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Original Research Article

Evaluation of antibacterial potential of some Indian honey samples against throat and skin infective pathogens

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A B S T R A C T

In this present study an attempt has been made to determine the antibacterial effect of 20 different Indian honey samples as they were tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. MIC values were ascertained by CFU count method. Bacterial growth curves were made by plating technique. SEM was used to study the morphology of treated and untreated bacterial cells. A two-way ANOVA was done with the MIC values using R(2.15.0) software which suggests strongly the interspecific variation in spite of intraspecific variation of honey samples. The result also suggests a better efficacy of antibacterial activity of honey on *Staphylococcus epidermidis* than other two tested bacterial strains. The bacterial growth curve confirms the bactericidal effect of honey which is also depicted with the result of SEM pictures. The study confirms the fact that honey can be used as an alternative medicine for various bacterial infections.

K e y w o r d s

Honey; Antibacterial potential; Therapeutic usage; Alternative medicine; CFU; Bactericidal mode of action.

Introduction

Honey is a traditionally used medicine for topical treatment, infected wounds and various other infections. It can be effective on antibiotic resistance strains of bacteria as well (Chute *et al*. 2010). Antibacterial activity of honey varies with origin and processes. Honey has been used as a folklore medicine for many different purposes: as a laxative, as a natural cure for diarrhea and upset stomach, for coughs and sore throats. Honey is an ancient remedy in the Middle East, India, China and Africa for many centuries and has been used in the treatment and prevention of the common cold and various upper respiratory tract infections (Molan, 1992, Zulma and Lulat, 1989).

The pure honey contains alkaloids, auterquinone glycosides, cardiac glycosides, flavonoids and reducing compounds and the antibacterial properties of honey includes the release of hydrogen peroxide when diluted. Some honey has additional phytochemical compounds which serve as antibacterial agents

(Dumronglert, 1983). The antibacterial property of honey is also due to osmotic effect of its high sugar content that is sufficient to inhibit the microbial growth (Dumronglert, 1983). Nutrients and secondary metabolites such as flavonoids, ascorbic acid, tocopherol, phenolics and many enzymes have been found in honey in small amounts which have known antioxidant activity (Frankel *et al*. 1998). It is reported that proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentration as low as 0.1%. In addition the acidic pH (between pH 3-pH 4) may assist in bacteria destroying action (Cavanagh, 1970, Mandal and Mandal, 2011). In recent investigations it has been claimed to be used in multi-drug-resistant microbial pathogens. Subrahmaniyam in 1993 reported that it can also be effective as a good preserving medium for skin-grafting (Subrahmaniyam, 1993).

Indiscriminate usage of antibiotics has led to the emergence of drug resistance strains for many diseases. Resistance to antimicrobial therapy is now more prevalent among *Pseudomonas* spp. (Cooper *et al*., 2002). In the present study an attempt has been made to evaluate the antibacterial activity of twenty different honey samples from different parts of India, against common throat and skin pathogens like, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Materials and Methods

Collection of honey samples

A total of 20 honey samples were collected from 10 different states of India (Table 1) in sterile container and stored in room temperature (26º± 2º C) for the test. Different dilutions of honey samples were made such as 20, 40, 60 and 80% (w/v) with sterile distilled water for the study.

Test organisms

Pure cultures of *Staphylococcus aureus* MTCC 96, *Staphylococcus epidermidis* MTCC 3068 and *Pseudomonas aeruginosa* MTCC 741 were used in the study and were procured from Microbial Type Culture Collection and Gene Bank, IM Tech., Chandigarh, India and were maintained in nutrient agar medium with regular transfer.

Determination of MIC

Different dilutions of each honey sample were tested against the selected bacterial strains by CFU count method in nutrient broth to study their minimum inhibitory concentration (MIC). Bacteria were treated in honey samples for overnight (10h), then diluted serially and plated on nutrient agar plates. The petri plates were incubated at 37ºC temperature for 24h. Each concentration of honey was tested in triplicates and the mean values of CFU counts were taken. The activity of oxytetracyclin was measured for positive control.

