

Antioxidants Properties of Stingless Bee Honey of the Oromia Region

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ABSTRACT

Stingless bee (*Meliponula beccarii*) honey forms a large group of bees that lack of a sting and they are spread throughout the tropical and subtropical areas of the globe. The aim of this study is to study the botanical origin and investigating antioxidants contents of stingless bee honey (*M. beccarii*) from different potential areas of Oromia. From the identified pollen, 15.4% were under families of Asteraceae and Fabaceae. The antioxidant contents and antioxidant activities of the honey were evaluated. Stingless bee honey excavated from Gera district had the highest total phenol content (897.59±151 mg GAE / 100 g). These results can show that stingless bee honeys have useful amounts of phenolic and flavonoid compounds that are able to act as natural anti-oxidants.

Keywords: Botanical origin, *Meliponula beccarii*, Phenol, Flavonoid, radical

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1. INTRODUCTION

Stingless bees are a primarily tropical group of over 500 species and are a vast monophyletic class of highly eusocial bees commonly found in abundance in warm humid forests around the world and their family Apidae, subfamily Apinae and tribe Meliponini Michener (2007). This species produces a rare honey that has gained attention in recent years, due to its particular characteristics and exotic flavor (Chuttong et al., 2016b; Ramón-Sierra et al., 2015; Sousa et al., 2016). Furthermore, it receives special attention because it shows resistance in the formation of 5-HMF when subjected to elevated temperatures Biluca et al. (2014). This feature increases the interest of pharmaceutical and food companies, since honey can be added as a complement to other products with no health risks due to excess 5-HMF.

Previous studies showed that stingless bee honey can act as anti-inflammatory), anticancer, antimicrobial and possessed antioxidant properties Batista et al (2016). Antioxidant inhibits oxidation, especially one used to counteract the deterioration of stored food products, removes potentially damaging oxidizing agents in a living organism.

Although free radicals of oxygen are a natural product of metabolism within the organism, they cause cellular damage and breakdown the structure of DNA Khalil et al (2010). Antioxidants components molecules in our bodies that get rid of such harmful by-products of normal metabolic functions by inhibiting destructive chemical reactions in our bodies It has been proposed that the antioxidant capacity of honey is due mainly to the phenolic compounds and flavonoids they contain, and there is a high correlation between polyphenols and honey antioxidant capacity Alzahrani et al (2012). Many studies have been done in different countries on the quality of honey based on physical characteristics and antioxidant properties (Alisi et al. 2012, Chua et al. 2013, Eleazu et al. 2013, Gorjanovic et al. 2013).

Modern science has found that most traditional practice of using stingless bee honey has great potential as an added value in modern medicine and considered to have a higher medicinal value than other bee species (Yaacob et al, 2017). In Ethiopia, honey produced by stingless bees is considered to be important in traditional treatments of wound, respiratory ailments, surface infection, diarrheal and various other diseases in line with treatments with other honey Ewnetu, Lemma et al (2013). For long tradition, stingless bee honey has been known as a product with high market demand, achieving higher prices than the honey produced by bees of the *Apis* genus, commercialized in different regions of Ethiopia.

Rural and urban communities attach high therapeutic value to stingless bees' honey making it a very popular traditional medicine (personal observation).

Although the use of honey has been of great importance in Ethiopia, there is little or no information on the antioxidant contents characteristics of stingless bee honey produced in Ethiopia, especially in potential areas of Oromia region. In addition, there is no enough research done and recent information on antioxidants properties of stingless bee honey of Ethiopia. Thus, this study will endeavor to quantify the levels of antioxidants of stingless bee honey which information will be used to add-on the present local knowledge on the beneficial use

of honeys as food and medicine. Therefore, the objectives of the study was to determine antioxidants contents and free radical scavenging activity of stingless bee honey from different potential areas of Oromia Regional state of Ethiopia. In addition, the aim of the work was to identify the botanical origin of stingless bee honey (*M. beccarii*) collected from the study areas.

2. MATERIALS & METHODS

2.1. Study area

Stingless bee honey samples were collected from potential districts of Jimma (Gera and Gomma), Ilu Ababora (Bacho, Didu and Alle) and West Shoa (Tokke Kuttaye and Chalia) zones of Oromia (Fig 1).

