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In-vitro Antibacterial Activity of Honey on Health Hazardous Microorganisms

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ABSTRACT

Honey finds an application in ayurvedic medicine since Vedic period. Chemical composition of honey keeps changing with respect to sugars, enzymes, acids, etc. This variation depends upon the honey bees and the surrounding vegetation from which the honey bees collect nectar to prepare honey, which plays an important role in nutritive value, color, taste, flavor and more with medicinal values. In this context, an attempt is made to study the antibacterial activity of honey samples collected from different places against health hazardous microorganisms under in vitro conditions. Three honey samples were collected from GKVK, Mudigere and Dhaka. They were tested against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.* by agar well diffusion assay technique at two concentrations- 25 and 50 %. GKVK honey sample could inhibit all the three tested bacteria at both concentrations (50 and 25%). GKVK honey is prepared mainly from/ by nectar collected from Eucalyptus flowers. Eucalyptus is known to possess some medicinal properties. Some of these principles may be present in nectar and might have gained presence in honey ultimately. Mudigere sample was effective on at 50%. Dhaka sample could inhibit *Staphylococcus aureus* at both 50 and 25% (29 and 17 mm diameter of inhibition zone respectively).

Keywords: Honey, Antimicrobial activity, Bacterial strains and Eucalyptus

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INTRODUCTION

Honey has been used as a medicine since ancient times in many cultures and is still used in folk medicine. Use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times and it is gaining acceptance as an antibacterial agent for the treatment of ulcers, bed sores and other skin infections [1].

The antibacterial activity of honey was first recognized in 1892 [2]. Honey is produced from many sources and its antimicrobial activity varies greatly with origin and processing [3]. Honey has been used as a medicine in many cultures for a long time [4]. It has been rediscovered by the medical profession and it is gaining acceptance as an antibacterial agent for the treatment of topical infections resulting from burns and wounds [5]. Numerous studies demonstrate that honey possesses antimicrobial activity, more recently honey has been reported to have an inhibitory effect on around 60 species of bacteria including aerobes and anaerobes, Gram-positive and Gram-negatives [6], it inhibits and destroys the growth of some pathogenic vegetative microorganisms [7]. An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium*, as well as all the common dermatophytes [8].

Honey possesses inherent antimicrobial properties, some of which are due to high osmotic pressure, low water activity, in which the low water activity of honey is inhibitory to the growth of majority of bacteria, many yeasts and moulds. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth. Even when diluted with water absorbed from wound, honey would be likely to retain a water activity sufficiently low to inhibit the growth of most bacteria. Honey is mildly acidic, with a pH between 3.2 and 4.5, gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid, the low pH and formation of hydrogen peroxide is inhibitory to many pathogenic bacteria and in topical application at last, could be sufficient to exert an inhibitory effect [9].

Honey is produced from many sources and its antimicrobial activity varies greatly with origin and processing [10]. The antibacterial compounds introduced by bees during the collection of material, the ripening process and the floral source of honey is also responsible for its antimicrobial activity [11]. The variation in activity of New Zealand honeys might be attributable to the floral source. Honey from Manuka (*Heptospermum scoparium*) demonstrated high antibacterial activity and this was shown to be a non-peroxide component [1].

MATERIAL AND METHODS

Collection and Preparation of Honey samples

The three honey samples used in this study were collected from the Department of Apiculture, GKVK (Gandhi Krishi Vignana Kendra), Bangalore, India; Dhaka (Bangladesh) and Mudigere, Karnataka, India. These were kept at 4° C away from direct sunlight. The honey samples were diluted to 50 % and 25 % (v/v) using sterile distilled water and the solution was shaken vigorously to dissolve honey.

Bacterial cultures

Three different bacterial species were collected from the Department of Agricultural Microbiology, GKVK, Bangalore viz., *Escherichia coli*, *Staphylococcus aureus* and *Salmonella sp.*, were inoculated into the nutrient broth and kept on a rotary shaker overnight for incubation. The broth cultures were subjected to standard plate count method to enumerate the population and the dilution having 10⁶cfu/ml were selected for antimicrobial test.

Antibacterial activity

The antibacterial activity was studied by agar well diffusion assay [12]. Different concentrations of honey samples were added to the wells viz., 50% (v/v) and 25% (v/v). Diameter of zone of inhibition around the well was measured.

