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The Antibacterial property of Bumble bee honey

Renuka Devi K. and M.S.Reddy
Centre for Apiculture Studies, Department of Zoology,
Bangalore University, Jnana Bharathi, Bengaluru-560 056
e-mail : renukaraj2k9@gmail.com, jenureddy@bub.ernet.in

ABSTRACT

Honey is increasingly valued for its antibacterial activity, honey is used on the basis of it being an antibacterial substance but knowledge regarding the mechanism of action is still incomplete. We assessed the bactericidal activity and mechanism of action, the antibacterial effects and minimum inhibitory concentration (MIC) of Bumble bee honey was evaluated. Honey have highly distinct compositions of bactericidal factors, resulting in large differences in bactericidal activity. Honey acts as an antibacterial agent against many bacteria .Four bacterial species viz., Escherichia coli, Bacillus subtilis, Pseudomonas fluoresces and Staphylococcus epidermidis which we isolated from pure cultures were used in this study. There are two sorts of antibacterial agents or so called "inhibines." One of them is heat- and light-sensitive and has its origin in the HP_2 produced by honey glucose oxidase. Some workers believe that hydrogen peroxide is the main antibacterial agent. Other authors find that the non-peroxide activity is the more important one. The H_2O_2 amount in honey is very small and it can be produced only after aerobic incubation of diluted honey solutions, which might mean that it is not very important for the antibacterial action of honey. However, a certain antibacterial test might be sensitive only to certain types of antibacterial substances. It was found that while in an agar disc diffusion test only the peroxide activity was measured, in a liquid medium test only the non-peroxide substances were active. The healing property of honey is due to the fact that it offers antibacterial activity.

Keywords: Bumble bee honey, antibacterial activity, minimum inhibitory concentration, agar disc diffusion

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INTRODUCTION

Honey is a rich source of carbohydrates mainly Fructose and Glucose. The chemical composition of honey varies depending on the plant source, season and production methods. Therefore the colour, concentration and compounds vary depending on the floral sources. Other compounds which can be found in Honey include Proteins and acids such as Gluconic Acid ($C_6H_{11}O_7$, also known as 2, 3, 4, 5, 6-pentahydroxyhexanoic Acid), Minerals and Anti-Oxidants such as Hydrogen Peroxide (H_2O_2) and Vitamins (B_6 and B_{12}), it is recognized that most types of honey have antibacterial activity and that this activity is dependent on physical and chemical factors [1-18]. The viscosity of honey is sufficiently high to create a physical barrel that inhibits the contamination of the wound by infectious agents present in the air. Due to its high sugar concentration, honey eliminates most bacteria by osmosis. The antibacterial activity can also be partially attributed to the acidity of honey, the presence of phytochemical components such as flavonoids and phthalic acids and, most importantly, the action of oxygen peroxide, produced in honey due to the presence of the glucose oxidase enzyme secreted by the hypopharyngeal glands of honeybees .Osmosis and hydrogen peroxide have long been considered as the main factors responsible for the antibacterial activity of honeys. However, the verification of non-peroxide antibacterial activity in honey diluted to low concentrations has brought attention to the presence of other antibacterial agents Among the chemical components in honey which could be responsible for the antibacterial activity, flavonoids and phenolic acids are the most studied. One reason for such interest is that these molecules present in numerous types of biological activity, including antibacterial properties. Several researchers have verified the antibacterial activity of flavonoids isolated from honey and prominent results have been reported honey. The use of honey as a traditional remedy for microbial infections dates back to ancient times Honey has broad spectrum activity against pathogenic and food-spoiling bacteria. The disc diffusion method is mainly a qualitative test for detecting the susceptibility of bacteria to antimicrobial substances;

however, the minimum inhibitory concentration (MIC) reflects the quantity needed for bacterial inhibition. In this study antibacterial activity of Bumble bee honey which is collected from the Apiarist, Daddaballapur was evaluated [19-22]. Honey has been shown to have antibacterial properties, in particular Bumble bee honey, had extensive research has been done on it, has been shown in many studies that Bumble bee Honey has antibacterial effects .

