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Investigation of the Antimicrobial Activity and Insight into the Physicochemical Properties of Honey from Egypt

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ABSTRACT The present study investigated the carbohydrate composition and the pollen content of the mostly produced and consumed honeys in Egypt and correlated these to their antimicrobial effect. Honey samples (clover, citrus, black seed and sider) were collected from beekeepers and/or markets depending on their availability. They were tested for physiochemical and sugar composition. Moreover, they were tested undiluted, and at 75, 50, 30 and 10% (w/v) dilution against *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Escherichia coli (E. coli) and Candida albicans (C. albicans)* to detect their antimicrobial activity using the agar well diffusion assay. Results indicated that all samples were complying with codex 1998,2001, European standard 2002 and Egyptian standard 2005, regarding their physicochemical parameters, but 62.5% were complying regarding their carbohydrate composition. According to pollen contents, all honey samples belonged to the class I of representivity (under-represented honeys, with less than 20,000 pollen/10g honey). Locally produced honeys were found to have activity against the tested pathogenic bacteria however, the greatest inhibition was seen at the undiluted form of honeys. Only *Candida albicans* was not inhibited. Pollen contents affected the antibacterial activity of honey, the higher the pollen, the higher is the antibacterial activity.

Key words: Honey, antimicrobial effect, sugars in honey, pollen grains

INTRODUCTION

Honey is a drug more than a nutrient. Honey was valued highly in the Middle East. It was mentioned in the Holy Quran 1400 years ago (And thy LORD taught the bee to build its cells in hills, on tree and in men's habitations, then to eat of all the produce of the earth and find with skill the spacious paths of its LORD, there issues from within their bodies a drink of varying colors, wherein is healing for men, verily in this is a sign for those who give thought). It was also mentioned in Holy Talmud and

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Holy bible. Hippocrates and Celsus used honey for wounds and ulcers. Prophet Mohamed (SAWW) had recommended honey for treatment of diarrhea. The antimicrobial activity of honey has been demonstrated *in vitro* and *in vivo*. Laboratory studies and clinical trials have shown that honey is an effective broad-spectrum antimicrobial agent.[1-8] The antimicrobial activity of honey has been attributed to hydrogen peroxide, osmolarity, acidity, aromatic acids and phenolic compounds.[9,10]

The high osmolarity of honey is due to the high content of sugar (average over 85% of honey) including fructose, glucose, maltose, sucrose and other types of carbohydrates.[11] Honey also contains oligosaccharides in small quantities. Shin and Ustunol,(2005) related the sugar composition of honeys from different floral sources to the growth inhibition of various intestinal bacteria.[12] Hydrogen peroxide, formed by glucose oxidase originating from the bees,[13] was tested against six food borne pathogens by Taormina, et al (2001).[14] It was shown that varying levels of antimicrobial activity were present depending on the variety of honey. The activity of honey was attributed not only to hydrogen peroxide but also to antioxidant compounds in honey.[14] Weston, (2000)[15], on the other hand, mentioned that hydrogen peroxide was the only antibacterial substance of any consequence in honey and that other substances such as propolis-derived phenolics, are insignificant in comparison to hydrogen peroxide which is produced by the enzyme glucose oxidase, when honey is diluted.[13]The oxidase originates from the hypopharyngeal glands of honey bees,[16] a fact from which one might expect a similar glucose oxidase level in most honeys world-wide. Weston, (2000)discussed the effect of the level of catalase - which occurs in flower pollen-in honey on the level of peroxide in a honey and concluded that this will depend on how

much pollen is collected by bees, the floral source of the pollen and also on the catalase activity of that pollen.[15]

Locally produced honeys have the advantage of ready availability and cheaper price than the high price and unavailable commercial, antibacterial honeys. Therefore the purpose of this study was to investigate the carbohydrate composition and the pollen content of mostly produced and consumed honeys in Egypt, and to correlate these with their antimicrobial effect against some pathogenic bacterial strains.

2. Material and Methods

2.1. Honey samples

Honey samples were collected from two sources. Four honey samples were purchased from the local market and 4 were purchased from the local beekeepers. Collected honeys were yellow except for one dark yellow (brownish) honey sample collected form one beekeeper (sider honey). Honey samples were mainly clover, and citrus as these are the most widely produced and consumed in Egypt. Markets samples included; 3 clover honey and 1 black seed honey whereas, beekeepers samples included; 1 clover honey, 2 citrus honey and 1 sider honey. Samples were collected in December 2008, they had different production dates - April and November 2008. For the antibacterial tests, honey samples were used undiluted and at 75, 50, 30 and 10% dilutions (grams of honey diluted to a final volume of 100ml). The number of collected samples was limited in order to be able to carry out the antibacterial test of all of the 8 samples in the same day to fix all conditions needed for the test.

