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Research Article

Antimicrobial Effects of Algerian Honey on Pathogenic Food-Related Bacteria

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Abstract: The purpose of the study was to characterize the physicochemical properties and the antibacterial activity of honey samples collected from different sites of Northeast Algeria. The antibacterial activity of honey against *Bacillus cereus* (*IPA*), *Staphylococcus aureus* (ATCC25923R), *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27893R) was evaluated by the disc diffusion method and determined as an equivalent of the inhibition zones diameters after incubation of the cultures at 37°C for 24 h. The moisture content, pH, Electrical conductivity and Ash were measured and the investigation of the polyphenol and flavonoid contents were done spectrophotometrically in each honey sample. Results showed that Algerian honey inhibited the growth of all examined microorganisms with the highest antimicrobial activity against the Gram positive bacteria. Physicochemical parameters were similar between the honey samples collected from different sites and *polyphenol* and flavonoid contents were variable, depending on the honey samples. The strong antimicrobial activity of Algerian honey may be due to high total phenolic and *flavonoid* contents and this study suggests potential use of honey in foods.

Keywords: Algerian honey, antimicrobial activity, polyphenol and flavonoid contents

INTRODUCTION

Microbial infections are the cause of a large burden of diseases and bacteria are listed in the first position among the common microorganisms responsible for opportunistic diseases. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics (Ahmed *et al*., 2013). Food-borne diseases caused by the consumption of contaminated foods have a wide economic and public health impact worldwide. Many pathogenic microorganisms (*Staphylococcus aureus*, *Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa*) have been reported as the causal agents of food-borne diseases (Erkmen, 2008; El-Bassiony *et al*., 2012). A variety of different chemical and synthetic compounds have been used as antimicrobial agents to inhibit bacteria in foods. There is great interest in controlling the growth or eliminating foodborne pathogens using natural antimicrobials (Taormina *et al*., 2001).

Honey, a natural product formed from nectar by honeybees, has attracted much attention in recent years as an useful ingredient applied in medicine (Mandal *et al*., 2010; DebMandal and Mandal, 2011; Alvarez-Suarez *et al*., 2013). Honey possesses biological properties including antioxidant (Erejuwa *et al*., 2010), fungicidal (Koc *et al*., 2011), anti-inflammatory (Kassim *et al*., 2010), reproductive (Mohamed *et al*., 2012; Zaid *et al*., 2010), hypoglycemic (Erejuwa *et al*., 2010) and antibacterial effects (Tan *et al*., 2009). Numerous reports describe the antibacterial properties of honey but there has been only limited research on antimicrobial activity of Algerian honey (Ouchemoukh *et al*., 2010; Ahmed *et al*., 2011, 2012).

Antimicrobial activity of honey varies greatly with processing and origin, depending on the natural vegetative flowers blooming in different seasons and in different places (Tan *et al*., 2009; Manyi-Loh *et al*., 2011). The healing effect of honey could be due to various physical and chemical properties (Snow and Manley-Harris, 2004). The floral source of honey plays an important role on its biological properties (Bogdanov, 2002; Molan, 2002). An extensive review of the antimicrobial activity of honey showed it to be derived from high sugar content low water content, acidity, the generation of hydrogen peroxide on dilution (Kacaniova *et al*., 2011; Hegazi and Abd Allah, 2012; Mistry and Shah, 2013) and phytochemical components (Snow and Manley-Harris, 2004; Kwakman and Zaat, 2012), aromatic acids and phenolic compounds (Alvarez-Suarez *et al*., 2010).

The present investigation was undertaken to evaluate the antibacterial potential of Algerian honey against a range of food-borne pathogenic bacteria and its physico-chemical properties.

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MATERIALS AND METHODS

Honey samples and preparation: Honey samples were gathered from honeybee colonies of the local strain *Apis mellifera intermissa* in four sites of Annaba, in Northeast Algeria: Seraidi (36°54'39.92"N, 7°37'36.24"E), Chetaibi (36°59'41.47"N,7°19'30.29"E), Berrehal (36°50'16.89"N, 7°26'56.51"E) and El-Bouni (36°49'35.92"N,7°39'51.45"E) and designed as SH, CH, BH and EH respectively. The four sampling sites belong to the same climatic level (sub-humide). Seraidi and Chetaibi are located far from road traffic and presented a rich floral diversity as compared to Berrehal and El-Bouni. All the samples were collected in september 2013 from the nests using sterile syringes, stored in dark glass bottles at room temperature. Initially, the honeys were subjected to sterilization by γ-irradiation at a dose of 25 kGy (Molan and Allen, 1996).

