



*Physico- chemical Properties and Antibacterial and Antioxidant Activity of Two Varieties of Honey from Algerian Steppe*

*Cezayir Bozkırından İki Bal Türünün Fiziko-Kimyasal Özellikleri ve Antibakteriyel ve Antioksidan Aktivitesi*

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## Abstract

Honey is a natural sweet substance produced by honey bees from the nectars of plant flowers or tree exudates. Natural honey has been valued in traditional medicine having demonstrated many antioxidant and antibacterial properties. The present study aimed to evaluate physicochemical characteristics and antibacterial and antioxidant activity of two varieties of honey from Algerian steppe. Physicochemical parameters, such as pH, moisture content, electrical conductivity (EC), total acidity, free acidity, ash and HMF were measured. The antibacterial activity was examined against the growth of *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC

## Özet

Bal, bal arıları tarafından bitki çiçeklerinin veya ağaç ekzudalarının nektarlarından üretilen doğal bir tatlı maddedir. Doğal bal, birçok antioksidan ve antibakteriyel özellik sergilediği için geleneksel tıpta değerlidir. Bu çalışma, Cezayir bozkırından iki bal çeşidinin fizikokimyasal özelliklerini ve antibakteriyel ve antioksidan aktivitesini değerlendirmeyi amaçlamıştır. pH, nem içeriği, elektriksel iletkenlik (EC), toplam asitlik, renk yoğunluğu, HMF gibi fizikokimyasal parametreler ölçüldü. Antibakteriyel aktivite, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862'nin büyümesine karşı agar katılma tekniği yöntemi

33862 by using the agar incorporation technique method and the determination of the minimum inhibitory concentration (MIC). The antioxidant activity was assessed by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP). Physico-chemical analysis of honey samples confirmed good quality of honey according to the standards set by European Union Commission and Codex Alimentarius Commission. All samples had ability to scavenge DPPH radicals and showed reducing potential analyzed by FRAP methods, with the highest performance obtained in *Euphorbia cheiradenia* honey. The result of the antibacterial effect of this study revealed that our honey samples have showed an important antibacterial activity against all the bacterial tested strains, *Noaea mucronata* honey has the better antibacterial effect against *Escherichia coli* and *Staphylococcus aureus*. The results of this study predict that Algerian honey possess natural compounds with antibacterial and antioxidant properties which can be used as natural agents in new drugs for therapy of diseases caused by pathogenic bacteria and oxidative stress.

**Keywords:** Honey, Physicochemical parameters, Antioxidant capacity, Antibacterial activity, Bioactive compounds.

**Abbreviations:** MIC, Minimum Inhibitory Concentration; FRAP, ferric reduction antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl ;HMF, Hydroxyméthylfurfural.

## 1. INTRODUCTION

Honey is a natural sweet substance produced by bees (*Apis mellifera*) from the nectar of flowers or tree exudates (Liu et al., 2013). It has been consumed for its high nutritive value and its contribution in human health. Honey has a very complex chemical composition, it is mostly dominated by sugars in the form of fructose and glucose (70–80%), water (10–20%) and other minor constituents such as organic acids (gluconic acid, acetic acid), mineral salts (potassium, calcium, sodium, phosphorus etc.),

ve minimum inhibitör konsantrasyonun (MIC) belirlenmesi ile değerlendirildi. Antioksidan aktivite 2,2-difenil-1-pikrilhidrazil (DPPH) ve demir indirgeyici antioksidan güç (FRAP) kullanılarak değerlendirildi. Bal örneklerinin fiziko-kimyasal analizi, Avrupa Birliği Komisyonu ve Codex Alimentarius Komisyonu tarafından belirlenen standartlara göre balın iyi kalitede olduğunu doğrulamıştır. Tüm numuneler DPPH'yi temizleme kabiliyetine sahiptir. *Euphorbia* balı elde edilen en yüksek performans ile FRAP yöntemleriyle analiz edilen indirgeme potansiyelini göstermiştir. Bu çalışmanın antibakteriyel etkisinin sonucu, bal örneklerimizin test edilmiş suşlara karşı önemli bir antibakteriyel aktivite gösterdiğini, *Noaea* balının *Escherichia coli* ve *Staphylococcus aureus*'a karşı daha iyi antibakteriyel etkiye sahip olduğunu ortaya koymuştur. Bu çalışmanın sonuçları Cezayir balının patojenik bakterilerin ve oksidatif stresin neden olduğu hastalıkların tedavisinde yeni ilaçlarda doğal ajanlar olarak kullanılabilir antibakteriyel ve antioksidan özelliklere sahip olduğunu öngörmektedir.

