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Assessment of Antioxidant Properties of Honeys from Tanzania

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Abstract
In this study, levels and properties of antioxidants, antiradical and iron chelating activities of honeybee and stingless bee honeys were determined using standard methods. The relationship between honey colour and the studied antioxidants was also explored. Phenolic content of stingless bees honey samples (mean value 847.6 mgGAE/100g) was significantly higher (P<0.0001) than that of raw (412.6 mgGAE/100g) and processed (218.4 mgGAE/100g) honeybee honey samples, respectively. Mean value of flavonoid content was 84.96 mgRE/100g for stingless bee honeys, 44.82 mgRE/100g for processed honeybee honeys and 41.60 mgRE/100g for raw honeybee honeys. β-Carotene and lycopene content varied among the three honey categories studied. Honey from stingless bees exhibited higher levels of the two antioxidants than honey bee honeys. Vitamin C content ranged from 20.4 – 66.7 mg/100g in processed honey, 42.5 – 61.6 mg/100g in raw honeybees honey and from 56.2 – 67.6 mg/100g in stingless bee honeys. The number of antiradical activity units in 1 mg of honey (EAU_{515}) varied significantly between the honey categories (P<0.0001). The mean values were 5.21 mg/100g for processed honeybee honeys, 6.8 mg/100g for raw honeybee honeys and 10.6 mg/100g for stingless bee honeys. DPPH radical scavenging and iron chelating activities were highest (lowest EC_{50}) in stingless bee honeys compared to honeybee honeys. EC_{50} mean value for DPPH was lowest (4.19 mg/ml) in stingless bee honeys, 12.93 mg/ml for raw honeybees honey and 18.03 for processed honeybees' honey. Similarly, iron chelating activity was highest in stingless bee honeys (EC_{50} 0.04 mg/ml) followed by raw honeybee honeys (EC_{50} 0.057 mg/ml) and processes honeybee honeys (EC_{50} 0.158). A diversity of honey colours ranging from light coloured to dark honeys was recorded in the samples studied. Majority of honeybee honey colours were described as white and light amber, while all the stingless bee honeys were between light amber and amber colours. There were strong positive correlations among the antioxidants studied ($r \geq 0.5$ at P<0.01). Honey colour intensity was found to correlate positively with all antioxidants studied. Consistently darker honeys were found to contain higher levels of antioxidants than lighter honeys. In overall, Tanzanian honeys studied contains high levels of antioxidants, depicting good quality characteristics for use as food and as medicine.
Key words: Antioxidants, DPPH, Antiradical activity, Phenolics, Flavonoids

1. Introduction

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 are 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. Researchers believe food products rich in antioxidants may prevent heart diseases, cancer and diabetes (Ames *et al.* 1993, Gutteridge and Halliwell 1994). Traditionally, honey in Tanzania has been used by local population as food, source of income and to a large extent as medicine in treatment of wounds, coughs and stomach discomforts (Tanzania Honey Council 2012).

The quality of honey as a functional food is attributed to a wide range of bioactive antioxidants components such as phenolic acids, ascorbic acid, flavonoids, enzymes and carotenoids among others (Ferreres *et al.* 1993, Khalil *et al.* 2010, Lachman *et al.* 2010). 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). In Tanzania, studies have been carried out on quality of honey based on physical and biochemical properties of honey such as colour, pH, total acidity, moisture content, suspended colloids, levels of hydroxymethyl furfural (HMF), sugar content and ash content among others (Gidamis *et al.* 2004, Muruke 2014). However there is no information on the quality of Tanzanian honey based on antioxidant and iron chelating properties. There is a widely shared view among Tanzanian rural and urban communities that attach high therapeutic value to stingless bees' honey (Muruke, 2014) making it a very popular traditional medicine. This study will thus endeavour to quantify the levels and properties of antioxidants namely total phenolic content, vitamin C content, total flavonoids, carotenoids, lycopenes and antiradical and iron chelating activities in honeybees honey samples and stingless-bee honey samples. This information will be used to complement the existing indigenous knowledge in Tanzania on the beneficial use of honeys as food and medicine.

