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Antimicrobial Activity of Fresh and Old Honey

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ABSTRACT

Honey is a thick sugary natural substance that is produced by honeybee from the nectar of flowers of different plants. The objective of this study was to determine the antimicrobial activity against different clinical isolates and to compare the activity of fresh honey with old honey. Unbranded honey samples were taken from a local market of Karachi. One sample was fresh and other was four year old. This activity was assessed by agar well diffusion method. Honey samples were diluted as 100%, 80% and 50% with saline as control. Undiluted and diluted old honey was inhibitory to Gram positive and Gram negative species. Fresh and old honey showed good antibacterial activity against *E. coli, Acinetobacter, S. paratyphi A, S. dysenteriae, K. pneumoniae, Citrobacter, S. aureus, S. saprophyticus, B. subtilis,* **with the strongest activity seen against** *P. fluorescens. S. typhi* **was resistant to both old and fresh honey.** *S. pneumonia* **was inhibitory to fresh honey only. Both honey samples also inhibit** *Candida albicans* **(yeast). Antibacterial activity of old honey is more than fresh honey.**

Keywords: Honey, antibacterial activity, agar well diffusion method, bacteria.

INTRODUCTION

Honey is a sweet food made by bees using nectar from flowers. The variety produced by honey bees (the genus *Apis*) is the one most commonly referred to and is the type of honey collected by beekeepers and consumed by humans. Honey bees transform nectar into honey by a process of regurgitation, and store it as a primary food source in wax honeycombs inside the beehive. Honey gets its sweetness from the monosaccharides fructose and glucose, and has approximately the same relative sweetness as that of granulated sugar. It has attractive chemical properties for baking, and a distinctive flavor that leads some people to prefer it over sugar and other sweeteners. Most microorganisms do not grow in honey because of its low water activity of 0.6. However, honey sometimes contains dormant endospores of the bacterium *Clostridium botulinum*, which can be dangerous to infants, as the endospores can transform into toxin-producing bacteria in the infant's immature intestinal tract, leading to illness and even death (Anonymous, 2011).

Honey is an ancient remedy for the treatment of infected wounds, gastroenteritis, gastric ulcers, diabetes etc. It can be effective against multi-drug-resistant strains of bacteria. The antibacterial properties of honey include the release of low levels of hydrogen per oxide while some honeys have an additional phytochemical antibacterial component (Molan, 2001).

In current study, the antimicrobial activity of local Pakistani ber/sidder honey was examined against 20 different bacterial strains.

MATERIAL AND METHODS

Honey sample:
Two samples of honey (1 fresh and one 3 years old) were used for antibacterial potential against different micro-organism.

Clinical isolates:
Twenty different isolates belonging to 11 genera of microorganisms viz., *E. coli* (5) *Pseudomonas*

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fluorescens (2), *Klebsiella pneumonia* (1), *Citrobacter* (1), *Salmonella typhi* (1), *Salmonella para typhi A* (1), *Acinetobacter* (1), *Shigella dysenteriae* (1), *S.aureus* (3), *Streptococcus pyogenes* (1), *B. subtilis* (1) and *Candida albicans* (2) were obtained from Essas's laboratory, Abbasi Shaheed hospital and Al-Khidmat Welfare hospital Karachi were included in study.

Dilution of honey sample:
100% (pure honey), 80%, 50% and 0% (control) dilutions of old and fresh honey samples were prepared as following protocol.

Honey sample	Sterile distilled water	Dilution (%)
10 ml	-	100%
8ml	2ml	80%
5ml	5ml	50%
-	10ml	Control

Preparation of Mcfarland nephlometer standard:
Mcfarland Nephlometer Standard tube no 5 was prepared by mixing 0.05 ml 1.175 % , Barium chloride and 9.95 ml 1% Sulfuric acid, to get bacterial density of 1.5×10^8 cfu / ml .

Prepration of inoculum:
Nutrient broth (5ml) was inoculated with given culture and incubated for 24 hours at 37 ^{o} C. After incubation, tubes were matched with 0.5 McFarland tube to standardize the inoculum.

Agar well diffusion method:
A lawn was prepared by using a sterile cotton swab dipped in standardized inoculum on Nutrient Agar plates. Wells were cut by sterile borer having 5mm diameter. Diluted samples were poured in each well. Plates were incubated at 37º C for 24 hours. Measure the zone of inhibition of respected wells.