**Anahtar kelimeler:** Bal, Fizikokimyasal parametreler, Antioksidan kapasite, Antibakteriyel aktivite, Biyoaktif bileşikler

vitamins (ascorbic acid, niacin), proteins, enzymes (invertase, glucose oxidase, catalase, phos-phatases), volatile chemicals, phenolic acids and flavonoids (Gulzar & Vikas, 2015). This multitude of minor components can be added by bees or comes directly from nectar due to the ripening process (Pauliuc et al., 2020). The composition and physico-chemical properties of honey are wholly dependent on the plant species visited by the honey bees as well as the processing, storage, geographical location and climatic conditions (Saxena et al., 2010).

The quality of honey is mainly determined by its sensory, physicochemical and microbiological characteristics. The criteria for the physicochemical quality of honey are well specified by the European Community Guidelines 2001/110 (Council Directive of the European Union, 2001). The main criteria of interest are moisture, electrical conductivity, ash, reducing and non reducing sugars, free acidity, diastase activity, and hydroxymethylfurfural (HMF) content (Azonwade et al., 2018). Moreover, the physicochemical data of honey is essential for storage, granulation, texture, flavor, nutritional and medicinal quality and marketing (Attri, 2011).

Honey is highly regarded for its nutritional value, healing properties and has been used in traditional medicine in many countries (Estevinho et al., 2008). There are several scientific reports indicating a great variety of pharmacological activities of honey, particularly antioxidant, antimicrobial and antiviral activity (Shahzad & Cohrs, 2012), treatment of wounds (Molan, 1999), burns (Molan, 2001), skin ulcers (Lasey & Van Rij, 1997) and inflammations. The therapeutic property of honey is due to its chemical composition. In Algeria honey is widely consumed and used in traditional medicine, there is not enough information on the physicochemical and biological properties of Algerian honeys. The present study was conducted to evaluate the physicochemical parameters and the antibacterial and antioxidant activity of two varieties of honey from Algerian steppe.

## 2. MATERIAL AND METHODS

### 2.1. Honey Samples

Two monofloral honey samples (*Euphorbia cheiradenia* and *Noaea mucronata*) were purchased from beekeepers in region of the steppe of Algeria during the flowering season of 2017 to 2018. Honey samples were kept away from sunlight at room temperature until the analysis.

### 2.2. Physico-Chemical Analyses

The physico-chemical parameters of honey samples were determined according to the harmonized methods of the International Honey Commission (Bogdanov et al., 1997). A pH meter (HI 98127, Hanna instruments, Mauritius) was used to measure the pH of a 10% (w/v) solution of honey prepared in double distilled water. For the water content an Abbe refractometer was used, ash was determined by incineration in a muffle furnace at 625 °C and for the electrical conductivity a Consort C951 conductivity meter was used. HMF was determined by the Winkler method, absorbance of the red colored reaction product was measured at 550 nm using a spectrophotometer UV visible type Shimadzu 1200. The free acidity is obtained by plotting the neutralization curve with a sodium hydroxide solution and determining the pH of the equivalence point (pHe).

The lactic acidity is obtained by adding an excess of sodium hydroxide to the honey solution and plotting the neutralization curve of the excess

sodium hydroxide by a back titration with sulphuric acid

### 2.3. Evaluation of the Antibacterial Activity

#### 2.3.1. Bacterial Strains and Inoculums Standardization

*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, were kindly provided by the university hospital Mustapha Pasha of Algiers (Algeria). Prior to the experiment the strains were maintained by subculture in the specific media; the inoculums suspensions were obtained by taking five colonies from 24-hour cultures. The colonies were suspended in 5 ml of sterile saline solution (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to  $1 \times 10^8$  cfu/ml).

