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Antibacterial activity of various honey types of Algeria against Pathogenic Gram-Negative Bacilli: *Escherichia coli* and *Pseudomonas aeruginosa*

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ABSTRACT

Objective: To assess the *in vitro* antibacterial activity of different honey types in Algeria on Gram negative organismes. **Methods:** Different concentrations (10, 30, 50, 70, 100 % v/v) of honey were studied *in vitro* using *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). 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. MIC was determined by both visual inspection and spectrophotometric assay at 620 nm. The antibacterial activity of these honey samples was determined by the disc and well diffusion method. **Results:** The zone diameter of inhibition of honey for *P. aeruginosa* and *E. coli* was 0-30 and 0-38 mm, respectively, while the MIC ranged 90-91% and 56-96%, respectively. **Conclusions:** The results show that Algerian honeys possess antibacterial activity against Gram negative bacilli, and it can be developed into antibacterial agents.

1. Introduction

The evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, is of great concern to the global health community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is traditional medicine[1]. The use of traditional medicine to treat infection has been practiced since the origin of mankind, and honey produced by *Apis mellifera* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments[2]. However, large variations in the *in vitro* antibacterial activity of various types of honey have been reported and thus hampered its acceptance in modern medicine[3]. The *in vitro* antimicrobial activity of honey was reported by Mohapatra *et al*[4] who observed that honey stopped the growth of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). Honey has a potent antibacterial activity and is very effective in protecting wounds from infection[5]. In some bee products, the antibacterial activity of honey is attributed to the presence of "inhibin", which acts as an antibacterial factor other than hydrogen peroxide. While in other products, several other factors play important roles like osmotic properties of honey which is saturated or super saturated solution of sugars with 84% being a mixture of fructose and glucose[6]. Thereby, the inhibitory activity caused by the osmotic effect of honey dilutions obviously depends on the species of bacteria.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[7]. The potential antimicrobial of diluted honey originating in several countries was already studied[8-11]. However, to our knowledge, no study was carried out before on algeria honey. The aim of the present study was to investigate the antibacterial activities of four different Algeria honey

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collected from different localities. They were tested 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.

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 areas 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 befoe testing by diluting honey to the required concentrations (10, 30, 50, 70 and 100%, v/v). All samples were then incubated for 30 minutes 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[12].

2.3 Test organisms

Micro-organisms were obtained from the Department of Biomedecine, the Institute of Sciences Veterinary University Ibn-khaldoun, Algeria. Two strains of the gram-negatives bacteria: *E.coli* and *P. aeruginosa*.

2.4 Preparation of test organisms

Stocked cultures of *E. coli* and *P. aeruginosa* used in this study were obtained from the Department of Microbiology, Ibn-khaldoun University, Tiaret, Algeria. The isolates were identified based on standard microbiological techniques, and sub-cultured in nutrient agar slopes at 37 ℃ for 24 h. Colonies of fresh cultures of the different microorganisms from overnight growth were picked with sterile inoculating loop and suspended in 3-4 mL nutrient broth contained in sterile test tubes and incubated for 2-3 h at 37 ℃. This was diluted with distilled water to set inoculum density used in this study.

2.5 Antibacterial activity

Three different methods were used to evaluate the antimicrobial activity of honey: well and disc diffusions, and Spectrophotometric assay[13].

Antibacterial activity of honey was tested using agar disc diffusion method against microorganisms.Fresh culture suspension of the test microorganisms (100 μL) was spread on Mueller Hinton agar plates. The concentration of cultures was 1×10^7 CFU/ mL. For screening, 5 mm sterile diameter filter paper disc were impregnated with 10 μL of honey equivalent to 0.1 mg of honey. The plates were placed 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 zone diameters of inhibition (ZDI) was measured in millimeter, including the diameter of disc. The controls were set up with equivalent quantities of water as control.

The well diffusion method was also employed. The honey samples were first inoculated separately on standard nutrient media 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. 10 uL of the different concentrations (10, 30, 50, 70, 100% v/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 mins for pre-diffusion to take place followed by an overnight incubation that lasted for 24 hrs at 37 ℃. The ZDI and the diameter of the well wre 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 and recorded in a spectrophotometer at 620 nm before incubation(T_0), 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 and recorded(T_{24}). The optical density for each replicate at T_0 was subtracted at determined using the formula:

Percentage inhibition = 1 − (OD test/OD control) x 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) x 100

2.7 Antibiotic susceptibility test

Susceptibility to a panel of antimicrobial agents was determined by the standardized disc diffusion assay on Mueller-Hinton agar with commercial antimicrobial susceptibility discs according to the recommendations of the Clinical and Laboratory Standards Institute CLSI/NCCLS. The antibiotics tested and their corresponding disc concentrations were as follows: penicillin G (10 IU), amoxicillin (25 μg), ampicillin (10 μg), amoxicillin (25 μg), gentamicin (10 μg), tobramycin (10 μg), chlo (30 μg), and erythromycin (15 IU). The plates were then incubated at 37 ℃ for 24 h to 48 h. The ZDI was recorded and the data was interpreted using CLSI standards[14].