2. Materials and Methods

2.1 Sample collection

A total of 22 honey samples grouped in three categories namely processed honey from honey bees (*Apis mellifera*), raw honey from honeybees and stingless bees' honey from *Mellipona* species used in this research were collected from ten regions in Tanzania (Table 1). Raw honey samples in combs were collected directly from beekeepers where as processed honey for commercial use were obtained from the Dar es Salaam International Trade Fair in July 2013 and July 2014. The origins of all processed honeys were determined by the manufacture's labels. All samples were kept in air-tight containers at room temperature (28-30ºC) before use.

Table 1: Honey samples and their places of origin

S/N	Sample name	Origin/Region	S/N	Sample name	Origin/Region
Processed honeybees honeys			*Raw honeybees honeys*		
1	P3 Uvinza	Kigoma	14	R23b Kondoa2	Dodoma
2	P9 Manyoni	Singida	15	R51 Tabora3	Tabora
3	P16 Ruangwa	Lindi	16	R65 Kondoa 3	Dodoma
4	P17 Mbeya1	Mbeya	17	R 66 Tabora4	Tabora
5	P18Dodoma	Dodoma	*Stingless bees honeys*		
6	P28 Mbeya2	Mbeya	18	S19 Urambo	Tabora
7	P52 Tabora1	Tabora	19	S20 Kibondo	Kigoma
8	P53 Tabora2	Tabora	20	S22 Biharamulo	Kagera
9	P55 Mugumu	Mara	21	S23a Usariver	Arusha
10	P56 Geita1	Geita	22	S57 Geita2	Geita
11	P58 Kasulu	Kigoma			
12	P59 Chunya	Mbeya			
13	P62 Kondoa1	Dodoma			

2.2 Determination of total Phenolic content

The total phenolic content of honey samples was analysed by using Folin-Ciocalteu reagent described by Singleton *et al.* (1999) with modifications by Chua *et al*. (2013). 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) was added and incubated for 2 hours at room temperature (28-30ºC). Then 0.8 ml of 7.5% sodium carbonate was added and the mixture was 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 (Uv-vis model 6305 Jenway UK). The concentration of total phenolic compounds in the honey samples was 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 (Tibuhwa *et al.* 2012).

2.3 Determination 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) were mixed with 5 ml of 2% Aluminium chloride ($AlCl_{13}$) and incubate for 10 minutes at room temperature (28-30ºC). The formation of Flavonoid-Aluminium complex was measured spectrophotometrically at 415 nm using Uv-vis model 6305 Jenway UK. Total concentration was calculated using quercetin standard curve, and expressed as Rutin equivalent/100 g of honey.

2.4 Determination of β – Carotene and Lycopene Contents

β-**C**arotene and Lycopene contents were measured using the method of Nagata and Yamashita (1992). One hundred (100) mg of a honey sample was shaken with 10 ml of Acetone-hexane mixture (92:3) for 1 minute and filtered through Whatman number 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. The β – carotene and lycopene content were calculated as:

Lycopene µg/mg $= 0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$

β–carotene µg/mg $= 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$

2.5 *Determination of Vitamin C*

The vitamin C content was determined by titration using 2,6 Dichloropheno Indophenols methods described by Plummer (1987). Known weight of honey sample was mixed with 25 ml of 5% metaphosphoric acid solution and shaken for 30 min. The mixture was then filtered with Whatman filter paper no.42 using suction pump. 10 ml was pippetted from the filtrate into 250 ml conical flask and titrated against 0.025% of 2,6 Dichlorophenol

Indophenol reagents. The amount of vitamin C in each honey sample was calculated from following equation:

$$\text{mg of ascorbic acid per } 100\text{ g} = \frac{(A * I * V_1 * 100)}{(V_2 * W)}$$ where:

A is quantity of ascorbic acid (mg) reacting with 1 ml of 2, 6 Indophenol, I is volume of Indophenol (ml) required for completion of the extract titration, V_1 is total volume of extract, V_2 is volume of aliquot and W is weight of honey sample used.

