Journal of Apitherapy
www.ejmscience.com
DOI: 10.5455/ja.20160507094104

Short Communication

Extraction and analysis of biologically active compounds in different Algerian honeys

Hamza Belkhodja, Asmaa Belmimoun, Boumediene Meddah

Laboratory of Bioconversion, Microbiology Engineering and Health Safety, Faculty SNV, University of Mascara, Algeria.

Address for correspondence:
Hamza Belkhodja,
Laboratory of Bioconversion, Microbiology Engineering and Health Safety, Faculty SNV, University of Mascara, Algeria.
hamzabelkhodja@yahoo.fr

Received: April 26, 2016
Accepted: May 02, 2016
Published: May 07, 2016

ABSTRACT

Aim: The aim of our work is to determine the qualitative and quantitative composition of honey from different regions in Algeria. **Method:** A qualitative and quantitative analysis was carried out on honey polyphenol extract with the characterization of the majors groups and the determination of the amount of total polyphenols. **Results:** The results indicate that the honey polyphenol extracts have different rates of phenolic compounds (Flavonoids, Tannins and Cardenolides). The polyphenol extracts present a concentration of 0.32, 0.4, 0.76 and 0.39 mg GAE/100 g for the extract of Mascara, Oran, Jijel and Tlemcen respectively. **Conclusion:** The different types of tested honey present a variable composition on phenolic compounds which allowed us to determine the different therapeutic properties of honey.

KEY WORDS: Apis mellifera; Honey, Polyphenols; Flavonoids; Tannins; Cardenolides.

INTRODUCTION

Nowadays, honey is one of the last remaining untreated natural foods, minimally affected by industrial technologies and highly varying consumer characteristics. Honey is one of the oldest and traditional sweetening agents for foods and it has still retained a "natural" image [1]. Honey is a sweet and flavorful natural product which has been consumed for its high nutritive value and its contribution in human health [2]. The composition of honey is rather variable and depends primarily on its floral source; however, certain external factors, such as seasonal and environmental factors and processing also play a role. Honey is a supersaturated solution of sugars, of which fructose (38%) and glucose (31%) are the main contributors, with phenolic compounds, minerals, proteins, free amino acids, enzymes, and vitamins acting as minor components [3]. Among minor honey constituents, polyphenols such as flavonoids and phenolic acids may function as natural antioxidants in our diet [4].

Honey contains phenolic compounds, which are recognised as natural antioxidants thus supporting the application of its use in specific health conditions. These compounds can also be used as the indicators in the studies of the floral and geographical origin of honey and propolis. Phenolic acids are important group of compounds with respect to appearance and functional properties. Phenolic compounds occurring in honey have been classified into three groups: flavonoids, cinnamic acids and benzoic acids [5]. The aim of the study was to determine the qualitative and quantitative composition of honey from different regions in Algeria. This work was carried out at the laboratory of the University of Mascara, beginning the month of May 2012.

MATERIALS AND METHODS

Extraction of Phenolic Compounds in Honey

15 g of honey sample was mixed with hydrochloric acid HCl (6M). Then, it hydrolyzed in 100 ml of ethanol-water [70-30; V/V]. The solution was added to 2.5 mL of Sodium metabisulfite. It was followed by stirring for 5 min and filtration. After drying at rotary evaporator at 40 to 70°C, we obtained the viscous residue witch present the phenolic extract [6].

Characterization of Phenolic Compounds

Flavonoids

The method for the characterization of flavonoids was based on Shibata reaction. 2 mL of polyphenol extract 10% is added to 5 mL of hydrochloric alcohol and 2 or 3 chips of magnesium. An orange or purplish pink color appearing that there is flavonoids [7].

Tannins

The reaction was expressed by the action of ferric chloride ($FeCl_3$) 5% on the extract of honey 10%. The appearance of a dark blue color or green indicated the presence of tannins [8].

Cardenolides

1 ml of the extract of honey was mixed with 10 mL ($CHCl_3$ and ethanol). The organic phase was evaporated and the rest

was dissolved in 3 ml of acetic acid. A few drops of $FeCl_3$ and 1 mL of concentrated sulfuric acid were added. The colored solution by a blue green color indicated the presence of cardenolides.

Polyphenols

Folin Ciocalteu was a yellow acid consisting of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It was reduced in the oxidation of phenols in a mixture of the blue oxides of tungsten and molybdenum [7]. The color produced in λ = 760 nm was proportional to the amount of polyphenols present in plant extracts [8].