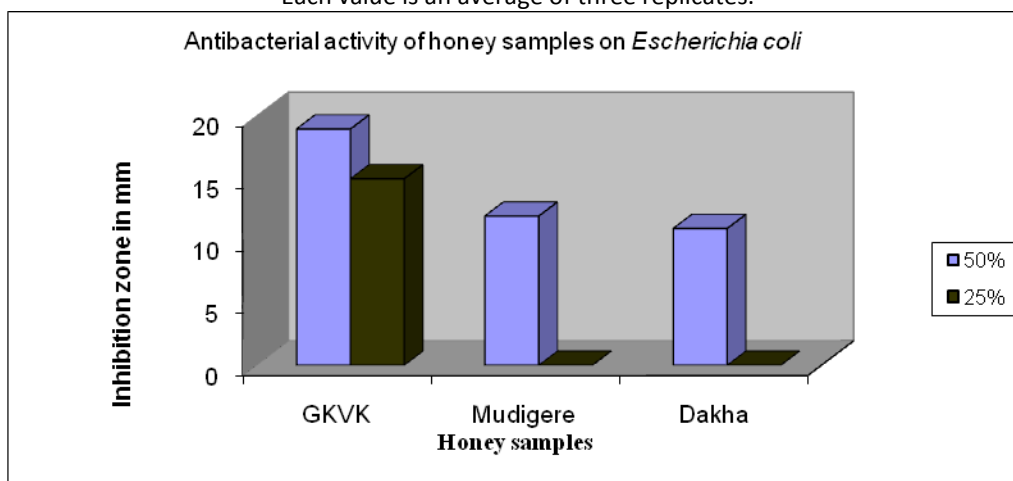
RESULTS AND DISCUSSION

The diluted honey 50% (v/v) had an antibacterial effect under in vitro on the tested health hazardous bacteria. All the three honey samples were found to be highly effective against Gram-positive bacteria *Staphylococcus aureus* (Graph 1) and less effective against Gram-negative bacteria *Escherichia coli* (Graph 2) and were less effective against *Salmonella sp.* (Graph 3) compared to *S. aureus* (Table 1). Diluted honey, 25% (v/v) also had an antibacterial effect on the tested bacteria. The GKVK honey sample was highly effective against *Staphylococcus aureus* compared to *Escherichia coli* and *Salmonella sp.* Mudigere honey sample was not effective and Dhaka honey sample was effective against *S. aureus* and not effective against *E. coli* (Table 1). Among all the three honey samples, the GKVK honey sample showed more effectiveness against the tested health hazardous bacteria.

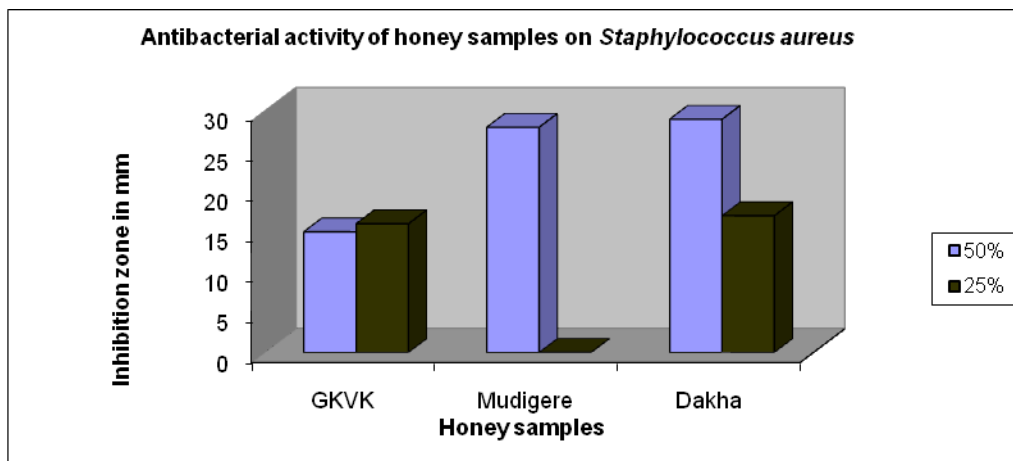
Table 1: Antibacterial activity of honey samples at different concentrations (v/v)

