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Full Length Research Paper

Nutrient composition, polyphenolic contents and free radical scavenging activities of five honey samples from NRCRI, Umudike and different locations in South Eastern Nigeria

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The dry matter, minerals, crude protein, polyphenolic contents and free radical scavenging activities of honey from Umudike farms (Eastern and Western) and different locations in the South Eastern parts of Nigeria (Nsukka, Amoaba and Ahiaeke) were carried out. Results indicate that except the honey from Nsukka, all other samples had high dry matter contents indicating their moisture contents were low. In addition, all the samples contained significant quantities of minerals and the total amount of minerals that was observed in the honey from Western farm was higher than that of other samples while that of honey from Nsukka, was the least. All the samples contained low quantities of proteins, total flavonoids, flavonols and flavones, but significant quantities of phenols and possessed strong reducing power and inhibitory actions on DPPH (2,2diphenyl-1-picrylhydrazyl radical), indicating their antioxidant potentials. There was a positive correlation between percentage inhibition of DPPH free radical and flavonol (0.641), phenol (0.757) and reducing power (0.889) but negative correlation with flavonoids (-0.681) and flavones (-0.712). Results show that these varieties of honey possess considerable nutritive potentials and free radical scavenging activities. In addition, the study showed that lighter honeys may have higher quantities of minerals than darker honeys. Finally, the honey that was produced from Western farm was the best in terms of inorganic minerals while that produced from Nsukka was the best in terms of total antioxidant activity.

Keywords: Nutrient, varieties, antioxidants, honey.

INTRODUCTION

Honey is a natural substance, produced by honey bees (*Apis mellifera*) which confers on it a high variable sensorial and physicochemical characteristics due to climatic and environmental conditions and diverse origin of plants from which it is harvested (Gheldof & Engeseth, 2002., Turkmen *et al.* 2005). Honeybees produce honey from the nectar of blossoms or from the secretion of living parts of plants (or excretions of plant sucking insects on the living parts of plants), which these honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature (Codex alimentarius,2001). Honey has been credited for many therapeutic purposes such as treatment of colds, skin wounds and various gastrointestinal diseases (The National Honey Board, 2003). It is therefore, not surprising to see indigent Nigerians who cannot afford the cost of proper medication, taking large amounts of honey for various therapeutic purposes.

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These medicinal values of honey have been attributed to its antibacterial and anti-inflammatory properties that could come from its high osmolarity, acidity as well as antioxidant composition (phenols and polyphenols) since most of these ailments come from free radical induced damages (Omafuvbe and Akanbi, 2012).

The processing, handling and storage of honey may influence its composition (Gheldof and Engeseth, 2002., Turkmen *et al.* 2005). Due to its potential and proven positive medicinal properties, honey is particularly recommended for children and sportsmen (Blasa, 2006). Because of that, it is quite important to determine the anti-oxidative potentials of honey bearing in mind that the knowledge of the antioxidant capacity of a plant can give a fair estimate of its therapeutic potentials (Eleazu *et al*., 2011).

The polyphenolic compounds that are present in honey were identified as: phenols, flavonoids and flavonides(Kaškoniene et al., 2009; Viuda-Martos et al., 2008).

In Nigeria, it is possible to have adulterated honeys, being sold commercially as genuine products. It now beholds on individuals to find a way of ascertaining the quality of honey that they buy.

Justification

Although reports on the physico-chemical and medicinal properties of honey from other countries (Singh and Kuar Bath, 1997., Anupama *et al*., 2003., Iurlina and Fritz, 1995) are quite numerous, there's paucity of such information on Nigerian honeys especially those from the South Eastern parts of Nigeria necessitating the need for this research. In addition, the nutritive and antioxidant activities of honey is affected by differences in climatic and geographical locations, underscoring the need for documentation of these parameters in Nigerian honeys.

This study was thus designed with an aim of evaluating the nutritive quality, phenolic contents, flavonoids, flavonols, flavones and antioxidant activities of 5 honey samples from different locations in South Eastern Nigeria.

MATERIALS AND METHODS

Harvesting of honey

Five varieties of honey, collected from the beehives of Umudike farms (Eastern farm- Light brown) and Western farm (Extremely light brown) and different locations in the South Eastern parts of Nigeria (Nsukka-Dark brown), Ahiaeke (Moderately dark brown) and Amoaba (Brown) were used for the study. The matured combs, laden with honey, were harvested into well covered containers.

