JOURNAL OF APITHERAPY, 2018
VOL 4, NO. 1, PAGE 1–8
10.5455/ja.20180621024230

eJManager

ORIGINAL ARTICLE

Open Access

Antibacterial activity of various honey monofloral and polyfloral from different regions of Algeria against uropathogenic Gram-Negative Bacilli

Banfitebiyi Gambogou[1,2], Hassina Khadimallah[1], Mabrouka Bouacha[1], Y. A. Ameyapoh[2]

[1]Laboratory of Biochemistry and Microbiology, Department of Biochemistry, Faculty of Sciences, University of Badji Mokhtar, Annaba, Algeria

[2]Laboratory of Microbiology and Food control quality, High School of Food and Biological Technic, University of Lomé, Lomé, Togo

ABSTRACT

Introduction: Multidrug resistant *Escherichia coli* and *Pseudomonas aeruginosa* pose treatment problem resulting in high morbidity, high mortality, and increased health care costs. The evolution of this resistance to antibiotics imposed the search for new molecules that are able to fight against bacterial infections. Natural honey is a sweet substance containing many compounds such as sugar, organic acids, enzymes, and phenolic compounds, which are responsible for its antimicrobial activity.

Aims: The aim of this *in vitro* study was to assess the antibacterial effect of Algerian natural honey.

Materials and Methods: Eight natural honey samples were collected from Skikda, El Taref, Tebessa, Oum EL Bouaghi, Djelfa, Khenchela in Algeria. The agar well diffusion assay and spectrophotometric analysis at 620 nm using the broth microdilution method were used on eight bacteria strains of *P. aeruginosa* and *Enterobacteria*, to determine the antibacterial effect and minimum inhibition concentrations (MICs) value.

Result: Antibacterial activity of the natural honey samples was revealed by the measurement of the zone of inhibition of bacterial growth with diameters of inhibition ranging from 18 to 60 mm in *Enterobacteria*, and from 17 to 44 mm in *P. aeruginosa*. The MICs ranging from 5% to 40% (v/v) in *Enterobacteria* and 10% to 40% (v/v) in *P. aeruginosa*.

Conclusion: According to its properties and antibacterial activity, honey is a potential source in the antimicrobial therapy of urinary tract infection caused by *P. aeruginosa* and *Enterobacteria*.

ARTICLE HISTORY

Received 21 June 2018
Accepted 20 August 2018
Published 29 August 2018

KEYWORDS

Honeybee; antibacterial activity; *Enterobacteria*; *P. aeruginosa*; Urinary tract infection; Algeria

Introduction

Honey is a natural substance derived from honey bees that assists in healing infected wounds, whether burns, ulcers, surgical incisions, or diabetic lesions [1,2]. The use of honey by the traditional medicine in the treatment of infections has been practiced since the origin of humanity, and honey is one of the oldest traditional remedies that medicine considers important in the treatment of several diseases [3,4].

In recent years, many bacteria have developed antimicrobial drug resistance; these include *Pseudomonas aeruginosa* and *Enterobacteriaceae* [5]. Antimicrobial resistance in *Enterobacteria* and *P. aeruginosa* has been reported worldwide and increasing rates of resistance is a growing concern in both developed and developing countries [6,7]. Bacterial resistance is attributed to an evolutionary response to the widespread and indiscriminate use of antimicrobials [5,8]. Multidrug resistant (MDR) bacteria poses treatment problem resulting in high morbidity, high mortality, and increased health care costs [9].

The evolution of resistance to antibiotics imposed the search and release of new molecules that should allow having a greater choice of products that are able to fight against bacterial infections. The antimicrobial properties of honey have been known for several centuries. Honey has inhibitory

Contact Banfitebiyi Gambogou ✉ dinhog39@gmail.com Laboratory of Biochemistry and Microbiology, Department of Biochemistry, Faculty of Sciences, University of Badji Mokhtar, Annaba, Algeria and Laboratory of Microbiology and Food quality control, High School of Food and Biological Technic, University of Lomé, Lomé, Togo.

Table 1. Different honey samples from different regions of Algeria.

