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Research Article

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STUDY OF INHIBITORY EFFECT OF HONEY ON VARIOUS PATHOGENIC AND NON-PATHOGENIC MICROORGANISMS

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Abstract

Honey is a sweet food made by bees using nectar from flowers. The variety produced by honey bees (the genus *Apis*). The purpose of the present study was to determine *in vitro* antibacterial activities of different honey against various pathogenic and non-pathogenic organisms. Antibacterial activity of honey was determine by using well diffusion method in which different concentrations (20, 40, 60, 80, & 100 % v/v) of honey were used against various test pathogen. These organisms also tested against artificial honey for study of effect of sugar on its antibacterial activity. The results of these study was shown that wide range of variation in Zone of Inhibition (mm) of each type of honey and only very few organism inhibited due to effect of sugar. That shows there are also other components other than the sugar present in honey which ultimately responsible for antimicrobial activity. Due to obtaining maximum level of antibacterial activity of each honey it allow further investigation for treatment various infection and in curing of disease.

Keyword: Honey, Antimicrobial activity, Pathogenic organism, artificial honey

Introduction

Honey is supersaturated solution of sugars produced by different *Apis* species. Natural honey (NH) is a sweet, flavorful liquid food of high nutritional value and immense health benefits. NH is produced by honey-bees as blossom honey by secreting nectars of flowers, and honeydew honey (forest honey) by secreting the exudates of plant sucking insects (Aphids). Honey was used in the medicine of many ancient communities (Molan, 2006), including the ancient Egyptians. The use of honey as a traditional remedy for microbial infections are the cause of a large burden of diseases and bacteria are listed in the first position among the common microorganisms responsible for opportunistic diseases. Therapy of bacterial infections is a frequent problem due to the emergence of bacterial strains resistant to numerous antibiotics. These shortcomings lead to an urgent global call for new antibacterial drugs, particularly from natural resources. Current research has been focused on herbal and aromatherapy products. However, a number of other products such as honey have shown therapeutic promise. Researches relating to honey show that pure honey is bactericidal for many pathogenic organisms, including various gram-negative and gram-positive bacteria. Honey has been used in treating different diseases as 2000 years ago. Honey is a natural product, well known for thousands of years for its high nutritive value and healing properties.

Also Honey has been considered as an important part of traditional medicines since ancient times.The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low pH (Kwakman and Zaat, 2012), the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey (Kačániová *et al*., 2011; Wahdan, 1998) and phytochemical factors. Numerous reports and clinical studies have demonstrated the antimicrobial activity of honey against a broad range of microorganisms, including multi-antibiotic resistant strains. The objective of this study was to investigate antimicrobial potency of the four different honey sample compared with laboratory synthesized artificial honey.

Material and Methods

Honey sample

Four different honey samples were collected. Among two type of honey was used in antimicrobial susceptibility testing. Raw honey A, B & C used in the study was taken from area (surat) produce by honey bee (*Apis mellifera*). Commercial honey D used in study was purchased from Dabur India Ltd. Different honey concentration (20%, 40%, 60%, 80%, & 100% v/v) were prepared by diluting honey followed by incubating at 37ºC for 30 minutes in a shaking water bath.

Test organisms

The following test organisms (bacteria) were used: *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi* A*, Salmonella paratyphi* B.

Preparation of test organisms

Stocked cultures of all these test organism used in this study were obtained from the Microbiology Department. The isolates were identified based on standard microbiological techniques, and sub-cultured in nutrient agar slopes at 37°C for 24 h. Colonies of fresh cultures of the different microorganisms from overnight growth were picked with sterile inoculating loop and suspended in 3-4 mL nutrient broth contained in sterile test tubes and incubated for 24 h at 37°C. This was used as inoculums in this study.

Artificial honey

Artificial honey was prepared by weighing and dissolving 33.5 g glucose, 1.5 g sucrose, 7.5 g maltose, 40.5 g fructose in 17 mL distilled water. This solution was heated briefly at 80°C in a water bath to aid dissolving. Check the Antibacterial activity of artificial honey against the all test organism using well diffusion method to study the effect of sugar on the test organisms.

Antibacterial Activity Assay

Antimicrobial activity was tested using well diffusion method against test microorganisms. Honey sample were first inoculated separately on standard nutrient media using pour plate method. The plates were drained and allowed to dry at 37°C for 30 minutes after which four equidistant wells of 5 mm in diameter were punched using a sterile cork borer at different sites on the plates. 10 uL of the different concentrations (20, 40, 60, 80, 100% v/v) of the honey samples were separately placed in the different punched wells with 1 mL sterile cork borer. The plates were allowed to stay for 15 minutes for pre-diffusion to take place followed by an overnight incubation that lasted for 24 hrs at 37°C. The resulting Zones of inhibition (ZOI) were measured in millimeter (mm) with the use of a caliper and recorded.

Result and Discussion

All the four samples of honey exhibited varying level of antibacterial activity against all the selected test organisms as indicating by the zone of inhibition of growth. The results of *in vitro* susceptibility of the various test organisms of four honey samples with five different concentration (20, 40, 60, 80 &100% v/v) were different that shown in Table 1. All honey samples shows low level antibactetrial activity in *S. typhi, S. typhi* A.

Another area of interest is that all of the test organisms were tested against artificial honey to compare with four honey sample. The result of antibacterial activity of artificial honey was highest on *E. coli, S. aureus* and *S.paratyphi*B (Table2)

Conclusion

From the result contained in this report it was concluded that all honey sample showed sensitivity against various tested organisms. Both type of honey, natural honey and commercial honey have similar antibacterial activity against pathogen. From these results it shown that all honey has different level of antibacterial activity which was observed by the various zone of inhibition that was shown that there are more than one component are responsible for inhibitory effect on pathogen. Ultimately all honey samples have highest inhibitory effect on all pathogen specially *S. typhi, S. paratyphi, P. aeruginosa, E.coli* that were allow to used honey for further therapeutic purpose. It was also concluded that in future it may be used as treating many incurable infection and disease in medical science.

Table 1: Diameter of ZOI (mm) of honey at different concentration against various pathogens by well diffusion method

Test Organisms	Honey concentration (v/v) %																			
	Honey A					Honey B					Honey C					Honey D				
	20	**40**	**60**	**80**	**100**	**20**	**40**	**60**	**80**	**100**	**20**	**40**	**60**	**80**	**100**	**20**	**40**	**60**	**80**	**100**
Escherichia coli	24	25	26	27	39	23	25	27	28	30	14	27	30	36	38	24	28	33	34	37
Bacillus subtilis	22	24	26	28	34	20	23	28	31	36	16	23	24	27	30	19	20	28	30	31
Pseudomonas aeruginosa	28	34	37	39	40	-	-	-	-	39	-	-	-	-	20	23	28	33	37	39
Staphylococcus aureus	10	14	20	23	36	-	25	27	28	33	25	28	30	35	38	15	20	25	27	39
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	-	11	13	22	-	-	-	-	-
Salmonella. paratyphi A	-	-	-	-	15	-	-	24	27	28	-	-	-	20	25	-	8	11	14	25
Salmonella. paratyphi B	-	24	29	31	32	10	23	31	34	35	20	28	35	38	40	25	27	29	32	39

Table 2: The diameter of ZOI against test organisms (Artificial honey)

Test organism	**Zone of inhibition (mm) (Artificial honey)**
E. coli	41
B. subtilis	-
B. megaterium	-
P. aeruginosa	-
S. aureus	44
S. marcescens	-
M. luteus	-
S. typhi	**-**
S. paratyphi A	-
S. paratyphi B	15

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