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Antioxidant capacity and sugar content of honey from Blue Nile State, Sudan

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Abstract

This study aimed to evaluate antioxidant capacity of honey samples that were collected from Blue Nile State, Sudan by determining total phenolic content (TPC) and total flavonoids content (TFC). Antioxidant activities were evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity and ferric reducing power assay (FRAP). High-Performance Liquid Chromatography (HPLC) was used for the determination of sugars content. The results showed that the highest TPC was (85.7±1 mg GAE /100g Fw), the highest TFC was found to be (55.14 ± 1.09 mg QE /100g Fw) using quercetin equivalent (QE) as standard and the inhibition value of (DPPH) was (52.93 ± 0.44%). The FRAP showed the highest value of (281 µM TE/100g Fw), also the results indicated that the honey contained fructose (38.6 ± 1.8 g/100g - 42.9 ± 1.3 gL100g Fw), and glucose (30.4 ± 0.75 - 31.7 ± 0.68 g/100g Dw). Protein content was found to be ranging between and 0.60% and 1.04%. In conclusion, the results showed that honey is a good source of antioxidants due to the presence of phenolic compounds, flavonoids and carotene. Also, an excellent source of the simple reducing sugars.

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Introduction

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market; serving as foreign exchange earner for many countries also use as a food store during the winter and this has been exploited by humans since ancient times. Honey has been reported to contain about 181 substances such as a complex mixture of sugars, but also small amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals (Anklam, 1998). Honey bees is also, considered to be an important part of traditional medicine (Ferreira *et al.*, 2009). The chemical composition of honey is complex, and according to the previously information it contains about 181 substances, including sugars, proteins moisture, vitamins, minerals, hydroxymethylfurfural (HMF), enzymes, flavonoids, phenolic acids, volatile compounds etc. (Busserolles *et al*., 2002, Alvarez-Suarez *et al*., 2010) However, the main constituents of honey are moisture, glucose, fructose and sucrose. The antioxidant properties of honey are well known because it contains a number of compounds such as flavonoids, phenolic acids, proteins, amino acids, ascorbic acid, HMF, and some enzymes (Dafalla *et al.*, 2014). Reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are known to play important roles in oxidative damage ,these ROS are considered to be important causative factors in the development of diseases such as cardiovascular diseases, cancer, liver disease, inflammatory diseases (Prior and Cao, 2000). Several studies have confirmed that natural antioxidants can effectively prevent and cure oxidative stress-related diseases (Vitaglione *et al*., 2005). The aim of this study was evaluated the phenolic and flavonoid contents and antioxidant properties of the honey samples those were collected from two locations in the Blue Nile State, Sudan.

Materials and methods

Honey samples were collected from two regions in the Blue Nile State, Sudan. All chemicals used in the analysis were of analytical and/or HPLC grade.

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Determination of total phenolic content

Total phenolic content (TPC) was determined according to the Folin–Ciocalteu colorimetric method using Gallic acid as standard. 20 µl of prepared ethanolic honey extracts (mg/ml) were mixed with 400 µl of 0.5 N Folin-Ciocalteu reagents and 680 µL of distilled water. Each solution was thoroughly mixed by vortex mixing and incubated for 3 min at ambient temperature, 400 µL of sodium carbonate solution (10%) was added to the each reaction mixture and further incubated for 2h at ambient temperature. The absorbance of the mixtures of each honey sample was measured at 760 nm using a spectrophotometer (Schott UVLine 9400, USA). The total phenolic content was determined by comparing with a standard curve prepared using Gallic acid (0.015–0.5 mg/ml) (Figure 1). The results were expressed as mg of Gallic acid equivalents per gram of dry weight of honey samples (Ertürk *et al*., 2014).

