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EXPERIMENTAL STUDY

Antibacterial activity of honey in north-west Pakistan against select human pathogens

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Abstract

OBJECTIVE: To investigate the antimicrobial activity of commercially available honey and raw honey samples in Khyber Pakhtunkhwa, Pakistan, against pathogenic bacterial strains.

METHODS: Well diffusion assays were performed to screen pure and diluted honey samples for antibacterial activity against six Gram-negative and six Gram-positive bacterial strains. Zones of inhibition were measured and compared with 10 mg Gentamycin.

RESULTS: When honey samples were diluted to 20%-70%, the honey samples showed no activity to mild antibacterial activity. The highest antibacterial activity was recorded when 90% and pure undiluted honey samples were tested and compared with a control Gentamycin disc (10 mg).

CONCLUSION: Commercially processed honey and raw honey samples from north-west of Pakistan possess good antimicrobial potential.

Key words: Anti-bacterial agents; Disk diffusion antimicrobial tests; Honey

INTRODUCTION

Since the beginnings of human civilization, apitherapy has been prescribed for many diseases and ailments.[1] Discorides described honey as a treatment for ulcers and as a carrier of therapeutic agents, while Avicenna claimed that honey could prolong life.[2] It was also administered to soldiers as an antiseptic woundtreatment.[3] Interestingly, Cleopatra used honey as part of her beauty regimen,[4] and honey was considered "food of the gods" in Greek mythology.[5] To authenticate these traditional uses for honey, the therapeutic features of this substance have been extensively studied.[6] Studies show that honey contributes to wound healing and controls wound infection.[7,8] Honey has also been reported in the treatment of respiratory infections.[9]

Honey is composed of carbohydrates, organic acids, minerals, aromatic acids, and waxes.[10,11] Unstable compounds such as enzymes, hormones, and vitamins may also be present.[12] At least 22 different types of sugar are found in honey, the majority being dextrose and laevulose. It is hypothesized that the chemical and physical properties of honey that promote antimicrobial activity and wound healing are due to sugar content, flavoring compounds, acids, and minerals.[13,14]

In recent years, over prescription, overuse, and misuse of antibiotics has led to increased microbial antibiotic resistance. As a result, the failure of prescribed antibiotics in controlling infection has sparked a need for alternative antimicrobial strategies.[15] In the present study, we evaluated the antibacterial potential of commercially available honey and raw honey samples found in Khyber Pakhtunkhwa, Pakistan, and compared these with a commonly prescribed antibiotic.

MATERIALS AND METHODS

Honey collection and storage

Fourcommercially available honey brands (Marhaba, Hamdard, Umm e Shifa, and Azka) and oneraw honey sample were collected from local markets inPeshawar, Khyber Pakhtunkhwa, Pakistan, and stored at 18℃. Dilutions (v/v) of each honey sample were made in sterilized ddH_2O to obtain final concentrations of 20%, 50%, 70%, 90%, and 100%.

Bacterial strains

All strains were grown in sterilized nutrient agar slants (Oxoid CM0309) and sub-cultured onto fresh nutrient agar media. The susceptibility of six Gram-negative bacterial strains (Pseudomonas aeruginosa, Xanthomonas campestris, Salmonella typhi, Salmonella typhimurium, Klebsiella pneumonia, and Escherichia coli) and six Gram-positive bacterial strains (Enterococcus faecalis, Clostridium perfringens type C, Clostridium perfringens type D, Clostridium chauvoei, Staphylococcus aureus, and Bacillus subtilis) was tested. The strains were procured from the Veterinary Research Institute at the Khyber Teaching Hospital, the Hayatabad Medical Complex, and the Centre of Biotechnology and Microbiology at the University of Peshawar (Peshawar, Pakistan). All strains were cultured on agar slopes and preserved at 4℃ before use.

Culture standardization

All bacterial cultures were standardized as described previously by Khalil *et al.* [16] Briefly, 1 mL of each nutrient broth culture (grown for 24 h) was placed into sterile test tubes containing 1 mL of nutrient broth. Sterilized dH_2O was added to the test tubes to visually adjust the culture turbidity to a 0.5 McFarland standard (Escherichia coli cell suspension).

