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Investigation of Antimicrobial Effect of Honey Collected from Various Regions of Turkey

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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. It was found that most of the honey samples at a concentration of 50% and above exerted inhibitory effects on bacteria but not on the yeast. Honey collected from Rize-Anzer region was found to be the most effective honey on clinically isolated bacteria.

Key words: Honey, honey bee, antibacterial effect, natural products

Introduction

Honey is a nutritionally rich food product that is consumed by human populations throughout the world. 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 (Subrahmanyam, 1991; Willix *et al.*, 1992; Farouk *et al.*, 1988; Russel *et al.*, 1990; Bogdanov, 1984; AlSomai *et al.*, 1994) and *Candida albicans* (Haspolat *et al.*, 1990).

The antibacterial activity of honey has been attributed both to physical factors: osmolarity (Molan, 1992; Bogdanov, 1997; Weston, 2000) and acidity (Bogdanov, 1997; Mato *et al.*, 2000; Weston *et al.*, 2000) and chemical factors: hydrogenperoxide (AlSomai *et al.*, 1994; Weston, 2000), cecropin-A and mellitin, methyl 3,5-dimethoxy-4-hydroxybenzoate, methyl-3,4,5-trimethoxybenzoate, 3,4,5-trimethoxybenzoic acid, 3,5-dimethoxy-4 hydroxybenzoic acid (syngic acid), tetracyclin, nectar, volatiles, propolis and unidentified substances from certain floral sources (Bogdanov, 1984; 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 different honey samples on clinically isolated bacterial species and evaluate the antimicrobial effect of honey.

Materials and Methods

Collection of honey: Honey samples, which were collected during the flowering seasons, were obtained from apiarists throughout Turkey and stored in airtight bottles at 10 °C in dark. Assayed samples might be stored as well as for 2 years (Allen *et al.*, 1991).

Preparation of honey samples: All honey samples were prepared aseptically and kept away from direct sunlight. Honey samples were diluted serially from 50 to 10%.

Assay of antimicrobial activity: Clinically isolated bacterial species such as *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative), *Staphylococcus aureus* (Gram positive), and *Candida albicans* (yeast) were used.

These species were kindly provided from Bacteriology Department of Ankara Numune 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 1 mL of bacterial samples were diluted 100x with sterile nutrient agar medium (Difco 28 g/L) (Farouk *et al.*, 1988; Russel *et al.*, 1990), mixed thoroughly and poured into the petri plates. Sabouraud Dextrose Agar (Difco, 65 g/L) was used for *Candida albicans* (Brooks *et al.*, 1995).

The prepared petri plates were kept at 4 °C until needed. Six wells with diameters of 8 mm were punctured and filled up with honey samples (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). Antibacterial activity was assumed if a zone formation around a well present.

Control plates were prepared for the antibacterial activity test.

Results and Discussion

Our study confirms the antibacterial effect of honey on various Gram-negative and Gram-positive bacteria. In particular, pure honey is a very potent inhibitor of growth of bacteria such as clinical isolates of *E. coli*, *K. pneumoniae* and *S. aureus*.

All bacterial pathogens failed to grow in honey at different concentrations depending on the honey samples (Tables 1,2,3). However, none of the honey samples had any effect on *C. albicans* in this respect (Data are not shown).

***Escherichia coli*:** Rize-Anzer A, B, C and D samples were more effective (at concentrations 30% and above) on *E. coli* bacteria than the other honey samples. However, other group that consists of samples from Bayburt-Armutlu, Eskisehir-Merkez, Kutahya-Domaniç, Nigde-Ulukisla and Sivas-Gokluce prevented bacterial growth at concentrations of 40% and above (Table 1). Honey samples collected from Ankara-Cubuk Karagol, Bolu-Onerler, Cankiri-Eskipinar, Erzincan-Ballikoy, Konya-Altinoba, Sivas-Yilanlikaya and Yozgat-Kocak Kumuk failed on *E. coli* even at a concentration of 50% (Table 1). However, most of the honey samples analyzed were inhibitory at 50% and above concentrations (Table 1).

***Klebsiella pneumoniae*:** Afyon-Anitkaya, Ankara-Cubuk Akkuzular, Bayburt-Armutlu, Bolu-Mudurnu-A, Erzincan-Ahmediye, Karaman-Sarivelliler, Kayseri-Pinarbasi, Konya-Karapinar, Rize-Anzer-A, B, C, D and Sivas-Yildizeli-C honey samples exhibited antibacterial activity at concentrations of 40% and above (Table 2), whereas honeys collected from Artvin-Borcka, Bolu-Mudurnu-B, Erzincan-Ballikoy, Izmir-Bergama, Kahraman Maras-Goksun, Kirsehir-Horozgedigi, Konya-Altinoba, Konya-Ilgin, Kutahya-Tavsanlı, Sivas-Gokluce and Tokat-Ugrak inhibited the growth of *K. pneumoniae* at only undiluted concentrations (Table 2).

