

Antimicrobial Activities of Natural Honey from Aromatic and Medicinal Plants on Antibio-resistant Strains of Bacteria

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ABSTRACT

Samples of natural honey from different aromatic and medicinal plants were studied for their antimicrobial activities on antibio-resistant strains of bacteria isolated from human pathology. Samples of honey from four different plants mainly: *Thymus broussonetti* Boiss, *Origanum vulgare*, *Eucalyptus globulus*, *Euphorbia resinifera* and multifloral were collected from different regions in Morocco. Dilutions of honey ranging from 1/2, 1/4, 1/8 and 1/16 were tested by the agar well diffusion method on various strains of bacteria including *E. coli*, *Staphylococcus aureus*, *Bacillus* and *Pseudomonas*. Results revealed that most of strains were inhibited by the dilution 1/2 and 1/4. The Gram negative bacteria were more sensitive to honey than Gram positive bacteria. No difference among the different origins of honey (plants) was observed. All the samples showed strong antimicrobial activities on the bacterial strains, which were first tested for their resistance to antibiotics.

Key Words: Honey; Antimicrobial activity; Antibio-resistant; Aromatic plants; Multifloral

INTRODUCTION

Honey has been used as a medicine since ancient times in many cultures, and is still used in folk medicine. Use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of ulcers and bed sores, and other skin infections resulting from burns and wounds (Allen *et al.*, 1991). However, little is known about the phenomena involved in the inhibition of microorganisms. In fact, honey has been found to be effective against microorganisms isolated from the urinary tract infection (Ibrahim, 1981), and in the treatment of infantile gastro-enteritis (Haffjee & Moosa, 1985). It also possesses beneficial anti-inflammatory and antimicrobial associated with the high antioxidant content (Molan & Cooper, 1999). Different aspects of the antimicrobial properties of honey have been extensively reviewed (Molan, 1992). A great deal of work has been done in attempt to identify the antimicrobial factors/compounds in honey and the range of organisms susceptible to this antimicrobial action. The word "inhibine" was introduced to describe these antimicrobial agents (Dold *et al.*, 1937) and this term is still used today.

Several mechanisms have been suggested to explain the antimicrobial activity of honey. This activity has been attributed to the hydrogen peroxide H_2O_2 (Adock, 1912) generated by the action of the enzyme glucose oxidase (White *et al.*, 1962). But, high antimicrobial activity of manuka honey has been entirely due to this non-peroxide compound (Allen *et al.*, 1991). Wahdan (1998) found that

hydrogen peroxide is not the only inhibiting compound in honey. In fact, inhibitors in honey include many other compounds, two important classes of which are flavonoids (Havsteen, 1983) and phenolic acids (caffeic acid & ferulic acid).

It is well assumed that pH of honey is quite low, ranging from 3.2 to 4.5. This low pH is attributed to the presence of gluconic acid (White, 1975). This value is considerably lower than the optimum pH required for the growth of most bacteria, which is around 7 (Molan, 1992). Additionally, non-dissociated organic acids also play an important role in the antimicrobial activity of honey (Marcis, 1975) since they are highly soluble in cell membranes. Molan and Russel (1988) reported that the floral source of honey is also responsible for its antimicrobial activity.

Honey is a saturated solution of sugars, with very low water content (Anonymous, 1988). This condition is inhibitory to the growth of bacteria. On the other hand, fungi are generally more tolerant than bacteria to the high osmotic effect (Molan, 1992). Other phenomena including lysozyme and volatile compounds identified in honey might play a role in the inhibition of microorganisms (Bogdanov, 1984).

This paper describes antimicrobial activity of honey against some bacteria.

MATERIALS AND METHODS

Sample collection. Thirty samples of honey were obtained from five different floral sources throughout Morocco. All samples were kept at 8°C in the dark.

Preparation of honey solutions. Solutions of honey were prepared by 50% (v/v). Each sample was diluted in sterile distilled water to give final concentrations of 6.25, 12.5, 25 and 50%.