Samples	Geographic origin	Floral origin
Sample 1	Skikda	*Eucalyptus amygdalina*
Sample 2	Skikda	*Eucalyptus piperata*
Sample 3	Khenchela	*Armoise*
Sample 4	Khenchela	*Jujubier, Mauve, Armoise, Pinus, Asperge, concombre d'âne, romarin, oxalis*
Sample 5	Tébessa	*Armoise, Eucalyptus, Pinus*
Sample 6	Djelfa	*jujubier (Zizyphus sativa*)
Sample 7	El Taref	*Eucalyptus, Pinus, aubépine, basilic, châtaignier.*
Sample 8	Oum EL Bouaghi	*Eucalyptus, Pinus, Armoise.*

effects towards approximately 60 bacterial species, including aerobes and anaerobes, Gram-positive and Gram-negative bacteria [10,11]. The factors responsible for the antimicrobial activity of honey include high osmolarity, acidity, hydrogen peroxide, and non-peroxide factors. The non-peroxide antimicrobial factors of honey include lysozyme, phenolic acids, and flavonoids [12]. Nonetheless, honey from different floral sources showed various levels of antimicrobial activity. It is within this framework that our work objective, which consists to evaluate the antibacterial activity of eight honey samples from different regions of Algeria and a honey sold on eight bacterial strains belonging to *Enterobacteria* and *P. aeruginosa*.

Materials and Methods

Samples

Table 1 shows different origins and the type of the examined honey. All honey samples were directly obtained from beekeepers. The beekeepers directly extracted the samples into 250 ml sterilized glass sample bottles with glass caps. The samples were then stored in a dry and dark place at a temperature of 20°C for 30 days. Subsequently, the honey samples were classified into eight categories as they belong to the following diverse floral origin.

Preparation of honey sample

The assays to evaluate the inhibitory activity of seven honey concentrations were prepared in sterile distilled water: 1%–75% honey (w/v). Samples were filtered with a pore size of 0.2 mm. The solution was heated briefly to 50°C to aid the dissolving of the sugars.

pH and optical density measurements

Honey samples were diluted at various concentrations, 1%, 5%, 10%, 25%, 50%, and 75% (w/v), in CO_2-free distilled water for measuring the pH value by the aid of a portable pH-meter. Ten grams of each honey sample was diluted with 100 ml of distilled water followed by centrifugation for 10 minutes at 3,000 *g* (*g*: gravity acceleration unit). The absorbance of filtrate supernatant was measured at 400 nm [13].

Color (optical density)

Ten grams of each honey sample was diluted with 100 ml of distilled water and centrifuged for 15 minutes at 3,000 *g* and the absorbency of the filtrate was measured at 620 nm using distilled water as a blank.

Bacterial strains growth and identification

The study was performed on a total number of eight uropathogenic bacteria: *Escherichia coli*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Citrobacter koseri*, and four strains of *P. aeruginosa* from inpatients and outpatients. All isolates were collected from the Laboratory of Microbiology of hospital Ibn Rochd, Annaba, Algeria. All MDR bacteria isolate, as well as *E. coli* standard strain [American Type Culture Collection 25922 (ATCC 25922)] and the standard strain *P*. *aeruginosa* ATCC 27853, were grown overnight in Trypticase Soy Broth at 37°C. All tested strains were identified by conventional methods of microbiology (Gram staining, oxidase and catalase test, analytical profile index (API) 20E, and API 20NE (Biomerieux, Paris, France).

The antibiotic susceptibility was performed using disk diffusion methods according to the Clinical and Laboratory Standards Institute [14] using Mueller Hinton agar medium (Biomerieux, Paris, France). Tested antibiotics: Ticarcillin/clavulanic acid (75/10 µg), piperacillin (75 µg), amoxicillin/clavulanic acid (AMC) (20/10 µg), ampicillin (10 µg), amoxicillin (AMX) (25 µg), cefalexin (CTX) (30 µg), cefotaxime (CTX) (30 µg), imipenem (IMP) (10 µg), kanamycin (K) (10 µg), tobramycin (10 µg), gentamicin (10 µg), doxycycline (30 µg), trimethoprim + sulfamethazole (125/23,75 µg), and nalidixic acid (AN) (30 µg) were supplied from (Oxoid, UK). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as a reference strain, and

Table 2. Bacteria isolated and identified from patients at Ibn Rchd hospital, Annaba, Algeria.

