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Antibacterial Activity of Egyptian Honey from Different Sources

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Abstract: With the rise in prevalence of antibiotic-resistant bacteria, honey is increasingly valued for its antibacterial activity. The antibacterial activity of honey samples provided by apiarists and different kind of honey obtained from local supermarket were tested against different microorganisms. The honey samples were tested without dilution and at 75, 50, 30 and 10% (w/v) dilution. The diameters of the inhibition zones generated by honey samples against some (Gram -ve and Gram +ve) bacteria as *Pseudomonas aeruginosa, Escherichia coli*, extended-spectrum β-lactamase producing *Klebsiella pneumoniae, Klebsiella oxytoca, Bacillus cereus, Bacillus subtlus, Bacillus pumilus, Bordetella brochisptica and Micrococcus luteus* indicatedthe presence of antibacterial activity. The diameters of the inhibition zones generated by honey samples provided by apiarists were similar to those generated by other honey. Aantibacterial activity of antibiotics used in treatment of infection with these bacteria were evaluatedt. On the other hand, two phenolic acids were found to be partially responsible for the activity of the tested honey samples. Ferulic acid was quantified 0.312 mg/kg honey and pinobanksin as 0.72 mg/kg honey. In conclusion, Honey can be recommended as an alternative treatment for infected wounds, especially those caused by antibiotic-resistant bacteria.

Key words: Antibiotic-resistant bacteria · Honey · Ferulic acid

INTRODUCTION

Honey forms part of the traditional medicine in many cultures according to G´omez-Caravaca and others [1] Antibiotic resistance emerged as major global problem by Amabile-Cuevas [2]. Honey is being used in a few hospitals, especially in the clinical treatment of ulcers, bedsores, burns, injuries and surgical wounds. The antibacterial properties of honey may be particularly useful against bacteria which have developed resistance to many antibiotics, e.g. *Staphylococcus aureus*, which is a major cause of wound sepsis in hospitals [3]. Honey is thus an ideal topical wound dressing agent in surgical infections, burns and wound infections [4].

The present study aimed to evaluate the antibacterial activity of Egyptian honey samples provided by apiarists and different kind of honey obtained from the local supermarket 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.

MATERIALS AND METHODS

Honey Samples: Honey samples were obtained from two sources; from a local apiary as well as four samples were obtained from Egypt market. Samples were stored at (23-25°C) in dark place. For antibacterial tests honey samples were used undiluted and at 75, 50, 30 and 10% dilution (grams of honey diluted to a final volume of 100ml).

Bacterial Strains: Strains of *Pseudomonas aeruginosa, Escherichia coli*, extended-spectrum β-lactamase producing *Klebsiella pneumoniae, Klebsiella oxytoca, Bacillus cereus, Bacillus subtlus, Bacillus pumilus, Bordetella brochisptica and Micrococcus luteus* were kindly donated by Microbiology Laboratory of NODCAR. Bacterial sub-cultured in nutrient broth and incubated for 18 hrs at 37°C.

Antibacterial Test: Agar-well diffusion assay was used according to National Committee for Clinical Laboratory Standards. Plates were inoculated with test organisms

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except two plates left as control. Five holes were drilled on the culture media and 50 μl of each honey dilution were added to each hole. The plates were incubated for 24hrs at 37°C. The diameters of inhibition zones obtained were measured according to CLSI [5].

Antibacterial Activity of Some Antibiotics: Ten antibiotic discs were tested (Oxoid); tobramycin, erythromycin, ampicillin, tetracycline, ciprofloxacine, amikacine, meropenem, cefotaxime and chloramphicol. The organisms were inoculated onto muller-Hinton agar. The cultured plates were incubated for 24hrs at 37°C. The diameters of inhibition zones obtained were measured according to CLSI [5].

High Performance Liquid Chromatography [HPLC]: A HPLC Perkin-Elmer system (USA) equipped with a binary LC-290 UV/vis was used.

Samples Preparation: 50ml of ethyl acetate in separated funnel six times. 300ml ethylactate extract was concentrated in a rotary evaporate under vacuum at 30°C to about 1ml. This concentration was taken up in methanol in sterile tube and stored at 0°C according to Sivam [6].

RESULTS

Activity of honey against six different pathogenic bacteria was carried out and the inhibition zones of apiary honey samples are shown in Table 1. It was observed that 100% of apery honey gives maximum antibacterial activity, especially against *Klebsiella* strains. The average diameter of the inhibition zones produced by these samples was 39.1mm.

