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Antioxidant Activity of Algerian Honey and Evaluation of its Inhibitory Action on *Candida albicans* Growth

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Abstract: Honey is known for a long time for its health benefits and biological activities. The aim of this work was to evaluate the antioxidant activity and *Candida albicans* growth inhibitory characteristics of honey of different botanical sources from Algeria. Colour intensity was also classified according to visual analysis and Pfund scale. The results indicated that total phenolic and total flavonoids ranged from 51 to 134 mg gallic acid equivalents (GAE)/100 g honey and from 29.6 to 187.1 mg QE/100 g honey respectively, antiradical activity expressed as % ranged from 61.28 % to 253.47 % and antioxidant activity expressed as FRAP value from 6.95 to 142.43. The honeys' color ranged from light to dark amber. Also, the percentage inhibition (%) for *C. albicans* (5.8 to 96.61). No significant correlation was established between total phenolic contents and antioxidant activity.

Key words: Honey, Algeria; Bioactive properties.

Introduction

Honey is a natural product produced primarily from the nectar secreted by flowering plants gathered and processed by the honeybee (*Apis millifera)*. Honey has been shown to present several biological activities such as: antibacterial [1-2]. antifungal [3], anti-inflammatory [4], antioxidant [5-6], antitumor [7], among others. These bioactivities are closely linked with the chemical composition, particularly with the richness in phenolic compounds. The antioxidant polyphenols ability is linked to several mechanisms, such as free radical-scavenging, hydrogen-donation, single oxygen quenching, metal ion chelation, and action as substrate for superoxide and hydroxyl radicals [8]. It was reported that the composition and antioxidant capacity of honey depend on the floral source used to collect nectar; seasonal and environmental factors, as well as processing may also have an effect on honey composition and antioxidant activity [9-10].

Several previous studies indicated that the source of the plant used in gathering nectar and pollen affected not only the sensory and physicochemical characteristics of the honey, but also the bioactive properties of honey [11-12]. There are different studies about the antifungal activity of honeys came from different origins and their action against yeasts [13-15].

Algeria has a very long tradition of beekeeping. Its favourable climate, good geographical conditions and a variety of botanical species provide great potential for the development of apiculture. Honey production in Algeria has very long traditions dating back to ancient times; however, little information is available on the composition and bioactive properties of honeys from floral sources in Algeria. To date, no data is available on the antioxidant properties of honey samples from western Algeria in the literature. In this study we determined the antioxidant properties and antifungal capacity of honeys from western Algeria.

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Material and methods

Sample collection

A total of 6 honey samples collected from different parts of western Algeria. Honey samples weighing 250 g, packed and sealed in glass bottles, were purchased from a local market, and stored at 4°C. The samples were analysed at the earliest in such a way that none of the samples exceeded the storage period beyond six months. The honey samples were kept at ambient temperature (26 ± 2°C) overnight before the analyses were performed.

Colour

The color intensity of honey samples was measured according to the Pfund classifier [16].

Analysis of antioxidant potentials

Determination of total phenolic contents

The total phenolic content was determined by the Folin-Ciocalteu (F-C) method [17]. Thirty microlitre of honey solution (0.1 g/ml) was mixed with 2.37 ml of milli Q water and 150 µl of 0.2 N Folin-Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min. at ambient temperature. Four hundred and fifty microlitre of sodium carbonate solution (0.2 g/ml) was added to the reaction mixture and further incubated for 2 h at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (0-200 mg/l). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

Determination of total flavonoid contents

The total flavonoid content (TFC) was determined using the aluminium chloride assay according to Amaral *et al* [18]. A 10 µl volume of a 10 % (v/v) honey solution was added to the wells of a 96 well plate; then 30 µl of a 2.5 % sodium nitrite, 20 µl of 2.5% aluminium chloride solutions and then 100 µl of a 2 % sodium hydroxide solution were sequentially added. The samples were mixed well and Abs at 450 nm was measured. TFC was expressed as mg catechin equivalents (CE)/100 g.