Apart from these two groups, all the other honey samples showed same results that 50% and above concentrations prevented the growth of *K. pneumoniae* (Table 2).

***Staphylococcus aureus*:** *S. aureus* failed to grow at a concentration of 40% and above in honeys collected from Afyon-Cayderesi, Ankara-Cubuk Akkuzular, Ankara-Polatli, Bayburt-Demirozu-A, Bolu-Mudurnu-A, Cankiri-Ortahoyuk, Erzincan-Kemah, Eskisehir-Merkez, Gumushane-Kelkit, Gumushane-Kosesemimli, Gumushane-Saricicek, Kastamonu-Merkez, Konya-Beysehir, Konya-Beleren, Rize-Anzer-A, B, C and Tekirdag-Cevrimkaya (Table 3).

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Table 1: Growth of *Escherichia coli* on plates containing varying concentrations of honey

Regions of collected honey samples	10 %	20 %	30 %	40 %	50 %	100 %	Control
Adapazari-Memnuniye	+	+	+	+	-	-	+
Adapazari-Pamukova	+	+	+	+	-	-	+
Afyon-Anitkaya	+	+	+	+	-	-	+
Afyon-Çay deresi	+	+	+	+	-	-	+
Afyon-Merkez	+	+	+	+	-	-	+
Ankara-Çubuk Akkuzular	+	+	+	+	-	-	+
Ankara-Çubuk Karagöl	+	+	+	+	+	-	+
Ankara-Polatli	+	+	+	+	-	-	+
Artvin-Borçka	+	+	+	+	-	-	+
Aydın-Söke	+	+	+	+	-	-	+
Balikesir-Susurluk	+	+	+	+	-	-	+
Bayburt-Armutlu	+	+	+	-	-	-	+
Bayburt-Demirözü-A	+	+	+	+	-	-	+
Bayburt-Demirözü-B	+	+	+	+	-	-	+
Bayburt-Söğütlük	+	+	+	+	-	-	+
Bodrum-Çamlık	+	+	+	+	-	-	+
Bolu-Mudurnu-A	+	+	+	+	-	-	+
Bolu-Mudurnu-B	+	+	+	+	-	-	+
Bolu-Ömerler	+	+	+	+	+	-	+
Burdur-Gökçebag	+	+	+	+	-	-	+
Çankiri-Eskipinar	+	+	+	+	+	-	+
Çankiri-Kursunlu	+	+	+	+	-	-	+
Çankiri-Ortahöyük	+	+	+	+	-	-	+
Çankiri-Sabanözü	+	+	+	+	-	-	+
Erzincan-Ballıköy	+	+	+	+	+	-	+
Erzincan-Kemah	+	+	+	+	-	-	+
Erzincan-Ahmediye	+	+	+	+	-	-	+
Erzurum-Kandilli	+	+	+	+	-	-	+
Eskisehir-Merkez	+	+	+	+	-	-	+
Eskisehir-Yakakayik	+	+	+	+	-	-	+
Gümüşhane-Kelkit	+	+	+	+	-	-	+
Gümüşhane-Kösesemimli	+	+	+	+	-	-	+
Gümüşhane-Sarıçiçek	+	+	+	+	-	-	+
Izmir-Bergama	+	+	+	+	+	-	+
Izmir-Menemen	+	+	+	+	-	-	+
Kahraman Maras-Göksun	+	+	+	+	-	-	+
Karaman-Adiller	+	+	+	+	-	-	+
Karaman-Sariveliler	+	+	+	+	-	-	+
Kastamonu-Merkez	+	+	+	+	-	-	+
Kayseri-Pınarbası	+	+	+	+	-	-	+
Kayseri-Yesilkent	+	+	+	+	-	-	+
Kirsehir-Horozgedigi	+	+	+	+	-	-	+
Konya-Altınoba	+	+	+	+	+	-	+
Konya-Beyşehir	+	+	+	+	-	-	+
Konya-Bozkir-A	+	+	+	+	-	-	+
Konya-Bozkir-B	+	+	+	+	-	-	+
Konya-Beleren	+	+	+	+	-	-	+
Konya-Çumra	+	+	+	+	-	-	+
Konya-İlgin	+	+	+	+	-	-	+
Konya-Karapınar	+	+	+	+	-	-	+
Kütahya-Domaniç	+	+	+	+	-	-	+
Kütahya-Tavsanlı	+	+	+	+	-	-	+
Mersin-Mut	+	+	+	+	-	-	+
Nigde-Hacibektaş	+	+	+	+	-	-	+
Nigde-Ulukisla	+	+	+	+	-	-	+
Rize-Anzer-A	+	+	-	-	-	-	+
Rize-Anzer-B	+	+	-	-	-	-	+
Rize-Anzer-C	+	+	-	-	-	-	+
Rize-Anzer-D	+	+	-	-	-	-	+
Sivas-Gemerek	+	+	+	+	-	-	+
Sivas-Göklüce	+	+	+	+	-	-	+
Sivas-Sarkisla	+	+	+	+	-	-	+
Sivas-Yılanlıkaya	+	+	+	+	+	-	+
Sivas-Yıldizeli-A	+	+	+	+	-	-	+
Sivas-Yıldizeli-B	+	+	+	+	-	-	+
Sivas-Yıldizeli-C	+	+	+	+	-	-	+
Sivas-Zara	+	+	+	+	-	-	+
Tekirdag	+	+	+	+	-	-	+
Tekirdag-Çevrimkaya	+	+	+	+	-	-	+
Tokat-Çamlıbel	+	+	+	+	-	-	+
Tokat- Merkez	+	+	+	+	-	-	+
Tokat-Ugrak	+	+	+	+	-	-	+
Yozgat- Koçak Kümük köyü	+	+	+	+	+	-	+