The growth of other bacteria was also inhibited by these honey samples, although to a lesser extent. *P. aeruginosa* was not inhibited except for only two (100 and 75%) dilutions of honey A.

Apery honey samples show antibacterial activity at even 10% concentration and the average diameter of the inhibition zones produced was 32 mm.

Activity of market honey (B, C, D) against six different pathogenic bacteria and the inhibition zones of these honey samples are shown in Table 2. Honey inhibited all types of bacteria used in this study. Honey samples C and D showed antibacterial activity at undiluted and 75% dilution, only against *P. aeruginosa*. No inhibition of bacterial growth was observed when honey was diluted at 50, 30 and 10%.

Honey samples B showed antibacterial activity at all dilution for the bacteria used, except for *Bordetella brochisptica* which were not affected at 30 and 10%dilution.

The activities of honey samples (A, B, C, D and E) against different Bacillus spp. are presented in Table 3. The honey (undiluted and diluted) samples shown antimicrobial activities against all *Bacillus* spp. tested except the 10% honey dilution of all tested honey which did not show any antimicrobial activities against *Bacillus cereus.*

Susceptibility of bacteria to antibiotic was tested as shown in Table 4. It was observed that Meropenem is the most effective antibiotic except for *Bacillus*. While *Ps. aeroginosa* was resistant to all antibiotics but Meropenem. *Salmonella.spp.* was resistant to Erhromycin, Ampicillin and Cefotaxime while it was susceptible to other antibiotics. The effect of antimicrobial agent decrease ampicillin AM, > Amikacin AK> tobramicin TOB> ciprofloxacin CIP> Rifampicin RD> cefotaxime CTX >tetracycline TE.

Table 1: Inhibition zones of apiary honey samples

Honey sample	Honey dilution	*K. oxytoca*	*M. luteus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*Bordetella brochisptica*
A	Undiluted	50	42	38	50	25	36
	75%	42	41	36	40	25	36
	50%	38	38	35	36	0	33
	30%	34	36	29	32	0	31
	10%	32	21	26	27	0	28
E	Undiluted	45	40	40	40	0	38
	75%	41	41	36	38	0	36
	50%	39	39	32	35	0	32
	30%	36	36	30	32	0	30
	10%	29	29	28	28	0	25

Table 2: Inhibition zones of different of market honey

Honey sample	Honey dilution	*K. oxytoca*	*M. luteus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*Bordetella brochisptica*
B	Undiluted	40	45	36	39	34	35
	75%	39	45	36	36	32	35
	50%	38	36	33	33	30	30
	30%	36	36	30	30	29	0
	10%	30	30	26	25	26	0
C	Undiluted	47	45	41	45	50	40
	75%	43	43	39	38	39	36
	50%	39	40	35	37	0	35
	30%	36	40	30	33	0	30
	10%	34	39	27	24	0	26
D	Undiluted	40	42	48	45	32	38
	75%	40	42	41	38	0	32
	50%	36	40	36	36	0	33
	30%	34	35	31	31	0	30
	10%	26	29	26	24	0	25

Table 3: Inhibition zones of different of honey samples against tested *Bacilus* spp

Bacteria	Honey dilution	A	B	C	D	E
Bacillus pumlus	100%	41	40	41	41	45
	75%	40	40	39	39	39
	50%	39	38	39	39	38
	30%	36	33	36	34	36
	10%	31	30	32	32	33
Bacillus subtilis	100%	40	43	34	34	17
	75%	40	40	35	34	36
	50%	32	38	37	35	37
	30%	31	35	40	34	40
	10%	17	25	35	30	33
Bacillus cereus	100%	40	36	4	36	40
	75%	40	35	38	38	35
	50%	36	35	33	32	33
	30%	35	30	27	27	0
	10%	0	0	0	12	0

Table 4: Inhibition zone in mm of bacteria to the tested antibiotic discs

	Antibiotics								
	CTX	MEM	RD	AK	CIP	TE Bacteria	AM	TOB	E
Bacillus	6 R	9 R	11 R	27	15	6 R	7 R	21	6 R
P. aeroginosa	0 R	18	0 R	0 R	0 R	11 R	8 R	0 R	0 R
Salm. Spp.	0 R	22	18	18	20	14	0 R	18	0 R
K. pneumoniae	12	20	7 R	13	16	10 R	7 R	18	6 R
S. aureus	6 R	34	10 R	23	6 R	8 R	14	18	6 R
M. luteus	12	25	10 R	27	3 R	7 R	0 R	18	0 R
S. epidermes	10 R	13	20	12	21	25	0 R	0 R	26