Bacteria	Number of bacteria (*n = 8*)
Pseudomonas aeruginosa	4
Escherichia coli	1
Citrobacter koseri	1
Enterobacter aerogenes	1
Proteus mirabilis	1

the result was interpreted according to the Clinical Laboratory Standards Institute, 2017.

Antibacterial activity of honey

After adjusting the inoculum to a 0.5 McFarland standard, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. The entire surface of Mueller Hinton agar was swabbed three times, rotating plates approximately 60° between streaking to ensure even distribution. The inoculated plate was allowed to stand for at least 3 minutes but no longer than 15 minutes before punching the wells in the agar plate. A hollow tube of 5 mm diameter was taken and heated. It was pressed on the inoculated agar plate and removed immediately after making a well in the plate. Likewise, four wells were made on each plate. Fifty microliters of honey sample was added to the respective wells on each plate. The plates were incubated within 15 minutes of compound application for 18–24 hours at 37°C. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to the nearest whole millimeter [14].

Determination of minimum inhibitory concentrations

Gram negatives *E. coli* ATCC 25922 and *P. aeruginosa* ATCC2 7853, were grown with different concentrations of the honey sample. Controls were constituted with honey without bacteria. All experiments were performed in triplicate [15]. The minimum inhibition concentration (MIC) of the honey sample was defined as the lowest concentration that completely inhibited the bacterial growth.

Up to 0.2 ml of the bacteria suspension was inoculated into 4 ml of honey concentration in a test tube. Inoculation of 4 ml volume of Muller Hinton broth with 0.2 ml of each bacteria suspension served as a negative control. The optical density (OD) was determined and recorded in a spectrophotometer at 620 nm before incubation (T_0), 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 ODs were again determined and recorded (T_{24}). The OD for each replicate at T_0 was subtracted at determined using the formula:

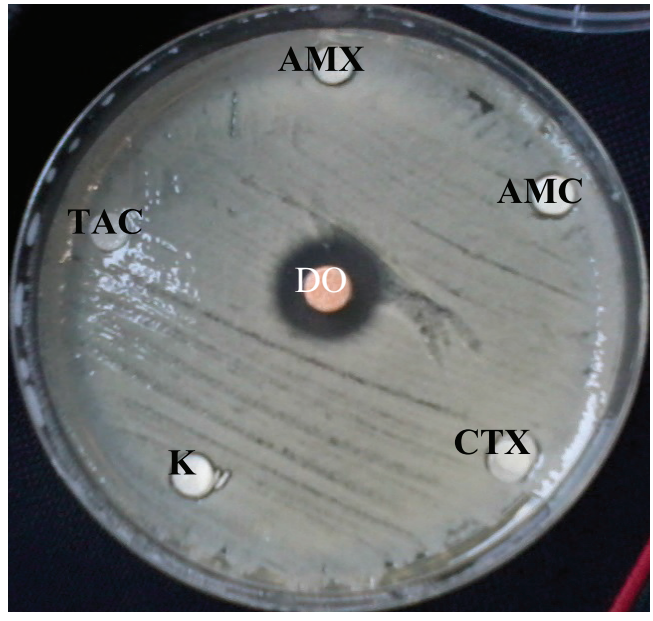

Figure 1. Antibiotic susceptibility of *E. coli*.

Percentage inhibition = 1 – (OD test/OD control) × 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) × 100

Determination of minimum bactericidal concentration

The minimum bactericidal concentration (MBC) demonstrates the lowest level of the antimicrobial agent resulting in microbial death. For determining MBC values, an aliquot (10 µl) from the broth dilution of MIC tests by subculturing to agar Mueller Hinton plates that do not contain the test agent was inoculated and incubated at 37°C for 24 hours [10]. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as ≥99.9%.

