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Antibacterial activity of various honey types of Algeria against *Staphylococcus aureus* and *Streptococcus pyogenes*

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ABSTRACT

Objective: To assess the *in vitro* antibacterial activity of honey from different geographical location on Gram negative organisms. **Methods:** Different concentrations (Undiluted honey, 10 %, 30%, 50% and 70% wt/vol) of honey were studied *in vitro* using *Staphylococcus aureus (S. aureus)* and *Streptococcus pyogenes (S. pyogenes)*, briefly, two-fold dilutions of honey solutions were tested to determine the minimum inhibitory concentration (MIC) against each type of microorganism, followed by more assays within a narrower dilution range to obtain more precise MIC values. MICs were determined by both visual inspection and spectrophotometric assay at 620 nm. These honey samples were compared with standard antibiotics like ampicillin, penicillin G, amoxicillin, gentamycin, tobramycin, erythromycin and chloramphenicol was determined by the disc diffusion method. **Results:** The diameter of zone of the inhibition (ZDI) of honey has various concentrations tested for the isolates ranged 0-46 mm for *S. aureus*, 0-44 mm for *S. pyogenes*. While the MIC (%) ranged 12%-95%, 25%-73% respectively. **Conclusions:** Algeria honey, *in-vitro*, possess antibacterial activity.

1. Introduction

The emergence in recent years of numerous resistant strains of pathogenic bacteria to a range of formerly efficient antibiotics constitutes a serious threat to public health[1]. Natural products have been traditionally used in the control of various diseases, because they are a source of many active compounds that show multiple therapeutic effects, in addition to constituting models for the synthesis of a large number of pharmaceuticals[2].

The use of honey as a traditional remedy for microbial infections dates back to ancient times. The antibacterial activity of honey refers to some bee products, presence of "inhibin" which acts as an antibacterial factor other than H_2O_2, several factors such as osmotic properties of honey which is saturated or super saturated solution of sugars, 84% being a mixture of fructose and glucose, so inhibition by osmotic effect of dilute solutions of honey obviously depends on the species of bacteria[3].

Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects[4].

Recently, many researchers have reported the antibacterial activity of honey against *Staphylococcus aureus (S. aureus)*, and *Streptococcus pyogenes (S. pyogenes)*[5,6].

The potential antibacterial of diluted honey originating in several countries was already studied[7-10]. Hence till date Algeria honeys has benn used mostly as home remedy. Due to lack of adequate scientific research and documentation the medicinal proprieties of Algeria honeys still remain mostly in dark.

The aim of the present study was to investigate the antibacterial activities of four different Algeria honey collected from different localities against different resistance pathogenic microorganisms. Also, antibacterial activities of certain antibiotics commonly used in the treatment of infections caused by these resistance pathogenic bacteria were evaluated.

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2. Materials and methods

2.1. Honey samples

During the 2011 flowering seasons, four honey samples were gathered and provided by various bee-keepers from two area different from the Algeria west. These honey samples were aseptically collected in sterile screwed cups and kept in a cool and dry place (at room temperature) overnight before they were finally transported to the laboratory.

2.2. Preparation of honey solutions

Honey solutions were prepared immediately prior to testing by diluting honey to the required concentrations (Undiluted, 10%, 30%, 50%, or 70%, wt/vol). All samples were then incubated for 30 min at 37 ℃ in a shaking water bath that allowed aeration of the solutions. Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive[11].

2.3. Test organisms

Micro-organisms were obtained from the department of biomedecine, of the institute of sciences vétériniares university Ibn-khaldoun, Algeria. Two strains of the gram-positives bacteria were *S. aureus* and *S. pyogenes*.

2.4. Preparation of inoculums

One single colony of each type of microorganism (from the nutrient agar stock culture) was inoculated with a sterile loop, and was transferred into 10 mL sterile nutrient broth (bioMérieux, Marcy l'Etoile, France). The broth cultures were incubated in a shaking incubator at 37 ℃ for 24 h.

2.5. Antibacterial activity

Three different methods were used to evaluate the antimicrobial activity of honey: well and disc diffusions Spectrophotometric assay, respectively[12].