#### 2.3.2. Minimum Inhibitory Concentration Measurement (MIC)

The Minimum Inhibitory Concentration (MIC) of honey samples was determined by using the incorporation method; concentrations of honeys between 5 % and 15% (vol/vol) 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 5mL. Then standard inoculums of 0.5 McFarland of each bacterial strain was inoculated and the plates were incubated at 37 °C for 24 h. The Minimum Inhibitory Concentration (MIC) was determined

by finding the plates with the lowest concentration of honey on which the strain would not grow.

### 2.4. Evaluation of The Antioxidant Activity of Honey

#### 2.4.1. Total Phenolic Content (TPC)

Total phenolic content (TPC) of honey samples was determined spectrophotometrically using the Folin-Ciocalteu reagent according to the method of Beretta et al. (2005). One gram of honey was treated with distilled water (10 mL), mixed and filtered using a qualitative filter No. 40 filter paper (Whatman, Cambridge, England). An aliquot of this solution (200  $\mu$ L) was mixed with Folin-Ciocalteu reagent (500  $\mu$ L, 10%) for 5 min and then 1500  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> solution (10%) was added. All samples were incubated at room temperature for 30 min in the dark and their absorbance was read at 765 nm. A standard curve of gallic acid was drawn with a concentration range of  $3.125 \times 10^{-3}$  to  $5 \times 10^{-2}$  mg/mL. The content of total phenolics was expressed as mg of gallic acid equivalents per 100g of sample (mg GAE/ 100g). All determinations were carried out in triplicates.

#### 2.4.2. Total Flavonoid Content (TFC)

The flavonoid content was measured using a colorimetric method, which based on the formation of a complex between the aluminum ion and the carbonyl and hydroxyl groups of flavonoids that produce a yellow color (Al Farsi, et al., 2018).

One milliliter (1 mL) of honeys solutions (0.2 g/mL) were mixed with 1 mL of a 2% aluminum chloride solution. Following incubation for 30 min, the absorbance of the reaction mixture was measured at 430 nm against a distilled water blank. A standard curve of quercetin was drawn with a concentration range of  $3.0 \times 10^{-4}$  to  $4.0 \times 10^{-3}$  mg/mL, and the results were expressed as mg quercetin equivalents per 100g of honey (mg QE/100g). All determinations were carried out in triplicates.

#### **2.4.3. Ferric Reducing Antioxidant Power (FRAP Assay)**

The ferric reducing power of honey samples was determined by the method of Yen & Duh (1993) with slight modifications. 2.5 ml of the honey samples solutions at various concentrations (16 mg/mL to 300 mg/mL) were mixed with 2.5 mL of potassium ferricyanide (1%) and phosphate buffer (2.5 mL, 0.2 M, pH 6.6). The mixtures were incubated for 20 min at 50 °C. After incubation, 2.5 mL of trichloroacetic acid (10%) was added to the mixtures, followed by centrifugation at 3000 rpm for 10 min. 1 mL of the upper layer was mixed with 1 mL of distilled water and 0.5 mL of ferric chloride (0.1%). Ascorbic acid and gallic acid were used as reference standards. The increase in absorbance provided an indication of higher reducing power of the samples being analyzed. The reducing potential of honey samples and standards (gallic acid and ascorbic acid) is expressed by the values of the effective concentrations 50% (EC<sub>50</sub>) that

correspond to the concentration of sample needed to give an absorbance equal to 0.5 at 700 nm. The lowest EC<sub>50</sub> corresponds to the most important activity.

#### **2.4.4. Free radical scavenging activity (DPPH test)**

The antioxidant scavenging activity was studied using 1,1-diphenyl- 2-picrylhydrazyl free radical (DPPH) as described by Blois (1958) with some modifications; 1.5 mL of various solution of the honey samples at various concentrations (16 mg/mL to 300 mg/mL) were mixed with 1.5 mL of a 0.2mM ethanolic DPPH solution. After an incubation period of 30 min at 25 °C, the absorbance at 517 nm, the wavelength of maximum absorbance of DPPH, were recorded as a (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation: % inhibition =  $100 (A(\text{blank}) - A(\text{sample}) / A(\text{blank}))$ . The antioxidant activity of honey was expressed as IC<sub>50</sub>, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid and Gallic acid were used as a standard. All measurements were performed in triplicate.