Microbial cultures. Sixteen isolates of bacteria were used: *Staphylococcus aureus* (4 strains), *Pseudomonas aeruginosa* (4 strains), *Escherichia coli* (4 strains), *Bacillus megaterium* (1 strain), *Bacillus subtilis* (1 strain), and *Bacillus sp* (2 strains).

Antibiotic sensitivity of microorganisms. Antibiotic sensitivity of bacterial isolates was performed by the disc diffusion method (Collins *et al.*, 1995). The antibiotics (Safoni Diagnostics Pasteur, France) used were E: Erythromycin 15 µg; ST: Sulfathiazol 0, 25 mg; AMX: Amoxycillin 25 µg; D: Doxycillin 30 µg; DA: Clindamycin 2 mg; CE: Cefalothin 30 µg; AM: Ampicillin 10 µg; TM: Tobramycin 10 µg; CP: Ciprofloxacin 5 µg. The bacterial suspension (100 µL) was inoculated onto Muller Hinton Agar plates (Biocar, France), and allowed for 15 to 20 minutes to solidify. The antibiotic discs were placed aseptically in the plates (5 discs/plate). These plates were then incubated at 37°C for 24 h. Diameter of the inhibitory zone around the discs was measured and recorded in millimeters.

Antimicrobial activity. The antimicrobial activity was studied by the agar well diffusion method (Collins *et al.*, 1995). The inoculum suspension of each strain of bacteria was prepared to give a concentration of 10^7 to 10^8 bacteria/mL. The bacterial strains were grown on Trypticase soy broth for 24 h. 100 µL of this culture was added to 9 mL of natural saline water (8.5 g/L) and 100 µL from this suspension was transferred to sterile petri dishes. 15 mL of Muller Hinton Agar (Biocar, France) was poured aseptically and the plates were kept for 15–20 minutes at room temperature to allow agar to solidify. Wells of 4 mm height and 4 mm in diameter were then made in the solid medium

Table I. Antibiotic sensitivity of the strains used in the antimicrobial activity of honey

	E	ST	AMX	D	DA	CE	AM	TM	CP
<i>S. aureus</i> 1	R	R	R	R	R	R	R	R	R
<i>S. aureus</i> 2	R	R	R	R	R	R	R	R	R
<i>B. subtilis</i>	R	R	R	S:13	R	R	R	R	R
<i>B. megaterium</i>	R	R	R	S:12	R	13	R	S:22	S:26
<i>P. aeruginosa</i>	S:11	R	R	R	R	R	R	R	R
<i>P. cecapaciae</i>	S:08	R	R	S:20	R	14	R	R	R
<i>E. coli</i> 1	R	R	R	S:24	R	R	R	R	R
<i>E. coli</i> 2	R	R	R	S:21	R	R	R	R	R

E: Erythromycin 15 µg; ST: Sulfathiazol 0,25mg; AMX: Amoxycillin 25µg; D: Doxycillin 30µg; DA: Clindamycin 2mg; CE: Cefalothin 30µg; AM: Ampicillin 10µg; TM: Tobramycin 10µg; CP: Ciprofloxacin 5 µg; R: Resistant; S: Sensitive

with a metallic device and filled with the different concentrations of honey of each sample (100 µL/ well). The plates containing bacteria were incubated at 37°C for 24 h. After incubation, the diameter of inhibitory zones was measured in mm.

RESULTS

All the strains used in this study were resistant to antibiotics (Table I) except for Doxycillin to which five strains were sensitive. These were isolated from human samples and may have involved in some attacks that would imply the use of antibiotics. The increase of the microbial resistance to antibiotics is now being more and more of concern throughout the world. New antibiotics are being processed for alleviating this situation and some research works are also being carried out on natural compounds for achieving the destruction of these microorganisms. As it could be pointed out, all the strains showed a normal growth on the medium and inhibition was observed around the culture for almost all the antibiotics. There was only one

Table II. Effect of different concentrations of honey from *Thymus broussonetti* Boiss and *Origanum vulgare* on different bacterial isolates