2.2. Compositional analysis of honey

Composition of honeys from the different floral sources (clover, citrus, black seed and sider) was evaluated. Moisture and ash contents of honeys were determined according to the respective Association of official analytical chemists

methods (AOAC,1995).[17] Optical rotation, free acidity and carbohydrate composition were determined according to the respective AOAC methods.[18] Oligosaccharide composition was estimated from the following equation: 100% - (Moisture + ash + fructose + glucose + maltose + sucrose) %.[12]

2.3. Pollen analysis

For the quantitative analysis, the method described by Maurizio (1979) was followed, where all the elements of botanical origin were counted from a sub-sample of 10 g of honey.[19] For the qualitative analysis, acetolysed slides were made.[20] Pollen grains were counted and identified. Botanical classification was achieved when the pollen spectrum contained >45% of the corresponding dominant pollen.[21]

2.4 Bacterial strains

Strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), in addition to *B. cereus* and the yeast *C. albicans* (isolated and identified in the Microbiology Department of the High Institute of Public Health-Alexandria university – Egypt according to the method described by Forbes, et al[22]) were utilized to assay antimicrobial activity of the collected honeys. Each culture was revised 48h prior to use on blood agar then suspensions were made in nutrient broths.

2.5 Assessment of antibacterial activity

The agar well diffusion method was employed to test the antimicrobial activity of the collected honey samples. [23] The opening of a 30ml sterilized test tube was used to make four wells (1.5cm diameter) on tryptic soy agar plates onto which 10µl of a suspension of a 24hour culture of one of the three bacterial strains or the yeast had been spread. One aliquot (1ml each) of each honey sample (undiluted) was deposited into one well. This was the method of Demera and Angert, (2004)[24] but with a slight modification as they were using lower aliquots of honey samples (0.6ml), lower bacterial age (16h culture)

and lower well size (4mm).

The plates were incubated aerobically at 37ºC for 24 hours. Plates for 75 %, 50 %, 30 %, and 10 % dilutions of honey samples were also prepared in the same manner.

2.6. Quantification of microbial growth inhibition

Quantification of microbial growth inhibition was determined by measuring the diameter of zones clear of microbial growth not including the wells in the agar (diameter of inhibition was measured on both sides of the well). Four measurements per well were made at 4 different directions (0º, 45º, 90º, and 135º).[24] In case of the absence of a clear inhibition zone, bacterial growth was rated using an inhibition score. A score of 0 indicated growth equivalent to the control with growth inhibition scored from 1-4 (e.g., 25% inhibition = 1, 50% inhibition = 2, etc.), growth greater than the control was scored in the same way and given a negative value (e.g., 25% increase = -1, 50% increase = -2, etc.).[25]

Table 1: Mean and standard deviation of some physiochemical parameters of market and beekeeper honeys .

Honey sample	Moisture (≤20%)	Optical rotation (negative)[b]	Ash (≤0.6%)[a]	Free acidity (≤ 50 meq/kg)
Market honey				
1. Clover				
Sample 1	19.0 ± 0.84	4.95 ± 0.07	0.28 ± 0.39	14.5 ± 0.70
Sample 2	19.4 ± 0.84	-11.1 ± 0.70	0.25 ± 0.35	15.0 ± 1.41
Sample 3	17.3 ± 0.28	22.95 ± 4.03	0.05 ± 0.07	11.5 ± 4.49
2. Back seed				
Sample 4	17.5 ± 0.0	3.95 ± 0.07	0.23 ± 0.32	9.5 ± 0.70
Beekeeper honey				
1. Clover				
Sample 5	18.4 ± 1.84	5.2 ± 0.07	0.025 ± 0.03	8.25± 0.35
2. Citrus				
Sample 6	17.8 ± 0.17	-0.5 ± 0.0	0.05 ± 0.07	10.5 ± 0.7
Sample 7	17.1 ± 0.28	-1.9 ± 0.49	0.0 ± 0.0	12.75 ± 1.06
3. Sider				
Sample 8	18.05 ± 1.06	3.7 ± 1.83	0.0 ± 0.0	12.75 ± 0.35
X^2 (p)	8.871 (0.262)	14.392* (0.045)	3.289 (0.857)	10.518 (0.161)

Each sample was run in duplicate.
Data in parentheses are the limits stipulated by codex 2001, European standard 2002 ,Codex 1998[a]and /or these of the Egyptian standards 2005[b].[26-29]
*Significant at p≤0.05

Table 2: Mean and standard deviation of sugar composition of market and beekeeper honeys.