### **3. RESULTS AND DISCUSSION**

#### **3.1. Physicochemical Analyses**

The results of physico-chemical analyses of our honey samples were indicated in the Table 1.

### 3.1.1. pH of honey samples

Table 1. Physico-chemicals properties of honey samples.

Parameter Honey	pH	Water content (%)	Electrical conductivity (mS/cm)	Ash (%)	Free acidity (meq/kg)	Lactonic Acidity (meq/kg)	Total Acidity (meq/kg)	HMF (mg/kg)
<i>Noaea mucronata</i> honey	4.87	14.6	0.31	0.17	9	2	11	19.29
<i>Euphorbia cheiradenia</i> honey	4.35	14.4	0.63	0.21	14	36	50	8.77

of 4.37 compared to *Noaea mucronata* honey with present a pH value of 4.87, so our results is consistent with international standards limit (pH 3.40–6.10) (Codex Alimentarius, 2001). Our finding is close to those previously reported for other honey samples from Algeria (Ouchemoukh et al., 2007) Turkish (Kayacier & Karaman, 2008) and Morocco (Abselami et al., 2018). The acidity of honey is due to a large number of organic acids. The main acid is gluconic, there are also formic, tartaric, maleic, citric, succinic, butyric, lactic, and oxalic acids as well as various aromatic acids (Mbogning, 2005). This acidity contribute to the flavor and the stability of honey against microbial spoilage.

### 3.1.2. Water content

In the present study, the examined honey samples present a similar water contents with a values of 14.6% for *N. mucronata* honey and 14.4% for *E.cheirdenia* honey, this values are included in the water range limits (<20%) approved by the

All honey samples are acidic, *Euphorbia cheiradenia* honey is the most acid with a pH value

international regulations (Codex Alimentarius, 2001). Our moisture values were within the values found in Algerian honeys (Ouchemoukh et al., 2007) and less than those found in Moroccan honeys (Abselami et al., 2018) and Estonian honeys (Kirs et al., 2011). The water content of honey depends on various factors such as degree of maturity reached in the hive, harvesting season, and climatic factors (Finola et al., 2007).

### 3.1.3. Electrical conductivity

In our study the electrical conductivity values of the honey samples were 0,31 mS/cm for *N.mucronata* honey and 0,63 mS/cm for *E.cheirdenia* honey, our results are similar to the findings previously reported by Saxena et al (2010) and Abselami et al (2018). Electrical conductivity is an important physicochemical parameter for the authentication of unifloral honeys (Khalil et al., 2012). Floral honeys should have electrical conductivity values below 0.8 mS/cm, whereas honeydew should have values above 0.8 mS/cm (Downey et al., 2005). All samples had conductivity measurements below

0.8 mS/cm which suggests that the two studied honeys were of a floral origin. Electrical conductivity varies with the mineral content, the botanical origin and depends on organic acids, proteins and (some complex sugars) (Terrab et al., 2003).

#### 3.1.4. Ash content

Ash content is a quality criterion for botanical and geographical origin of honey. The result of our study indicated that *E.cheirdenia* honey showed a high ash content (0.21%) compared to *N.mucronata* honey (0.17%). Ash content of our honey samples were within the acceptable range (0.6–1.2 %) indicated by codex range (Codex Alimentarius, 2001).

#### 3.1.5. Acidity

Acidity in honey is calculated as free, lactonic, and total acidity. Free acidity is the sum of all the free acids that exist in the both form ionized and un-ionized expressed in milliequivalents per kilogram of honey. Free acidity is due to the presence of organic acids, particularly gluconic acids, which are in equilibrium with the corresponding lactones and some inorganic ions such as phosphate or sulfate. Free acidity is an important parameter especially in the presence of hydrolysable ions. Lactonic acidity is considered as the acidity reserve when the honey becomes alkaline and total acidity is the sum of free and lactonic acidities (Terrab et al., 2002). The values of free acidity of our honey samples were 9 meq/kg in *N.mucronata* honey and 14 meq/kg in

*E.cheirdenia* honey. Our result fell within the permitted range proposed by the International regulations specify of no more than 50 milliequiv acid/kg (Codex Alimentarius, 2001; European Commission, 2002) indicating an absence of undesirable fermentations.