Determination of antiradical scavenging activity (DPPH)

The DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging effect (H/e- transferring ability) of honey samples was measured as per the method described by Chen [19]. The DPPH was dissolved in absolute ethanol to a 0.2 mM concentration. A 100 µl aliquot of honey solution (0.1 g/ml) was diluted to 500 µl with 70 % ethanol, and vigorously mixed with 400 µl of DPPH solution by vortexing. The mixture was incubated at room temperature for 15 min. and the absorbance of the solution (T1) was measured at 517 nm. Sample blank (B1) consisted of 600 µl of 70 % ethanol and 400 µl of DPPH whereas DPPH blank (B2) contained 100 µl of honey sample, 500 µl of 70% ethanol and 400 µl of absolute ethanol. The DPPH scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = $1- \{(T_1 - B_2) / B_1\} \times 100$

Where T1, B1, and B2 are the absorbencies of the sample, sample blank and DPPH blank, respectively.

Ferric ion reducing antioxidant power assay (FRAP assay)

The reducing power of the ethanolic extracts of honey was determined according to the method of Oyaizu [20]. A 1 ml aliquot of ethanolic honey extract (10 % v/v) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferri-cyanide (1 %). The mixture was incubated at 50°C for 20 min. After this, 2.5 ml of 10 % trichloroacetic acid was mixed by vortexing. The mixture was centrifuged at 3000 rpm for 10 min. A 2.5 ml aliquot of the supernatant was mixed with an equal amount of milli Q water and 0.5 ml of 0.1 % $FeCl_3$. The absorbance was measured at 700 nm using a spectrophotometer. Precipitation or flocculation was never observed. Assays were performed in triplicate. Ascorbic acid (1.0 mg/ml) was used as a reference standard. The increase in absorbance provided an indication of higher reducing power of the samples being analysed.

Antifungal activity

Honey solutions were prepared in three

concentrations: 100, 50 and 25 % (wt/vol). The samples of each honey (10 g) and sterile water were stored at 37°C for 30 min before mixing, to facilitate homogenization. The potential anti-fungal activity of 6 selected natural honeys, against *Candida albicans* was studied, using the spectrophotometric assay.

Culture media and inoculum

Candida albicans ATCC 10231 was grown on Sabouraud Dextrose Agar (SDA; Merck, Germany), for 24 h at 37°C. Yeast cells from at least five colonies (1 mm diameter) were suspended in 5 mL of sterile saline solution and the resulting yeast suspension was mixed for 15 s in a vortex. Then, the suspensions were adjusted by spectrophotometric method, adding saline solution, to reach the value of 0.5 in the McFarland scale corresponding to a final concentration of $3.0 \pm 2.0 \times 10^6$ cells/ mL.

Spectrophotometric assay

Up to 0.2 ml of the cell suspension was inoculated into 4 ml volume of honey concentration in a test tube, while inoculation of 4 ml volume of nutrient broth with 0.2 ml of the cell suspension, served as control. The optical density was determined in a spectrophotometer at 620 nm, prior to incubation (T_0) and recorded after which, the cultures were incubated for 24 hours in the dark at 37°C with constant shaking, to prevent adherence and clumping. After 24 hours of incubation, the optical densities were again determined (T_{24}) and recorded. The optical density for each replicate at T_0 was subtracted from the optical density for each replicate at T_{24}. The growth inhibition for the test at each dilution was determined, using the formula:

Percentage inhibition = 1 - (OD test/OD control) x 100.

Where the resulting measurement recorded a negative inhibition value (growth promotion), this was reported as stimulation using the formula:

Percentage inhibition = (OD test/OD control) x 100.

The minimum and maximum values were 0 % and 100 %.

Statistical analysis

Results were presented as mean values and standard deviations (mean ± SD). Data were tested using SPSS (version 9.0 for Windows 98, SPSS Inc.). Statistical analysis of the results was based on Kruskal-Wallis test and Pearson correlation analyses. Significant differences were statistically considered at the level of $p < 0.05$ otherwise given.

Results and discussion

The color characteristics are also presented in Figure1. In the tested honey samples colour values were very similar among them and ranged between 71 and 616 mm Pfund. Honey color depends on various factors, such as their mineral content.