MATERIAL AND METHODS

Antibacterial activity

The experiment method employed for this investigation was the Disc Diffusion Assay method, it was chosen because it was the easiest and the simplest method to use.

Chemicals

All the reagents and chemicals used in this study were of analytical grade.

Bacteria

Four different species of bacteria will be used in this study to explore the effectiveness of Bumble bee honey on the inhibition of growth; the bacteria chosen for this study are both Gram-Positive and Gram-Negative Bacteria, Aerobic and all four bacteria Genera have significance with interaction with humans (Homosapiens). The four bacterial species, which would beused in this study are: *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus,*

Media

In this investigation Nutrient agar and Nutrient Broth were used to culture four different bacterias pieces. The nutrient agar was used to isolate colonies and to observe the zone of inhibition around sterile absorbent discs. The nutrient broth was used in making liquid cultures from isolated colonies from the agar plates. The liquid cultures were then used in the disc diffusion assay, the maximum recovery diluents was used to dilute the honeys to make up the serial dilutions.

Culture Preparation

Four Universal bottles containing 9 ml each of nutrient broth were inoculated separately with *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* using an inoculum loop. The nutrient broth solutions which were inoculated with *Escherichia coli, Bacillus subtillis and Staphylococcus aureus*were then incubated at 37˚C for up to 48 hours. The nutrient broth solution which was inoculated with *Pseudomonas aeruginosa* was incubated at 25˚C for up to 48 hours. All the organisms used in the investigation were of level 1 classification, the inoculated culture plates were incubated at the temperatures which are stated in table 1, for up to 48 hours.

Table 1: Incubation Temperatures

S.No	Organism	Incubation Temperature (°C)
1	*Escherichia coli*	37
2	*Bacillus subtilis*	37
3	*Staphylococcus aureus*	37
4	*Pseudomonas aeruginosa*	25

These bacteria cultures were then stored at 4˚C

Disc Diffusion Assay

Five sets of four Nutrient Agar plates were set out; each agar plate in every set was inoculated separately with the bacteria *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylo coccus aureus*, by pipetting 100 Cl of each bacterium directly onto the agar surface of each plate of every set.

Using the spread plate technique, the bacteria samples were then spread across the surface using a glass spreader. The plates were left to dry for 15 minutes, whilst sterile absorbent discs were placed into each honey flask. The absorbent discs were left in the honey for 10 minutes to absorb the honey. An absorbent disc from honey was placed on every agar plate in each set.

The plates which were inoculated with *Escherichia coli*, *Bacillus subtili*s and *Staphylococcus aureus* with then incubated at 37˚C for up to 48 hours. The plates which were inoculated with *Pseudomonas aeruginosa* was then incubated at 25˚C for up to 48 hours.

Detection of Antibacterial Activity

After the plates had been incubated the inhibition of the bacteria was determined by the visual confirmation of a zone of inhibition. A zone of inhibition is a clear area surrounding the absorbent disc.

Detection of the Minimum Inhibitory Concentration

Honey Preparation : Honey samples were diluted in distilled water and were diluted using maximum recovery diluents (MRD), in which six dilutions were prepared. The concentration of each dilution was measured using weight in grams of honey against the volume in cm3 of MRD, grams/volume (g/vol.). Using universal bottles, the honey concentrations were prepared using the following measurements of honey and MRD as seen in table 2.

Table 2: Honey Dilutions

Percentage (%) Concentration	Weight in grams, of honey	Volume in cm3 of MRD
0	0	10
10	1	9
20	2	8
30	3	7
40	4	6
50	5	5

Each honey dilution was kept at room temperature out of direct sunlight

Disc Diffusion Assay

In this method, for each honey, four sets of six nutrient agar plates were set out, each set was then inoculated with one species of bacteria. In each set of nutrient agar plates, each agar plate was inoculated with bacteria by pipetting 100Cl of nutrient broth bacterial culture, directly onto the agar surface. Using the spread plate technique, the bacteria samples were then spread across the surface of the agar using a glass spreader.

The plates were left to dry for 15 minutes, whilst sterile absorbent discs were placed into each honey concentration of the two honeys. The absorbent discs were left in the honey dilutions for 10 minutes to absorb the honey. An absorbent disc from each honey dilution series was placed directly onto the surface of every agar plate in each set; this was done for each honey.

