Iraqi Journal of Science

THE ACTIVITY OF NATIVE HONEY AGAINST SOME MICROORGANISMS

Sinai Waleed

Tropical - Biological Research Unit, College of Science, University of Baghdad. Baghdad-Iraq.

Abstract

The present study was conducted to detect the susceptibility of six microorganisms isolated from patients suffering from urinary tract and wound infections against native honey, by the agar-well diffusion method. These isolates included: *Pseudomonas sp., Klebseilla sp., Escherichia coli, Proteus sp., Staphylococcus aureus* and *Candida albicans*.

The results showed that the bacteria isolates were resistant against most of the antibiotics uesd. Two isolates were sensitive to Amikacin and Co- trimoxazole, while all isolates were resistant to Gintamycin and Cephotaxime. In contrast, honey at 5% concentration was able to inhibit the growth of most microorganisms tested, with the exception of only *Pseudomonas sp.*, which was inhibited at concentration of 10%.

فعالية العسل الطبيعي ضد بعض الاحياء المجهرية

الخلاصة

أجريت الدراسة الحالية لمعرفة حساسية ست عزلات مجهرية تم الحصول عليها من مرضى يعانون من خمج المجاري البولية و الجروح ضد العسل الطبيعي الخام باتباع طريقة الانتشار بالحفر Agar-well diffusion، وهذه العزلات هي : *Pseudomonas sp., Klebseilla sp., Escherichia coli, Proteus sp., Staphylococcus aureus, Candida albicans.*

أظهرت النتائج ان العزلات البكترية كانت مقاومة لمعظم المضادات الحياتية المستخدمة في الدراسة, حيث وجد ان عزلتين من البكتريا كانت حساسة للمضادين Amikacin و Co- trimoxazole, بينما كانت جميع العزلات مقاومة للمضادين Gentamycin و Cephtaxime . وعلى العكس من ذلك فقد أظهر العسل في التركيز 5% فعالية تثبيطية لنمو معظم العزلات المدروسة ماعدا *Pseudomonas sp.* فقد ثبطت عند التركيز 10%.

Introduction

Honey is one of the oldest known natural products that have continued to be used up to present times in folk medicine [1]. It has been associated with curative and antimicrobial effects. Heart and liver diseases, coughs, constipation, gastrointestinal disturbances, measles, ulcers and wounds have all been treated with honey [2].

Other study has reported that honey has a higher antibacterial activity against infections in burn wounds, which was more than the antibacterial ointment silver sulphadiazine [3]. It has been also reported that honey showed antifungal activity against *Candida albicans* isolated from oral cavity of normal healthy humans [4].

It has been suggested that the antimicrobial properties of honey are related to the release of low levels of hydrogen peroxide, osmotic effect due to high carbohydrate concentration and some other phytochemical components [5].

The objective of this study was to examine the antimicrobial activity of single sample of honey

against microorganisms isolated from urinary tract and wound infections.

Materials and Methods

Isolation and identification

Samples were collected from urine and wound infections, from patients attending Al-Yarmouk Teaching Hospital during the period from 1/2/2004 to 1/5/2004. All samples were cultured directly on blood agar, MacConkey agar and sabouraud dextrose agar. The cultures were examined after overnight incubation at 25°C - 37°C. Identification of microorganisms was based on Gram stain, culture methods and biochemical tests [6, 7, 8].

- Identification of Gram negative bacteria was based on the following tests: Indole test, TSI test, citrate utilization test, urease test, motility test and oxidase test.
- Identification of *Staphylococcus aureus* was based on the following tests: catalase test, hemolysis test, manitol salt agar test and coagulase test.
- Identification of *Candida albicans* was based on the following tests: germ tube formation and chlamydospore production.

Antibiotics sensitivity test

It was carried out by using Muller-Hinton medium in which, 5-10 colonies of each isolate were picked up with a sterile loop and suspended in 2.5ml of sterile distilled water, and the suspension was taken by a sterile cotton swab, and then streaked the surface of all the plate in three different directions. Using sterile forceps, the antibiotic disks (Augmentine 30mcg, Amikacin 30mcg, Chloramphenicol 30mcg, Co-trimoxazole 30mcg, Cephotaxime 30mcg, Tetracyclin 30mcg and Gentamycin 10mcg) were placed on the inoculated plates and pressed gently on the surface of the agar and then incubated at 37°C for 18-24 hours. After incubation, the diameter of complete inhibition zones was measured [9]

Honey sample

A single randomly selected sample of honey was obtained from a local Apiary. It did not contain any additives or diluents, and was not heated. The honey was diluted with a sterile distilled water (v/v) in three concentrations (5, 10 and 20%).