2.6 Measurement of antiradical activity

DPPH (1,1-difenly-2-picrylhdrazyl) radical in its form has a characteristic absorbance at 515 nm, which disappears after its reduction by an antiradical compound (Sroka 2006). Thus, the reduction of DPPH by honey antiradicals was monitored spectrophotometrically by measuring decrease in its absorbance at 515 nm as described by Brand-Williams *et al.* (1995). In this study, 40 µl of honey samples (20 mg/ml) were added to 1460 µl of 0.0037% DPPH. Absorbance was measured at 515 nm at time 0 and after 1 minute. The antiradical activity unit (AU_{515}) of each extract was calculated according to the equation:

$AU_{515} = (A_0 - A_1) - (A_{0C} - A_{1C})$; where

AU_{515} is antiradical activity of the extract, A_0 is absorbance of the sample at the beginning of the reaction (0 minute), A_1 is absorbance of the sample after 1 minute of the reaction, A_{0C} absorbance of the control sample at the beginning of the reaction, and A_{1C} is absorbance of the control sample after 1 minute of the reaction.

The number of antiradical activity units per 1 mg (EAU_{515}) of each extract was calculated according the equation: $EAU_{515} = \frac{AU_{515}}{le}$ where, EAU_{515} = number of antiradical activity; le = amount of extract in the simple (mg) and AU_{515} = antiradical activity of the extract.

2.7 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 then incubated for 15 minutes at 25 ^{0}C and the absorbance measured at 517 nm. The percentage of DPPH radical scavenging activity of each extract was determined at the various concentrations and was calculated as:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \left(\frac{Ac - As}{Ac}\right) * 100$$

Where Ac and As are the absorbance of control and sample, respectively. The concentration of honey sample required to scavenge 50% of DPPH (EC_{50}) was determined from the plotted graph of scavenging activity against the concentration of extracts.

2.8 Iron chelating ability

Chelating ability ferrous ions was determined according to the method of Dinis *et al.* (1994). Different concentration of each honey sample (ranging from 0.05 to 0.4 mg/ml) in water (1 ml) were mixed with 3.5 ml of methanol and 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by the addition of 2 ml of 5mM ferrozine. After 10 minutes at room temperature, the absorbance of the mixture was measured at 562 nm against a blank. EC_{50} value (mg/ml extract), which is the effective concentration at which ferrous ions were chelated by 50% was obtained from the plotted graph of chelating activity against the concentration of honey samples.

2.9 Honey colour analysis

This analysis was carried out in order to assess the influence of honey colour on other parameters studied. Honey samples were 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.

2.10 Statistical analysis

All analyses were performed in triplicates and data was presented as mean standard deviation. Differences in performance between individual/group of honey samples were analyzed using One-way analysis of variance

(ANOVA) determined according to the INSTAT PACKAGE 3.0. The correlation between studied parameters was analysed by MINITAB Statistical software. Differences at $P \leq 0.05$ were considered statistically significant.

3 Results and Discussion

3.1 Phenolic and Flavonoid contents

The antoxidant activity of honey is generally attributed to its phenolic compounds and flavonoids (Beretta *et al.* 2007, van den Berg *et al.* 2008, Khalil *et al.* 2011, Kishore *et al.* 2011). Table 2 presents mean values of phenolic and flavonoid contents of honey samples from Tanzania grouped in three categories namely; processed honey from honeybees, raw honey from honeybees and honey from stingless bees. Phenolic content of processed honeybee honeys ranged from 38 to 618 mgGAE/100g, raw honeybee honeys from 220.4 to 655.6 mgGAE/100g and stingless bee honeys from 660.4 to 1,309.4 mgGAE/100g honey. Six of the thirteen processed honeybee honey samples had very low phenolic content. Significant variations were observed in the phenolic content of different honey samples within the category (one-way ANOVA, $P < 0.001$) and extremely significant ($P < 0.0001$) among the three categories.

