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PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF ALGERIAN HONEYS AND THEIR ANTIBACTERIAL POTENCY AGAINST THREE STRAINS OF *E. COLI**

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Key words: Algerian honey, physicochemical, antioxidant, antibacterial properties, entero-pathogenic, bacteria.

Abstract

The aim of the present study was to investigate the physicochemical, the antioxidant and the antibacterial properties of three Algerian honeys (*Eucalyptus*, Wild carrot and Multifloral). Several physicochemical parameters including moisture content, pH, electrical conductivity (EC), Hydroxymethylfurfural (HMF), invertase number and diastase number were measured. Total phenolic contents, reducing power and ABTS scavenging activity were determined. The agar incorporation method was used to determine the antibacterial activity of honeys against three strains of *E. coli* isolated from diarrhea in young calves. The results showed that moisture contents vary from 15.4% to 18.0%, pH values ranged between 4.19 and 4.34, HMF contents ranged between 11.2 and > 100 mg kg^{-1}, invertase number showed values of 3.2 and 20.7, electrical conductivity ranged between 0.38 and 1.1 mS cm^{-1} and diastase number was detected only in Wild carrot honey (11.3). This honey showed the highest level of polyphenols (850.48 ±167.29 mg gallic acid/kg) and the highest reducing power (0.771 ± 0.141), while *Eucalyptus* honey showed the best ABTS scavenging activity (1.7637 ± 0.8596 mmol Eq Trolox/L^{-1}). A strong

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correlation was found between total phenolic content and reducing power (r value was 0.875 and $P < 0.001$). All honeys were effective against all the tested strains with Minimum Inhibitory Concentrations (MIC) of 7% and 8%. *Eucalyptus* honey was bactericide against all the tested strains. This study demonstrated remarkable variation in antioxidant properties of honey depending on its botanic or geographic origin. It also revealed that Algerian honeys exhibit a strong antibacterial activity.

FIZYKOCHEMICZNE I ANTYOKSYDACYJNE WŁAŚCIWOŚCI ALGIERSKICH MIODÓW ORAZ ICH POTECJAŁ ANTYBAKTERYJNY WOBEC TRZECH SZCZEPÓW *E. COLI*

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Słowa kluczowe: miód algierski, właściwości fizykochemiczne, antyoksydacyjne, antybakteryjne, enteropatogenne szczepy bakterii.

Abstract

Celem badań było określenie fizykochemicznych, antyoksydacyjnych oraz antybakteryjnych właściwości trzech gatunków algierskich miodów (eukaliptusowego, z dzikiej marchwi oraz wielokwiatowego). W miodach oznaczono: zawartość wody, pH, przewodność elektryczną, zawartość hydroksymetylofurfuralu (HMF), aktywność inwertazy, liczbę diastazową, całkowitą zawartość związków polifenolowych oraz aktywność przeciwutleniającą. Określono również właściwości antybakteryjne analizowanych miodów wobec trzech szczepów *E. coli*. Stwierdzono, że zawartość wody w badanych miodach wynosiła od 15.4% do 18.0%, a wartości pH – od 4.19 do 4.34, natomiast zawartość HMF – od 11.2 do > 100 mg kg^{-1}. Liczba inwertazowa wynosiła od 3.2 do 20.7, a przewodność elektryczna od 0.38 do 1.1 mS cm^{-1}, natomiast liczbę diastazową określono jedynie dla miodu z dzikiej marchwi (11.3). Ten gatunek miodu charakteryzował się najwyższą zawartością polifenoli ogółem (średnio 850.48 mg kg^{-1} w przeliczeniu na kwas galusowy) oraz najwyższą siłą redukującą (średnio 0.771). Z kolei miód eukaliptusowy wykazał najwyższą aktywność przeciwrodnikową w układzie ABTS (średnio 1.7637 mmol Eq Trolox L^{-1}). Silne związki korelacyjne stwierdzono między całkowitą zawartością związków fenolowych i siłą redukującą (r = 0.875; $P < 0.001$). Wszystkie badane miody wykazywały aktywność antybakteryjną wobec testowanych szczepów bakterii (MIC 7% i 8%), przy czym miód eukaliptusowy charakteryzował się aktywnością antybakteryjną wobec wszystkich trzech szczepów *E. coli*. Wykazano zróżnicowanie właściwości przeciwutleniające badanych miodów zależne od ich pochodzenia botanicznego i geograficznego. Stwierdzono również, że algierskie miody wykazują silne właciwości antybakteryjne.

