

Full Length Research Paper

# Antibacterial efficacy of raw and commercially available honey

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**In vitro** antibacterial activity of raw and commercially available honey was tested against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp. and *Salmonella* spp.). Both types of honey showed antibacterial activity against test organisms with the zone of inhibition ranging from 8.13 to 30.85 mm, while *E. coli*, *S. aureus*, and *Shigella* spp. showed sensibility towards both types of honey. Raw honey possesses more inhibitory activity against *S. aureus* and *Shigella* spp., than commercially available honey. On the other hand, commercially available honey possesses more inhibitory activity than raw honey against *E. coli*. Both types of honey showed no effects on *Salmonella* spp. The potency of honey at 100% concentration was found to be higher than all other concentrations tested. However, no effect was observed at concentration of 6.25% v/v honey in the case of both samples.

**Key words:** Honey, antibacterial activity, raw honey, commercially available honey, minimum inhibitory concentration (MIC).

## INTRODUCTION

Honey is a popular sweetener and a common household product throughout the world. It is nonirritant, nontoxic, available and cheap (Bansal et al., 2005). It has been used from ancient times as a method of accelerating wound healing (Van den Berg et al., 2008; Mullai and Menon, 2007). Traditional importance and use of honey as therapeutics has been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago (Maryann, 2000). Ibne Sina, the Prince among Muslim physicians listed several beneficial uses of honey in his monumental work of "The Canon of Medicine" and Hippocrates, the father of Western Medicine, used honey to treat a number of diseases (Khalil et al., 2006). Ancient users did not know its antibacterial properties; they only knew it as an effective remedy. During the twentieth century, it was reported that honey have good antimicrobial properties together with therapeutic potential in wound healing (Medhi et al., 2008; Pieper and Caliri, 2003; Moore et al., 2001). Various studies have species) have antibacterial properties (Lusby et al., 2005;

Patton et al., 2006).

It has been shown that natural unheated honey has some broad-spectrum antibacterial activities when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria (Mundo et al., 2004). On the other hand, bacteria are not uniformly affected by honey. Furthermore, it has been shown that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source (Wilkinson and Cavanagh, 2005).

Since both raw honey and commercially available honey is used extensively in Bangladesh, the purpose of the present study was to evaluate in vitro antibacterial activity of these two types of honey against five human pathogenic bacterial cultures such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp. and *Salmonella* spp. which were isolated from patients.

## MATERIALS AND METHODS

### Honey samples

Two kinds of honeys were used in this study. They are raw honey

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and commercially available honey. Raw honey was collected from the comb and commercially available honey was bought from the market. The samples were kept in the dark at room temperature. Sterility of honey was checked by spreading a loopful quantity on blood agar medium (Mulu et al., 2004).

### Bacterial species

Five species of bacteria which are pathogenic to human were collected from The General Hospital of Kushtia city. They include Gram negative *P. aeruginosa*, *E. coli*, *Salmonella* spp., *Shigella* spp. and Gram positive *S. aureus*. These bacteria were isolated from the infected individuals' urine, stool and pus samples and identified by standard methods (Harley and Prescott, 2002). Pure cultures of isolates were preserved at 4°C on nutrient agar slants. In order to confirm the identity of the test bacterial isolates, morphological characteristics and conventional biochemical tests were performed according to Harley and Prescott (2002).

### Susceptibility test

The antibacterial susceptibility was determined by Kirby and Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute Guidelines using commercially available antimicrobial discs. The following antibiotics were used: Amoxicillin (10 µg), tetracycline (10 µg), chloramphenicol (30 µg), streptomycin (5 µg) and Kanamycin (30 µg).

### Determination of minimum inhibitory concentration (MIC)

The MIC was determined as described by Mulu et al. (2004). Mueller Hinton Agar (Merck) was sterilized and held in a water bath (45 to 50°C). Honeys were briefly heated to 40°C to reduce viscosity and known volumes of honey were measured into 20 ml of molten media to give final concentrations of 100, 75, 50, 25, 12.5 and 6.25% (v/v). The plates were poured, allowed to set and seeded with bacteria adjusted at  $1 \times 10^4$  CFU/ml before incubation at 37°C for 24 h. The MIC was recorded as the lowest concentration of honey at which visible bacterial growth was completely inhibited. This experiment was performed in triplicate to ensure the reproducibility of the results.

## RESULTS

The results of the antibacterial activity assays indicated that both types of honey have inhibitory activity against *E. coli*, *S. aureus* and *Shigella* spp. On the other hand, *P. aeruginosa* is only susceptible to commercially available honey. However, no inhibitory activity was observed against *Salmonella* spp. Mean results of antibacterial activity assays are represented in Table 1.

Raw honey possesses more inhibitory activity against *S. aureus* and *Shigella* spp., than commercially available honey. On the other hand, commercially available honey possesses more inhibitory activity than raw honey against *E. coli*. However, both honeys showed no inhibitory activity on *Salmonella* spp. Table 1 also shows that *Shigella* spp. and *P. aeruginosa* showed multiple drug resistance pattern and these two microbes are susceptible to commercially available honey.

