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TOTAL PHENOLS AND ANTIOXIDANT ACTIVITIES OF NATURAL HONEYS AND PROPOLIS COLLECTED FROM DIFFERENT GEOGRAPHICAL REGIONS OF ETHIOPIA

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ABSTRACT. In this study, ten honey and five propolis samples from different geographical origins were tested. Both honey and propolis samples showed high content of total phenolic compounds (330-610 mg gallic acid equivalent (GAE)/100 g honey; 365-1022 mg GAE/g ethanol extract of propolis (EEP). The total flavonoids ranged from (15.1-42.6 mg catechin equivalent (CE)/100 g for honey; to 123-74 mg CE/g for EEP. These honeybee products of Ethiopia had high total radical scavenging properties with respect to 2,2-diphenyl-1-picryl-hydrazyl (DPPH); 18.1-59.8% and 48.6-87.8% for honey and EEP respectively. Furthermore, the hydroxymethylfurfural (HMF) of the honey samples was found to be low with a mean value of 4.8 mg/kg suggesting that the samples were of good quality. The antioxidant properties of the products showed a good correlation (r^2 = 0.50-0.82) with their polyphenolic contents.

KEY WORDS: Ethiopia, Honey, Propolis, Polyphenols, Flavonoids, Antioxidants

INTRODUCTION

Honey and propolis are easily accessible honeybee products which are becoming increasingly popular due to their potential role in contributing to human health [1]. Honey is a natural substance produced by honeybees (*Apis mellifera*) from the nectar of blossoms and secretions of plants. It is known to have both enzymatic and non-enzymatic antioxidant activities [2, 3]. Though, honey is a highly supersaturated solution of a complex mixture of sugars, it also contains small amount of other constituents including minerals, proteins, vitamins, organic acids, flavonoids, phenolic compounds, and enzymes; catalase, peroxides, glucose oxydase and other phytochemicals [3-5].

5-Hydroxymethyl-2-furaldehyde (HMF) is an aldehyde that is often used as an indicator for the honey quality. HMF and pH of honey are considered as important physicochemical parameters to determine the status of honey samples [6]. Because, HMF formation increases as the result of bad storage and heating, it is an excellent indicator of the honey's freshness and proper storage [7]. According to ANNEXII-Composition Criteria for Honey of the EU Council Directive 2001/110/EC, the HMF content of honey should be under 40 mg/kg in general, and under 80 mg/kg for honeys from regions of tropical climate [8].

Depending on the geographical and climatic conditions, different types of honey contain a wide range of phytochemicals including polyphenols and phenolic acids which act as antioxidants [9]. The main polyphenols in honey are the flavonoids with contents varying between 60 and 460 μg/100 g of honey [2]. Recent studies on honeys indicated that the biological actions of honey can be ascribed to its polyphenolic contents, which are elucidated by its antioxidant, anti-inflammatory, anti-proliferative and antimicrobial actions [10].

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Propolis is a natural honeybee product with sticky and resinous nature. It is collected by honey bees (*Apis mellifera*) from the buds and barks of different trees and enriched in thehive by the addition of salivated secretions and wax [11]. Propolis is widely used as a popular remedy in folk medicine and it does have high potential for use in human and veterinary medicine [11]. Like honey, propolis chemical variability is due to its plant origin and different geographic locations of the source plants [12]. Kumazawa *et al.* [13] have reported that more than 300 compounds including different flavonoids, polyphenolic esters, terpenoids, steroids, amino acids, caffeic acids and their esters, and inorganic compounds have been identified in propolis samples. Phytochemical investigations of propolis have demonstrated the presence of flavonoids and polyphenolic components as the main active ingredients having potent antioxidant activities. The antioxidant property of propolis seems to be responsible for its anti-carcinogenesis and hepatoprotective activities [14, 15].

The use of analytical methods for the determination of phenolic acids and flavonoids individually or as a group at the same time, has been related to the floral and geographical origins of honey and propolis. Folin-Denis and Folin-Ciocalteu reagents were widely used for estimation of plant phenols through color changes. Folin-Denis colorimetry was considered the best and “official” method but was subject to precipitations that interfered with colorimetry, yet gives good result [16]. One of the important functions of antioxidants in the living system is preventing the disturbance and functional loss of biological membranes and enzymes by scavenging the free radicals that otherwise induce oxidation of lipids, proteins, and DNA [17]. The antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents [18, 19]. To analyse the antioxidant activity of honey and propolis, the analytical methods commonly used refers to the sample's reducing capacity using the Folin-Ciocalteu or Folin-Denis and ferric reducing antioxidant power (FRAP) tests as well as antiradical activity with the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assays [4].