Introduction

Antibiotic-resistant bacteria pose a very serious threat to public health. Nowadays, the most critical problem facing modern medicine is the rapid emergence of many strains of antibiotic-resistant bacteria (WANG et al. 2012). In addition, the problem of drug resistance is not restricted to pathogenic bacteria but it also involves the commensal bacterial flora that may become a major reservoir of resistant strains (ERB et al. 2007). A number of epidemiological studies demonstrated that resistance does not only concern hospitals but resistant bacteria continue to occur among various groups in the community including pig-breeding, chicken, and cattle, ... etc.

Diarrhea of neonatal calves is a major problem in breeding farms because they often recorded heavy losses and higher rate of morbidity and mortality during calving period (AKAM et al. 2011). The most commonly pathogens incriminated in neonatal calf scours include viral (*rotavirus* and *coronavirus*), protozoal (*Cryptosporidium parvum*) and bacterial pathogens (enterotoxigenic *Escherichia coli* K99 and *Salmonella* spp.) (IZZO et al. 2011). Among bacteria, enterotoxigenic *Escherichia coli* (ETEC) can cause severe diarrhea in newborn calves via the production of a heat-stable enterotoxin (STa). The most common observed fimbriae on ETEC in calves with diarrhea are K99 (F5) and F41, although, strains with F17 fimbriae have been also isolated (NGUYEN et al. 2011).

The use of honey as a traditional remedy for microbial infections dates back to ancient times (BOUKRÂA and BELLIK 2011). The Holy Hadith records the Muslim prophet Mohammed instructing a man afflicted with diarrhea to take honey. The Roman physician Celsus, (c. 25 AD) used honey as a cure for diarrhea (MOLAN 1999). The healing property of honey is due to its chemical composition (ARVANITOYANNIS et al. 2005). Honey contains abundant amounts of polyphenols and flavonoids which confer it good antimicrobial properties. The antimicrobial action is due to its high osmolarity, low pH, hydrogen peroxide content, and some minor uncharacterized compounds (ALZAHRANI et al. 2012a, BERETTA et al. 2005).

The antibacterial nature of honey is dependent on various factors working either singularly or synergistically, the most salient of which are: hydrogen peroxide (produced by the glucose oxidase added to honey by bees), phenolics and aliphatic hydroxy acids of royal jelly and unsaturated dicarboxylic acids, acidity of honey, and the osmotic pressure exerted by honey (ISODOROV et al. 2015). KWAKMAN et al. (2010) reported that the bactericidal activity of honey is due to its high sugar concentration, H_2O_2, the 1,2-dicarbonyl compound methylglyoxal (MGO), the cationic antimicrobial peptide bee defensin-1 and the low pH.

The objective of the present study is to investigate the physicochemical, the antioxidant properties and the antibacterial activity of three varieties of Algerian honeys from different botanical and geographical origin.

Material and Methods

Honey samples

Three local Algerian honey samples (AH1, AH2 and AH3) were purchased from beekeepers in three different geographic area of Algeria (Tlemcen district, Mostaganem district and Chelef district, respectively), and classified according to their botanical origin using acetolysis according to the Erdtman acetolysis method (ERDTMAN 1969). The studied honeys were *Eucaluptus* honey (AH1), Multifloral honey (AH2) and *Daucus carota* or Wild carrot honey (AH3). The three honey samples used in this study were stored at room temperature (22–24°C) in airtight plastic containers until analysis.

Physicochemical analysis

Physicochemical analysis was realized in CARI ASBL (Beekeeping Center for Research and Information, Louvain-la-Neuve, Belgium). Briefly, moisture in honeys was determined using a refractometer at 20°C. The pH value was measured by a pH meter. Hydroxymethylfurfural (HMF) content was measured according to the method of Winkler and the results were expressed in milligrams per kilogram [mg kg^{-1}]. Invertase number was determined by a spectrophotometer at 400 nm. Diastase number was measured according to the method of Phadebas and electrical conductivity was determined with a conductivity meter, the result was expressed in mS cm^{-1}.

