International Digital Organization for Scientific Research ISSN: 2579-0811

IDOSR JOURNAL OF BIOCHEMISTRY, BIOTECHNOLOGY AND ALLIED FIELDS 1(1): 9-14, 2016.

Total phenolic, Flavonoid Contents and Antioxidant Activities of Honey and Propolis Collected From The Region of Laghouat (South of Algeria)

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ABSTRACT

The aim of this study was to determine total phenolic, flavonoid contents and to evaluate antioxidant activities of two honeys and one propolis samples, collected from the region of Laghouat (South of Algeria). Total phenolic contents were determined by using Folin-Ciocalteu reagent as gallic acid equivalent and flavonoids using $AlCl_3$ method as Rutin equivalent. Antioxidant activities of the honeys and propolis were examined by two different methods, namely scavenging of free radical 2,2-diphenyl-1-picrylhydrazyl and Reducing power. The antioxidant activities were compared with standard antioxidants such as Ascorbic acid, BHT and Trolox. The highest level of phenolic was 2385 mg Gallic acid per 100g sample, the highest level of flavonoid was 379 mg Rutin per 100g sample and the highest protein content was 1177 mg per 100g sample, DPPH (0.026 mg/ml) and TEAC (0.0015) were detected especially the propolis sample, indicating good antioxidant properties. A strong positive correlation was found between phenolics, flavonoids, DPPH and TEAC indicating that in addition to total phenolic, flavonoid and protein concentrations are good indicators of the antioxidant potential of propolis.

Keywords: Honey, Propolis, Phenolic Extracts, Antioxidant Activity, DPPH, FRAP.

INTRODUCTION

Honey and propolis are easily accessible honeybee products which are becoming increasingly popular due to their potential role in contributing to human health (Gómez-Caravaca et al., 2006)[1]. Honey is the organic, natural sugar, produced from the nectar and exudation of plant by honey bees (Sataruba and Subha 2014)[2]. It is mainly composed by sugars (fructose and glucose), water and also contains small amounts of other constituents like proteins, vitamins, minerals, flavonoids, phenolic acids, enzymes, numerous volatile compounds and other natural products (Baroni et al., 2006 ; Kuçuk et al., 2007 ; Khalil et al., 2011)[3],[4],[5]. Propolis is a resinous material that bees collect from the buds and bark of some trees, especially coniferous trees (Debab et al., 2016). The chemical composition of propolis is very complex and depends

on the specificity of the local flora and thus on the geographic and climatic characteristics of this site. This fact results in the striking diversity of propolis chemical composition (Popova et al., 2007)[6]. The antioxidant properties of honey and propolis believed to be at the heart of their polyphenolic compounds. The Algerian 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[7],[8],[9],[10].

The objectives of the present study were to determine the total phenolic content, total flavonoid content and antioxidant activities of the of two honey (*Zizyphys lotus* and *Peganum harmala*) and one propolis samples, collected from the region of Laghouat (South of Algeria). The total phenolic content was estimated by Folin-Ciocalteau method and total flavonoid content was estimated by $AlCl_3$ method respectively. Finally, the antioxidant activities of all samples were evaluated by DPPH assay and Reducing power.

MATERIALS AND METHODS

SAMPLES COLLECTION

Honey and propolis were collected starting in December 2016 until February 2017 from the region of Laghouat (South of Algeria) and were kept in the dark at 4°C until used.

HYDRO-ALCOHOLIC EXTRACT

Propolis (1g) was chopped into small pieces and extracted with 10 ml 80% ethanol and left for 96 h at 37ºC, under agitation (200 rpm) and then filtered and evaporated until a constant volume. The same procedure was repeated with ethanol 50 % for honey.

TOTAL PHENOLIC CONTENT (TPC)

Total phenolics (TPC) of the samples were determined spectrophotometrically by the Folin-Ciocalteu reagent according to the method of **Singleton and Rossi, 1965.** The content of total phenolics was expressed as mg of gallic acid equivalents per 100g (mg GAE/ 100g) of sample. All determinations were carried out in triplicates.

TOTAL FLAVONOID CONTENT (TFC)

Total flavonoids (TFC) of the samples were measured by the aluminum chloride spectrophotometric assay (Ahn et al. 2007)[11]. Total flavonoids content was expressed as mg of rutin equivalents per 100g (mg RE/ 100g) of sample. All determinations were carried out in triplicates.

TOTAL PROTEIN CONTENT

Protein content was determined by spectrometry at 750 nm by the method of Lowry et al., (1951)[12] with bovine serum albumin as the standard. The results were expressed as mg/100g of sample.All determinations were carried out in triplicates.

SCAVENGING OF FREE RADICAL (DPPH) ASSAY

Reduction of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by extracts followed the procedure of Brand-Williams et al. (1995)[13]. The scavenging ability of the samples was expressed as IC_{50} value, which is the effective concentration at which 50% of DPPH radicals were scavenged. The IC_{50} values were calculated from the relationship curve of scavenging activities (%) versus concentrations of respective sample.

REDUCING POWER

The reducing power of the samples was determined by the method of Oyaizu (Oyaizu et al, 1986)[14]. Ascorbic acid was used as a reference standard. The increase in absorbance provided an indication of higher reducing power of the samples being analyzed.