Bacterial growth curve

Pseudomonas aeruginosa MTCC 741(Gram negative) and *Staphylococcus epidermidis* MTCC 3068 (Gram positive) were selected for the test of bacterial growth curve to ascertain the mode of action of honey on these bacteria. Platings were done in every 2h intervals for 32h in triplicates and kept for incubation at 37ºC for 24h.

Preparation for SEM study

Scanning electron microscopy (SEM) was used to study the morphology of bacterial cells. The specimens were prepared as follows: Cells were harvested by centrifugation at 10,000×g for 10 minutes. Bacterial pellets were washed thrice with normal saline and prefixed with a mixture of 3% glutaraldehyde and 5% DMSO in 0.05 M acetate buffer, pH 5.0, for 30 min, cells were then harvested by centrifugation at 10,000×g for 10 min and the pellets were washed thrice with 0.1 M sodium acetate buffer, pH 5.0. The pellets were then post-fixed with osmium tetraoxide solution for 30 min. Cells were collected by centrifugation at 10,000×g for 10 min and were dehydrated with a series of ethyl alcohol starting with 30% via 40, 50, 60, 70, 80, 85, 90 and 95% and finally with 100% with 10 min of dehydration in each grade. The cells were then spread on a clear glass slide (1 sq.cm.). The slide was mounted on a stub with double slide adhesive tape and silver dag and coated slowly with a very thin (2-5 nm) layer of gold in a sputtering unit prior to examination under scanning electron microscope (Philips, Model PSEM-500, Holland) following the method of Sarem-Damerdji *et al*. (Sarem- damerdji *et al*. 1995).

Statistical analysis

A two-way ANOVA was performed with the MIC values of the honey samples against these selected bacterial strains using R (2.15.0) software.

Results and Discussion

Different dilutions of twenty different honey samples were tested against the common throat pathogen *Pseudomonas aeruginosa* MTCC 741and against two skin pathogens viz., *Staphylococcus aureus* MTCC 96 and *Staphylococcus epidermidis* MTCC 3068 and the results are presented in the table (Table1). Numbers of bacterial colonies at different dilutions were counted to find out the MIC values of the honey samples against the bacterium. The dilution, at which a drastic decrease of CFU followed by almost declining trend at further higher concentration of honey sample occurred (Fig. 1 & 2), was considered as MIC of that honey sample. Increase in treatment hours with honey sample reduced CFU count further. This was well depicted during the study of mode of action (Fig. 4 & 5). The studied MIC values (Fig. 3) for *Pseudomonas aeruginosa* MTCC 741 were varied from 50 to 60% (w/v) among the selected 20 honey samples with a value of 0.01 mg/ml of oxitetracycline as positive control (Table 1). For *Staphylococcus aureus* MTCC 96 and *Staphylococcus epidermidis* MTCC 3068, the MIC value ranges between 30-60% and 25-40% (w/v) respectively and the positive control of oxytetracycline is 0.02 and 0.018 mg/ml respectively (Table 1, Fig.3). Honey samples showed a better inhibitory effect for *Staphylococcus epidermidis* than the other two strains. Honey sample from Joka (H2) showed a low range of MIC values for all the three bacterial strains which was 60% for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and for *Staphylococcus epidermidis* it was 40% which suggest lower efficacy than other honey samples (Table 1). In most of the samples like H1, H3, H4, H5, H6, H7, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18 and H20, the similar MIC values are observed (50%) for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*, whereas it is 30%

Table.1 List of honey samples with their MIC values along with positive control of oxytetracyclin