Honey type	Sugar (%)						
	Fructose	Glucose	Fructose/ glucose ratio (≥1.06)[b]	Fructose + glucose (≥60%)	Sucrose (≤5%)	Maltose	Oligosaccharides
Market honey							
1. Clover							
Sample 1	30.79±2.13	29.41±2.84	1.04 ±0.029	60.2 ± 4.98	8.87 ± 0.89	2.03 ± 0.30	9.63 ± 5.71
Sample 2	39.76±0.93	37.33±0.95	1.076±2.03	77.09 ± 1.9	2.25 ± 013.	0.95 ± 0.07	0.54 ± 0.76
Sample 3	26.54±11.00	27.31±11.38	0.97 ± 2.44	53.85±22.38	4.41 ±1.96	3.03 ± 1.32	21.36±25.46
2. Black seed.							
Sample 4	26.61±3.24	24.92±3.37	1.06 ± 0.01	51.53 ±6.60	7.34 ± 0.90	0.88 ± 0.28	22.53±7.56
Beekeeper honey							
1. Clover							
Sample 5	36.6 ± 4.70	32.23±4.26	1.14 ± 4.34	68.83 ±8.95	7.44 ± 0.85	2.16 ± 0.76	5.98 ± 8.46
2. Citrus							
Sample 6	36.77±3.86	32.42±3.20	1.13 ± 7.31	69.19±7.06	0.5 ± 0.03	1.97 ± 0.33	10.49±7.57
Sample 7	50.90±16.21	46.39±20.44	1.13 ± 0.15	97.29±36.45	1.97 ± 0.49	3.27 ± 0.90	3.395±4.80
3. Sedr							
Sample 8	24.12±5.32	28.38±3.59	0.84 ± 0.08	52.50±8.93	14.99±5.70	0.0±0.0	14.47±13.54
X^2 (p)	10.809 (0.147)	8.868 (0.262)	11.366 (0.123)	11.074 (0.135)	14.547†(0.042)	12.415 (0.088)	-

Each sample was run in duplicate.

Data in parentheses are the limits stipulated by codex 2001,European standard 2002 ,Codex 1998 and /or these of the Egyptian standards 2005b.(26,27,28,29)

† Significant at $p \leq 0.05$

Table 3: Pollen analysis of market and beekeeper honeys

Honey type	Qualitative method (/10g honey)	Quantitative method (/10g honey)
1. Clover (*Trifolium sp.*)		
Market		
Sample 1	***Trifolium* sp,** *Gosspium sp.*	8500
Sample 2	***Trifolium* sp.,** *Gosspium sp.*	9350
Sample 3	***Trifolium* sp,** *Gosspium sp.*	10090
Beekeeper		
Sample 5	***Trifolium* sp.,** *Citrus sp., Eucalyptuses sp.,*	9000
2. Black seed *(Nigella sativa)*		
Market		
Sample 4	***Tifolium* sp,** *Gosspium sp.*	12000
3. Citrus (*Citrus sp.*)		
Beekeeper		
Samples 6	***Citrus* sp.,** *Trifolium sp.*	13000
Sample 7	***Citrus* sp. ,***Trifolium sp, Eucalyptus sp.*	9800
4. Sider		
Beekeeper		
Sample 8	***Trifolium* sp.**	5220

Each sample was run in duplicate.

Table4: The inhibition score for each honey sample against different bacterial and yeast strains.

Honey sample	Honey dilution	*S. aureus*	*B. cereus*	*E. coli*	*C. albicans*
1 (Market clover)	Undiluted	2	4 (0.5± 0.1)	2	0
	75%	2	0	2	0
	50%	2	0	2	-1
	30%	2	0	1	-1
	10%	1	0	0	-2
2 (Market clover)	Undiluted	2	4 (0.5 ± 0.1)	2	0
	75%	2	0	2	0
	50%	2	0	2	-1
	30%	2	0	1	-1
	10%	1	0	0	-2
3 (Market clover)	Undiluted	4 (0.8± 0.2)	4 (0.93± 0.3)	2	0
	75%	2	0	2	0
	50%	2	0	2	-1
	30%	2	0	1	-1
	10%	1	0	0	-2
4 (Market black seed)	Undiluted	4 (1.8 ± 1.11)	4 (2.46 ± 0.58)	4 (2.2 ±0.14)	0
	75%	4 (1.5 ± 0.57)	4 (2.2 ± 0.14)	2	0
	50%	4 (1.2 ±0.28)	4 (1.7± 0.45)	2	-1
	30%	2	4 (1.1± 0.07)	1	-1
	10%	1	0	0	-2
5 (Beekeeper clover)	Undiluted	4(0.2±0.0)	4 (1.4 ±0.52)	2	0
	75%	2	0	2	0
	50%	2	0	2	-1
	30%	0	0	1	-1
	10%	0	0	0	-2
6 Beekeeper citrus)	Undiluted	4 (1.85 ± 0.57)	4 (0.8±0.28)	2	0
	75%	2	0	2	0
	50%	2	0	2	-1
	30%	2	0	1	-1
	10%	1	0	0	-2
7 (Beekeeper citrus)	Undiluted	4 (0.3±0.14)	0	2	0
	75%	2	0	2	0
	50%	2	0	2	-1
	30%	0	0	1	-1
	10%	0	0	0	-2
8 (Beekeeper sider)	Undiluted	0	0	2	0
	75%	0	0	2	0
	50%	0	0	2	-1
	30%	0	0	1	-1
	10%	0	0	0	-2

Data in parentheses represent the average diameter of the inhibition zone in cm.
Score of 0= growth equivalent to control, 1 = 25%inhibition
2 = 50% inhibition 4 = 100% inhibition, -1=25% increase.
-2 = 50% increase in growth.