The MIC of five tested bacterial cultures are shown in

Table 2. The mean MIC of raw honey was recorded as 12.5% (v/v) against *S. aureus* and *Shigella* spp. and commercially available honey as 25% (v/v) against *Shigella* spp. (Table 2). The potency of honey at 100% concentration was found to be higher than all other concentrations tested. However, no effect was observed at concentration of 6.25% v/v honey in the case of both honey samples.

## DISCUSSION

In general, all types of honey have high sugar content as well as low water content and acidity, which prevent microbial growth. Osmotic effect, effect of pH and hydrogen peroxide are represented as an "inhibition" factor in honey (Postmes et al., 1993). Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide (Schepartz and Subers, 1964). Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their variable antimicrobial effects (Molan, 1992). Moreover, nonperoxide factors also play critical role. The content of nonperoxide factors are related to the floral source and sometimes account for the major part of the antibacterial activity in honey (Molan and Russell, 1988). Besides its antimicrobial properties, honey can clear infection in a number of ways, including boosting the immune system, having anti-inflammatory and antioxidant activities, and via stimulation of cell growth (Al-Jabri, 2005).

The Tables 1 and 2 show the results of *in vitro* susceptibility and minimum inhibitory concentration of raw and commercially available honey having varying degree of antibacterial activities against Gram-positive as well as Gram-negative bacteria. Our results resembles that of others (El-Amari and Ben-Gweirif, 2010; Mulu et al., 2004; Cooper et al., 1999, 2002; French et al., 2005; Nzeako and Hamdi, 2000; Agbaje et al., 2006; Basson and Grobler, 2008) who found that honey inhibited the growth of *S. aureus*, *E. coli*, *Shigella* spp. and *Pseudomonas* sp. and 100% concentrated honey is more effective than other concentrations (El-Amari and Ben-Gweirif, 2010). In the case of *Shigella* spp., our results differ from the result of other researchers (Mulu et al., 2004).

Our results also showed that the test organisms exhibited varying degrees of multidrug resistance to standard antibiotics, which were used in this study. However, when honey was administered, they show susceptibility. These findings resemble the previous researchers' findings (Agbaje 2006; Patel et al., 2010).

## Conclusion

The present study showed that certain organisms are

**Table 1.** Antibacterial susceptibility of raw and commercially available honey.

Test organism	Raw honey	Commercially available honey	A <sub>10</sub>	T <sub>10</sub>	CiP <sub>30</sub>	Stp <sub>5</sub>	Kn <sub>30</sub>
<i>Escherichia coli</i>	8.13 ± 1.7	23.85 ± 0.9	16.67 ± 2.2	15.07 ± 1.2	17.14 ± 3	15.21 ± 1.8	12.07 ± 4
<i>S. aureus</i>	30.85 ± 3	20.01 ± 6.1	14.75 ± 1.7	12.09 ± 9	16.67 ± 3.5	15.11 ± 1.6	14.65 ± 1.8
<i>Salmonella</i> spp.	-	-	7.25 ± 2.3	7.28 ± 4.3	8.35 ± 1.9	6.27 ± 3.3	-
<i>Shigella</i> spp.	20.60 ± 13.5	13.19 ± 9	14.20 ± 9	-	13.10 ± 1.3	17.15 ± 4	-
<i>P. aeruginosa</i>	-	10.13 ± 0.41	9.32 ± 1.5	10.03 ± 0.51	16.01 ± 0.81	-	-

A<sub>10</sub> = Amoxicillin (10 µg), T<sub>10</sub> = tetracycline (10 µg), CiP<sub>30</sub> = chloramphenicol (30 µg), Stp<sub>5</sub> = streptomycin (5 µg), Kn<sub>30</sub> = kanamycin (30 µg) and “-” = no antibacterial activity. Values are the mean of triplicate determinations, and shown as Mean ± SD.

**Table 2.** Minimum inhibitory concentration of raw and commercially available honey.

Test organism	Mean of inhibition zone (diameter in mm)											
	Raw honey						Commercially available honey					
	Concentration (v/v %)											
	100	75	50	25	12.5	6.25	100	75	50	25	12.5	6.25
<i>S. aureus</i>	30±0.0	20±0.0	19±0.0	6±0.0	5±0.0	-	20±0.0	23±0.0	-	-	-	-
<i>E. coli</i>	8±0.5	6±0.0	5±0.5	-	-	-	23±0.0	15±0.0	7±0.5	-	-	-
<i>Shigella</i> spp.	20±0.0	8±0.0	7±0.0	7±0.0	6±0.5	-	13±0.0	8±0.0	6±0.0	6±0.0	-	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	10±0.5	8±0.0	-	-	-	-
<i>Salmonella</i> spp.	-	-	-	-	-	-	-	-	-	-	-	-

“-” = No antibacterial activity; MIC values are the mean of triplicate determinations, and shown as Mean ± SD.

sensitive to honey and honey can be used as alternative therapy against certain bacteria. Therefore, it is necessary to characterize the active antimicrobial components of honey. In addition, more investigation is needed to explore the possible benefits of the use of honey among therapies in the treatment of bacterial infections.

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