The antioxidant properties of honey and propolis believed to be at the heart of their polyphenolic compounds. The Ethiopian natural honey and propolis are thought to be of different varieties due to the unique and highly diverse flora of the country because of its rich variety of environmental features ranging from semi-desert to mountain forests and its wide range of ecological, edaphic, and climatic conditions. There are over 7000 flowering plants species recorded, of which 12% or more are probably endemic to Ethiopia [20]. To the best of our knowledge, no study has been reported on the phenolic contents and antioxidant properties of Ethiopian honey and propolis samples to date. Therefore, this study was designed to determine the total phenolic and flavonoid contents, and evaluate the radical scavenging activities of ten honey samples and five propolis samples collected from different geographical sources of Ethiopia.

EXPERIMENTAL

Equipment and reagents

Equipment. Lyophilizer (Operon, OPR-FDU-5012, Korea), Double beam UV/VIS NIR spectrometry (Perkin Elmer, Lambda 950, Waltham, MA 02451, USA) and Rotavapor (Buchi, Switzerland) were used.

Reagents. All the chemicals and reagents used in this study were of analytical grade reagents. Anhydrous sodium carbonate, zinc acetate and sodium bisulfite (Research Lab Fine Chem Industries, Mumbai, India), orthophosphoric acid (85%) and sodium molybdate dehydrate (98%) (BDH, England), anhydrous $AlCl_3$ and Na_2NO_2 (Fluka, Switzerland), sodium tungstate ($Na_2WO_4.2H_2O$) and phosphomolybdic acid (Scharlau Chememia, USA), ethanol and methanol (Alpha Chemika, India), gallic acid, chatechin, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH),

and ascorbic acid (Sigma-Aldrich, USA) were used as received. Folin-Ciocalteu was prepared in the laboratory.

Preparation of Folin-Ciocalteau reagent

Into 100 mL of round bottom flask, 10 g of sodium tungstate and 2.5 g of sodium molybdate were dissolved in 70 mL of distilled water. To the mixture 5 mL of 85% phosphoric acid and 10 mL of concentrated hydrochloric acid were added. After the solution was refluxed for 10 hours, 15 g of lithium sulfate, 5 mL of distilled water and 1 drop of bromine were sequentially added to the solution and refluxed for 15 min. The resulted mixture was allowed to cool at room temperature and was diluted to 100 mL with distilled water.

Honey samples

Honey samples were collected from six different geographical areas namely Agarfa Agricultural Technical and Vocational Education Training (ATVET) college (Bale zone, Oromia region), Alage ATVET college (West Arsi Zone Oromia region), Wolaita Soddo ATVET college (Wolaita zone, Southern Nation Nationality peoples, SNNP region), Holeta (West Showa zone, Oromia region), Bore (Guji zone, Oromia region), and Addis Ababa (Akaki-Kality and Yeka Kifle-Ketema of Addis Ababa City Administrative region) from May, 2011 to November, 2012 (Table 1). All samples were stored in capped airtight plastic and glass jars at room temperature until analysis.

Table 1. The ten honey samples, the site and dates of their collections.

Sample code	Site of collection	Harvest date	Type of honey	Production type
AAKal 1 (July)	Addis Ababa Kality area	July, 2012	Multi floral	Traditional
AAKal 2 (Sept.)	Addis Ababa Kality area	September, 2012	Multi floral	Traditional
AAQab (June)	Addis Ababa Qabenna area	June, 2012	Multi floral	Modern
Alage (July)	Alage ATVET	July, 2011	Multi floral	Modern
Agarfa (May)	Agarfa ATVET	May, 2011	Multi floral	Modern
Agarrfa (Oct.)	Agarfa ATVET	October, 2011	Multi floral	Modern
Bore (May)	Bore	May, 2011	Multi floral	Modern
Holeta (July)	Holeta Bee research center	July, 2012	Multi floral	Modern
Wolaita 1 (July)	Wolaita Sodo ATVET	July, 2011	Multi floral	Modern
Wolaita 2 (Nov.)	Wolaita Sodo ATVET	November, 2011	Multi floral	Modern

ATVET = Agricultural Technical and Vocational Education Training; AAK = Addis Ababa Kality area; AAQab = Addis Ababa Qabana area; Modern = improved beehives and honey production systems employed; Traditional = traditional basket beehives and honey production used.

