International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Volume 3, Issue 8 - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-8-23

Antimicrobial activity of various types of honey against throat infection

T.Selvamohan[1*], N.K.Parameswaran[1], Helan Subitha[2]

[1]Department of Zoology, Rani Anna Government College for Women, Gandhi nagar, Tirunelveli-8
[2]Department of Microbiology, Sivanthi Aadithanar College, Pillayarpuram, Nagercoil.
*Corresponding author: *tsmohan71@gmail.com*

Abstract

In traditional system of medicine honey is used forthe treatment and prevention of various illness of human beings. Antimicrobial activity of the different honey samples produced by honey bees as *Apis dorsata* (Rock bee) *Apis florae* (little bee) *Apis indica* (Indian bee) were assayed using standard technique. The standard techniques of well method, well diffusion method, disc diffusion method, minimal inhibitory concentration (MIC) and minimal bacterial concentration (MBC). All honey samples were tested at different concentration (15%, 25%, 50%, and 100%) against throat infection. The throat infection organism are *Staphylococcus aureus, Pseudomonas aueroginosa* and *Klebsiella pneumoniae.* Honey is one of the important medicinal product. Honey play a significant role in Ayurvedic and Unani systems of medicine. Some uses of honey are blood purifier, preventive against cough, cold and fever, malnutrition, heart attack, impaired digestion, ulcers and sore throat. Because it has been attributed to its high osmotic effect high acidic nature, hydrogen peroxide concentration and its phytochemical nature. Honey bees has both bacteriostatic and bactericidal activity. In this present study deals with the microbial activity against the throat infectious agents by using the numerous sorts of honey.

Keywords: Honey, Hydrogen peroxide, Antimicrobial activity, Throat Pathogens.

Introduction

Honey is the most primitive nourishing and healing agent. It is a thick, syrupy, translucent, pale yellow or yellowish brown liquid deposited in the honey comb. The use of honey for the treatment of many human disease can be found in general magazines, bee keeping journals and natural products leaflet, an suggesting a wide variety of unfounded properties. Honey shows positive results for the control of pathogens or the improvement of human health [1].Honey is acceptable in the medical profession as an antibacterial agent for the treatment of some disease and infection. The low pH of honey is inhibitory of many human pathogens. .Honey gains its broad antimicrobial activity from hydrogen peroxide generated by a glucose oxidase deposited into the honey by bees and by the high osmolarity due to the sugar content. Honey is a complex mixture of potentially beneficial natural products such as flavonoids and other phytochemicals. Honey has been demonstrated to be effective against several human pathogens, including *Staphylococcus aureus Pseudomonas aueroginosa, Klebsiella pneumoniae.* Clinical trials involving patients with superficial burns demonstrated the anti-inflammatory effect of honey in human [2].

Honey is more effective than a chemical anti-cough syrup. Many health promoting and curative properties attributed to it are the basis for some traditional folk medicine treatment throughout the world today.

Honey is a healthful product recognizing it as a pure natural products [3].Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including catalase, ascorbic acid, flavonoids and alkaloids. Different honey have different flavonoid profile, depending on the floral source for the nectar. Nectar is a thin, easily spoiled sweet liquid that is changed by honey bee to a stable, high density, high energy food. In general higher antioxidant content was found in darker honey and in honey with higher water content. Honey contains a number of enzyme including glucose, oxidase, invertase, diastase (amylase) catalase and acid phosphatase. Honey contains many other organic acids – butyric acid, acetic acid, formic acid, lactic acid, succinic acid, malic acid, citric acid, maleic acid, oxalic acid and pyro glutamic .(Honey Health and Therapeutic Qualities).Apitheraphy or therapy with bee product like honey is an age - old tradition. Honey used for baldness, contraception, wound healing, laxative action, cough and sore throat, eye disease, topical antisepsis, prevention and treatment of scars[4].Honey seeds have a great medicinal importance and have been reported to exhibit many pharmacological effects that include anti-parasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities. Its oil is used as condiment, carminative, food preservative, analgesic and to treat many ailment [5].