Extraction procedure

The Crushing method was employed for the extraction of the various honey varieties from their different combs and this method is ideal for processing the bee wax. Each of the honey combs, laden with honey, was broken into trunks and tied up in a cheese cloth which was positioned into a press that was screwed with the aid of a jack to release the honey into a receptacle. The released honey was stored under room temperature (27-30^{o}c) using glass jars. The stored honeys were collected, oven dried at 60^{o}c for 24 hrs before further analysis.

Extract preparation

Six mls each of the different varieties of honey was dissolved with 2mls of methanol, made up to 60mls with water, and left overnight. The mixtures were filtered using Whatmann No 1 filter paper and stored in a refrigerator for the analysis of phenols, flavonoids and reducing power assay.

Nutrient analysis

The atomic absorption spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) was used in the analysis of magnesium and calcium, the flame photometric method was used for analysis of sodium and potassium, the molybdate method (Onwuka, 2005) was used for the analysis of phosphorous, the Kjeldahl method was used for determination of the protein contents while the AOAC method (1990) was used in the analysis of the dry matter and ash contents of the honey varieties.

Phenolic assay

The method of Singleton and Rossi (1965) was used with modifications. Briefly, to 0.1ml each of the extracts of the different honey samples was added 50µl of Folin-ciocalteau reagent and the whole set up was shaken for thorough mixing. After 3mins, 0.3mls of 20% Na_2CO_3 was added to the reaction mixture and the whole setup was shaken and incubated for 15mins at room temperature. One ml of distilled water was added to the reaction mixture and the absorbance was read at 725nm using an UV spectrophotometer (Genesys 10 VIS Thermo Electron Corporation) against the reagent blank. The total phenolic content of the samples was determined using the standard curve of gallic acid at 0.4-2.0 mg/mL concentrations and results were expressed as Mg Gallic Acid Equivalent/100g.

Assay of total flavonoids

The method of Meda *et al*., 2005 was used with modifications. To 0.5ml of the extract were added 0.5ml of methanol, 50µl of 10% $AlCl_3$ (in ethanol), 50µl of 1mol/l of potassium acetate and 1.4mls of water. The mixture was incubated at room temperature for 30mins and the absorbance read using an Ultra-violet spectrophotometer at 420nm against the reagent blank. The same procedure was followed for the Quercetin standard quercetin standard (100mg/ml) which was diluted to the following concentrations: 10, 20, 30, 40 and 50 mg/ml and the amount of flavonoids in the samples was extrapolated from the equation of the standard curve of Quercetin Y = 0.005x + 0.050 (R^2 = 0.421).

Assay of flavonols

The method of Kumaran and Karunakaran (2007) was used with modifications. One ml of honey was dissolved with 1ml of ethanol, made up to100mls with water and left overnight. It was filtered, centrifuged and the supernatant was collected. One ml of each extract + 1ml of 2% AlCl3 (in ethanol) + 1ml of sodium acetate solution (2g in 40mls of water) were thoroughly mixed together and left in a water bath at 20^{o}c for 10 mins. The absorbance was read at 440nm against the reagent blank that contained 1ml of ethanol. The same procedure was followed for the standard quercetin (1mg/ml) which was diluted to the concentrations: 0.2, 0.4, 0.6, 0.8 and 1mg/ml and the amount of flavonols in the sample was extrapolated from the equation of the curve Y = 0.223x + 0.017.

Determination of flavones

The total amounts of flavones in all the varieties of honey investigated was determined by the difference between the total flavonoids contents and the total flavonol contents.

2,2-Diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay

The method of Blois (1985) was used with modifications. A measured amount (0.5ml) each of the samples was dissolved in 200mls of methanol to give a concentration of 2.5mg/ml and the mixture was filtered with Whatmann No 1 filter paper. Then, 0.1, 0.2, 0.3, 0.4 and 0.5mls each of the filtrates were further diluted with methanol to give final concentrations of 125, 250,375, 500 and 625 µg/ml respectively. Finally, 0.1ml of 0.3mM DPPH in methanol was added to each of the reaction mixtures and the whole setup was well shaken and left in the dark for 30mins before the absorbance was read spectrophotometrically at 517nm against the reagent blank that contained 1ml of methanol. The same procedure was followed for standard quercetin (2.5mg/ml in methanol) which was diluted to the concentrations: 125, 250, 375, 500 and 625µg/ml respectively. The percentage scavenging activity was calculated as:

% Scavenging activity = [(Absorbance of control – Absorbance of sample) / Absorbance of control] x 100

Reducing power assay

The method of Pulido *et al.* (2000) was used with modifications. Each of the extracts (1.25, 2.5, 3.75, 5 and 6.25mls) was mixed with 2.5mls of sodium phosphate buffer (0.2m pH 6.6) and 2.5mls of potassium ferri-cyanide (1% in water) in a test tube and reacted for 20mins at 50^{o}C.The mixture was cooled using crushed ice and 0.5ml of trichloroacetic acid (10% in water) was added and the set up was centrifuged for 10mins. One ml of the supernatant was collected and an equal volume of water was added + 0.2ml of (0.1% in water) ferric chloride. The absorbance was read at 700nm against the reagent blank. Quercetin was used as the control. Increased absorbance reading indicates increased reducing power.

Statistical analysis

Data was subjected to analysis using the statistical package for social sciences (SPSS), version 15.0. Results are presented as mean ± standard deviations. One way analysis of variance (ANOVA) was used for comparison of the means. Differences between means were considered to be significant at $P < 0.05$ using the Duncan Multiple Range Test

RESULTS AND DISCUSSION

Minerals, as inorganic elements, function as co-factors in enzyme catalyzed reactions, regulation of acid-base balance, nerve conduction, muscle irritability and structural elements of the body.

Determination of the calcium contents of the different varieties of honey was of importance as calcium is an important macronutrient that plays critical roles in skeletal development and neuromuscular functions with its deficiency resulting in muscle spasms, cramps and eventually osteoporosis (Andre et al., 2007). Values obtained for calcium in all the five honey samples investigated as shown in table 1, were higher than reported values for calcium in honey (Annon, 2003a and b). In addition, honey from Western farm which had the lightest color was observed to contain the highest amounts of calcium (2.03 ± 0.04 %) while honey from Nsukka with the darkest color, had the least amount of calcium (1.02±0.02 %) which is a significant finding in this study. Our results conflict with earlier reports of Annon (2001-2004) who stated that minerals are usually present

Table 1. Percentage mineral composition of 5 honey samples from different locations

Locations	Calcium	Magnesium	Phosphorous	Potassium	Sodium
Ahiaeke	1.21±0.02^{c}	0.37±0.00^{b}	1.09±0.01^{e}	0.3±0.00^{a}	0.15±0.05^{b}
Eastern	0.62±0.01^{a}	0.24±0.00^{a}	0.65±0.01^{c}	0.29±0.01^{a}	0.11±0.01^{a}
Amoaba	1.03±0.04^{b}	0.25±0.02^{a}	0.66±0.01^{d}	0.56±0.01^{d}	0.25±0.04^{d}
Western	2.03±0.04^{d}	0.61±0.01^{c}	0.50±0.00^{a}	0.36±0.02b	0.22±0.01^{c}
Nsukka	1.02±0.02^{b}	0.36±0.03^{b}	0.59±0.01^{b}	0.40±0.02^{c}	0.11±0.01^{a}

Values with the same superscripts along each vertical column are not significantly different (P > 0.05). N = 5.

Table 2. Proximate composition of 5 honey samples from different locations (%)

Locations	Dry Matter	Ash	Crude protein
Ahiaeke	77.07±4.09^{c}	9.05±0.07^{c}	2.84±0.00^{e}
Amoaba	75.86±2.28^{ab}	9.35±0.07^{d}	2.63±0.01^{d}
Nsukka	70.91±1.40^{a}	1.68±0.04^{a}	2.21±0.01^{c}
Eastern	76.63±0.62^{ab}	7.96±0.06^{b}	1.76±0.01^{b}
Western	78.75 ±0.78^{c}	10.27±0.04^{e}	1.31±0.00^{a}

Means with different superscripts along each vertical column are significantly different (P < 0.05) (N = 5).

in very small quantities in honeys with potassium being the most abundant and dark honeys being the richest in mineral content. This variation in the calcium contents of the samples compared with that given by Annon, could be attributed to the source of honey in addition to differences in environmental, climatic conditions and geographical locations as these have been reported to affect the mineral contents of foods (Lowell, *et al*., 2012).