Total flavonoids content

The total flavonoid content (TFC) of honey samples was determined based on the method of Isla *et al*. (2011) with some modification. A 5 mL of honey solution (0.1g/ml) was mixed with 5 mL of 2% aluminum chloride ($AlCl_3$). Flavonoids- aluminum complex was formed after 10 min of incubation time at 25°C. The formation of the complex was measured at 430nm by using a UV-Visible spectrophotometer (Schott UVLine 9400, USA). Quercetin (0–100mg/L) was used as a standard chemical for calibration curve preparation (Figure 2). The TFC was reported as the mean value of triplicate assays and expressed as milligram of quercetin equivalent (QE) in a gram of honey (Pontis *et al.* 2014).

Total carotenoid content

Total carotenoid content (TCC) was performed by using the method described by (Boussaid *et al.*, 2014) with slight modification. Briefly, 2 g of each honey sample was added to 10 ml of n-hexane – acetone mixture at a ratio of 6:4. The mixture was centrifuged (600 rpm) for 12 minutes at room temperature and was then filtered through Whatman No. 4 filter paper. After that, the absorbance of the filtrate was taken at 450 nm in comparison to a blank one using a spectrophotometer (Schott UVLine 9400, USA). The total carotenoid content of the honey samples was then extrapolated from a curve prepared using the absorbance of the standard β carotene (0.015 – 0.3 µg/ml, $R^2 = 0.982$). Thus, results were expressed as a microgram of β carotene equivalents (mg β carotene /100g Fw honey).

Determination of protein content

Total protein content was determined according to the method proposed by Liberato and coworkers (Liberato *et al*., 2013). To a 0.1 mL solution of protein extract (honey sample 50% w/v) were added 5 mL of Coomassie Brilliant Blue (200 mg of Coomassie Brilliant Blue G-250 dissolved in 100 mL 95% ethanol, and then 200 mL 85% H_3PO_4 were added. The resulting solution was diluted to a final volume of 2 L. The Coomassie Brilliant Blue forms a protein-dye complex. After 2 minutes of incubation, absorbance was measured at 595 nm against an albumin standard solution of bovine serum (5-100 µg/0.1 mL) in 0.15 M NaCl.

Antioxidant activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical

The DPPH solution (20 mg/l) was prepared by dissolving 2 mg of DPPH in methanol (100 mL). A 0.75 ml of the methanolic honey solution at different concentrations, ranging from 20 to 40 mg /ml was added to 1.5 ml of DPPH solution. The absorbance was measured at 517 nm after 15 min of incubation at 25°C. Ascorbic acid was used as positive control. The ability to scavenge the DPPH was calculated using equation [1]:

$$\text{DPPH scavenging activity (\%)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100 \quad [1]$$

Where A is absorbance (Isla, *et al*. 2011).

Ferric reducing antioxidant power (FRAP)

FRAP assay was conducted by the following the method as described by Md. Ibrahim Khalil and co-workers (Ibrahim Khalil *et al*., 2012) with a slight modification. About 300 mM acetate buffer pH 3.6 was prepared by mixing 3.1 g of sodium acetate trihydrate ($CH_3COONa.3H_2O$) with 16 mL of glacial acetic acid. Distill water was then added up to 1.0 L while adjusting the pH to 3.6 and mixed using magnetic stirrer. The solution was kept cool at 4°C and the pH was checked prior to use. Next, 40 mM HCl was then prepared using 1.46 mL of concentrated HCl and make it up to one liter using distilled water. Subsequently, 10 mM (TPTZ) 2,4,6 –tri (2-pyridyl)–s-triazine was prepared by dissolved 0.031 g of TPTZ with 10 ml of 40 mM HCl. The solution was freshly prepared and kept in water bath at 50°C throughout the analysis. Finally, ferric chloride solution was also freshly prepared by dissolved 0.054 g of iron (III) chloride hexahydride ($FeCl_3.6H_2O$) with10 mL distill water. The fresh working solution of FRAP reagent was prepared by mixing 200 mL of 300 mM acetate

Table 1. Total antioxidant capacity, total phenolic content, protein, carotene, and sugar of natural honey samples*