The plates which were inoculated with *Escherichia coli, Bacillus subtilis and Staphylococcus aureus* were then incubated at 37˚C for up to 48hours. The plates which were inoculated with *Pseudomonas aeruginosa* was then incubated at 25˚Cfor up to 48 hours.

The 0 percent honey dilution for each honey, which contained only MRD as stated in table 1 is a negative control for inhibition. The amount of inhibition was recorded by measuring the diameter of the zone of inhibition, in millimeters (mm), this was measured using a ruler. The measurement included the diameter the diameter of the absorbant disc.

Table 3: Bacterial Inhibition at 100% concentration of honey

S.No	Organism	Bumble bee Honey
1	*Bacillus subtilis*	+
2	*Staphylococcus aureus*	+
3	*Pseudomonas aeruginosa*	_
4	*Escherichia coli*	_

'+' Positive Inhibition; '-'Negative Inhibition

The results in table 3 show that at 100% concentration of honey have antibacterial activity which inhibits all four bacteria species; this shows that this honey acts on both gram positive and gram negative bacteria.

Identification of Minimum Inhibitory Concentration

Table 4: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Escherichia coli*

Concentration of Honey	Bumble bee Honey
0%	0±0
10%	0±0
20%	7.32±0.0786
30%	6±0.8815
40%	7.65±0.576
50%	8.65±0.175

According to table 4, these results show that with concentrations of honey up to 50%, the Bumble bee honey have antibacterial activity against the gram negative bacteria *Escherichia coli* with a minimum inhibitory concentration of 10%. And showed the largest zones of Inhibitionat the concentration of 50%.

Table 5: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Bacillus Subtilis*

Concentration of Honey	Bumble bee Honey
0%	0±0
10%	21±0.086
20%	25±0.041
30%	28.65±0.07
40%	30.32±0.102
50%	31.32±0.093

These results (Table 5) show that with concentrations of honey up to 50%, Bumble bee honey have antibacterial activity against the gram positive bacteria *Bacillus Subtilis* with a minimum inhibitory concentration of 10% and had the largest zones of inhibition out of all the honeys at 50%.

Table 6: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Staphylococcus aureus*

Concentration of Honey	Bumble bee Honey
0%	0±0
10%	20.3±0.015
20%	21.3±0.037
30%	26±0.134
40%	10.66±0.0514
50%	11±0.0175

According to table 6 above, these results show that the Bumble bee honey had antibacterial activity against the gram positive bacteria *Staphylococcus aureus* had the largest zones of inhibition at 30%and had a minimum inhibitory concentration of 10%.

Table 7: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Pseudomonas aeruginosa*

Concentration of Honey	Bumble bee Honey
0%	0±0
10%	0±0
20%	9±0.111
30%	8.6±0.066
40%	8±0.125
50%	7.66±0.0619

According to table 7, both the honeys have shown antibacterial activity against the gram negative bacterium *Pseudomonas fluorescens*. The minimum inhibitory concentration was found to be 10% and the largest zones of inhibition at 20%.

DISCUSSION

The results obtained shows that as expected the honeys, exhibited a level of antibacterial activity which generally increased with increasing concentration. The degree of antibacterial activity varied according to the type of bacteria and type of honey. The minimum inhibitory concentration (MIC) has been observed to lie between 10% and 20% for both the honeys against all the bacteria used in this investigation. These results are in agreement with Ali ATM *et. al;* [5] who found that the honey concentration 20 % was sufficient to inhibit the growth of a range of isolates. The expected range of the minimum inhibitory concentration was between 10-50 %as shown by the research done by Barret J *et. al;* [2] who observed an MIC of 5-10 % and by Al-Waili NS, who observed an MIC of 30-50 %.

CONCLUSION

It is clear from this study that different honeys act differently on the same microorganism. This is to be expected since the composition of each honey is different. The composition would be different for each honey according to the different floral sources and the species of bee.