Results

Identification and antibiotics susceptibility

Table 2, and Figures 1 and 3, shows the eight different bacteria that were isolated and identified from

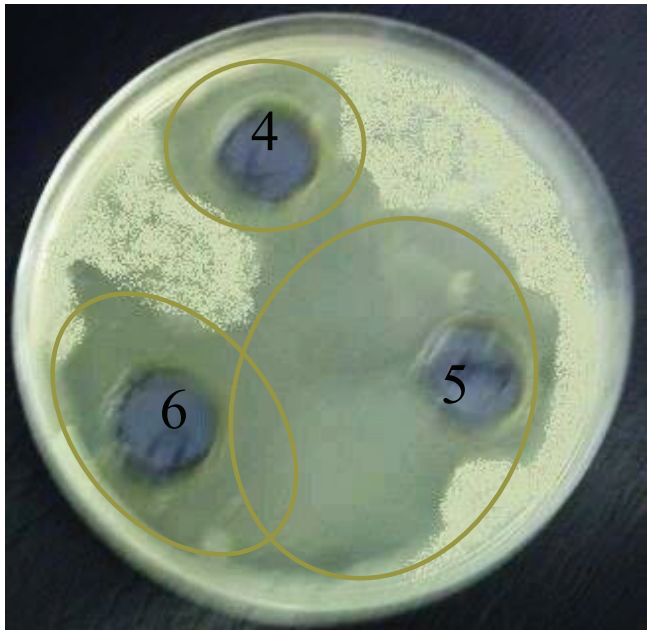

Figure 3. Antibacterial activity of honey on *P. mirabilis*.

patients and testing their antibiotics susceptibility. Five types of Gram-negative bacteria, namely *E. coli*, *E. aerogenes*, *C. koseri*, *P. mirabilis*, and *P. aeruginosa*. All bacteria were 100% resistant to AMC and AMX. The results showed (Table 3) that all tested bacteria exhibited varying degrees of multidrug resistance of standard antibiotics used. The uropathogenic strains revealed the presence of high levels of multiple antimicrobial resistance.

Color, pH, and optical density of different honey used

Table 4 presents the pH, color, and OD of different honey used in this study. The pH of a culture is one of the main factors that determine the survival and growth of microorganisms during its preparation and storage, but the color of the honey samples may be due to the presence of the elements that compose it or its environment. The pH of our honey varied from 3.54 to 3.94. The high pH (3.94) was observed in honey polyfloral (*Armoise*, *Eucalyptus*, and *Pinus*) from Tebessa (sample 5) and the low pH (3.54) was observed with honey monofloral (*Eucalyptus amygdalina*) from Skikda (sample 1). Honey samples in this study gave us at 350 nm a wavelength of 0.545–2.533. The honey such as sample 4 from Khenchela gave the OD = 2.533 is a dark brown color and sample 8 from Oum EL Bouaghi which has a low OD of 0.545 is brown, so it can be said that the color varies from light brown to dark brown.

Antibacterial activity

Table 5, Figures 2 and 4, presents the antibacterial properties of different honey use in this study against *P. aeruginosa* and *Enterobacteria.* Our honey samples show different antimicrobial activities against *P. aeruginosa* and *Enterobacteriaceae* strains. The different honey used against *Enterobacteriaceae* strains show diameters of zone inhibition from 18 ± 1 mm to 60 ± 2 mm. High-antibacterial activity is obtained by the sample 7 on *P. mirabilis* and *E. aerogenes*. The diameters of the zones of inhibition vary from 17 ± 1 mm to 44 ± 2 mm for antibacterial activity of different honey used against *P. aeruginosa* strains, and the most effective honey is the sample 5.

Determination of MICs and MBCs

Table 6 presents the MIC and MBC values of different honey samples against *P. aeruginosa* and *Enterobacteria* strains. The MIC and the MBC values

Table 3. Antibiotics resistance of *P. aerugnosa* and *Enterobacteria* strains.

Strains	AMX	AMC	TAC	PIP	CN	CTX	IMP	K	TM	GM	DO	SXT	AN
Pseudomonas aeruginosa 1	R	R	R	R	R	S	S	R	R	R	R	R	R
Pseudomonas aeruginosa 2	R	R	R	R	R	R	R	S	R	R	R	R	R
Pseudomonas aeruginosa 3	R	R	R	R	S	R	R	R	R	S	R	R	R
Pseudomonas aeruginosa 4	R	R	R	R	R	R	S	R	R	S	R	R	R
Escherichia coli	R	R	R	R	S	R	S	R	S	R	S	S	S
Citrobacter koseri	R	R	S	S	R	S	S	R	S	R	R	R	R
Enterobacter aerogenes	R	R	R	S	S	R	R	R	S	S	R	R	S
Proteus mirabilis	R	R	R	S	S	S	R	R	S	S	R	R	S
Susceptibility percentage (%)	0	0	12,5	37,5	50	37,5	37,5	12,5	50	50	12,5	12,5	37,5

TAC = Tircalcillin/Clavulanic acid; PIP = Piperaclin; TM = Trobamycin; DO = Doxicyclin; SXT = Triméthoprime/Sulfaméthazole; R = Resistance; S = Susceptibility.