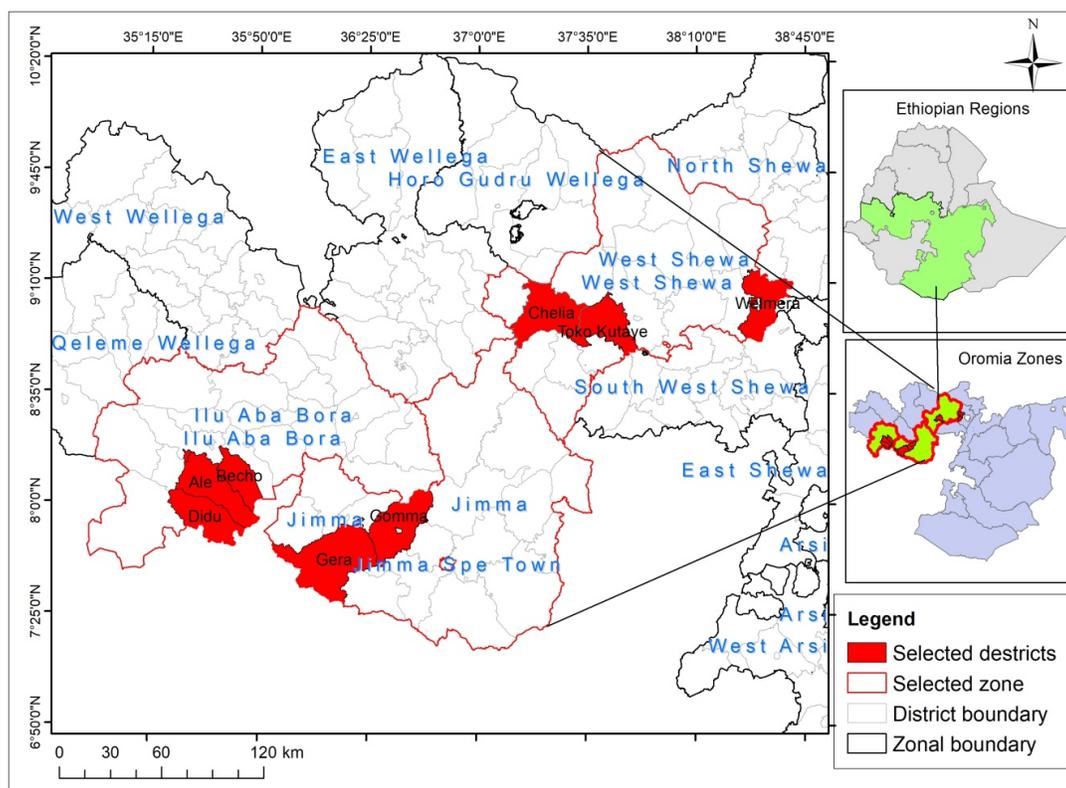




Fig 1: a) *M. Beccarii* on the entrance before excavated b) Stingless bee honey in honey pots after excavated c) and d) pure stingless bee honey harvested

2.2. Sample collection

The stingless bee (*M. beccarii*) honey samples were collected purposively from each potential district of each zone. The samples in honey pots were collected directly from cerumen pots from stingless bee honey hunters of the zones. Fresh and pure honey samples of (*M. beccarii*) were harvested directly from sealed honey pots with disposable syringes. Totally 32 samples was sampled and stored in contaminant free honey containers till laboratory analyses at about 4 °C in refrigerator.

b)

2.3. Pollen analysis

Botanical origin of honey was identified and counted using Louveaux et al (2015). Glycerine jelly, distilled water; centrifuge, vortex, microscope, heating plate, sterile slides and cover slips will be used. Ten grams (10g) of honey were weighed into a 50ml centrifuge tube. 20 ml of distilled water was added to dissolve the honey. It is centrifuged for 10 min at 1000 rpm, the supernatant liquid decanted and 20 ml of water was added, centrifuged and decanted again. Then it was transferred to slide on a heating plate and spread evenly and left to dry, glycerol was added and slip covered. Then, botanical origin was identified using digital microscope and quantified.

2.4. Honey colour analysis

This analysis was carried out in order to assess the influence of honey colour on other parameters studied. Honey samples was placed in clean and clear glass bottles and observed against the colour grading chart by Panaromic Hill Honey Collective (2013). Honey colour intensity was given a rank according to USDA Honey Colour Grading Chart USDA (1985). The relationship between honey colour and other honey parameters reported in this study was explored by conducting Pearson Correlation Tests.