Results

Results of the physiochemical parameters of the collected beekeeper and market honeys shown in table 1 revealed that all samples were complying with codex 2001,Codex 1998, European standard 2002 and Egyptian standards 2005 regarding moisture (≤ 20%) , ash (≤ 0.6%) and free acidity (≤ 50 meq/kg) contents. However, only the 2 beekeeper citrus samples (6 and 7) and of market clover sample (2) showed significant levorotatory polarization, hence, were complying with the Egyptian standards (optical rotation must be negative).

Results of the analysis of sugar composition of beekeeper and market honeys shown in table 2 demonstrated that 5 out of the 8 honey samples (62.5%) were complying with Egyptian standards 2005 regarding the fructose/ glucose ratio, except for two market clover honeys (samples 1 and 3) and the beekeeper sider honey sample (sample 8). These had ratios < 1.06. Moreover samples 3 and 8 were also the only samples not complying with codex 2001,1998, European standard 2002 and Egyptian standards 2005 regarding their contents of fructose + glucose (<60%). The same applied for sample 4 (market black seed honey) which although had fructose/glucose ratio = 1.06 ± 0.01, its fructose + glucose content was 51.53% ± 6.60. Sucrose content of > 5% was obviously detected in 4 out of the 8 examined samples with 0.5% ± 0.03 and 1.97 ± 0.49 being the lowest sucrose content detected in the two beekeeper citrus honey (sample 6 and 7).

The pollen analysis of the examined honey samples is presented in table 3. The dominant pollen (> 45% of pollen spectrum except for citrus which is known as under represented pollen and ranges from 2-42%) is quoted first (italised and boldfaced) followed by the most important accompanying pollen. The table also shows that the pollen grains of *Tirfolium* sp.

were always very predominant in all samples. Citrus honeys contained 2-11% pollen of *citrus* sp., whereas clover honey contained 84-98% pollen of *Trifolium* sp. *Eucalyptus* sp. pollen was seen in only 2 samples (5% pollen).

The results of the inhibition tests run with honey samples are shown in table 4. It was observed that clear inhibition zones were seen mainly with the undiluted honeys. The other used honey dilutions (75%, 50%, 30% and 10%) resulted only in zones containing lower microbial growth than the surrounding and than the control, hence, they were given scores according to the level of inhibition.

B. cereus was the most inhibited bacterial strain; 6 honey samples inhibited its growth. The mean diameter of the inhibition zones produced by all of the 6 undiluted honey samples ranged from 0.5 ± 0.1 to 2.46±0.58 cm. *S.aureus* was inhibited by 5 honey samples. The mean diameter of the inhibition zones produced by the undiluted honey samples ranged from 0.2 ± 0.0 to 1.85 ± 0.57. Honey sample 4 (market black seed) showed the highest antibacterial activity at undiluted, 75, 50% and 30% dilutions against *B. cereus* and at undiluted, 75% and 50% dilutions against *S. aureus*. Following were honey samples 3, 5 and 6 which showed antibacterial activity at undiluted form against both *S. aureus* and *B. cereus* with the highest inhibition zone diameter against *S. aureus* for sample 6 (1.85 ± 0.57cm) and that against *B. cereus* for sample 5 (1.4 ± 0.52 cm).

4. Discussion

4.1 Physicochemical composition of honey

Clover (*Trifolium* sp.) and citrus (*Citrus* sp.) are the most famous and the most commonly produced and consumed honeys by the Egyptians thus the present study focused on these 2 types of floral honeys. Honey from black seed (*Nigella sativa*) is produced at a smaller scale while sider honey is a mixture of Egyptian clover and other imported honeys prepared by

some beekeepers.

The physicochemical parameters including; moisture, ash and free acidity of the honey samples complied with the limits stipulated by codex 2001, 1998, European standard 2002 and Egyptian standards 2005[26-29] as these were ≤ 20%, ≤ 0.6 and ≤ 50meq/kg, respectively. However, the values of these parameters for beekeepers samples were lower insignificantly than those of markets (Table 1). The moisture content was as low as 17.1 ± 0.28 (sample 7) and was as high as 19.4 ± 0.84 (sample 2). This range was higher than that of Spanish unifloral honeys (16.00 ± 0.40 to 18.75 ± 0.78) [21] and was nearly in the range of that of Moroccan honeys (16.8 ± 0.9 to 20.3 ± 3.7). [30] For ash content, it was as low as 0.0 ± 0.0 (sample 7 and 8) and as high as 0.28 ± 0.39 (sample 1). This range was nearly similar to that of the Spanish unifloral honeys (0.06 ± 0.02 – 0.29 ± 0.01) . [21] Ash in sourwood, alfalfa and sage honeys was found to have an average of 0.3 ± 0.1. [12]