Phosphorus helps to control acid-alkaline reaction of the blood (Norman and Joseph, 1996) in addition to playing critical roles in bone formation and neuromuscular function.

Magnesium, sodium and potassium play central roles in the regulation of blood pressure (Karppanen, 1994), control of arterial resistance (Altura and Altura, 1999), regulation of the fluid balance of the body and thus, regulate the cardiac output. A lower than normal dietary intake of magnesium can be a strong risk factor for hypertension, cardiac arrhythmias, ischemic heart disease, atherogenesis and sudden cardiac death (Altura and Altura, 1999). A diet that is low in sodium but high in potassium is encouraged as it is associated with the lowest blood pressure levels and lower disposition to stroke. The magnesium contents of all the five honey samples as obtained in Table 1,were higher than the values given by Annon (2003a and b), indicating the therapeutic potentials of these honey samples as their consumption may prevent the disposition to cardiovascular diseases such as hypertension, ischemic heart disease, etc. Also, the low in sodium: calcium ratio is of advantage as it will lead to a lower disposition to stroke. This is bearing in mind that sodium and potassium are responsible for the maintenance of the electrolyte balance, which the body achieves through the Na+K+ATPase that keeps the intracellular sodium concentration low.

Dry matter relates to cooking qualities of food. Higher dry matter means lower moisture contents and vice versa. With the exception of the honey from Nsukka, the dry matter contents of other honey samples were investigated as observed in Table 2, fell within the range reported by Crane, (1975). The honey from Nsukka as shown in Table 2, contained lower amounts of dry matter than reported and this highlights the need for investigation of the microbial quality of the honey from this source.

Ash is the non-volatile inorganic residue remaining after the ignition of an organic compound. In addition, dry ash reflects the total mineral contents of the samples. The ash contents of all the five honey samples studied ranged from 1.64 to 10.31 with Western farm having the highest ash content (10.27±0.04 %) while Nsukka farm had the least (1.68±0.04 %). The values obtained for all the samples investigated as shown in Table 2, were higher than reported values for ash contents in honey (Anonn, 2003., Anonn, 2001-2004., Crane, 1975). In addition, the study shows that lighter honeys may have higher mineral contents than darker honeys. The reason behind this cannot really be explained but it worth's being noted.

All honey samples studied as shown in Table 2, contained low quantities of proteins with Ahiaeke honey having the highest protein content (2.84±0.00 %) while the honey from Western farm had the least (1.31±0.00%).

The therapeutic effect of various plants extracts in the management of diseases associated with oxidative stress is attributed to their bioactive substances. These bioactive substances which include: alkaloids, tannins, flavonoids, phenols and minerals have been credited with

Table 3. Polyphenolic composition of 5 honey samples from different locations (Mg/100g)

Locations	Flavonoids(mg QE/100g)	Flavonols(mg QE/100g)	Flavones(mg QE/100g)	Phenols(mg GAE/100g)
Western	5.043±0.000^{ab}	1.934±0.016^{a}	3.110±0.020^{a}	75.430±0.140^{a}
Amoaba	5.057±0.020^{b}	2.013±0.032^{ab}	3.040±0.020^{a}	75.710±0.080^{a}
Ahiaeke	5.018±0.000^{a}	2.145±0.064^{b}	2.870±0.090^{a}	79.100±0.140^{b}
Eastern	5.055±0.007^{b}	2.170±0.099^{b}	2.890±0.130^{a}	75.870±0.000^{a}
Nsukka	5.039±0.013^{ab}	2.102±0.094^{ab}	2.940±0.110^{a}	107.000±1.410^{c}

Means with the same superscripts in each vertical column are not significantly different from each other (P > 0.05). QE = Quercetin equivalence; GAE = Gallic acid equivalence.

biological and medicinal values such as: anti-inflammatory, anti-diabetic, antimicrobial, anti-atherosclerotic and anti-carcinogenic properties (Girish *et al*., 2008).