Honey Sample	Place of collection (State)	MIC values (mg/ml)		
		Pseudomonas aeruginosa	***Staphylococcus aureus***	***Staphylococcus epidermidis***
H1	Bardhaman (West Bengal)	50(40-60)	50(40-60)	30(20-40)
H2	Joka (West Bengal)	60	60	40
H3	Moipith (West Bengal)	50(40-60)	50(40-60)	30(20-40)
H4	Majhdia (West Bengal)	50(40-60)	50(40-60)	30(20-40)
H5	Bishnupur (West Bengal)	50(40-60)	50(40-60)	25(20-40)
H6	KSH I (Jammu & Kashmir)	50(40-60)	50(40-60)	25(20-40)
H7	KSH II (Jammu & Kashmir)	50(40-60)	50(40-60)	25(20-40)
H8	Keonjhor (Orissa)	50(40-60)	30(20-40)	30(20-40)
H9	Shimlipal (Orissa)	50(40-60)	50(40-60)	30(20-40)
H10	Solan (Himachal Pradesh)	50(40-60)	50(40-60)	25
H11	Sundarnagar (Himachal Pradesh)	50(40-60)	50(40-60)	30(20-40)
H12	Bisalgarh (Tripura)	50(40-60)	50(40-60)	30(20-40)
H13	Belonia (Tripura)	50(40-60)	50(40-60)	30(20-40)
H14	Mujaffarpur (Bihar)	50(40-60)	50(40-60)	30(20-40)
H15	Panchmari (Madhya Pradesh)	50(40-60)	50(40-60)	25
H16	Kanha (Madhya Pradesh)	50(40-60)	50(40-60)	30(20-40)
H17	Kurg (Karnataka)	50(40-60)	50(40-60)	30(20-40)
H18	Rudraprayag (Uttarakhand)	50(40-60)	50(40-60)	30(20-40)
H19	Puna (Maharastra)	50(40-60)	30(20-40)	30(20-40)
H20	Aurangabad (Maharastra)	50(40-60)	50(40-60)	25
Oxytetracycline		0.01	0.02	0.018

Table.2 Result of two-way ANOVA test of MIC values of twenty different honey samples against the selected three bacterial pathogens.

Source of Variation	Df	Sum of Squares	F value	p-value
Honey.samples	19	590	31.1	0.034
Bacterial.strain	2	5643	2821.7	<0.001*
Residual	38	590	-------	----------
Total	20	6823	-------	--------

Figure.1 MIC values of twenty different honey samples against *Pseudomonas aeruginosa* MTCC 741, *Staphylococcus aureus* MTCC 96 and *Staphylococcus epidermidis* MTCC 3068.

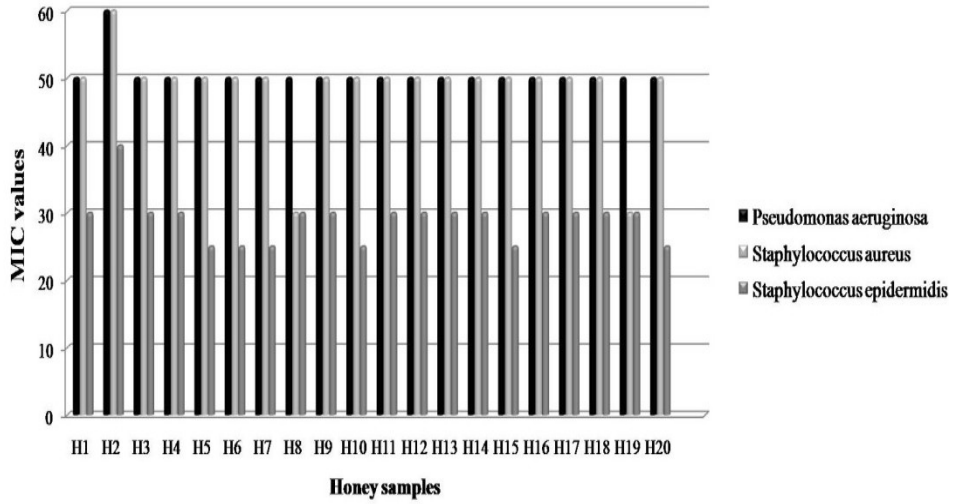


Figure.2 Effect of honey sample (KSH I, 40%) on CFU count of actively growing *Pseudomonas aeruginosa* MTCC 741