Honey has been used in treating different diseases as for as 2000 year ago. Honey is used as a medicine [6]. Sugar solution and pastes have a high osmolarity but theyare therapeutically inefficient. The low pH of honey, ranging from 3.5 to 4.5[7].Honey has been used as medicine in many cultures for long time. The enzyme content of fresh honey is one of the characteristics that make it beneficial to human health, but processing, heating and prolonged storage can lower enzyme activity [8].Antibiotic resistant bacteria possess a very serious threat to public health. There is a large variation in the antimicrobial activity of honey collected form natural environments, which is a concern from the view of clinical application. Antibacterial activity is present in all higher organisms and some foods to protect against their natural flora of bacteria. Antimicrobial agents includes those derived from animals, plants, and microorganisms. The effectiveness of antimicrobial agents depends on some factors like the effect of pH or acid dissociation and the specific effect of the antimicrobial agent. Antimicrobial activity, including water activity, moisture content, temperature, osmotic pressure and composition of the food, as well as the presence of essential nutrients for growth. Honey is a super saturated solution of sugars and present low pH and low water activity. It produced from many sources and its antimicrobial activity varies greatly with origin and processing. Essential oils from different plants have been recently assayed for activity against strains of bacteria, fungi, virus and cytotoxic activity [9].

Honey is a complex mixture and present very great variations in composition and characteristics due to its geographical and botanical origin. The composition and quality of honey also depend on several environmental factors during production such as weather and humidity inside the hive, nectar conditions and treatment of honey during extraction and storage. Honey has numerous uses and functional application worldwide such as food system, religious and magical ceremonies as well as in human and veterinary medicine. Antibacterial action of honey is well established, the mechanism of its action is still a matter of debate. There is a common belief that the antibacterial activity of honey lies partially in its high osmolarity due to its high sugar content and in its acidity due mostly to the presence of gluconic acid. A number of organic components with antibacterial activity have been identified in the ether extract of honey these include 3, 5dimethoxy -4- hydroxy benzoic acid and methyl 3, 5dimethoxy -4- hydroxyl benzoate by using high performance liquid chromatography (HPLC) some other flavonoids and phenolic acids have also been identified in different honey [10].Honey is an important and unique food product containing bioactive compounds derived from bees and plants. Honey possess antimicrobial activity it destroy or inhibits the growth of some pathogenic vegetative microorganisms. The unique composition of honey contributes to its antimicrobial properties. The enzymatic production of hydrogen peroxide is considered to be a major factor antibacterial honey effects. The amount of this bactericidal compound in honey depends on the amount of catalase, with different types of plants contributing to different concentration of catalase honey with high hydrogen peroxide activity in sensitive to heat and light because the main enzymes which generates hydrogen peroxide is inactivated [11].

Honey stipulates a pure product that does not allow for the addition of any other substance. This includes, but is not limited to water or other sweetness. The remaining 14% contains small amounts of disaccharides, trisaccharides, oligosaccharides, minerals, vitamins and enzymes. The acid pH of honey 3.9 and high sugar concentration. The medical importance of honey is being used as therapeutic agent

and Ayurvedic medicine in India.Nutrient with known antioxidant properties such as tocopherols, alkaloids, ascorbic acid, flavonoids, phenolics and various enzymes in small amount of honey. Nectar contains large quantities of flavonoids plant pigments and flavoring compounds with antioxidant properties [12].

Materials and Methods

Fifteen throat samples were collected from private hospital Nagercoil. Kanyakumari District. The sample were collected in a pre-sterilized peptone water and aseptically transported to the laboratory in an ice cold condition and processed within 4 hours for further analysis.

Isolation and Identification of Pathogen from Throat Samples

For isolation of throat infection causing organisms a loopful of throat samples was streaked on Macconkey agar plate, Mannitol salt agar plate and, Cetrimide agar plates and incubated at 37° c for 24hrs. After incubation the colonies were selected and characterized on the basis of morphological, cultural and biochemical characteristics and were identified with the help of Bergey's Manual of Systematic Bacteriology. The identified organisms were maintained on nutrient agar slants at 4°c and subculture were made for every 30 days.

Collection of Honey

To perform this experimental studies three honey were collected from the honeybroad Marthandam, Nagercoil.

Antimicrobial Activity of Various Types of Honey Using Agar Well Diffusion Method

The 20ml of sterilized Muller Hinton Agar was poured into sterile petri plates, after it get solidified 100µl of fresh culture of pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher and various concentration (20, 40, 60, 80,100 µl) of each honey samples were added to the wells. The plates were incubated for 24hours at 37°c. After incubation, the diameter of inhibition zones formed around each wells were measured and expressed in millimeter (mm).

