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# Antimicrobial potential of honey on some microbial isolates

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# صلاحية العسل كمضاد حيوي ضد بعض الجراثيم

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**الملخص: الهدف:** تقييم فعالية العسل المضادة للبكتيريا. **الطريقة:** لقد تم في هذا البحث دراسة لصلاحية احدى عشرة نوعا من العسل تباع في منطقة مكة المكرمة ( تسعة أنواع مستوردة ونوعان محليان ) كمضادات حيوية ضد الم22كورات العنقودية البرتقالية، ألأشريكية المعوية والزائفة الزنجارية. **النتائج:** تمت دراسة فاعلية ستة أنواع من العسل على عدة أنواع من البكتيريا والفطريات المعزولة في المختبر. وأثبت البحث أن الفاعلية تختلف حسب نوع العسل كما أن بعض الجراثيم مقاومة لمفعول العسل. أيضا أثبت البحث أن العسل لا يفقد فاعليته كمضاد حيوي حتى بعد غليه لمدة خمس عشرة دقيقة أو حفظه في درجة حرارة بين 2 – 8 درجة مئوية ولمدة ستة أشهر. كما تبين أن عسل الغابة السوداء الألماني يحتوي على أعلى فاعلية يتبعه العسل التركي وعسل زهور البرتقال وعسل الغابة وأخيرا عسل زهور الصيف. **الخلاصة:** أظهر البحث أن للعسل خواصا مضادة للحيوية كما للمضادات الحيوية ألأخرى، فبعض الكائنات الحية حساسة له بينما غيرها مقاوم له، وتختلف هذه الحساسية إعتمادا على نوعية العسل.

**ABSTRACT:** ***Objective*** – To assess the antimicrobial potential of honey against certain microbial isolates. ***Method*** – Samples of commercial honeys sold in Makkah area of Saudi Arabia were checked for their antimicrobial activities using standard organisms, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The minimal inhibitory concentration end points of six honey samples found to possess antimicrobial activities were used to determine the sensitivity patterns of some isolates from the laboratory. The temperature stabilities of the honey samples were also determined. ***Results*** – The six honey samples had differing levels of antimicrobial activities with the standard organisms and with the laboratory isolates. Black Forest honey showed the highest activity followed respectively by Turkish, Orange Flower, Forest Honey and Summer Flower. The antimicrobial activities of the samples were stable after storing at 2–8° C for six months and after boiling for 15 minutes. ***Conclusion*** – The study shows that honey, like antibiotics, has certain organisms sensitive to it while others are resistant, and the sensitivity varies depending on the source of the honey.

KEY WORDS: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* honey, antibiotics, sensitivity, antimicrobial.

Honey was found by some workers to possess antibacterial activity where antibiotics were ineffective.[1,2,13,14] Some chronic debilitating conditions resulting from pressure sores, infected wounds, burns and fournier's gas gangrene have been found to respond favourably to honey treatment.[1,2,3] Radwan[4] attributed this antibacterial activity to specific chemicals in honey. The nature of these chemicals and the mechanisms of their action are not fully understood even though thin layer chromatography (TLC), polyacrylamide gel electrophoresis (PAGE) or high performance liquid chromatography (HPLC) have shown that honey contains seven tetracycline derivatives, fatty acids, lipids, amylases and ascorbic acid.[5,6,7,8]

Allen[9] showed that there are many types of honey with and without antibacterial activity and postulated that the type of the flower that was the source of the nectar determines the nature of the antibacterial activity of the honey. While the empirical application of honey on open wounds, burns or use of honey in syrups does show that it stops the growth of many microorganisms, the latter have seldom been isolated and identified.[2,3]

Efem[10] found that undiluted honey stopped the growth of *Candida* species while *Pseudomonas aeruginosa*, *Clostridium oedematiens*, *Streptococcus pyogenes* remained resistant. Wellford[11] found that some species of *Aspergillus* did not produce aflotoxin in various dilutions of honey. Radwan[4] observed that honey stopped the growth of *Salmonella*, *Escherichia coli*, *Aspergillus niger* and *Penicillium chrysogenium*. However, these investigations were not conducted with standard organisms of known sensitivity to common therapeutic agents. Conclusions were mostly

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drawn from the results from one sample of honey.[9]

Since honey is used extensively in the Arabian Gulf region, the authors felt it desirable to scientifically determine the antimicrobial activities of the honeys in common use. This study was conducted to assess the antimicrobial potential of a few commercial honey samples on some laboratory isolates of known sensitivities to common antibiotics.

The investigation hoped to determine the temperature stability of the active agent(s) in honey since the ambient temperature might affect both its shelf life and its killing potential. This knowledge could be effectively utilised in hospital practice and in primary health care where open skin lesions are routinely treated.

## METHOD

Six brands of commercial honey available in Saudi Arabia were used in the study: *Black Forest*, *Orange Flower* and *Summer Flowers* produced by Biophar, Germany, *Black Forest* and *Forest Honey* produced by Langaneza, Germany, and *Turkish* from Turkey (figure 1).