(+) Growth (-) No Growth

Table 2: Growth of *Klebsiella pneumonia* on plates containing varying concentrations of honey

Regions of collected honey samples	10 %	20 %	30 %	40 %	50 %	100 %	Control
Adapazari-Memnuniye	+	+	+	+	-	-	+
Adapazari-Pamukova	+	+	+	+	-	-	+
Afyon-Anitkaya	+	+	+	+	-	-	+
Afyon-Çay deresi	+	+	+	+	-	-	+
Afyon-Merkez	+	+	+	+	-	-	+
Ankara-Çubuk Akkuzular	+	+	+	+	-	-	+
Ankara-Çubuk Karagöl	+	+	+	+	-	-	+
Ankara-Polatli	+	+	+	+	-	-	+
Artvin-Borçka	+	+	+	+	+	-	+
Aydın-Söke	+	+	+	+	-	-	+
Balikesir-Susurluk	+	+	+	+	-	-	+
Bayburt-Armutlu	+	+	+	+	-	-	+
Bayburt-Demirözü-A	+	+	+	+	-	-	+
Bayburt-Demirözü-B	+	+	+	+	-	-	+
Bayburt-Söğütlük	+	+	+	+	-	-	+
Bodrum-Çamlık	+	+	+	+	-	-	+
Bolu-Mudurnu-A	+	+	+	+	-	-	+
Bolu-Mudurnu-B	+	+	+	+	+	-	+
Bolu-Ömerler	+	+	+	+	-	-	+
Burdur-Gökçebag	+	+	+	+	-	-	+
Çankiri-Eskipinar	+	+	+	+	-	-	+
Çankiri-Kursunlu	+	+	+	+	-	-	+
Çankiri-Ortahöyük	+	+	+	+	-	-	+
Çankiri-Sabanözü	+	+	+	+	-	-	+
Erzincan-Ballıköy	+	+	+	+	+	-	+
Erzincan-Kemah	+	+	+	+	-	-	+
Erzincan-Ahmediye	+	+	+	+	-	-	+
Erzurum-Kandilli	+	+	+	+	-	-	+
Eskisehir-Merkez	+	+	+	+	-	-	+
Eskisehir-Yakakayik	+	+	+	+	-	-	+
Gümüşhane-Kelkit	+	+	+	+	-	-	+
Gümüşhane-Kösesemimli	+	+	+	+	-	-	+
Gümüşhane-Sarıçiçek	+	+	+	+	-	-	+
Izmir-Bergama	+	+	+	+	+	-	+
Izmir-Menemen	+	+	+	+	-	-	+
Kahraman Maras-Göksun	+	+	+	+	+	-	+
Karaman-Adiller	+	+	+	+	-	-	+
Karaman-Sariveliler	+	+	+	+	-	-	+
Kastamonu-Merkez	+	+	+	+	-	-	+
Kayseri-Pınarbası	+	+	+	+	-	-	+
Kayseri-Yesilkent	+	+	+	+	-	-	+
Kirsehir-Horozgedigi	+	+	+	+	+	-	+
Konya-Altınoba	+	+	+	+	+	-	+
Konya-Beyşehir	+	+	+	+	-	-	+
Konya-Bozkir-A	+	+	+	+	-	-	+
Konya-Bozkir-B	+	+	+	+	-	-	+
Konya-Beleren	+	+	+	+	-	-	+
Konya-Çumra	+	+	+	+	-	-	+
Konya-İlgin	+	+	+	+	+	-	+
Konya-Karapınar	+	+	+	+	-	-	+
Kütahya-Domaniç	+	+	+	+	-	-	+
Kütahya-Tavsanlı	+	+	+	+	+	-	+
Mersin-Mut	+	+	+	+	-	-	+
Nigde-Hacibektaş	+	+	+	+	-	-	+
Nigde-Ulukisla	+	+	+	+	-	-	+
Rize-Anzer-A	+	+	+	+	-	-	+
Rize-Anzer-B	+	+	+	+	-	-	+
Rize-Anzer-C	+	+	+	+	-	-	+
Rize-Anzer-D	+	+	+	+	-	-	+
Sivas-Gemerek	+	+	+	+	-	-	+
Sivas-Göklüce	+	+	+	+	+	-	+
Sivas-Sarkisla	+	+	+	+	-	-	+
Sivas-Yılanlıkaya	+	+	+	+	-	-	+
Sivas-Yıldizeli-A	+	+	+	+	-	-	+
Sivas-Yıldizeli-B	+	+	+	+	-	-	+
Sivas-Yıldizeli-C	+	+	+	+	-	-	+
Sivas-Zara	+	+	+	+	-	-	+
Tekirdag	+	+	+	+	-	-	+
Tekirdag-Çevrimkaya	+	+	+	+	-	-	+
Tokat-Çamlıbel	+	+	+	+	-	-	+
Tokat- Merkez	+	+	+	+	-	-	+
Tokat-Ugrak	+	+	+	+	+	-	+
Yozgat- Koçak Kümük köyü	+	+	+	+	+	-	+