Preparation of Sterile Disc

Whattman's No: 3 filter paper were punched into 5mm disc form. The discs were sterilized and each sterile disc were incorporated individually with various concentration (20, 40, 60, 80,100µl) of honey samples using micropipettes. Then the discs were allowed for drying, after sometimes another dose of honey samples were applied. Then the preparation disc were stored at 4°c

Antimicrobial Activity of Various Types of Honey Using Agar Disc Diffusion Method

The 20ml of sterilized Muller Hinton Agar was poured into sterile petri plates after it get solidified 100ml of fresh culture of pathogens were swabbed on the respective plates. The discs were kept over the agar plates using sterile forceps at various concentration (20, 40, 60, 80,100 µl). The plates were incubated for 24hours at 37°c. After incubation, the diameter of inhibition zones formed around each discs were measured and expressed in millimeter (mm).

Determination of Minimal Inhibitory Concentration (MIC)

To determine the minimal inhibitory concentration (MIC), each strains were grown in nutrient broth for 24hours, then 100µl of each culture was in inoculated in test tubes with nutrient broth supplemented with different concentration (20,40,60,80,100 µl) of honey samples respectively. Afterwards the tubes were incubated for 24hours at 37°c. The minimal inhibitory concentration of each sample was determined by measuring the optical density in the spectrophotometer (620nm).

Antibiotic Susceptibility Testing

The antimicrobial activity of various types of honey were compared with the commercially available antibiotics. For this study sterile Muller Hinton agar plates were prepared and the test organisms (100µl) were swabbed over the surface of agar plates using sterile cotton swab. Then the antibiotic disc such as Amikacin, Chloramphenicol and Penicillin were placed on the surface of the plates. The plates were incubated at 37°c for 24 hours. After incubation the diameter of inhibition zones were measured in millimeter (mm).

Results

The antimicrobial activity of various types of honey (*Apis dorsata*, *Apis florae* and *Apis indica*) were investigated against throat infection using agar well diffusion method, agar disc diffusion method, determination of minimal inhibitory concentration (MIC) and antibiotic susceptibility testing against throat infection causing pathogens. All the examined honey showed varying degrees of antimicrobial activity against pathogens.

The antimicrobial activity of various types of honey against throat infection causing agar well diffusion method The anti-microbial activity of agar well diffusion method the *Apis florae* (Little bee) honey showed the maximum zone of inhibition against *Klebsilla* sp (22mm) and the minimum zone of inhibition against (6mm) was observed against *Pseudomonas* sp. The antimicrobial activity of various types of honey against throat infection causing pathogens by agar disc diffusion method. The antimicrobial activity of agar disc diffusion method the *Apis florae* (Little bee) & *Apis dorsata* (Rock bee)showed the zone of inhibition (18mm) against *Pseudomonas* sp and *Klebsiella* and the minimum zone of inhibition against (6mm) against *Staphylococcus* sp.

The antimicrobial activity of various types of honey against throat infection causing pathogens by minimal inhibitory concentration (MIC). In minimal inhibitory concentration the maximum microbial growth was observed in *Apis florae* (Little bee) honey (OD620:0.22) at 20µl concentration against *Klebsiella* sp. The minimum microbial growth was observed in *Apis indica* (Indian bee) and *Apis florae* (Little bee) honey (OD620:0.5) at 100µl concentration against *Pseudomonas* sp.

The antimicrobial activity of various types of honey against throat infection causing pathogens by commercially available antibiotics was tested against the throat infection causing pathogens. The maximum inhibitory zone was observed in chloramphenicol (18mm) against *Staphylococcus* sp and the minimum inhibitory zone was found in Amikacin (9mm) against *Pseudomonas* sp.