R= resistance

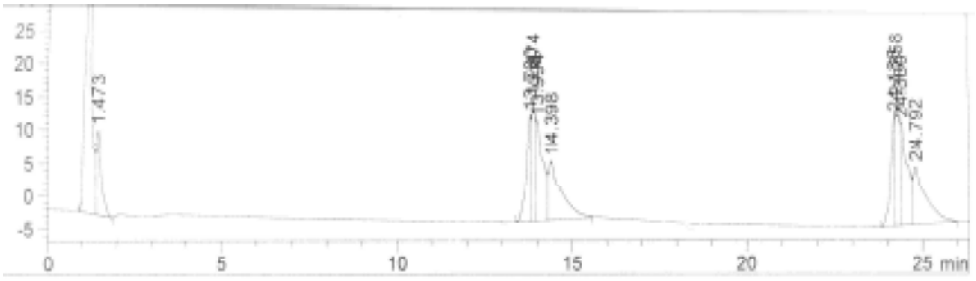

Fig. 1: Honey chromatogram

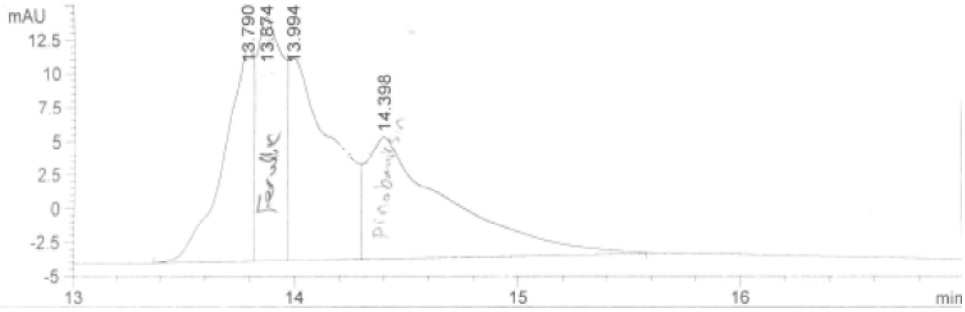

Fig. 2: Honey chromatogram and identified phenol compounds

Phenol Composition of Honey Samples: HPLC, which is the method of choice for food phenolic analysis, was used in this study for the identification of honey phenolics.

In the present study, Ferulic acid was quantified as 0.312 mg/kg honey and pinobanksin as 0.72mg/kg honey as shown in Figures (1, 2).

DISCUSSION

The antibacterial capacity of honey was, first reported in 1980 and, is currently being revised. Two main theories have been proposed to explain this capacity: one is that well documented by Dustmann [7] who reported that it is due to the action of the hydrogen peroxide in honey that is produced by glucose oxidase in the presence of light and heat. The other is that well documented by Roth and others [8] who interpreted that it is the nonperoxide activity, which is independent of both light and heat, that inhibits microbial growth. In this respect, Molan and Russell [9] observed that this nonperoxide activity, which remains unaltered even during long storage times, depends on the flower source of the nectar used and so not all honeys possess this activity.

The major components of honey are sugars, which themselves possess antibacterial activity due to the osmotic effect [10], although studies carried out to test this antimicrobial activity use concentrations at which the sugars are not osmotically active. It is also well known that honey contains lysozyme, a powerful antimicrobial agent Bogdanov [11]. Other researchers attributed the antimicrobial capacity of honey to a combination of properties, such as its low pH and high osmolarity or to the presence of certain volatile substances, although this has not been studied in great depth according to many investigators [12,13].

The antimicrobial activity of both honey and propolis is basically against Gram-positive bacteria [14]. Burdock [15] attributed this capacity to the presence of aromatic acids and esters, while, Takaisi and Schilcher [16] suggested that it is due to the action of the flavonone pinocembrin and the flavonol galangin and caffeic acid phenethyl ester, whose action mechanism is based on the inhibition of bacterial RNA polymerase. Cushnie and Lamb [17] reported that other flavonoids such as galangin also present antibacterial action. The action mechanism involves degrading the cytoplasm membrane of the bacteria, which leads to a loss of potassium ions and the damage caused provoking cell autolysis.