Antibacterial activity of honey as evaluated using agar disc iffusion method against test microorganisms. A total of 100 μL of fresh culture suspension of the test microorganisms was spread on respective media Mueller Hinton agar plates (bioMérieux, Marcy l'Etoile, France). The concentration of cultures was 1×10^7 CFU/ mL. For screening, sterile, 5 mm diameter filter paper disc were impregnated with 10 μL of honey equivalent to 0.1 mg of honey after being placed on the surface of the inoculated media agar plates. The plates were stood at 4 ℃ for 2 h before being incubated under optimum conditions for 24 h. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. The diameters of the inhibition zones were measured in millimeter, including the diameter of disc. The controls were set up with equivalent quantities of water as control.

The agar diffusion technique (well diffusion method) was employed. The honey samples were first inoculated separately on standard nutrient media (bioMérieux, Marcy l'Etoile, France). With no test organisms so as to evaluate their possible contamination.Thereafter, solidified nutrient agar plates were separately flooded with the liquid inoculums of the different test organisms using the pour plate method. The plates were drained and allowed to dry at 37 ℃ for 30 mins after which four equidistant wells of 5 mm in diameter were punched using a sterile cork borer at different sites on the plates. 50 μL of the different concentrations (undiluted, 30%, 50% and 70% wt/v) of the honey samples were separately placed in the different punched wells with 1 mL sterile syringe. The plates were allowed to stay for 15 min for pre-diffusion to take place followed by an overnight incubation that lasted for 24 h at 37 ℃. The diameter of zones, including the diameter of the well, was recorded. Each assay was carried out in triplicate.

2.6. Minimum inhibitory concentration (MIC) determination.

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

$$\text{Percentage inhibition} = 1 - (\text{OD test}/\text{OD control}) \times 100$$

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

$$\text{Percentage inhibition} = (\text{OD test}/\text{OD control}) \times 100$$

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

2.7. Antibiotic susceptibility assay

Antibiotic susceptibility for the pathogens and their reference strains was detected using the disk diffusion method, according to the standards set by the CLSI[13]. An aliquot of 100 mL of an overnight culture was diluted in saline solution to about 1.5×10^7 CFU/mL (0.5 Units of McFarland turbidity standard). Mueller Hinton agar (bioMérieux, Marcy l'Etoile, France) plates were flooded with this suspension to give confluent colonies.

For the *S. pyogenes* the Susceptibility to antibiotics was determined using the disc diffusion assay on Muller Hinton agar plates (MHA; BioMérieux, Marcy l'Etoile, France) supplemented with 5% defibrinated sheep blood. The inoculated plates were allowed to stand at room temperature for 15 min prior to dispensing the paper disks and the plates incubated at 37 ℃ for 24 h. The diameters of the clear zones around each disk were measured after incubation.

3. Results

Antibacterial activity of the four honey samples were done with four concentrations 10% (wt/v), 30% (wt/v), 50% (wt/v), 70% (wt/v) and undiluted used in this study are shown in Table 1.

Table 1
Antibacterial activity (zone of inhibition in mm) and MIC% (vol/vol) of honeys at different concentrations against *S. aureus* and *S. pyogenes*.

Honey dilution		Gram-positive bacteria					
		S. aureus			*S. pyogenes*		
		Well (mm)	Disc (mm)	Spectrophotometry MIC% (vol/vol)	Well (mm)	Disc (mm)	Spectrophotometry MIC% (vol/vol)
Honey A	Undiluted	36	40	68	39	22	68
	10%	0	0	84	0	0	25
	30%	0	0	12	25	7	>100
	50%	37	34	>100	25	19	>100
	70%	0	0	>100	27	0	>100
Honey B	Undiluted	40	43	62	44	35	73
	10%	0	0	87	0	0	30
	30%	0	0	24	30	0	>100
	50%	38	35	>100	25	17	>100
	70%	0	0	>100	32	16	>100
Honey C	Undiluted	46	41	73	39	27	74
	10%	0	0	95	0	0	47
	30%	0	0	>100	27	14	>100
	50%	37	38	>100	25	20	>100
	70%	0	0	>100	31	18	>100
Honey D	Undiluted	38	38	54	41	27	59
	10%	0	0	66	0	0	55
	30%	0	0	>100	29	14	>100
	50%	34	34	>100	20	17	>100
	70%	0	0	>100	33	19	>100

The sensitivity of *S. aureus* and *S. pyogenes* against the honey samples studied was screened. Table 1 shows the diameter values of inhibition of *S. aureus* and *S. pyogenes* growth in presence of honey concentrations (Undiluted, 10%, 30%, 50% and 70%) wt/vol. The antibacterial activity was classified as: no sensitive, for diameters lower than 8 mm; sensitive, for diameters from 8 to 14 mm; very sensitive, for diameters from 15 to 19 mm; extremely sensitive, for diameters higher than 20 mm.

