INVESTIGATION OF ANTIMICROBIAL EFFECT OF HONEY COLLECTED FROM VARIOUS REGIONS OF TURKEY

H. SELCUK, K. NEVIN

Hacettepe University, Faculty of Science, Department of Biology 06532-Beytepe/Ankara, TURKEY
E-mail: selcukhazir@hotmail.com

Summary

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.

A total of seventy three honey samples collected from various regions of Turkey were tested for their antimicrobial activity. 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 the most effective honey on clinically bacterial isolates.

Key words: *Honey/honeybee/antibacterial effect/natural products*

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[16; 19; 8; 15; 4; 1] and *Candida albicans*[9].

The antibacterial activity of honey has been attributed both to physical factors: osmolority[14; 5; 17] and acidity[5; 11; 18] and chemical factors: hydrogenperoxide[1,17], cecropin-A and mellitin, methyl 3,5-dimethoxy-4-hydroxybenzoate, methyl-3,4,5-trimethoxybenzoate, 3,4,5-trimethoxybenzoicid, 3,5-dimethoxy-4 hydroxybenzoic acid (syringic acid), tetracyclin, nectar, volatiles, propolis and unidentified substances from certain floral sources[4; 12; 6; 15; 2; 3; 19; 18].

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.

Material and Method

Collection of honeys

Honey samples, which were collected during the flowering seasons, were obtained from apiarists throughout Turkey. The honey samples were stored in airtight bottles at 10°C in dark. Assayed samples might be stored as well as for 2 years[2].

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

We used clinically isolated bacterial species such as *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative), *Staphylococcus aureus* (Gram 'positive), and *Candida albicans* (yeast).

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[8; 12; 2]

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[10]. Following the calculation 1 ml of bacterial samples were diluted 100x with sterile nutrient agar medium (Difco, 28 g/l)[8;15], mixed throughly and poured into Petri plates. Sabouraud Dextrose Agar (Difco, 65 g/l) was used for *Candida albicans*[7].

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[10; 2; 8; 12].

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 clinically isolates of *E. coli, K. pneumoniae* and *S. aureus*.

All bacterial pathogens tested failed to grow in honey at a different concentration depending on the honey samples (Table I, II, III). However, none of the honey samples had an effect on *C. albicans* in this respect (Data are not shown).

Escherichia coli

We observed that Rize-Anzer A, B, C and D samples were more effective (at a concentration of 30% and above) on *E. coli* bacteria than the other honey samples. However, other group that consist of the samples collected from Bayburt-Armutlu, Eskisehir-Merkez, Kutahya-Domanic, Nigde-Ulukisla and Sivas-Gokluce prevented to bacterial growth at a concentration of 40% and above (Table I).

Table I

Growth of *Escherichia coli* on plates containing varying concentrations of honey

Regions of collected honey samples	%10	%20	%30	%40	%50
1. Adapazari-Memnuniye	+	+	+	+	-
2. Adapazari-Pamukova	+	+	+	+	-
3. Afyon-Anitkaya	+	+	+	+	-
4. Afyon-Çay deresi	+	+	+	+	-
5. Afyon-Merkez	+	+	+	+	-
6. Ankara-Çubuk Akkuzular	+	+	+	+	-
7. Ankara-Çubuk Karagöl	+	+	+	+	+
8. Ankara-Polatli	+	+	+	+	-
9. Artvin-Borçka	+	+	+	+	-
10. Aydin-Söke	+	+	+	+	-
11. Balikesir-Susurluk	+	+	+	+	-
12. Bayburt-Armutlu	+	+	+	-	-
13. Bayburt-Demirözü-A	+	+	+	+	-
14. Bayburt-Demirözü-B	+	+	+	+	-
15. Bayburt-Sögütlük	+	+	+	+	-
16. Bodrum-Çamlik	+	+	+	+	-
17. Bolu-Mudurnu-A	+	+	+	+	-
18. Bolu-Mudurnu-B	+	+	+	+	-
19. Bolu-Ömerler	+	+	+	+	+
20. Burdur-Gökçebağ	+	+	+	+	-
21. Çankiri-Eskipinar	+	+	+	+	+
22. Çankiri-Kurşunlu	+	+	+	+	-
23. Çankiri-Ortahöyük	+	+	+	+	-
24. Çankiri-Şabanözü	+	+	+	+	-
25. Erzincan-Balliköy	+	+	+	+	+
26. Erzincan-Kemah	+	+	+	+	-
27. Erzincan-Ahmediye	+	+	+	+	-
28. Erzurum-Kandilli	+	+	+	+	-
29. Eskişehir-Merkez	+	+	+	-	-
30. Eskişehir-Yakakayik	+	+	+	+	-
31. Gümüşhane-Kelkit	+	+	+	+	-
32. Gümüşhane-Köseşeminli	+	+	+	+	-
33. Gümüşhane-Sariçiçek	+	+	+	+	-
34. Izmir-Bergama	+	+	+	+	+
35. Izmir-Menemen	+	+	+	+	-
36. Kahraman Maraş-Göksun	+	+	+	+	-
37. Karaman-Adiller	+	+	+	+	-
38. Karaman-Sarivelier	+	+	+	+	-
39. Kastamonu-Merkez	+	+	+	+	-
40. Kayseri-Pinarbaşi	+	+	+	+	-
41. Kayseri-Yeşilkent	+	+	+	+	-
42. Kirşehir-Horozgediği	+	+	+	+	-
43. Konya-Altinoba	+	+	+	+	+
44. Konya-Beyşehir	+	+	+	+	-
45. Konya-Bozkir-A	+	+	+	+	-
46. Konya-Bozkir-B	+	+	+	+	-
47. Konya-Beleren	+	+	+	+	-
48. Konya-Çumra	+	+	+	+	-
49. Konya-Ilgin	+	+	+	+	-