Table 2: Phenolic and Flavonoid contents
(Values are means of three different measurements)

	Phenolic content (mgGAE/100g)		**Flavonoid content (mgRE/100g)**
Processed honey from honeybees			
		Mean±SD	Mean±SD
1	3	68.163±3.652	45.244±0.365
2	9	37.970±3.111	35.960±0.346
3	16	118.833±2.220	87.320±0.402
4	17	60.935±2.738	58.370±0.497
5	18	38.205±1.353	35.180±0.269
6	28	42.206±1.312	23.581±0.669
7	52	82.047±3.891	56.500±0.383
8	53	618.138±2.170	61.673±0.566
9	55	451.873±2.974	45.330±0.148
10	56	399.121±1.814`	39.442±0.337
11	58	260.688±1.572	26.752±0.605
12	59	423.085±3.791	42.931±0.521
13	62	238.506±2.958	24.530±0.155
Raw honey from honeybees			
14	23b	547.294±5.383	55.209±0.250
15	51	655.576±3.675	65.507±0.042
16	65	220.381±1.418	22.531±0.832
17	66	225.378±3.552	23.194±0.190
Honey from Stingless bees			
18	19	660.404±3.080	66.507±0.841
19	20	882.516±4.027	88.878±0.228
20	22	705.691±9.673	71. 095±0.401
21	23a	950.222±5.430	94.565±0.463
22	57	1,039.394±3.412	103.749±0.666

Phenolic content of stingless bee honey samples (mean value 847.6 mgGAE/100g) was two times and four times higher than that of raw (412.6 mgGAE/100g) and processed (218.4 mgGAE/100g) honeybee honey samples, respectively. In the overall, phenolic content values for honeybee honeys are comparable with those reported on Nigerian honey by Alisi *et al.* (2012), 106 to 130 mgGAE/100g and Malaysian honey by Khalil *et al.* (2010), 4.6 to 79.6 mgGAE/100g honey. Comparatively lower phenolic content values have been reported on Bangladesh honey, 15.2 to 68.9 mgGAE/g (Islam *et al.* 2012), Cuban honeys, 21.4 to 34.8 mgGAE/100g (Alvarez-Suarez *et al.* 2010) and Slovenian fir and forest honey, 24.1 and 23.4 mgGAE/100g (Bertoncelj *et al.* 2007).

Flavonoid content expressed in mg Quercetin equivalent per 100 g was highest in honey samples from stingless bees (mean value 84.96 mgRE/100g) than in processed honeybee honeys (mean 44.82 mgRE/100g) and raw honeybee honeys (mean 41.60 mgRE/100g). Values for honeybee honeys reported in this study are comparable to those reported by Alisi *et al.* 2012 on Nigerian honeys and Chua *et al.* (2013) on Malaysian

honeys but are much higher than those reported by Lachman *et al.* (2010) on honey from Czech Republic and Moniruzzaman *et al.* (2013) on Malaysian honeys. Like with phenolic content above, significant variations were observed in the flavonoid content of different honey samples within the category (one-way ANOVA, $P \leq 0.001$) and between categories ($P \leq 0.0001$). Similarly, stingless bees honeys had much higher values compared to honeybees honeys. Variation in vegetation and other environmental conditions in different geographic locations from which bees forage could be a contributing factor to the difference observed. In addition, the way honey is prepared by the two different groups of insects, the honey bees and stingless bees could bring about fundamental differences in the contents of their honeys.

3.2 β-Carotene and Lycopene contents

β-Carotene and Lycopene contents have been reported as powerful antioxidants, which play an important role in animals (Rao and Agarwal 2000, Pal *et al.* 2010). In this study, honey samples have been shown to contain appreciable amounts of β-Carotene and lycopenes (Table 3), which are higher than those reported on some mushrooms (Pal *et al* 2010, Muruke 2014). There were significant differences in β-Carotene and lycopene content among the three honey sample categories studied ($P \leq 0.0001$). Nevertheless, β-Carotene did not show significant differences within the honey categories. Honey from stingless bees exhibited higher levels of the two antioxidants than honeybee honeys.