2.5. Antioxidant content and activity

2.5.1. Total Phenolic content (natural compounds) determination

The total phenolic content of honey samples was analyzed by using Folin-Ciocalteu reagent. 0.5 ml of honey solution (0.5g/ml) was mixed with 2.5 ml of Folin–Ciocalteu reagent for 5 minutes. Thereafter, 2 ml of Sodium Carbonate solution (75g/l) were added and incubated for 2 hours at room temperature (28-30°C). Then 0.8 ml of 7.5% sodium carbonate were added and the mixture were agitated for 30 min in the dark, followed by centrifugation for 5 minutes at 3300 g. Absorbance of the honey samples and a prepared blank were measured at 765 nm using spectrophotometer. The mean of three readings was used. The concentration of total phenolic compounds in the honey samples were expressed as milligram of gallic acid equivalents (GAE) per 100 g weight of honey using linear equation obtained from the standard Gallic acid calibration curve.

2.5.2. Determination of total flavonoid content

The total flavonoid content of honey samples was determined based on the method of Isla *et al.* (2011) with some modifications as described by Chua *et al.* (2013). For each sample, 5 mls of honey solution (0.5g/ml) was mixed with 5 ml of 2% aluminium chloride (AlCl₃) and incubate for 10 minutes at room temperature (28- 30°C). The formation of Flavonoid-Aluminium complex was measured spectrophotometrically at 415 nm using Uv-vis spectrophotometer. A total concentration was calculated using quercetin standard curve, and expressed as Rutin equivalent/100 g of honey.

2.5.3. Determination of free radical scavenging activity

The free radical scavenging activity of honey was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay described by Pal *et al.* (2010), Isla *et al.* (2011) with minor modifications by Chua *et al.* (2013). The DPPH solution (20 mg/l) was prepared by dissolving 2 mg of DPPH in 100 ml methanol. A 0.75 ml of methanolic honey solution at different concentration, ranging from 2.5 to 40 mg/ml, was added to 1.5 ml of DPPH solution. The mixture was incubated for 15 minutes at 25 0C and the absorbance measured at 517 nm. The percentage of DPPH radical scavenging activity of each extract was determined at the various concentrations. The radical scavenging activity was calculated as follows as:

$$\% \text{ Inhibition} = [(\text{blank absorbance} - \text{sample absorbance}) / \text{blank absorbance}] \times 100$$

2.6. Statistical analyses

All analyses were being performed in triplicates and data were presented as mean standard deviation. Differences in performance between individual/group of honey samples were analyzed using General Linear Model. Correlation between studied parameters was analyzed by SPSS Statistical software.

3. RESULT and DISCUSSION

3.1. Botanical origin

The result of pollen analysis was as shown in Table 1. The predominant pollen types were recorded for *Coffea arabica*, *Eucalyptus globulus*, *Eucalyptus camaldulnesis*, *Guizotia scabra*, *Maesa lanceolata*, *Trifolium semipilosum*, *Schefflera abyssinica*, and *Vernonia amygdalina*. *Maesa lanceolata* and *Coffea arabica* identified as secondary pollen source. Important minor pollen source recorded were *Brassica carinata*, *Acacia abyssinica*, and *Croton macrostachys*. From the identified pollens 15.4% under families of Asteraceae and Fabaceae followed by other (Fig 3). Multivariate analysis of honey using principal component analysis (PCA) has been widely used in classification of honey types based on honey pollen analysis Ahmad *et al.* (2015b).

District	Predominant pollen Source (> 45%)	Secondary pollen Source (16-45%)	Important minor pollen Source (3-15%)	Minor pollen source (< 3%)
Tokkee Kuttaye	Eucalyptus globules and Guizotia scabra		Grass spp	Plantago lanceolata
Gedo	Eucalyptus glubulus, Trifolium semipilosum and Guizotia scabra		Brassica carinata	Coriodrum sativam and Plantago lanceolata
Wolemera	Eucalyptus globules and Guizotia scabra		Acacia abyssinica	Brassica carinata
Alle	Schefflera abyssinica		Coffea arabica, Croton macrostachys and Vernonia amygdalina	
Didu	Guizotia scabra, Schefflera abyssinica and Maesa lanceolata	Coffea arabica	Eucalyptus camaldulnesis and Vernonia amygdalina	
Bacho	Schefflera abyssinica and Eucalyptus camaldulnesis	Maesa lanceolata	Guizotia scabra	Croton macrostachys and Vernonia amygdalina
Gera	Vernonia amygdalina and Eucalyptus camaldulnesis	Coffea arabica	Schefflera abyssinica and Croton macrostachys	verpris dainelli
Goma	Guizotia scabra and Coffea arabica		Vernonia amygdalina and Croton macrostachys	