		<i>Thymus broussonetti</i> Boiss. 1				<i>Thymus broussonetti</i> Boiss. 2				<i>Origanum vulgare</i>			
		1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
<i>S. aureus</i>	1	21	0	0	0	18	0	0	0	40	34	0	0
	2	20	12	0	0	26	0	0	0	33	24	0	0
	3	26	22	0	0	32	28	12	0	42	34	20	0
	4	30	20	0	0	32	24	0	0	40	32	20	11
<i>Bacillus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	20	15	0	0	0	0	0	0	26	0	0	0
	3	20	0	0	0	16	12	0	0	24	0	0	0
	4	22	10	0	0	20	12	0	0	26	0	0	0
<i>E. coli</i>	1	38	30	26	12	36	30	24	0	38	25	12	0
	2	32	28	23	15	42	36	0	0	31	24	10	0
	3	23	10	0	0	28	18	0	0	26	15	0	0
	4	24	19	13	0	40	32	20	0	34	28	22	18
<i>P. aeruginosa</i>	1	29	25	19	0	36	28	12	0	34	24	10	0
	2	30	22	10	0	24	12	0	0	37	30	22	0
	3	21	12	0	0	38	26	12	0	32	20	10	0
	4	32	22	0	0	36	24	0	0	36	28	12	0

Table III. Effect of different concentrations of honey from *Euphorbia resinifera* on different bacterial isolates

		Sample 1				Sample 2				Sample 3				Sample 4			
		1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
<i>S. aureus</i>	1	24	0	0	0	44	36	24	10	36	24	10	0	28	12	0	0
	2	30	24	0	0	42	36	24	0	36	24	0	0	23	13	0	0
	3	40	21	0	0	28	12	0	0	34	22	0	0	18	10	0	0
	4	32	28	0	0	36	24	0	0	32	26	0	0	43	34	12	0
<i>Bacillus</i>	1	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	23	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0
	3	26	12	0	0	13	0	0	0	20	0	0	0	32	24	0	0
	4	27	16	0	0	22	0	0	0	30	10	0	0	24	11	0	0
<i>E. coli</i>	1	40	34	28	16	42	32	22	0	40	32	26	0	35	28	17	12
	2	38	30	26	0	42	32	26	0	42	34	22	0	30	24	12	0
	3	30	22	0	0	30	20	0	0	36	22	0	0	28	12	0	0
	4	24	10	0	0	46	36	24	0	42	34	26	0	40	32	20	0
<i>P. aeruginosa</i>	1	36	28	12	0	34	22	0	0	30	18	10	0	37	28	17	12
	2	34	28	14	11	32	24	16	0	36	26	16	0	29	23	13	0
	3	36	26	13	0	20	11	0	0	28	14	0	0	40	26	0	0
	4	34	24	0	0	41	32	0	0	31	22	0	0	22	10	0	0

isolate of *Bacillus megaterium* which was sensitive to Tobramycin and Ciprofloxacin and the isolates of *Pseudomonas* which were sensitive to Erythromycin.

Different types of honey are naturally produced on different types of flowers. The most frequent types are *Thymus broussonetti* Boiss, *Origanum vulgare*, *Eucalyptus globulus*, *Euphorbia resinifera* and the seasonal multifloral honey or wild plants flowers. Four types of honey from *Eucalyptus globulus*, *Thymus broussonetti* Boiss., multifloral and *Euphorbia resinifera* were studied.

Table II shows results of honey from thym (*Thymus broussonetti* Boiss. -2 samples- and *Origanum vulgare* -1 sample-). All the strains were inhibited by the dilution 1/2 and most were inhibited by the dilution 1/4. Gram negative strains were unexpectedly more sensitive than Gram positive bacteria, and among the gram negative bacteria, *Pseudomonas* was more sensitive than *E. coli*. Four strains were inhibited by the dilution 1/2 for the former and only 3

strains out of 4 were inhibited by the same dilution for the later. Differences in the inhibition between the samples were not so important for both Gram – and Gram + bacteria regarding the dilution, but for the diameter of the inhibition zone, differences were observed for all the strains. This is due to the diffusion of the antimicrobial components in the medium. Table III shows results of the antimicrobial activity of honey samples from *Euphorbia resinifera*. This plant is different from thym and its active principles may also be different from those of thym. However the same profile of inhibition was observed, that is, all the strains were inhibited by the dilution 1/4. Gram negative strains were more sensitive than Gram positive ones. But the same sensitivity among the Gram negative was observed for all the strains of *Pseudomonas* and *E. coli*.

