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## Indian Honey: A Natural Product with Antibacterial Activity Against Antibiotic Resistant Pathogens, an *in vitro* Study

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**Abstract:** The present study designed to investigate the antibacterial activity of honey obtained from different state of India. A total of 10 honey samples (five from Uttaranchal state and five from Uttar Pradesh state) were investigated for their antibacterial activity against antibiotic resistant bacterial isolates of *S. epidermidis* and *E. coli* using the disc diffusion method. Marked variations were observed in the antibacterial activity of different sample of honey. Three (60%) of the five Uttaranchal samples and two (40%) of the five Uttar Pradesh samples showed excellent antibacterial activity against gram-positive and gram-negative bacteria. Both Uttaranchal state and Uttar Pradesh state honey samples possess *in vitro* antibacterial activity against antibiotic resistant isolates of *S. epidermidis* and *E. coli* bacteria at 400 uL/disc quantity of 60% concentration (v/v).

**Key words:** Indian honey, antibacterial activity, antibiotic resistant pathogens, gram-positive bacteria, gram-negative bacteria

### INTRODUCTION

According to the definition of National Honey Board, honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honeybees. The definition of honey stipulates a pure product that does not allow for the addition of any other substance. This includes, but is not limited to water or other sweeteners.

The composition of honey is mainly due to three major components, fructose (38%), glucose (31%) and water (17%)<sup>[1]</sup>. The remaining 14% contains small amounts of some 22 disaccharides, trisaccharides, oligosaccharides, minerals, vitamins and enzymes. The acidic pH of honey (average 3.9), the high sugar concentration and the long equilibration times all favor, revision and may be responsible for the formation of some rare sugars in honey that are not ordinarily found in nature. The potential nutritional value of many of these sugars is an area of research that has remained largely unexplored. There is also a growing interest in the reintroduction of healthy bacteria into the colon following diarrheal diseases or the extended use of antibiotics<sup>[2]</sup>.

Nutrients with known antioxidant properties such as tocopherols, alkaloids, ascorbic acid, flavonoids, phenolics and various enzymes, have been noticed in honey in small amounts. Nectar contains large quantities of flavonoids, plant pigments and flavoring compounds with antioxidant properties<sup>[3]</sup>.

The medical importance of honey is well understood and honey is being used as therapeutic agent in Ayurvedic medicine in India<sup>[4,5]</sup>. The therapeutic value of honey has been demonstrated in various countries such as China<sup>[6]</sup>, the Middle East<sup>[7]</sup>, Africa<sup>[8]</sup>, India<sup>[9]</sup>. In the Middle East and China, honey is considered a traditional remedy to treat the common cold and various respiratory tract infections<sup>[6,7]</sup>.

The antibacterial activity of honey is well documented and its therapeutic value has been useful in the treatment of infected surgical wounds and ulcer<sup>[10-12]</sup> and burns<sup>[9]</sup>. Although, anti-fungal activity of honey has been reported, but a very little information available about its anti-mycotic activity<sup>[13-15]</sup>. Subrahmanyam<sup>[16]</sup> reported that honey is a good preserving medium for skin grafts. Recent investigation uses it against multi-drug -resistant microbial pathogens<sup>[6,17]</sup>.

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Due to the possible antibacterial activity of Indian honey, the present study was designed to investigate the antibacterial activity of Indian honey against antibiotic resistant bacteria i.e. *S. epidermidis* (gram-positive) and *E. coli* (gram-negative).

## MATERIALS AND METHODS

**Collection and preparation of honey samples:** Total of 10 honey samples {(five from Uttaranchal state (Hardwar) and 5 from Uttar Pradesh state (Meerut), India)} were collected in sterile containers and kept at room temperature (24-26°C) for 30 days before experimentation. Each sample was prepared in various concentrations (20, 40, 60, 80 and 100% (v/v) with sterile distilled water and was soaked in 5 mm diameter of paper discs (Whatman filter paper-42) for 2 h at room temperature.

**Honey samples test for sterility:** The sterility of honey was tested by inoculation of honey samples as described by James *et al.*<sup>[18]</sup> and modified method version<sup>[5]</sup>. In brief, a loopful of prepared honey sample was streaked on blood agar and Sabouraud agar medium. Blood agar plates were incubated at 37°C for 24 h and Sabouraud agar plates were incubated at 25°C for 7 days.

**Test organisms:** Pure isolates of *S. epidermidis* and *E. coli* were isolated from Indian patients and were confirmed for antibiotic resistant in our laboratory. Both test organisms were therefore considered to be multidrug-resistant.

**Determination of antibacterial activity:** Prepared concentrations of honey samples were tested against antibiotic resistant bacteria (*S. epidermidis* and *E. coli*) by the disc diffusion method<sup>[19]</sup>. In the suspension of bacterial colony was mixed thoroughly ( $10^6$  bacterial cell/100 uL) with nutrient agar (15 mL/plate)

and poured into a petri dish. After 30 min, soaked paper discs were placed on the medium to check the antibacterial potency of various concentrations of honey and then the Petri plates were incubated at 37°C temperature for 24 h. Each concentration of honey was tested in triplicate and the mean was calculated.

## RESULTS AND DISCUSSION

**Sterility of honey samples:** Prepared concentrations of honey were tested for its sterility and were observed to be sterile for bacterial growth on blood agar medium and for fungal growth on Sabouraud agar medium.