Table 1. Antimicrobial activity of various types of honey against Agar well diffusion method

	Agar well diffusion method														
Concentration (µl)	**20**				**40**		**60**			**80**			**100**		
Organisms	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***
Apis dorsata (Rock bee)	10	9	14	11	11	15	13	12	16	16	14	18	20	14	20
Apis florae (Little bee)	9	6	7	11	8	9	12	12	16	13	13	19	20	14	22
Apisindica (Indian bee)	7	8	9	8	10	11	10	9	16	12	11	17	13	13	18

Table 2. Antimicrobial activity of various types of honey against Agar disc diffusion method

	Agar disc diffusion method														
Concentration (µl)	**20**				**40**		**60**			**80**			**100**		
Organisms	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***
Apis dorsata (Rock bee)	8	8	9	10	9	10	12	11	12	14	14	15	16	17	18
Apis florae (Little bee)	6	11	7	12	13	11	13	14	12	14	15	14	16	18	17
Apis indica (Indian bee)	10	8	7	11	10	8	13	11	10	14	12	13	17	13	16

Table 3. Antimicrobial activity of various types of honey against minimal inhibitory concentration method

	Minimal inhibitory concentration method														
Concentration (µl)	**20**				**40**		**60**			**80**			**100**		
Organisms	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***	***Stap***	***Pseu***	***Kleb***
Apis dorsata (Rock bee)	0.19	0.19	0.19	0.19	0.15	0.14	0.18	0.15	0.13	0.14	0.13	0.12	0.07	0.08	0.10
Apis florae (Little bee)	0.20	0.19	0.22	0.19	0.18	0.14	0.15	0.14	0.13	0.13	0.13	0.10	0.10	0.05	0.06
Apis indica (Indian bee)	0.18	0.17	0.15	0.14	0.15	0.13	0.09	0.13	0.12	0.09	0.10	0.10	0.08	0.05	0.07

Honey samples: *Apis dorsata* (Rock bee); *Apis florae* (Little bee); *Apis indica* (Indian bee).

Table 4. Antimicrobial activity of various types of honey against commercially available antibiotics

Commercially available antibiotics			
Antibiotics / Organisms	**Amikacin**	**Chloramphenicol**	**Penicillin**
Staphylococcus	16	18	-
Pseudomonas	9	16	-
Klebsiella	11	15	-

Discussion

Now a days the development of resistance by a pathogen to many of the commonly used antibiotics provides impetus for further attempts to search for new antimicrobial agents. In the present investigation revealed that the honey can be effective antibiotics. In this present study, preliminary screening for antimicrobial activity showed that the *Apis florae* (Little bee) exhibited the maximum inhibitory zone against *Klebsiella* sp while the *Apis indica* (Indian bee) showed least inhibitory activity against *Pseudomonas* sp. Malika *et al*., (2004) described dilutions of honey ranging from 1/2, 1/4, 1/8, 1/16 were tested by the agar well diffusion method on various strains of bacteria include, Staphylococcus sp and Pseudomonas sp. The most of strains were inhibited by dilution ½ and ¼. The antimicrobial activities of honey were assayed using well diffusion method. Honey samples were tested at four concentration 5%, 25%, 50% and 100% against *Staphylococcus aureus, Pseudomonas aueroginosa* and *Klebsiela pneumoniae*.The inhibitory effect of both honey samples was higher on *Escherichia coli* and followed by *Staphylococcus aureus.* In this present study, the antimicrobial activity of *Apis florae* (Little bee) and *Apis dorsata* (Rock bee) exhibited the maximum zone of inhibition against *Pseudomonas* sp and *Klebsiella* sp while the *Apis indica* (Indian bee) showed least inhibitory activity against *Klebsiella* sp. The disc diffusion assay demonstrated that Methicillin resistant *Staphylococcus aureus* (MRSA) strains were completely inhibited at 4mg. However a concentration of 0.5mg per disc failed to inhibit any of these strains.

The quality of the extract required to inhibit bacteria was low in agar dilution assay as compare to the disc diffusion technique. A wide range of bacteria showed positive result in disc diffusion test. The ranges for honey of 40, 60, and 80% was considered as 0.2, 0.6, 7 and 8mm respectively. In the present study the antimicrobial activity of *Apis dorsata* (Rock bee) exhibited the maximum microbial growth against *Klebisella* sp at 20µl concentration while *Apis florae* (Little bee), and *Apis indica* (Indian bee) showed least microbial growth against *Pseudomonas sp* at 100µl concentration. Motior Rahman et al., (2010) suggested the minimal inhibitory concentration is very effective against *Staphylococcus aureus* at 2.74mgml^{-1} minimal inhibitory concentration values ranged from 0.080 to 0.100mgml^{-1} in *Staphylococcus aureus.* The minimal inhibitory concentration of honey was effective on *Staphylococcus aureus* 4% and *Pseudomonas aueroginosa* minimal inhibitory concentration of the honey was effective at 5%. Andargarchew The MIC

