*. (2013), the physicochemical properties for a given honey is influenced by the nectar types that the honey bee used, and Agro- ecology.

Honey has been traditionally used for different purposes and has a great potential to serve as a natural food and source of antioxidant. Now a days, the demand for antioxidant supply in the food is highly increasing. Honey is becoming popular as a source of antioxidant since it is rich in phenolic acids and flavonoids and other antioxidants as an effective protection against oxidative damage the human cell (Meda *etal.*, 2005).The antioxidant activity of honey, however, varies greatly depending on the honey floral source (Liu *etal.,* 2013).

Pollen is the male reproductive cells of flowers and the source of protein to honey bees and it affects growth, and ability to rear brood. Bee pollen is rich in proteins, essential amino acids, fatty acids, vitamin complexes, lipids, trace elements, phenols and polyphenols, which are responsible for its antioxidant property. As result pollen has great potentials for use in human diet (Gupta *etal.*, 2011).

The chemical and nutritional composition of pollen varies according to floral source and geographical origin, in addition climatic conditions, soil type, processing and handling are known to influence the pollen chemistry (Sattler *et al.*, 2016). Due to its high lipid, protein and amino acid contents, the use of bee pollen as a dietary supplement by humans has rapidly increased (Ulusoy *et al.*, 2014). In Ancient time, honey bee collected pollen is intentionally consumed in different countries because of its therapeutic abilities (Reinhard and Adii, 1994).

Health benefits of bee pollen is due to the presence of bioactive compounds associated with their potential to inhibit the growth of microorganisms and prevention of oxidative stress resulting for the development of chronic degenerative diseases such as cancer, cardiovascular diseases, and neuronal degeneration (De-Melo *etal.*, 2015 ; Sattler*etal.,* 2015).

Bee pollen is one of the very valuable commodities on international market and its unit price is much more than the price of honey. This indicates the potentiality of pollen collection and selling as source of income for beekeepers. Even though the pollen trapping equipment was developed based on the African bees size In Ethiopia the pollen trap designed from local available material and its efficiencies and its effect honey and brood rearing was studied (Adgaba *et al.*, 2015) but in Ethiopia pollen is used for natural pollination and pollen analysis of honey (Lemessa, 2006).The pollen analysis mainly used for botanical identification (Melissopalynology) of honey (Von Der Ohe *et al*., 2004; Adgaba, 2007).

Through pollen analysis and field observation Adgaba *et al*.(2001)¸ have identified 181 plant species in West Showa zone as an important nectar and pollen source plants. However there is no study on nutritional use and health benefit of other bee products like pollen. Today, for sustainable development of the sub sector and also to increase and diversify the incomes of farmer beekeepers, it becomes very essential to efficiently utilize the available apicultural resources.

Today, the basic concept of nutrition transformed from the classical concept of adequate nutrition to optimal nutrition, which are engaged in preventing non communicable diseases and improve the physical and mental well-being of the consumers (Siro *etal.*,2008). This can possibly be used to facilitate developing market for natural food antioxidants based on two aspects: well documented protective effect against cancer and cardiovascular diseases of these natural antioxidants and a general rejection of synthetic antioxidants by the consumers (Saura-Calixto, 1998).

Ethiopia is the leading honey producer in Africa, and one of the ten largest honey producing countries in the world (Mengistu , 2016). Likewise, West Shoa is one of the honey producing potential area, which accommodate about 204,449 bee colonies (CSA, 2014). The most widely known honey plants, which are used as a source of nectar and pollen are *Guizotia scabra (Mech/Tuffo) and Guizotia. abyssinica (Noug), Eucalyptus globulus, Sheffleria abyssinica (Geteme), Vernonia species (Grawa), Syzygium guineense (Dokma),Acacia species, Croton machrostachyus, and Erica arborea (Asta)(Leggesse* , 2013).This is mainly attributed to the presence of diversified agro-climatic conditions and biodiversity, which favored the existence of diversified honeybee flora and large number of honeybee colonies (Adgaba, 2007).

In Ethiopia, few studies were done on botanical origin, physicochemical propriety of Ethiopian honey (Fichtl and Adi, 1994; Adgaba, 2007; Belay *et al.,* 2015). Even though, West Shoa is endowed with a huge apicultural potential and it is known in contributing a large volume of honey, there is no study done on honeys botanical origin from field flowers, herbs and shrubs; which are used as a typical source of honey in this study area.

Therefore, this research finding is certainly contributes in investigating the physicochemical properties and antioxidant propriety of honey, which ultimately add value for Ethiopian honey value chains.

1.2. Statement of the problem

Of the total honey production in Africa and globally, Ethiopia shares around 23.58% and 2.13%, respectively (Beyene *et al.,* 2014). Ethiopia has the potential to produce 500,000 tons of honey per year, but currently production is limited to 54,000 tons of honey (CAS ,2017). According to CSA (2016/17) livestock and livestock characteristics report Ethiopia, honey production from all types of bee hive was 47.7million kilogram. Because 95% of the beekeepers use traditional method of beekeeping practice with no improved techniques or technologies, the quality of Ethiopian honey is affected and graded as poor (Beyene *et al.*, 2014).

Honey has different properties this can be: physicochemical, botanical and antioxidant. This property of honey has a role in determining the quality property of honey and its nutritional values. However, only little information are available on botanical origin and physicochemical properties of Ethiopian honey and there is a knowledge gap regarding antioxidant proprieties of Ethiopian honey that need to be studied. In view of the growing interest of pollen as nutritional and api-therapeutic substance, identification of major source plants and evaluating their quality is a paramount for promoting bee pollen as food supplement for the benefit of health. In this regard, Ethiopia has the great potential for production of a quantity and quality of pollen since the country is endowed with great diversity of flora and high population density of *Apis mellifera,*which creates

suitable conditions to gathering all year-round. West Shoa, being one of the major potential honey production area, there is a strong need for such a study. Therefore, studying the botanical origin, physicochemical and antioxidant proprieties of West Shoa honey will contributes to fill the gap in knowledge regarding the above mentioned proprieties of honey and also adds value to the quality of Ethiopian honey.

Different studies were undertaken in the study area regarding production, marketing and value chain development of honey. The studies by Mekonnen, (2015) reported the different factors contribute to the low quality honey produced in the study area. Of the problems indicated, botanical information, physicochemical and antioxidant properties of honey and pollen was described in this study.

Due to the growing interest of pollen as nutritional and api-therapeutic substance, the identification of major pollen source plants used by bees and the evaluation of their quality as paramount for promoting this product as food supplement are required to improve health. Bee pollen antioxidant activity and pollen nutritional composition of bee pollen is not studied for Ethiopia and regarding the study area. In Ethiopia the presence of relatively good vegetation coverage and a high honeybee colony population, facilitates significant amount of pollen collection and supplying to food supplements for local and international markets (Adii *et al* ., 2017) .

Therefore, these research findings certainly contribute in defining the botanical origin, physicochemical and antioxidant proprieties of honey and pollen, which ultimately add value for Ethiopian honey value chain development. The ultimate goal of characterizing

the physicochemical and antioxidant proprieties of honey and pollen is to get a good quality of honey and pollen their contribution to the human health benefits as this mainly depends on botanical origin of honey.

1.3. Objectives

1.3.1. General Objective

The objectives of the study is to investigate the botanical origin, physicochemical and antioxidant proprieties of West Shoa honey and pollen of Ethiopia.

1.3.2. Specific Objectives

The specific objectives of this study were:

- To identify the botanical origin of honey and honeybee collected pollen samples based on botanical origin
- To characterize the physicochemical proprieties of honey and bee pollen.
- To examine antioxidant propriety of honey and bee pollen.

2. LITERATURE REVIEW

2.1. Floral origin of honey

Melisopallynology is the identification and quantification of pollen grains in honey sediment it is the most important method for determining the botanical origin of honey (Anklam, 1998).Honeys that are made from nectar belong to a single plant an extent of at least 45% comprising pollen count percentage is called monofloral. Monofloral honeys, originating predominantly from a single botanical source, which has higher demand from the consumer, or have a higher commercial value for the producer.

Variations in nectar content, together with other factors such as climatic conditions, soil type, beekeeper activities, contribute to the existence of different types of honeys (Anklam, 1998). Differences honey composition, is due to differences in the organoleptic andnutritional properties of these honeys (Feás*etal.*,2010).The physico-chemical parameters of natural honeys,suchasmoisture, sucrose and Hydroxymehylfurfural(HMF) contents, acidity and specific conductivity, are constitute the quality indicators which characterize individual honey varieties (Feás *et al*., 2010).

2.2. Physicochemical proprieties of honey

The physicochemical properties for a given honey is influenced by the nectar types that the honey bee used, geographical ecology (climatic and soil) and postharvest honey handling practices. Analysis of the physicochemical properties of honey is used to verify the genuineness of the product and to tell the possible presence of artificial components or adulterants (Meda *et al.*,2005; Belay *et al.,* 2013)

Physicochemical parameters which includes moisture, reducing sugar, sucrose, water insoluble, ash, free acid, Hydroxymehylfurfural contents, pH, electrical conductivity and specific rotation. The color of the honey collected by the bees varies according to the floral source and its mineral content, which usually ranges from water white to dark amber. Flavor of the honey depends upon the color, generally the darker the honey the stronger the flavor and quality Khalil and Boukraa .(2010).

2.3. Antioxidant properties of honey

Honey is a supersaturated solution of sugars, of which fructose (38%) and glucose (31%) are the main contributors of the honey composition. A lot of minor constituents is also present in honey, many of which are known to have antioxidant properties. According to Meda *et al.*(2005); Khalil and Boukraa. (2010) honey has been found to contain significant antioxidant, antioxidative activity which include both enzymatic (catalase, glucose oxidase) and non-enzymatic substances (organic acids, maillard reaction products, amino acids, proteins, flavonoids, phenolics, a-tocopherol, flavonols, catechins, ascorbic acid and carotenoids). The antioxidant activity of phenolic compounds might significantly contribute to the human health benefits and protect key cell components from damage by neutralizing the free radicals.

Honey has been used in ethno medicine since the early humans and in more recent times its role in the treatment of burns, gastrointestinal disorders, asthma, infected and chronic wounds, skin ulcers, cataracts and other eye ailments has been ‘‘rediscovered”(Ferreira *et al.*, 2009). However, since some of these diseases are a consequence of oxidative

damage, it seems that part of the therapeutic properties of honey is due to its antioxidant capacity. Antioxidants are nutritive and nonnutritive agents that can retard biologically destructive chemical reactions in foods and living organisms. These compounds are considered to protect humans from disease, in part, through their ability to scavenge oxidants and free radicals, absorbing molecular damage that might otherwise compromise the function of essential lipids, proteins, and nucleic acid (Schramm *et al.,* 2003).

Natural antioxidants can be phenolic compounds (tocopherol, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyl derivatives, amino acids, and amines), carotenoids as well as ascorbic acid. Phenols are very proficient scavengers of peroxyl radicals, because of their molecular structures which include an aromatic ring with hydroxyl groups containing mobile hydrogens. Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion which catalyse lipid peroxidation (Al-Mamary *et al.*, 2002). Significant differences in both composition and content of phenolic compounds have been found in different unifloral honeys (Khalil *et al*., 2011). Also the antioxidant activity depends on the botanical origin of honey and varies to a great extent in honeys from different botanical sources(Liu *et al*., 2013).

2.4. Pollen grain nutrient rich food

Pollens grains are the male reproductive cells of flowers and pollen is a fine, powder like material produced by flowering plants and gathered by bees (Llnskens and Jorde, 1997) and Morais *et al*.(2011) and pollen mainly collected by the honey bee *Apis mellifera* for the purpose of feeding its larvae in the early stages of development, Campos *et al.*(2008). Bee collected pollens composition can vary due to their botanical and geographic origin

contain nutritionally essential substances like carbohydrates, proteins, amino acids, lipids, vitamins, mineral substances and trace elements but also significant amounts of polyphenol substances mainly flavonoids, The early Egyptians and ancient Chinese used pollen as rejuvenating medicinal agent. Hassan.(2011) and Morais *et al*.(2011) and Bee pollen is considered to be a nutrient rich perfect food and is promoted as a commercially available supplement.

Bee collected pollen is also a very important source of vitamins and polyphenolic compounds, particularly flavonoids, which may act as potent antioxidants (Campos *et al.*, 2003; Arráez-Román *et al.,* 2007). Among natural products, honeybee pollen has been applied for centuries in traditional medicine as well as in food diets and supplements . In ancient times bee pollen is used to cure conditions such as colds, flu, ulcers, premature aging, anemia, colitis, allergic reactions and enteritis. Bee pollen is also used in the field of cosmetics for its contribution of vitamins to cold creams (Ulusoy *et al.*, 2014).