Free acidity, on the other hand, was as low a 8.25 ± 0.35 (sample 5) and as high as 15.0 ± 1.41 (sample 2). These data were much lower than those of Spanish unifloral honeys (14.0 ± 0.35 – 26.9 ± 0.67)[21] and than that of Moroccan honey (19.5 ± 5.31 – 88.6 ± 23.4).[30]Concerning optical rotation, it was only negative in 3 honey samples (citrus beekeeper samples 6 and 7, and clover market sample 2). Hence, these 3 samples were the only samples out of the 8 tested ones that complied with the Egyptian standards which necessitates a levorotatory polarization for complying samples.[29] Optical rotation is not stipulated in the international standards.[26-28]

4.2 Sugar composition of honey

Five tested samples (2,4,5,6and7) were complying with standards[26-29] regarding fructose/glucose ratio (≥1.06). Concerning sum of fructose + glucose (≥60%); samples 1, 2, 5,6 and 7 were complying with standards, and for sucrose (≤5%),samples

2, 3, 6 and 7 were complying. The fructose /glucose ratio ranged from 1.06 to 1.14 in complying samples.This was much lower than that of Spanish unifloral honey which was from 1.11 ± 0.04 to 1.33 ± 0.06, [31] and from 0.93 ± 0.07 to 1.41 ± 0.04.[21] It was also lower than Colombian honey with a reported ratio of 1.21 and 1.26. [32] However, it was consistent with American honey where the ratio was from 1.03 to 1.09.[12]

Concerning the sum of fructose + glucose (reducing sugars) samples 1,2,5,6 and 7 were ≥60% [ranging from 60.2 ± 4.98 (sample 1) to 97.29±36.45 (sample 7)] as recommended by standards.[26-29] This was consistent with other studies as it was from 65.5 to 65.9% in Colombian honey [32] as well as from 62.22, to 73.4%[21] and from 60.0 to 74.8% in Spanish honey.[31] In American honey it was from 69.0% to 76.4%.[12]

Regarding sucrose content, it was ≤5% in samples 2, 3, 6 and 7. These samples were hence complying with International and Egyptian standards. [26-29] Sucrose content was found to range in complying samples from 0.5 ± 0.03 (sample 6) to 4.41 ± 1.96 (sample 3). This was consistent with other reports where sucrose content ranged from 0.07%± 0.03 to 3.19% ± 0.51,[21] and from 0.062% ± 0.048 to 4.45% ± 3.3 in Spanish unifloral honeys[31], from 1.6% ± 0.2 to 3.1% ± 0.4 in American honey[12] and was 3.29%in Colombian honey[32].

No limits were set for either maltose or oligosaccharides content for honey in codex 2001, 1998, European standard 2002 or Egyptian standards 2005.[26-29] Generally, maltose content of examined Egyptian honey samples was found to range from zero (sample 8) to 3.27% (sample 7). This range was very much lower than those found in Spanish unifloral honeys where it ranged from 6.69% ± 0.17 to 7.93% ± 1.36 [21] and those of American honey where it ranged from 9.8% ± 1.5 to

11.76% ± 1.4,[12] ,however, it was consistent with that of another study of Spanish honey where maltose ranged from 2.7% ± 0.3 to 4.9% ± 0.8 . [31]

For oligosaccharides, honey samples showed percentages ranging from 0.54% ± 0.76 to 22.53% ± 7.56. This range was higher than that for American honey report where it ranged from 3.8% ± 0.6 to 10.9% ± 1.1.[12]

4.3 Pollen analysis

The results of microscopical analysis of the sediment for the honey used in this work are presented in table 3. It was noticed that clover pollen grains were found in all samples and at counts much greater than citrus pollen grains. Concerning the quantitative analysis, the highest pollen count detected was 13000 pollen/10 g of citrus honey in sample 6 followed by 12000/10 g of black seed honey in sample 4, 10090/10 g of clover honey in sample 3, 9800/10 g of citrus honey in sample 7, 9350/10 g of clover honey in sample 2, 9000/10 g of clover honey in sample 5 and 8500/10 g of clover honey in sample 1. The lowest pollen count was for sider honey, a mixture of honey prepared by some beekeepers (5220 pollen/10g honey). All honey samples, hence, belonged to the class I of representatively (under – represented honeys, with less than 20,000 pollen grains in 10 g honey, according to Louveau, 1978). [33]

4.4 Antimicrobial activity of honey

In this study we have demonstrated that locally produced honeys had activity against some pathogenic bacteria. Our data showed that all honeys tested had some antimicrobial action at concentrations as low as 10%, however, the greatest inhibition was seen with the undiluted honey. The growth of only *C. albicans* was not inhibited by the honeys (Table 4). Similarly, *C.albicans* was not inhibited by Australian honeys.[25] However, this yeast species was inhibited by the honey

produced from different phytogeographic regions of Costa Rica [24] and by honey from United Arab Emirates when honey was added 2 to 6 hours after inoculation of the yeast into the broth. Honey reduced growth of *C. albicans* from 5+ to 1+ when added between 8 and 24 hours after inoculation.[34]

S. aureus, on the other hand was inhibited completely by the black seed honey (sample 4) whether undiluted or diluted up to 75% and 50% giving inhibition zones of 1.8±1.11cm, 1.5±0.57cm, and 1.2±0.28cm, respectively. It was also inhibited completely by the undiluted honey of samples 6, 3, 7 and 5 with inhibition zones of 1.85±0.57cm, 0.8±0.2cm, 0.3±0.14cm, and 0.2±0.0cm, respectively.

