

Antibacterial Activity of Pakistani Honey

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Abstract. The current study was conducted to determine the antibacterial activity of 50 crude and processed honey samples produced by *Apis mellifera* and stingless bee. All the honey samples were collected from different places of Districts Dir, Swat and Oghi of Khyber Pakhtunkhwa Province, Pakistan and were tested against the six ATCC bacterial species including *E. coli* ATCC number 25922, *Pseudomonas aeruginosa* ATCC number 27853, *Staphylococcus aureus* ATCC number 6538, *Enterococcus faecalis* ATCC number 19433, *Salmonella typhi* ATCC number 19943 and *Klebsiella pneumoniae* ATCC number 27736. The honey samples showed variable zones of inhibition by using Agar well plate technique. *E. coli* showed 17-23 mm, *S. typhi* 31-37 mm, *E. faecalis* 28 mm, *P. aeruginosa* 14-15 mm, *K. pneumoniae* 20-24 mm and *Staph. aureus* 19-25 mm. Most of the honey samples used in this study showed broad spectrum antibacterial activity.

Keywords: antibacterial activity, honey, zones of inhibition, agar well diffusion method

Introduction

Honey produced by honey bees (*Apis mellifera*) is one of the oldest traditional medicines used against several human diseases (Manisha *et al.*, 2011). Honey inhibits the growth of many pathogenic bacteria including, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*Staph. aureus*) and *Salmonella typhi* (*S. typhi*) (Zumla and Lulat, 1989). It has been reported that honey has a bacteriostatic and bactericidal effect against various species of gram positive and gram negative bacteria (Nzeako, 2000; Molan and Betts, 2000). The antibacterial activity of honey may be due to the presence of hydrogen peroxide (Molan, 1992).

Antibiotics are very important agents in reducing global burden of bacterial infections. However, the effectiveness of drugs is reduced due to the development of drugs resistant bacteria (WHO, 1999), which is an increasing problem in the world (Shears, 2000).

Globally, due to lack of access to health care, infectious diseases account for 25% of mortality (Mandal *et al.*, 2011). Currently, Herbal and modern scientific medicine has gained importance globally (WHO, 2000). The purpose of this research was to evaluate the antibacterial

activity of Pakistani honey produced by *Apis mellifera* against various pathogenic bacteria.

Materials and Methods

A cross-sectional study was performed to evaluate the antibacterial activity of Pakistani honey against different bacterial species. Locally manufactured honeys were collected from different beekeepers and super markets of Districts Dir, Swat and Oghi, Pakistan and brought to the Microbiology Laboratory, Hazara University, Mansehra, Pakistan for antibacterial analysis. Out of total 50 honey samples collected, each sample was filtered through a sterile mesh to remove debris followed by streaking on blood agar culture media plate and was then incubated overnight to check for microbial purity.

Bacterial isolates were procured from the American Type Culture Collection (ATCC), Veterinary Research Institute (VRI) Abbottabad, Pakistan. A total of six bacterial strains were used including, *E. coli* with ATCC number 25922, *Pseudomonas aeruginosa* with ATCC number 27853, *Staphylococcus aureus* with ATCC number 6538, *Enterococcus faecalis* with ATCC number 19433, *Salmonella typhi* with ATCC number 19943 and *Klebsiella pneumoniae* with ATCC number 27736. The bacterial isolates were confirmed through standard microbiological techniques and drug susceptibility tests

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following standard agar disc diffusion method. For further use, bacterial isolates were stored in the laboratory on nutrient agar slopes at 4 °C.

Morphologically identical bacterial colonies from overnight growth culture tubes were picked up with sterilized wire loop and were then suspended in 3 mL of nutrient broth following incubation for 3 h at 37 °C. Bacterial dilution was prepared with normal saline to a turbidity of 0.5 McFarland standards (108 colony forming unit/mL). Agar well diffusion technique was used for checking antibacterial activity of honey against the recommended bacterial strains. A wire loop full of prepared bacterial suspensions (1x10⁴ cfu/mL) were applied to the center of a sterile Mueller Hinton culture media plate and spread evenly throughout the plates by using a sterile dry cotton wool.

Different concentrations, 10 , 20, 50 and 75 % v/v of each honey sample were made in sterile distilled water. These were obtained by dissolving 0.1, 0.2, 0.5 and 0.75 mL of each honey into desired volumes of water. Similarly, one hole was drilled at the center of culture media plate and 50 µL of each honey dilution. Negative control well only contained 50 µL of sterile distilled water and all the culture plates were incubated at 37 °C for 24 h. The diameters of inhibitory zones were measured in millimeter. This experiment was repeated three times to ensure reproducibility and to be able to calculate an average value for each honey.

Results and Discussion

In the present research work, all the honey samples were found pure following streaked for purity. The antibacterial activity of honey samples were checked against *E. coli*, *S. typhi*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae* and *Staph. aureus* (Fig.1). Following Agar well plate technique, the highest degree zone of inhibition was observed against *E. coli* (17-23mm) as shown in Fig.1. Similarly, the zone of inhibition of each honey sample against *S. typhi* (31-37mm), *E. faecalis* (28mm), *K. pneumoniae* (18-21mm), *Staph. aureus* (19-25mm) and *P. aeruginosa* (14-15mm) was also reported as shown in Table 1.

Antibacterial activity of honey has been reported since long time especially for wound healing which may be due to its complex composition and ability to generate hydrogen peroxide by the bee-derived enzyme glucose oxidase (Vallianou *et al.*, 2014; Lu *et al.*, 2014).