Bacterial analysis

Bacterial strains and inoculum standardization

The antibacterial properties of the honeys samples were tested against *Escherichia coli* F5, *Escherichia coli* F17 and *Escherichia coli* CS31A isolated from neonatal calves with diarrhea. Prior to the experiment the strains were inoculated onto the surface of Mac Conkey agar media; the inoculum suspensions were obtained by taking five colonies from 24 h cul-

tures. The colonies were suspended in 5 mL of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to $1–5 \cdot 10^8$ cfu mL^{-1}).

Minimum Inhibitory Concentration (MIC) measurement

By using the incorporation method, concentrations of honey between 5% and 10% (v/v) were added into Mueller Hinton agar media to test their efficiency against bacteria. The final volume of honey and media in each plate (60 mm) was 5 ml. The plates were inoculated and incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC) was determined by recording the plates with the lowest concentration of honey on which the strain would not grow. Tests were achieved in triplicate. All MIC values are expressed in percentage (v/v).

Antibiotic susceptibility test

Susceptibility to a panel of antimicrobial agents was determined by the standardized disc diffusion assay on Mueller-Hinton agar using commercial antimicrobial susceptibility discs according to the recommendations of the Standardization of Antimicrobial Susceptibility Testing in the Veterinary Medicine at the national level, according to WHO recommendations (MOARD 2008 and 2011). The plates were inoculated and the antibiotic discs were placed on their surface. The tested antibiotics and their corresponding disc concentrations were as follows: Amoxicillin+ acid clavulanic (20/10 µg), Ampicillin (10 µg), Gentamicin (10 µg), Tetracycline (10 µg), Colistin (10 µg), Trimethoprime/sulfamethoxazole (1.25/23.75 µg), Ofloxacine (5 µg) and Cifotaxime (30 µg). The plates were then incubated at 37°C for 24h to 48h. The zone of inhibition was recorded and the data was interpreted using the Standardization of Antimicrobial Susceptibility Testing in the Veterinary Medicine at the national level, according to WHO recommendations (MOARD 2008 and 2011).

To establish whether the antibacterial activity of the tested honey samples was bacteriostatic or bactericidal, the plates where bacterial growth, with the corresponding concentration of honey, was inhibited were scraped by sterile swabs and plated on to nutriment agar. Plates with visible colony growth were considered to correspond to bacteriostatic honey activity while those with no growth were recorded as representing bactericidal honey activity.

Antioxidant activity

Total Phenol Content (TPC)

Total phenolic content was determined using Folin-Ciocalteu method as described by BERETTA et al. (2005). One g of honey was treated with distilled water (10 mL), mixed and filtered using a qualitative filter (filter paper Whatman No. 40, Cambridge, England). An aliquot of this solution (200 µL) was mixed with Folin-Ciocalteu reagent (500 µL, 10%) for 5 min, and then a solution of Na_2CO_3 (1500 µL, 7.5%) was added. All samples were incubated at room temperature in the dark conditions for 30 min, and the absorbance of blue mixtures was recorded at 765 nm using a double beam UV-Visible spectrophotometer (Shimadzu UV-Vis 1202, Japan). Total phenolic content was expressed as mg gallic acid equivalents (GAE)/kg of honey from a calibration curve using the equation: $y = 0.0094\,x + 0.029$ ($R^2 = 0.998$) where y is absorbance and x the concentration.

Reducing power

Ferric reducing power of honey varieties was determined by the method of YEN and DUH (1993). Each sample of honey was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated for 20 min at 50 °C. After incubation, 2.5 ml of trichloroacetic acid (10%) was added to the mixture followed by a centrifugation at 3000 rpm for 10 min. The upper layer (1 ml) was mixed with 1 ml of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance of the obtained solution was measured by spectrophotometer (Shimadzu UV-Vis 1202, Japan) at 700 nm. A higher absorbance indicates a higher reducing power.