Results relative to the inhibitory activity of honey from *Eucalyptus* are reported in Table VI. These are comparable to those of thym and *Euphorbia resinifera* in terms of concentrations, but the same sensitivity of Gram negative and Gram positive bacteria was observed except for sample 2 which was more inhibitory for Gram negative and Gram positive bacteria, for 1 isolate of *Pseudomonas* (1) and 2 isolates of *E. coli* (1 and 2).

The multifloral honey is considered by people as a low quality honey compared to honey from *Thymus broussonetti* Boiss and *Euphorbia resinifera* but more than that of *Eucalyptus*. This is not true at least for the antimicrobial activity, since the four samples of multifloral honey showed almost the same profile of bacterial inhibition as the other types of honey. All the strains of bacteria were inhibited by the dilution 1/2 and most of them were inhibited by the dilution 1/4 (Table V). This was only one exception for the strains 1 and 2 of *Bacillus*, which were not inhibited and the sample 4, which has not inhibited the four strains of *Bacillus*.

Table IV. Effect of different concentrations of honey from *Eucalyptus globulus* on different bacterial isolates

		Sample 1				Sample 2			
		1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
<i>S. aureus</i>	1	30	26	12	0	34	24	0	0
	2	32	28	10	0	22	18	0	0
	3	36	28	10	0	36	26	12	0
	4	38	30	22	0	32	24	0	0
<i>Bacillus</i>	1	20	0	0	0	0	0	0	0
	2	22	0	0	0	0	0	0	0
	3	27	16	12	0	20	0	0	0
	4	0	0	0	0	18	14	0	0
<i>E. coli</i>	1	36	30	26	0	36	33	24	15
	2	36	32	28	0	40	30	22	12
	3	34	22	0	0	26	18	0	0
	4	32	21	10	0	36	29	16	0
<i>P. aeruginosa</i>	1	30	14	0	0	32	25	19	15
	2	36	30	26	16	26	16	0	0
	3	16	0	0	0	30	16	0	0
	4	16	12	0	0	28	14	0	0

Table V. Effect of different concentrations of multifloral honey samples on different bacterial isolates

		Sample 1				Sample 2				Sample 3				Sample 4			
		1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16	1/2	1/4	1/8	1/16
<i>S. aureus</i>	1	30	0	0	0	32	20	0	0	34	0	0	0	33	0	0	0
	2	32	26	10	0	34	22	0	0	36	18	0	0	32	22	0	0
	3	32	33	0	0	22	0	0	0	28	12	0	0	31	0	0	0
	4	33	0	0	0	32	22	0	0	31	0	0	0	24	14	0	0
<i>Bacillus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	17	11	0	0	19	12	0	0	24	0	0	0	0	0	0	0
	4	11	0	0	0	22	0	0	0	28	0	0	0	0	0	0	0
<i>E. coli</i>	1	34	26	16	0	38	32	26	0	36	30	0	0	29	25	0	0
	2	36	24	0	0	36	28	0	0	36	32	16	0	32	28	18	0
	3	28	12	0	0	40	34	14	0	38	22	0	0	32	30	18	0
	4	28	16	0	0	38	22	0	0	42	32	18	0	34	24	0	0
<i>P. aeruginosa</i>	1	34	22	0	0	36	22	12	0	32	20	0	0	0	0	0	0
	2	36	22	12	0	36	24	10	0	32	20	0	0	36	24	15	13
	3	32	20	0	0	36	26	12	0	34	22	0	0	24	12	0	0
	4	32	26	0	0	32	22	0	0	24	11	0	0	22	12	0	0