Table 4. The pH, color, and OD of different honey from different regions.

Honey	Color	pH	OD
Sample 1	Dark Brown	3.54	1.064
Sample 2	Dark Brown	3.55	1.394
Sample 3	Dark Brown	3.61	1.738
Sample 4	Dark Brown	3.77	2.533
Sample 5	Light Brown	3.94	1.087
Sample 6	Light Brown	3.86	0.783
Sample 7	Dark Brown	3.88	1.575
Sample 8	Brown	3.83	0.545

are ranging from 5% to 40% for all *Enterobacteria* strains, the low MIC (5%) is obtained by sample 7 *P. aeruginosa* strains the MICs are ranging from 10% to 40% and the MBCs are ranging from 20% to 80%. The low MIC (10%) was observed in samples 4, 5, and 7 on *P. aeruginosa*.

Discussion

Antibacterial susceptibility testing, as illustrated in Table 1, revealed that all tested uropathogenic bacteria exhibited a high level of resistance to standard antibiotics used in the treatment of urinary tract infections; similar to results previously reported [10]. However, some strains of *Enterobacteriaceae* and *P. aeruginosa* had poor sensitivity to gentamycin (GM), tobramycin, and CTX (50%). The indiscriminate use of antibiotics has developed many resistant microorganisms creating immense clinical problems in the treatment of infections such as those caused by *Enterobacteria* and *P. aeruginosa* strains in urinary tract infection. Therefore, there is a need to develop alternative antimicrobial agents for the treatment of these infections. A non-antibiotic approach to the treatment and prevention of urinary tract infections includes the application of honey. Honey is derived from many different floral sources and its antimicrobial activity varies from origin and processing [12,16,17]. Considering the enormous potential for using honey in a clinical setting, it is important that research continues not only using honey that are commercially available but also those of local origin with a dearth of information on their antimicrobial potential.

From Table 4, the analysis of honey samples showed that all of the tested Algerian honey samples were acidic, with pH values that varied between 3.54 and 3.94. It was reported previously that the pH values of Algerian honey ranged from 3.19 to 4.54 [10] and 3.96 to 4.34 [12]. The acidity of any honey is directly related to the floral sources that created it.

The OD of 50% (w/v) honey solutions was varied from 783 to 1,087 mAU for the light honey and from 545 to 1,738 mAU for the brown and dark brown honey. Honey samples from Algeria and other countries were reported to have absorbance values between 352 and 1,965 mAU in Algeria [10] and between 524 and 1,678 mAU in Sahara honey [19]. This difference in color intensity might be a reliable index of the presence of pigments with antioxidant activities, such as carotenoids and some flavonoids, which are known to have antioxidant properties [19].

Table 5. Antibacterial activity of different honey from different regions against *P. aeruginosa* and *Enterobacteria*.

Strains	Diameter of inhibition (mm ± SD)							
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Pseudomonas aeruginosa ATCC 27853	30 ± 2	24 ± 1	28 ± 0.5	40 ± 1	44 ± 2	24 ± 1	30 ± 1	28 ± 1
Pseudomonas aeruginosa 1	30 ± 1	24 ± 2	27 ± 1	25 ± 1	20 ± 1	23 ± 2	34 ± 2	17 ± 1
Pseudomonas aeruginosa 2	36 ± 2	25 ± 1	26 ± 1	24 ± 2	26 ± 1	24 ± 1	26 ± 0.5	30 ± 2
Pseudomonas aeruginosa 3	19 ± 1	22 ± 1	18 ± 1	20 ± 1	25 ± 2	24 ± 2	19 ± 2	25 ± 2
Pseudomonas aeruginosa 4	24 ± 3	30 ± 2	25 ± 2	36 ± 1	30 ± 1	32 ± 1	20 ± 1	24 ± 1
Escherichia coli ATCC 25922	30 ± 2	22 ± 2	23 ± 1	28 ± 2	20 ± 1	20 ± 2	22 ± 1	20 ± 0.5
Escherichia coli	30 ± 1	22 ± 1	22 ± 2	18 ± 2	20 ± 2	20 ± 2	18 ± 2	20 ± 1
Citrobacter koseri	20 ± 3	22 ± 0.5	24 ± 1	20 ± 1	26 ± 3	23 ± 1	20 ± 1	28 ± 1
Enterobacter aerogenes	22 ± 2	22 ± 1	20 ± 3	20 ± 1	18 ± 2	18 ± 2	60 ± 2	36 ± 1
Proteus mirabilis	36 ± 1	40 ± 3	40 ± 2	36 ± 2	30 ± 1	36 ± 1	60 ± 1	28 ± 1