Total of Protein, Carotene, and sugar in honey		
Type of Analysis	**Honey samples**	
	Sample 1	**Sample 2**
DPPH %	52.93 ± 0.4	49.67 ± 0.8
FRAP mg/100g Fw	281.08 ± 1.4	273.33 ± 1.4
TPC mg/100g Fw	85.77 ± 1.0	86.57± 0.8
TFC mg/100g Fw	55.14 ± 1.09	53.24 ± 1.81
Total of Protein, Carotene, and sugar in honey		
Protein mg/100g Dw	2.35 ± 1.31	3.10 ± 1.24
Carotene mg/100g Fw	6.72 ± 0.01	7.31 ± 0.02
Fructose g/100g Dw	42.9 ± 1.3	40.5 ± 1.4
Glucose g/100g Dw	30.4 ± 0.75	31.7± 0.68

*Results are average of three replicates ± standard deviation

buffer pH 3.6, 20 mL of 10 Mm TPTZ and 20 mL of 20 mM $FeCl_3.6H_2O$. Prior to sample analysis, three mL of FRAP reagent (TPTZ) was taken into a cuvette and absorbance was measured by UV-Vis-spectroscopy (Schott UVLin9400, USA) at 593 nm and considered as blank. Then, 0.4 ml of each diluted solution (0.1g /ml) from the tested sample was mixed with 3 ml of freshly prepared FRAP reagent and the reaction mixtures were incubated at 37°C for 30 min before taking the absorbance at the same wavelength. The change in absorbance was compared with a standard curve (Sochor *et al*., 2010). The standard curve was linear between 100 to 1000 µM Trolox/L. The FRAP values were expressed as µM Trolox equivalent (µM Trolox /100g honey).

Determination of sugars content

High-performance liquid chromatography (HPLC, Shimadzu-Japan) was used for determination of sugars content in the various samples of honey which was operated according to the following conditions: Mobile phase : Acetonitrile 75% , water : 25%, flow rate :1ml/min., detector: refractive index, RID- 10A, stationary phase: Column: shodex Asahipak, NH2P - 50 4E and injection volume: 10µl .In the process, 1 g of sample was taken and diluted with water, the mixture was introduced to ultra some water bath to aid solubility then the sample was filtered through membrane filter 0.45 µm.10 µl of the filtrate was injected into HPLC system (Moniruzzaman *et al*., 2013).

Results and discussion

Table 1 shows the total antioxidant capacity, total phenolic content, protein, carotene, and sugar of natural honey samples. The results of DPPH of the examined honey samples from Sudan were 52.93±0.44% and 49.67±0.8%. Both are higher than that reported for some Bangladesh honey samples (Sulieman *et al.*, 2013).

The result showed that the total phenolic content (TPC) of honey from Sudan determined were 85.77 ± 1.0 and 86.57 ± 0.8 mg GAE /100g Fw, respectively (Table 1). That was different than the amount of TPC of Slovenian honey (24.1 to 23.3) mg of Gallic acid per equivalent 100 g of honey and significantly higher amount compared to that of East Black Sea Region honey, which was found to be in the range of 0.058 to 0.396 mg of Gallic acid per equivalent 100 (Bertoncelj *et al*., 2007). The flavonoids content was 55.14 ± 1.09 mg of quercetin per equivalent 100 g of honey compared with the flavonoids content obtained from Roraima, Brazil honey (9.0 to 48.6 mg) (Moniruzzaman *et al.*, 2013). The regression equation (y = 0.0007 x – 0.0036) and determination coefficient (R^2) of 0.9283 (Figure 2) revealed a good linearity response for the method developed, since the R^2 for standard curve is >0.92 indicating that the calculated line could explain more than 92% of the experimental data.

Antioxidant assay based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) standard curve is shown in Figure 3. Degree of inhibition in (%) was measured using the absorbance at 517nm as a function of the concentration of ascorbic acid. Since the regression equation (y = 6.9317x + 16.117) and determination coefficient (R^2) of 0.9802 (Figure 4) showing a good linearity response for the method developed, since the R^2 for the standard curve is >0.98 indicating that the calculated line could explain more than 98% of the experimental data of the honey samples.