Currently the pollen, is increasingly used as health food supplements and is marketed widely in Europe and Asia as a tonic primarily with appeal to the elderly to meliorate the effects of ageing. The consumption of bee pollen or its derivative products, as a dietary supplement, and can be considered as a potential source of energy for human consumption has been rapidly increasing (Solange *et al.,* 2007) due to its high lipids, sugars, proteins, amino acids, vitamins, carotenoids, polyphenolics such as flavonoids ,carbohydrate and amino acid contents (Ulusoy *et al.*, 2014; Graikou *et al.*, 2011).

Proteins are made up of building blocks called amino acids, composed of carbon, hydrogen, oxygen and nitrogen (amino group).Proteins from different food sources contain different amounts of amino acids. Essential amino acids are those that the body cannot synthesis and must therefore be provided from outside. Proteins are required to build new tissue, particularly during the rapid growth period of infancy and early childhood, during pregnancy and nursing, and after infections or injuries. Excess protein is burned for energy (WHO/FAO, 2002). The quality of the diet depends to a large extent on the amount of protein it contains. This is because protein foods are usually carriers of other important nutrients such as vitamins and minerals (Cameron and Hofvander,1983).Generally foods eaten in developing countries contain high levels of carbohydrate with low or no protein due to the high cost of protein rich food Amankwah *et al.*(2009).

2.5. Pollen trapping

Now a day bee products like bee collected pollen become very important to increase and diversify the incomes of beekeepers. To increase the harvesting of pollen from local bees; improving of the efficiency of pollen trap is very important. Therefore pollen trap with 4.5mm opening its efficiency and its effect on honey yield was evaluated (Adgaba *et al* ,. 2015) and the result of study shows that the highest pollen yield (4.7gm/day/colony = 141gm/month/colony) and10.9kg/colony average honey yield obtained from pollen trap with opening size of 4.5mm. Using pollen trap with 4.5mm opening size is very suitable to local bees to collocate pollen without affecting the honey yield.

Figure 1. Pollen trap

3. MATERIALS AND METHODS

3.1. Study Area Description

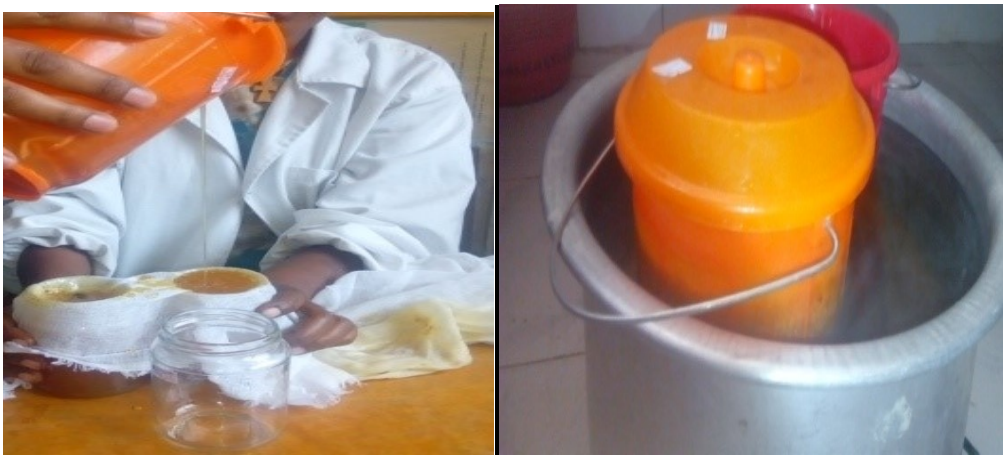
The Study was conducted in Ada'a Berga and Ejere districts of West Shoa zone, Ethiopia and the districts are selected purposively based on their beekeeping potential. Ada'a Berga District is located in central Ethiopia and 64 km away from Addis Ababa, with an area of 798.35 sq. kilometers. Its altitude ranges from 1,400 to 3,500 m and its agro-ecology is divided into lowland (37%), midaltitude (34%) and highland (29%) with an annual rainfall of 918 mm to 1,368 mm while Ejere district is located on 40kms away from Addis Ababa. The elevation of the Ejere district range from 2060-3185 m a.s.l and it lies between the coordinate of 38°15'E-38''30'E latitude and 9°0'0''-9°15''N longitudes. The area receives between 25% 900-1200mm annual rain fall and have mean temperature ranges from 22-28°C. The climatic condition of the area is divided into highland (45%) and mid land (55%) condition also the district covers about 56918 hectares of land.

3.2. Harvesting and Honey Sampling

Fifty beekeepers, twenty five from each district, randomly selected from the study area. Thirty six honey samples, eighteen from each district, was collected using lottery sampling methods, at the farm gate (Figure 1). Honey samples was collected during the major honey flow season from November 27 to December 30,2017.The honey samples were collected both from traditional, transtional and frame hive and the honey combs of the traditional or transitional hive were broken into pieces and strained using honey sieve and the honey from modern hive were extracted using honey extractor.

The honey transported to temporary extraction places using empty super box in beekeepers home. The sealed frame combs decapitated using uncapping fork and inserted into the honey extractor. Through centrifugation, the honey was drained from the cell and taken from the outlet of the honey extractor. The honey samples was immediately separated from other impurities using straining and settling, and poured in a food grade glass cup, according to Belay *et al.*(2017) (Figure 2.a and b and Figure 3).

Figure 2: Honey samples collected from Ada'a berga and Ejere districts



a b

Figure 3.a and b. honey samples separation from impurities and strain

Figure 4. Honey storage in Holeta Bee Research center in sample preparation room

3.3. Pollen Sampling

Pollen sample was collected from Holeta Bee Research Center in Muger sub-site. Pollen loads, trapped using pollen traps, having 16% pollen trapping efficiency which was fitted at the entrance of beehives. The pollen samples were placed the clean paper bags and left for 24h to dry at room temperature for drying. Collected pollen pellets was weighed for fresh and dry weights and sorted by color. The fat content was washed out using ether to enhance the clearness of pollen grains. The slides were covered with a cover slip and examined under a light microscope having 400X magnifications. Photos of pollen grain morphology was made using a light microscope linked with the computer program and pollen grains identified to genus or species level using the pollen atlas of Ethiopia (Adgaba, 2002).

Figure 5. Pollen samples collected

3.4. Analysis of Botanical origin

Pollen analysis was done to determine the botanical origin of honey (louveax *et al* 1978).Ten gram of honey, in centrifuge tube, was dissolved in 20 ml of warm distilled water at temp ranged 20-40^{0}C. The solution was centrifuged for 10 minutes and the supernatant was decant. Distilled water of 20 ml again was added to completely dissolve the remaining sugar crystals and centrifuged again for 5 minutes and supernatant was removed completely. The sediment was spread evenly using a micro spatula on microscope slide and the sample was dried for while then one drop of glycerin jelly was added to the cover slip and then the sample was examined under the microscope and the picture of the pollen was taken by the camera connected to the microscope (Carl ZEISS microscope Germany). The pollen source plant was identified using reference slides and publications of pollen atlas (Adgaba, 2007); and frequency occurrences of pollen was determined by counting 500 pollens from a single slide. The pollen count was converted into percent to calculate the relative dominance, secondary, tertiary and quaternary enrichment of honey plant species of the honey sample.

3.5. Color determination

Colors of the honey samples were measured using a Pfund grader (Koehler Bohemia. NY).Hundred (100 g) of honey was poured into the sample holder of the Pfund grader. Determination was based on the matching of the honey sample colors with the color indexes present in the glass Pfund grade.

3.6. Honey Analysis

3.6.1. Physiochemical properties

3.6.1.1. Moisture

Moisture content was determined using Abbe refractometer (ABBE- 5 Bellingham Stanley. Ltd, United Kingdom,) at 20^{o}C. Directly after homogenization of honey, r the surface of the prism was evenly covered with the sample of honey and after 2 minutes the reading of the refractive index. Distilled water (1.3330) was used as a reference. The refractive index reading was converted to moisture content (g/100 g) using (AOAC, 1990)

3.6.1.2. Sugars

Honey sugars were determined using High performance liquid chromatography (HPLC-1260 Infinity Series Agilent Technologies, Germany). Five gram of honey was dissolved in 40 ml water, 25ml of acetonitrile pipetted into a 100ml volumetric flask and the honey solution quantitatively was transferred to the flask and filled to the mark with water and syringe filter (0.45 µm) each solution before chromatographic analysis. The HPLC separation system was composed of analytical stainless steel column, 4.6 mm in diameter, 250 mm length, containing amine modified silica gel with 5-7 µm particle size. Flow rate 1.3 ml/min, mobile phase Acetonitrile: water (80:20, v/v) and sample volume

10 µl. The sugars was detected by a Refractive Index Detector thermo stated at 30^{o}C temperature regulated column oven at 30^{0}C. The identification of honey sugars was obtained by comparison of their retention times with those of the standard sugars as described in harmonized IHC (Bogdanov *et al*., 2002)

3.6.1.3. Hydroxymehylfurfural

Hydroxymethylfurfural (HMF) content was determined using 6800 UV–Vis spectrophotometer (JENWAY, United Kingdom). Different reagents was prepared first for the HMF determination Carrez solution I 15g of potassium hexacyanoferrate(II), $K_4Fe(CN)6•3H_2O$ was dissolved in water and make up to 100 ml. Carrez solution II 30g of zinc acetate, $Zn(CH_3.COO)_2.2H_2O$ was diluted and make up to 100 ml. 0.20 g of solid sodium hydrogen sulphite $NaHSO_3$, (metabisulphite, $Na_2S_2O_5$), in water and diluted to 100 ml. Carrez solution is clarification reagents to remove these interfering compounds from the analytes.

Five gram of honey was weighed into a 50 ml beaker and dissolved the sample in 25 ml of water and transferred quantitatively into a 50 ml volumetric flask. A 0.5 ml of Carrez solution I was added with 0.5 ml of Carrez solution II, mixed well and made up to the mark with water and filtered through filter paper; the first 10 ml of the filtrate was rejected. Pipette five ml was pippted in each of two 2 test tubes and five ml of water was added to one of the test tubes and mix well (the sample solution). Five ml of sodium bisulphite solution 0.2% added to the second test tube and mixed well. By subtracting the absorbance measured at 284 nm for HMF in the honey sample solution against the

absorbance of reference (the same honey solution treated with sodium bi-sulphite, 0.2%) at 336 nm as described in harmonized method (AOAC, 1990method).

HMF content of honey was calculated:

HMF in mg/kg honey = $(A_{284}- A_{336})$ x 149.7 x 5 x D/W

Where A284= absorbance at 284 nm, A336= absorbance at 336 nm,

Factor = 149.7= (126/1683) (1000/10)(1000/5)

126 = molecular weight of HMF, 16,830 = molar absorptive of HMF at 284 nm,

1000 = conversion of g into mg, 10 = conversion of 5 into 50 mL,

1000 = conversion g honey into 1000 g (kg),

5 = nominal sample weight, D= Dilution factor and W= weight in

g of the honey sample

3.6.1.4. Free acidity and pH

Free acidity of honey is the content of all free acids, expressed in mill equivalents /kg honey (meq of acid/1000 g) was determined using pH meter (METTLER TOLEDO, CHINA). About 10g of honey sample was dissolved in 75 ml distilled water in 250 volumetric flask and the standardized 0.1M NaOH was prepared one gram of NaOH was dissolved in 250 ml of distilled water and the dissolved honey sample was titrated with standardized 0.1M NaOH to pH 8.3 using pH glass electrode attached to pH meter as end point indicator , pH was determined by using glass electrode after calibration with standard buffer solution pH 4, 7 and 10 (AOAC, 1990method)

3.6.1.5. Ash

Ten gram of the honey samples (m_0) was weighed into a pre-weighed crucible (m_2) and then two drops of olive oil was added to the honey sample. Then ash dish (m_1) with honey was place in hotplate to remove water from the honey at low heat rising to 350 - 400^0 C and after the preliminary ashing, and the dish was placed in the preheated a muffle furnace at 600ºC until ashing complete (BioBase JKKZ .5.12GJ Muffle Furnace, Shandong.ldt, China) and was heated for at least 1 hour and the ash dish Cooled down in the desiccators(m_1) and weighed as described in harmonized IHC(Bogdanov,2009).

The proportion of ash (W_A) in g/100g honey was calculated using the following formula:

$W_A = (m_1 - m_2)/m_0)100$

Where: m_0 = weight of honey sample,

m_1 = weight of crucible with ash,

m_2 = weight of dish.

3.6.1.6. Electrical conductivity

The electrical conductivity of a solution of 20g dry matter of honey in 100 ml distilled water was measured using an electrical conductivity cell (BANTE Instrument- 520 conductive and temperature meter, China). A 0.745g of potassium chloride (KCl), was dried at 130°C, dissolved in freshly distilled water in a 100 ml flask and filled to volume with distilled water. Forty ml of the potassium chloride solution was transferred to a beaker and the conductivity cell connected to the conductivity meter, the cell rinsed thoroughly with potassium chloride solution and immerse the cell in the solution, together with a thermometer and reading of the electrical conductance of the solution in

mS after the temperature has equilibrated to 20^0C was taken as described in harmonized IHC (Bogdanov, 2009)

The cell constant K, was calculated using the following formula:

K=11.691x1/G

Where:

K=the cell constant in cm^{-1}

G= the electrical conductance in mS, measured with the conductivity cell.

