International Journal of Microbiological Research 4 (1): 50-55, 2013
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2013.4.1.71174

Comparative Studies on Antimicrobial Activities (AMA) of Different Types of Honey Using Bacteria from Animal Origin

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Abstract: The antimicrobial activities (AMA) of commercially available honeys (clover, citrus, flowers, Marjoram, cotton, Nigella sativa and mountain) obtained from apiarists and honey packers as raw honeys were in-vitro analyzed and evaluated. AMA was performed against 4 types of bacteria that were isolated from animal origin *(Salmonella enteritidis, Pseudomonas aeruginosa, Staphylococcus aureus and E. coli)* using agar well diffusion method. Clover, flower and mountain honeys were the best types of honey that can be used for infections with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enteritidis*. While citrus and mixed honey were the best for *Salmonella enteritidis* infections and *E. coli* was most sensitive with flower honey. Diluted different types of honey exhibited variable degrees of antibacterial activity.

Key words: Isolation % *Salmonella enteritidis* % *Pseudomonas aeruginosa* % *Staphylococcus aureus and E. coli.*

INTRODUCTION

Honey is an extraordinarily healthy highly nutritious yellowish brown sweet viscid natural supersaturated fluid product of honey bees of the genera Apis and Meliponinae produced from the nectar of flowers. Honey is recognized for medicinal properties because it is rich in about 300 biologically active substances including proteins (containing 9 essential amino acids), salts, carbohydrates, organic acids, enzymes and 30 trace elements including minerals and vitamins [1]. Furthermore, honey has been found to harbor an antioxidant activity [2]. The antioxidant activity of honey comes from the phenolics, peptides, organic acids and enzymes. Honey improves physical performance, resistance of fatigue and increases mental efficiency [3]. Honey may exhibit antimicrobial activity against the major causes of bacterial gastroenteritis. The antimicrobial action of honey may be due to H_2O_2 and non- peroxide antibacterial factors. The activity of H_2O_2 works when honey is diluted. Moreover, the hygroscopic feature of honey causes withdrawal of moisture from the surroundings by osmosis leading to microbial death. H_2O_2 is produced when glucose oxidase which is secreted from hypopharyngeal gland of the bee converts glucose in the nectar into gluconic acid and H_2O_2 [1]. Other antimicrobial factors of honey include low pH and the presence of inhibins [4, 5].

Variations in the type and level of antimicrobial activity of honeys are chiefly associated with their floral source. However, while some floral sources appear to be associated with particular levels of hydrogen peroxide activity, variations in this activity among honeys from within the same floral species have also been observed by Hamid and Saeed [6]

Recently, the use of honey as a therapeutic substance has been rediscovered and accepted as an antibacterial agent for treatment of different illnesses including ulcers, bed sore and surface wound infection [7, 8], bacterial gastroenteritis especially in infants [9] and liver disease [10]. However the establishment of the effectiveness of different types of honey as antimicrobial is our goal. Thus the aim of the present study was to analyze and assess the antimicrobial activities (AMA) and MIC of different types of commercially available honeys.

MATERIALS AND METHODS

Sampling: Thirty four samples of commercially available honey (clover, citrus, flowers, Marjoram, cotton, *Nigella sativa*, mountain and mixed) purchased from apiarists and honey packers were used and all honey samples were kept at 4°C in dark jars till used.

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Isolation and Identification of Bacteria: For *Salmonella* isolation, the collected animal samples were enriched in selenite F broth, streaked on brilliant green agar and identified by API -20 of Enterobacteriaceae. For *Pseudomonas aeruginosa* isolation, the samples were inoculated onto cetramide agar medium and confirmed biochemically [11]. For *Staphylococcus aureus* isolation, the animal samples were inoculated onto mannitol salt agar medium and the biochemical identification was done by API of *Staphylococcus*. For *E. coli* isolation, the collected animal samples were inoculated onto eosin methylene blue agar medium and confirmed biochemically [11].

Honey Examination:

- Microbial colony count for every honey type was performed by direct streaking a loopful from each type of honey and its dilutions on brain heart infusion agar plates and the plates were then incubated aerobically and anaerobically [12].
- Separate swabs from honey types were inoculated into tubes of brain heart infusion broth (BHIB) and cooked meat broth (CMB), then the tubes of BHIB and CMB were incubated aerobically and anaerobically respectively at 37°C for 24 hours. The turbidity of broth was checked and samples from the broth were microscopically examined by Gram stain. Positive tubes of BHIB and CMB (presence of turbidity) were then sub cultured on brain heart infusion agar and blood agar with and without neomycin and incubated aerobically and anaerobically. Significant grown bacterial colonies if present were identified [13, 14]). Honey swabs were cultured on Sabaroud agar medium for detection of fungal growth [15].