Agar – well diffusion method

Antimicrobial activity of honey was determined by agar well diffusion method [10]. Wells (6 mm diameter) were made in Mueller-Hinton agar. Plates were seeded with 0.1 ml of 24 hours inoculated broth of tested microorganisms. The different concentrations of honey (equal volum) were added to the wells. The inoculated plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measuered for each plate and the average reading of two replicates for each microorganism was taken. The standard Amikacin disk (30 mcg) and Co-trimoxazole disk (25 mcg) were used as a control.

Statistical analysis: One way Anova test was used in the analysis of results.

Results and Discussion

Identification of isolated microorganism

After the different biochemical tests were done, five bacterial isolates (*Pseudomonas sp., Klebseilla sp., Proteus sp., E. coli* and *Staphylococcus aureus*) and one yeast isolate (*Candida albicans*) were isolated (Tables 1, 2 and 3).

Antibiotic sensitivity

The standard disk diffusion method was used to determine the antibiotic resistance pattern of bacteria isolates against seven different antibiotics (Table 4).

Generally, a resistance was detected among most bacteria isolates against the antibiotics used. It was found that the effective antibiotics against these isolates were Amikacin (aminoglycosid group) and Co-trimoxazole (sulphonamides and trimethoprim group), in which two isolates were sensitive to them, while only three isolates were resistant. Followed by Augmentine (β-lactam-pencillin group), chloramphenicol, Tetracycline, in which only one isolate was sensitive to them, while four isolates were resistant. The less effective antibiotics (all isolates were resistant to them) were Cephotaxime (β-lactam-ephalosposporin gorup) and Gentamycin (aminoglycosid group). Development of antibiotic resistance may be explained by different mechanisms; it may either involve a modification in DNA gyrase, the target enzyme of quiolones, or due to a modification in the bacterial outer membrane proteins, rendering the drug unable to penetrate inside the bacteria [11].

Honey activity

The present investigation showed that the honey at 5% concentration inhibited the growth

of tested microorganisms, with the exception of *Pseudomonas sp.*, which was inhibited at 10% (Table 5). These findings agreed with Badawy *et al.*[12] but they are not in agreement with Subrahmanyam *et al.*[13], which found that the inhibitory concentraion of honey was 30% while Mulli and Menon [14] found that the inhibitory concentration of honey was 11%.

Figure (1) shows that increasing the concentration of honey lead to increase inhibition zones of all microorganisms, and such differences were significant ($P \leq 0.05$).

The antimicrobial activity of honey appears to be concentration dependent due to osmotic effect, because honey has high concentration of carbohydrates such as glucose and fructose, resulting in a very low water content. This condition inhibits the growth of bacteria by removing the water from bacteria cell. Also, the pH (3.2 – 4.5) which is low enough to be inhibitory to many pathogens. Another antimicrobial factors are hydrogen peroxide (H_2O_2) and phytochemical factors (1, 4). In this study, it was demonstrated that the employed honey concentrations (5 - 20%) were the effective in inhibiting the growth of all isolated microorganisms.

Table (1): Biochemical tests for the identification of Gram negative bacteria

Bacterial isolates	Indole test	TSI test	H_2S production	Citrate utiliztion test	Urease test	Motility test	Oxidase test
E. coli	+	A/AG	-	-	-	+	-
Pseudomonas sp.	-	Alk /Alk	-	-	-	+	+
Proteus sp.	-	Alk /AG	+	+	+	+	-
Klebsiella sp.	-	A /AG	-	+	+	-	-

A = acid, Alk = alklain, G = gas, + = positive, - = negative

Table (2): Biochemical tests for the identification of *Staphylococcus aureus*.

Biochemical tests	*S. aureus*
Gram stain	+
Catalase test	+
Hemolysis	β
Manitol salt agar	+
Coagulase	+

Table (3): Morphological identification of *Candida albicans*.

Test	*C. albicans*
Gram stain	+
Shape	Spheroid-ovate
Germ tube formation	+
Chlamydospore production	+