The result of our study indicated that Lactonic and total acidity of *E.cheirdenia* honey are largement higher compared to *N.mucronata* honey (Table 1). Variations in total acidity have been attributed to harvest season and the floral source (Ojeda de Rodríguez et al., 2004). The acidity honey contributes to its flavor, its stability against microbial spoilage and improves antioxidant activity (Cavia et al., 2007).

#### 3.1.6. HMF

Hydroxymethylfurfural (HMF) is a quality parameter to verify the honey freshness and high temperature processing (Tosi et al., 2002). Our honey varieties showed an HMF level lower than the limit (40 mg/kg), recommended by the Codex Alimentarius (Codex Alimentarius, 2001). The low HMF concentrations of the examined Algerian honeys confirm that these samples are of good quality, raw and unprocessed. *N.mucronata* honey showed a high HMF level (19.29 mg/kg) compared to *E.cheirdenia* honey (8.77 mg/kg). The HMF values of the analyzed honey samples were consistent with the values reported by Mondragon-Cortez et al (2013) and Abselami et al (2018). The HMF content in honey can be affected by heat temperature and time, storage

conditions, pH and floral source (Fallico et al., 2004).

### 3.2. Antioxidant Activity of Honey Samples

#### 3.2.1. Total polyphenol content

The total polyphenol content of our honey samples is shown in Figure 1.

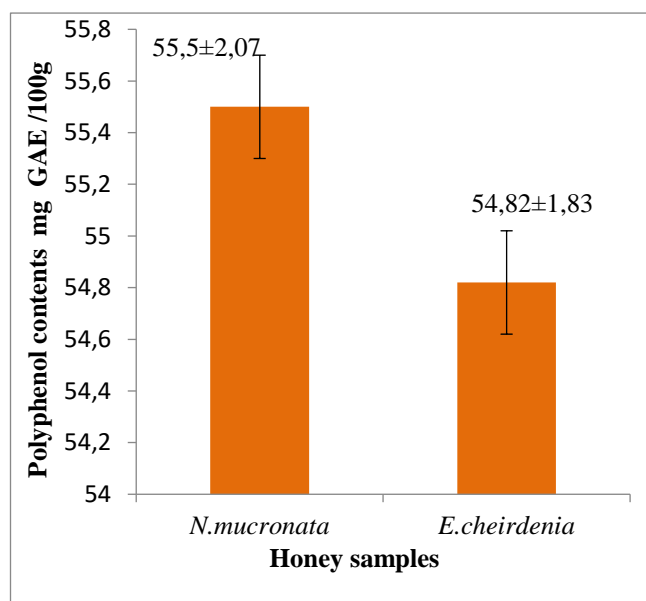


Figure 1. Polyphenols contents of honey samples

Polyphenols are one of the most important classes of substances found in honey. The result of the present study indicated that *N.mucronata* honey present the highest polyphenols content ( $55.5 \pm 2.07$  mg GAE/100g) when compared to *E.cheirdenia* honey ( $54.82 \pm 1.83$  mg GAE/100g). The total polyphenol content of the tested Algerian honeys is higher than those reported by Khalil et al. (2012), Juszczak et al. (2016) and Pauliuc et al. (2020). Our polyphenols result is lower to those previously reported for other honey samples from Malaysia (Chua et al., 2013) and Oman (Al-Farsi et al., 2018).

The concentration and type of phenolic in honey depended on several factors, such as flower source of the nectar, season and environmental factors, such as soil type and climate, genetic factors and processing methods (Ruiz-Navajas et al., 2011).

#### 3.2.2. Flavonoid content

Flavonoids are low molecular weight phenolic compounds that are vital components of the aroma and which at the same time possess antioxidant properties of honey. Flavonoids in honey may originate from nectar, pollen or propolis (Hamdy Ismail et al., 2009). Figure 2 represents the flavonoid content of our honey samples.