of honey for 90% 0f test organisms was 6.25% and 7.5% for *Pseudomonas auerоginosa*. In the present study antimicrobial activity of commercially available antibiotics against isolated pathogens shows less effective when compared to honey, it showed moderately week results were obtained. Penicillin did not show any activity against *Staphylococcus* sp *Pseudomonas* sp and *Klebsiella* sp. Malu *et al*.,(2003) concluded that honey samples shows antimicrobial activities on *Pseudomonas aeruginosa, Pseudomonas mirabilis* and other bacteria ,which were resistant to some common antibiotics discs such as penicillin, ampicillin, chloramphenicol, cotrimoxal and gentamycin. Comparing these results we can found out that honey is much better than commercially available antibiotics.

Conclusion

Honey has been used as a medicine since ancient times in many cultures and used in folk medicine. Use of honey as a therapeutic substance by medical profession. Little is known about the phenomenon involved in the inhibition of microorganisms. Honey has been found to be effective against microorganisms isolated from the urinary tract infection and treatment of infantile gastro-enteritis. Non dissociated organic acids play an important role in the antimicrobial activity of honey since they are highly soluble in cell membranes. Lysozyme and volatile compounds identified in honey might play a role in the inhibition of microorganisms. This research was proved the efficiency of the three different honey (*Apis dorsata, Apis indica, Apis florae*) using various procedure against the isolated throat infection pathogens.

Access this Article in Online	
	Website: www.ijarbs.com
	Subject: Antimicrobials
Quick Response Code	

References

1. Jeffrey, A.E, and Echazarreta, C.M.1996. RevBiomed**7** : 43-49
2. Davis, S.Cand Peroz, R. 2009. Clinies in Dermetology**27**:502-506.
3. Stefan Bogdanov. 2010. Bee product science. **8**:1-23.
4. Bansal,V,B. Medhi, Pandhi, P. 2005. Medical Journal. **3(3)**:305-309.
5. Abdul Hannan, Sidrah Saleem, Saadia Chaudhary, Muhammad Barkaat, and Muhammad Usman Arshad. 2008. J. Ayub Med coll Abbottabad .**20(3)**:72-74.
6. Osho,A and Bello, O.O. 2010. Asian J. Exp. Biol. Sci.**1 (4)**:875-880.
7. Vorlova, L., R. Karpiskova, I. Chabiniokova, K. Kalabova and Z. Brazdova.2005J.Anim. Sci.8:376 -384
8. Badawy, O.F.H, S.S.A, Shafii, E.E Tharwat and A.M. Kamal. 2004. Rev Sci Tech off. Int. Epig. **23(3)**:1011-1022.
9. Dastouri,M.R, Kamran Fakhimzadeh, Jalal Shayeg, JalilDolgari– Sharaf, Valilou .M.R and NaserMaheri-Sis. 2008. Journal of Animal & Veterinary Advances**7(4)**:409-412.
10. Aljadi, A. M, and Yusoff, K.M. 2002. Turk J Med Sci. **33:** 229-236.Baltrusaityte, V, Venskutonis, P.R and VioletaCeksteryte. 2007. Food Techol. Biotechnol. **45(2)**:201-208
11. Emi Temaru, Satoshi Shimura, Kuzuhiro Amano and Tadahiro Karasawa. 2007. Polish Journal of Microbiology. **56(4):**281-285
12. Malik,N. Faid Mohamed and E.L Adlouni Chakib. 2004. International journal of Agriculture and Biology. **6(2)**:289-293.
13. Motior Rahman., M, Allan Richardson, and M. SofianAzirun. 2010. African Journal of Microbiology Research.**4 (16)**:1872-1878.
14. Andargarchew Mulu, Belay Tessema, FeteneDerbie. 2003. Ethiop J. Health Dev. **18(2)**: 107-111.

How to cite this article:
T.Selvamohan, N.K.Parameswaran, Helan Subitha. (2016). Antimicrobial activity of various types of honey against throat infection. Int. J. Adv. Res. Biol. Sci. 3(8): 136-141.