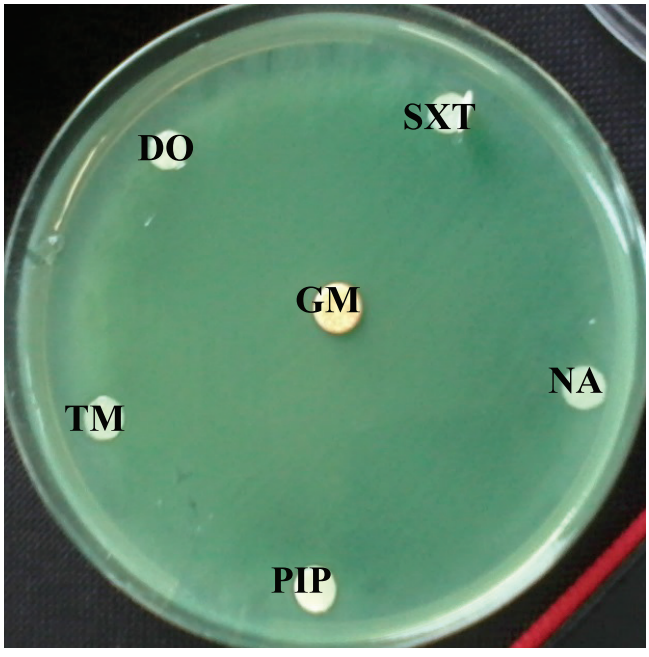

Figure 2. Antibiotic susceptibility of *P. Aeruginosa* 2.

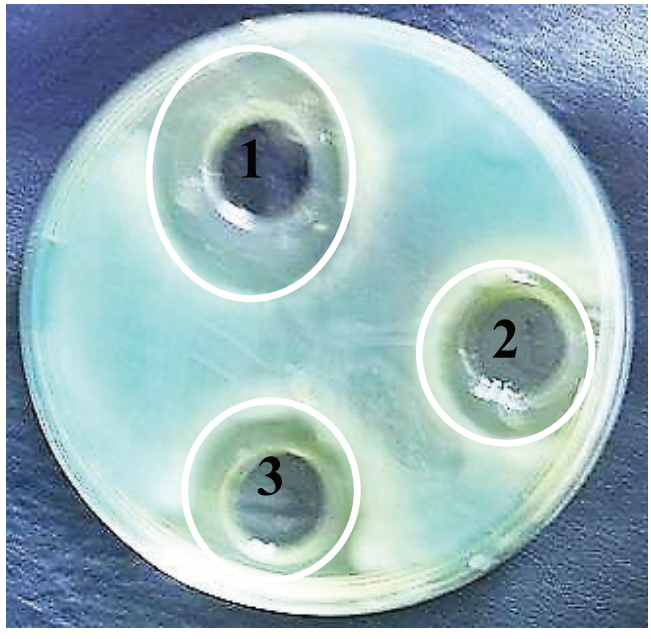

Figure 4. Antibacterial activity of honey on *P. aeruginosa* 3.