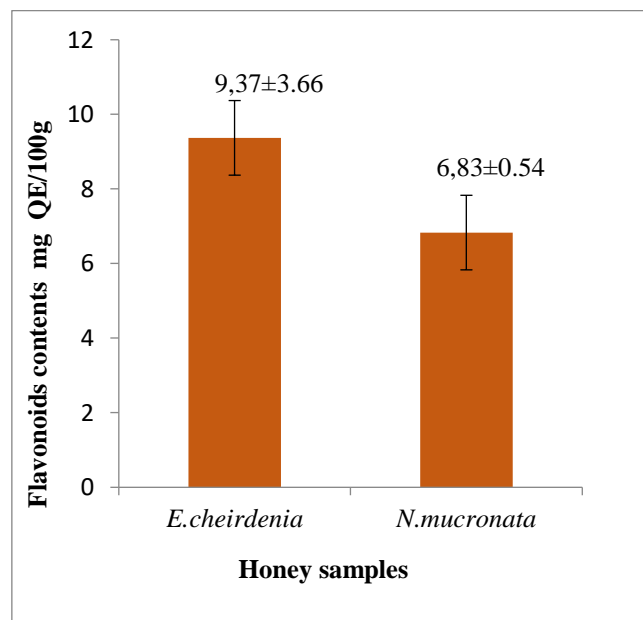


Figure 2. Flavonoid content of the honey samples

The present result demonstrated that *E.cheirdenia* honey has showed the higher flavonoid content ( $9.37 \pm 3.66$  mg QE/100g) compared to *N.mucronata* honey ( $6.83 \pm 0.54$  QE/100g).



The flavonoid content of the tested honey is higher than those reported by Khalil et al. (2012), Juszczak et al. (2016) and Pauliuc et al. (2020). Our flavonoid result is lower to those previously reported for other honey samples from Malaysia (Chua et al 2013) and Oman (Al-Farsi et al., 2018).

The variation in the flavonoid content of honey depends on its botanical and geographical origin and also on the collection season (Mouhoubi et al, 2016).

### 3.2.3. Ferric reducing antioxidant power (FRAP)

The antioxidant activity of honey is a biological property of great interest because there is a strong need for effective antioxidants from natural sources as alternatives to synthetic food additives in order to prevent deterioration of foods drugs and cosmetics. The result of the antioxidant activity of our honey samples evaluated by the FRAP assay is showed in Figure 3. The antioxidant activity of the tested honeys evaluated by reducing potential test revealed that *E.cheirdenia* honey and *N.mucronata* honey have a greater antioxidant activity with EC<sub>50</sub> values in the ordre of 159.37±3.91mg /ml and 176.93±4.65 mg /ml respectively. The reducing power of the examined honeys is much lower than that of gallic acid (EC<sub>50</sub> = 0.021±0.00236 mg/mL) and of ascorbic acid (EC<sub>50</sub>= 0.068±0.00436 mg/mL). *E.cheirdenia* honey has the best reducing power this is probably due to its richness in flavonoids (9.37±3.66mg EQ/100g) compared *N.muconata* honey (6.83±0.54 mg EQ /

100g). These results are lower to those previously reported for other honey samples from Palestine (Imtara et al., 2018) and Morocco (El-Haskoury et al., 2018).