Table1. Botanical origin of stingless bee honey

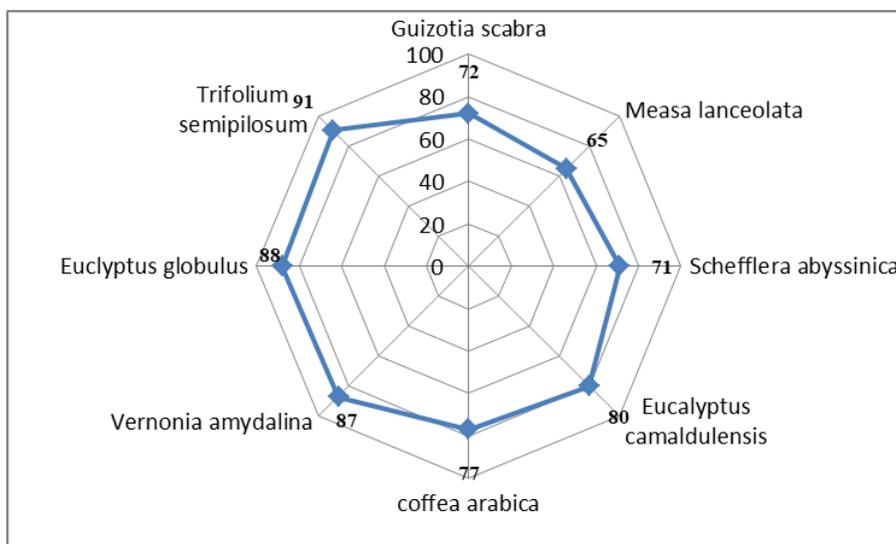


Fig 2: Major bee forage plants of monofloral honeys in the study area

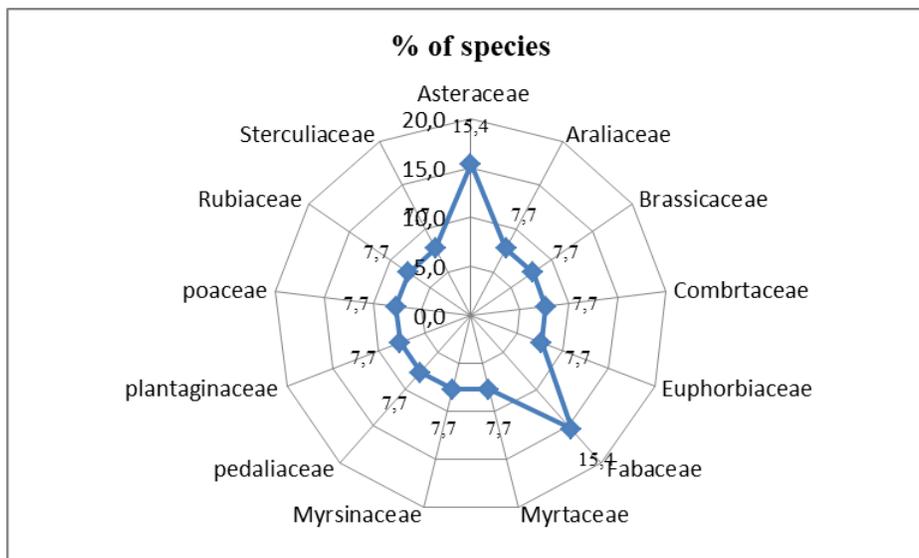


Fig 3: Percentages of plant species from different botanical families identified in stingless bee honey samples collected from different zones of Oromia region.

3.2. Color of the honey

All the stingless bee honeys were dark colored between Extra light amber and amber. Honey colors are varies due to many factors including the type of vegetation from which bees forage, soil and associated minerals, age of honey, storage factors and honey processing. Dark honeys have been associated with high phenolic content and antioxidant activities.

3.3. Antioxidant contents and activities

3.3.1. The total phenol content (TPC)

Table 2. Total phenol, Flavonoid contents and Radical scavenging activities

Distriicts	TPC mg GAE/ 100 g of honey	TFC mg QE /100 g of honey	RSA mg /100g of honey
Tokke Kutayye	213.10±98 ^c	290.05±62.5 ^{bc}	713.02±257.60 ^c
Chaliya	321.62±52 ^d	287.08±53.8 ^{bc}	797.26±100.11 ^c
Wolemera	468.56±152 ^c	419.15±101.8 ^a	848.15±295.60 ^c
Allee	473.97±16 ^c	290.24±84.9 ^{bc}	1112.46±82.87 ^b
Didu	486.18±80 ^c	331.31±136.1 ^{ab}	1091.47±88.31 ^b
Bacho	633.23±65 ^b	191.57±132.5 ^c	1317.24±11.19 ^a
Gera	897.59±151 ^a	289.1894±171.0 ^{bc}	1240.66±95.73 ^{ab}

Where QE=quercetin equivalent, AAE= Ascorbic acid equivalent and GAE= Gallic Acid Equivalents, TPC=Total Phenol Content, TFC=Total Flavonoid Content, RSA=Radical scavenging activities.