3. Results

The results of the assays of antibacterial activity of the four honey samples with five concentrations (10, 30, 50, 70, 100% v/v) used in this study are shown in tables 1. The susceptibility of bacteria to antibiotic was tested as shown in Table 2 .

The sensitivity of *E. coli* and *P. aeruginosa* against the honey samples studied was screened. Table 1 shows the ZDI of *E. coli* and *P. aeruginosa* growth in presence of honey concentrations(10, 30, 50, 70 100% v/v). 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.

Table 1
Antibacterial activity ZDI (mm) and MIC of honeys at different concentrations against *E. coli* and *P. aeruginosa*.

Honey type	Concerntration(%)	*E. coli*			*P. aeruginosa.*		
		Well	Disc	MIC	Well	Disc	MIC%
Honey A	10	0	0	56	8	0	90
	30	8	0	>100	0	0	>100
	50	13	19	>100	9	33	>100
	70	12	20	>100	0	0	>100
	100	38	37	96	27	22	91
Honey B	10	0	0	22	10	0	73
	30	12	0	>100	0	0	>100
	50	10	5	>100	10	30	>100
	70	11	18	>100	0	0	>100
	100	31	31	64	30	16	97
Honey C	10	0	0	64	7	0	63
	30	9	0	>100	0	0	>100
	50	1	15	>100	7	28	>100
	70	13	20	>100	0	0	>100
	100	35	32	82	30	22	98
Honey D	10	0	0	81	9	0	90
	30	10	0	>100	0	0	>100
	50	12	19	>100	9	25	>100
	70	17	22	>100	0	0	>100
	100	34	17	97	26	17	94

Table 2 .
Antibiotic susceptibility of *E. coli* and *P. aeruginosa*.

Antibiotic	*E. coli*	*P. aeruginosa*
Penicillin G	R	R
Ampicillin	R	R
Oxacillin	R	R
Gentamycin	S	I
Chloramphenicol	I	S
Erythromycin	S	I
Tobramycin	S	S

R: resistance; I: intermediately susceptible; S: sensitivity

4. Discussion

The emergence of resistant Gram negative bacteria presents a major challenge for the antimicrobial therapy of infectious diseases and increases the incidence of mortality and morbidity[15]. Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic therapy[16]. The antibacterial activity of honey is dependent on various factors working either singularly or synergistically, the most salient of which are hydrogen peroxide, phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey. 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[16]. In this study, we attempted to assess the value of honey from different

botanical sources as an antimicrobial therapeutic agent.

The effect of honey on Gram-negative bacteria was explained by Taormina *et al*[17] who attributed it to the presence of hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, which is unsuitable for bacterial growth and to the presence of phenolic acids, lysozyme and flavanoids. Chauhan *et al*[18] reported that the most suspceptible bacteria included *E.coli* and *P.aeruginosa* with MIC of honey in the range of 0.625-5 .000 mg/mL, and ZDI for the isolates ranged 6.94-35.95 mm, respectively.

AI-Namma[19] also observed that honey has a greater inhibitory effect on Gram negative bacteria. *S. typhi*, *P.aeruginosa*, and *E. coli* are more susceptible than opter test organisms, and honey may have potential as therapeutic honeys. Similarly,Wilkinson and Cavanagh[20] compared the activity of 13 honeys at four concentrations (10, 5, 2.5, and 1% v/v) with corresponding dilutions of an artificial honey, a solution containing the principal sugars found in honey and using *E. coli* and *P.aeruginosa* as the test organisms. Nzeako and Hamdi[21] found that *E.coli* and *P. aeruginosa* were inhibited at a concentration of 40% among the studied six commercial honeys.

In the present study, the antibacterial activity was tested using the well and disc-agar diffusion assay and the honey samples were tested at 100, 70, 50, 30 and 10% (v/v) concerntration. Most of the honey samples inhibited the growth of *E. coli* and *P. aeruginosa*.

This study provided a sight on the antibacterial activity honey of Algeria and proved that many honeys have the potential for the therapeutic use as antibacterial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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