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THE INHIBITORY ACTIVITY OF HONEY AND "HONEY PASTES" AGAINST SELECTED FOODBORNE BACTERIAL PATHOGENS

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ABSTRACT

Twenty nine samples of locally produced honey and twenty two samples of honey pastes were collected from retail outlets in Jeddah, Saudi Arabia. Their antibacterial activity against some Gram positive and Gram negative foodborne bacterial pathogens using the agar well diffusion method was studied. In addition, the minimal inhibitory concentrations (MIC) were determined for honey pastes samples using the dilution method. All samples of honey had antibacterial activity against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157: H7 and *Salmonella typhimunium* on plates of tryptic soy agar with varying diameters of inhibition zones. Samples of honey pastes showed also antibacterial effect against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157: H7 and *Salmonella typhimunium* adopting the previous technique. MIC varied between honey pastes samples where it was sample dependent.

Key Words: Honey, Honey pastes, Inhibitory activity

INTRODUCTION

Honey pastes are those paste-like food commodities that mainly consisted of honey mixed with various different ingredients. Available brands mostly contained, in addition to honey, the black seed (*Nigella sativa*), royal jelly, propolis, pollen grains, ginseng and natural herbs. These popular products are claimed to have nutraceutical benefits and to positively affect the consumer health. Retail outlets and groceries in Saudi Arabia sell different brands of honey pastes either imported or locally processed.

Honey had antibacterial properties as reviewed in depth by **Molan (1992a & b)** and briefly by **Armstrong & Otis (1995); McCarthy (1995) and Molan (1995).** Expectedly, honey pastes might have the same properties in regards to the antibacterial activity as honey was their main ingredient. In addition, other ingredients included in honey pastes were shown to possess antibacterial activity as well. The black seed was reported to have antagonistic effect **(Hanafy and Hatem,**

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1991). Propolis has similar properties to honey **(Sato and Miyata, 2000).** The antibacterial, antifungal and antiviral activities of propolis were documented by **Kujumgiev *et al* (1999).** Royal jelly had high antibacterial activity **(Abd-Alla *et al* 1995)**. Ginseng (*Parax notoginseng*) contains saponins which reflected antibacterial properties **(Cowan, 1999).** Since honey pastes are widely consumed and due to lacking of information, research studies concerning the safety, microbiological quality and antibacterial activity of these commodities were undertaken. Separate parts of the results of these studies constituted in-press articles **(Al-Hindi, 2005 a & b)** while the remaining part of the results were the subject of the present report.

MATERIAL AND METHODS

Samples collection

a) Honey samples

A total of 29 locally produced honey samples were obtained from retail sites in Jeddah, Saudi Arabia. Honey samples were of different floral origin. Composition of honey samples was reported elsewhere (Al-Hindi, 2004). Samples were supplied in airtight glass or plastic containers and kept at 10°C in the dark.

b) Honey paste samples

Twenty two honey paste samples were collected from different retail outlets and groceries throughout Jeddah city, Saudi Arabia. Samples resembled mostly all available marketed brands of honey pastes. These contained beside honey, a cocktail of additives which varied from a brand to another as stated on their containers labels. Propolis, pollen grains, royal jelly, ginseng and black seeds were the mostly present ingredients in honey pastes samples studied. Samples were supplied in airtight glass or plastic containers and kept at 10°C in the dark until examined.

Bacterial strains and preparation of inocula

Four foodborne bacterial pathogens, 2 of which were Gram negative (G-ve) while the others resembled Gram positive (G+ve) species, were used during this study. *Escherichia coli* O157: H7, *Salmonella typhimurium* and *Listeria monocytogenes*, *Staphylococcus aureus* resembled both G-ve and G+ve bacteria, respectively. All strains were kindly provided by Prof. S. Abdl El Ghani, National Research Center, Dairying department, Dokki, Giza, Egypt. All pathogens were maintained frozen at –18°C and were propagated twice in tryptic soy broth (TSB; Difco, USA) at 37°C for 24h before being used as inocula.