The results also indicated that the honey samples

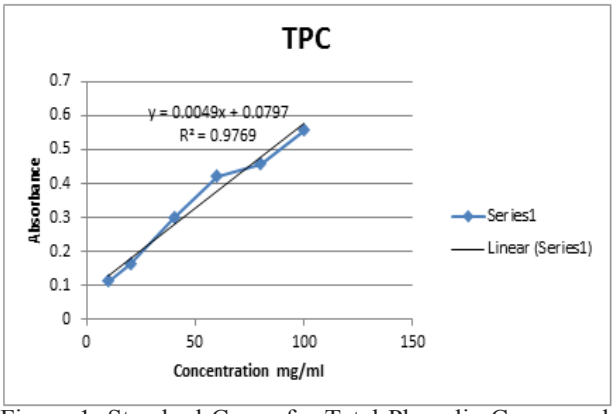

Figure 1. Standard Curve for Total Phenolic Compounds (TPC) As Garlic acid concentration measured at 760 nm using a spectrophotometer

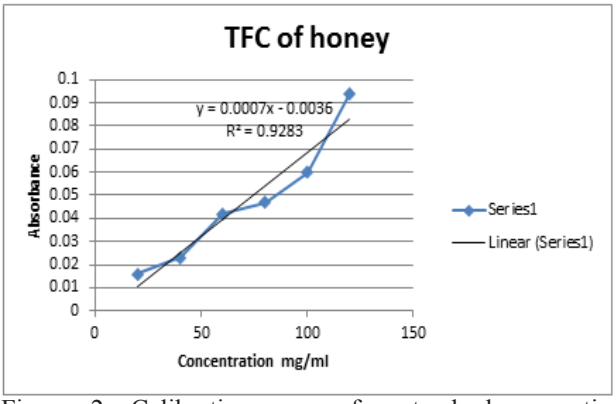

Figure 2. Calibration curve for standard quercetin-aluminum chloride complex spectrophotometrically assayed at 430 nm for the determination of Total Flavonoid Compounds (TFC)

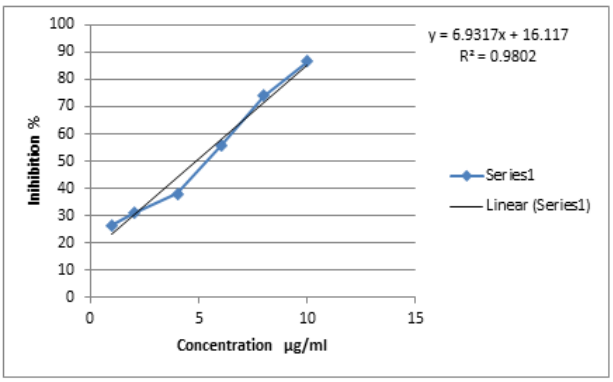

Figure 3. Standard Curve for DPPH: Degree of inhibition of the absorbance at 517nm as a function of the concentration of ascorbic acid

contained fructose (40.5 ± 1.4 and 42.9 ± 1.3) compared to Al-Damazain (a city in the same region) honey (39.01%) Ground honey had fructose (14.8%) and glucose is found to be (30.4 ± 0.75 and 31.7 ± 0.68) in the two samples, respectively, compared to the Mountain honey had (35.0%); the Ground honey had (14.1%) glucose.

Many researchers reported that the protein in honey is low and disagrees. The value of nitrogen content is about 0.04%, which represents about 0.20 to 0.25% protein with the high standard deviation (White Jr and Rudyj, 1978). As reported by the same previous author in another article (White 1978) proline is the dominating amino acid in holey representing more than 50% of the total amino acids in the honey protein.

Conclusion

It could be concluded that natural honey bee collected from the Blue Nile State in Sudan is a good source of antioxidants due to the presence of phenolic compounds, flavonoids and carotene. Also, an excellent source of the simple reducing sugars. Future studies are advised to be conducted to identify and quantify individual flavonoids and phenolic acids of honey samples from the region and may be other places.

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