Table 2. The five Propolis samples, and the site and date of collections.

Name of sample	Site of collection	Time of collection
Alage	Alage ATVET	July, 2011
Agarfa	Agarfa ATVET	June, 2011
Holeta	Holeta Bee research	June, 2011
Gedo	Gedo area	June, 2011
Wolaita	Wolaita Sodo ATVET	July, 2011

ATVET = Agricultural Technical and Vocational Education Training.

Collection of the propolis samples

Five propolis samples were collected from honeybee hives in the apiary sites of Agarfa ATVET, Alage ATVET, Wolaita Sodo ATVET, Holeta and Gedo areas of West Showa zone from May

2011 to November 2011 by trained technical experts. The propolis samples were collected from different geographical areas all from the same sites as honey samples except one sample from Gedo (Table 2).

Extraction of the propolis samples

The crude propolis samples were dried in ice, crushed into pieces and cleaned from impurities by thinning between fingers with hand so that the pure propolis is sticky and elastic. Each propolis sample was weighed and mixed with 70% ethyl alcohol in a ratio of 1 g : 5 mL (w/v) and the mixture was sealed in a container with intermittent shaking twice a day for 20 days [21]. The supernatant liquid was filtered with Whatman filter paper No. 1; the alcohol evaporated with a Rota vapor under vacuum and freeze dried by lyophilizer. The ethanol extract of propolis (EEP) was kept in a clean, airtight brown bottle in a refrigerator at -20 ^{o}C until used for analysis.

Determination of hydroxymethyl furfural (HMF) of the honey samples

Five gram of each honey sample was dissolved in 25 mL of distilled water to determine the HMF of the honey samples. The solution was quantitatively transferred to 50 mL flask and 0.5 mL of Carrez Solution I (150 mg/mL potassium ferrocyanide) and 0.5 mL of Carrez Solution II (300 mg/mL zinc acetate) were added. After the mixture was vigorously shaken and mixed well, the solution was brought to a final volume of 50 mL with distilled water. Aliquots of 5 mL were put in two test tubes; to the first test tube 5 mL of distilled water was added (sample solution); and to the second one 5 mL of 0.2% sodium bisulfite solution was added (reference solution). Then the HMF content of the ten honey samples were determined according to the White method by measuring the absorbance of the solutions at 284 and 336 nm with a UV-VIS spectrophotometer as suggested by International Honey Commission [22]. The HMF content was calculated using the formula;

$$HMF = (A_{284} - A_{336}) * 149.7 * 5 * \frac{D}{W} (mg / kg)$$

WhereA_{284} = absorbance at 284 nm, A_{336} = absorbance at 336 nm, $149.7 = \frac{126*1000*1000}{16830*10*5}$ = constant, D = dilution factor and W = weight of honey sample (g) [22].

Determination of total phenolic compounds in honey samples

The total phenolic compounds of the ten honey samples were determined according to the Folin-Ciocalteau method [23]. The solution of each honey sample was prepared by dissolving 2.5 g honey in 50 mL distilled water to obtain 0.05 mg/mL solution. One milliliter of properly diluted honey solution was mixed with 0.5 mL of Folin-Ciocalteau reagent and then 2.5 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added to the mixture. The mixture was kept in the dark for 90 min for reaction to occur. Then the absorbance of a blue colored mixture was measured at 740 nm using double beam UV/VIS NIR spectrometry. Gallic acid was used as a reference standard. The measurements were done in triplicate and the absorbance was read at 740 nm with a double beam UV/VIS NIR spectrometry. The results were expressed as mg gallic acid equivalents (GAE) per 100 g of honey.

Determination of total phenolic compounds in ethanol extracts of propolis (EEP)

The total phenolics in EEP were determined spectrophotometrically by the Folin-Ciocalteau method as for honey samples employing gallic acid as a standard [16]. Ten milligram of EEP was dissolved in 25 mL of 70% ethanol. One milliliter (0.4 mg/mL) of the solution was mixed with 0.5 mL of Folin-Ciocalteau reagent and 1.5 mL of 4% Na_2CO_3. This formed a deep blue colored solution which was kept in dark at room temperature for 2 h and then the absorbance was measured at 740 nm with a double beam UV/VIS NIR spectrometry and results were expressed as mg gallic acid equivalent (GAE)/g ethanol extract of propolis (EEP).