Total antioxidant status (ABTS scavenging activity)

Total antioxidant status was measured by using radical cation decolorization assay (RE al. 1999). This assay based on the inhibition by antioxidants of the absorbance of the free radical cation from ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6- sulfonic acid) diammonium salt. ABTS was incubated with potassium persulfate in order to produce the free radical cation ($ABTS^{\circ+}$). In brief, ABTS was dissolved in deionized water to make a 7 mmol L^{-1} concentration solution. $ABTS^{\circ+}$ was produced by mixing ABTS stock solution with 2.45 mmol L^{-1} potassium persulfate (final concentration) and the mixture was allowed to stand in the dark at room temperature for 12–16 h before use. In our study, the $ABTS^{\circ+}$ solution was

diluted with PBS (Phosphate buffer solution), pH 7.4, to an absorbance of 0.70 (± 0.02) at 734 nm. After addition of 2 mL of diluted $ABTS^{\circ+}$ to 20 µL of honey sample in PBS, the absorbance reading was taken exactly 6 min after initial mixing. PBS blank were run in each assay. Trolox was used as standard. Radical scavenging activity was expressed as mmol Eq Trolox L^{-1}.

Statistical analysis

All experiments were carried out in triplicate and the results were expressed as the mean values with standard deviations (SD). The significant differences were obtained by a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test ($P < 0.05$). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P < 0.01$). These correlations were calculated using Systat 12 (version 12.00.08).

Results

The results of the pollen analysis obtained by acetolysis according to the Erdtman acetolysis method (ERDTMAN 1969) for three honey samples are shown in Table 1. The frequency classes of pollen grains are given as predominant pollen (> 45%), secondary pollen (16–45%) and minor pollen (< 15%). The botanical families Myrtaceae, Apiaceae, Cistaceae and Asteraceae were the most frequent in the honey samples.

Table 1

Pollen types present in the honey samples [%]

Samples	Samples predominant pollen > 45%	Secondary pollen 10–45%	Minor pollen < 10%
AH1 : Tlemcen district	Eucalyptus (75%)	–	Acacia, Brassicaceae, Ericaceae, Cistaceae, Lamiaceae, Asteraceae Type Dandelion, Rosaceae, Apiaceae, Asteraceae
AH2 : Mostaganem district	–	Apiaceae (42%), Asteraceae (22%) and Cistaceae (16%)	Chenopodiaceae, Plantaginaceae, Poaceae, Fabaceae, Myrtaceae, Rosaceae, Brassicaceae
AH3 : Chelef district	Wild carrot (74%)	Myrthacées (12%)	Acacia, Chenopodiaceae, Cistaceae, Plantain, Poaceae, Renunculaceae, Asteraceae, Brassicaceae, Rosaceae, Apiaceae

Table 2 summarizes the physicochemical values of the studied honey varieties. AH1 (*Eucalyptus* honey) presented the lowest moisture and HMF contents and the highest values of pH and electrical conductivity. On the contrary, AH2 (Multifloral honey) showed the lowest pH and the highest value of moisture content, HMF and invertase number. While AH3 (Wild carrot honey) presented the lowest invertase number and moisture content and the highest amount of HMF and diastase number.

Table 2

Physicochemical properties of the studied honey

Honey Variety	pH		Moisture content [%]		HMF mg kg^{-1}	
	Value	Difference	Value	Difference	Value	Difference
AH1	4.34	AH1-AH2	15.4	AH1-AH2	11.2	AH1-AH2***
AH2	4.19	AH1-AH3	18.0	AH1-AH3***	18.2	AH1-AH3***
AH3	4.31	AH3-AH2	15.4	AH3-AH2	> 100	AH3-AH2***
Honey variety	Invertase number		Diastase number		Electrical conductivity mS cm^{-1}	
	Value	Difference	Value	Difference	Value	Difference
AH1	16.7	AH1-AH2	ND	AH1-AH2	1.1	AH1-AH2***
AH2	20.7	AH1-AH3	ND	AH1-AH3	0.81	AH1-AH3***
AH3	3.2	AH3-AH2***	11.3	AH3-AH2	0.38	AH3-AH2***

ND – no determined
In each column, difference with "***" indicate significant differences at $P < 0.001$.