Table 3: β-Carotene and Lycopene contents
(Values are means of three different measurements)

	β-Carotene (mg/100g)		Lycopene content (mg/100g)
Processed honey from honeybees			
		Mean±SD	**Mean±SD**
1	P3 Uvinza	0.034±0.008	0.268±0.046
2	P9 Manyoni	0.126±0.042	0.135±0.028
3	P16 Ruangwa	0.184±0.010	0.144±0.012
4	P17 Mbeya1	0.023±0.010	0.050±0.008
5	P18Dodoma	0.0134±0.009	0.107±0.015
6	P28 Mbeya2	0.110±0.022	0.070±0.007
7	P52 Tabora1	0.129±0.002	0.135±0.034
8	P53 Tabora2	0.015±0.007	0.054±0.007
9	P55 Mugumu	0.019±0.013	0.048±0.013
10	P56 Geita1	0.133±0.011	0.097±0.013
11	P58 Kasulu	0.031±0.011	0.031±0.005
12	P59 Chunya	0.172±0.014	0.116±0.009
13	P62 Kondoa1	0.036±0.023	0.035±0.013
	Raw honey from honey bees		
14	R23b Kondoa2	0.136±0.020	0.109±0.010
15	R51 Tabora3	0.199±0.015	0.127±0.013
16	R65 Kondoa 3	0.014±0.004	0.049±0.007
17	R 66 Tabora4	0.019±0.006	0.034±0.021
	Honey from Stingless bees		
18	S19 Urambo	0.111±0.013	0.116±0.006
19	S20 Kibondo	0.215±0.025	0.255±0.010
20	S22 Biharamulo	0.191±0.005	0.429±0.010
21	S23a Usariver	0.224±0.012	0.233±0.038
22	S57 Geita2	0.207±0.007	0.217±0.034

3.3 Antiradical activity and Vitamin C content

Results on the antiradical activity of honey samples are presented in Table 4. Significant differences were observed in the antiradical activity units (AU_{515}) and number of antiradical activity units in 1 mg of honey (EAU_{515}) between stingless bee honey samples and honeybee honey samples in both raw and processed honeys (one-way ANOVA, $P \leq 0.0001$). The mean values were 5.21 mg/100g for processed honeybee honeys, 6.8 mg/100g for raw honeybee honeys and 10.6 mg/100g for stingless bee honeys. However, there was no significant difference ($P \geq 0.05$) between raw and processed honeybee honeys and within each of the three categories, that is, processed honeybees honey, raw honeybees honey and stingless bee honey samples despite

the fact that these samples originate from different and distant geographical locations within Tanzania. In the literature there was virtually no report on the antiradical activity units and number of antiradical activity units per 1 gram of honey obtained in this study, which could offer plausible comparisons. Nevertheless the values for honey in this study were closely comparable to those reported on plants and mushrooms (Sroka 2006, Muruke 2014).

Table 4: Antiradical activity (AU_{515}) and Vitamin C content
(Values are means of three different measurements)

	Antiradical activity units (AU_{515})		Number of antiradical activity units per mg honey (EAU_{515})	Vitamin C (mg/100g)
	Processed honey from honeybees			
		Mean±SD		Mean±SD
1	P3 Uvinza	0.380±0.032	4.75	20.440±0.864
2	P9 Manyoni	0.324±0.039	4.05	22.177±0.502
3	P16 Ruangwa	0.713±0.010	8.91	63.683±0.520
4	P17 Mbeya1	0.339±0.043	4.24	61.660±0.719
5	P18Dodoma	0.279±0.018	3.49	32.727±0.674
6	P28 Mbeya2	0.355±0.026	4.44	32.163±0.321
7	P52 Tabora1	0.384±0.512	4.80	66.710±0.551
8	P53 Tabora2	0.468±0.029	5.85	52.427±0.871
9	P55 Mugumu	0.287±0.030	3.59	42.433±0.335
10	P56 Geita1	0.520±0.013	6.50	63.190±0.098
11	P58 Kasulu	0.488±0.034	6.10	28.697±0.566
12	P59 Chunya	0.142±0.040	1.78	22.067±0.500
13	P62 Kondoa1	0.737±0.028	9.21	31.380±0.401
	Raw honey from honey bees			
14	R23b Kondoa2	0.867±0.059	10.84	61.557±0.627
15	R51 Tabora3	0.616±0.043	7.70	46.880±0.720
16	R65 Kondoa 3	0.323±0.030	4.04	42.513±0.509
17	R 66 Tabora4	0.378±0.048	4.73	51.457±0.741
	Honey from Stingless bees			
18	S19 Urambo	0.904±0.025	11.30	61.223±0.458
19	S20 Kibondo	0.873±0.082	10.91	64.710±0.594
20	S22 Biharamulo	0.796±0.069	9.95	61.107±0.629
21	S23a Usariver	0.867±0.040	10.84	67.623±0.645
22	S57 Geita2	0.781±0.033	9.76	56.177±0.447