Determination of total flavonoids in honey samples

The total flavonoids content in the honey samples was determined according to calorimetric assay methods [24, 25]. The solution of each honey sample was prepared by dissolving 2.5 g honey in 50 mL distilled water. One milliliter of properly diluted honey (0.05 g/mL) was mixed with 4 mL of distilled water and 0.3 mL of (5% w/v) $NaNO_2$ was added. Then, 0.3 mL of (10% w/v) $AlCl_3$, and 2 mL of 1 M solution of NaOH were added after 5 min and 6 min, respectively. The mixture was diluted to 10 mL by the addition of distilled water. The mixture was shaken vigorously and the absorbance of the mixture was read at 503 nm with a double beam UV/VIS NIR spectrometry. Catechin was used as a calibrator standard. The results were expressed as mg catechin equivalents (CE) per 100 g of honey.

Determination of total flavonoids in ethanol extracts of propolis (EEP)

The total flavonoid contents of different EEP were determined using the aluminium chloride colorimetric method [24, 25]. To 1 mL of the EEP solution, 0.3 mL of (5% w/v) $NaNO_2$, 0.3 mL of 10% $AlCl_3$ and 2 mL of 1 M solution of NaOH were added sequentially as mentioned above. Catechin in 70% ethanol solution was used as standard calibrator. Flavonoid contents were expressed as catechin equivalents (CE/g of EEP).

Determination of radical scavenging activities of honey and propolis extract

The antioxidant capacity of the honey samples and EEP was measured by evaluating the free radical-scavenging effect of the samples with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical using the method reported by Ferreira *et al.* [26]. Ascorbic acid was used as standard for both honey and EEP. The solution of each honey sample was prepared by dissolving 1.5 g honey in 20 mL distilled water. One milliliter of properly diluted honey solutions (0.075 g/mL) was mixed with 0.5 mL of DPPH in methanol (0.342 mg/mL) and then diluted with 3 mL methanol. The mixture was shaken vigorously and left for 1 h in the dark. The reduction of the DPPH radical was then determined by measuring the absorbance of the mixture at 517 nm with a double beam UV/VIS NIR spectrometry. A control solution was prepared containing the same amount of methanol and DPPH. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\% \text{ RSA} = [(A_{control}-A_{sample})/A_{control}] \times 100$ [26]. The antioxidant activity of the honey samples was evaluated through the free radical scavenging effect against DPPH radicals, and was compared with radical-scavenging activity of ascorbic acid as a standard substance. For ethanol extract of propolis (EEP), lyophilized extract was dissolved in 70% ethanol (0.5 mg/mL) and 1 mL of properly diluted sample was taken and treated as above. The antioxidant role of the EEP was also tested as its free radical inhibition power against DPPH using ascorbic acid as control substance.

Statistical analysis

All analyses were carried out in triplicate and the data were expressed as means ± standard deviations (SD). Data were analyzed using SPSS Ver. 16 and MS Excel 2007. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test was used to compare the phenol contents and DPPH scavenging activity of different honey types. Differences between means at the 95% ($p < 0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSIONS

Determination of HMF of the honey samples

Hydroxymethyl furfural (HMF) is one of most important physicochemical parameters of honey [27]. The mean value of HMF of the tested honey samples ranged from 0.1 mg/kg for honey

sample from Alage to 11.9 mg/kg for samples from the Addis Ababa Kality area. The mean value for the ten honey samples tested in this experiment was 4.8 mg/kg. These values were very low in general indicating the storage conditions and harvesting processes are good and the honey samples used in this study were of good qualities. According to International Honey Commission, the limit for HMF in honey samples from tropical regions like Ethiopia is set to be 80 mg/kg while that from temperate region is 40 mg/kg [22].

Ethanol extraction of the propolis samples

As crude propolis samples contain plant resins, bees wax and insoluble material, the use of 70% ethanol for extracting the bioactive components (such as polyphenolic compounds) and to remove the bees wax of the samples is reasonable. This is because one of the factors that affects the yield and composition of hydro-ethanolic extracts of propolis is the concentration of ethanol used; solvents containing 70% of ethanol or more extracted more propolis resins by maceration [28]. Accordingly, the yield of the five propolis samples were obtained after macerating the samples for 20 days with intermittent shaking and lyophilazation after evaporating the alcohol component of the supernatant solution with a Rota vapor under vacuum. The yield ranged from 11.7% for sample from Agarfa (Bale) to 16.7% for sample from Wolaita Soddo (Table 3).