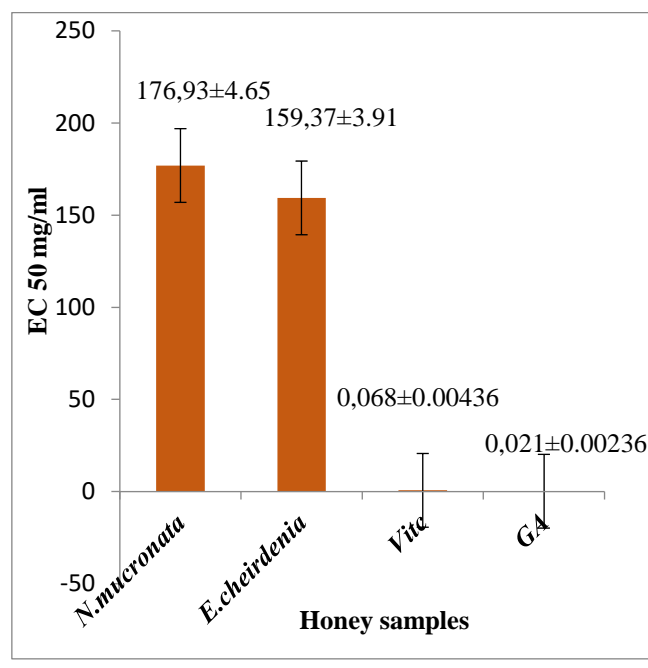


Figure 3. Reductive potential of honey samples

### 3.2.4. Free radical scavenging activity (DPPH test)

The result of DPPH test of this study was showed in the following Figure 4. The results of DPPH test of this study showed that the studied honeys have an important antioxidant activity with IC<sub>50</sub> values of 60.67±1.81 mg/mL for *E. cheirdenia* honey and 91.08±0.84 mg/ ml for *N.mucronata* honey. This result is less than that of the standards antioxidant, ascorbic acid, and gallic acid, which exhibit IC<sub>50</sub> of the order of 0.00854±0.75 mg/mL and 0.00426±0.185 mg/mL, respectively. We found that *E.cheirdenia* honey has the best scavenging activity this is may be

due to its high flavonoids content ( $9.37\pm 3.66$ mg EQ/100g) compared to *N. mucronata* honey ( $6.83\pm 0.54$ mg EQ/100g). Our DPPH results are lower to those previously reported by Alzahrani et al. (2012b), Kishore et al. (2011) and Almeida et al. (2016). Previous studies showed the antioxidant activity of Algerian honey.

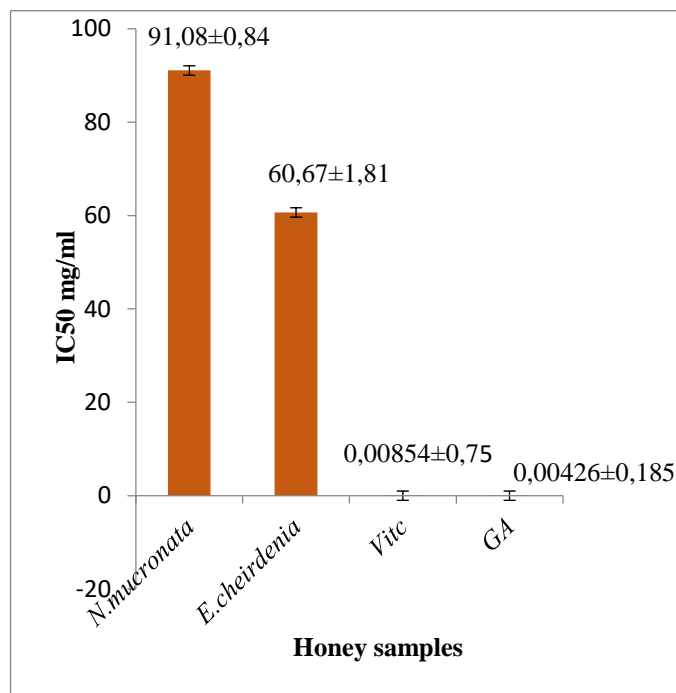


Figure 4. Free radical-scavenging capacities of honey samples

Khalil et al. (2012) reported that Algerian honey has a high antioxidant potential evaluated by FRAP and DPPH test this study revealed that Algerian honey is a good source of antioxidants. In a study done Mouhoubi et al. (2016) the reducing power results showed that all Algerian honey samples exhibit a high antioxidant activity. Rebiai et al. (2017) found in their study that the phenolic extracts of Algerian honey from *Zygyphyllum album* floral sources have an antioxidant activity evaluated by ferric

reducing/antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The antioxidant activity of honey is attributed to several types of compounds including carotenoids, ascorbic acid, tocopherols, phenolic acids and flavonoids, sugars, proteins, amino acids, carotenes, organic acids, Maillard reaction products, and other minor components also contribute to antioxidant effect (Ahmed et al., 2018). The enzymes glucose oxidase and catalase contribute to the antioxidant activity through their ability to eliminate oxygen from the media (Ruiz-Navajas et al., 2011). The antioxidant activity of honey depends on a variety of factors such as geographical origin, environmental factors (temperature, humidity and soil composition) as well as post-harvest condition (Yanishlieva-Maslarova, 2001).

