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ARAŞTIRMA MAKALESİ/RESEARCH ARTICLE

INVESTIGATION OF ANTIMICROBIAL EFFECT OF HONEY AND THE ROLE OF OSMOLORITY

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ABSTRACT

The antimicrobial effect of honey was evaluated by an in vitro study testing the growth of various Gram-negative and Gram-positive bacteria and a yeast in media containing varying concentrations of honey.The bacteria used were *Escherichia coli, Klebsiella pneumonia* and *Staphylococus aureus* and *Candida albicans* as a yeast.Two honey samples collected from various regions of Turkey were tested for their antimicrobial activity. It was found that the more honey was concentrated the more was the inhibition zone on bacteria but not on the yeast.

The possible mechanism of this effect is suggested to be the osmolority of invert sugar which is present naturally in honey.

Key Words: Honey, Antibacterial activity, Osmosis.

BALIN ANTİMİKROBİAL ETKİSİNİN İNCELENMESİ VE OSMOLORİTENİN ROLÜ

ÖZ

Balın antimikrobial etkisi farklı konsantrasyonlarda bal içeren ortamlarda Gram negatif ve Gram pozitif bakteriler ve bir maya üzerinde in vitro olarak test edilmiştir. Kullanılan bakteriler *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* ve bir maya olan *Candida albicans*'tır. Türkiyenin farklı bölgelerinden elde edilen iki bal örneği antimikrobial aktiviteleri açısından test edilmiştir. Bal konsantrasyonu arttıkça bakteriler üzerinde oluşan inhibisyon zonlarının da arttığı ancak maya ortamında bunun gerçekleşmediği bulunmuştur.

Bu etkinin muhtemelen balın doğal yapısında mevcut olan invert şekerin ozmoloritesinden kaynaklandığı düşünülmektedir.

Anahtar Kelimeler: Bal, Antibakteriyal aktivite, Ozmozis.

1. INTRODUCTION

Honey is a nutritiously rich food product that is consumed by human populations throughout the world. Besides its nutritional value, it also contains antibacterial agents with different floral activity. It was reported that honey had antimicrobial activity, against a number of Gram positive and Gram negative bacteria (Farouk *et al.*, 1988; Russel *et al.*, 1990; Subrahmanyam, 1991) and Candida albicans (Haspolat *et al.*, 1990).

The antibacterial activity of honey has been attributed both to physical factors: osmolority (Molan, 1992; Bogdanov, 1997; Weston, 2000) and acidity (Mato *et al.*, 2000; Weston *et al.*, 2000) and chemical factors: hydrogen peroxide (Weston, 2000), cecropin-A and mellitin, methyl 3,5-dimethoxy-4-hydroxybenzoate, methyl-3,4,5-trimethoxybenzoate, 3,4,5-trimethoxybenzoicacid, 3,5-dimethoxy-4 hydroxybenzoic acid (syringic acid), tetracyclin, nectar, volatiles, propolis and unidentifed substances from certain floral sources (Molan and Russell., 1988; Boman, *et al.*, 1989; Russel, *et al.*, 1990; Allen *et al.*, 1991; Andreu, *et al.*, 1992; Willix, *et al.*, 1992; Weston *et al.*, 2000).

The purpose of the present study was to test the antibacterial effects of the honey samples on clinically isolated bacterial species, and evaluate its antimicrobial effect.

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2. MATERIAL AND METHODS

2.1 Collection of honeys

Honey samples were obtained from Bolu and Erzurum region of Turkey. Invert sugar, Sucrose, Hydroxymethylfurfural (HMF) and the number of total pollen analyses were performed for each honey sample (Table 1).

2.2 Preparation of honey samples

All honey samples were prepared aseptically, and kept away from direct sunlight. Honey samples were diluted using sterile distilled water serially from 50% to 10%.

2.3 Assay of antimicrobial activity

The clinically isolated bacterial species which were used in the study were *Escherichia coli, Klebsiella pneumoniae* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Candida albicans* (yeast) provided by the bacteriology department of Ankara hospital.

The antimicrobial activity of the honey samples was assayed using the agar well diffusion method (Farouk, *et al.*, 1988; Molan and Russell, 1988, Allen *et al.*, 1991).

Bacteria were cultured in liquid Tryptic Soy Broth (Difco 30 g/L) and the measurements of the bacterial growth were calculated using Mc Farland 0.5 method (Jeddar *et al.*, 1985). Following the calculation, 1ml each of bacterial samples was diluted 100x with sterile Nutrient Agar medium (Difco 28 g/L) (Farouk *et al.*, 1988; Russel *et al.*, 1990), mixed throughly and poured into petri plates. Sabouraud Dextrose Agar (Difco, 65 g/L) was used for Candida albicans (Brooks *et al.*, 1995).