Flavonoids are the largest group of polyphenolic compounds found in higher plants and synthesized from the shikimic acid and malonic acid pathways (Rupasinghe, 2008). Flavonoids possess free radical scavenging activities which prevent oxidative cell damage, have anti-inflammatory, anticancer activities as well as protection against the different levels of carcinogenesis. Honey has been reported to contain flavonoids of approximately 2mg/100g (Ferreres *et al*., 1994). The darker honeys (Nsukka and Ahiaeke) were observed to have lower flavonoid contents than the lighter honeys. The higher amounts of total flavonoids obtained for all the five samples investigated as shown in Table 3 could be attributed to their floral source, environmental factors, seasonal and method of processing as these have been reported to affect the composition of honey (Gheldof *et al*., 2002). The amounts of flavonoids recorded for all the five honey samples investigated explains their biological functions against allergies, ulcers, inflammation, platelet aggregation and these biological functions are some of the uses to which these varieties of honey are being put to.

Flavonols are phytochemical compounds found in high concentrations in a variety of plant-based foods and beverages. Based on their structure (3-hydroxyflavone backbone), flavonols are classified as flavonoids and include the following compounds: quercetin, kaempferol, and myricetin. The flavonoids in honey were identified as flavones and flavonols (Anklam, 1998) and this justified the assay of the flavonol and flavone contents of these varieties of honey. Results obtained in Table 3 show that all the samples contained low quantities of flavonols.

The results of the flavone contents of all the five samples of honey studied, indicate that the honey from Western farm had the highest amounts of flavonoes (3.11±0.02 Mg QE/100g) while the honey from Ahiaeke farm had the least (2.87±0.09 Mg QE/100g).

The presence of phenolic compounds with antioxidant activities is believed to be responsible for the free radical scavenging activities of many medicinal plants. Results obtained in Table 3 indicate that all the five samples of honey studied contained significant amounts of phenol. Nsukka and Ahiaeke, which had the darkest color amongst the varieties of honey studied, recorded the highest phenolic content while Western farm, which had the lightest color, recorded the least phenolic content.

The DPPH assay is a widely accepted method for the determination of the antioxidant activities of various food substances. This is because, DPPH is a stable free radical in methanol or aqueous solution and accepts an electron or hydrogen radical to turn into stable diamagnetic molecule, in addition to producing a strong absorption band at 517 nm in the visible region of the electromagnetic radiation. The color change from purple to yellow is observed as the molar absorptivity of the DPPH reduces from 9660 to 1640 at 517 nm when the odd electron of DPPH becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH (Vinson *et al*., 1995). Thus, the lower the DPPH absorbance and IC_{50} values, the higher the antioxidant activity of the sample analyzed.

As observed in Table 4, the scavenging activities of all the five honey samples analyzed and standard quercetin decreased in the following order: Quercetin > Nsukka honey > Ahiaeke honey > Eastern farm honey > Amoaba honey> Western farm honey. The darker honeys from Nsukka and Ahiaeke had higher scavenging activities on DPPH radical while the lightest honey (Western) had the lowest scavenging activity. Similar reports have been given previously by other authors (Meda *et al*., 2005., Aleksandra, 2010).

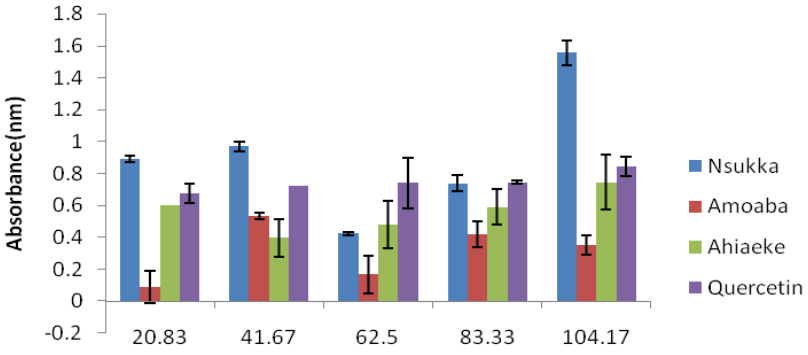
The DPPH assay has the limitation of color interference and sample solubility (Dorman and Hiltunen, 2004., Oboh *et al*., 2008) and this informed the assay of the reducing power of all the samples.