11.691= the sum of the mean value of the electrical conductivity of freshly distilled water in $mS.cm^{-1}$ and the electrical conductivity of a 0.1M potassium chloride solution, at 20°C.

3.6.1.7. Crude protein content of honey

The determination of protein content was carried out using the Kjeldahl method with some modification. The total nitrogen content was first estimated from which the protein content was calculated using the 6.25 conversion factor for protein nitrogen using the AOAC Official Method 1990.

Sample preparation

One gram of honey sample was measured in four tecator tubes and were placed in the tecator rack. Three blanks were used in order to avoid over estimations of the results due to nitrogen from reagents. About 6 ml of concentrated sulfuric acid was added in to the tubes containing the sample using a pipette and then, mixed carefully and 3.5 ml of hydrogen peroxide was added step by step in to each sample tube. The tubes were shaken for a few times after the violent reaction has ceased and put back into the rack. A 3 g of

copper sulfate and potassium sulfate catalytic mixture were added into the sample tubes and the tubes were let to be stand for 15 minutes before digestion.

a. Digestion

The sample tubes were placed in a digester after the working temperature (370 ^{o}C) has reached and the digestion process has continued until the clear solution was observed. The sample tubes were taken out, placed in the rack and allowed to cool in fume hood.

b. Distillation

Twenty ml of distilled water was added into the sample tubes in order to avoid precipitation of sulphate. Forty ml of sodium hydroxide solution was added into the digested and diluted solution. Two hundred fifty ml conical flask containing 25 ml of boric acid, 25 ml of distilled water and an indicator solution were placed under the condenser of the distiller with its tip immersed into the solution. The distillation step is continued until the volume become between 200 ml and 250 ml. The tip of the distiller was rinsed with a few milliliters of water before the receiver was removed.

c. Titration

The solution containing an indicator, ammonium ion and borate ion was titrated using 0.1 N HCl till the color of the solution changes to reddish and the total volume of the HCl required to reach the endpoint of the titration was recorded. The volume of the HCl consumed during titration was adjusted by subtracting the average volume of HCl consumed by the blank from HCl consumed by each sample.

$$\%\text{Nitrogen} = \frac{\text{V HCl} \times \text{N HCl} \times 14.0 \times 100}{1000 \times \text{Wo}}$$

$$\%\text{Protein} = 6.25 \times \%\text{Nitrogen}$$

Where;

V-volume of HCl consumed (ml) to the endpoint of titration

N-the normality of the HCl used

Wo-Sample weight on dry matter basis

14-the molecular weight of atomic nitrogen

6.25-conversion factor

3.6.1.8. Fat content of honey

The fat content was determined by using acid hydrolysis method based on the (AOAC, Official Method 1990) with some modification. The weight extraction cylinder was taken (W1), and about 5 gm of honey sample was measured in the thimbles (W) and was covered with a layer of fat free cotton and the thimbles were put in the extraction chamber then extraction cylinders were taken out of the desiccators and put on the bracket. Fifty ml of ether was added into the extraction cylinders and moved into the heating plank and the extraction was left to go on for about 4 hours.

The extraction cylinders were disconnected and were put in a drying oven at 70^{o}C for about 30 minutes. The cylinders were taken out of the oven and cooled in desiccators for 30 minutes. The weight of the cylinders was measured immediately after they are taken out of the desiccators (W2). The percentage of fat in the sample was calculated using the following formula:

$$\%\text{Fat} = \frac{W2 - W1}{W} \times 100$$

Where;

W_1 = Weight of the extraction cylinder

W_2 = Weight of the extraction cylinder plus the dried crude fat

W = Weight of the sample

3.7. Pollen Analysis

3.7.1. Physiochemical properties

3.7.1. 1.Crude protein content

The determination of protein content was carried out using the Kjeldahl method. The total nitrogen content was first estimated from which the protein content was calculated using the 6.25 conversion factor for protein nitrogen using AOAC Official Method, 2005.

3.7.1.2. Fat content of pollen

The fat content was determined by using acid hydrolysis method based on the (AOAC Official Method 1984, number14.019).

3.7.1.3. Ash content of pollen

Ash content determined by AOAC, 2000 method

3.7.1.4. Pollen moisture content

Moisture contents were determined according to AOAC, 2000

3.7.1.5. Carbohydrate value of the pollen content

Carbohydrate value of the pollen samples were estimated using the method of Charrondiere *et al*. (2004).

%Carbohydrate = 100% – (%Moisture + Crude Fat + Protein + %Ash)

3.8. Antioxidant properties

Antioxidants was examined for its the antioxidant content (phenol and flavonoids) and antioxidant activity (Radical scavenging activity and Ferric reducing antioxidant power (FRAP) assay.

3.8.1. Antioxidant content

The Folin–Ciocalteu method was used to determine total phenolic content using Singleton & Rossi (1965) with some modifications. Each honey sample (5 g) was diluted to 50 ml with distilled water and filtered through Whatman No. 1 paper. The sample solution (0.5 ml) then mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent for 5 min and 2 ml of 37.5g/500 ml sodium carbonate (Na_2CO_3) then added and 0.01mg/ ml gallic acid prepared. After incubation at room temperature for 2h, the absorbance of the reaction mixture was measured at 760 nm using a UV- 7804C VIS spectrophotometer against a methanol blank. Gallic acid (0–200 mg/l) was used as standard to produce the calibration curve. The mean of three readings used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

The total flavonoids content of honey samples was determined based on the method of aluminium chloride (AlCl3), which was specific for flavones and flavonols methods measure the formation of colored complex substances quantitatively, after reacting

flavonoids with aluminium ion (III) (Liu *et al,* 2013).The total flavonoids contents of the honey samples were determined using aluminum chloride, and the results were expressed as mg quercetin/100g honey (Jonierison *et al*, 2014).The honey solutions were prepared at the concentration of 10 g/ml. Two milliliters of the stock solution were mixed with 3 mL of a 5% aluminum chloride solution. Following incubation for 30 minutes, the absorbance of the reaction mixture was measured at 437 nm using a UV-7804C VIS spectrophotometer against a methanol blank. Quercetin 0.1mg/ml was used as standard to produce the calibration curve.

3.8.2. Antioxidant activity

3.8.2.1. Ferric reducing antioxidant power assay

The reducing power of the ethanolic extracts of honey was determined by Saxena *et al*. (2010) with some modifications. One ml aliquot of ethanolic honey extract (10% v/v) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferri-cyanide (1%). The mixture was incubated at 50 ^{0} C for 20 minutes. After this, 2.5 ml of 10% trichloroacetic acid was mixed by vortexing. The mixture was centrifuged at 3000 rpm for 10 minutes. A 2.5 ml aliquot of the supernatant was mixed with an equal amount of milli Q water and 0.5 ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm using a UV- 7804C VIS spectrophotometer. Assays was performed in triplicate and ascorbic acid (0.0125mg/ml) was used as a reference standard. Ferric reducing antioxidant power (FRAP) values were expressed as micromoles of ferrous equivalent (μM Fe [II]) per g of honey. The increase in absorbance provided an indication of higher reducing power of the samples was analyzed.

3.8.2.2. Radical scavenging activity

The scavenging activity of honey samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by (Meda *et al*., 2005) with some modifications. Honey samples dissolved in methanol at a concentration of 2.65–170 mg/ml, and 0.75 ml of each sample was mixed with 1.5 ml of DPPH in methanol (0.09 mg/ml), methanol serving as the blank sample. The mixtures were left for 30 min at room temperature and the absorbance then measured at 517 nm using a UV- 7804C VIS spectrophotometer. Ascorbic acid (0 - 0.04 mg/ml) was used as positive control.

The radical scavenging activity was calculated as follows as % Inhibition = [(blank absorbance - sample absorbance)/blank absorbance] x 100.

3.8.3. Pollen Antioxidant content and activity

3.8.3.1. Preparation of pollen extract

Pollen samples were extracted according to the procedures developed by Ferreira *et al.* (2007) with some modification. Two grams of dried pollen powder was extracted by stirring with 25 ml of methanol and 25 mL of distilled water and placed at 25^0C for 60 minutes maceration using temperature shaker incubator and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 25 ml portions of methanol as described above. The combined methanol extracts were evaporated at 40°C to dryness using a rotary evaporator and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4 °C for further use.

3.8.3.2. Pollen Antioxidant content

Total phenolic content of pollen methanol extracts (PME) was quantified according to the Folin-Ciocalteu spectrophotometric method using galic acid as reference standard (Carpes *et al*., 2007).In each sample of 0,5 ml, two ml (1:10 dilution) of Folin-Ciocalteu reagent and after 8 minutes, 2 ml of Na_2CO_3(4%w/v) was added, stored this mixture in dark at room temperature for 2hours. The absorbance of all samples was measured at 740 nm using a UV- 7804C VIS spectrophotometer. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight of pollen (mg GAE/g dw) and are presented as the mean of triplicate analyses.

The total flavonoid content was determined using by the method described Rebiai *et al*. (2005). The methanol extract of pollen retaken and 1ml of methanol and treated with AlCl3 (2%, 1ml) in methanol solution. After 30 minutes, the sample were well mixed and absorption readings was undertaken at 430 nm (UV- 7804C VIS spectrophotometer). Quercetin was used to calculate the standard curve (0.1 and 0.02) and expressed as mg of Quercetin equivalent (QEs) per gram of extract.

3.8.3.3. Pollen Antioxidant activity

3.8.3.3.1. Radical Scavenging Assay

Free radical scavenger activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Cosmulescu *et al*., 2015) with some modifications. Briefly, 50 µl of sample extracts were mixed with 3 ml of ethanolic solution containing DPPH radicals (40 mg/L). The mixture was kept in dark for 30 minutes, and the absorbance was measured at 517nm using a UV- 7804C VIS spectrophotometer. All assays were

conducted in triplicate. Anti-oxidant capacity was expressed in mg ascorbic acid equivalents per gram (mg Ascorbic /g).

3.9. Statistical Analysis

Data were analyzed using SPSS version: 20.Analysis of variance (ANOVA) was used to test for statistical significance difference between physicochemical, and antioxidant properties. Differences at a 95% ($p< 0.05$) confidence level were considered statistically significant. Correlations between the parameters evaluated were obtained using Pearson's correlation coefficient (r).

4. RESULTS AND DISCUSSION

4.1. Botanical origin

Melissopalynology was an early branch of palynology (study of pollen and spores). Honey mostly includes numerous pollen grains, mainly from the plant species when they forage (Belay *et al*., 2015). Honey origin was confirmed by qualitative and quantitative microscopic pollen analyses. Honey is considered as predominant from a given botanical origin (unifloral honey), if the relative frequency of the pollen of that taxon exceeds 45% (Ohe., 2004). In addition to predominant frequency, different levels of abundance of given pollen type in nectar such as secondary, tertiary and quaternary enrichment are required for botanical description of honey (Belay *et al*., 2015). Accordingly, the relative pollen count found in honey samples indicated that *Guizotia scabra* was a predominant monofloral honey source plant and have higher relative frequency (38.9%) than the other species from Ada'aa Bargaa district while Trifolium species was the most frequent monofloral honey plant in Ejere district as indicated in Table.1. Multiflora honeys pollen have higher percentage in honey sample collected from Ada'a Barga than from Ejere district. Even though Eucalyptus plant starts to flower in April and honey harvested in May/June which is in line with the second season of honey harvesting, the flowers were found in the first season honey sampling sometimes the honey not harvested during honey harvesting season and passed as it is stored to first. The multiform honey is the collection of different honey plant species these are *Guizotia scabra, Hypoestes spp, vicia faba, plantago lanceolata, Ceasealpina spp, Rumex* spp and unidentified plant species.