The other tested honey samples (samples 1and 2) reduced the growth of *S. aureus* to 50% (score 2) at 75%, 50% and 30% of honey dilution and to 25% (score 1) at 10% of honey dilution (Table 4).

In another study, *S. aureus* was the most inhibited bacterial strain by Argentinan honeys especially in its undiluted form, and at dilutions of 75% and 50% in only 2 of the tested honey samples.[35] Similarly, this bacterium was inhibited by honey from United Arab Emirates at 50%, 60%, 70%, 80%, 90% and 100% concentrations of honey.[34] On the other hand, *S. aureus*, was not susceptible to honey from different phytogeographic regions of Costa Rica[24] and an unexpected overall poor activity of Australian honeys was observed against this organism .[25]

In the present study, *B. cereus* was the most inhibited bacterial strain, as it was affected by 6 honey samples out of the 8 tested ones. The growth of this bacterium was inhibited mainly by honeys in their undiluted forms (samples 1, 2, 3, 4, 5, and 6). The highest inhibition zone measured was for sample 4 (2.46±0.58cm) which showed the highest antibacterial activity against *B. cereus* as the inhibition occurred

also at dilutions of 75% (inhibition zone = 2.2±0.14cm), 50% (inhibition zone = 1.7±0.45cm) and at 30% (inhibition zone = 1.1±0.07cm (Table 4). *B. cereus* was also susceptible to the effect of honey in another study.[24]

All honey samples were found to reduce to the growth of *E.coli* to 50% (score 2) at the undiluted form of honey, and at dilutions of 75% and 50%, however, at 30% dilutions; 25% only of growth was inhibited (score 1). The only exception was for sample 4 (undiluted form) as it inhibited the growth of this bacterium (inhibition zone of 2.2±0.14cm).

E. coli was inhibited by Australian honeys. At 20% dilutions of honey, *E .coli* was reduced to 75% inhibition (score 3), and at higher concentrations there was a progressive increase in inhibition as honey concentration increased.[25] Argentinian honeys, on the other hand, inhibited the growth of *E. coli* to a lesser extent (4 samples out of the 15 tested ones).[35] Complete inhibition for *E .coli* growth at dilutions as high as 30% of honey was from United Arab Emirates .[34]

4.5 Correlation of the physiochemical and the sugar composition of honey to its antibacterial activity

Honey samples examined in this study are the most commonly consumed and produced in Egypt, especially clover and citrus. Results obtained indicates that the quality of honey is acceptable as most of the samples were complying with codex 2001,1998, European standard 2002 and Egyptian standards 2005 concerning their physiochemical and sugar composition, in addition they were consistent with other reports. This also, included its antibacterial activity which showed mainly a reduction in bacterial growth. This means that Egyptian honey sold in markets or bought from beekeepers is useful for the health of consumers. This usefulness might be maximized if honey contained higher number of pollen grains. This is indicated

after comparing the bacterial inhibition and/or reduction by the different examined honey samples where samples having the highest pollen counts (samples 6 ,4, and 3 exhibited the highest antibacterial activity (table 4).

The undiluted form of sample 6 (citrus honey) had remarkable antibacterial activity against *S. aureus* (inhibition zone = 1.85±0.57 cm) and against *B. cereus* (inhibition zone = 0.8±0.28 cm), in the mean time it caused a reduction of 50 % in growth of *E. coli*. Also dilutions of 75%,50% and 30% caused similar reduction for both S*. aureus* and *E. coli* but caused only a reduction of 25% in *B. cereus* growth. This honey sample was complying with codex 2001, 1998, European standard 2002 and Egyptian standard 2005 in all physiochemical and sugar composition (tables 1, 2). This was not however, the case of samples 3 and 4 which although fructose/ glucose ratio (sample 3) and the sucrose content (sample 4) were out of standards they exhibited considerable antibacterial activity. Sample 3 had almost similar effect as sample 6, in addition, its pollen count (10090 pollen/10g honey) approached that of sample 6 (13000 pollen/ 10 g honey) (table3). Sample 4, on the other hand exhibited the most powerful antibacterial activity against *S*. *aureus* and *B. cereus* undiluted and at 75%, 50% and 30% dilutions with varying sizes of inhibition zones as low as 1.1 and as high as 2.46 cm although its sucrose content was out of standards. The high pollen count (12000 pollen/ 10 g honey) of this sample together with the known bactericidal effect of the black seed might be the cause for this powerful antibacterial activity. The provenance of honey could determine its antibacterial properties.[35] Following was sample 5 (9000 pollen /10 g honey) which similarly inhibited *S. aureus* (inhibition zone = 0.2 ±0.0 cm) and *B. cereus* (inhibition zone = 1.4 ±0.52 cm). This sample had no complying sucrose