Antibacterial well diffusion assays

Well diffusion assays were performed to evaluate the antibacterial activity of each sample as described previously.[7] Briefly, uniform lawns were produced using a bent spreader, and excess inoculum was removed using sterile cotton swabs. A 6 mm sterilized borer was used to make wells in the nutrient agar plates and 50 µL of a test dilution was introduced into each well. A standard Gentamycin (Sigma-Aldrich, St. Louis, MO, USA) discs (10 µg) served as a positive control while sterile dH_2O served as a negative control. Zones of inhibition i.e. susceptibility were measured in "mm" using Vernier calipers.

Statistical analyses

Assays were performed in triplicate and all statistical analyses were performed with SPSS 21.0 software (IBM corp. Armonk, NY, USA). All the data were given mean ± standard deviation (*SD*). A probability value $P<0.05$ was taken as significant with 95% confidence interval.

RESULTS

The antibacterial activity of four commercially available honey brands (Marhaba, Hamdard, Umm e Shifa, and Azka) and one raw honey sample from Khyber Pakhtunkhwa, Pakistan was measured against 12 human pathogens. Different concentrations (20%, 50%, 70%, 90%, and 100%) of each honey sample were assayed. The zone of inhibition relative to its activity whether it is significant, good or mild were according to the literature.[16] At the lowest concentration (20%) of each honey sample assayed, all bacterial strains were resistant and Hamdard had the largest zone of inhibition [(1.39±0.26) mm; Figure 1A]. As expected, the antimicrobial activity of Gentamycin (positive control) was observed with all tested strains, and the largest zone of inhibition measured was with Klebsiella pneumonia [(20.36±0.55) mm].

When the honey samples were diluted to 50%, the highest zone of inhibition recorded was Umm e Shifa brand with Staphylococcus aureus [(4.33 ± 0.41) mm], while all other bacterial strains were resistant. The largest zone of inhibition was observed with Klebsiella pneumonia [(20.50±0.60) mm], followed by Clostridium perfringens type C [(19.90±0.41) mm; Figure 1B]. The largest zone of inhibition measured for Marhaba was with Staphylococcus aureus [(9.33±0.33) mm; Figure 1C], followed by Hamdard against Xanthomonas campestris[(6.73±0.30) mm].

At a concentration of 90%, significant antibacterial activity was observed for all honey samples against all bacterial strains tested (Figure 1D). Zones of inhibition ranged from (5.26 ± 0.75) mm for Umm e Shifa with Clostridium chauvoei, to (32.8 ± 0.41) mm for Marhaba with Escherichia coli. All undiluted honey samples (100%) showed significant antibacterial activity against the tested bacterial strains when compared to Gentamycin. The highest zone of inhibition recorded was for Marhaba with Staphylococcus aureus [(35.20 ± 1.01) mm], followed by Umm e Shifa with Salmonella typhi [(31.4±0.96) mm; Figure 1E].

DISCUSSION

It is well established that honey confers antimicrobial activity. In the present study, we demonstrated that processed and raw honey samples diluted to 20% do not inhibit growth of Pseudomonas aeruginosa, and these data are in agreement with the findings reported by Tumin *et al.* [6] Our data also support previous studies demonstrating that undiluted honey samples of Marhaba, Umm e Shifa, and Hamdard inhibit growth of Salmonella typhi.[6,7] Similarly, the zones of inhibition for Marhaba and Hamdard against Staphylococcus aureus [(35.2 ± 1.01) and (24.8 ± 0.20) mm, respectively] were in accordance with findings previously described by