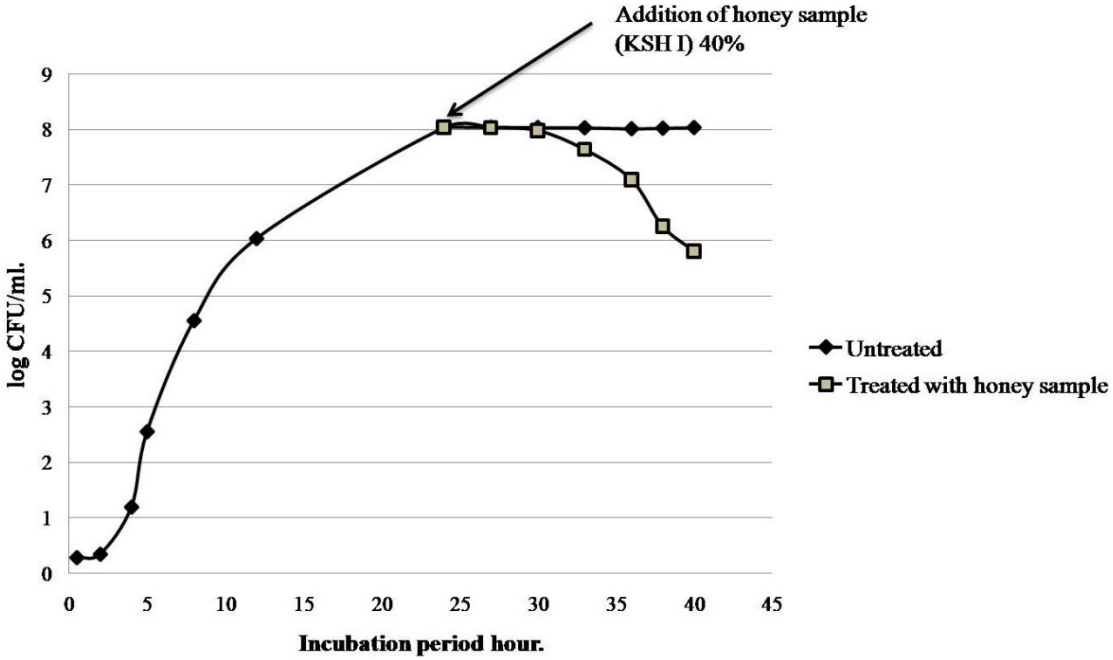


Figure.3 Effect of honey sample (KSH I, 40%) on CFU count of actively growing *Staphylococcus epidermidis* MTCC 3068

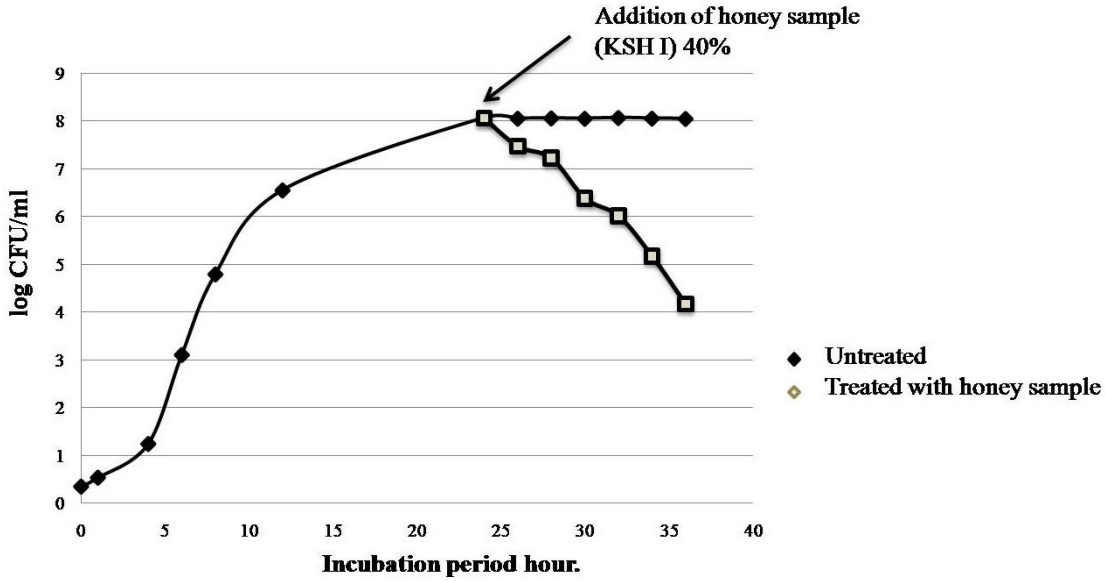
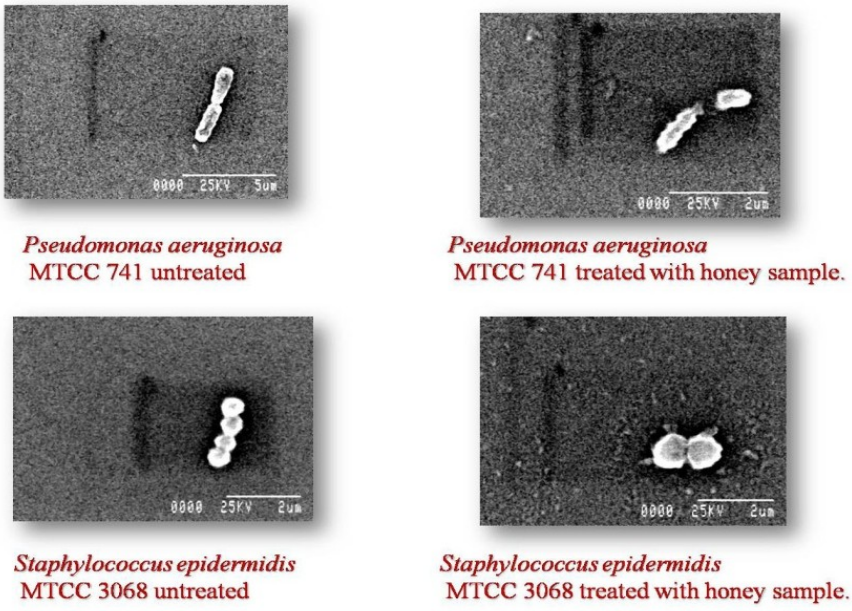