Six wells with diamaters of 8 mm were punctured and filled up with honey samples of 0.2 ml (10% up to 50%). One out of 6 wells was filled with undiluted honey sample. These plates were then incubated at 37°C for 18 h. (Jeddar, *et al.*, 1985., Allen, *et al.*, 1991, Farouk *et al.*, 1988, Molan and Russell., 1988).

Additionally, sour-cherry jam samples of commercial type were used in the same manner as honey in the experiments.

Zone diameters were measured manually in mm units.

2.4 Control plates

Control plates were prepared in identical dilutions and appropriate concentrations as in the antibacterial activity test (Table 1). As agents of putative antimicrobial activity other than honey we used hydroxymethylfurfural, sucrose, invert sugar and jam. Dilution procedures and the loadings were the same for all of the control compounds as for honey.

3. RESULTS AND DISCUSSION

Studies with *K. pneumoniae, S. aureus,* and *E. coli* showed that the concentrations of 50 and 100 % of honey were not perfectly tolerated. We observed that despite the zone-like circles that were formed around the wells containing 10, 20, 30 and 40 % dilutions (among which the 40 % dilution group had circles around), the bacteria could reproduce in those areas. As for the wells that contained undiluted or 50 % diluted honey samples, it was observed that they had minute real zones around them. None of the honey samples had an effect on at any level.

When the control plates were incubated for 18 h at 37°C, the plates containing HMF (a decaying product formed in the heating procedure of the sugars such as glucose and fructose at acidic pH, (Vorwohl *et al.*, 1989), and sucrose had no zone, but the results obtained from the experiments with invert sugar were found to be identical to those found with honey. The same results were obtained in many repeated experiments (Table 2).

Preliminary results of zone formations in our experiments resembled to those of previous investigations reported by Jeddar *et al.*, (1985) and Farouk, *et al.*, (1988).

High content of sugar in the wells creates a hypertonic environment for bacteria. In this case, the bacteria continuously lose water, which results in separation of cell membrane from cell wall, and ultimate plasmolysis occurs (Brock *et al.*, 1994). In water-deficient cells, metabolic activities and cell division reduce. Contrary to the procaryotic cells, the yeast *Candida albicans* is thought to have different response to such osmolority and therefore are still reduced (Frazier and Westhoff, 1978, Cemeroğlu and Acar, 1986).

Typical zone circles such as these were routinely obtained with honey, jam, and invert sugar tests. We conclude that the high osmolority in the honey filled wells was an effective cause for the bacteria to lose water irreversibly, blocking their reproduction.

As a conclusion, our results confirm the osmolority of the invert sugar which is a natural component of honey and among other causes, is mainly responsible for this activity. Finally, our results are in agreement with those of Yatsunami and Echigo (1984) and Jeddar *et al.*, (1985).

Table 1. The Analysis of Tested Honey Samples.

Region of the collected honey samples	Invert sugar	Sucrose	HMF	Pollen*
Bolu-Omerler	54.12	8.61	2.38	1400
Erzurum-Kandilli	77.69	1.24	3.36	159000

* Total number of pollen in 10 gr honey.

Table 2. Comparison of Inhibition Zone Ranges of Honey, Invert Sugar, Sucrose, HMF and Jam Against Bacterial Isolates.

	Escherichia coli (inhibition zone mm) Concentrations						*Klebsiella pneumonia* (inhibition zone mm) Concentrations						*Staphylococcus aureus* (inhibition zone mm) Concentrations					
	%10	%20	%30	%40	%50	%100	%10	%20	%30	%40	%50	%100	%10	%20	%30	%40	%50	%100
Honey (Bolu)	3	5.	6	10	13	15.5	-	6	8	11	13	15	4	6.	9	12	13	14.5
Invert sugar	3	4.5	6	10	13	15	-	5	6	10	12	14	3	5	8	10	11.5	14
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HMF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Honey (Erzurum)	4.5	7	8	9	11.5	14.5	-	4	7	10	12	15	4.5	8	12	13	15.5	18
Invert sugar	3.5	5	7	8	11	13.5	-	3	6	10	11	14	4	7	11	12	13	16.5
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HMF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jam	2	4.5	5	7	9.5	13	-	2	5.5	8	9.5	13	3	5	7.5	9.5	10	14

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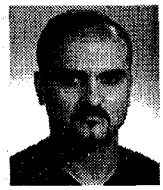

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