**Antibacterial activity of honey samples against antibiotic resistant bacteria:** Out of 10 honey samples, only 5, 3 (60%) from Uttaranchal state and 2 (40%) from Uttar Pradesh state, India) exhibited antibacterial activity against *S. epidermidis* and *E. coli* bacteria (Table 1). Samples UH-H-1 (from Uttaranchal state) and UP-H-3 (from Uttar Pradesh state) showed the maximum zone of inhibition at 60% concentration against *S. epidermidis* (18 and 16 mm diameter) and *E. coli* (16 and 14 mm diameter), respectively.

Antibiotic resistant isolates of *S. epidermidis* and *E. coli* were found highly sensitive to honey samples, collected from Uttaranchal state as compared to those obtained from Uttar Pradesh state. Additionally, all potential honey samples of both states showed good antibacterial activity against *S. epidermidis* than *E. coli* bacteria.

Selected concentration (60% v/v) of honey samples (UH-H-1 and UP-H-3) were tested at different concentration i.e. 100, 200, 300, 400 and 500 uL/disc for antibacterial efficacy against both antibiotic resistant isolates. Table 2 shows the highest zone of inhibition observed against both isolates was with 400 uL/disc of 60% concentration of UH-H-1 (18.0 mm for *S. epidermidis*

Table 1: Anti-bacterial activity of Indian honey against antibiotic resistant bacteria

Antibiotic resistant bacteria	Various concentrations of honey samples (% v/v)	Mean of inhibition zone (mm) of honey from different sates of India									
		Uttaranchal (UH-H)					Uttar Pradesh (UP-H)				
		1	2	3	4	5	1	2	3	4	5
<i>S. epidermidis</i>	20	9	-	7	6	-	-	6	8	-	-
	40	12	-	9	7	-	-	8	9	-	-
	60	18	-	14	12	-	-	13	16	-	-
	80	14	-	11	9	-	-	11	12	-	-
	100	11	-	8	8	-	-	8	9	-	-
<i>E. coli</i>	20	6	-	6	7	-	-	8	9	-	-
	40	10	-	9	8	-	-	10	11	-	-
	60	16	-	13	11	-	-	12	14	-	-
	80	11	-	10	9	-	-	9	11	-	-
	100	9	-	8	6	-	-	6	9	-	-

Table 2: Quantitative activity of 60% (v/v) concentration of selected (high potency) honey samples against antibiotic resistant bacteria

Different quantity of honey samples (uL/disc)	Mean of inhibition zone (mm) of selected honey samples against antibiotic resistant bacteria			
	UH-H-1		UP-H-3	
	<i>S. epidermidis</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>E. coli</i>
100	10	9	8	7.5
200	12	12	11	12.0
300	14	13	13	12.5
400	18	17	15	14.5
500	17	15	14	14.0

and 17.0 mm for *E. coli*) and UP-H-3 (15.0 mm for *S. epidermidis* and 14.5 mm for *E. coli*).

In India, plants and their products are used as therapeutic agent in Ayurvedic medicine system<sup>[20-22]</sup>. Honey is produced from floral sources and its antimicrobial property has been well documented<sup>[4-6]</sup>. Since Dold *et al.*<sup>[23]</sup> first discovered the antimicrobial activity of honey in 1937 and later many other researchers worked on this issues and discussed this property in detail<sup>[4,5,8]</sup>. Honey has been reported to have anti-*S. epidermidis* and anti-*E. coli* activity<sup>[14]</sup>, but there is no report regarding the antibacterial activity of Indian honey against antibiotic resistant isolates of *S. epidermidis* and *E. coli* bacteria. In this study, Indian honey samples showed the antibacterial activity against antibiotic resistant isolates of *S. epidermidis* and *E. coli*.

The honey concentration at 40% was reported to possess strong anti-bactericidal activity against various gram-positive and gram-negative bacteria, in particular against *Salmonella*, *Shigella*, enteropathogenic *E. coli* and *Vibrio cholera*<sup>[24]</sup>.

Present study shows that Indian honey samples (UH-H-1 and UP-H-3) had the greatest activity out of all samples tested against antibiotic resistant isolates such as *S. epidermidis* and *E. coli*. This antibacterial activity was demonstrated at 60% (v/v) concentration of honey samples for gram-positive and gram-negative bacteria. This study indicates the broad-activity at 60% concentration of Indian honey. UH-H-1 and UP-H-3 samples were tested at different quantity of 60% concentration and 400 uL/disc quantity of honey showed the maximum zone of inhibition against *S. epidermidis* and *E. coli*.

When we compare the antibacterial activity of Uttaranchal samples with those obtained from Uttar Pradesh samples, it seems that the degree of activity depends on the geographical location of honey samples<sup>[25]</sup>.

The present study indicates that honey obtained from Uttaranchal state (mountainous area) would show better antibacterial activity (60% honey samples) than honey obtained from Uttar Pradesh state (only 40% honey samples).

Although honey is known to act against different bacteria, including those that are highly resistant to antibiotics<sup>[6,16,17]</sup>. From the present study, it is clear that Indian honey possesses considerable antibacterial potency and can be used to treat infections of antibiotic resistant bacterial isolates of *S. epidermidis* (gram-positive) and *E. coli* (gram-negative).

Honey was used, as a remedy for burns, cataracts, ulcers and wound dressings from long time, perhaps due to it's soothing during its initial application to open wounds. In near future, it can be used as therapeutics for treating bacterial infection specially related with *S. epidermidis* and *E. coli*. The results are encouraging but further there is a need to continue to investigate this issue in detail.

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