Physicochemical analysis: The determination of moisture (AOAC, 1990) was ascertained by refractometry. All measurements were performed at 20°C, after waiting for 6 min for equilibrium and obtaining the corresponding % moisture from the refractive index of the honey sample by consulting a standard table for the purpose (Zerrouk *et al*., 2011).

The pH value was determined in a solution containing 10 g of honey in 75 mL of distilled water. Determination of pH was done with pH-meter mode (AOAC, 1990).

Honey Electrical conductivity was determined by conductimetric assay from a solution containing 10 g of honey in 75 mL of distilled water (Sancho *et al*., 1992).

Ash content was indirectly determined using the measured Electrical conductivity and applying the following equation:

$$X1 = (X2-0.143) /1.743$$

where,
X1 = Ash value
X2 = Electrical conductivity in mS/cm at 20°C (Piazza *et al*., 1991)

All physicochemical tests were performed in triplicate.

Polyphenols of honey: Total *polyphenol* contents of honey were determined by the Folin-*Ciocalteu* colorimetric method (Singleton *et al*., 1999; Vit *et al*., 2009). Honey sample (0.1 mL) was mixed with 0.5 mL of the Folin-*Ciocalteu* reagent and 0.5 mL of 7.5% (w/v) Na_2CO_3 and the reaction was kept in the dark for 1h, after which the absorbance was read at 725 nm. The total polyphenol content was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid Equivalent (GAE) per 100 g of sample.

Flavonoids of EEP: Total flavonoid contents in honey were determined by the method of Woisky and Salatino (1998) with minor modifications (Vit *et al*., 2009). To 0.1 mL of the honey sample solution (10% w/v), 0.5 mL of 20 mg $AlCl_3$/mL ethanol 96% (v/v) solution was added. After 1h at room temperature, the absorbance was measured at 420 nm. Total flavonoids are calculated as mg quercetin equivalents QE/100 g honey from a calibration curve.

Antimicrobial activity test: Antimicrobial activity of honey samples were investigated by the disc diffusion method (Bauer *et al*., 1966). The antimicrobial screening was performed using Mueller-Hinton agar. The bacteria tested were graciously provided by Pasteur Institute of Algiers (Algeria) and included two Gram-positive bacteria [*Bacillus cereus (IPA)*: *Staphylococcus aureus* (ATCC 25923R)] and two Gram-negative bacteria [*Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC 27893R)] strains. Agar disc diffusion method was employed for the determination of antimicrobial activities of Algerian honey. Suspensions of tested microorganisms (0.5 Mac Farland scale) were spread into solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 20 µL of each honey sample and with distilled water (control) and the inoculated plates were incubated at 37°C for 24 h. Diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate.

Statistical analysis: The results are reported as mean±standard deviation (m±SD). One-way ANOVA and Tukey post hoc multiple comparison tests were used to analyze data. P value less than 0.05 were considered significant.

RESULTS

Physicochemical parameters: The results of the analysis of quality parameters, such as moisture, pH, Electrical conductivity and Ash content are summarized in Table 1. There were no significant differences ($p>0.05$) in all physicochemical parameters between honey samples of different sites. Moisture is a parameter related to the maturity degree of honey and temperature. In the present study, moisture values are between 17.88 and 18.38% and all the Algerian honeys analyzed were found to be acidic in character. Their pH values ranged from 3.77 to 4.6 (Table 1). The Electrical conductivity (mS/cm) in honey samples, varied in the range of 0.29 to 0.41 and the Ash content is generally small and its values were ranged from 0.08 to 0.15%.

Total polyphenol and flavonoid contents: Total polyphenol and flavonoid contents of honey samples from the four sites of Algeria were investigated (Table 2). Results showed that there was a significant difference between the sites for total polyphenol contents ($F = 16.48$, $df = 3$, $p<0.001$) and also for flavonoid contents ($F = 15.42$, $df = 3$, $p<0.001$).