Table 3. The ethanol extract of propolis (EEP) product yield.

Sample name	Amount soaked (g)	Yield (g)	Yield (%)
Alage	110	13.2	12.0
Agarfa	80	9.4	11.7
Holeta	90	12.6	14.0
Gedo	120	15.4	12.8
Wolaita Soddo	100	16.7	16.7

Determination of total phenolic compounds in honey samples

The total polyphenol content of the tested honey samples ranged from 330±38 mg GAE/100 g (sample from Bore) to 610±5 mg GAE/100g (sample from Woliata Soddo) area both from Southern region of Ethiopia. The two honey samples collected from Wolaita Soddo ATVET showed the highest total polyphenols (604 and 610 mg GAE/100 g) followed by two honey samples collected from Addis Ababa Kalityarea (566 and 470 mg GAE/100 g) while the lower values were recorded for samples collected from Bore, Holeta, Addis Ababa Qabana area and Agarfa ATVET (330, 332, 337, and 377 mg GAE/100 g, respectively) (Table 4). The colors of these honey samples showed clear differences. The honey sample from Bore was white colored while Holeta and Addis Ababa Qabana area samples were light yellowish colored. The samples from Agarfa and Alage were brown in color while Wolaita Soddo and Addis Ababa Kality area samples were observed to be dark brown colored. This study confirmed that the darker honey samples have higher total phenolic contents compared to the light colored ones. Results of the present study showed that the tested Ethiopian honey samples contained the higher phenolic contents compared to the Malaysian Tualang and Gelam honeys (877±4 mg GAE/kg and 484±4 mg GAE/kg, respectively) and also much higher than the Manuka honey (435 mg GAE/kg) of the New Zealand which is well investigated [4]. Even though, the Folin Ciocalteu assay method is commonly used to evaluate the total phenolic compounds in plant extracts and honey samples, it results in overestimation of the compounds due to the phosphotungstic acid and phospho-molibdic acid mixture that react with other nonphenolic reducing compounds like ascorbic acid, some sugars and amino acids that are known to interfere with the test results [4, 29, 30]. Nevertheless, the method remains useful and is largely used to evaluate the relative contents of total polyphenolic compounds in varieties of honey samples from diverse floral origins.

Table 4. [*]Total phenolic, flavonoid contents and antioxidant activity of honey samples.

Honey type	Total phenols (mg GAE[a]/100 g honey)	Total flavonoids (mg CEQ[b]/100 g honey)	% DPPH inhibition (% RSA)	DPPH (mg AAE[c] /100 g honey)
AAKal 1 (July, 2012)	470 ± 38	42.2 ± 2.4	51.4 ± 2.6	174 ± 9
AAKal 2 (Sept., 2012)	566 ± 10	39.2 ± 2.0	46.0 ± 2.0	155 ± 7
AAQab (June, 2012)	377 ± 37	18.0 ± 1.5	33.1 ± 2.3	109 ± 8
Alage (July, 2011)	419 ± 37	19.1 ± 0.86	43.4 ± 2.9	146 ±10
Agarfa (May, 2011)	441 ± 36	22.6 ± 1.4	49.6 ± 3.8	168 ±14
Agarfa (Oct., 2011)	337 ± 30	25.3 ±1.92	22.8 ± 2.1	72.7 ± 7.6
Bore (May, 2011)	330 ± 38	20.6 ± 2.0	18.4 ± 1.6	57.2 ± 5.8
Holeta (July, 2012)	332 ± 21	18.4 ± 1.0	23.0 ± 1.3	73.7 ± 4.3
Wolait1(July, 2011)	604 ± 63	34.5 ±3.4	58.9 ± 2.5	201 ± 9
Wolaita 2 (Nov., 2011)	610 ± 5	31.0 ±0.75	43.9 ± 3.8	147 ± 14
Mean value	449 ± 28	27.1 ±1.7	39.1 ± 2.5	130 ± 9

[a]Gallic acid equivalent; [b]Catechin equivalent; [c]Ascorbic acid equivalent; % RSA = percent radical scavenging activities; [*](Mean ± SD, n = 3).

