Antibacterial potential of honey samples of Apis cerana indica

M. Baskaran* and K. Thiyagesan

Division of Wildlife Biology, P.G. and Research Department of Zoology, A.V.C. College, (Autonomous), Mayiladuthurai - 609 305, Tamilnadu, India

Abstract

Antibacterial potentials of honey samples collected from hill and plain regions of Tamilnadu, south India when tested against human pathogenic bacteria *viz., Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, Escherichia coli and Pseudomonas aeruginosa,* showed region-wise differences only with regard to *S. aureus, B. cereus* and *C. perfringens.* However, different concentrations of honey samples differed significantly (ANOVA; F<0. 05) in their efficacy against all the bacterial species tested.

Keywords: antibacterial, Apis, honey, inhibition zone, pathogenic bacteria

INTRODUCTION

Honey is the combination of nectar secretions from the flowers of some plants and other sweet plant deposits that are gathered and modified by honeybees. Bees store honey in honey combs and then use it for food in winter. Since, it is a natural sweetener because of its high glucose and fructose content; it is widely used in candies, cereals, and baked goods. There are more than 300 types of honeys varying in flavor and colors (from pale yellow to dark amber), depending on the type of blossoms visited by the honey bee. Honey has been known to humans for centuries to have antimicrobial potential (Efem, 1993). Since Dold and Dziao's (1937) first report on antibacterial potential of honey there were numerous reports of the antimicrobial activities of honey which have been comprehensively reviewed by Radwan et al. (1984). Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram- negatives (Molan, 1992a, b).

The high antimicrobial activity of honey has been attributed to its properties such as osmotic effect, acidity, hydrogen peroxide and other phytochemical factors (Radwan *et al.*, 1984). Honey is characteristically acidic with a pH of between 3.2 and 4.5 which is low enough to be inhibitory to many animal pathogens (Molan, 1998, 2001). The minimum pH values for growth of some common pathogenic species are: *Escherichia coli* (4.3), *Salmonella sp.* (4.0) *Pseudomonas aeruginosa* (4.4), *Streptococcus pyogenes* (4.5). As such in the undiluted honey the acidity is a significant antibacterial factor, and its concentration in medical preparations using honey is a vital factor in their therapeutic values. Further more, it appears that the honey from certain plants has better antibacterial activity than from others

(Radwan *et al.*, 1984). So honey samples from different regions with different flowereing plants should differ in their antibacterial potentials. This paper explains the efficacy of honey samples of *Apis cerena indica* collected from two different regions of Tamilnadu, south India *viz.*, samples collected from hills and plains, against five bacterial pathogens *viz.*, *Streptococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Escherischia coli* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Collection and preparation of honey samples

Samples of honey of *A.C. indica* collected from two different geographical regions *i.e.*, Hill (Ooty) and Plain (Mayiladuthurai), were collected in sterile containers and kept at room temperature ($24-26^{\circ}$ c) for 30 days before experimentation. Each sample was diluted to various concentrations (25, 50, 75 and 100% in v/v) in order to test their antibacterial potential.

Determination of antibacterial Potential:

The antibacterial activity was evaluated by well diffusion assay of Bauer et al. (1996). Suspensions of bacterial colony were mixed thoroughly (106 bacterial cell/ 100 ml) with nutrient agar (15 mc /plate) and poured into Petri dishes. Wells were made in Petri dishes and 0.2ml of honey sample of different concentrations were poured in each well (Limm, 1998; Kumar et al., 2005). The Petri plates were incubated at 37°c temperature for 24hrs. Each concentration of honey was tested in triplicate and the diameters of the zones of inhibition were measured in mm from one edge to the opposite edge as the complete inhibition of growth appears to the unaided eye. The mean values were calculated and analysis of variance (ANOVA) was performed to evaluate the effects of concentration and place of collection on antibacterial activity.

*Corresponding Author email: *beebaskaran@rediffmail.com*

RESULTS AND DISCUSSION

	MEAN	DIAMETER	OF ZONE OI	IS∓X) X±SI	N (mm) AT (()(=3))	VARIOUS DI	IO SNOLLNT	'HONEY		R	AUON	
TestOrganism		ITIH	REGION			PLAINS	REGION		Reg	tion	Concentr	ation
	25 %	50 %	75 %	100 %	25%	50 %	75 %	100 %	F 4	64	E4	4
Staphyloc occus aunus	9.5±0.50	146±0.57	23.0±1.00	26.7±0.08	9.1 ± 0.17	12.2 ± 0.72	22.3±1.53	26.1±0.23	5.36	0.032	236.75	0.000
Bac ill te cerete	8.3±0.57	12.8 ± 0.23	20.2 ± 0.29	25.3 ± 0.57	8.7 ± 0.26	16.4±0.69	19.8±0.7 6	25.7 ± 0.57	5.99	0.024	333.25	0.000
Clostridium perfringens	9.2±0.76	16.5 ± 0.50	21.2 ± 1.04	30.7±0.56	11.6 ± 0.53	20.0±1.00	25.7±0.58	27.3±1.04	5.26	0.033	102.35	0.000
Eschrischia coli	ó.8±0.7ó	14.5 ± 0.50	18.0±1.00	25.0±1.00	8.0 ± 0.10	14.0 ± 1.00	20.0±1.00	22.0±1.00	0.02	0.883	151.80	0.000
Pæud on onæ ængi nosa	8.8±0.76	10.2±0.72	14.3 ± 0.57	31.2±1.05	8.0 ± 0.35	11.7±0.57	21.5±1.00	29.0±1.00	2.47	0.133	120.12	0.000