Assay for inhibitory activity

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The antibacterial activity of honey and honey pastes against foodborne pathogens was assayed using the agar well diffusion method as described by **Allen *et al* (1991).** Plates seeded with either *E. coli* O157: H7, *S. typhinurium*, *S. aureus* or *L. monocytogenes* were prepared by adding 100 µl of a 18-h culture of target bacteria (in TSB) to 15 ml of sterilized tryptic soy agar (TSA) cooled to 45°C. The plates were poured on a level surface immediately after mixing and allowed to solidify at 4°C for 1h. Wells were cut in the agar using a cooled flamed (8mm) cork borer. Honey and honey pastes samples were tempered to 50°C in a water bath (to become semi-liquid) and were pipetted in 0.5-ml aliquots into the wells. The plates were incubated at 37°C for 24h and examined for inhibition zones caused by the samples. Diameters of zones of inhibition were measured in (mm).

Minimal inhibitory concentration (MIC)

MIC for honey pastes was carried out according to the method described by **Stahly and Ryan (1995).** A series of tubes containing 5 ml of TSB supplemented with appropriate amounts of the sample to yield concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 percent. Tubes were then inoculated by 0.2 ml of an 18h-culture of the four bacteria used in the study. Growth after incubation at 37°C for 24 or 48h was observed visually. The lowest concentration showed no growth was recorded as the minimal inhibitory concentration (MIC). A loop from the recorded minimal inhibitory concentration was streaked on the surface of a TSA plate and incubated at 37°C for 24h. Negative result with no growth confirmed the appropriate minimal inhibitory concentration.

RESULTS AND DISCUSSION

1- Antibacterial activity of honey

Table (1) showed the inhibitory activity of honey samples. The samples were grouped in three categories according to the measured diameters of inhibition zones for the four pathogens tested. For Gram-ve bacteria namely, *E. coli* and *S. typhimurium*, 21 and 22 samples showed diameters between 2 to 4mm, 6 and 5 samples had diameters between >4 to 6mm, and 2 samples for each pathogen had >6mm diameters, respectively. On the other hand, for Gram+ve species, 14 and 13 samples had diameters between 2 to 4mm, 7 and 6 samples with diameters between >4 to 6mm, and 8 and 10 samples had >6mm diameters for *L. nonocytogenes* and *S. aureus*, respectively.

Table 1. Results of antibacterial activity of 29 locally produced honey samples against four bacterial species.

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Diameter of inhibition zones in (mm)	No. of samples			
	E. coli O157:H7	*S. typhimurium*	*L. monocytogenes*	*S. aureus*
2 to 4	21 (72.4%)	22 (75.9%)	14 (48.3%)	13 (44.8%)
>4 to 6	6 (20.7%)	5 (17.2%)	7 (24.1%)	6 (20.7%)
>6	2 (6.9%)	2 (6.9%)	8 (27.6%)	10 (34.5%)

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From the obtained results it could be concluded that all honey samples showed antibacterial activity against the four foodborne pathogens employed. However, there was a difference in the effect between G-ve and G+ve bacteria. The antibacterial inhibitory effect was more pronounced in the latter based on the data cited in Table (1). The reason may be attributed to the different response of the outer membrane in both groups of bacteria to the antibacterial agents present in honey. In this respect, the antibacterial components in honey itself have clearly not been fully defined **(Al-Waili, 2005).**

2- Antibacterial activity of honey pastes

Table (2) reported the inhibitory activity of 22 honey pastes samples. As have been discussed with honey, the samples were also grouped in 3 categories according to the measured diameters of inhibition zones. It appeared from Table (2) that for G-ve bacteria, there were 14 and 13 samples in the first category of 2 to 4mm diameter, 7 and 7 samples in the second category of >4 to 6mm and 1 and 2 samples in the >6mm category. In case of G+ve bacteria, as seen in the same Table, 4 and 6 samples fell in the category of 2 to 4mm, 12 and 7 samples in >4 to 6mm and 6 and 9 samples in the third one being >6 mm in diameters. Also here, the antibacterial inhibitory effect was more pronounced on the G+ve bacteria. Compared to the concomitant figures in Table (1), it was clear that honey pastes had more vigorous antibacterial activity. This could be attributed to the occurrence of other ingredients in honey pastes with antibacterial activity as reported in the introduction section. More recently, honey mixture apparently could inhibit growth of *S. aureus* and *Candida albicans* as reported by **Al-Waili (2005)** which coincided with the current results.