Determination of total phenolic compounds in ethanol extracts of propolis (EEP)

The total phenolic contents of the ethanol extracts of propolis (EEP) in this study ranged from 365±37 mg GAE/g EEP (for sample from Woliata Soddo) to 1022±60 mg GAE/g EEP (for Alage propolis extract). The mean value for the total phenolic contents for the five propolis samples tested in this experiment was 617 mg GAE/g EEP (Table 5). According to this study the Ethiopian propolis showed the highest phenolic contents compared to Indian propolis extracts (48.6 mg GAE/g EEP) [31] and Portuguese propolis (329 mg GAE/g EEP) [32]. The total phenolic content in the ethanol extracts of Ethiopian propolis samples were different according to the floral origin of the region. This holds true for propolis samples obtained from different regions in Brazil depending on the location of the hives and local floral sources [33].

Table 5. [*]Total phenolic, flavonoid contents and antioxidant activity of ethanol extracts of propolis (EEP).

Type of propolis	Total phenols (mg GAE[a]/g EEP)	Total flavonoids (mg CEQ[b]/g EEP)	% DPPH inhibition (% RSA)	DPPH (mg AAE[c]/g EEP)
Alage	1022 ± 60	574 ± 101	87.8 ± 0.1	455 ± 1
Agarfa	666 ± 28	139 ± 1	49.9 ± 1.8	253 ± 10
Gedo	597 ± 21	170 ± 5	86.4 ± 0.2	448 ± 1
Holeta	433 ± 32	123 ± 4	71.7 ± 1.9	369 ± 10
Wolaita	365 ± 37	218 ± 4	48.6 ± 0.9	247 ± 5
Mean value	617 ± 36	224 ± 5	68.9 ± 0.9	354 ± 5

[a]Gallic acid equivalent; [b]Catechin equivalent; [c]Ascorbic acid equivalent; % RSA = percent radical scavenging activities; [*](Mean ± SD, n = 3).

Determination of total flavonoids inhoney samples

Total flavonoid contents of the honey samples were lower than phenolic content and ranged from 18.0± 1.5 mg CEQ/100 g for honey sample from Addis Ababa Qabana area to 42.2 ± 2.4 mg CE/100 g for honey sample from Addis Ababa Kality area. This is attributable to the differences in the type of honey samples, floral origin and season of collection to a large extent (Table 4). The two honey samples from Addis Ababa Kality area showed the highest flavonoid content followed by samples from Wolaita Soddo ATVET College. These honey samples were darker in colour but collected during different seasons (from May, 2011 to November, 2012) and also differed in their storage time (from 18 month to 2 month). The yellowish light coloured honey samples from Addis Ababa Qabana area, Holeta, Bore, Alage and Agarfa ATVET

colleges were found to have lower flavonoid content ranging from 18.0 to 25.3 mg CE/100 g honey. The flavonoid contents of the honey samples in this study are generally higher than that of Malaysian Tualang and Gelam honeys (234 mg CE/kg and 34.3±1.2 mg CE/kg, respectively) and also higher than of the Manuka honey (85.1 mg CE/kg) of the New Zealand [4].

Determination of total flavonoids inPropolis extracts (EEP)

The total flavonoid contents for the propolis samples showed the highest value (574±10 mg CE/g EEP) for the sample from Alage ATVET college located in the Southern region of Ethiopia while the sample from the central high land area of Holeta found to be the lowest (123 ± 4 mg CE/g EEP) of all the five propolis extract analyzed in this study (Table 5). The mean value for the flavonoid contents of all the five propolis extracts was 224 mg CE/g EEP. Paviani *et al.* [34] had reported similar results (311 mg CE/g EEP) for Brazilian green propolis. It has to be noted that the propolis extract from Alage ATVET College showed the highest total phenolics (1022 mg GAE/g EEP) and flavonoids (574 mg CE/g EEP) than any other sample. However, the Wolaita Soddo ATVET propolis extract has shown the median value of flavonoid content though it was found to have the lowest total phenolic content.

In this study, Ethiopian propolis samples generally showed the highest total phenolic and total flavonoid contents compared to samples from Japanese [13] and propolis samples from Greek and Cyprus which ranged from 80.2 to 338 mg GAE/g EEP [35].

Determination of radical scavenging activities of honey and propolis extract

In human health, the radical scavenging antioxidants play their roles by scavenging reactive free radicals to protect biologically essential molecules from oxidative modification; though their effects on chronic diseases found to be contradictory and confusing; partly due to the complex effects of oxidative stress on pathogenesis [36]. The radical scavenging activities of the honey samples and propolis extracts were compared to ascorbic acid which was used as a standard antioxidant agent. The antioxidant activity of honey samples and propolis extracts were shown by their radical scavenging ability expressed as percentage of inhibition against DPPH radical.