Table 1.Relative frequency of enriching nectariferous species from West Shoa in district (% distribution)

Location	Pollen identified			
	Guizotia scabra	*Trifolium Species*	*Eucalyptus globulus*	*Multiflora*
Ada'aa Bargaa	38.9%	23.1%		38%
Ejere	16.7%	61.1%	11.1%	11.1%

Table 2. Relative frequency of nectariferous species from west shoa honey in each sample (% distribution)

Sample code	*Trifolium species*	*Guizotia scabra*	*Eucalyptus golobulus*	*Plantago lanceolata*	*Rumex*	*unknown*	*Hypostes trifolia*	*Caesalpina decepetal*
AB1	58	18			1			
AB2	8	71.8		0.2	3.4	21		14.8
AB3	60	21	1	11	1			
AB4	52	50		10				
AB5	70	14	24.5	10.8	19.2			
AB6	44.8	28		1			2	2
AB7	17	79.6%	7.6					
AB8	8.2	24.8	30.4	18.6	9.4	14.2	9.6	
AB9	49.8	43.8	20	8.8			21.6	
AB10	52.8	30	19					
AB11	8.2	86	0.2	4.8				
AB12	15	50		15			10	
AB13	36	54.6	11.4					
AB14	70	20		10				
AB15		65	1	25	2		5	
AB16		60	5	10	10	2	5	
AB17	15	65		5	10			
AB18	20	80						
EJ1	12.4	6	81.6					
EJ2	22	10	78					
EJ3	70	14.4	15.6					
EJ4	65	10	25					
EJ5	75	15	7	3				
EJ6	20	65	2	10	3		5	
EJ7	72	39.4	2.4					
EJ8	30	52	5					
EJ9	10	78		10			2	
EJ10	50	0.2	47	0.6				
EJ11	65	20	10					
EJ12	70	20	2	8				
EJ13	93.6		5.4					
EJ14	50	31	1.8	32				
EJ15	72	18	10					
EJ16	80	18					2	
EJ17	88		2	10				
EJ18	15	75	3	5			2	

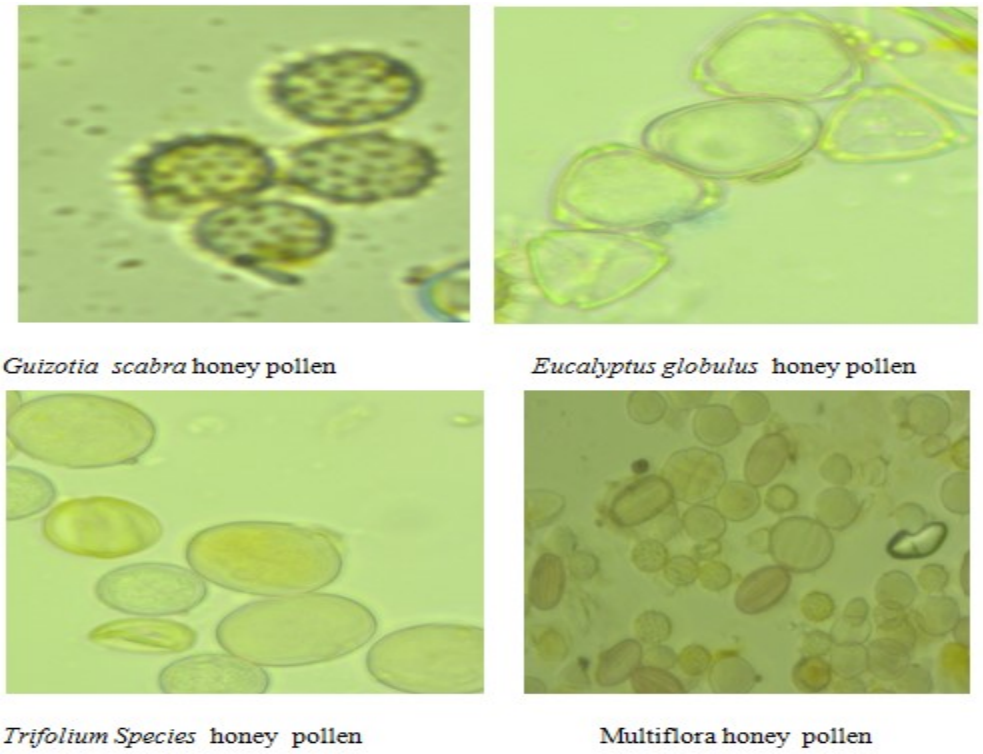


Figure 6. Pollen grain morphology identified from honey sample

Identification of the major plant taxa from in the honeybee collected pollen were characterized as *Plantago lanceolata and Eucalyptus globulus* during the flowering season.

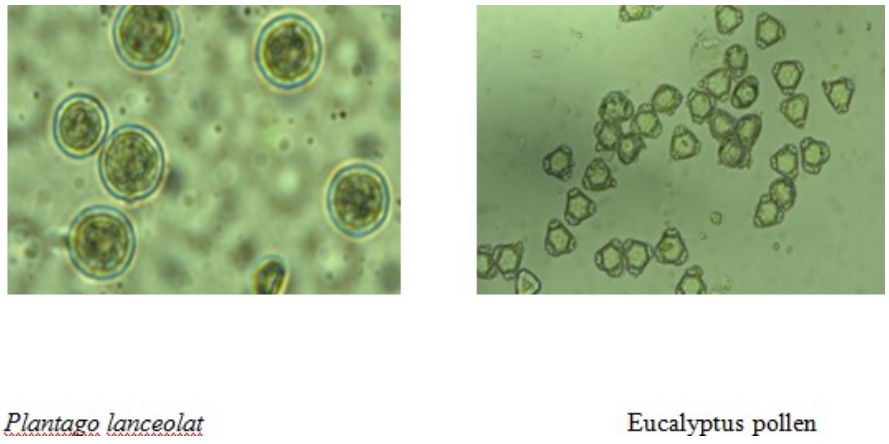


Figure 7.Pollen grain morphology identified from pollen trap

4.2. Color

The colour of honey is a useful parameter for the characterization of the product. colour is the single most important factor determining import and wholesale prices (Belay *et al*., 2015). The colour of the west Shoa honey ranged from 50 to 96 mm Pfund scale, grouped as extra light amber to light amber *Guziotia scabra* honey was found to be extra light amber to Ambe, *Trifolium* species was light amber and *Eucalyptus globulus* was light amber and Multiflolar honey extra light amber colors. The variation among the honey types was due to the botanical origin of the honey. This was in agreement with the findings of Belay *et al*. (2015).

4.3. Physico-chemical properties of honey and pollen

4.3.1. Moisture content different honey Types

The moisture content is a quality parameter, which is important for honey shelf life. It has a minor contribution for the characterization of unifloral honeys (Bogdanov *et al*., 2004). However, depending on the production season and the climate, unifloral honeys show some typical differences in moisture content, which affect the physical properties of honey and moisture content can be artificially changed during honey processing (Bogdanov *et al*., 2004) and the higher the moisture content the lower the quality of honey (Alemayehu, 2011).

Moisture content of honey depends on the ripeness of the honey before honey harvest or maturity in the hive, harvesting techniques and on the extraction material storage condition and storage materials and also based on the hive to Chefrour *et al.*, (2009), honeys having a water content higher than 18% are regarded as lower quality

Table 3.Physico-chemical properties of honey of the West Shoa zone

Variables	Honey type				Ethiopian honey standard
	*Trifolium*species	*Giuzotia scabra*	*Eucalyptus globulus*	Multiflora	
Moisture content (%)	16.54±1.68[a]	15.77±1.07[a]	15.90±0.62[a]	15.65±0.93[a]	≤21
Electrical conductivity (mS/cm)	0.39±0.21[b]	0.33±0.17[b]	0.69±0.42[a]	0.40±0.16[b]	≤0.8 mS/cm
PH	4.07±0.25[a]	4.16±0.22[a]	4.44±0.92[a]	4.13±0.17[a]	
Free Acid (meq/kg)	29.66±6.12[a]	27.6±5.34[a]	15.5±4.95[b]	29.1±5.73[a]	≤40
Ash (%)	0.21±0.15[b]	0.21±0.09[b]	0.50±0.07[a]	0.27±0.14[b]	≤0.6
HMF (mg/kg)	1.87±0.21[b]	2.6±0.38[b]	10.0±0.28[a]	0.03±0.01[b]	≤40
Protein content (g/100g)	0.35±0.00[b]	0.34±0.01[b]	0.18±0.00[c]	0.53±0.00[a]	
Fat content(g/100g)	0.02±0.01[a]	0.01±0.01[a]	0.00±0.00[a]	0.08±0.02[a]	

Different letters across raw showed significant difference ($p < 0.05$)

The results of the present study showed that the highest moisture content were seen in *Trifolium* spp honey (16.54 ± 1.68%) with a range of 13.9 –20% while that of multiflora honey had lowest moisture content (15.65±0.93%) and ranging from 14.83-17.96% as indicated in (Table 3). No significant difference was observed among the honeys in moisture content ($P<0.05$). This might be due to most of the time moisture variability depends on climatic factors, season of production and maturity of honey than the botanical origin of the honey.

The report of the present study showed that all honey samples of the study areas had low moisture content, it might be attributed to low relative humidity of the area when the honey samples were harvested (November) and major or peak honey flow season,

October-November, has relatively low humidity as compared to the minor season,(May-June)(Alemayehu, 2011).

The low moisture content of honey is an important factor for determination of its qualities which also protects honey from being degraded by microorganisms. The shelf life of honey depends on the moisture content of the honey. Moisture a very important factor for the shelf life of honey during storage; and the quality of honey deteriorated due to moulds and yeasts growth in honey. When the moisture content is high, especially if it is > 19%, it can lead to undesirable honey fermentation (El Sohaimy *et al*., 2015; Ibrahim *etal*., 2012; Sisay *et al*.,2012). Therefore, the lower the moisture content, the longer the shelf life and the higher quality of the honey. Generally, all of the honey samples were of good quality and might have longer shelf life.

The results reported in this study is lower than the average moisture content of honey from southwestern Ethiopia which ranged from 20.4 % to 24.8 % (Getachew *et al*., 2016). The result of this study was supported by different pervious findings of (Meda *et al*., (2005); Gangwar *et al*., (2010); Alemayehu, (2011); Belay *et al*., 2015).Moisture content multiflora honey(15.6±0.93), was in line with the report of multiflora honey reported by Pridal & Vorlova, (2002) which has a moisture content of 15.6%. The moisture content of all honey samples in this study, was lower than the mean moisture content reported for Ethiopian honey which was 21%/100 g (Adgaba,1999) and below the maximum limit of Codex Alimentarius and European Union (Bogdanov, 2002).

4.3.2. Electrical conductivity

The electrical conductivity of honey is defined as that of a 20% weight in volume solution in water at 20^{o}C, where the 20% refers to honey dry matter. The highest electrical conductivity (EC) was *Eucalyptus globulus* honey (0.69 ±0.42mS/cm) with range of 0.66-0.76mS/cm and the lowest was in *Guizotia scabra* honey (0.33±0.11 mS/cm) with range of 0.22-0.61 mS/cm (Table 3). The mean EC of honey from *Eucalyptus globulus* was significantly ($P<0.05$) higher than the mean EC values of honey from other sources and the variation might be due to the soil type and botanical origin of honey. The increase in ash content of *Eucalyptus globulus* from was accompanied by the increase electrical conductivity, as previously reported by Diafat *et al*. (2017). EC correlates well with the mineral content of honey (Bogdanov *et al*., 2004).

Electrical conductivity is one of the most important factors for determining the physical characteristics of honey and it is also an important physicochemical measurement for the authentication of unifloral honeys with the exception of a single sample (0.806 mS/cm), the electrical conductivity values of samples were within the allow Codex and EU (<0.8 mS/cm).Electrical conductivity depends on ash, organic acids, proteins, and varies with botanical origin (Chefrour *et al*.,2009; khalil *et al*.,2012). The electrical conductivity values of this study are lower than that reported by the in (Ibrahim *et al*., 2012) and (Elsohaimy*etal*.,2015).According to Codex Alimentarius Committee (2001) on sugars and European Commission (2002), the botanical origin of honeys is often classified into two classes: blossom and honeydew honey based on the measurement of the electrical conductivity.

Honey with electrical conductivity values higher than 0.8 mS/cm are considered as honeydew honeys, while those with lower values than 0.8 mS/cm are blossom honeys or blends of blossom with honeydew honey (Bogdanov, 2007). Honey samples in this study were categorized as blossom honeys.

4.3.3. pH

Honey is naturally acidic irrespective of its geographical origin, which may be due to the presence of organic acids that contribute to its texture, flavor and its stability against microbial spoilage (Bogdanov *et al.,* 2004). Trifolium species honey had the lowest pH (4.07±0.25) while the highest was *Eucalyptus globulus* honey samples (4.44 ± 0.09) as indicated in (Table 3). There was no statistically significant difference ($p < 0.05$) in pH among honey samples due to honey type as show in (Table 3).

All honeys are acidic with a pH-value generally ranging between 3.5 and 4.5 and the low pH of honey inhibits the presence and growth of microorganisms (Terrab *et al.*, 2004).The result of this study is in line with studies (Gangwar *et al.*, 2010; Alemayehu, 2011). Multiflora honey samples were acidic in nature which concurs the reported values of khalil *et al*. (2012) which was (3.00 to 4.00), Kumar *et al.* (2013) (3.30 to 4.13) and Cimpoiu, (2013) (3.43 to 4.96)

The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: flavour and stability against microbial spoilage (Bogdanov *et al*., 2008).All the honey samples were acidic and within the standard limit (pH 3.40–6.10) (Codex Alimentations, 2001),

which confirm the freshness of honey samples. In general, honey is acidic in nature irrespective of its variable geographical origin.