50. Konya-Karapinar	+	+	+	+	-
51. Kütahya-Domaniç	+	+	+	-	-
52. Kütahya-Tavşanli	+	+	+	+	-
53. Mersin-Mut	+	+	+	+	-
54. Niğde-Hacibektaş	+	+	+	+	-
55. Niğde-Ulukişla	+	+	+	-	-
56. Rize-Anzer-A	+	+	-	-	-
57. Rize-Anzer-B	+	+	-	-	-
58. Rize-Anzer-C	+	+	-	-	-
59. Rize-Anzer-D	+	+	-	-	-
60. Sivas-Gemerek	+	+	+	+	-
61. Sivas-Göklüce	+	+	+	-	-
62. Sivas-Şarkişla	+	+	+	+	-
63. Sivas-Yilanlikaya	+	+	+	+	+
64. Sivas-Yildizeli-A	+	+	+	+	-
65. Sivas-Yildizeli-B	+	+	+	+	-
66. Sivas-Yildizeli-C	+	+	+	+	-
67. Sivas-Zara	+	+	+	+	-
68. Tekirdağ	+	+	+	+	-
69. Tekirdağ-Çevrimkaya	+	+	+	+	-
70. Tokat-Çamlibel	+	+	+	+	-
71. Tokat-Merkez	+	+	+	+	-
72. Tokat-Uğrak	+	+	+	+	-
73. Yozgat-Koçak Kümük köyü	+	+	+	+	+

(+) = Growth; (-) = No growth

Honey samples collected from Ankara-Cubuk Karagol, Bolu Omerler, Cankiri-Eskipinar, Erzincan-Ballikoy, Konya-Altinoba, Sivas-Yilanlikaya and Yozgat-Kocak Kumuk failed on *E. coli* even at a concentration of 50% (Table I).

However, most of the honey samples analyzed were inhibitory at 50% and above concentrations (Table I).

Klebsiella pneumoniae

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

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

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,

Table II

Growth of *Klebsiella pneumoniae* on plates containing varying concentrations of honey

Regions of collected honey samples	%10	%20	%30	%40	%50
1. Adapazari-Memnuniye	+	+	+	+	-
2. Adapazari-Pamukova	+	+	+	+	-
3. Afyon-Anitkaya	+	+	+	-	-
4. Afyon-Çay deresi	+	+	+	+	-
5. Afyon-Merkez	+	+	+	+	-
6. Ankara-Çubuk Akkuzular	+	+	+	-	-
7. Ankara-Çubuk Karagöl	+	+	+	+	-
8. Ankara-Polatli	+	+	+	+	-
9. Artvin-Borçka	+	+	+	+	+
10. Aydin-Söke	+	+	+	+	-
11. Balikesir-Susurluk	+	+	+	+	-
12. Bayburt-Armutlu	+	+	+	-	-
13. Bayburt-Demirözü-A	+	+	+	+	-
14. Bayburt-Demirözü-B	+	+	+	+	-
15. Bayburt-Sögütlük	+	+	+	+	-
16. Bodrum-Çamlik	+	+	+	+	-
17. Bolu-Mudurnu-A	+	+	+	-	-