Staphylococcus aureus

Bacillus cereus



Clostridium perfringens











Figure 6. Efficacy of different concentrations of honey samples collected from two different regions of Tamilnadu, South India, on different bacterial species

Antibacterial potentials of honey samples of hill and plain regions against five bacterial species have been given in the table 1 and shown in figures 1-6. All the bacterial species showed significant differences in their susceptibility with regard to different concentrations of honey samples whereas for *E.coli* and *P. aeruginosa*, the variation due to sample collection sites (regions) was not significant (Table 1).

From the present study, it is evident that Indian honey possesses considerable antibacterial potency and honey offers advantages in controlling bacterial growth and in the treatment of certain health problems. Allen and Molan (1991) studied the antibacterial activity of various honeys against S. aureus and found that it was comparable with that of phenol, a standard antiseptic and to range from the equivalent of 2% phenol to 58% phenol. The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature (pH. 3.2-4.5), hydrogen peroxide concentration and phytochemical nature i.e. its content of tetracycline derivatives, peroxides, amylase, fatty acids, phenols, ascorbic acid, fluorides, sulfathiazole, trepenes, benzyl alcohol and benzoic acid (Bogdanov, 1984; Molan, 1992a,b; and Heering et al., 1998). However, according to N.H.B. (1994), the production and type or nature of honey produced by honey bee is dependent on geographical regions and various natural vegetative flowers blooming in different seasons. Thus the flowers from which bees gathered nectar to produce the honey may contribute to the differences in the antibacterial potential of honey. In this study two varieties of honey samples *i.e.* from plain region and hill region, were tested for their antibacterial potential of selected bacterial species. Thus the source of honey i.e., the geographical region has contributed to the differences in antibacterial potential of honey as the floral source that determines many of the attributes of honey, such as flavour, aroma, colour and phytochemicals composition is dependent on the habitat of a plant.

The low pH of honey is inhibitory to many animal pathogens (Molan, 1992a, b). Under experimental conditions, especially with heavily diluted honeys, the growth medium tends to neutralize the acidity of the honey so that it does not cause inhibition (Molan 1992a,b, 1998, 2001). As such the significant reduction in the efficacy of honey samples when they were diluted, as observed in the present study might perhaps be due to the changes in pH due to dilution.

REFERENCES

- Allen, K.L. and Molan, P.C. 1991. A survey of antibacterial activity of some New Zealand honey. J. Pharmacy and Pharmacol., 43: 817-822.
- Bauer, A.W., Kirby, W. M. M., Truck, H. and Sherries, J.C. 1996. Antibiotic susceptibility testing by standardized single

disc Method. Am. J. Clin. Pathol., 45: 493-496.

- Bogdanov, S. 1984. Charactrization of antibacterial substance in honey. *Lebensm Wiss. Technol.*, 17: 74-76.
- Dold, D.H. and Dziao, S.T. 1937. The antibacterial heat and light sensitivity inhibitory substances, inhibine in natural honey. Z. Hyg. Infektionskrankh, 121:333.
- Efem, S.E. 1993.Recent advances in the Management of Fournier's gangrene: Preliminary Observations. *Surgery*. 113: 200-204.
- Heering, W., Usleber, E., Dietrich, R. and Martlbaver, E. 1998. Immunochemical Screening for antimicrobial drug residue in commercial honey. *The Analyst* 123(12): 2759-2762.
- Kumar, A., Kaushik, R. Kashyap, A. and Kashyap, M.K. 2005.
 Indian Honey: A Natural Product With Antibacterial Activity Against Antibiotic Resistant Pathogens, an *in vitro* Study. *Pakistan Journal of Biological Sciences*. 8: 190-193.
- Limm, D. 1998. Micro biology, 2nd Ed., WCB/Mc Graw Hill, New York.
- Molan, P.C. 1992a. The antibacterial activity of honey. The nature of antibacterial activity. *Bee World*, 73: P. 5-28.
- Molan, P.C. 1992b. The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World* 73: 59-76.
- Molan, P.C. 1998. A brief review of honey as a clinical dressing Primary intention. J. Apic. Res., 6: 148-158.
- Molan, P.C. 2001. Why honey is effective as a medicine. The Scientific explanation of its effects. *Bee World*. 73: 59-76.
- Molan, P.C. and Betts, J. 2000. Using honey dressings. The practical considerations. *Times*. 96: 36-37.
- Molan, P.C. and Russell, K.M. 1988. Non-Peroxide antibacterial activity in some New Zealand Honeys. J *Apic.* Res., 21: 62-67.
- N.H.B. (National Honey Board) 1994. Honey Definitions Document. *American bee Journal*. 117-118.
- Radwan, S., Essway, A. and Sarhan, M.M. 1984. Experimental evidence for the occurrence in honey of specific substances active against Microrganisms. *Zentrall. Mikrobiologie.*, 139: 249-255.