4.3.4. Free acidity

The highest mean free acid content was in Trifolium species honey samples (29.66 ± 6.13 meq/kg; 15–36 meq/kg) and the lowest was Eucalyptus *globulus* honey samples is 15.5±4.95 meq/kg with the range of 12.00-19.00 meq/kg. Furthermore, there is a significant difference between *Eucalyptus globulus* honey and other honey types due to their botanical origin at ($p<0.05$) as shown in(Table 3).Variation in free acidity among different honeys can be attributed to floral origin or to variation in the harvest season (Perez- Arquillue *et al* ., 1994). All honeys are acidic, indicating the absence of undesirable fermentation and such results indicate the freshness of the honey samples the analyzed honey samples and the acidity of honey is important for taste (Bogdanov , 2011 ; Diafat *et al.*, 2017) .

Acidity of honey is due to the presence of organic acids and inorganic ions such as the gluconic acid with their lactones or esters, phosphate and chloride. Acid measurement is useful for evaluation of honey fermentation, authentication of unifloral honeys and differentiating nectar from honeydew honeys (Nandaa *et al.*, 2003). Free Acidity content indicate the freshness of the honey samples and absence of unwanted fermentation in the honey samples and the acid content of honey is relatively low but it is important for the honey taste and most acids are added by the bees and the main acid is gluconic acid, a product of glucose oxidation by glucose oxidase Bogdanov ,(2011) .

These results were in agreement with the findings of Alemayehu.(2011). The mean free acid value of the current study shows that all honey types were below the national average, (39.9meq/kg) (Adgaba, 1999), and satisfies the CA, EU and Ethiopian standards. The EU and Ethiopian standards have more restrict norms compared to CA standard. The maximum limit for free acid set by the CA is 50 meq/kg of honey, while the EU and Ethiopian standard is 40 meq/kg of honey (Bogdanov, 2007).

4.3.5. Hydroxymehylfurfural

The HM contents of honey stated in Table 3.The highest HMF value recorded in *Eucalyptus globulus* honey ranged 9.8-10.20 mg/kg with the average HMF content of 10.0±0.28 mg/kg while multiflora honey recorded lowest HMF content of 0.03±0.10mg/kg with the range of 0.17-0.91 mg/kg of honey. There is significance difference between HMF content of *Eucalyptus globulus* and other honey types at ($p < 0.05$).

HMF is not a criterion for the botanical classification of honey however, before determining storage dependent parameters like enzyme activity and colour, one should ensure that honeys are fresh and unheated (Bogdanov *et al.,* 2004). The amount of HMF in honey is one of the important indicators of honey quality. Fresh honey contains Hydroxymehylfurfural (HMF) present only in few amounts and its concentration increases with storage and prolonged heating of honey (Bogdanov *et al*., 2004; Bogdanov, 2011; Getachew *et al.,* 2016).

HMF formation results from the acid-catalyzed dehydration of hexose sugars with fructose being particularly susceptible and it is thus an essential parameter used to

indicate honey purity (Bogdanov, 2011).The result of this study indicate the freshness of the honey. The maximum acceptable limit for HMF content of Ethiopian honey is ≤40 mg/kg (Adgaba, 1999) while the maximum acceptable HMF limit of honey reported by the European Union is ≤40 mg/kg (Bogdanov *et al*., 2002) .

4.3.6. Ash content

The highest mean ash content was seen in *Eucalyptus globulus* honey (0.50±0.70%) which ranged from 0.45-0.55% and the lowest in *Guizotia scabra* honey (0.21 ±0.94%) with range of 0.05-0.34%. There is significance difference in ash content between *Eucalyptus globulus* honey and the other honey type at ($p<0.05$) as shown in (Table 3). The difference in ash content between the two samples in the current study could be attributed to soil type and concentration of minerals found in the nectar on different apiaries (Diafat *et al*., 2017).These results were in agreement with other study of (Belay *et al.*, 2013 and Alemayehu,2011) and comparable to Saxena *et al*. (2010).

The maximum limit of 0.6% is set for ash content of honey by EU, CA and QSAE. The average ash content of the honey samples analyzed was within the national and international limits for ash content of honey. The ash content of all the analyzed honey samples fell within the 0.01-1.2% range reported by the Ethiopian Quality and Standards Authority (QSAE, 2005) and 0.6% maximum limit reported by the International Honey Commission (Bo g d an o v, 2002) which satisfied the limit set by Codex Alimentarius Standard specifications maximum limit and country standard(Adgaba,1999).

The ash content (%) is an indicator of the mineral content and is considered as a quality criterion indicating the possible botanical origin of honey (Claudia, 2013) and also the percentage ash content is an indicator of the mineral content and mineral content is an important indicator of possible environmental pollution and an indicator of the soil types of the area . The ash content of honey depends on the material contained in the pollen collected by the bees during foraging on the flora (Sisay *et al*., 2012).

According to Mairaj *et al.*(2008), ash value indicates the botanical origin; the blossom honey has lower mineral content than honeydew honey. Ash varies from 0.02 to slightly over 1 percent for a floral honey (Bogdanov, 2007; White, 1975).

4.4. Total protein content of different honey types

Total Protein content of *Guziotia scabra* honey averaged(0.34 ±0.04 g/100g) with range of 0.34-0.35 g/100g,*Trfolium* species honey which ranged from 0.35-0.35 g/100g with mean of 0.35 ±0.00 g/100g.The mean content of *Eucalyptus globulus* honey protein content was 0.18±0.00 g/100g with range of 0.18-0.18 g/100g and the mean multiflora honey protein content is 0.53 ±0.01 which ranged from 0.52-0.53 g/100g.The was a significant difference at ($p<0.05$) among honey types on their protein content due to their botanical origin.

The honey proteins are mainly enzymes, honey bees add different enzymes during the process of honey ripening from those diastase (amylase) digests starch to maltose and is relatively stable to heat and storage and invertase (saccharase, α-glucosidase), catalyses mainly the conversion of sucrose to glucose and fructose. The other two main enzymes are glucose oxidase and catalase regulate the production of H_2O_2, one of the honey

antibacterial factors. Diastase and invertase play an important role for judging of honey quality and are used as indicators of honey freshness (Bogdanov¸ 2011). Honey contains a trace amount of protein usually originated from pollens which is a natural and protein-rich food source and some enzymes such as glucose oxidase invertase and diastase. The variability in protein content of different types of honey might refer to the origin of honey and the type of pollens ElSohaimy *et al.* (2015).

During honey consumption, the intake of proteins is low that is because these nutrients are related to the presence of enzymes and free amino acids, some of which are introduced by bees or may be are derived from the nectar (Olga *et al*., 2013).

These values are lower than that reported by Buba *et al.*(2013) (0.67 ± 0.25 g/100g) and ElSohaimy *et al.* (2015), Kashmiri honey has protein content of 4.67 ± 0.171 mg/g and protein content of Egyptian honey was 1.69 ± 0.015 mg/g. These values are higher than the values reported in the current study.

4.5. Fat content of different honey types

The highest fat content of honey were recorded in multiflora honey (0.08±0.02g/100g) ranged 0.02-0.22g/100g while the lowest fat content was the *Eucalyptus globulus* honey mean fat content 0.00±0.00g/100g.There was no significant difference at *($p<0.05$)* between *Trifloium species,Guizotia scabra, Eucalyptus* and multiflora *globulus* honey base on their fat content. Fat content of the *Eucalyptus globulus* honey similar with previous report of Chua and Adnan (2014).

4.6. Sugar content

The highest mean Fructose content was obtained in honey from *Eucalyptus globulus* (39.09±0.30g/100g) ranged from 38.81-39.24 g/100g and the lowest was *Guizotia scabra* in honey (37.56 ±2.32g/100g) ranged 31.45-40.01g / 100g (Table 4). The highest glucose was recorded in Guzotia *scabra* honey (36.48±1.94g/100g) with the range of 32.32-38.67g/100g, while the lowest glucose content recorded in *Trifolium* species (34.36±3.98g/100g) ranged from 21.32 to 38.34g/100g. The mean *Trifolium* species honey had a maltose content of 0.46±0.29g/100g with range of 0.19-1.06g/100g, *Guzotia scabra* honey maltose content was 0.43±0.28g/100g range 0.17-1.06g/100g and multiflora honey maltose content was ranged from 0.17-0.91g/100g with the mean content of 0.36±0.23g/100g. *Eucalyptus globulas, Trifolum species, multiflora and Guzotia scabra* honeys exhibited sucrose content 1.54±0.11g/100g ranged 1.46 to 1.62g/100g, 0. 86±0.43g/100g ranged 031-1.83g/100g, 0.77±0.27g/100g ranged 0.37 to 1.11g/100g and 0.71±0.29g/100g ranged0.31-1.11g/100g, respectively. The result of the study showed that *Eucalyptus globulas* is significantly different ($p<0.05$) from other honey on their sucrose content at due to their botanical origin.

Table 4. Sugar content of West Shoa honey

	Honey type			
Parameter	Trifolium	Guizotia	Eucalyptus	Multiflora
Fructose g/100g)	37.48 ±3.56[a]	37.56±2.32[a]	39.02±0.30[a]	38.09±0.93[a]
Glucose g/100g	34.36% ±3.98[a]	36.48±1.94[a]	34.67±1.28[a]	34.67±1.28[a]
Maltose g/100g	0.46±0.29[a]	0.43±0.28[a]	0.41±0.01[a]	0.36±0.23[a]
Sucrose g/100g	0.86±0.43[b]	0.71±0.29[b]	1.54±0.11[a]	0.77±0.27[b]
Fructose + glucose (g/100g)	71.84	74.04	74.01	72.73
Fructose /Glucose	1.09	1.03	1.13	1.18

According to the result the study, sucrose content of *Eucalyptus* honey is significantly different from the sucrose content in other honey types. The sucrose content of *Trifolium species, Guzotia scabra*, *Eucalyptus globulus* and Multiflora honeys are in the limit of the Codex Alimentarius and to the EU standards (≤5 g/100 g) (Bogdanov *et al*., 2015) and the result this study also shows that all of the honey types have low sucrose content which indicates the complete conversion of sucrose into glucose.

Specific sugar content determination by using specific sugar standard will be used for honey control. Thus, the fructose/glucose ratio and the sucrose concentrations can be criteria for differentiating between different unifloral honeys and also specific sugar spectrum give information on honey authenticity and sugar adulteration (Bogdanova *et al*., 2015). According to El Sohaimy *et al*.(2015) ; Chua *et al*.(2014), the F/G ratio indicates the ability of honey to crystallize, since the glucose is less soluble in water than fructose and honey crystallization is faster when the F/G ratio is below 1.0 and it slows when this ratio is more than one. Furthermore, the value of F/G ratio could be used as a favorable indicator because fructose is sweeter than sucrose and glucose. Accordingly, the F/G in the study samples is above 1.0 which means all of the honey types will crystallize slowly El Sohaimy *et al*. (2015).

These results were in agreement with other studies (Buba *et al*., 2013) and Hana'a Yousif Abd Elaziz Hussien,(2007). The study also reveals that different monofloral and multiflora honey sample in the study area has low sucrose and maltose contents might indicate the purity of the honey sample from adulteration El Sohaimy *et al*.(2015).

4.7.Pollen protein content

The proximate composition of pollen was stated in (Table 5). *Plantago lanceolata* pollen protein was 21.8±0.30 with range of 21.5 to 22.10% and *Eucalyptus globlus* pollen protein was 26.6±2.55 with range of 24.70 to 29.5%. There is significance difference *($p<0.05$*) between the pollen protein contents. The variability of protein content found in bee pollen can be partly explained by the natural variation of the composition that is a consequence of the distinct source plants also reported by Nogueira *et al*.,(2012); Adii *et al.,* (2017) and the result of the study was in line with Adii *et al.*(2017) (15.87-27.09 %).

Table 5. Proximate composition of pollen

Parameters	unit	*Plantago lanceolata*	*Eucalyptus globulus*
Protein	%	21.8±0.30[a]	26.6±2.55[a]
fat content	%	0.3 ±0.01[b]	0.9±0.01[a]
moisture	%	5 .27±0.31[a]	4.16±0.28[a]
Ash	%	0.51±0.32[a]	0.43±0.35[a]
Carbohydrate	%	72.4±0.28[a]	68.71±2.86[a]

The protein content of the pollen for different plant species were significantly different and often used as an indicator of the nutritional quality of pollen because it influences several morphological, physiological and behavioral aspects in honey bees and also it is the major nutrient in pollen, it is a valid source of easily digestible peptides for humans Conti *et al*. (2016).

4.8. Pollen fat content

Plantago lanceolata had a pollen fat content of 0.15 ±0.21% with range of 0.3 to 0.4 % and the *Eucalyptus globulus* pollen fat had 0.9±0.01% ranged from 0.1 to 0.9%. There was a significant difference $p<0.05$) based on the fat content between the *Plantago lanceolata* and *Eucalyptus globulus* pollen grains (Table 5) and less than the reports by of the Adii *et al.*(2017) (2.7-5.8%), and Feás *et al.* (2012)(5.2%) this variation might be because of the botanical origin of the pollen.