Either *S. aureus* or *S. pyogenes* were susceptible to Gentamycin (GM), Chloramphenicol (CHL) and Tobramycin (TBO) but showed resistance to Penicillin G (P), Ampicillin (AM), Oxacillin (OX) and Erythromycin (ERY)

4. Discussion

Disease causing bacteria have always been considered a major cause of morbidity and mortality in humans. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents[14]. The emergence of resistant gram positive bacteria presents a major challenge for the antimicrobial therapy of infectious diseases and increases the incidence of mortality and morbidity. Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria[15,16].

The antibacterial nature of honey is dependent on various factors working either singularly or synergistically, the most salient of which are H_2O_2, phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey[17]. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects[18].

Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source[19-21].

Dilution of honey was observed by Basualdo *et al*[22] who found honey inhibited the growth of *S. aureus* even at 50% dilution. Undiluted honey samples also inhibited the growth of *Staphylococcus uberis* (*S. uberis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) , although to a lesser extent.

Agbagwa and Frank- Peterside[23] examined different honey samples: Western Nigerian honey, Southern Nigerian honey, Eastern Nigerian honey and Northern Nigerian honey, and compared their abilities to inhibit the growth of *S. aureus*, *P. aeruginosa*, *Escherich coli (E. coli)* and *Proteus mirabilis (P. mirabilis)* with an average of ZDIs (5.3-11.6) mm, (1.4-15.4) mm, (4.4-13.5) mm and (9.1-17) mm, respectively, and with honey concentrations of 80%-100%.

Also, Nzeako and Hamdi[24] in their studies of six commercial honeys found that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* did not occur at honey concentrations less than 40% (wt/vol). Similarly, Iurlina and Fritz[24-27] found that honey diluted to concentrations from 75% to 1% (w/v) of full-strength honey showed total antibacterial activity. Also Ahmed *et al*[28] evaluated the *E. coli* and *S. aureus* dde growth in media containing different concentrations of honey and found that the two bacteria failed to grow in honey at a concentration between 5% and 70%. The Tualang honey has been reported to be effective against *E. coli*,

Salmonella typhi (S. typhi) and *S. pyogenes*[29], and thus, when taken orally in its pure undiluted form, this honey may help speed up recovery from such infections. In practice, when undiluted honey is applied to wounds, it is diluted by exudates and its antimicrobial activity at low concentrations is therefore, crucial. For clinical use, the selection of honeys with high levels of antibacterial activity is indicated to maximize therapeutic effects[30]

Some of the standard antibiotics as penicillin, ampicillin, oxacilline, and erythromycin were not effective on the test bacterial isolates.

In the present study, the antibacterial activity was tested using the well and disc-agar diffusion assay and The honey samples were tested without dilution and at 70%, 50%, 30% and 10% (w/v) dilution Most of the undiluted honey samples inhibited the growth of *S. aureus* and *S. pyogenes*.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Raymond Mouokeu S, Rosalie Ngono AN, Paul Lunga K, Martin Koanga M, Alambert Tiabou T, Guy Njateng SS, et al. Antibacterial and dermal toxicological profiles of ethyl acetate extract from Crassocephalum bauchiense (Hutch.) Milne-Redh (Asteraceae). *BMC Complemen Altern Med* 2011; **11**: 43.

[2] Matos FJA. *Farmácias vivas: sistema de utilização de plantas medicinais projetado para pequenas comunidades*. 4th ed. Fortaleza: Editora UFC; 2002.

[3] Saadia Hassanein M, Hassan M Gebreel, Abdel-Rahman Hassan A. Honey compared with some antibiotics against bacteria isolated from burn-wound infections of patients in Ain Shams University Hospital. *J Am Sci* 2010; **6**(10): 510.

[4] Molan PC. The antibacterial activity of honey: the nature of the antibacterial activity. *Bee World* 1992; **73**(1): 5-28.

[5] Hassanain AT, Alyaa AK, Karim AJ. Antimicrobial effect of Malaysian honey on some human pathogens: an *in vitro* Study. *IMJM* 2010; **9**(2) : 98.