**TABLE 1**

*Examined commercial honey samples and their sources.*

| | Name | Source |
|---|---|---|
| 1 | Black Forest | Germany (Biophar) |
| 2 | Orange Flower | Germany (Biophar) |
| 3 | Black Forest | Germany (Langaneza) |
| 4 | Forest honey | Germany (Langaneza) |
| 5 | Summer Flowers | Germany (Biophar) |
| 6 | Turkish | Turkey |

The samples were collected and marked randomly by one investigator while the experiments were performed blindly by the other. Each honey sample was collected in a sterile universal container and kept at 2–8°C until tested. Each sample was checked for purity on blood agar plates and was diluted to 75, 50, 40, 20, and 10% of its original concentration using physiological saline.

Three control organisms, *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used to determine the antimicrobial activity of each sample of honey. Three colonies ($1.1\times10^6$ organisms per ml, equivalent to Brown's opacity tube 3) of each standard organism were emulsified in 4ml of distilled water and used to swab Mueller Hinton sensitivity agar plates. Fifty microlitres (50µl) of each honey dilution were applied on each plate using 1ml sterile syringe without the needle. Each dilution was done in triplicate. The plates were left at room temperature till the honey seeped into the agar. After incubation, the inhibition zones were measured in millimetres (mm) and the average of the inhibition zones recorded. The end point of antimicrobial activity of each honey was defined as the highest dilution (lowest concentration) producing an inhibition zone with the control organisms.

Using Stokes[12] method, some multiresistant organisms isolated from hospital patients were subjected to sensitivity test using the honeys at their antimicrobial activity end points (lowest concentration end points). The sensitivities of *Proteus spp.* to honey samples were read after 4 hours up to 12 hours duration to check for swarming activities. Organisms showing inhibition zones equal to or greater than that of the control organisms were regarded as sensitive to honey samples.

A stability test was also conducted as follows: Each honey sample was divided into two aliquots. The first aliquots were stored for six months at 2–8° C while the second aliquots were boiled for 15 minutes and allowed to cool. Each aliquot was retested for antimicrobial activity as before.

## RESULTS

Table 2 shows the zones of inhibition of the six honey samples with the standard organisms. These depended on the species of the control organism. Turkish honey (sample 6) had highest activity with *Staph. aureus* and least with *Pseudomonas aeruginosa* while Black Forest honey (sample 3) had highest activity with *Pseudomonas* and least with *Esch. coli*. All the samples showed zones of inhibition of 10 mm or more at 50% dilution with *Staph. aureus, Ps. aeruginosa* and *Esch. coli* except samples, 4 and 5 with *Staph. aureus* and *Esch. coli* respectively and sample 2 with *Esch. coli* and *Pseudomonas aeruginosa* respectively.

All the laboratory isolates were found sensitive to the honey samples except *Proteus mirabilis*, *Aspergillus niger*, *Aspergillus fumigatus*, *Enterococcus faecalis* and *Streptococcus pyogenes* (Table 3). Some *Pseudomonas aeruginosa* and *Acinetobacter* species found resistant to amikacin, ceftriaxone, tobramicin, aztreonam, gentamicin and imipenem were also found sensitive to all the honey samples.

The honey samples retained their antimicrobial activities with the control organisms even after storage at 2–8°C for six months and after boiling for 15 minutes, though the activity on *Esch. coli* was destroyed at 50% dilution of honey, but retained at neat (undiluted honey).

## DISCUSSION

The six commercial samples of honey showed differing antimicrobial activities with organisms isolated from the laboratory. Commercial Black Forest honey, followed by Turkish honey (sample 6), had the highest antimicrobial activity (Tables 2 and 3).

**TABLE 2**

*Minimal inhibitory concentration of various honey samples with control organisms*