The results on Vitamin C content of various honey samples (Table 4) show significant differences among the 3 honey categories studied ($P \leq 0.0001$) and within each category ($P \leq 0.001$). In processed honey, vitamin C ranged from 20.4 – 66.7 mg/100g honey, with a mean value of 41.5 mg/100g while that of raw honeybees honey ranged from 42.5 – 61.6 mg/100g with mean value of 50.6 mg/100g. Stingless bees honey samples exhibited higher values ranging from 56.2 – 67.6 mg/100g, with a mean value of 62.2 mg/100g. These values are much higher than those reported by Khalil *et al.* (2012) on Algerian honey, Buba *et al.* (2013) on honey samples from Nigeria and Moniruzzaman *et al.* (2013) on Malaysian honeys. However, higher vitamin C content values have been reported by Adefagha and Aboh (2011) on some tropical green leafy vegetables (321.4 – 842.0 mg ascorbic equivalent/100g), Tibuhwa (2012) on termitarian mushrooms (200 – 480 mg/100g); while comparable values have been reported by Mshandete and Cuff (2007) and Muruke (2014) on mushrooms.

3.4 DPPH radical scavenging and Iron Chelating activities

All honey samples showed a pattern of increased radical scavenging ability with increase in honey concentration from 2.5 to 40 mg/ml. Highest DPPH radical scavenging ability was displayed by stingless bee honey samples compared to honeybee honey samples. For example at a concentration of 10 mg/ml honey, DPPH scavenging ability of all 5 stingless bee honeys ranged from 75 – 85% where as only two out of thirteen processed honeybees samples (P16Ruangwa and P62Kondoa1) had scavenging ability of 71% and 73%, respectively while the remaining had less than 50%. Similarly, two of the four raw honeybee honey samples studied (R51 and R23b) showed radical scavenging ability of 59% and 80%, respectively. Radical scavenging ability of honeybee honey samples reported in this study are slightly higher than those reported by Moniruzzaman *et al.* (2013) on Malaysian honeys but comparable to those reported by Alisi *et al.* (2012) on 8 Nigerian honey samples.

The total antioxidant required to decrease the initial DPPH radical concentration by 50 percent (EC_{50} value) followed the above trend, being lowest in all stingless bee honeys (mean value: 4.19 mg/ml) signifying that they display a very high scavenging ability in comparison with raw honeys (mean EC_{50} value: 12.93 mg/ml) and processed honeys (mean EC_{50} value: 18.03 mg/ml) from honeybees (Table 5). Uniquely, two honeybee honey samples namely P62Kondoa1 (processed) and R23bKondoa2 showed very low EC_{50} values (high radical scavenging ability), which compares with those from singles bees group. Comparable EC_{50} values were reported by Chua *et al.* 2013 on Malaysian honey with EC_{50} values ranging from 15.68 to 48.90 mg/ml. Earlier on, Kishore *et al.* 2012 had reported lower EC_{50} values on Malaysian honeys from Tualang and Gelam of 5.8 and 6.7 mg/ml, respectively. It follows that the very high antioxidant levels and activities in stingless bee honeys support the relevance of the traditional practices that attaches high values of stingless bees honey as medicine.