Different letters down the column showed significant difference (p <0.05).

The total phenolic content of the honey samples ranged from 213 to 897mg GAE / 100 g, honey from Gera stingless bee honey have the highest total phenol content (897.59±151 mg GAE / 100 g) ranges from 828.49 to 66.69 milligram of gallic acid equivalents (GAE) per 100 g honey and Tokke kuttaye has the lowest total phenol (213.10±98 mg GAE/ 100g) with the range of 153.25 to 272.94 milligram of Gallic acid equivalents (GAE) per 100 g weight of honey content than the other districts. Since phenolic compounds are derived from plants which come from different area, the phenolic contents in honey are greatly affected by the nectar source chosen by the

bees and the bee species (Shamsudin et al., 2019, Silva et al., 2013, Biluca et al., 2017). In addition to these, there are several other factors that contribute to the phenolic contents such as harvest season, weather and processing conditions. The study shows that there is a significant difference ($P < 0.05$) between the honey samples, Gera stingless bee (*M. beccarii*) honey is highly significantly different from the other honey samples. However, stingless bee (*M. beccarii*) honey samples from Wolemera, Alle and Didu are not significantly different from each other in terms of their total phenolic content as shown in Table 1. The TPC level of this study was higher than that of the six stingless bee honey samples from Malaysia (27.33 and 55.86 mg GAE/100 g) reported by Shamsudin et al. (2019) and Amazon stingless bee honey (0.6 mg/100 g) reported by Bastos et al. (2009).

3.3.2. The total flavonoid content (TFC)

There are two different spectrophotometric methods in determining the total flavonoid content, which both measure the formation of coloured complex substances quantitatively. Chua et al. (2013). The difference between the two methods is the compound used to react with flavonoid, which one uses aluminium ion (III), usually from aluminium chloride ($AlCl_3$), while the other uses 2,4-dinitrophenylhydrazine (DNP). However, certain flavonoids such as flavones and flavonols could not react with DNP, thus suggesting that the former method is preferred in determining the total flavonoid content.

According to the result of this study, the total flavonoid content of stingless bee (*M. beccarii*) honey from different places shows that stingless bee honey from Wolemera has the highest flavonoid content 419.15 ± 101.8 and the lowest was recorded in stingless bee honey samples collected from Bacho district and it was found to be 191.57 ± 132.5 mg quercetin equivalent (QE) /100 g of honey. There is also a significant difference ($P < 0.05$) between the samples of honey in total flavonoid content which may be due to place or soil type or altitude differences of the collected honey samples. The flavonoid content of stingless bee honey of this study is higher than that of stingless bee, *Mellipona* species honey of Tanzania (44.82 mg RE/100g) reported by Muruke (2014).

3.3.3. The antioxidant activity

The free radical scavenging activity (RSA %) of honey was determined by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and DPPH is used in a radical scavenging assay that is based on electron-transfer; it assesses the scavenging potential of a given substance. The assay produces an intense violet solution that is stable at room temperature. When a DPPH solution is mixed with a test compound that is able to donate a hydrogen atom, this changes the color of the compound to colorless or light yellow as the free radicals are scavenged. In this study, varying levels of DPPH scavenging activity ranging from 713.02 to 1317.72 mg/100g (Table 1). Specifically, Bacho stingless honey exhibited the highest DPPH 1317.24 ± 11.19 mg/100g with a range of 1196.12-1438.38 and also it is significantly different ($P < 0.05$) from all study area honey samples collected. According to the result of this study, the DPPH of stingless honey from different agro-ecologies of Oromia is higher than stingless bee honey from Malaysia DPPH ($47.40 \pm 3.18\%$) Tuksitha et al. (2018).

3.3.4. The correlation

According to the result of this study, there is a significant strong positive correlation between the total phenol content and the antioxidant activity ($r = 0.675$) of stingless bee honey, this means the antioxidant activity depends on the total phenolic content of the honey, whereas total flavonoid content has a weak and reverse correlation between antioxidant activity and the total phenol content.

4. CONCLUSION and RECOMMENDATIONS

Recently, there has been increased interest shown in the use of natural antioxidants as a form of protection against oxidative damage in the human body. The consumption of stingless bee honey therefore can increase antioxidant defenses against cancer-causing agents. According to the result of this study, most of the samples collected from Jima and Iluu Ababora Zones from different districts have high antioxidant activity. These results recommend that stingless bee honeys have useful amounts of phenolic and flavonoid compounds that are able to act as natural anti-oxidants.

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