### 3.3. Antibacterial activity of honey samples

The results of the antibacterial activity of our honey samples were showed in the Table 2. The result of the antibacterial effect of this study revealed that our honey samples have showed an important antibacterial activity against all the bacterial tested strains, they have a similar effect against *Pseudomonas aeruginosa* with a MIC value of 6%, while *N.mucronata* honey has the better antibacterial effect against *Escherichia coli* and *Staphylococcus aureus*, this is most probably due to its high phenolic content ( $55.5\pm 2.07$  mg GAE/100g) compared to *E.cheiradenia* honey. Gram-negative bacteria are more resistant than Gram-positive bacteria. This may be due to the

nature of the Gram negative bacteria wall which is formed mainly of lipoprotein, lipopolysaccharide and lipid. These compounds play a barrier role and limit the penetration of antimicrobial agents through the bacterial wall unlike *S. aureus* which has a Gram-positive wall, free of these compounds (Larpent & Gourgaud, 1985). These results are similar to those obtained by Matzen et al. (2018) and Anand et al., (2019).

Table 2. Antibacterial effect of the tested honeys

Tested Strains	<i>Escherichia coli</i> ATCC 25922	<i>Staphylococcus aureus</i> ATCC 33862	<i>Pseudomonas aeruginosa</i> ATCC 27853
<i>Noaea mucronata</i> honey	6%	4%	6%
<i>Euphorbia cheiridonia</i> honey	8%	5%	6%

The antimicrobial activity of Algerian honey has been demonstrated by many studies. In a study by Abdellah et al. (2012) found that Algerian wild carrot honey has an antibacterial effect against three pathogenic bacteria, namely *Staphylococcus aureus* OxaR ATCC 43300, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, while Alzahrani et al. (2012) reported that *Daucus carota* honey obtained from an Algerian beekeeper has a greater antimicrobial effect against *Staphylococcus aureus* 43300 (Oxa R), *Staphylococcus aureus* 25923 (Oxa S) and *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans*. Alzahrani et al. (2014) demonstrated that *Daucus carota* honey from Algeria has an antifungal effect against

*Aspergillus niger* and *Aspergillus flavus*. The result of a study done by Nedji & Loucif (2014) showed that Algerian honey inhibited the growth of the foodborne pathogens bacteria: *Bacillus cereus* (IPA), *Staphylococcus aureus* (ATCC 25923R), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27893R). In a study done by Belaid et al. (2014) they found that honeys from northern Algeria inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results obtained in a study done by Bouacha et al. (2018) revealed that six honey samples collected from different localities in the east of Algeria exhibited potent antibacterial activity Against 11 multidrug-resistant bacterial strains, isolated from urinary tract infections of pregnant women. The antibacterial effect of honey is attributed to presence of inert antibiotic factors in it. These factors include its acidic pH, osmotic effect of sugars, and production of H<sub>2</sub>O<sub>2</sub> by peroxidase. Some non peroxidase substances also support antibacterial activity which include flavonoids, phenolic acids, and lysozyme (Bogdanov, 2011). Botanical origin plays an important role in influencing a honey's antimicrobial activity (Taormina et al., 2001).

#### 4. CONCLUSION

In this study physico-chemical analysis of honey samples confirmed good quality of Algerian honey according to the standards set by European Union Commission and Codex Alimentarius

Commission. Evaluation of the biological activities of these honey samples confirms antibacterial potential of honeys against all bacterial strains *Noaea mucronata* honey has the better effect against *S.aureus* and *E.coli*. While *Euphorbia cheiradenia* honey has the better antioxidant effect. The results of our study may suggest that Algerian honey possess natural compounds with antibacterial and antioxidant properties which can be used as natural agents in new drugs for therapy of diseases caused by pathogenic bacteria and oxidative stress.

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