Table 2. Results of antibacterial activity of 22 honey pastes samples against four bacterial species.

Diameter of inhibition zones in (mm)	No. of samples			
	E. coli O157:H7	*S. typhimurium*	*L. monocytogenes*	*S. aureus*
2 to 4	14 (63.6%)	13 (59.1%)	4 (18.2%)	6 (27.3%)
>4 to 6	7 (31.8%)	7 (31.8%)	12 (54.5%)	7 (31.8%)
> 6	1 (4.5%)	2 (9.1%)	6 (27.3%)	9 (40.9%)

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3- Minimal Inhibitory Concentration (MIC)

Table (3) cited the percent (w/v) concentration of honey paste which inhibited bacterial growth in a tube containing the sample and pathogen in a model system (TSB). The samples were grouped in four groups according to the MIC of honey pastes samples for the four pathogens tested. For the G-ve bacteria resembled by *E. coli* O157: H7 and *S. typhimurium*, 10% concentration of 4 and 6 tested samples was competent to bacterial growth,

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Table 3. Results of minimal inhibitory concentration of 22 honey pastes samples against four bacterial species.

% conc. of honey paste (w/v)	No. of samples			
	E. coli O157: H7	*S. typhimurium*	*L. monocytogenes*	*S. aureus*
10	4	6	5	4
15	12	10	8	9
20	6	6	3	4
25	-	-	6	5

respectively. The same concentration from 5 and 4 samples of honey pastes inhibited *L. monocytogenes* and *S. aureus*, respectively. Worthy mention, as shown in Table (3), that the higher concentration of 25% from all honey pastes samples was required to inactivate the two G+ve bacteria tested. Honey pastes samples containing different ingredients in possibly quite varied concentrations could withstand as an explanation for such findings.

On conclusion, honey pastes samples showed more pronounced antibacterial activity against tested foodborne pathogens due to the ingredients used in their preparation. The antibacterial activity of honey pastes could be attributed to their contents of spices and medicinal herbs possessing antioxidant, antibacterial, antifungal and antiviral activities **(Al-Waili, 2005).**

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النشاط التثبيطي لعسل "السدر" ومعاجين العسل ضد بعض البكتيريا الممرضة المنقولة بواسطة الأغذية

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جمعت تسع وعشرون عينة من سدر محلياً واثنان وعشرون عينة من معاجين العسل من الأسواق بمدينة جدة بالمملكة العربية السعودية. معاجين العسل سلع غذائية تتكون أساساً من العسل مخلوطاً معه محتويات عديدة تختلف في تكوينها من نوع لآخر. بشكل عام احتوت أنواع معاجين العسل المتوافرة بالأسواق بالاضافة للعسل على حبة البركة، والسوداء والغذاء الملكي والبروبوليس وحبوب اللقاح والجنسنج وأعشاب طبيعية.

التراكيز المثبطة الدنيا من معاجين العسل باستخدام طريقة التخفيف.

دلت النتائج أن عينات العسل ومعاجين العسل تحت الدراسة كان لها نشاط تثبيطي واضح ضد أربعة من البكتيريا المستهدفة، اثنتان منها موجبة لصبغة جرام وهي *Listeria monocytogenes* و *Staphylococcus aureus* و اثنتان منها سالبة لصبغة جرام وهي *Escherichia coli* O157: H7 و *Salmonella* typhimurium. و بشكل عام

كان الهدف من هذه الدراسة تقدير النشاط التثبيطي ولوحظ أن القدرة على التثبيط كانت تختلف باختلاف الدراسات على عينات العسل ومعاجين العسل ضد بعض البكتيريا أكثر فعالية من عينات العسل لمعاجين العسل المضاف إليها الأعشاب الطبيعية والمواد الأخرى المضافة لمعاجين العسل ذات قدرة تثبيطية للبكتيريا. المنقولة بواسطة الأغذية وذلك باستخدام طريقة الانتشار بالآبار. تم أيضاً تقدير

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