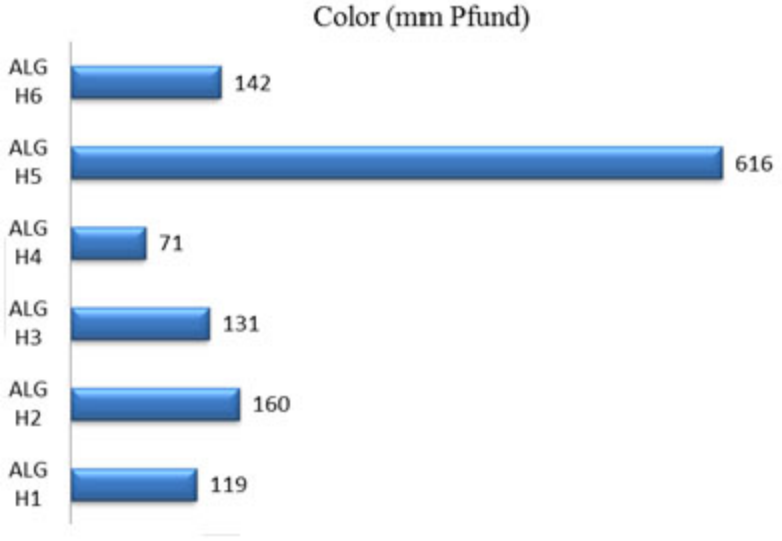

Figure 1. Color characteristics of different Algerian honeys

Total polyphenol and total flavonoid contents

Phenolic compounds are secondary metabolic products of plants that bees transfer to honey. Many authors have studied the phenolic and flavonoid contents of honey to determine their beneficial effect in human health and whether a correlation exists with floral origins [21-22].

The mean total phenolic contents of the 6 examined honey samples was 155.55 mg GAE/kg (Figure 2) .The minimum detected amount was 63.93±0.97 mg GAE/kg, while the maximum one was 97.35±1.78 mg GAE/kg. The total phenolic compounds is sensitive to phenol and polyphenol entities and other electron-donating antioxidants such as ascorbic acid and vitamin E. The total flavonoid contents, it was determined at a mean of 8.41 mg QE/kg, with a minimum of 5.41±0.52 mg QE/kg and a maximum of 11.72±0.29 mg QE/kg (Figure 3). Flavonoids are low-molecular-weight phenolic compounds that affect the aroma and antioxidant properties of honey. The total flavonoid and total phenolic contents vary between different honey samples depending on the geographical location of the different floral sources. Flavonoids are the predominant phenolic class present in honeybee-collected pollen and are best described for their ability to act as antioxidants [23].

Antioxidant activity

In this study, antioxidant properties of monofloral and multifloral honeys from the western region were evaluated using two parameters. Thus, the antioxidant capacities were

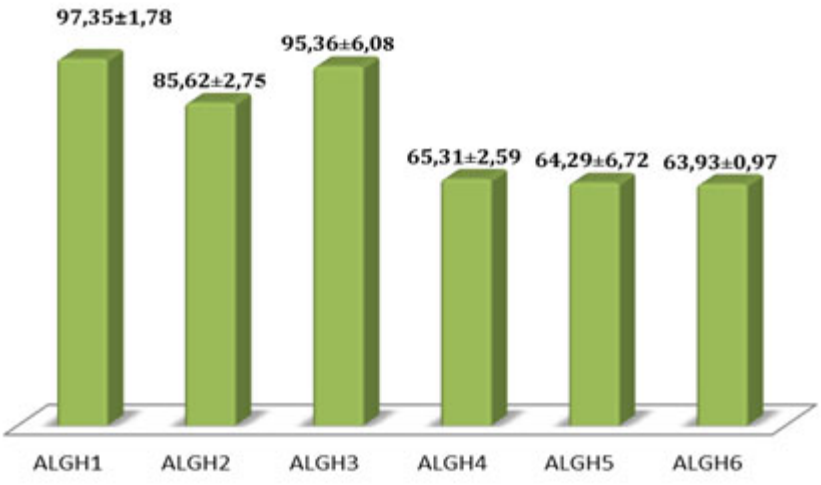

Figure 2. Total phenolic contents of Algerian honey sample

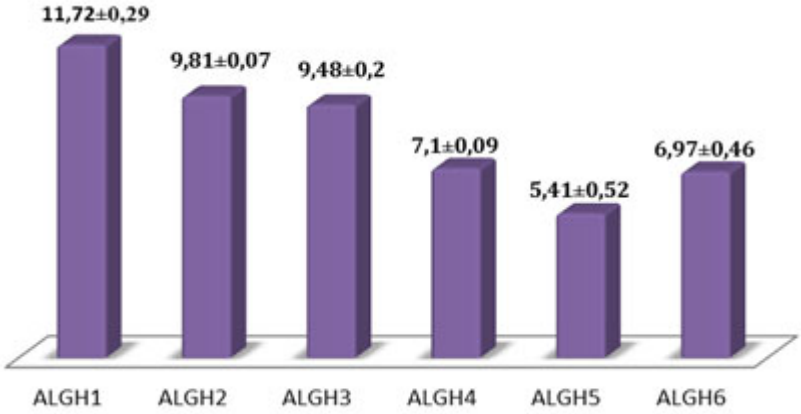

Figure 3. Total flavonoid content of Algerian honey sample

measured by the DPPH radical and FRAP methods.