| Honey dilution | *Staphylococcus aureus* | | | | | | *Escherichia coli* | | | | | | *Pseudomonas aeruginosa* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Honey samples & zones of inhibition (mm) | | | | | | Honey samples & zones of inhibition (mm] | | | | | | Honey samples & zones of inhibition (mm) | | | | | |
| Sample | **1** | **2** | **3** | **4** | **5** | **6** | **1** | **2** | **3** | **4** | **5** | **6** | **1** | **2** | **3** | **4** | **5** | **6** |
| Neat | 22.00 | 21.00 | 18.00 | 13.00 | 15.00 | 15.00 | 20.00 | 15.00 | 20.00 | 19.00 | 15.00 | 20.00 | 25.00 | 14.00 | 20.00 | 15.00 | 18.00 | 18.00 |
| 75 % | 17.00 | 15.00 | 14.00 | 11.00 | 11.00 | 12.00 | 19.00 | 11.00 | 15.00 | 15.00 | 12.00 | 17.00 | 16.00 | 11.00 | 16.00 | 14.00 | 13.00 | 14.00 |
| 50 % | 12.00 | 11.00 | 12.00 | 9.00 | 11.00 | 10.00 | 14.00 | 9.00 | 13.00 | 12.00 | 9.00 | 13.00 | 13.00 | 10.00 | 13.00 | 11.00 | 11.00 | 12.00 |
| 40 % | 6.00 | 0.00 | 6.00 | 0.00 | 0.00 | 8.00 | 9.00 | 0.00 | 3.00 | 0.00 | 0.00 | 9.00 | 8.00 | 0 | 12.00 | 6.00 | 7.00 | 5.00 |
| 20 % | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 % | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The antimicrobial effects of the honey samples were more with *Pseudomonas* and *Acinetobacter* species than with the other bacteria tested. The reason for this is not clear. It is possible that the low redox potential of ascorbic acid[5] in honey affects aerobic organisms such as the *Pseudomonas* and *Acinetobacter* species. Jeddar[15] found honey inhibitory to the growth of microorganisms at 40% dilution. This observation is not in conformity with our results; some honey samples tested by us had no activity at 40% dilution. Our findings also disagree with Radwan[5] who found *Aspergillus niger* sensitive to honey and Efem[10] who found *Pseudomonas aeruginosa* resistant. The reason for *Staphylococcus aureus* being sensitive to honey and *Streptococcus spp*. resistant is not understood. However, it is known that *Streptococcus spp.* are lactic acid bacteria while staphylococci are not. If lactic acid accumulates in the areas containing honey samples as one of the microbial metabolic products of streptococcal growth, the activity of honey may be altered since high acidity affects the inhibition zones produced by various antibiotics.[16] Our findings agree with Obaseiki Ebor[17] who found *Candida albicans* sensitive. Our honey samples also exerted antimicrobial activities on *Pseudomonas and Acinetobacter* species, which were resistant to some antibiotics.

The ability of honey to kill microorganisms has been attributed to its high content of tetracycline derivatives, peroxidases, fatty acids, phenols, ascorbic acids and amylases.[1,5,18–20] In this study, the antimicrobial substances in the honeys were not estimated. However, the fact that Black Forest honey had more activity than Turkish or other honeys, highlights the finding that the sources of the nectars may have contributed to the differences in their antimicrobial activities.[9]

The experiment showed that the antimicrobial substances in honey can withstand refrigeration temperatures for six months and are heat stable at 100°C. This shows that its antimicrobial activity is not dependent alone on its tetracycline derivatives, ascorbic acid, peroxidase or amylase activities as claimed by other workers, for these agents are heat labile. Takeba[19] and Joerg[21] attributed the antibacterial effect of honey to its phenolic content. Phenol is heat stable and may be an active agent but its concentration in honey appears too low (1.3–5.0μg/l) to be solely responsible. The antimicrobial agent therefore may depend on the integrity of a particular honey sample.

## CONCLUSION

In this experiment, we attempted to assess the value of honey as an antimicrobial therapeutic agent. We have found some samples to have high broad-spectrum antimicrobial activity, even after the honey has been exposed to boiling or refrigerating temperatures. This makes honey unique since many topical antibiotics used in open skin lesions are heat labile. Among our samples, Black Forest honey had the highest antimicrobial activity followed respectively by Turkish, Orange Flower, Forest Honey and Summer Flower. The study also shows that some organisms are sensitive to some types of honey while others are resistant. However, much remains unknown, which makes this a fertile field for further research.

**TABLE 3**

*Effect of honey samples on different laboratory organisms.*

| Organism | No. tested | Honey samples with Average Inhibition Zones (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| *Pseudomonas aeruginosa* | 31 | 19.00 | 17.00 | 18.00 | 17.00 | 18.00 | 18.00 |
| *Klebsiella pneumonia* | 13 | 14.00 | 16.00 | 20.00 | 15.00 | 15.00 | 16.00 |
| *Acinetobacer spp.* | 4 | 16.00 | 15.00 | 20.00 | 22.00 | 18.00 | 19.00 |
| *Candida albicans* | 3 | 12.00 | 10.00 | 19.00 | 12.00 | 12.00 | 13.00 |
| *Staphylococcus aureus* | 15 | 10.00 | 17.00 | 20.00 | 15.00 | 19.00 | 18.00 |
| *Escherichia coli* | 14 | 22.00 | 21.00 | 20.00 | 18.00 | 20.00 | 14.00 |
| *Serrratia spp.* | 4 | 14.00 | 15.00 | 16.00 | 12.00 | 14.00 | 10.00 |
| *Enterobacter cloacae* | 4 | 17.00 | 18.00 | 17.00 | 18.00 | 14.00 | 16.00 |
| *Proteus mirabilis* | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus niger* | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aspergillus fumigatus* | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Streptococcus faecalis* | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Strep pyogenes* | 5 | 0 | 0 | 0 | 0 | 0 | 0 |

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