Table 5: PPPH and Iron Chelating ability [EC_{50} (mg/ml)]

S/N	**Sample No.**	**EC_{50} DPPH**	**EC_{50} Iron Chelation**
Processed honey from honeybees			
1	P3 Uvinza	29.00	0.190
2	P9 Manyoni	32.7	0.355
3	P16 Ruangwa	5.25	0.133
4	P17 Mbeya1	31.00	0.280
5	P18Dodoma	21.25	0.165
6	P28 Mbeya2	20.00	0.045
7	P52 Tabora1	16.50	0.145
8	P53 Tabora2	17.00	0.055
9	P55 Mugumu	19.25	0.130
10	P56 Geita1	9.75	0.275
11	P58 Kasulu	13.75	0.115
12	P59 Chunya	14.20	0.060
13	P62 Kondoa1	4.80	0.110
Raw honey from honey bees			
14	R23b Kondoa2	4.25	0.050
15	R51 Tabora3	7.50	0.072
16	R65 Kondoa 3	17.50	0.055
17	R 66 Tabora4	22.50	0.052
Honey from Stingless bees			
18	S19 Urambo	4.30	0.001
19	S20 Kibondo	3.80	0.010
20	S22 Biharamulo	4.50	0.005
21	S23a Usariver	3.75	0.001
22	S57 Geita2	4.60	0.055

Iron chelators are important free radical scavengers which play an important role in detoxifying metal ions and prevent poisoning in our bodies (Pal *et al.* 2010). Iron chelating ability of honey samples reported in this study (Table 5) increased with increase in honey concentration from 0.05 mg/ml 0.4 mg/ml. As with DPPH, iron chelating ability differed significantly among the processed honeybee honeys, raw honeybee honey and stingless bee honey samples. The results obtained demonstrated that honey possesses strong Fe (II) chelating activity, with stingless bee honeys being the strongest iron chelators. At minimal concentration of 0.1mg/ml honey, stingless bee honey chelating ability ranged from 61 - 91%, raw honeybee honeys from 56 – 61% and processed honey bee honeys from 31 – 64%. The EC_{50} values (Table 5) followed the same trend, being lowest in stingless bees honey (mean value: 0.014 mg/ml) followed by raw honeybees honey (mean value: 0.057 mg/ml) and processed honey bees honey (mean value: 0.158 mg/ml). In the overall, the iron chelating ability of stingless bee honeys is by far stronger than those reported in mushrooms by Pal *et al.* (2010) and Muruke (2014), and in plants by Batool *et al.* (2010). Strong radical scavenging and iron chelating abilities of the honey samples may explain their preferred usage traditionally as medicine for wound healing and for treating other diseases.

3.5 Honey colour analysis

A diversity of honey colours was recorded in the samples studied, which ranged from light coloured to dark honeys. The ranking of colour intensity of the honey samples revealed an array of colours of Tanzanian honeys (Table 6). On the average, colours of processed and raw honeybee honeys were lighter than those of stingless bees honey samples with the rank mean values of 3.9, 4.5 and 9.0, respectively. While majority of honeybee

honeys were described as white and light amber, all the stingless bee honeys were dark coloured between light amber and amber. Honey colours are a function of 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 (Meda *et al*. 2005, Eleazu *et al*. 2012, 2013). In a study by Muruke (2014), honey colour and moisture content were reported as the two most important physicochemical parameters that can be used to gauge the quality of honey.

Table 6: Colour analysis of honey samples

S/N	Sample No.	**Colour**	**Colour scale**	**Rank**
Processed honey from honeybees				
1	P3 Uvinza	White	30-55mm	3
2	P9 Manyoni	Extra Light Amber	50-90 mm	5
3	P16 Ruangwa	Light Amber	90-250 mm	9
4	P17 Mbeya1	Extra Light Amber	50-90 mm	5
5	P18Dodoma	Extra White	20-40 mm	2
6	P28 Mbeya2	White	10-30 mm	3
7	P52 Tabora1	White	10-30 mm	3
8	P53 Tabora2	Light Amber	60-120 mm	6
9	P55 Mugumu	Light Amber	90-250 mm	5
10	P56 Geita1	Water White	10-30 mm	1
11	P58 Kasulu	Extra Light Amber	40-70 mm	4
12	P59 Chunya	Extra White	20-40 mm	2
13	P62 Kondoa1	White	10-30mm	3
Raw honey from honey bees				
14	R23b Kondoa2	Light Amber	70-150 mm	7
15	R51 Tabora3	Light Amber	90-250 mm	5
16	R65 Kondoa 3	White	10-30mm	3
17	R 66 Tabora4	White	10-30mm	3
Honey from Stingless bees				
18	S19 Urambo	Light Amber	60-120 mm	6
19	S20 Kibondo	Amber	100-300 mm	10
20	S22 Biharamulo	Light Amber	80-200 mm	8
21	S23a Usariver	Amber	110-400 mm	11
22	S57 Geita2	Light Amber	80-200 mm	8