4.9. Pollen moisture content

Moisture content is one of the parameter, which have great importance during the storage of pollen as it influences its texture, stability and shelf life. The low moisture content inhibits the presence and growth of microorganisms and makes pollen compatible with many food products (Feás *etal.*,2012).The mean *plantago lanceolata* moisture content is 5 .27±0.31% with range of 5.00 - 5.20%, and the mean moisture content of *Eucalyptus globulus* pollen is 4.16±0.28 ranged from 4.00 to 4.50 %. There was no significance difference ($p<0.05$) between the two pollen sample base on their moisture content (Table 5). The moisture content of pollen were within the international maximum limit (6%) (Bo g d an o v , 2004).

According to Carpes *et al.* (2009) moisture could favor microbiological contamination, (particularly fungi and yeasts) as pollen has hygroscopic properties and possibly affected by environmental conditions. The maximum allowed humidity varies from country to country:(Brazil, 4 %, Switzerland 6 %, Russia 8-10 %, Bulgaria 10 %). More than 10 %

of moisture makes the pollen susceptible to fermentation. The examination of the sensory quality in Switzerland concluded that humidity of less than 6 % makes the pollen too dry and less acceptable from sensory point of view (Bogdanov, 2017).

4.10. Pollen ash content

Ash is an inorganic matter present in bee pollen. The ash content is influenced by different factors such as soil type, geographical origin, flora species and capacity of the plant to accumulate minerals. The ash content may increase due to the presence of mineral impurities due to inefficient cleaning procedures. *Plantago lanceolata* pollen ash content ranged between 0.49 to 0.55 with mean of 0.51±0.32% and *Eucalyptus globulus* was 0.43±0.35 with range of 0.40 to 0.47%. The result revealed that there is no significance difference ($p<0.05$) between pollen sample based on their ash content (Table 5) and the result of this study is less than the reports by Carpes *et al*.(2009) 2.9± 0.5% and Nogueira *et al*. (2012) (3.16 ± 0.03%).

4.11. Pollen Carbohydrate content

Carbohydrate content varies widely in pollen source plants and is significantly lower in fresh than in stored pollen. The carbohydrate content of *plantago lanceolata* pollen mean content was 72.4±0.28 with the range of 72.12- 72.68%.The mean carbohydrate content of *Eucalyptus globulus* was 68.71±2.86 ranged from 65.45 to 70.80%.There was no significance difference ($p<0.05$) between *plantago lanceolata* and *Eucalyptus globulus* pollen based on their carbohydrate content (Table 5). The result of this study was in agreement with reports of Nogueira *et al*. (2012) which ranges (69.68 ± 2.08 to 84.25 ± 0.58) .

4.12. Antioxidant content

4.12.1. Total phenolic contents and activity of honey

The concentration and type of phenolic substances depend on the floral origin of the honey and are mainly responsible for its biological activities (Mohammed *et al*., 2014). The highest phenolic content was recorded in multiflora honey (196.54±6.47), which ranged from 183.5 to209.57 mg of gallic acid equivalents (GAE)/100 g, while lowest recorded in *Trifolium* species honey was (161.16±6.46), with range of 148.12-174.19 mg GAE/100 g of honey (Table 6).

Table 6. Antioxidant content and activities of different honey type

Parameters	Honey type			
	Guzotia scabra	*Eucalyptus globulus*	Trifolium species	Multiflora
Total phenol (mg of GAE/100g)	185.92±5.29^{a}	177.83±7.48^{ab}	161.15±6.48^{b}	196.54±6.47^{a}
Flavonoids (mg quercetin/100g)	13.6±3.07^{a}	12.31±2.46^{ab}	12.20±0.67^{ab}	10.17±0.85b
RSA (%)	70.83±4.13^{bc}	70.56±0.85^{cb}	73.05±4.85^{a}	67.74±3.12^{d}
FRAP (μM Fe (II)/100 g)	70.76±4.79^{a}	62.85±6.78^{ab}	59.77±5.87^{ab}	41.69±5.87^{b}

These results of this study showed that there was significance difference ($p<0.05$) among the honey types in their total phenolic content (Table 6). The total phenolic contents of *Guizotia scabra and Multiflora* honey were significantly different from the total phenolic contents of *Trifolium and Eucalyptus globulus honey.* But there is no significant difference ($p<0.05$) between *Guzotia scabra and Multiflora honey.* This might be due to

their botanical origin. The concentration and type of polyphenolic substances depend on the floral origin of honey and are major factors responsible for biological activities, including antioxidant, antimicrobial, antiviral, and anticancer activities (Küçük *et al*., 2007).

These values were higher than that reports by Kumazawa *et al* . (2012) (51.1±2.0 mg /100g),Meda *et al*. (2005) (32.59–114.75 mg GAE/100g) and Saxena *et al*. (2010) 47 to 98 mg GAE/100g honey. The result was in line with report of Liu *et al*. (2013) which was 110.394 to 196.500 mg GAE/100 g honey. In general the concentration and type of polyphenolic substances depend on the floral origin of honey (Küçük *et al*., 2007; Saxena *et al*., 2010)

According to Mohammed *et al*. (2014), flavonoids are low molecular weight phenolic compounds responsible for the aroma and the antioxidant potential of honey. According to Liu *et al,* (2013) the predominant flavonoid in honey samples as from the group of flavonols (45% quercetin). *Guziotia scabra* honey recorded the highest flavonoids content ranged from 13.43 to 13.81 with a mean content of 13.62 ±3.09 mg quercetin/100g honey and the multiflora honey had the lowest flavonoids content ranged from 9.94 to 10.42 with a mean content of 10.17±0.85 mg quercetin/100g honey as indicated in (Table 6). There is a significance difference ($P<0.05$) among the honey group in flavonoids content, which might be due to the botanical origin of the honey.

4.12.2. Antioxidant activities of different honey type

Antioxidant activities of the honey were evaluated in terms of their anti- radical power (ARP) as assessed by DPPH and antioxidant power (TAP), as assessed by FRAP assay.

DPPH were determined in terms of RSA and the RSA mean content of *Guizotia scabra* honey mean content was 70.83±4.13 with the range of 69 -72 % and *Trifolium* species honey RSA content ranged from 70.82 to 75.28 with average content of 73.05 ± 4.85 % . The *Eucalyptus globulus* honey mean content of RSA was 70.56±0.85 % with range of 67.98 -73.13% and the multiflora honey RSA mean content is 67.74±3.12 ranged from 65.15 to 69.97%). The Result shows that Multiflora honey and *Trifolium* species honey were significantly different ($P<0.05$) from other honey. These values were in line with RSA content of the analyzed honey samples which was between of 21.79 to 84 % (Khalil *et al*., 2011).

The FRAP assay of all the west Shoa honeys tested are displayed in (Table 6). A relatively higher absorbance value indicated more reduction of ferric ions to ferrous ions. Samples having a higher reducing recorded was in *Guizotia scabra* honey (70.76±4.79) with range 61.12-80.41 followed *Eucalyptus globulus* honey (62.85±6.78) with range of 49.21-76.50, *Trifolium* species honey had a mean of 59.77±5.87 ranges of47.95-71.59 and the multiflora honey FRAP mean content was 41.69±5.87 ranged from 29.88 to 53.52 (Table 6). The result showed that *Guizotia scabra* honey is significantly different from other honey by their FRAP because of the botanical origin of the honey ($P<0.05$). *Guizotia scabra* honey was a relatively higher absorbance value indicated more reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) this was in agreement of the report of (Saxena *et al*., 2010; Moniruzzaman *et al* ., 20 Antioxidant power (TAP), as assessed by FRAP assay. The FRAP assay measures the reducing potential of an antioxidant that reacts with a ferric tripyridyltriazine (Fe3+- TPTZ) complex to produce a colored ferrous tripyridyltriazine (Fe2+- TPTZ). Generally, the reducing properties are associated with

the presence of compounds, which exert their action by breaking the free radical chair through the donation of a hydrogen atom (Hussein *et al.*, 2011).

4.12.3. Total phenolic content and antioxidant activity of pollen

The total phenol content of *Eucalyptus globulus* a mean total phenol content was 6.49±0.65 with ranges of 6.45-6.57 mg of gallic acid equivalents (GAE)/100 g of pollen. the *Plantago lanceolata* pollen total phenol content was ranged 3.75-4.22 with mean of 4.06±0.27 mg of gallic acid equivalents (GAE)/100 g of pollen using the standard curves of gallic acid(R^2= 0.996).According to the result there was significant difference between the pollens on their total phenol content ($p<0.05$) origin as indicated in (Table 7).The difference total phenol in content between the two samples in the current study could be attributed to the botanical origin. The result of the study was in line with value reported by Carpes *et al.* (2007) which varies 3.6 to 8.1 mg of GAE /100 g of pollen.

Table 7.Antioxidant content and activities of different pollen type

Parameters	*Plantago lanceolata*	*Eucalyptus globulus*
Total phenol mg (GAE)/100 g	4.06±0.27[b]	6.49±0.65[a]
Flavonoids mg quercetin/100g	5.06±0.12[a]	3.12 ±0.41[b]
RSA %	70.71±0.39[a]	22.12±0.43[b]

According to Carpes *et al*, 2009 ; Aličić *et al*, *2014* pollen collected honey bees generally show characteristic amounts of total polyphenols with some variations due to its botanical origin and total phenolic compounds extracted from pollen is solvent dependent.

Eucalyptus globulus pollen mean flavonoids content was 3.12 ±0.41 with range of 3.07-3.15 mg quercetin/100g pollen and the mean *Plantago lanceolata* for flavonoids content was 5.06±0.12 with range of 5.05-5.08 mg quercetin/100g pollen. *Eucalyptus globulus* was significantly different from *Plantago lanceolata* on their flavonoids content ($p<0.05$) based on the botanical origin of the pollens (Table7). The result of this study found that total flavonoids content of *Eucalyptus globulus* pollen was similar with a finding of (Feás et al., 2012) which was 5.8 ± 0.8 CAEs.

The DPPH radical is one of the few stable organic nitrogen free radicals; it has been widely used to determine the free radical scavenging ability of the various samples and the free radical-scavenging activity of the extracts was attributed to their hydrogen-donating ability Morais *et al*,(2011).DPPH is measured as Radical scavenging activities and the RSA of Eucalyptus globulus pollen mean content was 22.12±0.43 with range of 21.63-22.42% and *Plantago lanceolata* pollen RSA ranged from 70.33-71.12 with mean of 70.71±0.39%. The result of this study showed that *Plantago lanceolata* pollen is significantly different ($p<0.05$) from *Eucalyptus globulus* pollen based their Radical scavenging activities .the variability might be due to botanical origin. The *Plantago lanceolat* pollen showed higher radical scavenging activities than pollen *Eucalyptus globulus*.

4.13. Correlation between the physicochemical, antioxidant activity of and its total phenolic honey and pollen

The correlation between the antioxidant content and antioxidant activities was stated in Table 8. The correlation between the two sets of antioxidant contents of honey was (r=0.902). The correlation between FRAP and total flavonoids (r=0.924) was strong,

while correlation of RSA to total phenol was a reversely correlated. It is known that where similar phenolic levels occur, these do not necessarily correspond to the same antioxidant responses. This means the RSA of a sample cannot be predicted on the basis of its total phenolic content. In the case of honey, the antioxidant capacity is the result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes, Maillard reaction products and possibly other minor components (Gheldof *et al*., 2002).This negative linear correlation proves that when the total phenolic content is higher, the RSA will be lower.

Correlation between Protein and total phenol r=0.660.Protein can also work as an effective antioxidant as reported by Sivapriya and Srinivas, (2007) as cited by Saxena *et al*. (2010).

The FRAP values correlated well with the phenolic content and Total flavonoids with a correlation value of 0.077 and 0.924 respectively as indicated (Table 8). Previous studies onhoney indicate that the presence of compounds such as polyphenols and flavonoids may function as potential natural antioxidants (Saxena *et al*., 2010). The antioxidant activity of Phenolics is mainly due to their redox properties which can play an important role in neutralizing free radicals Aličić *et al.* (2014).

Table 8. Correlation between the antioxidant activity of honey and its total phenolic

Parameters	Total phenol	Total flavonoids	RSA	FRAP	Protein
Total phenol	1	0.902	-0.780*	0.077*	0.660*
Total Flavonoids		1	0.105	0.924*	-0.430
RSA			1	-0.062	-0.589
FRAP				1	-0.401
Protein					1

RSA: Radical Scavenging Activities: FRAP: Ferric Reducing Antioxidant Power. * Correlation is significant at 0.01 levels

There is strong correlation between total phenol and RSA of pollen while there is a reverse correlation between total flavonoids and RSA. According to Carpes *et al*. (2009), the antioxidant activity of pollen is largely as a result of the phenolic compounds also according to Almaráz-Abarca *et al*. (2007) as cited by (Vasconcelos *et al* ., 2017) regarding the identification of antioxidant activity, the content of phenolic compounds in pollen is more important than the flavonoids content as show (Table 9).