Sl. No.	Honey sample	Diameter of inhibition zone in mm					
		E. coli		S. aureus		Salmonella sp.	
		Dilution (v/v)					
		50%	25%	50%	25%	50%	25%
1	GKVK	19	15	15	16	24	10
2	Mudigere	12	-	28	-	24	-
3	Dhaka	11	-	29	17	23	10
4	Control	-	-	-	-	-	-

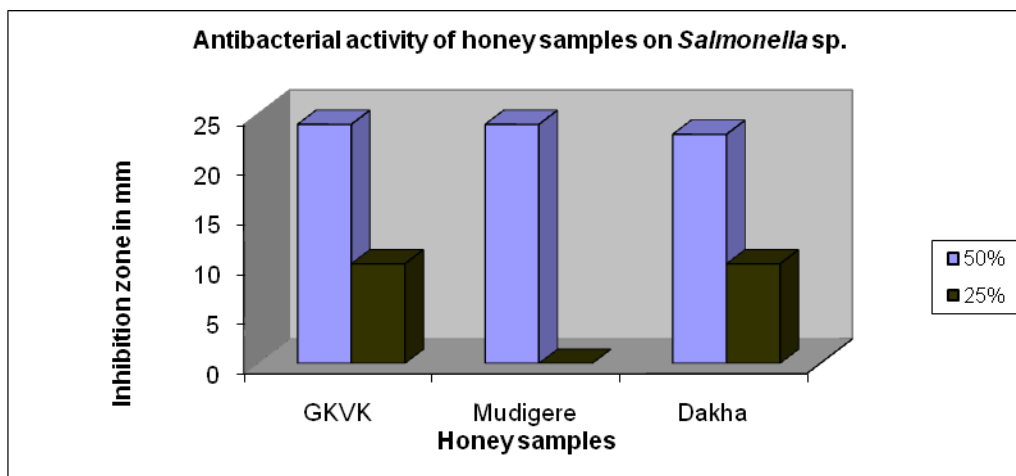
Each value is an average of three replicates.



Graph 1: Antibacterial activity of honey samples on *Escherichia coli*



Graph 2: Antibacterial activity of honey samples on *Staphylococcus aureus*



Graph 3: Antibacterial activity of honey samples on *Salmonella* sp.

The increasing trend of microbial resistance to antibiotics is an alarming concern throughout the world. New antibiotics are being processed for alleviating this situation and more research work is also being carried out on natural compounds for achieving the destruction of these microorganisms. Therefore, this study was taken up to investigate in vitro antibacterial activity of honey against health hazardous bacteria. Honey samples showed a potential activity against both Gram-positive and Gram-negative bacteria. A demonstration of the antibacterial activity was first carried out by Dold et al., who first suggested the possibility of hydrogen peroxide as the principle factor for the antibacterial activity of honey [13]. Jedder reported that honey is inhibitory to the growth of microorganisms at 50% (v/v) dilution [14]. These observations are in concurrence with our results.

Among all the three honey samples, the GKVK honey sample showed more effectiveness against the tested health hazardous bacteria. This honey sample was collected from Eucalyptus plantation. Therefore, honey must have acquired the medicinal properties of Eucalyptus. These results were in agreement with the results of Molan and Russel reported that the antibacterial activity of honey depends on its origin of collection i.e., floral source and ripening process [11]. The honey samples showed antimicrobial activity against *Staphylococcus aureus* and this result is in agreement with Molan who found the *S. aureus*, as one of the bacterial species most susceptible to the antibacterial activity of honey [9]. Lavie found another group of light sensitive, heat stable factors in honey which inhibited the growth of *E. coli*, *Salmonella* sp. and *S. aureus*. Taorima et al., (2001) studied the antimicrobial effect of honey on Gram-negative bacteria and attributed it to the presence of tetracycline derivatives, hydrogen peroxide and powerful antioxidants, as also naturally low pH, which is unfavorable for bacterial growth and to the presence of phenolic acids, lysozyme and flavonoids [16].

CONCLUSION AND RECOMMENDATION

Honey sample showed a potential activity against Gram-positive bacteria compared to Gram-negative bacteria. Honey can be used as an antibacterial agent against the health

hazardous microorganisms such as *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli*. The antibacterial activity of honey depends on its origin of collection and ripening process. Further research is therefore recommended to isolate and characterize the chemical compound responsible for the activity.

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