Table 3 summarizes the results concerning total phenolic contents, reducing power and total antioxidant status of the tested honeys. AH3 presented the highest phenolic content and ferric reducing capacity, but the lowest scavenging activity against $ABTS^{\bullet+}$ free radical. We noticed that AH1 showed the best scavenging activity against $ABTS^{\bullet+}$ free radical even its total phenolic content and reducing power were the lowest when compared to the other honey samples (AH2 and AH3)

Table 3

Total phenolic contents (TPC), reducing power (PR) and total antioxidant status (TAS) of the tested honeys

Honey Variety	TPC [mg gallic acid kg^{-1}]		Reducing power (PR) [$ABS_{700\ nm}$]		TAS [mmol Eq Trolox L^{-1}]	
	Mean ± SD	Difference	Mean ± SD	Difference	Mean ± SD	Difference
AH1	679.49 ± 90.17	AH1-AH2	0.6819 ± 0.052	AH1-AH2	1.7637 ± 0.8596	AH1-AH2
AH2	736.95 ± 115.09	AH1-AH3*	0.7372 ± 0.081	AH1-AH3	1.3678 ± 0.3369	AH1-AH3*
AH3	850.48 ± 167.29	AH3-AH2	0.771 ± 0.141	AH3-AH2	0.9623 ± 0.2691	AH3-AH2

SD – standard deviation
In each column, difference with "*"indicate significant differences at $P < 0.05$.

A positive and statistically significant correlation ($P < 0.001$) was observed between total phenolic content and reducing power (Table 4).

Table 4

Correlation matrix showing the interrelation between total phenolic content, reducing power and total antioxidant status

Specification	TPC	PR	TAS
TPC	1.000	–	–
PR	0.875***	1.000	–
TAS	-0.112	-0.239	1.000

*** Correlation is significant at the $P < 0.001$.

In terms of the antibiotic susceptibility, all tested strains were susceptible to Cifotaxime but they exhibited resistance to the majority of the tested antibiotics (Table 5).

Table 5

Antibiotic susceptibility of the tested strains

Antibiotic	*E. coli* F5	*E. coli* F17	*E. coli* CS31A
Ampicillin (10 µg)	R	R	R
Amoxicillin + acid clavulanic (20/10 µg)	R	R	R
Gentamicin (10 µg)	R	R	S
Tetracycline (10 µg)	R	R	R
Colistin (10 µg)	R	R	R
Trimethoprime/sulfamethoxazole (1.25/23.75 µg)	R	R	R
Ofloxacine (5 µg)	R	R	R
Cifotaxime (30 µg)	S	S	S

Table 6 summarizes the MIC [%] of the studied honey varieties against three strains of *E. coli* responsible of diarrhea in young calves. The MIC of all honey varieties against the tested strains was 7%, except that AH2 on *E. coli* CS31A which was 8%. The bactericidal or the bacteriostatic effect of honeys varied considerably according to the variety of honey (Table 7). AH1 showed a bactericidal effect against the three strains of bacteria. Whereas AH2 exhibited a bactericidal effect against *E. coli* F5 and *E. coli* F17 and a bacteriostatic effect against *E. coli* CS31A. AH3 exerted a bactericidal effect only on *E. coli* F17 and a bacteriostatic effect against *E. coli* F5 and *E. coli* CS31A.

Table 6

The antibacterial potency of honeys against the tested strains

Honey variety	MIC% of the three varieties against the tested microbes		
	E. coli F5	*E. coli* F17	*E. coli* CS31A
AH1	7	7	7
AH2	7	7	8
AH3	7	7	7

Table 7

Bacteriostatic/bactericidal activity of honeys against the tested strains

Honey variety	Bacteriostatic/bactericidal activity of honeys against the tested microbes		
	E. coli F5	*E. coli* F17	*E. coli* CS31A
AH1	bactericidal	bactericidal	bactericidal
AH2	bactericidal	bactericidal	bacteriostatic
AH3	bacteriostatic	bactericidal	bacteriostatic

Discussion

Honey is rich in properties that result from its chemical composition. The variation of the physicochemical properties of honey depends on the nectar and pollen of the plant source, color, moisture, and protein and minerals contents (WHITE 1978). The pH value has great importance during honey extraction and storage, due to influence on texture, stability and endurance (TERRAB et al. 2002). In our study, all of the investigated honey samples were acid (pH 4.19–4.34). Among all the honey types, Multifloral honey was the most acidic (pH 4.19). Our results are similar to those reported by other researches for Algerian (OUCHEMOUK et al. 2007), Moroccan (NAMAN et al. 2005), Slovak (KASPEROVÁ et al. 2012), Indian (SAXENA et al. 2010), and Portuguese (GOMES et al. 2010) honeys with values ranging between 3.49–4,43, 3.8–4.5, 3.83–4,72, 3.7–4.4 and 3.7–4.3, respectively. The obtained results were slighthly higher to those reported by MONIRUZZAMAN et al. (2013a) and MONIRUZZAMAN et al. (2013b) with values ranging between 3.53–4.03 and 3.83–4.10 and KHALIL et al. (2012) who obtained pH values of 3.7–4.0.