Reducing power assay is a novel method that is used in the assay of the antioxidant activities of various medicinal plants and it employs the reduction of Fe^{3+} to $Fe^{2+.}$ This is because antioxidants are strong reducing agents. The reducing power of all the samples as shown in Figures 1 and 2, indicated that they had a strong antioxidant activity. The reducing power of all the samples and quercetin, decreased in the following order: Nsukka honey > quercetin > Ahiaeke honey > Eastern farm honey > Western farm honey > Amoaba honey. However, quercetin which had a higher free radical scavenging activity than Nsukka honey, was observed to have a lower reducing power while the honey from Amoaba farm was observed to have the least reducing

Table 4. Free Radical Scavenging Activities of 5 samples of Honey from different locations

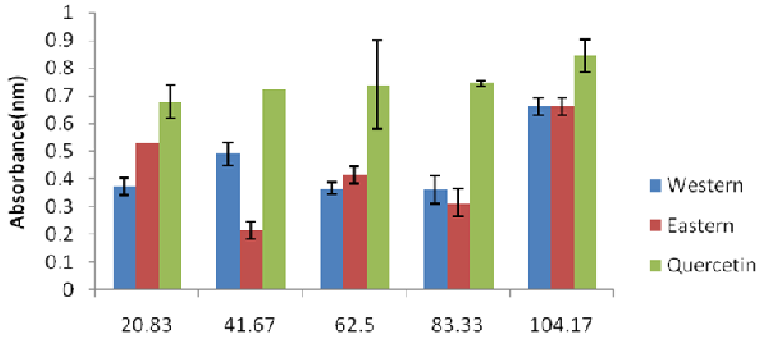
Sample	Mean scavenging activity (%)	IC_{50}(µg/ml)
Nsukka	80.67±12.28	47.48
Ahiaeke	78.12±10.48	54.91
Amoaba	65.33±16.33	157.36
Western	64.60±18.23	188.88
Eastern	69.27±14.43	131.90
Quercetin	83.64±9.65	32.90

(1) Linear equation for Nsukka: Y = 36.60x - 11.36 (R^2 = 0.675); (2) Linear equation for Ahiaeke: Y = 36.30x - 13.15 (R^2 = 0.912); (3) Linear equation for Amoaba: Y = 48.35x -56.22(R^2 = 0.660); (4) Linear equation for Western: Y = 61.41x -89.78 (R^2 = 0.862); (5) Linear equation for Eastern farm: Y = 48.90x - 53.68 (R^2 = 0.872); (6) Linear equation for quercetin: Y = 33.75x -1.211 (R^2 = 0.929)



Concentration(mg/ml)

Figure1. Reductive capacity of Nsukka, Amoaba, Ahiaeke and Quercetin



Concentration(Mg/ml)

Figure 2. Reductive capacity of Western, Eastern farm and Quercetin

power. Despite these, we also recorded a high correlation (r =0.889) between percentage inhibition of DPPH free radical and the reducing power of the samples.

Correlation analysis revealed that there was a positive correlation between percentage inhibition of DPPH radical versus flavonols (r =0.641), higher correlation

Table 5. Pearson correlation between percentage inhibition versus polyphenols and reducing power

	Flavonol	Flavones	Flavonoids	Phenol	Reducing power
Percentage inhibition	0.641	-0.712	-0.681	0.757	*0.889

*Correlation is significant at 0.05 level

between percentage inhibition and phenols (r =0.757), while there was a negative correlation between percentage inhibition versus total flavonoids (r =-0.681) and flavones (r =-0.712) (Table 5). The positive correlation recorded with phenols and flavonols versus percentage inhibition of DPPH radical, suggest that both (phenols and flavonols) could be responsible for the antioxidant activities of honey samples, although the phenols could have a higher contribution. In addition, the negative correlation recorded between percentage inhibition of DPPH radical versus flavones, suggest that the later may not contribute to the antioxidant activities of these varieties of honey. Similar reports of negative correlation between percentage inhibition of DPPH radical and total flavonoids have been given by several authors (Meda *et al*., 2005., Mohammed *et al*., 2010).

CONCLUSION

All the samples analyzed, contained low quantities of proteins, but significant quantities of minerals, indicating their nutritive potentials. The samples had strong reducing power and inhibitory actions on DPPH radical, indicating their antioxidant properties. In addition, the higher correlation that was recorded between percentage inhibition versus phenol and flavonols, compared with total flavonoids suggest to us that the phenolic and flavonol compounds that are present in these varieties of honey may have a higher contribution to their antioxidant activities than their total flavonoid contents. Lighter honeys were observed have higher mineral and flavonoid contents than darker honeys. Finally, the study highlights the effect of location on the composition and antioxidant potentials of honey.

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