The dosage of total polyphenols was carried out by using a Shimadzu UV-2401PC UV spectrophotometer with λ = 760 nm. For the determination of total polyphenols, we used the Folin-Ciocalteu. 0.2 ml of the honey polyphenol extract was added to 1 ml of Folin Ciocalteu reagent with Bicarbonate (CO_3Na_2) 4.25%. The control solution was prepared with distilled water. Then, the solutions were brought in a water bath at 70 °C for 20 minutes. After cooling, the optical density was determined at 760 nm compared to the control. The use of a standard range was established under the same conditions with Gallic acid (0 to 1 g / L). The Folin Ciocalteu was expressed in g of Gallic acid / L. To ensure that the results were reliable, the dosage of each phenolic compound was carried out in three trials. Then, we calculated the average optical density measured [8].

RESULTS AND DISCUSSION

Phenolic Compounds

Each sample of honey has recovered a precise amount of polyphenolic extract (table 1).

Characterization of phenolic compounds of honeys allowed detecting the types of polyphenols according to the qualitative properties. From the table below, the four samples of honey present an important characteristic which is the abundance of flavonoids especially in honey of Jijel and Oran explained by the brown color of these extracts. Flavonoids vary quantitatively and qualitatively according to season and stage of plant development [9]. However, tannins and cardenolides are rares. This amount was related to the botanical origin of each variety of honey [10].

Table 1. Color and aspect of honey extracts.

Samples	Colors	Aspect	Yields (%)
Jijel	Brown	Viscous	18,5 ± 2
Oran	Brown	Viscous	20 ± 2,9
Tlemcen	Yellow	Liquid	13 ± 1,5
Mascara	Yellow	Liquid	20,2 ± 2,3

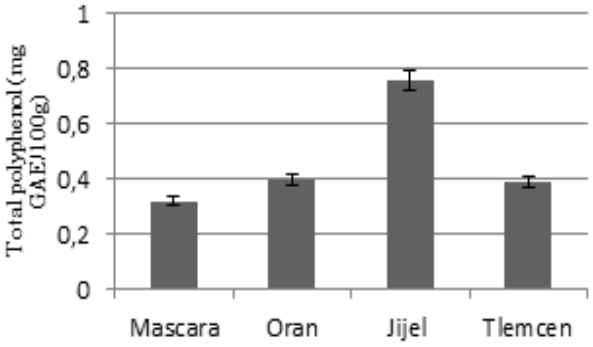

Figure 1. Rate of total polyphenols of different honeys

Polyphenols

After preparing the calibration range of Gallic acid (0.25 g / L, 0.5 g / L, 0.75 g / L, 1g / L), the measurement of the optical density was performed at λ=760 nm. The absorbance obtained was plotted against concentrations; the calibration curve obtained showed the linearity of the detector response as a function of different concentrations. The mean absorbance of phenolic extracts of honey (Jijel, Mascara, Oran, and Tlemcen) was shown in table 3.

The concentration of each extract was 0.32, 0.4, 0.76 and 0.39 mg GAE/100 g for the extract of Mascara, Oran, Jijel and Tlemcen respectively (figure 1). This large difference was probably due to the storage conditions of the original honey and by the different solids content of honey.

According to Harris [11], the different levels of phenolic compounds in the extracts of honey were explained by

Table 2. Phenolic compounds detected in honey extracts.

Compounds	Identification	Jijel	Oran	Mascara	Tlemcen
Flavonoids	Orange / purplish pink	+++	++	+	++
Tanins	Dark blue / green	+	+	-	-
Cardenolides	Blue green	-	+	+	-

Table 3. The optical densities of polyphenolic extracts.

Extract	Jijel	Oran	Mascara	Tlemcen
Optical Density (λ=760 nm)	0.721	0.401	0.286	0.360

the effect of influential factors on the nature of honeys, the main ones: light, precipitation, the rate of HMF and season. In this regard, Macheix *et al* [12] reported that the concentration of polyphenols varied from one variety to another and declined steadily during ripening and storage. This variation was based on several factors including temperature, pH and the quantitative and qualitative changes in phenolic compounds contained in the original plants [13].

CONCLUSION

The results of this study indicate that the honey polyphenol extracts have different rates of phenolic compounds (Flavonoids, Tannins and Cardenolides). It presented a concentration of 0.32, 0.4, 0.76 and 0.39 mg GAE/100 g for the extract of Mascara, Oran, Jijel and Tlemcen respectively. The different types of honey presented a variable composition on phenolic compounds which allowed us to determine the different therapeutic properties of honey.

ACKNOWLEDGEMENTS

We express our sincere gratitude to all staff of the Directorate of Education of Mascara.

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Source of Support: Nil, Conflict of Interest: None declared