Determination of Antibacterial Activity of Tested Honeys: Agar well diffusion test [12] was used for determination of AMA. Muller Hinton agar medium was the medium of choice used and a uniformly circular zone of inhibition with a confluent lawn of growth was measured in millimeters [16].

RESULTS

Honey Examination: All tested honey samples didn't show pathogenic bacterial or fungal growth.

Antibacterial Activity of Tested Honeys: 34 tested honey samples were used in their original concentrations as (1) and diluted forms as (2) and (3) with ratio (1:2) and (1:5) respectively. .

In Table (1), clover honey exhibited AMA against tested bacteria ,two samples out of 7 with percentage of (28.5%) for E.coli, 3 out of 7 (42.8%) for *Pseudomonas aeruginosa*, 3 out of 7 (42.8%)for *Staphylococcus aureus* and 3 out of 7 (42.8%) for *Salmonella enteritidis*, so all tested bacteria were more sensitive than *E.coli* to clover honey and no significant zone of inhibition was detected upon dilution of the examined clover honey.

In Table (2), flowers honey exihibted AMA against tested bacteria 1 out of 3 with percentage of (33.3%) for *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and 100 % for *Salmonella enteritidis*, so *Salmonella entritidis* was the most sensitive bacterium to flowers honey and no significant zone of inhibition was detected upon dilution of the examined flowers honey.

In Table (3), citrus honey exihibted AMA against tested bacteria two samples out of 5 with percentage of (40%) for *E.coli*, 3 out of 5 (60%) for *Pseudomonas aeruginosa*, 3 out of 5 (60%)for *Staphylococcus aureus* and 4 out of 5 (80%) for *Salmonella enteritidis*, so *Salmonella entritidis* was the most sensitive bacteria to citrus honey and variable zone of inhibition were found upon diluting citrus honey.

In Table (4), marjoram honey exihibted AMA against tested bacteria 1 sample out of 4 with percentage of (25%) for *E.coli*, 2 out of 4 (50%) for *Pseudomonas aeruginosa*, 1 out of 4 (25%)for *Staphylococcus aureus* and 2 out of 4 (50%) for *Salmonella enteritidis*,so *Salmonella enteritidis* and *pesudomonas aeruginosa* were the most sensitive bacteria to marjoram honey and variable zones of inhibition were found upon diluting marjoram honey.

In Table (5), mixed honey exihibted AMA against tested bacteria with 0 sample out of 4 with percentage of (0%) for *E.coli* and *Pseudomonas aeruginosa* while 1 out of 4 (25%)for *Staphylococcus aureus* and 3 out of 4 (75%) for *Salmonella enteritidis*,so *Salmonella enteritidis* was the most sensitive bacterium to mixed honey and no significant zone of inhibition was detected upon dilution of the examined mixed honey.

In Table (6), nigella sativa honey exihibted AMA against tested bacteria with 0 sample out of 4 with percentage of (0%) for *E.coli* and *Pseudomonas aeruginosa* while 1 out of 4 (25%) for *Staphylococcus aureus* and *Salmonella enteritidis*, so both

Table 1: The antibacterial activity of clover honey

Isolate	Sample number	Zone of inhibition 1(original)		2(1:2)		3(1:5)	
E.coli	7	+	1.4	+	1.2	+	1.0
	34	+	1.6	+	1.2	+	1.0
	10,15,25,30,31	No detection of AMA and MIC for these honey samples					
Pseudomonas aeruginosa	7	+	1.4	+	1.2	+	1.0
	34	+	1.6	+	2.0	+	1.0
	10	+	2.2	+	1.2	+	1.0
	15,25,30,31	No detection of AMA and MIC for these honey samples					
Staphylococcus aureus	7	+	1.6	+	1.4	+	1.2
	10	+	1.0	+	1.0	+	1.0
	15	+	2.0	+	1.4	+	1.4
	25,30,31,34	No detection of AMA and MIC for these honey samples					
Salmonella enteritidis	7	+	1.6	+	1.6	+	1.4
	10	+	1.8	+	1.2	+	1.0
	15	+	2.2	+	1.2	+	1.0
	25,30,31,34	No detection of AMA and MIC for these honey samples					