Table 9. Correlation between the antioxidant activity of pollen and its total phenolic content

Parameters	Total phenol	Total flavonoids	RSA
Total phenol	1	-0.999*	1*
Total Flavonoids		1	-0.999
RSA			1

RSA: Radical Scavenging Actives. * Correlation is significant at 0.01 levels

Table **10**. Correlation between the physicochemical properties of honey

Parameters	pH	Ash	MC	FA	EC	HMF
pH		0.286	-0.278	-0.732	0.589*	0.488*
Ash			-0.227	-0.312	0.644*	0.182
MC				0.187	-0.126	0.116
FA					-0.515*	-0.426
EC						0.236
HMF						1

MC= moisture content; EC = electrical conductivity; HMF = hydroxymethylfurfura; FA=Free Acidity.* correlation is significant at 0.01 level

The positive correlation between Ash and electrical conductivity this due to electrical conductivity depends on the ash and acid content of honey; the higher their content, the higher the resulting conductivity(Bogdanov *et al*.,1999).There is a linear relationship

between the ash content and the electrical conductivity and negative correlation between electrical conductivity and free acidity. It is however difficult to explain the strong positive correlation between pH and Electrical conductivity and between pH content and HMF levels observed in this study.

5. CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

In West Shoa *Giuzotia scabra, Eucalyptus globulus and Trifolium Species types* monofloral honey and multiflora honey can be possibly produced. West Shoa honey and pollen moisture, ash, free acid, pH, HMF, electrical conductivity sugar, protein, fat and carbohydrate content values satisfied the CA, EU and Ethiopian standards. The antioxidant activity of *Giuzotia scabra* honey was significantly higher than other honey types which was due to the difference in their phenolic contents and consequently their floral sources. Antioxidant activates *Eucalyptus globulus* pollen have higher Radical scavenging activities than *Plantago lanceolat* pollen, which is due to pollen collected by bees generally showed characteristic amounts of total polyphenols with some variations due to its botanical origin and total phenolic compounds extracted from pollen. It can be summarized that the overall antioxidant property in the studied west Shoa honey can be attributed to various contributing factors such as, phenolics, flavonoids and proteins as shown by the correlation value. It can be concluded that West Shoa honey and pollen as a good quality bee products for local and international, market which can fetch foreign currency ultimately add value for Ethiopian honey and pollen value chain .

5.2. RECOMMENDATIONS

Based on this finding, it is possible to recommend the following West Shoa honey and pollen

- As one of a good quality honey and pollen with high antioxidant source of food.

- The west Shoa honey and pollen satisfied the CA, EU and Ethiopian standards in all parameters so that it can be recommended for further study on for their value addition. Further study was recommended anticancer and bacterial properties of honey.

-Further study also recommend for pollen particularly with post-harvest management, processing, on its allergies and product development.

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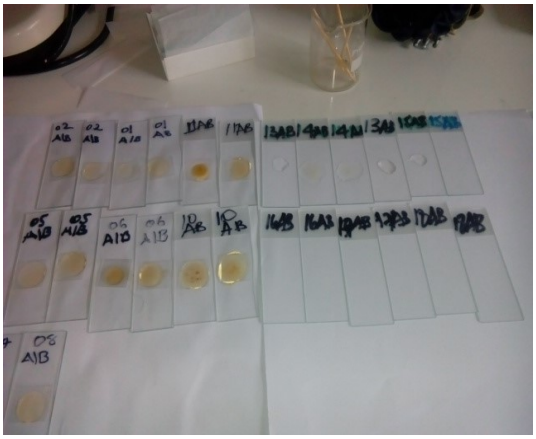
APPENDIX

APPENDIX.1

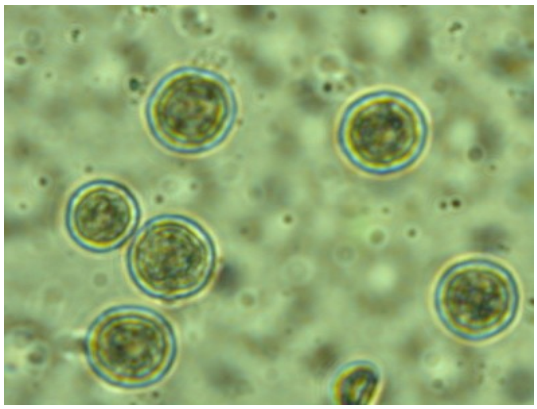
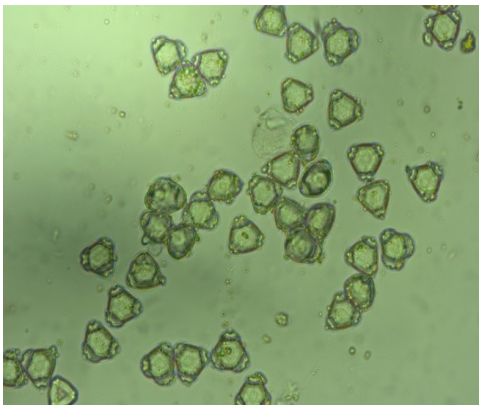
Botanical origin of honey identification (pollen analysis)

Pollen sample

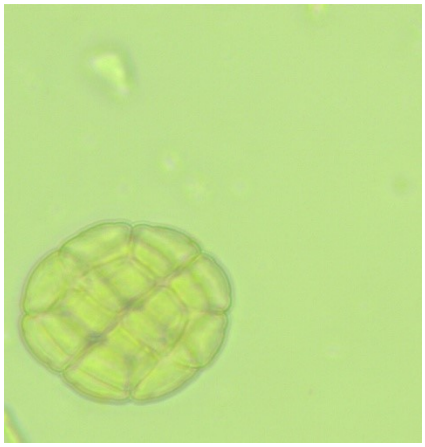
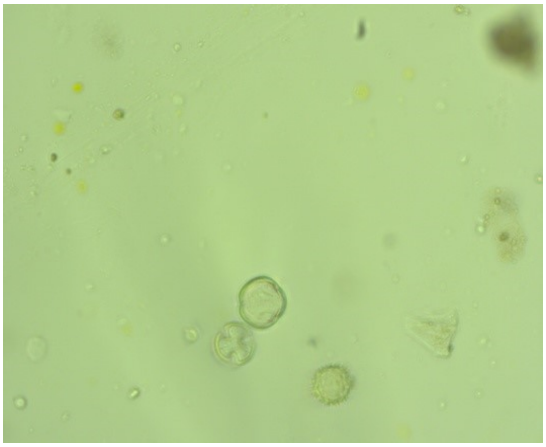


Slide Preparation

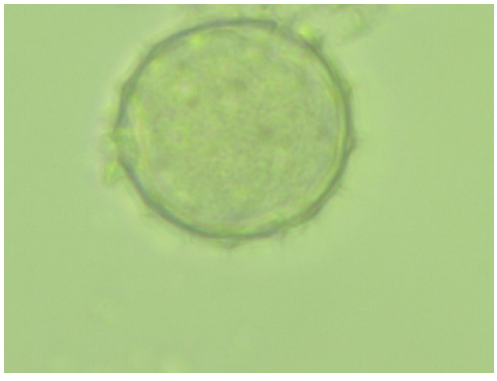
Pollen count for botanical origin identification

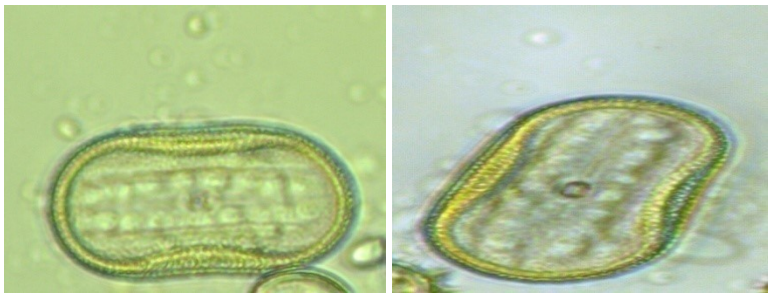
*Plantago lanceolata*Eucalyptus pollen

Acacia abyssinica Caesalpinia decepetal




Dombiya torrida Hypoestes trifora



Vicia faba

Different pollens identified in Multiflora honey

APPENDIX.2

Physico-chemical analysis

Sugar Analysis using HPLC

Sample preparation for sugar analysis filtering the sample

Running honey samples for sugar analysis on HPLC

peaks of standards and samples

Data File C:\CHEM32\1\DATA\25-08-2017\MESERET HONEY SAMPLE 2017-08-28 11-26-15\004-0401.D
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Acq. Instrument	: HBRC_1260_HPLC	Location	: Vial 4
Injection Date	: 8/28/2017 12:05:21	Inj	: 1
		Inj Volume	: 10.000 µl
Acq. Method	: C:\CHEM32\1\DATA\25-08-2017\MESERET HONEY SAMPLE 2017-08-28 11-26-15 \MESERET HONEY SAMPLE.M		
Last changed	: 8/28/2017 11:26:15 by SYSTEM		
Analysis Method	: C:\CHEM32\1\METHODS\19032018.M		
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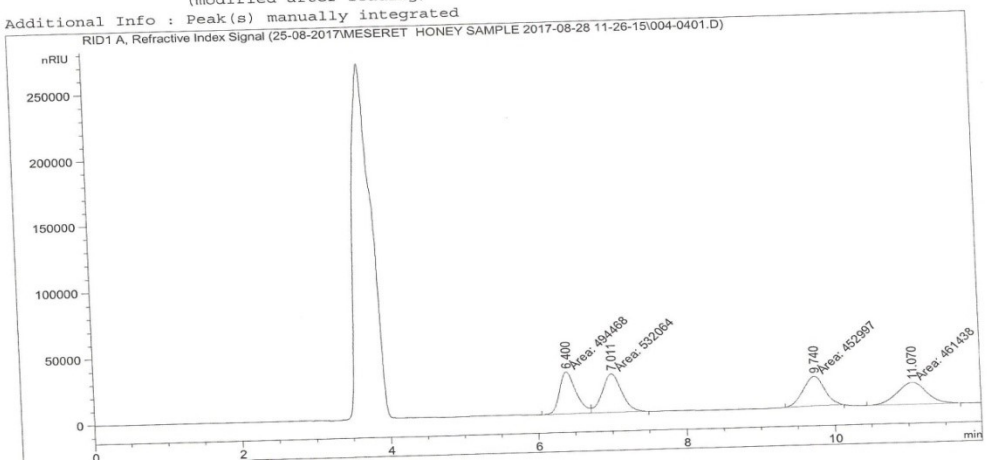
Additional Info	: Peak(s) manually integrated		



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Location : Vial 5
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Inj Volume : 10.000 µl

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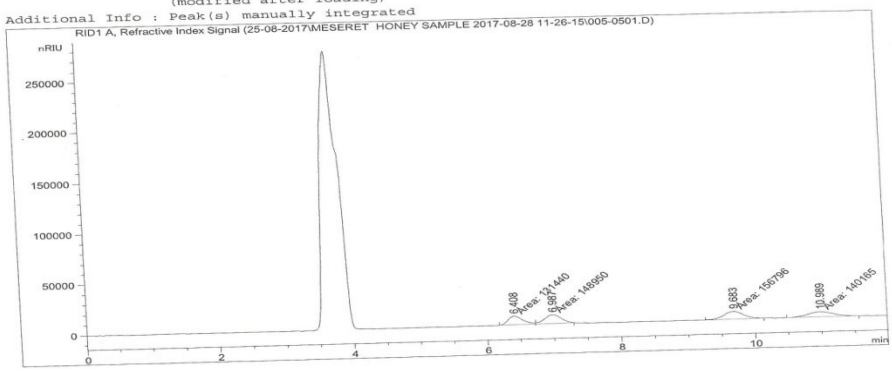

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Location : Vial 2
Inj : 1
Inj Volume : 10.000 µl