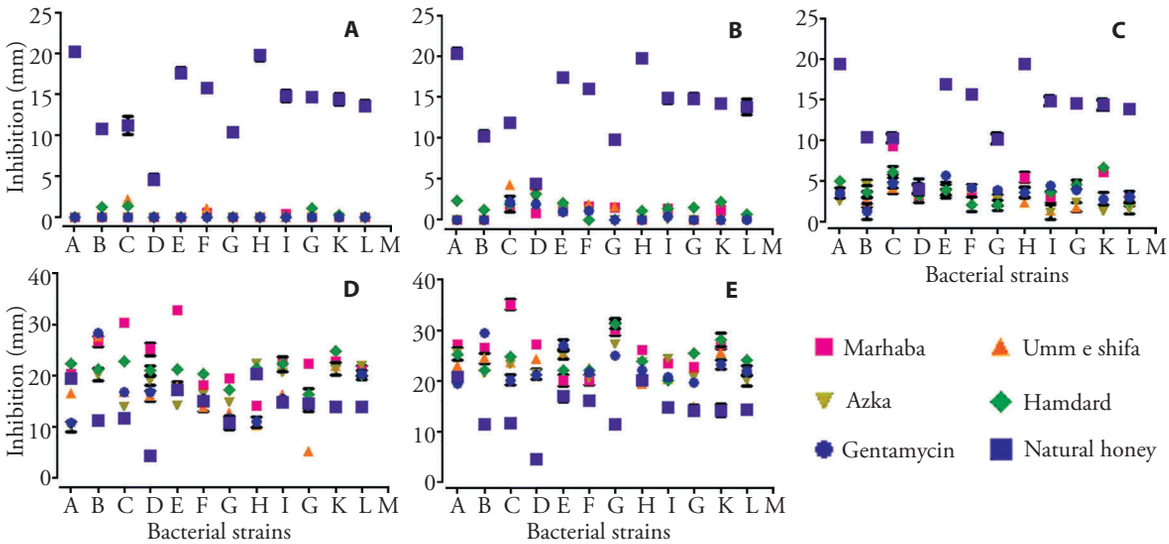

Figure 1 Antibacterial activity of honey sample concentrationsagainst selected bacterial strains
A: antibacterial activity of honey samples at 20%; B: antibacterial activityof honey samples at 50%; C: antibacterial activityof honey samples at 70%; D: antibacterial activityof honey samples at 90%; E: antibacterial activity of honey samples at 100%. Selected bacterial strains assayed were: A: Klebsiella pneumonia; B: Enterococcus faecalis; C: Staphylococcus aureus: D: Pseudomonas aeruginosa; E: Escherichia coli; F: Salmonella typhimurium; G: Salmonella typhi; H: Clostridium perfringens type C; I: Clostridium perfringens type D; J: Clostridium chauvoei; K: Xanthomonas campestris; L: Bacillus subtilis. Zone of inhibition showed by test samples with respect to activity: above 18: significant activity; 16-18: good activity; 13-15: low activity; 9-12: non significant activity; below 8: no activity.

Sheikh *et al*. [7] However, Sheikh *et al* [7] also demonstrated that undiluted Marhaba and Hamdard samples resulted in no growth inhibition for Bacillus subtilis, while the undiluted Marhaba and Hamdard samples in this study yielded zones of inhibition measuring (22.5± 0.4) and (24.2 ± 0.4) mm, respectively (Figure 1E). In addition, several reports have demonstrated that honey samples diluted to 20% inhibit growth of Escherichia coli and Salmonella typhi,[17] yet no such observations were many for any of the honey samples analyzed in the current study with Escherichia coli. Finally, this study is the first to report the antibacterial activity of Marhaba and Hamdard honey brands against Klebsiella pneumonia.

Variations between these data and earlier reports may be attributed to many factors. For instance, Tumin *et al* [6] have hypothesized that geographical location, seasonal variation, and humidity alter experimental conditions. Floral sources also alter honey composition and antimicrobial properties, [18] while pH, sugar content, hydrogen peroxide concentrations, flavonoids, tannins, and organic acid concentrations may be other contributing factors.[19-22] Honey processing methods can further alter chemical and physical properties.[23]

In conclusion, the present study demonstrates that several processed and raw honey samples collected from north-west Pakistani sources have significant antibacterial properties. However, further characterization of the active bactericidal and/or bacteriostatic properties displayed by these samples and other honey sources is needed. Nonetheless, this study contributes to the authentication and broadening of indigenous knowledge for traditional apitherapy.

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