Table 2: The antibacterial activity of Flowers honey

Isolate	Sample number	Zone of inhibition 1(original)		2(1:2)		3(1:5)	
E.coli	13	+	1.4	+	1.2	+	1.0
	21,22	No detection of AMA and MIC for these honey samples					
Pseudomonas aeruginosa	13	+	1.2	+	1.0	+	0.8
	21,22	No detection of AMA and MIC for these honey samples					
Staphylococcus aureus	13	+	1.2	+	1.0	+	0.8
	21,22	No detection of AMA and MIC for these honey samples					
Salmonella enteritidis	13	+	1.7	+	1.4	+	1.2
	21	+	1.8	+	1.6	+	1.6
	22	+	1.8	+	1.6	+	1.4

Table 3: The antibacterial activity of citrus honey:

Isolate	Sample number	Zone of inhibition 1(original)		2(1:2)		3(1:5)	
E.coli	6	+	1.2	+	1.2	+	1.0
	33	+	1.0	+	0.8	+	0.6
	9,14,26	No detection of AMA and MIC for these honey samples					
Pseudomonas aeruginosa	6	+	1.4	+	1.0	+	0.8
	9	+	1.2	+	1.6	+	1.8
	33	+	1.2	+	0.8	+	0.6
	14,26	No detection of AMA and MIC for these honey samples					
Staphylococcus aureus	6	+	1.4	+	1.2	+	1.0
	9	+	1.0	+	1.4	+	1.6
	33	+	1.2	+	1.0	+	0.8
	14,26	No detection of AMA and MIC for these honey samples					
Salmonella enteritidis	6	+	1.6	+	1.2	+	1.2
	9	+	1.2	+	1.4	+	1.6
	33	+	1.2	+	0.8	+	0.6
	26	+	1.0	+	1.4	+	1.4
	14	No detection of AMA and MIC for these honey samples					

Table 4: The antibacterial activity of Marjoram honey:

Isolate	Sample number	Zoone of inhibition 1(original)		2(1:2)		3(1:5)	
E.coli	8	+	1.4	+	1.6	+	2.0
	2,3,20	No detection of AMA and MIC for these honey samples					
Pseudomonas aeruginosa	3	+	0.4	+	0.8	+	1.6
	8	+	2.4	+	1.4	+	12
	2,20	No detection of AMA and MIC for these honey samples					
Staphylococcus aureus	8	+	1.2	+	1.6	+	1.6
	2,3,20	No detection of AMA and MIC for these honey samples					
Salmonella enteritidis	3	+	1.2	+	0.8	+	0.6
	8	+	1.2	+	1.4	+	2.2
	2,20	No detection of AMA and MIC for these honey samples					

Table 5: The antibacterial activity of mixed honey

Isolate	Sample number	Zone of inhibition 1(original)		2(1:2)		3(1:5)	
E.coli and *Pseudomonas aeruginosa*	12,16,23,24	No detection of AMA and MIC for these honey samples					
Staphylococcus aureus	16	+	0.6	+	1.2	+	1.2
	12,23,24	No detection of AMA and MIC for these honey samples					
Salmonella enteritidis	16	+	1.8	+	1.4	+	1.2
	23	+	1.6	+	1.6	+	1.4
	24	+	1.6	+	1.6	+	1.4
	12	No detection of AMA and MIC for these honey samples					

Table 6: The antibacterial activity of Nigella sativa honey

Isolate	Sample number	Zone of inhibition 1(original)		2(1:2)		3(1:5)	
E.coli and *Pseudomonas aeruginosa*	1,4,17,28,32	No detection of AMA and MIC for these honey samples					
Staphylococcus aureus and	4	+	1.4	+	1.2	+	1.0
Salmonella enteritidis	1,17,28,32	No detection of AMA and MIC for these honey samples					