(+) Growth (-) No Growth

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Table 3: Growth of *Staphylococcus aureus* on plates containing varying concentrations of honey

Regions of collected honey samples	10 %	20 %	30 %	40 %	50 %	100 %	Control
Adapazari-Memnunye	+	+	+	+	-	-	+
Adapazari-Pamukova	+	+	+	+	-	-	+
Afyon-Anitkaya	+	+	+	+	-	-	+
Afyon-Çay deresi	+	+	+	-	-	-	+
Afyon-Merkez	+	+	+	+	-	-	+
Ankara-Çubuk Akkuzular	+	+	+	-	-	-	+
Ankara-Çubuk Karagöl	+	+	+	+	-	-	+
Ankara-Polatli	+	+	+	-	-	-	+
Artvin-Borçka	+	+	+	+	-	-	+
Aydın-Söke	+	+	+	+	-	-	+
Balikesir-Susurluk	+	+	+	+	-	-	+
Bayburt-Armutlu	+	+	+	+	-	-	+
Bayburt-Demirözü-A	+	+	+	-	-	-	+
Bayburt-Demirözü-B	+	+	+	+	-	-	+
Bayburt-Söğütlük	+	+	+	+	-	-	+
Bodrum-Çamlık	+	+	+	+	-	-	+
Bolu-Mudurnu-A	+	+	+	-	-	-	+
Bolu-Mudurnu-B	+	+	+	+	-	-	+
Bolu-Ömerler	+	+	+	+	-	-	+
Burdur-Gökcebag	+	+	+	+	-	-	+
Çankiri-Eskipinar	+	+	+	+	-	-	+
Çankiri-Kursunlu	+	+	+	+	-	-	+
Çankiri-Ortahöyük	+	+	+	-	-	-	+
Çankiri-Sabanözü	+	+	+	+	-	-	+
Erzincan-Ballıköy	+	+	+	+	+	-	+
Erzincan-Kemah	+	+	+	-	-	-	+
Erzincan-Ahmediye	+	+	+	+	-	-	+
Erzurum-Kandilli	+	+	+	+	-	-	+
Eskisehir-Merkez	+	+	+	-	-	-	+
Eskisehir-Yakakayik	+	+	+	+	-	-	+
Gümüşhane-Kelkit	+	+	+	-	-	-	+
Gümüşhane-Kösesemimli	+	+	+	-	-	-	+
Gümüşhane-Sarıçiçek	+	+	+	-	-	-	+
Izmir-Bergama	+	+	+	+	-	-	+
Izmir-Menemen	+	+	+	+	-	-	+
Kahraman Maras-Göksun	+	+	+	+	-	-	+
Karaman-Adiller	+	+	+	+	-	-	+
Karaman-Sarıveliler	+	+	+	+	-	-	+
Kastamonu-Merkez	+	+	+	-	-	-	+
Kayseri-Pınarbası	+	+	+	+	-	-	+
Kayseri-Yesilkent	+	+	+	+	-	-	+
Kirsehir-Horozgedigi	+	+	+	+	-	-	+
Konya-Altınoba	+	+	+	+	-	-	+
Konya-Beyşehir	+	+	+	-	-	-	+
Konya-Bozkir-A	+	+	+	+	-	-	+
Konya-Bozkir-B	+	+	+	+	-	-	+
Konya-Beleren	+	+	+	-	-	-	+
Konya-Çumra	+	+	+	+	-	-	+
Konya-İlgin	+	+	+	+	-	-	+
Konya-Karapınar	+	+	+	+	-	-	+
Kütahya-Domaniç	+	+	+	+	-	-	+
Kütahya-Tavsanlı	+	+	+	+	-	-	+
Mersin-Mut	+	+	+	+	-	-	+
Nigde-Hacıbektas	+	+	+	+	-	-	+
Nigde-Ulukisla	+	+	+	+	-	-	+
Rize-Anzer-A	+	+	+	-	-	-	+
Rize-Anzer-B	+	+	+	-	-	-	+
Rize-Anzer-C	+	+	+	-	-	-	+
Rize-Anzer-D	+	+	+	+	-	-	+
Sivas-Gemerek	+	+	+	+	-	-	+
Sivas-Göklüce	+	+	+	+	-	-	+
Sivas-Sarkisla	+	+	+	+	-	-	+
Sivas-Yılanlıkaya	+	+	+	+	-	-	+
Sivas-Yıldizeli-A	+	+	+	+	-	-	+
Sivas-Yıldizeli-B	+	+	+	+	-	-	+
Sivas-Yıldizeli-C	+	+	+	+	-	-	+
Sivas-Zara	+	+	+	+	-	-	+
Tekirdag	+	+	+	+	-	-	+
Tekirdag-Çevrimkaya	+	+	+	-	-	-	+
Tokat-Çamlıbel	+	+	+	+	-	-	+
Tokat-Merkez	+	+	+	+	-	-	+
Tokat-Ugrak	+	+	+	+	-	-	+
Yozgat- Koçak Kümüük köyü	+	+	+	+	-	-	+