DPPH Radical Scavenging Activity Assay

The DPPH assay is a widely accepted method for the determination of the antioxidant activities of various food substances. The DPPH radical is one of the few stable organic nitrogen free radicals; it has been widely used to determine the free radical scavenging ability of the various samples [24]. The DPPH radical-scavenging effect of the honey samples ranged from 21.03±3.66 % to 42.65±22.34% (Figure 4) Similar observa-tions have also been observed for Polish honeys [25]. Also, Léon-Ruiz *et al*. [26] reported that the radical scavenging activity of honey samples from Spain was from 3.51-96.29 % in a DPPH reaction system.

Ferric reduction-antioxidant power (FRAP)

The reducing power test, in which the capacity of breaking radical chain reactions reflected, was considered to be a good indicator of antioxidant capacity [27]. The mean FRAP value of Algerian honey samples was 398.58 ± 38.92 µM Fe II/100 g. (Fig. 5). The highest was 838.13 ± 59.66 µmol Fe (II)/100 g) (ALGH2), and the lowest was 45.19±22.19 µmol Fe II/100g (ALGH5) (Figure 5). Gorjanovic *et al*.[28] reported lower FRAP values (between 0.04 and 4.98 mmol TE/g) in Serbian honey samples, as did Šaric *et al*. [29] (18.83 to 319.41 µmol Fe II/100g), which is different

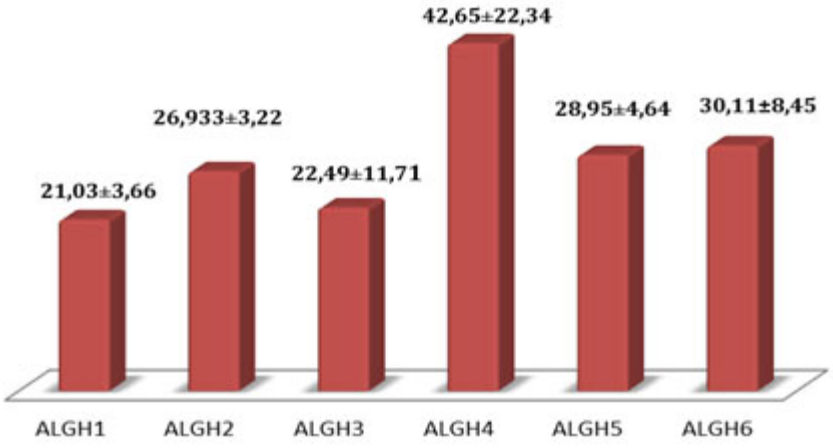

Figure 4. DPPH scavenging effect % for honey samples

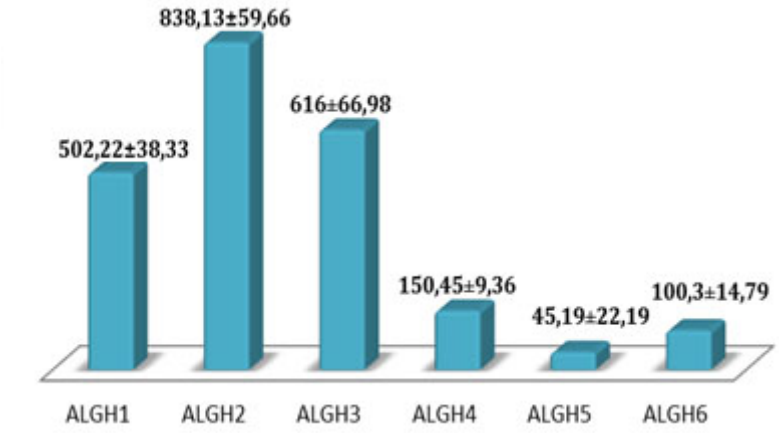

Figure 5. FRAP value (ì M Fe^{2+}) of Algerian honey sample

from our observations data. These variations may be due to the different source of the honey samples collected.

