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Antioxydant activity of some algerian honey and propolis

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ABSTRACT

Honey and propolis are easily accessible honeybee products which are becoming increasingly popular due to their potential role in contributing to human health. The present study was performed on some honey and propolis samples, harvested in Bejaia (Algeria). Firstly, total phenolic compounds, flavonoids, carotenoids and ascorbic acid were quantified. To maximize the extraction of polyphenols, four solvents (water, 50% ethanol, 85% ethanol and 50% methanol) were tested. After that, the antioxidant capacity of the samples was estimated by determining the reducing power. The total phenolics and flavonoids contents of propolis were significantly ($^*P < 0.05$) different from those of honey. Water permits a better extraction of polyphenols from honey, whereas 85% ethanol was the best solvent of polyphenol extraction from propolis. The reducing power results showed that all the samples exhibit a high antioxidant activity.

1. Introduction

Many degenerative diseases related to aging including cancer, cardiovascular disease, cataracts and diabetes are the result of oxidative damage caused by free radicals (Aljadi and Kamaruddin, 2004). These substances are chemical species containing one or more unpaired electrons; they are highly reactive and unstable. They can be trapped or neutralized by antioxidant substances naturally present in medicinal plants, fruits and vegetables (Al-Mamary et al., 2002; Schramm et al., 2003). Phenolic compounds constitute one of the major groups of compounds known to act as primary antioxidants or free radical terminators and inhibit some molecular targets of proinflammatory mediators in inflammatory responses (Fawole et al., 2009).

Since honey and propolis are hive products made from plants, it is quite normal that they contain these antioxidant substances. Indeed, therapeutic virtues of these products are mainly due to their antibacterial and antioxidant activity (Aljadi and Kamaruddin, 2004).

Honey is a natural substance produced by honeybees (*Apis mellifera*) from the nectar of blossoms and secretions of plants. It is known to have both enzymatic and non-enzymatic antioxidant activities (Codex Alimentarius, 2001). Honey is a highly supersaturated solution of a complex mixture of sugars, it also contains small amount of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic compounds, and enzymes (catalase, peroxides, glucose oxydase) (Blasa et al., 2006). Depending on the geographical and climatic conditions, different types of honey contain a wide range of phytochemicals including polyphenols and phenolic acids which act as antioxidants (Jaganathan and Mandal, 2009). Recent studies on honeys indicated that the biological actions of honey can be ascribed to its polyphenolic contents, which are elucidated by its antioxidant, anti-inflammatory, anti-proliferative and antimicrobial actions (Alvarez-Suarez et al., 2013).

Propolis, or bee glue, is animal products which denote a group of resinous substances, gummy and balsamic harvested by honeybees foraging on bark (Jean-Proste and Le Conte, 2005) and buds mainly from various plants such as birch, poplars, oaks, willows, conifers and many others. Propolis is a natural substance produced by bees from resins and gums; it is a mixture of salivary bee secretions and wax (Bankova et al., 2000). Propolis is widely used as a popular remedy in folk medicine and it does have high potential for use in human and veterinary medicine (Bankova et al., 2000). Like honey, propolis chemical variability is due to its plant origin and different geographic locations of the source plants. Kumazawa et al. (2004) have reported that more than 300 compounds including different flavonoids, polyphenolic esters, terpenoids, steroids, amino acids, caffeic acids and their esters, and inorganic compounds have been identified in propolis samples.

The use of analytical methods for the determination of phenolic acids and flavonoids individually or as a group at the same time, has been related to the floral and geographical origins of honey and propolis. A Folin–Ciocalteu reagent was widely used for estimation

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of plant phenols through color changes (Singleton and Rossi, 1965). One of the important functions of antioxidants in the living system is preventing the disturbance and functional loss of biological membranes and enzymes by scavenging the free radicals that otherwise induce oxidation of lipids, proteins, and DNA (Niki, 2011). The antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents (Mihai et al., 2011). To analyze the antioxidant activity of honey and propolis, the analytical methods commonly used refers to the sample's reducing capacity ferric reducing antioxidant power (FRAP) tests as well as antiradical activity with the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assays (Khalil et al., 2011).

The objective of this study is to assess the antioxidant properties of honey and propolis extract from different regions of Algeria. The content of polyphenols, flavonoids, ascorbic acid and carotenoids was assayed. The antioxidant effect of these compounds was determined by ferric reducing antioxidant power.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and were supplied from Sigma–Aldrich Química S.A. (Sintra, Portugal) and from Sigma (represented by Algerian Chemical Society, Setif, Algeria).