STATISTICAL ANALYSIS

All data were presented as means ± S.D. Statistical analysis of all the assay results was done using the Microsoft Excel program (2007).

RESULTS AND DISCUSSION

Table 1 shows the total polyphenol, flavonoids, protein contents and antioxidant activities of honey and propolis samples. While the amount of phenolic compounds is very low in honey samples (38–86 mg/100g sample), it is very high in propolis sample (2385 mg/100g sample). Ahn et al. (2007) and Kumazawa and Nakayama (2004)[11],[15] reported that the polyphenols content of ethanolic extracts from European and Chinese propolis were approximately 200–300 mg/g samples. Besides, the honey phenolic contents have been found higher than other floral honeys in many investigations[16],[17].

The protein content of honey and propolis samples was between 85 and 133 mg/100g for honey samples and 1177mg/100g for propolis (Table1). Relatively higher protein levels ranging from 370 to 940 mg/g have also been reported in Algerian honey samples (Ouchemoukh *et al.,* 2007)[18], whereas for honey samples from India, the content was reported to be lower (40 mg/g). The protein content can be attributed to the presence of different types of enzymes and other derived products that were introduced by the bees from the flower nectar. Protein levels in honey are dependent on the type of flora on which the bees forage[19].

The antioxidant activities of the honey and propolis samples were examined by comparing them with the known antioxidants (Ascorbic acid, BHT and Trolox) by employing the following two complementary in vitro assays: Reducing power and DPPH radical scavenging. Ascorbic acid has been used as a natural antioxidant and BHT and Trolox as artificial standard antioxidants. The propolis extracts had stronger antioxidant activity than the same floral honey extracts.

DPPH scavenging is widely used to test the free radical-scavenging activity of several natural products (Ahn et al. 2007)[11]. DPPH is a stable free radical and any molecule that can donate an electron or hydrogen to $DPPH^{\cdot}$ can react with it and bleach the DPPH· absorption at 517 nm (Huang et al. 2005)[20]. There is a reverse correlation between IC_{50}

values and DPPH scavenging activity. The DPPH radical-scavenging activity of the samples is presented in Table 1.The radical-scavenging activities of the samples and the standards were found to be in the order of Ascorbic acid > BHT > Propolis >Trolox > Honey 2> Honey 1. The better DPPH scavenging activity may be related to the higher phenolic contents. Although the honey and propolis samples may be said to have a superior level of antioxidant activity, it is not convenient to compare our individual results with literature data due to the lack of standardization in the methods.

FRAP is a widely used method for antioxidant determination and has been used for the assessment of the antioxidant and reducing power of honey (Aljadi and Kamaruddin 2004)[15]. The FRAP assay gives a direct estimation of the antioxidants or reductants present in a sample based on its ability to reduce the Fe^{3+}/Fe^{2+} couple. The mean TEAC value of the honey samples was 0.30 The highest was 0.32 (Honey 1), and the lowest was 0.0015 (Propolis) (Table1).

The evidence about the antioxidant activity of honey and propolis and its relationship with total polyphenol content, and especially flavonoid concentration, is numerours. Honey and other bee products, such as royal jelly and propolis may be used as functional foods because of their naturally high antioxidant potential. Apart from sugars, honey contains many minor components with antioxidant activity, among them amino acids and proteins, carotenes, phenolic compounds and flavonoids, ascorbic acid, organic acids, and Maillard reaction products (Al-Mamary et al., 2002; Gheldof et al., 2002)[16],[21]. According to Aljadi and Kamaruddin (2004)[17], the antioxidant capacity of honey and propolis is due to the content of phenolic compounds and flavonoids, and there is a high correlation between them and the antioxidant capacity of honey, although a synergism between several compounds is present (Johnston et al., 2005; Kücük et al., 2007)[22],[23]. Propolis also contains amino acids, phenolic acids, flavonoids, terpenes, steroids, aldehydes, and ketones which account for its antioxidant activity[24].

Table 1 : The total polyphenol, flavonoid and protein contents of honey and propolis samples

	TPC (mg GAE/ 100g sample)	TFC (mg RE/ 100g sample)	Protein (mg/100g)	DPPH IC_{50} (mg/ml)	Reducing power TEAC
Honey 1 (*Z. lotus*)	38±0.009	5±0.003	85±0.11	10.94±1.90	0.32±0.08
Honey 2 (*P.harmala*)	86±0.008	8±0.002	133±0.05	6.60±1.50	0.31±0.06
Propolis	2385±2.90	379±0.54	1177±0.66	0.026±0.001	0.0015±0.0002
Ascorbic acid	-	-	-	0.015±0.001	0.80±0.1
BHT	-	-	-	0.020±0.002	0.62±0.1
Trolox	-	-	-	0.032±0.005	

CONCLUSION

As a conclusion, total phenolic ; flavonoids, protein contents and antioxidant activities of the propolis sample were higher than the same-floral honey samples. Thus, propolis and honeys may protect humans from deleterious oxidative processes as a result of their

antioxidative activity. Because of their high phenolic constituents, they may also possess anticancer activities. The polyphenolic-rich natural products such as honey and propolis can be suggested for regular consumption and use in food industries.

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