In this study, no significant correlation was found between antioxidant activity and phenolic contents, indicating that phenolics are not the only components responsible for the antioxidant effect of honey, but obviously other factors are involved.

Inhibitory activity evaluation

The results of the antifungal activity (Percentage of inhibition PI %) of the honey samples ALGH1, ALGH2, ALGH3, ALGH4, ALGH5 and ALGH6 are presented in Figure 6. All samples exhibited antifungal capacity against the tested microorganism. The PI % produced by the undiluted honeys against *C. albicans* ranged from 5.8 % to 96.61 %. The PI % of honey with 50 and 25 % concentration varied from (85.59-93-22 %) and (14.4-22.88 %) respectively.

It is well documented in the literature that *C. albicans* has high degree of pathogenicity and responsible for wide range of diseases [30]. The antimicrobial activity of honey against several pathogens and its dependence on the floral origin has been widely reported [31-32]. Several researchers have concluded that the major antimicrobial factors in honey are hydrogen peroxide, catalase, and glucose oxidase [33]**.** Non-peroxide factors also contribute to the antimicrobial properties of honey, including lysozyme and phytochemical compounds.

Ahmed *et al*.[34] also found high antimycotic activity against *C. albicans* with Algerian honey. Irish *et al*. [35] also reported that honey has significant antifungal activity against clinical isolates of *C. albicans*, *C. glabrata* and *C. dubliniensis*. Al-Waili [36] found that honey concentration ranging from 30 % to 50 % inhibited the growth of several pathogenic microorganisms, including *C. albicans.* Khosravi *et al***.** [37] reported that honey had antifungal activity against Candida species such as *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *Candida kefyr*, *C. glabrata*, and *C. dubliniensis*. Honey may inhibit yeast growth for several different reasons. High sugar concentration, low pH, hydrogen peroxide generation, flavonoid extract, proteinaceous compounds, these factors have various toxic effects on microorganisms that directly affect their metabolism and structure [38]. The emergence of resistant *C. albicans* has a major impact on public health and the economy. Drug-resistant *C. albicans* strains have been

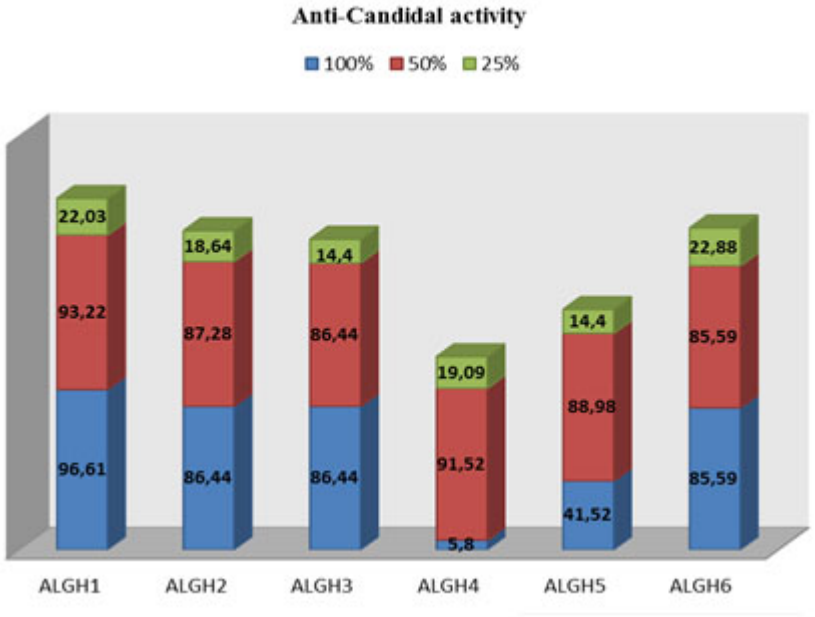

Fig. 6. Percentage of inhibition (PI %) of Algerian honey against *C. albicans* ATCC 10231 at different concentrations

frequently isolated from patients therefore, ; there is an urgent need to develop alternative treatments for Candida infections that are safe, effective and inexpensive. Many antifungals have been isolated from naturally occurring substances over the years. Honey is a honeybee product known for its biological and pharmacological properties for centuries. It has been extensively used in traditional medicine and also, because of it is antimycotic activity, in complementary medicine. In conclusion, the phenolic compounds in honey may render it a good source of antioxidants beside their antiradical and antifungal activity

Conflicts of interest

All authors have none to declare.

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