Mirzoeva and others [18] reported that Quercetin, which is also found in honey, increases membrane permeability and dissipates its potential, leading the bacteria to lose their capacity to synthesis ATP, their membrane transport and motility. While the antibacterial capacity of honey is clear, there seems to be no one clear-cut cause, suggesting that there is a combined or synergistic effect at work.

Antibiotic resistance of bacteria is on the rise, thus the discovery of alternative therapeutic agents is urgently needed. Honey possesses therapeutic potential, including wound healing properties and antimicrobial activity.

This study showed that the growth inhibition is complete in the media containing 100%, partial in media containing 75, 50 and 30% and no inhibition was produced by 10% honey.

The effect of honey on Gram-negative bacteria was explained by Taormina *et al*. [19] who attributed it to the presence in bee honey 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.

Apery honey has highest antibacterial activity against *Micrococcus* and *Klebsiella* isolates and lowest activity against *Pseudomonas i*solate. Douglas *et al*. [???] stated that *Pseudomonas aeruginosa* continued to be a serious cause of infection and septic mortality in burn patients, particularly when nosocomially acquired and that all recent efforts were directed to solve this problem.

All concentrations of honey B have antibacterial activity against *Micrococcus* and *Klebseilla* isolates and lowest activity against *E. coli* and *Pseudomonas* isolate especially in concentration 10% It is however possible that the hydrogen peroxide production was not at its peak at the time of testing. It has been shown that hydrogen peroxide production can peak at different times for different honeys. Some may take as long as 24 hours [20].

In the present study, honey C had similar antibacterial activity to that of honey B except for it has high activity at concentration 10% to *K. oxytoca, M luteeus*. Other researchers attributed the antimicrobial capacity of honey to hydrogen peroxide was produced at the very low dilutions and at concentrations of ± 1 mg/liter. These concentrations are too low to have any bacteriostatic effect. Concentrations of at least 10 mg/litre are required to inhibit bacterial growth [21].

In the present study, honey D has antibacterial activity in undiluted, 75 and 50% but start to decrease with decreasing the dilution percentage particularly against *E coli, K pneumoniae P. aeruginosa and Bordetella*. It has been reported that the antimicrobial activity of honey may range from concentrations lower than 3% to concentrations of 50% and higher according to many investigators [22-24].

In the present study, an interesting point was observed, whereas *Bacillus* spp. Were found to be sensitive to all kind of honey at the concentrations undiluted, 75, 50, 30 and 10% except *Bacillus cereus*. *B. cereus* causes. The antibacterial activity of honey was attributed to the presence of organic components originating from floral source [10].

In the present study, The eluted compounds were detected at 290nm because and most of the phenolic compounds showed reasonably high absorbance at this value [14]. Ferulic acid averaged as 0.312mg/kg honey and pinobanksin as (0.72 mg/kg honey as shown in Figures 1 and 2. This finding agree with those of Weston *et al.* [25] and Weston *et al.* [26]. Most of these organic components are phenolics (flavoids or phenolic acids) in nature and are of plant origin Therefore, the variation in the antibacterial activity of honeys could be attributed to their phenolics [10]. Phenolic substances that include cinnamic acid derivatives (mainly prenylated compounds) and some flavonoids are detected in honey and propolis [14].

The antibacterial activity of honey samples provided by apiarists and honey packers was tested against microorganisms usually isolated from skin wounds.

The antibacterial activity was tested using the well-agar diffusion assay. The honey samples were tested without dilution and at 75, 50, 30 and 10% (w/v) dilution. Most of the undiluted honey samples inhibited the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Some honey samples provided by apiarists also inhibited the growth of *S. aureus* even at 50% dilution.

Undiluted honey samples also inhibited the growth of *Staphylococcus uberis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*, although to a lesser extent. No inhibition of *Micrococcus luteus* and *Enterococcus faecalis* growth was detected. The diameters of the inhibition zones generated by honey samples provided by apiarists were larger than those generated by honey samples provided by honey packers. This observation may be explained by considering the provenance of the honey samples [27].follow by infections with *K pneumoniae, E coli* and other pathogen microorganisms [28].

It was concluded that the honey samples were tested without dilution and at 75, 50, 30 and 10% (w/v) dilution have antibacterial activity. Also detected in honey Ferulic acid averaged as 0.312mg/kg honey and pinobanksin as (0.72 mg/kg honey. Therefore, the variation in the antibacterial activity of honeys could be attributed to their phenolics.

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