Table 7: The antibacterial activity of Mountain honey

Isolate	Sample number	Zone of inhibition					
E.coli	29	+	1.0	+	0.8	+	0.4
	5,11,18,19	No detection of AMA and MIC for these honey samples					
Pseudomonas aeruginosa	5,19	No detection of AMA and MIC for these honey samples					
	11	+	2.4	+	1.2	+	1.0
	18	+	1.8	+	1.4	+	1.2
	29	+	2.0	+	1.6	+	1.2
Staphylococcus aureus	5,19	No detection of AMA and MIC for these honey samples					
	11	+	1.2	+	0.8	+	0.6
	18	+	1.8	+	1.2	+	1.2
	29	+	1.4	+	1.4	+	1.2
Salmonella enteritidis	5,19	No detection of AMA and MIC for these honey samples					
	11	+	1.4	+	1.2	+	1.0
	18	+	1.6	+	1.2	+	1.0
	29	+	1.6	+	1.2	+	1.2

Staphylococcus aureus and *Salmonella enteritidis* were the most sensitive bacteria to *Nigella sativa* honey and upon diution of this honey no detected zone of inhibition was seen.

In Table (7), mountain honey exihibted AMA against tested bacteria 1sample out of 5 with percentage of 20 % for *E.coli*, 3 samples out of 5 with percentage 60 % for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enteritidis*, so all previous bacteria were sensitive more than *E.coli* to mountain honey and and no significant zone of inhibition was detected upon dilution of the examined mountain honey.

The Result of AMA of Cotton Honey: *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enteritidis* were resistant to cotton honey and no zone of inhibition was observed.

DISCUSSION

In this study the AMA and MIC of different types of commercially available honey (clover, citrus, flowers, Marjoram, cotton, *Nigella sativa*, mountain and mixed) were demonstrated against 4 types of bacteria that were isolated from animal origin (*Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*). It was found that *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enteritidis* were more sensitive than *E. coli* to clover and Mountain honey with percentage (42.8 and 60%) respectively. Furthermore, upon diluting the honey samples and subjected to the test no significant zone of inhibition could be detected. On contrary of our results, Molan [17] and Turner [18] found that upon diluting the honey, its zone of inhibition is increased. Also, Mohsen *et al*. [19] found that *P. aeruginosa* does grow rapidly in low concentrations of honey, whereas in higher concentrations, the bacterial growth is different.

The results of loss of antimicrobial activitiy of honey upon dilutions may be attributed to the long storage. Moreover, in some cases peroxide activity of honey can be destroyed by heat or the presence of catalase [20]. It is suggested that the antimicrobial activity of clover and mountain honeys against *Salmonella entritidis, Pseudomonas aeruginosa and Staphylococcus aureus* could be due to non peroxide factors such as the presence of methyl syringate and methylglyoxal. Generally, antimicrobial activity of honey may be due to peroxide (H_2O_2) and non peroxide factors such as phenolics that include cinnamic acid derivatives (mainly prenylated compounds) [21]. Most types of honey generate H_2O_2 when diluted, because of the activation of the enzyme glucose oxidase that oxidizes glucose to gluconic acid and H_2O_2, which thus attributes the antimicrobial activity. Hyslopp *et al*. [22] stated that *E. coli* growth is mainly inhibited by hydrogen peroxide. As a whole the effect of honey on Gram negative bacteria was attributed to H_2O_2, antioxidants, low pH, phenolic acid and lysozyme.

In addition, *Salmonella enteritidis* was found to be the most sensitive bacteria to flower, citrus and mixed honey (multifloral) with percentages (100, 80 and 75%) respectively.

Our results of variations of antimicrobial activity of different types of honey may be attributed to many factors including the different floral sources of honey.Hodgeson [23] found that *Staphylococcus aureus* and *Pseudomonas aeruginosa* are inhibited by ling heather and Manuka honeys while inhibition of *E. coli* is only seen with Manuka honey.

Honey at concentrations of 17-100% by volume will completely kill or inhibit growth of microorganisms because of its intrinsic enzymatic glucose oxidation reaction and other physico-chemical properties [24,25]. Jeddar *et al*. [26] found that most pathogenic bacteria (Gram positive and Gram negative) fail to grow in honey at a concentration of 40% or above. However, Nzeako and Hamdi [27], in their study of six commercial honeys, found that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa* does not occur at honey concentrations of 40%. On the other side, Basualdo *et al*. [28] found that honey inhibits growth of *S. aureus* even at 50% dilution.

It can be concluded that clover, flower and mountain honeys were the best types of honey that can be used for infections with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella enteritidis*. While citrus and mixed-honeys were the best for *Salmonella enteritidis* infections and *E. coli* was most sensitive with flower honey.

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