18. Bolu-Mudurnu-B	+	+	+	+	+
19. Bolu-Ömerler	+	+	+	+	-
20. Burdur-Gökçebağ	+	+	+	+	-
21. Çankiri-Eskipinar	+	+	+	+	-
22. Çankiri-Kurşunlu	+	+	+	+	-
23. Çankiri-Ortahöyük	+	+	+	+	-
24. Çankiri-Şabanözü	+	+	+	+	-
25. Erzincan-Balliköy	+	+	+	+	+
26. Erzincan-Kemah	+	+	+	+	-
27. Erzincan-Ahmediye	+	+	+	-	-
28. Erzurum-Kandilli	+	+	+	+	-
29. Eskişehir-Merkez	+	+	+	+	-
30. Eskişehir-Yakakayik	+	+	+	+	-
31. Gümüşhane-Kelkit	+	+	+	+	-
32. Gümüşhane-Köseşeminli	+	+	+	+	-
33. Gümüşhane-Sariçiçek	+	+	+	+	-
34. Izmir-Bergama	+	+	+	+	+
35. Izmir-Menemen	+	+	+	+	-
36. Kahraman Maraş-Göksun	+	+	+	+	+
37. Karaman-Adiller	+	+	+	+	-
38. Karaman-Sarivelier	+	+	+	-	-
39. Kastamonu-Merkez	+	+	+	+	-
40. Kayseri-Pinarbaşi	+	+	+	-	-
41. Kayseri-Yeşilkent	+	+	+	+	-
42. Kirşehir-Horozgediği	+	+	+	+	+
43. Konya-Altinoba	+	+	+	+	+
44. Konya-Beyşehir	+	+	+	+	-
45. Konya-Bozkir-A	+	+	+	+	-
46. Konya-Bozkir-B	+	+	+	+	-
47. Konya-Beleren	+	+	+	+	-
48. Konya-Çumra	+	+	+	+	-
49. Konya-Ilgin	+	+	+	+	+
50. Konya-Karapinar	+	+	+	-	-
51. Kütahya-Domaniç	+	+	+	+	-
52. Kütahya-Tavşanli	+	+	+	+	+
53. Mersin-Mut	+	+	+	+	-
54. Niğde-Hacibektaş	+	+	+	+	-
55. Niğde-Ulukişla	+	+	+	-	-
56. Rize-Anzer-A	+	+	+	-	-
57. Rize-Anzer-B	+	+	+	-	-
58. Rize-Anzer-C	+	+	+	-	-
59. Rize-Anzer-D	+	+	+	-	-
60. Sivas-Gemerek	+	+	+	+	-
61. Sivas-Göklüce	+	+	+	+	+
62. Sivas-Şarkişla	+	+	+	+	-
63. Sivas-Yilanlikaya	+	+	+	+	-
64. Sivas-Yildizeli-A	+	+	+	+	-
65. Sivas-Yildizeli-B	+	+	+	+	-
66. Sivas-Yildizeli-C	+	+	+	-	-
67. Sivas-Zara	+	+	+	+	-
68. Tekirdağ	+	+	+	+	-
69. Tekirdağ-Çevrimkaya	+	+	+	+	-
70. Tokat-Çamlibel	+	+	+	+	-
71. Tokat-Merkez	+	+	+	+	-
72. Tokat-Uğrak	+	+	+	+	+
73. Yozgat-Koçak Kümük köyü	+	+	+	+	-

(+) = Growth; (-) = No growth

Bayburt-Demirozu-A, Bolu-Mudurnu-A, Cankiri-Ortahoyuk, Erzincan-Kemah, Eski-sehir-Merkez, Gumushane-Kelkit, Gumushane-Koseseminli, Gumushane-Saricicek, Kastamonu-Merkez, Konya-Beysehir, Konya-Beleren, Rize-Anzer-A, B, C and Tekir-dag-Cevrimkaya (Table III).

Only undiluted honey obteined from Erzincan-Ballikoy inhibited the growth of *S. aureus* (not 50% and below) (Table III).

Depending on the tested bacterial strain, 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-Ballikoy exhibited rather weak antibacterial activity on all three bacterial species. This honey sample inhibited the growth of bacterial cells at only 100% concentration (Table I, II, III).