REFERENCES

1. Yates BD and Yates JD; (1996). Beekeeping study notes for the BKA examination: Modules 1,2,3 and 4. vol. 1, Bee Books New and Old.
2. Barret J, Bernnan J, Moran N and Patton T; (2006). Use of a spectrometric bioassay for determination of microbial sensitivity to Manuka honey, Journal of Microbiological Methods, 64(1):84-95.
3. Allen KL, Molan PC, Reid GM; (1991). A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol, 43: 817-822.
4. Mundo MA, Padilla-Zakour OI and Worobo RW; (2004). Growth inhibition of food borne pathogens and food spoilage organisms by selected raw honeys. International Food Journal, 98: 1-8.
5. Ali ATM, Chowdhury MNH, Humayyd MSA; (1991). Inhibitory effect of natural honey on Helicobacter pylori. Tropical Gastroenterology, 1991; 12(3): 139- 143.
6. Al-Waili NS. (2004). Topical honey applications vs. Acyclovirfor the treatment of recurrent Herpes simplex lesions.Diagnosis and Medical Technology, 10(8):45-49
7. Kwakman, P. Zaat, S. (2012). Antibacterial components of honey. In IUBMB Life, vol.64, no. 1, p. 48-55.
8. Lin, S. Molan, P. Cursons, R. (2011). The controlled in vitro susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey. In European journal of clinical microbiology & infectious diseases, vol. 30, 2011, no. 4, p. 569-574.
9. Lusby P.E. and Coombes, A.L. (2005). Wilkinson JM: Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res., Int J Food Microbiol. 36:464-467.
10. Maddocks, S. Lopez, M. Rowlands, R. Cooper, R. (2012). Manuka honey inhibits the development of Streptococcus pyogenes biofilms and causes reduced expression of two fibronectin binding proteins. In Microbiology, vol. 158, 2011, no. 3, p. 781-790.
11. Molan, P., (2002). Not all honeys are the same for wound healing. Bull. Eur. Tissue Rep.Soc., 9: 5-6.
12. Mullai, V. and T. Menon, (2007). T Bactericidal Activity of Different Types of Honey against Clinical and Environmental Isolates of *Pseudomonas aeruginosa*. J. Altern.Complement. Med., 13: 439-442.
13. Mundo, M. A., Padilla-Zakour, O. I. and Worobo, R. W. 2004. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. , 97:1-8.
14. Snow, M. and M. Manley-Harris, (2004). On the nature of non-peroxide antibacterial activity in New Zealand Manuka honey. Food Chem., 84: 145-147.
15. Voidarou, C. Alexopoulos, A. Plessas, S. Karapanou, A. Mantzourani, I. Stavropoulou, E. Fotou, K. Tzora, A. Skoufos, I. Bezirtzoglou, E. (2011). Antibacterial activity of different honeys against pathogenic Bacteria. In Anaerobe, vol. 17, 2011, no.6, p. 375-379.
16. Wahdan, H. (1998). Causes of the antimicrobial activity of honey. In Infection, vol. 26,1998, no. 1, p.26-31.
17. Abd-Elaal, A. M., El-Hadidy, M.R., El-Mashad, N.B., and El-Sabaie, A.H. (2007). Antimicrobial effect of bee honey in comparism to antibiotics on organisms isolated from infected burns. Annals of Burns and Fire Disaster, 20(2): 91-94.
18. Al-Somail, N., Coley, K.E., Molan, P.C., and Hankock, B.M. (1994). Susceptibility of Helicobacter pylori to the antibacterial activity of Manuka honey. J.Royal Society Med.,87: 9-12 .
19. Dustman, J. H. (1979). Antibacterial effect of honey. Apiacta, 14: 7-11.
20. Harrigan, W. F., and McCance, M. E. 1990. Laboratory Methods in Food and Dairy Microbiol.,8th edition, Academic Press Inc., London. pp. 7-23, 286-303.
21. Molan, P. C. (1992). The antibacterial activities of honey. Bee World, 73: 5-22.
22. Molan, P. C. (1998). Honey as a dressing for wounds, burns and ulcers: A Brief Review of Clinical Reports and Experimental Studies. Primary Infection, 6(4): 148-158.

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