content (> 5%) and had slightly lower pollen grains (9000 / 10 g) than that of the complying sample 2 (9350 / 10 g) which inhibited only *B. cereus* with a much lower inhibition zone (0.5± 0.1 cm). This might be attributed to the lower free acidity of this sample (8.25 ± 0.35 meq/ kg) (table 1). Although sample 7 had higher pollen grains (9800 pollen/10g), it only caused inhibition of S. aureus (0.3 ± 0.14 cm inhibition zone) at undiluted form and reduction in growth at 75 and 50% dilutions. This sample in particular showed a considerably high sum of fructose + glucose (97.297±36.65) suggesting intensive and extended bee-feeding with sugar syrup especially that maltose was the highest (3.27±0.090) this sample compared to the other ones. It is known that intensive and extended bee-feeding with sugar syrups results in chemical modification of honey quality similar to direct insertion of sugar syrup into honey, however in that case the maltose amount starts increasing and in parallel to this the sucrose level starts decreasing due to the bee's enzymatic hydrolysis as the hydrolyzed sucrose is stored on the maltose form.[36] This suggests that bee-feeding with sugar syrup affects its antibacterial activity even in the presence of a higher pollen grains.

Although sample 8 was as sample 4, i.e., not complying with codex 2001,1998, European standard 2002 and Egyptian standards 2005 [26-29] concerning sum of fructose + glucose (< 60%) and sucrose content (>5%) in contrast to sample 4 it exhibited no antibacterial activity against *S.aureus* and *B. cereus* but caused only a reduction of 50% (score 2) in *E .coli* growth at undiluted form, 75% and 50% of honey dilutions (Table4). Both samples differed in their content of pollen grains which was lowest in sample 8 (5220 pollen/10g). The higher pollen content in sample 4 might be a contributing factor to this higher antibacterial activity.

As the glucose oxidase in honey

originates in bees, one might expect a similar glucose oxidase level in all examined honey samples, since bees control the ripening of honey within narrow limits .[15] This coupled with our results that the higher the pollen grains in honey the higher is the antibacterial activity may suggest that catalase in pollen grains did not destroy the hydrogen peroxide in honey. Hence, we agree with the suggestion by Weston (2000)[15] that hydrogen peroxide might react with the benzoic acids of honey to create peroxyacids, which are more stable than hydrogen peroxide. These acids will escape destruction when catalase is added to a solution of honey prior to an antibacterial assay, due to the selectivity of the catalase, which is specific for hydrogen peroxide and does not destroy alkyl peroxides or peroxycarboxylic acids. Peroxycarboxylic acids are more powerful antimicrobial agents than hydrogen peroxide and this fact might compensate for the low level of the carboxylic acids in honey.[15] Other studies might be needed on commercial antibacterial honeys from Arabic regions and on that containing much higher counts of pollen grains content.

Conclusion

1. Honey samples having sucrose content > 5% found in both beekeepers and markets honey indicate adulteration using added sugar syrups.
2. Honey samples – including not complying ones – exerted some antibacterial activity against the tested organisms.
3. The higher the pollen grain content of honey, the higher is the antibacterial activity of that honey.
4. Intensive and extended bee-feeding on sugar syrups results in chemical modification of honey quality similar to direct insertion of sugar syrup into honey, in addition reduces greatly the antimicrobial activity of honey even in

the presence of higher pollen grains count.