The percent inhibition or percent radical scavenging power (% RSA) of the honey samples ranged from 18.4% to 58.9% for samples from Bore and Wolaita, respectively. If evaluated in terms of ascorbic acid antioxidant activity the equivalent values ranged from 57.2 mg AAE/100 g honey for Bore sample to 201 mg AAE/100 g honey for Wolaita sample (Table 4). On average the % RSA of all honey samples tested in this experiment is 39.1% and is equivalent to 130 mg ascorbic acid per 100 g honey sample analyzed. Piljac-Zegarac *et al.* [37] reported that the mean scavenging potential of heterofloral Croatian honey was found to be 16.7 mg AAE/100 g. The DPPH radical scavenging capacities of Ethiopian honey samples were found to be very high compared to those Croatian honeys. Two Tualang honey types with the total polyphenols content (877 mg GAE/kg and 652 mg GAE/kg) showed the best radical scavenging properties with respect to DPPH· (81.6% and 77.3%) of the analyzed Malaysian honey samples [4].

The percentage inhibition (% RSA) of the EEP ranged from 48.6% to 87.8% or 247 mg AAE/g EEP to 455 mg AAE/g EEP. On average the % RSA of EEP is 68.9% which corresponds to 354 mg AAE/g EEP indicating that the propolis extracts showed good in vitro antioxidant properties (Table 5).

Correlations between antioxidant activity, total phenols and flavonoids content in the samples

Correlation between total phenolic and flavonoid contents and radical scavenging activity of honey and propolis extracts were analyzed. Results are depicted in Tables 6 and 7 with correlation coefficient (r^2) of 0.50-0.82. The total flavonoid contents correlates with the total phenolic contents with correlation coefficient (r^2) of 0.82 (Tables 6 and 7; $p < 0.05$). In general, extracts with a high radical scavenging activity showed a high phenolic and flavonoids content as well, but good correlations could not be found among them (Tables 6 and 7). The capacity of

free radical scavenging does not always correlate well with the capacity to inhibit oxidation. A direct correlation between radical scavenging activity and phenolic and flavonoids content of the samples failed to demonstrate by linear regression analysis. This lack of relationship is in agreement with other literatures [10, 38]. It is known that only phenolic compounds with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [39].

Table 6. Correlation between polyphenolic contents and their radical scavenging effect of whole honey samples.

	Total phenols	Total flavonoids	DPPH
Total phenols	1	0.71	0.82
Total flavonoids	0.71	1	0.63
DPPH	0.82	0.63	1

Table 7. Correlation between polyphenolic contents and their radical scavenging effect of propolis extracts.

	Total phenols	Total flavonoids	DPPH
Total phenols	1	0.82	0.56
Total flavonoids	0.82	1	0.50
DPPH	0.56	0.50	1

CONCLUSIONS

This study has reported the results of Ethiopian honey and propolis samples on their phenolic and flavonoid contents and their radical scavenging activities. The total phenolic contents of the tested Ethiopian honey and propolis samples were found to be high (average 449 mg GAE/100 g for honey and 617 mg GAE/g for EEP) compared to similar studies done elsewhere. The total flavonoid composition was 27.1 mg CE/100 g for honey on average while that of propolis samples was 224 mg CE /g for EEP. Honey sample from Wolaita Soddo showed the highest total phenolic content (610 mg GAE/100 g) while the highest total flavonoid value (42.2 mg CE/100 g) was recorded for the sample from Addis Ababa Kality area. The Alage propolis sample showed the highest total phenolic and flavonoid content (1022 mg GAE/g EEP and 574 mg CE/g, respectively) compared to all other propolis samples.

The antioxidant activities of both honey and propolis sample expressed in terms of their radical scavenging power with respect to DPPH varied depending on their origin. The higher radical scavenging power was observed for the Wolaita Soddo sample (58.9%) while for propolis the Alage samples showed the best antiradical activity (87.8%). It can be noted that the antioxidant activities of both honey and propolis samples collected from different geographical origin in Ethiopia appeared to depend on their total phenolic composition. Thus, these honeybee products can be considered as easily accessible and valuable natural sources of antioxidants and dietary supplement.

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