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Last changed : 8/28/2017 11:26:15 by SYSTEM
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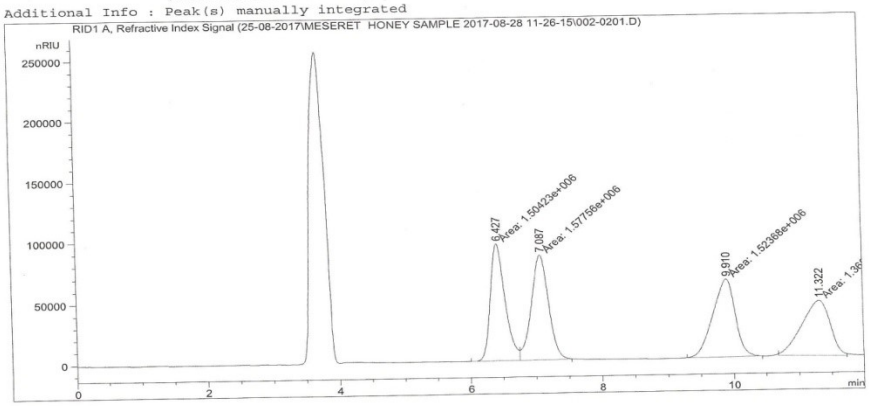
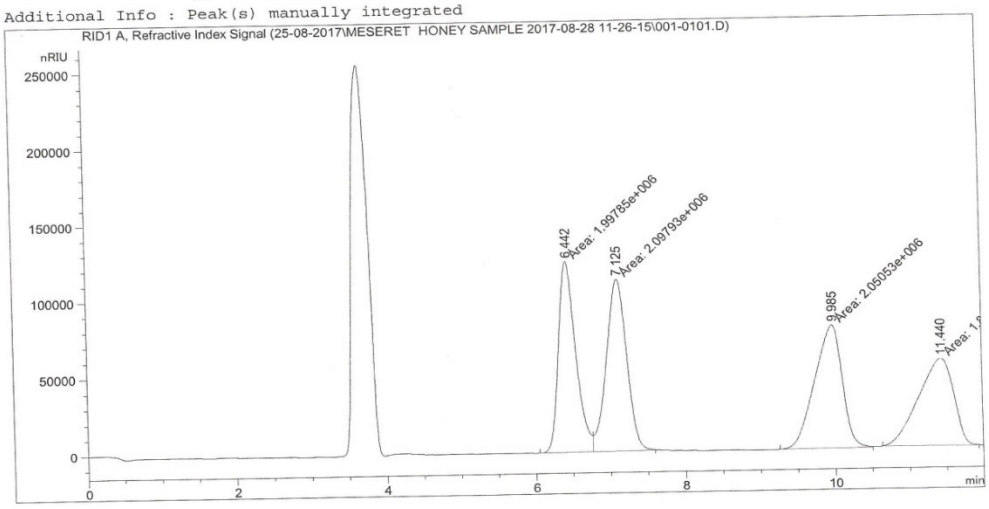
Additional Info : Peak(s) manually integrated



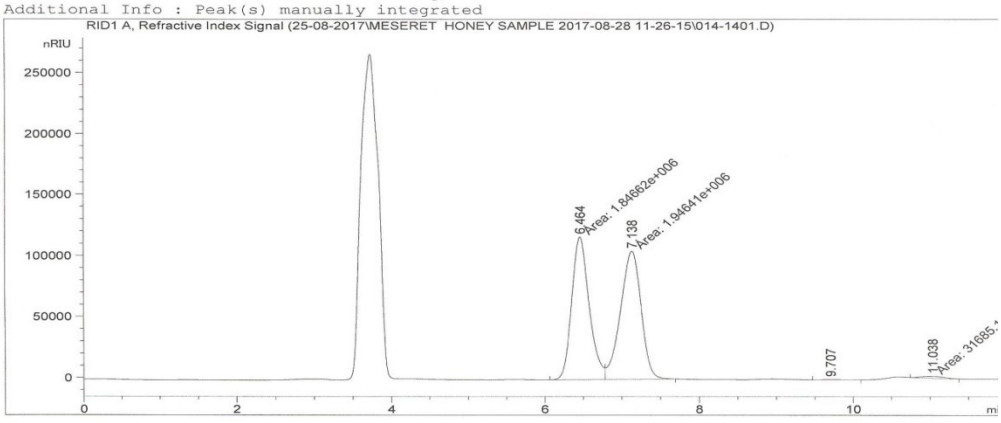
Data File C:\CHEM32\1\DATA\25-08-2017\MESERET HONEY SAMPLE 2017-08-28 11-26-15\001-0101.D
Sample Name: STANDARD SUGAR 2 GM

Acq. Operator	: SYSTEM	Seq. Line	: 1
Acq. Instrument	: HBRC_1260_HPLC	Location	: Vial 1
Injection Date	: 8/28/2017 11:26:59	Inj	: 1
		Inj Volume	: 10.000 µl
Acq. Method	: C:\CHEM32\1\DATA\25-08-2017\MESERET HONEY SAMPLE 2017-08-28 11-26-15 \MESERET HONEY SAMPLE.M		
Last changed	: 8/28/2017 11:26:15 by SYSTEM		
Analysis Method	: C:\CHEM32\1\METHODS\19032018.M		
Last changed	: 3/21/2018 09:26:20 by SYSTEM (modified after loading)		
Additional Info	: Peak(s) manually integrated		



Sample Name: SAMPLE AB8

Acq. Operator	: SYSTEM	Seq. Line	: 14
Acq. Instrument	: HBRC_1260_HPLC	Location	: Vial 14
Injection Date	: 8/28/2017 14:13:18	Inj	: 1
		Inj Volume	: 10.000 µl
Acq. Method	: C:\CHEM32\1\DATA\25-08-2017\MESERET HONEY SAMPLE 2017-08-28 11-26-15 \MESERET HONEY SAMPLE.M		
Last changed	: 8/28/2017 11:26:15 by SYSTEM		
Analysis Method	: C:\CHEM32\1\METHODS\19032018.M		
Last changed	: 3/19/2018 11:03:06 by SYSTEM (modified after loading)		
Additional Info	: Peak(s) manually integrated		



Multiflora honey

Sample Name: SAMPLE EJ1

```
======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   7
Acq. Instrument : HBRC_1260_HPLC                 Location : Vial 7
Injection Date  : 9/5/2017 12:45:08                   Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\05-09-2017\MESERET  EJERE HONEY SAMPLE 2017-09-05 11-27-45
                  \MESERET EJERE HONEY SAMPLE.M
Last changed    : 9/5/2017 11:27:45 by SYSTEM
Analysis Method : C:\CHEM32\1\METHODS\19032018.M
Last changed    : 3/19/2018 11:03:06 by SYSTEM
                  (modified after loading)
Additional Info : Peak(s) manually integrated
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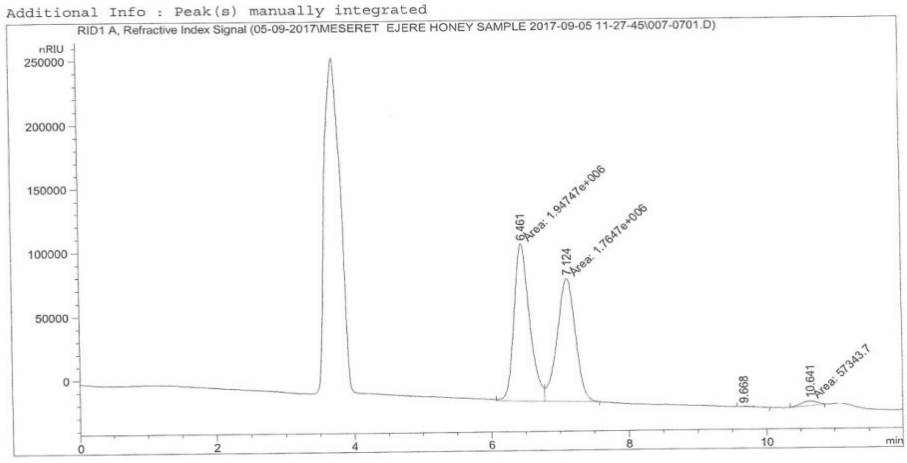

Eucalyptus globulus honey sample

Sample Name: SAMPLE EJ 13

```
Acq. Operator   : SYSTEM                          Seq. Line :  19
Acq. Instrument : HBRC_1260_HPLC                   Location : Vial 19
Injection Date  : 9/5/2017 15:18:24                    Inj :   1
                                                  Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\05-09-2017\MESERET  EJERE HONEY SAMPLE 2017-09-05 11-27-
                  45\MESERET EJERE HONEY SAMPLE.M
Last changed    : 9/5/2017 11:27:45 by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\MESERAT HONEY CALIBRATION.M
Last changed    : 9/22/2017 08:52:26 by SYSTEM
Additional Info : Peak(s) manually integrated
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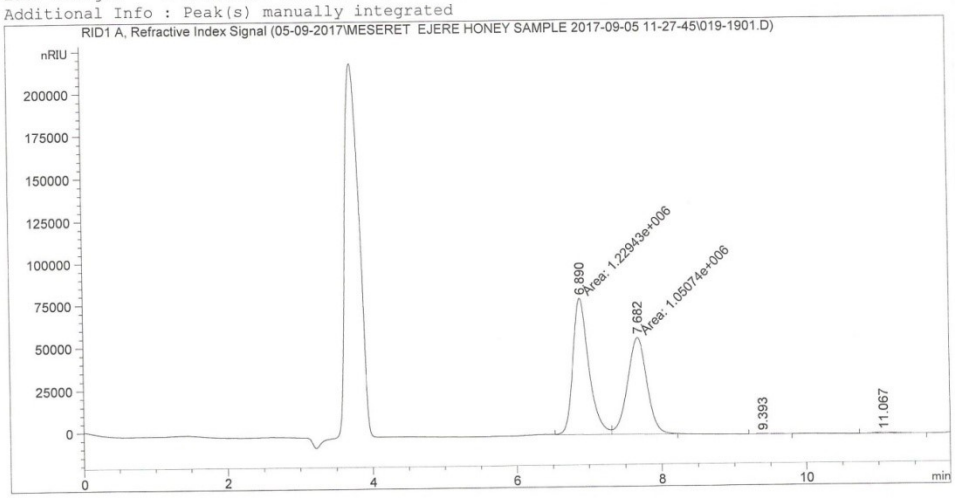
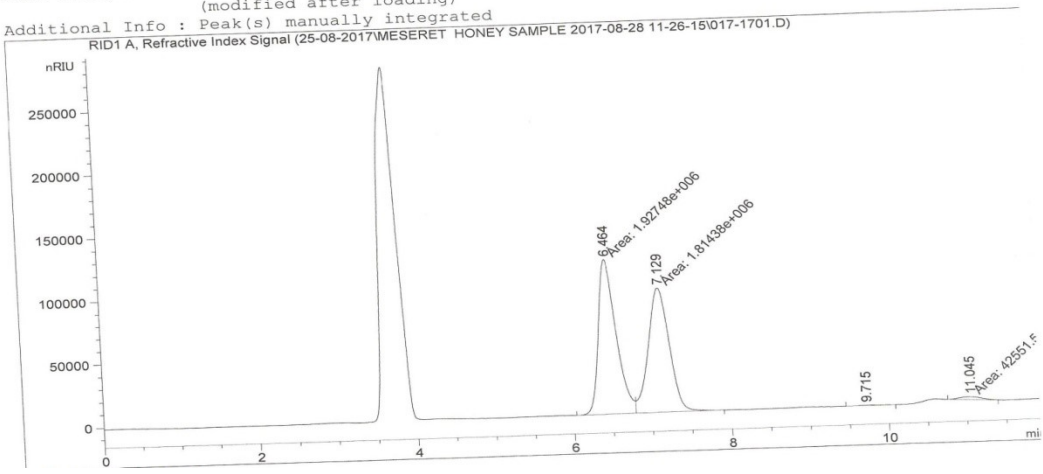


Trifolium honey sample

Sample Name: SAMPLE AB11

```
Acq. Operator   : SYSTEM                          Seq. Line :  17
Acq. Instrument : HBRC_1260_HPLC                   Location : Vial 17
Injection Date  : 8/28/2017 14:51:38                   Inj :   1
                                                  Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\25-08-2017\MESERET  HONEY SAMPLE 2017-08-28 11-26-15
                  \MESERET HONEY SAMPLE.M
Last changed    : 8/28/2017 11:26:15 by SYSTEM
Analysis Method : C:\CHEM32\1\METHODS\19032018.M
Last changed    : 3/19/2018 11:03:06 by SYSTEM
                  (modified after loading)
Additional Info : Peak(s) manually integrated
```



Guizotia scabra honey sample

Ashanalysis of honey using muffle furnace

carbonation of honey samples using not plate

Ash of honey samples

Ashing using Muffle Furnace

HMF content determination using UV- spectrophotometer

Honey Sample preparation

Reading absorbance honey sample for HMF detection using UV- spectrophotometer

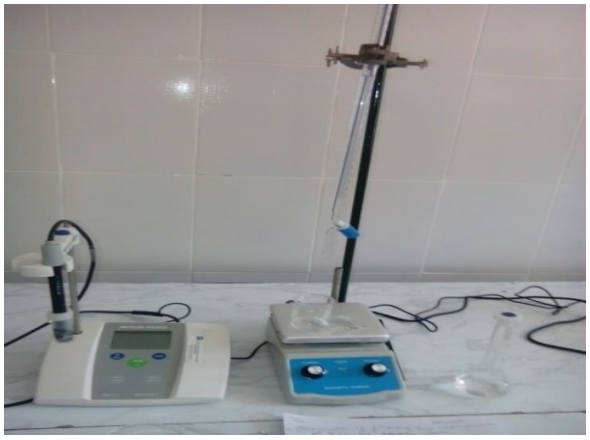
Electrical conductivity honey analysis

Honey sample preparation

Reading electrical conductivity of honey samples

PH and free acidity analysis of honey samples

sample preparation



PH meter

Reading of the PH content of the honey samples