Figure.4 SEM photographs of *Pseudomonas aeruginosa* MTCC 741 and *Staphylococcus epidermidis* MTCC 3068 in untreated and treated condition with honey sample (KSH I, 40%).



for honey samples H8 and H19. On the other hand, in *Staphylococcus epidermidis* the MIC value is 30% for the honey samples H1, H3, H4, H8, H9, H11, H12, H13, H14, H16, H17, H18 and H19, 25% for H5, H6, H7, H10, H14 and H20 and 40% for H2 (Table 1). It has been found that in *Pseudomonas aeruginosa* and *Staphylococcus aureus*, bacterial growth is increased in lower dilutions of honey like 20% or lower values. This might be justified as because in lower dilutions, honey may act as nutrient rather than inhibitory agent and enhances the bacterial growth.

The growth kinetics of two test organisms namely, *Pseudomonas aeruginosa* (Fig. 4) and *Staphylococcus epidermidis* (Fig. 5) under treated and untreated conditions were performed which clearly showed a gradual decline in the number of CFU upon treatment of actively growing cultures, indicated a bactericidal mode of action (Ray *et al*., 1999).

The results of the SEM study (Fig. 6) showed the morphological changes of these two selected bacterial strains when treated with honey and with the untreated condition as well. The study suggested the swelling and lyses of the bacterial cells upon exposure with honey. This indicates the effect of honey sample on direct cellular integrity of the bacterial cell. Similar action of compounds on bacterial cells was also noticed in other bacteria (Sawer *et al*., 2005).

The result of two-way ANOVA (Table 2) with the MIC values of the honey samples against these selected bacterial strains stated that there is an interspecific variation in 0.001% significance level without having any intraspecific variation. On the other hand, in 0.005% significance level both inter and intraspecific variation was found. Thus, it can be concluded that MIC values varies with the 3 different bacterial strains, but not with the different honey samples in 0.001% significance level. However, in a broader aspect (at 0.005% significance level), there is a little variation of MIC values with different honey samples.

The study firmly supports the antibacterial property of the selected honey samples on the three bacterial strains responsible for throat or skin infections. It also suggests that MIC values do not vary greatly with different honey samples; rather they vary with different bacterial strains that have been proven statistically. Bactericidal mode of action was confirmed with the growth kinetics and also from SEM photographs. It can be concluded that the therapeutic usage of honey as an alternative medicine for different diseases caused by these organisms can enhance traditional remedies of health care option.

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