3.6 Correlation analysis

In this study attempt has been made to correlate honey colours with antioxidant properties reported in the previous sections. Pearson correlation coefficient (r) values between the studied parameters are presented in Table 7. In all categories of honey studied, colour intensity was strongly positively correlated with all the parameters. Of interest to note is the strongest correlation between colour intensity and flavonoid content (r = 0.846, P = 0.000), DPPH scavenging ability and antiradical activity. The results are in conformity with levels and activities of honey antioxidants studied which are always higher in stingless bee honeys. Dark honeys have been associated with high phenolic content and antioxidant activities (Meda *et al.* 2005, Eleazu *et al*. 2012, 2013). Therefore a strong correlation between honey colour and all antioxidants studied makes it a reliable indicator in predicting quality of honey.

Table 7: Pearson Correlation analysis on studied parameters

	Colour intensity	DPPH radical scavenging	Iron Chelation	Vitamin C content	Flavonoid content	Phenolic content	Antiradical activity (AU_{515})	Lycopene content
DPPH radical scavenging	0.721 (0.000)[2]							
Iron Chelation	0.614 (0.002)[1]	0.622 (0.002)						
Vitamin C content	0.573 (0.005)	0.560 (0.007)	0.396 (0.068)					
Flavonoid content	0.846 (0.000)	0.698 (0.000)	0.452 (0.035)	0.652 (0.001)				
Phenolic content	0.670 (0.001)	0.739 (0.000)	0.712 (0.000)	0.490 (0.021)	0.717 (0.000)			
Antiradical activity (AU_{515})	0.740 (0.000)	0.959 (0.000)	0.621 (0.002)	0.609 (0.003)	0.656 (0.001)	0.687 (0.000)		
Lycopene content	0.536 (0.010)	0.495 (0.019)	0.394 (0.070)	0.253 (0.256)	0.605 (0.003)	0.453 (0.034)	0.454 (0.034)	
Carotenoid content	0.539 (0.010)	0.959 (0.000)	0.369 (0.091)	0.370 (0.090)	0.688 (0.000)	0.538 (0.010)	0.506 (0.016)	0.630 (0.002)

Key: 1. P-values (in brackets): Significant correlation when P≤ 0.01, marked in yellow

2. Highly significant when P = 0.000, marked in green

DPPH radical scavenging ability was also positively correlated with all parameters except lycopene content. Very high correlation (r = 0.959, P = 0.000) between DPPH radical scavenging ability and antiradical activity (AU515) is expected because the two parameters are related and measure the same thing. The high correlation between DPPH radical scavenging ability and carotenoids content in honey (r = 0.959, P = 0.000) is worth noting. Colour intensity factor appears to play an important role in these relationships. However, further studies are needed to attest this general conclusion.

4.0 Conclusion

This study reported for the first time the levels and activities of antioxidants in 22 honey samples from Tanzania. In overall, the results reveal that Tanzanian honey samples studied contains high levels of antioxidants, comparable to those reported on honeys from other countries. Consistently darker honeys were found to contain higher levels of antioxidants studied than lighter honeys. Very high antioxidant levels and activities of stingless bee honeys support inclusion as medicine in traditional practices. Coincidentally, stingless bee honeys are much darker than honeybee honeys which may be explained by the presence in higher levels of minerals, antioxidants and other useful compounds.

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