Table (4): Antibiotic sensitivity to tested bacteria

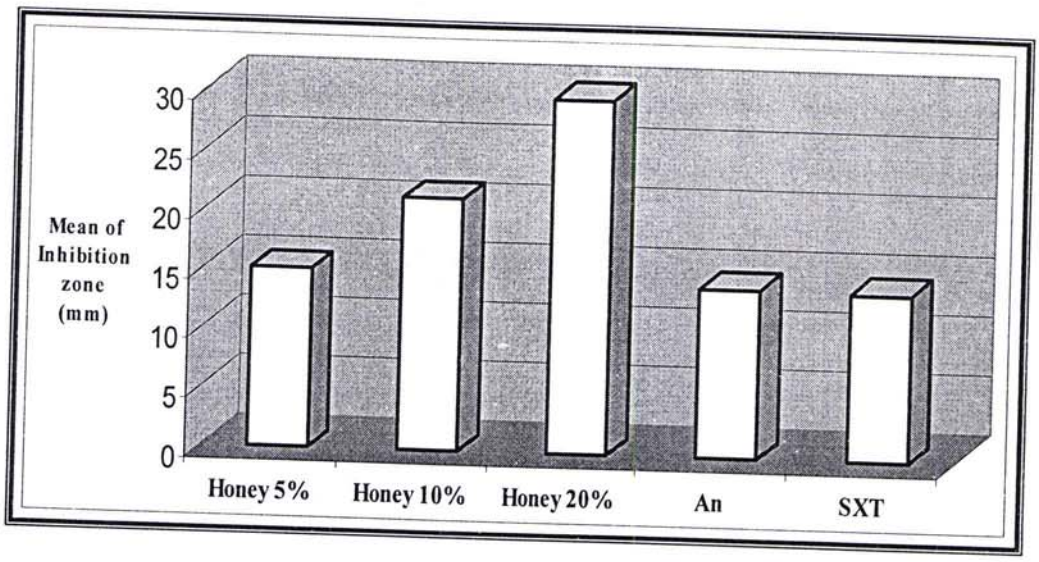
Bacteria	Augmentine 30mcg	Amikacin 30mcg	Chloramphenicol 30mcg	Co-trimoxazole 25mcg	Cephotaxime 30mcg	Tetracycline 30mcg	Gentamycin 10mcg
Pseudomonas sp.	R	R	R	R	R	R	R
Klebsilla sp.	R	R	R	R	R	R	R
E. coli	R	S	S	R	R	R	R
Proteus sp.	S	R	I	S	R	R	R
Staph. aureus	R	S	R	S	R	S	I

R = resistant, S = sensitive, I = intermediate

Table (5): Minimum inhibitory concentrations of honey measured in millimeters.

Microorganisms	Diameters of inhibition zone (mm)				
	Honey concentration			Antibiotics	
	5%	10%	20%	An 30mcg	SXT 25mcg
Pseudomonas sp.	0	6	10	10	11
Klebsiella sp.	15	20	30	12	9
E. coli	19	26	34	18	10
Staphylococcus aureus	21	30	35	20	18
Proteus sp.	20	25	35	11	22
Candida albicans	15	20	35	ND	ND

An= Amikacin, SXT= Co-trimoxazole, ND= not done, ANOVA test = $P < 0.05$



An= Amikacin, SXT= Co-trimoxazole

Figure (1): Inhibition zones (mm) for honey and two antibiotics against the investigated microorganisms.

References

1. Malon, P. C., **2001,** " *Honey as a topical antibacterial agent for treatment of infected wounds*" World wide wounds, 1-3.
2. Cliver, D. O., Snowdon, J. A., **1996,** "*Microorganisms in honey*" International J. Food Microbiol., 31: 1-26.
3. Molan, P., **2000,** "*The Curative Property of Honey*" Waikato University Press, New Zealand, 10-15,
4. Theunissen, F., Grobler, S. and Gedalla, I., **2001,** " *The antifungal action of three south african honeys on Candida albicans*" Apidologie, 32, 371-379.
5. Malon, p., **2001,**" *Honey as a topical agent for treatment of infected wounds*" World wide wounds, 1-13.
6. Gupte, S., **1982,** "*The short textbook of medical microbiology*" JayPee Broth., Del., 330.
7. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T., **1994,** "*Bergey's manual of determinative bacteriology*" 9th edn. William. Wilk., Maryl, 285-289
8. Kobayashi, G.S., Pappagianis, D., **1980,** "*The Mycoses*" In: Grad whole's clinical laboratory methods and diagnosis. Edited by Sonnenwirth A. C. and Jaratt L. 8th ed. The C.V. Mosby Company. St. Louis. Toronto. London, pp: 2211-2239
9. Atlas, R. M., Brown, A. E. and Parks, L. C., **1995,** "*Laboratory manual of experimental microbiology*" Mosby, Missour., 565
10. National Committee for Clinical Laboratory Standards (NCCLS), **1993,** "*Performance standards for antimicrobial disk susceptibility tests*" NCCLS, Pennsylvania, USA, M2-A5.
11. Hussein, H. K., **2007,** "*Detection the occurrence of Helicobacter pylori among dyspeptic pateint and susceptibility to antibiotic agents*" J. of Al-Nahrain Unv., Vol.10(2), 98-106.
12. Badawy, O. F. H., Shafii, S. S. A., Tharwat, E. E. and Kamal, A. M., **2004,** "*Antibacterial activity of bee honey and its therapeutic usefulness against Escherichia coli O157: H7 and Salmonella typhimurium infection*" Rev. sci. tech. off. Int. Epiz., 23 (3), 1011 – 1022.
13. Subrahmanyam, M. and Hemmady, A., **2001,** "*Antibacterial activity of honey on bacteria isolated from wounds*" Annals of Burns and Fire Disasters, Vol. XIV-n.1, 1-4.
14. Mullai, V., and Menon, T., **2005,** "*Antibacterial activity of honey against Pseudomonas aeruginosa*" Indian journal of pharmacology, Vol. 37 (6), 403