(+) Growth (-) No Growth

Only undiluted honey obtained from Erzincan-Ballıköy inhibited the growth of *S. aureus* (not 50% and below) (Table 3).

Depending on the tested bacterial strains, the degree of efficiency of tested honey samples collected from Rize-Anzer region were found more effective than the other honey samples.

On the contrary to that of Rize-Anzer, honey from Erzincan-Ballıköy exhibited rather weak antibacterial activity on all three bacterial species. This honey sample inhibited the growth of bacterial cells only at 100% concentration (Tables 1,2,3).

However, none of the honey samples had any effect on *C. albicans* in this respect (data are not shown).

Contents of honey are determined by many factors such as bee species and flora containing the nectar. Sugars, pollens, minerals, enzymes and antibacterial substances called inhibine constitute the structure of honeys.

Honeys collected from Rize-Anzer region was more effective to impede the growth of bacteria than the other honey samples, which are very famous in Turkey. This honey is used as a folk medicine and sold in natural health product centers in Turkey.

In this study we investigated the antimicrobial activity of honey samples collected from various regions of Turkey. It was determined that some honeys prevent the growth of bacteria at a concentration of 30% and above, some 40% and above, some 50% and above depending on bacterial isolates. Our data are supported by Jeddar *et al.* (1985) who reported that most pathogenic bacteria failed to grow in honey at a concentration of 40% and above.

A remarkable feature of present data is a range of variation in antibacterial activities among the honey samples from different collection sites.

The antibacterial activity of honey has both physical and chemical factors, such as acidity, osmolarity, hydrogen peroxide, volatiles, beeswax, nectar, pollen and propolis (Gil *et al.*, 1995; Mato *et al.*, 2000; Weston *et al.*, 2000). In most cases these factors can be related to different floral sources and bees of different origin (Russel *et al.*, 1990, Weston *et al.*, 1999, 2000). In this respect our results may indicate differences in floral distribution and bee types in our collection sites. However, it should be noted that we have not identified particular sources and do not give any observation in relation to the relative weight of a floral or a bee-type origin for our sample sites. After a detailed source analysis this issue would have been resolved in reference to particular phenomena.

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