Table 1: Collection sites and physicochemical parameters of honey samples; Data are expressed as means±SD

Honey	Collection site	Moisture content (%)	pH	Electrical conductivity(mS/cm)	Ash (%)
SH	Seraidi	17.88±0.22[a]	3.77±0.05[a]	0.36±0.06[a]	0.12±0.03[a]
CH	Chetaibi	17.94±0.63[a]	3.83±0.05[a]	0.29±0.05[a]	0.08±0.03[a]
BH	Berrehal	18.24±0.60[a]	3.89±0.18[a]	0.38±0.11[a]	0.14±0.06[a]
EH	El-Bouni	18.38±0.51[a]	4.6±0.08[a]	0.41±0.02[a]	0.15±0.01[a]

Means with the same superscript letters within a column are not significantly different (p>0.05)

Table 2: Total polyphenol and flavonoid contents in honey samples

Honey	Total polyphenol* (mg GAE/100 g of honey)	Flavonoid** (mg EQ /100 g of honey)
SH	189.2±16.01[a]	95.94±5.97[a]
CH	131.3±13.64[b]	69.99±7.36[b]
BH	99.03±3.97[b c]	64.47±1.39[bc]
EH	92.14±5.23[c]	44.56±3.88[C]

*: Total polyphenol contents were determined by the Folin-*Ciocalteu* method; Value is mean±standard deviation; **: Flavonoid contents were determined by $AlCl_3$ coloration; Means with different superscript letters within a column are significantly different at p<0.05 (ANOVA followed by a post-hoc Tukey test)

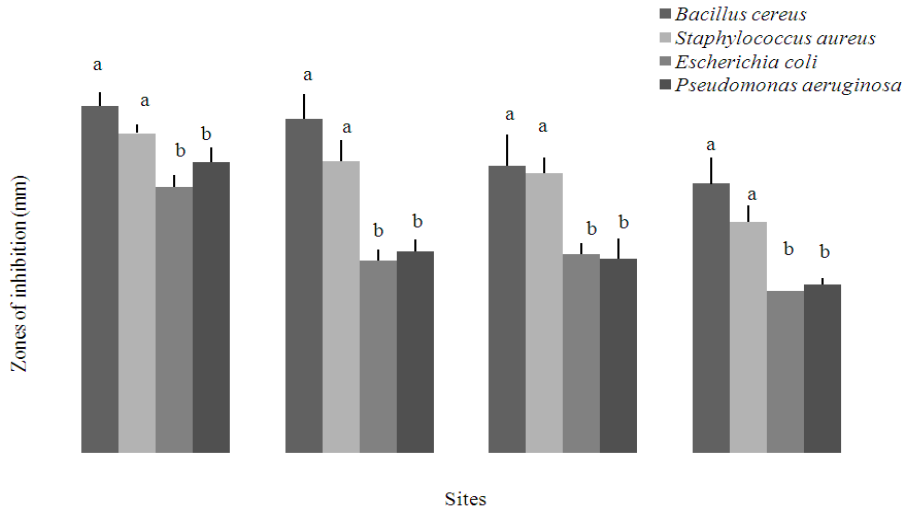

Fig. 1: Diameters of the zones of inhibition (mm) of the growth bacteria tested according to the honey samples

The total polyphenol contents of honey studied were ranged between 92.14 to 189.2 mg GAE/100 g of honey. Honeys also contained flavonoids at levels of 44.56-95.94 mgQE/100 g of honey, with the higher values observed in honey samples from Seraidi and Chetaibi sites.

Antibacterial activity assay: The disc diffusion method was used to determine the inhibition zones of the different honey from the four sites. The two Gram positive and two Gram negative bacteria have been used. According to the results in Fig. 1, different honey samples showed antibacterial activity against all bacteria with the highest antibacterial activity against the Gram positive bacteria.

In our results, the antimicrobial activity of honey from the four sites varies according to the origin of the honey. The largest inhibitory zones of the growth bacteria were noticed for the honey of Seraidi and Chetaibi sites which showed also the highest values of polyphenol and flavonoid contents.

DISCUSSION

In the present study, the analysis of some parameters of Algerian honey, such as moisture, pH, Electrical conductivity and Ash content was evaluated. Moisture values were within the values found in Moroccan honeys by Chakir *et al*. (2011) (14.64-18.59%) and Terrab *et al*. (2003) (14.5-23.6%). The moisture content of honey depends on various factors such as harvesting season, degree of maturity reached in the hive and climatic factors (Finola *et al*., 2007). Also, the moisture content of honey is a highly important factor contributing to its stability against fermentation and granulation during storage (Al *et al*., 2009; Saxena *et al*., 2010).

In general, honey is acidic in nature, irrespective of its variable geographical origin. The pH values of Indian, Morocco, Argentinean honeys and Saudi honeys, have been found to vary between 3.7 to 4.4, 3.91 to 4.93, 3.25 to 3.32 and 3.48 to 6.06, respectively (Azeredo *et al*., 2003; Ouchemoukh *et al*., 2007). This parameter is of great importance, during extraction and

storage of honey, as it influences the texture, stability and shelf life of honey (Terrab *et al*., 2004). The values obtained in this study were similar to those previously reported for other honey samples from India, Brazil, Spain and Turkey, which were reported to have pHs between 3.49 and 4.70 (Azeredo *et al*., 2003; Saxena *et al*., 2010). The pH values of Algerian honey samples previously reported were ranged from 3.49 to 4.43 (Ouchemoukh *et al*., 2007), 3.29 to 4.37 (Chefrour *et al*., 2009) and 3.70 to 4.00 (Khalil *et al*., 2012).