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

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

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

Table III

Growth of *Staphylococcus aureus* on plates containing varying concentrations of honey

Regions of collected honey samples	%10	%20	%30	%40	%50
1. Adapazari-Memnuniye	+	+	+	+	-
2. Adapazari-Pamukova	+	+	+	+	-
3. Afyon-Anitkaya	+	+	+	+	-
4. Afyon-Çay deresi	+	+	+	-	-
5. Afyon-Merkez	+	+	+	+	-
6. Ankara-Çubuk Akkuzular	+	+	+	-	-
7. Ankara-Çubuk Karagöl	+	+	+	+	-
8. Ankara-Polatli	+	+	+	-	-
9. Artvin-Borçka	+	+	+	+	-
10. Aydin-Söke	+	+	+	+	-
11. Balikesir-Susurluk	+	+	+	+	-
12. Bayburt-Armutlu	+	+	+	+	-
13. Bayburt-Demirözü-A	+	+	+	-	-
14. Bayburt-Demirözü-B	+	+	+	+	-
15. Bayburt-Sögütlük	+	+	+	+	-
16. Bodrum-Çamlik	+	+	+	+	-
17. Bolu-Mudurnu-A	+	+	+	-	-
18. Bolu-Mudurnu-B	+	+	+	+	-
19. Bolu-Ömerler	+	+	+	+	-
20. Burdur-Gökçebağ	+	+	+	+	-
21. Çankiri-Eskipinar	+	+	+	+	-
22. Çankiri-Kurşunlu	+	+	+	+	-
23. Çankiri-Ortahöyük	+	+	+	-	-
24. Çankiri-Şabanözü	+	+	+	+	-
25. Erzincan-Balliköy	+	+	+	+	+
26. Erzincan-Kemah	+	+	+	-	-
27. Erzincan-Ahmediye	+	+	+	+	-
28. Erzurum-Kandilli	+	+	+	+	-
29. Eskişehir-Merkez	+	+	+	-	-
30. Eskişehir-Yakakayik	+	+	+	+	-
31. Gümüşhane-Kelkit	+	+	+	-	-
32. Gümüşhane-Köseşeminli	+	+	+	-	-
33. Gümüşhane-Sariçiçek	+	+	+	-	-
34. Izmir-Bergama	+	+	+	+	-
35. Izmir-Menemen	+	+	+	+	-
36. Kahraman Maraş-Göksun	+	+	+	+	-
37. Karaman-Adiller	+	+	+	+	-
38. Karaman-Sarivelier	+	+	+	+	-
39. Kastamonu-Merkez	+	+	+	-	-
40. Kayseri-Pinarbaşi	+	+	+	+	-
41. Kayseri-Yeşilkent	+	+	+	+	-
42. Kirşehir-Horozgediği	+	+	+	+	-
43. Konya-Altinoba	+	+	+	+	-
44. Konya-Beyşehir	+	+	+	-	-
45. Konya-Bozkir-A	+	+	+	+	-
46. Konya-Bozkir-B	+	+	+	+	-
47. Konya-Beleren	+	+	+	-	-
48. Konya-Çumra	+	+	+	-	-
49. Konya-Ilgin	+	+	+	+	-
50. Konya-Karapinar	+	+	+	-	-
51. Kütahya-Domaniç	+	+	+	+	-
52. Kütahya-Tavşanli	+	+	+	+	-
53. Mersin-Mut	+	+	+	+	-
54. Niğde-Hacibektaş	+	+	+	+	-
55. Niğde-Ulukişla	+	+	+	+	-
56. Rize-Anzer-A	+	+	+	-	-
57. Rize-Anzer-B	+	+	+	-	-

58. Rize-Anzer-C	+	+	+	-	-
59. Rize-Anzer-D	+	+	+	+	-
60. Sivas-Gemerek	+	+	+	+	-
61. Sivas-Göklüce	+	+	+	+	-
62. Sivas-Şarkişla	+	+	+	+	-
63. Sivas-Yilanlikaya	+	+	+	+	-
64. Sivas-Yildizeli-A	+	+	+	+	-
65. Sivas-Yildizeli-B	+	+	+	+	-
66. Sivas-Yildizeli-C	+	+	+	+	-
67. Sivas-Zara	+	+	+	+	-
68. Tekirdağ	+	+	+	+	-
69. Tekirdağ-Çevrimkaya	+	+	+	-	-
70. Tokat-Çamlibel	+	+	+	+	-
71. Tokat-Merkez	+	+	+	+	-
72. Tokat-Uğrak	+	+	+	+	-
73. Yozgat-Koçak Kümük köyü	+	+	+	+	-

(+) = Growth; (-) = No growth

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 isolate. 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.

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