2.2. Honey and propolis samples

In this study, four samples of honey (H1-4) and three samples of propolis (P1-3) were supplied by companies that use permanent beehives and standardized procedures for collecting apiaries products. These samples were collected in July 2007 in different regions of north Algeria; Adekar (H1), Bejaia (H2, P1, and P3), Oued ghir (H3, P2) and Elkseur (M4). All samples were kept at 0–5 °C and protected from light until analysis.

2.3. Extract preparation

Four solvents (water, 50% ethanol, 85% ethanol, and 50% methanol) were used for the extraction of phenolic compounds from honey and propolis samples. A quantity of 3 g of honey was mixed with 20 ml of solvent. In the other hand, raw materials of propolis samples were frozen at −20 °C overnight and then rapidly ground in a mortar to obtain homogeneous powders. Extracts were obtained by maceration of ground propolis sample (0.5 g) in 20 ml of solvent. All the solutions were protected from light and stirred for 15 h at room temperature (25–30 °C). After centrifugation at 2000 × *g* for 20 min, the supernatant was recovered, filtered paper filters and stored at 6 °C until analysis.

2.4. Total phenolic content

Total phenolic compounds content was assayed using the Folin–Ciocalteu reagent, following Velioglu et al. method (1998). An aliquot (0.1 ml) of extract was added to 1 ml of distilled water and 0.2 ml of the Folin–Ciocalteu reagent (diluted 1/10). The mixture was shaken and allowed to stand for 5 min. After that, 1 ml of sodium carbonate solution (6%) was added to the mixture. The absorbance was read at 760 nm after 30 min of incubation in dark. Total phenolic content were expressed as gallic acid equivalents in mg per 100 g of product (mg GAE/100 g).

2.5. Flavonoid content

The concentration of flavonoids was achieved using the method described by Marquele et al. (2005) by using the aluminum chloride reagent. A volume of 1.5 ml of extract was mixed with 1.5 ml of aluminum chloride solution (10% in methanol). After incubation for 30 min at room temperature, the absorbance was read at 415 nm and concentrations of flavonoids were determined from a calibration curve obtained with chrysin. The results were expressed as chrysin equivalents in mg per 100 g of product (mg CE/100 g).

2.6. Carotenoid content

Determination of the carotenoid content was performed according to the method of Soto-Zamora et al. (2005). An aliquot of 0.1 g (honey) and 7 g (propolis) was added to 10 ml of a mixed solvent (hexane: 2, ethanol: 1, acetone: 1) and 0.5 ml of KOH solution (1 M). After 90 min of stirring, the solution was centrifuged at 2000 × *g* for 15 min at 15 °C. Carotenoid content was determined by measuring the absorbance of hexane extracts at 470 nm. The results were expressed as β-carotene equivalents in mg per 100 g of product (mg β-CE/100 g).

2.7. Ascorbic acid content

Ascorbic acid content was measured according to the method recommended by Toor and Savage (2005). Ascorbic acid was extracted from 2.5 g (honey) and 1 g (propolis) with 10 ml of citric acid (3%). The mixture was then stirred for 15 min and centrifuged at 2000 × *g* for 20 min. 2 ml of supernatant were homogenized with 1 ml of DCIP (2,6-dichloroindophenol). A control was prepared in parallel by replacing the supernatant by citric acid. The absorbance was measured at 515 nm. The results were expressed as percentages of reduction of DCIP by the following formula, and the concentration of vitamin C was expressed as ascorbic acid equivalents in mg per 100 g of product.

$$R(\%) = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{samples}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100$$

where R (%) is the percentage reduction of DCPIP, $\mathrm{Abs}_{\mathrm{control}}$ is the absorbance of the control reaction (a reaction with all the reagents except the sample extract) and $\mathrm{Abs}_{\mathrm{sample}}$ is the absorbance of the sample extract.

2.8. Determination of reducing power

The reducing power was measured according to Beretta et al. (2005). A volume of 0.5 ml of sample was homogenized with 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium ferricyanide [K3Fe (CN) 6] (1%). After incubation in a water bath (50 °C/20 min), 1.25 ml of trichloro acetic acid (10%) was added to the mixture. which was then centrifuged at 2000 rpm for 20 min. The upper layer of the solution (1.25 ml) was mixed with distilled water (1.25 ml). The absorbance was read at 700 nm after the addition of 0.25 ml of iron chloride (1%). The results were expressed as antioxidant (gallic acid) equivalents in mg per 100 g of product (mg AGAE/100 g).

2.9. Statistical analysis

Data were presented as mean ± standard deviation (SD) of three determinations. Statistical analyses were performed using a one way analysis of variance (ANOVA) in the software STATISTICA 5.5. Differences were considered significant at $^{*}P < 0.05$.