5. The floral spectrum of the locally produced Egyptian honeys consists mainly of *Trifolium* sp. pollen (84-98 %).

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REFERENCES

1. Al-Waili N, Saloom K. Effects of topical honey on post-operative wound infections due to gram positive and gram negative bacteria following caesarean sections and hysterectomies. Eur j Med Res. 1999; 4: 126-30.
2. Al-Waili N. Topical honey application vs, acyclovir for the treatment of recurrent herpes simplex lesions. Med Sci Monit. 2004; 10(8): MT94-MT98.
3. Al-Waili N. Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. J Med .Food. 2004; 7: 210-22.
4. Molan P. Honey as a topical antibacterial agent for treatment of infected wounds. 2001. Available from: http://www.worldswidewounds.com/2001/november/Molan/honey-as-topical-agent.htm.
5. Molan P. Using honey dressing the practical consideration. Nurs. Times. 2000; 96: 36-7.
6. McInerney F. Honey-a remedy rediscovered. J Royal Sec Med. 1990;83: 127-28.
7. Zaghloul A, El-shattawy H, Kassem A. Honey, a prospective antibiotic: extraction, formulation, and stability. Pharmazie. 2001;56:643-47.
8. Zumla A, Lultat A. Honey a remedy rediscovered. J Royal Soc Med. 1989,82: 384-85.
9. Molan PC. The antibacterial activity of honey: 1. The nature of the antibacterial activity. Bee World. 1992a; 73: 5-28.
10. Molan PC. The antibacterial activity of honey: 2. Variation in the potency of the antibacterial activity. Bee world. 1992b;73: 59-76.
11. White Jr JW. Composition of honey. In: Crane, E (Ed.). Honey: a comprehensive survey. New York: Crane, Russak & Company. Inc; 1975; p 157-206.
12. Shin H, Ustunol Z. Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria. Food Research International. 2005; 38: 721-28.
13. White Jr JW, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochemica et Biophysica Acta. 1963;73 :57-70.
14. Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen perioxide and level of antioxidant power. Inter J food Microbiol. 2001;69: 217-25.

15. Weston RJ. The contribution of catalase and other natural products to the antibacterial activity of honey: a review. Food Chemistry. 2000;71: 235-39.
16. Gauhe A. Über englucoseoxydierendes Enzyme in der pharnyxdrüse der Monigbiene. Zeitschrift für vergleichende physiologie. 1941;28(3): 211-53.
17. Association of official analytical chemists (AOAC). Official methods of analysis (16th ed.) Arlington, VA: Association of official analytical chemists. 1995.
18. Association of official analytical chemists (AOAC). Official methods of analysis (15th ed), Arlington, VA: Association of official analytical chemists. 1990
19. Maurizio A. Microscopy of honey. In E Crane (Ed), Honey: a comprehensive survey. London: Heineman in cooperation with international Bee Research Association. 1979; p. 240-57.
20. Erdtman G. The acetolysis method. Svensk Botanisk Tidskrift. 1960;54: 561-4.
21. Pèrez-Arquilluè C, Conchello P, Arifio A, Juan T, Herrera A. Physiochemical attributes and pollen spectrum of some uniffloral Spanish honeys. Food Chemistry. 1995; 54: 167-72.
22. Forbes BA, Sahm DF, Weissfeld AS. Bailey & Scott ´s diagnostic microbiology. 10 th Edition, St. Louis: Mosby; 1998.
23. Sommeijer MJ, Beetsma J, Boot WJ, Roberts EJ, de Vries R. Perspectives for honey production in the tropics, proc. Symp. Organized by the Netherlands Expertise Centre for Tropical Agricultural Research (NECTAR), Utrecht, Netherlands, Dec. 18. 1995
24. Demera JH, Angert ER. Comparison of the antimicrobial activity of honey produced by *Tetragonisca angustula* (Meliponinae) and *Apis mellifera* from different phytogeographic regions of costa Rica. Apidologie. 2004;35: 411-7.
25. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Archives Med Res. 2005; 36:464-7.
26. Codex Alimentarius standard of honey. No.12-1981. Rev. 1(1987),Rev.2 (2001). FAO and WHO, Rome.
27. European Commission Council Directive 2001/110/CE concerning honey. Off J Eur Communities. 2002, L10/47-52.
28. Codex Alimenation. Codex Alimentarius draft revised for honey at step 6 of the codex procedure. CX 5/10.2, CL 1998/12-S.FAO and WHO, Rome; 1998. Egyptian standards 2005. Honey and methods of analysis part 1: Honey, No. 355. 1998.
29. Egyptian standards: Honey and methods of analysis part 1: Honey, No. 355, Egyptian Organization for standardization and Quality Control, Egypt, 2005.
30. Terrab A, Diez MJ, Heredia FJ. Characterization of Moroccan unifloral honeys by their physiochemical characteristics. Food chemistry. 2002; 79: 373-9.
31. Mateo R, Bosch-Reig F. Sugar profiles of Spanish unifloral honeys. Food Chemistry. 1997; 60(1): 33-41.
32. Torres A, Garedew A, Schmolz E, Lamprecht I. Calorimetric investigation of the antimicrobial action and insight into the chemical properties of “angelita” honey-a product of the stingless bee tetragonisca angustula from Colombia. Thermochimica acta. 2004;415: 107-13.

33. Louveaux J, Maurizio A, Vorwofl G.. Methods of melissopalynology, Bee World. 1978; 59: 139-57.
34. Al-waili NS, Akmal M, Al Waili FS, Saloom KY, Ali A. The antimicrobial potential of honey from United Arab Emirates on some microbial isolates. Med Sci Monit. 2005;11(12), BR433-BR438.
35. Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microbiol. 2007;124: 375-81.
36. Cordella C, Militão JSLT, Clément MC, Drajnudel P, Cabrol-Bass D.. Detection and quantification of honey adulteration via direct incorporation of sugar syrups or bee-feeding: preliminary study using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and chemometrics. Analytica Chimica Acta. 2005;531: 239-48.