A linear relationship is known to exist between the electrical conductivity and the Ash content. Similarly, a correlation has been found between the electrical conductivity and Ash content for some Algerian honeys (Ouchemoukh *et al*., 2007; Khalil *et al*., 2012). The electrical conductivity shows great variability according to the floral origin and is important for differentiating honeys of different floral origin (Terrab *et al*., 2004). Ash content is a parameter that has been associated with botanical and geographical origins of honey samples. The Ash content in honey is generally small and depends on nectar composition of predominant plants in their formation (Adams *et al*., 2008). Our results are in agreement with those of some authors (Al-Khalifa and Al-Arify, 1999; Sudhanshu *et al*., 2010).

Polyphenols are an important group of compounds which influence the appearance and the functional properties of honey (Khalil *et al*., 2011; Cimpoiu *et al*., 2012).

The total polyphenolic and flavonoid content of honey samples has been determined in different countries. Dong *et al*. (2013) has reported that total phenols in Chinese honey were 9.41 to 102.1 mg GAE/100 g and in Romanian honey samples, values were 23.0-125.0 mg GAE/100 g (Al *et al*., 2009; Bobis *et al*., 2011). Total phenols of Burkina Faso honey were 32.59-114.75 mg GAE/100 g (Meda *et al*., 2005) while in Paraguay, it varied between 125.17 and 176.50 mg GAE/100 g honey (Vit *et al*., 2009). For Indian and Croatian honeys, the phenolic content ranged from 48 to 99 and 31.72 to 80.11 mg GAE/100 g, respectively (Saxena *et al*., 2010; Krpan *et al*., 2009). Total phenols of Algeria were 63.93 - 95.36 mg GAE /100 g (Ahmed *et al*., 2013).

The concentration and type of polyphenolic substances depend on the floral origin of honey and are major factors responsible for biological activities (Bobis *et al*., 2010; Mărghitas *et al*., 2010), including antimicrobial activities (Al-Mamarya *et al*., 2002). Also, flavonoid contents in Romanian honey samples were 38.96-65, 98 mg QE/100 g of honey (Bobis *et al*., 2011). In samples from Venezuela, flavonoid content were ranged from 2.6 to 31.0 mg QE/100 g of honey (Rodríguez-Malaver *et al*., 2009) and Socha *et al*. (2009) has reported that total flavonoid content ranged from 6.9 to 28.5 mg QE/100 g. Total flavonoid contents varied from 41.88 to 211.68 mgQE/kg of Italian honeys (Pichichero *et al*., 2009) and flavonoids varied between 2.52-27.21 mgQE/100 g for honey samples of Nigeria. Flavonoid contents were ranged between 27.07-71.78 mg/kg of honey in sample from Algeria (Khalil *et al*., 2012).

In this study, the antimicrobial activity of honey was investigated and showed antibacterial activity against all bacteria tested with high antimicrobial activity against Gram+bacteria. The antimicrobial activity of the honey from the four sites varies according to the origin of the honey. The honey samples of Seraidi and Chetaibi had strong antimicrobial activity as compared to those from Berrehal and El-Bouni. This strong antimicrobial activity is related with high values of total polyphenol and flavonoid contents in SH and CH. The rich floral diversity of the two sites (Seraidi and Chetaibi) seems to play an important role in polyphenol and flavonoid contents, influencing the honey´s antimicrobial activity. In fact, honey from different regions varied in its ability to inhibit the growth of bacteria suggesting that botanical origin plays an important role in influencing a honey´s antimicrobial activity (Taormina *et al*., 2001; Alvarez-Suarez *et al*., 2010; Silici *et al*., 2010; Liu *et al*., 2013). The total content of phenolic compounds is dependent on the botanical source of honey and its collection region (Socha *et al*., 2009).

Natural products are promising natural antimicrobial agents with potential applications in pharmaceutical or food industries for controlling the pathogenic bacteria. The strong antibacterial effects of Algerian honey against foodborne pathogens such as *B. cereus* and *S. aureus* suggests potential use as a food preservative against pathogenic food-related microorganisms. Other research will be pursued to determine the plant origin of Algerian honey and its qualitative chemical composition.

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