RESULTS

Table 1. Antimicrobial activity of fresh honey

Organism	Zone Of Inhibition (mm)				
	No of isolates	Control (0%)	100 %	80%	50%
Gram positive					
S. aureus	02	-	30	20	15
S. saprophyticus	01	-	41	32	30
S. pneumoniae	01	-	13	12	-
B. substilis	01	-	23	20	19
Gram negative					
Salmonella paratyphi A	01	-	23	22	19
Shigella dysenteriae	01	-	20	20	21
E .coli	05	-	26	20	17
K.pneumoniae	01	-	-	-	-
P. florescense	02	-	27	25	40
S. typhi	01	-	-	-	-
Citrobacter	01	-	15	16	-
Acinetobacter	01	-	20	20	11
Yeast					
Candida albicans	02	-	28	28	-

Table 2. Antimicrobial activity of old honey

Organism	Zone Of Inhibition (mm)				
	No of isolates	Control (0%)	100 %	80%	50%
Gram positive					
S. aureus	02	-	20	15	22
S. saprophyticus	01	-	30	30	20
S. pneumoniae	01	-	32	-	-
B. substilis	01	-	24	21	20
Gram negative					
S. paratyphi A	01	-	30	25	20
Shigella dysenteriae	01	-	30	20	15
E. coli	05	-	34	27	27
K. pneumoniae	01	-	25	20	26
P. florescense	02	-	28	35	36
Salmonella typhi	01	-	-	-	-
Citrobacter	01	-	35	20	30
Acinetobacter	01	-	25	20	15
Yeast					
Candida albicans	02	-	25	18	-

DISCUSSION

In this study two dark coloured berry honey samples were used. They were analysed by agar well diffusion method against different Gram negative and positive bacteria. *Ziziphus jojoba* is another economically important plant in Pakistan. It is commonly known as 'sidder' or 'ber' and is indigenous plant species to Pakistan it is a bush like tree found in Karak, Kohat and Bannu districts of N.W.F.P, Attock, Chakwal and ÊMianwali districts of Punjab, Karachi, Hyderabad and Nawabshah districts of Sindh province (Qamer *et al*., 2007).

Most bacteria showed similar growth inhibition patterns for both honey tested, but some variations were detected. Fresh and old honey showed good antibacterial activity against *P. fluorescens* .The zone

of inhibition obtained in this study indicated the 50% (minimum) concentration of honey needed to kill 50 % of bacteria. All honey solutions were freshly prepared before each assay. The above information shows that in microbiological and clinical tests, honey offers advantages in controlling bacterial growth and treatment of certain health problems. Even in modern day society, the medical use of honey still has a place.

Both of the honey samples (old and fresh) inhibited *S. aureus* at concentrations of 100 %, 80 % and 50 % dilutions of honey. *E. coli* was sensitive against both honey samples at concentrations of 100%, 80 % and 50 % dilutions. In the present study, the activities against *S. aureus* and *E. coli* were confirmed; however some Omani and African honey samples also showed activity against *P. aeruginosa* (Zulma *et al* ., 1989).

Although honey has been reported to have antifungal activity against C. *albicans*, our sample shows the zone of inhibition at concentrations of 100 % and 80 %.

Different formulations of honey has significantly inhibited growth of pathogenic microorganisms, *S. aureus* , *E. coli*, *C. albicans* and *A. nigar* when compared to control group, which is an evidence that honey is a therapeutic agent being used since ancient time throughout the world (Gulfraz *et al* ., 2010).

The antimicrobial activity against *S. saprophyticus* was almost equal in fresh and old honey samples. *Strept. pneumoniae* was resistant to both honey samples at concentration 50% . Both of the honey samples (old and fresh) inhibited *B. subtilis* at 80%, 50% and 100% dilutions of honey.

The highest zone of inhibition for old and fresh honey was *P. fiuorescens*. *S.typhi* was resistant in both old and fresh honey. Both of the old and fresh honey inhibited *S. paratyphi* A at concentrations of 100%, 80% and 50%. *Sh. dysenteriae* was inhibited at concentrations 100%, 80% and 50% in both old and fresh honey samples. *K. pneumoniae* was inhibited in old honey while resistant in fresh honey.

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