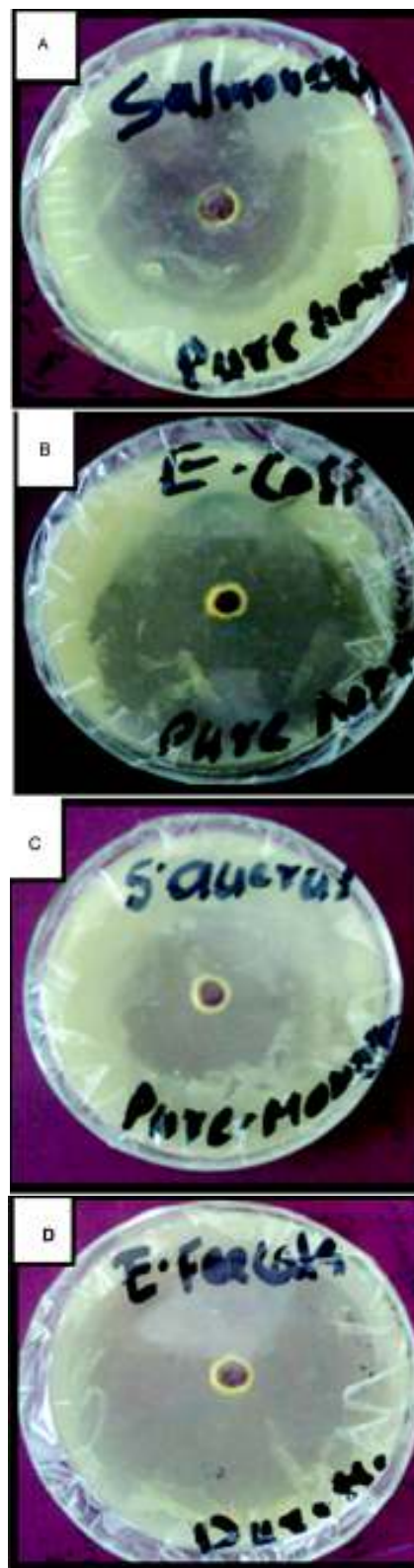


Fig. 1. Visible zone of inhibition of honey samples against (A) *S. typhi*, (B) *E. coli*, (C) *Staph. aureus* and (D) *E. faecalis*.

Table 1. Zone of inhibition of honey against *E. coli*, *S. typhi*, *E. faecalis*, *K. pneumoniae*, *Staph. aureus* and *P. aeruginosa*.

Location	Zone of inhibition of honey (mm)					
	<i>E. coli</i>	<i>S. typhi</i>	<i>E. faecalis</i>	<i>K. pneumoniae</i>	<i>Staph. aureus</i>	<i>P. aeruginosa</i>
Dir Lower	22.6	31.4	28.3	20.9	24.6	15.4
Swat	17.6	35.2	28.7	22.9	19.8	14.7
Oghi (Mansehra)	23.6	37.3	28.8	24.4	25.2	15.9

Hegazi (2011), reported the antibacterial activity of different honey (Acacia honey, Citrus honey, Clover honey, Coriander honey, Cotton honey, Palm honey, Sesame honey and Saudi Seder honey) against *K. pneumoniae*, *P. aeruginosa* and *E. coli*. Honey has antibacterial activity against *Staph. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. typhi* (Mohapatra *et al.*, 2011). The bacteriostatic or bactericidal activity depends on the type of honey samples (Lu *et al.*, 2014). It is reported that antibacterial effect exhibited by honey was related to the levels of hydrogen peroxide present in the honey (Irish *et al.*, 2011). Researchers at the Waikato Honey Research Unit (2012) in New Zealand attributed the antibacterial activity of honey to its acidic PH (3 to 4.5) as most bacteria can best grow at neutral PH (7-7.4). All the honey brands used in this study were effective against *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae* and *Staph. aureus* which concur with several similar reports (Melissa *et al.*, 2004; Taormina *et al.*, 2001).

Present results showed that Swat honey was effective against *E. coli* (17-23mm), *S. typhi* (31-37mm), and *Staph. aureus* (24-29mm) zone of inhibition observed for each bacterial strain. It was also observed the same results when our research data was compared to the previous reports in which *E. coli* showed 15-20mm, *S. typhi* 30-50mm and *S. aureus* showed 17-20mm zones of inhibition (Dilnawaz *et al.*, 1995). In another similar reports, *E. coli* showed 17-23mm, *S. typhi* 30-35mm zone and *Staph. aureus* showed 19-25mm zone of inhibition (Nuriza-Tumin *et al.*, 2005). While our results show 17-23mm for *E. coli*, 24-29mm for *S. typhi*, 19-25mm for *Staph. aureus*. Similarly, antibacterial activity of honey against *E. coli*, *Staph. aureus* showed 8-45 and 12-45mm zones of inhibition, respectively. Honey has also antibacterial activity against *Shigella* species (Omoya and Akharaiyi, 2010). Antibacterial activity of

honey against *E. coli*, *Staph. aureus*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae* and *S. typhi* have been also reported in many previous research studies (Molan and Betts, 2000).

Conclusion

Honey is widely researched and has well-known antibacterial activity. It is very effective against several gram negative and gram positive bacteria. This study demonstrates that honey has antibacterial activity. Therefore, it is necessary to study healing property of honey *in-vivo* as well as its mechanism of action.

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Conflict of Interest. The authors declare no conflict of interest

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