[6] Rajneeta S, Vincent B, Dhana R, Prashant S, Peter M. The antimicrobial efficacy of Fijian honeys against clinical isolates from diabetic foot ulcers. *J ApiProduct ApiMedical Sci* 2009; **1** (3): 64-67 .

[7] Nur-Azida MN, Ahmad SH, Kirnpal-Kaur BS, Ananda AD, Mehru- Nisha MH. Antimicrobial properties of tualang honey and its effect in burn wound management: a comparative study. *BMC Complemen Altern Med* 2010; **10**: 31.

[8] Voidarou C, Alexopoulos A, Plessas S, Karapanou A, Mantzourani I, Stavropoulou E, et al. Antibacterial activity of different honeys against pathogenic bacteria. *Anaerobe* 2011; **1**: 1-5.

[9] Sherlock O, Anthony D, Rahma A, Alice P, Georgina G, Seamus C, et al. Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa. BMC Complemen Altern Med* 2010; **10**: 47.

[10]Hayam M Hamouda, Dalia SM. Antibacterial activity of Egyptian honey from different sources. *Int J Microbiol Res* 2011; **2** (2): 149-155.

[11]White JW, Subers MH. Studies on honey inhibine. effect of heat. *J Apic Res* 1964b; **3**(3):45-50.

[12]Patton T, Barrett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *J Microbiol Met* 2006; **64**: 84-95.

[13]National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disc susceptibility tests*. PA: NCCLS Publications; 1993, p. M2-A5.

[14]Roula AM, Elias A, Elias B, Ziad D. Antibacterial activity of the etracts obtained from *Rosmarinus officinalis, Origanum majorana*, and *Trigonella foenum-graecum* on highly drug resistant gram Negative bacilli. J Botany 2010, Article ID: 464087, 1-8 doi:10.1155/2010/464087.

[15]Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res* 2005; **36**: 464-467.

[16]Mundo MA, Padilla-Zakour OI, Worobo RW. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int J Food Microbiol* 2004; **97**: 1-8.

[17]Manisha Deb M, Shyamapada M. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 2011; **2**: 154-160.

[18]Molan PC. The antibacterial nature of honey. The nature of the antibacterial activity. *Bee World* 1992; **73**: 5-28.

[19]Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res* 2005; **36**: 464-467.

[20]Mundo MA, Padilla-Zakour OI, Worobo RW. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int J Food Microbiol* 2004; **97**: 1-8.

[21]Wilkinson JM, Cavanagh HM. Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *J Med Food* 2005; **8**: 100-103.

[22]Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet Microbiol* 2007; **124**: 375-381.

[23]Agbagwa OE, Frank-Peterside N. Effect of raw commercialhoneys from Nigeria on selected pathogenic bacteria. *Afr J Microbiol Res* 2010; **4**: 1801-1803.

[24]Nzeako Hamdi. Antimicrobial potential of honey. *Med Sci* 2000; **2**: 75-79.

[25]Moussac 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); 211-214.

[26]Ravikumar S, Gokulakrishnan R, Boomi P. *In vitro* antibacterial activity of the metal oxide nanoparticles against urinary tract infectious bacterial pathogens. *Asian Pac J Trop Dis* 2012; **2**(2); 85-89.

[27]Iurlina MO, R Fritz. Characterization of microorganisms in Argentinean honeys from different sources. *Int J Food Microbiol* 2005; **105**: 297-304.

[28]Ahmed M, Aissat S, Djebli N, Boulkaboul A, Abdelmalek M, Khiati Baghdad. The influence of starch of Ginger on the antibacterial activity of honey of different types from Algeria against *Escherichia coli* and *Staphylococcus aureus*. *Int J Microbiol Res* 2011; **2**(3): 258-262.

[29]Tumin N, Halim NA, Shahjahan M, Noor Izani NJ, Sattar MA, Khan AH, et al. Antibacterial activity of local Malaysian honey. *Malaysian J Pharma Sci* 2005; **3**: 1-10.

[30]Nagi A AL-Haj, Amghalia E Mariana, Shamsudin N, Rasedee A, Rahmah M, Zamberi S. Antibacterial activity of honey against methicillin resistant *Staphylococcus aureus. Res J Biol Sci* 2009; **4** (8): 943-947.