Therefore, in this study, eight locally produced honey were screened for their antimicrobial activity against *P. aeruginosa* and *Enterobacteria* strains. Our data showed that all honey tested demonstrated antibacterial action. Algerian honey samples have significant antibacterial activity on *Enterobacteriaceae* strains and *P. aeruginosa* strains. Although there was no statistically significant difference between values of zone diameters inhibition (in Table 5) of the different honey against *P. aeruginosa* strains (p: 0.929) or *Enterobacteria* strains (p: 0.811). These variations may be attributed to the difference in antimicrobial potential because bees are able to obtain nectar from different sources [11] and many medical plants are screen in Algeria for it antimicrobial properties [20]. The distribution of vegetative flowers and plant species from which honeybees collect nectar and sweet deposits to produce honey are affected by several factors including climatic conditions,

Table 6. MICs and MBCs of different honey sample against *P. aeruginosa* and *Enterobacteria* strains.

Strains	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Pseudomonas aeruginosa ATCC 27853	20	40	40	80	20	40	10	20	10	20	40	80	20	40	20	40
Pseudomonas aeruginosa 1	20	40	20	40	20	40	20	40	20	40	40	80	10	20	20	40
Pseudomonas aeruginosa 2	10	20	20	40	20	40	20	40	40	80	40	80	40	80	20	40
Pseudomonas aeruginosa 3	40	80	40	40	40	80	40	80	40	80	40	80	40	80	20	40
Pseudomonas aeruginosa 4	40	80	20	40	20	40	20	40	20	40	20	40	40	80	20	40
Escherichia coli ATCC 25922	20	40	40	40	40	40	20	20	40	40	40	40	20	40	40	40
Escherichia coli	20	40	40	40	40	40	40	40	40	40	40	40	20	40	20	40
Citrobacter koseri	40	40	40	40	40	40	20	40	40	40	40	40	20	40	20	40
Enterobacter aerogenes	40	40	40	40	40	40	40	40	40	40	40	40	5	5	20	40
Proteus mirabilis	40	40	10	10	10	20	10	20	20	40	20	40	5	5	20	40

among others [10,12] imparting different properties on honey from different locations due to differences in their chemical composition of fatty acids, lipids, amylases, ascorbic acid, peroxidases, and fructose [3,18,19]. Although it not investigated in this study.

As shown in Table 6, the MIC and MBC are ranging from 10% to 40% (w/v) against *Enterobacteria* and 10% to 80% (w/v) against *P. aeruginosa*. It corroborates the finding of Guendouze-Bouchefa et al. [18] and Bouacha et al. [10] who has reported *in vitro* antibacterial activity of both Algeria honeys, with 5% to 20% (w/v) and 20% to 80% (w/v) of the honey samples showing excellent activity against *Enterobacteria* and *P. aeruginosa*, respectively. Furthermore, our results are in line with the studies of Moussa et al. [4] and Abdelmalek et al. [21] that reported potent activity against Gram-negative bacteria in Algeria. The MIC values determined by visual inspection may not have been accurate because impurities in the honey extracts could result in disturbance and imprecision in the readings. Consequently, we also used spectrophotometry for MICs determination. This is in accordance with the study of Almasaudi et al. [22] that equally reported lower MIC values obtained spectrophotometrically at 620 nm for honey against their isolates. These honey samples may contain compounds with anti-*Enterobacteria* such as anti-*P. mirabilis* and anti-*P. aeruginosa* activity and therefore, call for more elaborate phytochemical studies to isolate and characterize the compounds.

Conclusion

The study allowed us to evaluate the potential antibacterial activity of natural honey samples from the different locations in Algeria. The results indicate that all natural honey samples tested have an antibacterial effect on *P. aeruginosa* and *enterobacteria* strains. This confirms to us that bee origin honey has an important antibacterial activity and can be used in modern medicine for treatment.

The pressure in recent years for the search for a new antibiotic drug, more work is required in order to evaluate the antibacterial ability of honey against various pathogenic microorganisms. Thus, the results obtained with the honey samples open very interesting perspectives to continue this work on the evaluation of its antiparasitic activity, the synergy action with other antibiotics and the *in vivo* test.

References

[1] McLoone P, Warnock M, Fyfe L. Honey: a realistic antimicrobial for disorders of the skin. J Microbiol Immunol Infect 2016; 49(2):161–7.

[2] Zainol MI, Yusoff KM, Yusof MYM. Antibacterial activity of selected Malaysian honey. BMC Complement Altern Med 2013; 13(1):129.