Moisture is a parameter related to the maturity degree of honey and temperature. In the present study, moisture values ranged between 15.4% and 18%. All tested honeys showed moisture contents below 20%, which is the maximum prescribed limit (≤ 20%) by European Community regulations (The Council of the European Union 2002). The moisture contents

of the analyzed honey samples were consistent with those previously reported for Algerian honeys with values ranging between 14.64 to 19.04% (OUCHEMOUK et al. 2007) and 11.59 to 14.13% (KHALIL et al. 2012), Malaysian honey (14.16 to 17.53%) (MONIRUZZAMAN et al. 2013b), Portuguese honey (15.9–17.2%) (GOMES et al. 2010), Moroccan honey (14.3 to 20.2%) (CHAKIR et al. 2016), and Indian honey (17.2–21.6%) (SAXENA et al. 2010). It is worth noting that the moisture content of honey can be affected by climate, season and moisture content of plant source (OUCHEMOUK et al. 2007).

Hydroxymethylfurfural (HMF) content is widely recognized as a parameter of honey samples freshness because it is absent in fresh honeys and tends to increase during processing and/or aging of the product. Several factors influence the levels of HMF such as temperature and time of heating, storage conditions, pH and floral source; therefore HMF provides an indication of overheating and storage in poor conditions (GOMES et al. 2010, MONIRUZZAMAN et al. 2013a). AH3 contained a high level of HMF (>100 mg kg^{-1}), which exceeded the limit established by Codex Alimentarrius (ALINORM 01/25 2000); this honey was probably stored for more than one year. The concentrations of HMF for AH1 and AH2 were 11.2 mg kg^{-1} and 18.2 mg kg^{-1}, respectively; these concentrations were within the recommended range set by the Codex Alimentarrius at 80 mg kg^{-1}. Others studied Algerian honeys and reported higher concentrations of HMF (15.23–24.21 mg kg^{-1}) (KHALIL et al. 2012). Likewise, MAKHLOUFI et al. (2010) recorded a higher concentration of HMF for *Eucalyptus* honey (25.63 mg kg^{-1}) and a low concentration of HMF for Multifloral honey (17.18 mg kg^{-1}).

In the present study, diastase number (DN) was not detected in *Eucalyptus* (AH1) and Multifloral (AH2) honeys but in Wild carrot honey (AH3) it was 11.3. Similar result regarding diastase number of Wild carrot honey (8.3) was reported by ALZAHRANI et al. (2012a). However, MAKHLOUFI et al. (2010) obtained higher levels of DN (18.0 and 15.9 for Multifloral and *Eucalyptus* honeys, respectively). Besides, the results showed an invertase number of 16.7, 20.7 and 3.2 for *Eucalyptus*, Multifloral and Wild carrot honeys, respectively. These results were higher to those obtained by MAKHLOUFI et al. (2010) for *Eucaluptus* honey (9.64) and Multifloral honey (7.9). The possible explanation for variation in the diastase and invertase number could be attributed to variations in geographical origin of honey as well as the time of harvest.

Electrical conductivity (EC) is a key physicochemical parameter for the authentication of unifloral honeys. The EC value depends on the ash and acid content in honey. According to the EU directive (EUROPEAN COM-

MISSION 2002) nectar honey should have a conductivity of no more than 0.8 mS cm^{-1}. Higher values are considered as belonging to honeydew honey or mixtures of honeydew and nectar honey. There are however some exceptions to this limit, *Eucalyptus* honey being one of them.