DISCUSSION

Almost all the strains used in the present study were resistant to the antibiotics studied (Table I). It could be pointed out that, except for Doxycillin (30 µg), which was active on the majority of strains, all the other antibiotics did not show an inhibition activity on most of the isolates. Some isolates such, as *B. megaterium* was sensitive to Cefalothin, Tobramycin and Ciprofloxacin. Honey samples showed a potential activity against the growth of both Gram positive and Gram negative bacteria which was resistant to antibiotics. This is a very important phenomenon to apply in the field of therapeutics and food preservation as well as other applications where microorganisms should be controlled. This would be a very interesting approach to control more dangerous species of microorganism in medical sciences. Because of the development of resistance by the microorganisms to common antibiotics, it has become necessary to search for an alternative approach dealing with this situation. It has been demonstrated in earlier studies that honey could accelerate healing many diseases and may also have bactericidal properties (Efem *et al.*, 1992), and it has been found active in controlling wound infections caused by *S. aureus*, *E. coli* and *Pseudomonas sp.* (Efem *et al.*, 1992). Honey samples were found to inhibit the growth of a wide range of microorganisms that were resistant to antibiotics. The samples of honey showed varied degrees of antimicrobial activity. This activity was higher against Gram negative bacteria than Gram positive bacteria. It is possible that the low redox potential of ascorbic acid in honey affects aerobic microorganisms such as *Pseudomonas* and *Acinetobacter* species. Jeddar (1985) reported that honey is inhibitory to the growth of microorganisms at a 40% (W/V) dilution. These observations are in discordance with our results. Our findings were also contrary to the results reported by

Radwan (1984) who found that *Pseudomonas* was resistant to honey, but our results agree with those reported by Zaghloul (2001). Molan and Cooper (1999) determined the use of honey as an antiseptic dressing for wounds infected by *Pseudomonas*. The bases for the antibacterial activity of honey are controversial. A demonstration of the antibacterial activity was first carried out by Dold *et al.* (1937) who first suggested the possibility of hydrogen peroxide as the principal factor for the antibacterial activity in honey. Subrahmanyam *et al.* (2001) showed that the minimal inhibitory concentration of honey was around 30% (W/V) for all the organisms including coagulase positive and coagulase negative isolates that caused burn wound infection.

The antimicrobial activity of honey has been attributed to hydrogen peroxide which is produced by glucose oxidase, and phenolic compounds. White *et al.* (1962) found that the predominant acid in honey was gluconic acid due to this enzyme (Bogdanov, 1997). The addition of catalase to honey results in the breakdown of H₂O₂. Bogdanov (1984) extracted a substance belonging to a group of flavonoids noted for their antimicrobial activity from honey. Molan and Russel (1988) have reported that the floral source of honey may also be responsible for the antibacterial activities of honey.

The antimicrobial activity of honey is not to be demonstrated but there is a wide difference between results reported by the above mentioned works especially for the honey concentration. If the honey can give a safe results when it is diluted, the use would be more beneficial and this is due to the origin of honey which is mainly the type of flowers and the region as well as some other intrinsic and extrinsic factors such as the nature of bees and the breeding techniques. Lavie (1968) found another group of light sensitive, heat stable factors in honey which inhibited the growth of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas*

*pyocyane*s, *Salmonella* and *Staphylococcus aureus*.

Taormina (2001) found that the antimicrobial properties of honey containing hydrogen peroxide and characterized by a range of antioxidant power need to be validated using model food systems. Investigations have shown that honey is more effective than other products in the management of partial burns (Subrahmanyam, 1991). Early burn wounds healed quickly and without bacterial infection in presence of honey.

Almost all the works carried out focused on the hydrogen peroxide acidity and compounds such phenolic acids. To our knowledge, little is known about the role of the plant from which honey is made. Some types of honey can be made from some medicinal plants in Morocco, such as *Thymus broussonetti* Boiss, *Origanum vulgare*, *Eucalyptus globulus* and *Euphorbia resinifera*. These honeys are very known for their therapeutic and medicinal activities and they are preferred for other types of honey such as multifloral. In the present study we demonstrated that the antimicrobial activities of both types of honey was similar.

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