[3] Draiaia R, Dainese N, Borin A, Manzinello C, Gallina A, Mutinelli F. Physicochemical parameters and antibiotics residuals in Algerian honey. Afr J Biotechnol 2015; 14(14):1242–51.

[4] Moussa, A, Noureddine D, Abdelmelek M, Saad A. Antibacterial activity of various honey types of Algeria against Pathogenic Gram–Negative Bacilli: *Escherichia coli* and *Pseudomonas aeruginosa*. Asian Pac J Trop Dis 2012; 2(3):211–4.

[5] Wiedemann B, Heisig A, Heisig P. Uncomplicated urinary tract infections and antibiotic resistance- epidemiological and mechanistic aspects. Antibiotics (Basel) 2014; 3(3):341–52.

[6] Sabtu N, Enoch DA, Brown NM. Antibiotic resistance: what, why, where, when and how? Br Med Bull 2015; 116(1):105–13.

[7] Rasamiravaka T, Sheila H S, Rakotomavojaona T, Rakoto-Alson A O, Rasamindrakotroka A. Changing profile and increasing antimicrobial resistance of uropathogenic bacteria in Madagascar. Med Mal Infect 2015; 45(5):173–6.

[8] Petty NK, Ben Zakour NL, Stanton-Cook M. Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci USA 2014; 111:5694–9.

[9] Neupane S, Pant ND, Khatiwada S, Chaudhary R, Banjara MR. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta-lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Nepal. Antimicrob Resist Infect Control 2016; 5(1):5.

[10] Bouacha M, Ayed H, Grara N. Honey bee as alternative medicine to treat eleven multidrug-resistant bacteria causing urinary tract infection during pregnancy. Sci Pharm 2018; 86(2):14.

[11] Bachir RG, Benali M. Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against *Escherichia coli* and *Staphylococcus aureus*. Asian Pac J Trop Biomed 2012; 2(9):739–42.

[12] Abdulkhaliq A, Swaileh KM. Physico-chemical properties of multi-floral honey from the West Bank, Palestine. Int J Food Prop 2017; 20(2):447–54.

[13] Beretta G; Granata P, Ferrero M, Orioli M, Facino RM. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta 2005; 533:185–91.

[14] National Committee for Clinical Laboratory Standards. Methods for determining bactericidal

activity of antimicrobial agents. Tentative Guidelines, M26-TNCCLS,Villanova, PA, 2017.

[15] Patton T, Barett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination ofmicrobial sensitivity to manuka honey. J Microbiol Methods 2005; 64:84–95.

[16] Laallam H, Boughediri L, Bissati S, Menasria T, Mouzaoui MS, Hadjadj S, et al. Modeling the synergistic antibacterial effects of honey characteristics of different botanical origins from the Sahara Desert of Algeria. Front Microbiol 2015; 6:1239.

[17] Alzahrani HA, Alsabehi R, Boukraâ L, Abdellah F, Bellik Y, Bakhotmah BA. Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules 2012; 17(9):10540–9.

[18] Guendouze-Bouchefa N, Madani K, Chibane M, Boulekbache-Makhlouf L, Hauchard D., Kiendrebeogo M, et al. Phenolic compounds, antioxidant and antibacterial activities of three Ericaceae from Algeria. Indus Crops Prod 2015; 70:459–66.

[19] Elamin MM. Phytochemical and ethnobotanical study about Tamarisk Gallicain a North Africa South-West of Algeria. Global J Med Res 2017; 17(1):1–9.

[20] Chermat S, Gharzouli R. Ethnobotanical study of medicinal flora in the North East of Algeria-An empirical knowledge in Djebel Zdimm (Setif). J Mater Sci Eng 2015; 5:50–9.

[21] Abdelmalek M, Moussa A, Noureddine D, Saad A. Antibacterial activity of honey alone and in combination with Nigella sativa seeds against *Pseudomonas aeruginosa* infection. Asian Pac J Trop Dis 2012; 2:S428–30.

[22] Almasaudi SB, Al-Nahari AA, El Sayed M, Barbour E, Al Muhayawi SM, Al-Jaouni S, Harakeh S. Antimicrobial effect of different types of honey on *Staphylococcus aureus*. Saudi J Biol Sci 2017; 24(6):1255–1261.