EC values of all honey samples were 0.38–1.1 mS cm^{-1} (Table 2). Two of honey samples (AH1 and AH2) were upper to the allowed parameters set by Codex Alimentarius. The EC values of some Algerian honeys were reported to be 0.21–1.61 mS cm^{-1} (OUCHEMOUK et al. 2007). Our results were higher to the findings reported by KHALIL et al. (2012) and SAXENA et al. (2010).

Polyphenols are an important group of compounds that were reported to influence not only the appearance but also the functional properties of honey (MONIRUZZAMAN et al. 2013b, CIMPOIU et al. 2013). The concentration of phenolic compounds in honey is highly dependent on its plant source (KHALIL et al. 2012). The total phenolic contents in the studied honeys varied greatly according to the type of honey. Wild carrot honey contained the highest level of polyphenols (850.48 ± 167.92 mg gallic acid/kg) (Table 3). This concentration is higher than that reported by ALZAHRANI et al. (2012a) for the same type of honey (503.09 ± 8.29 mg gallic acid/kg). In general, the levels of total phenolic content of all tested honeys were higher than that reported by KHALIL et al. (2012) for some Algerian honeys with values ranging between 411.10 ± 1.55 to 498.16 ± 1.32 mg gallic acid/kg, but lower than that reported by OUCHEMOUK et al. (2007) with values ranging between 64 mg gallic acid/100 g to 1304 mg gallic acid/100g. Many researchers found that a honey with high level of total phenol content indicates their high antioxidant proprieties.

Reducing power is a widely used method for antioxidant determination of plants and natural products and has been successfully applied for the assessment of the antioxidant capacity of honey. The reducing power 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 (ISLAM et al. 2012). A relatively higher absorbance value corresponds to a more reduction of ferric ions to ferrous ions. The reducing power of honey samples varied from 0.6819 ± 0.052 to 0.771 ± 0.141. The obtained results were greatly higher than those reported by SAXENA et al. (2010) and ALZAHRANI et al. (2012a).

ABTS is a measure of antioxidant activity in contrast to antioxidant concentration, which might include a proportion of biologically inactive antioxidants. ABTS permits the measurement of antioxidant activity of mixtures of substances, hence helping to distinguish between additive and synergistic effects (MONIRUZZAMAN et al. 2012). Despite the fact that hon-

eys showed important phenolic contents, which involves the presence of many hydroxyl groups capable of chelating free radicals, the studied honeys showed low scavenging activity against ABTS free radical. Our results disagree those reported by ALZAHRANI et al. (2012b) and other previous studies dealt on TAS of honey.

Statistical tool is a useful complimentary approach to investigate the relationship between the antioxidant activities of honey and its biochemical composition. From the Table 3, it can be seen that total phenol content was strongly correlated with reducing power (r = 0.885), indicating that polyphenol compounds also contribute to the antioxidant capacity of honey. This statistically significant correlation was in agreement with the previous findings (MONIRUZZAMAN et al. 2013a, ALZAHRANI et al. 2012a, ISLAM et al. 2012).

The important finding from this study is that all tested bacterial strains were inhibited by the three honey samples. While most of the used strains were resistant to the tested antibiotics (Table 3). It could be pointed out that, except for Cifotaxime (30 μg), which was active on the majority of strains, all the other antibiotic did not show an inhibition activity on the most of the isolates. The antibacterial activities of different brands of honeys were proved to be efficient against all strains of *E. coli*. The average MIC value was 7% (v/v). This antibacterial effect is greatly higher than those reported in the literature for *E. coli* strains (JIMENEZ et al. 2016, SHERLOCK et al. 2010, TAN et al. 2009). Regarding the bactericidal and bacteriostatic effect of the different varieties of honey, it was found that *Eucalyptus* honey exhibited bactericidal effect against all the tested strains of *E. coli*. Multifloral honey was bactericide on *E. coli* F5 and *E. coli* F17, while Wild carrot honey was bactericide only on *E. coli* F5.

Conclusions

In conclusion, this study showed that Algerian honeys contained a significant amount of polyphenols that can produce the high antioxidant activity. In addition, these honeys (*Eucalyptus*, Multifloral and Wild carrot) exhibited a potent antibacterial activity against pathogenic *E. coli* causing diarrhea in young calves. These findings may afford useful basis for the alleged therapeutic effects of honey and support its application as an alternative treatment, however, further *in vivo* studies are needed to confirm the findings of the present study.

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