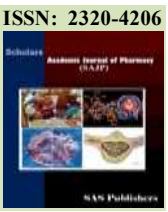
ISSN: 2320-4206
Scholars Academic Journal of Pharmacy (SAJP)
Sch. Acad. J. Pharm., 2013; 2(1):5-11

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com



Research Article

In-vitro comparative antimicrobial activity of commercial and raw honey against various bacteria isolated from ear discharge

E. Venkata Naga Raju[1]*, Dr. Divakar.Goli[2]
[1]Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522 510, India
[2]Department of Biotechnology & Microbiology, Acharya & B.M.Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta, Banglor-560090, India

Corresponding Author's Email: venkatanagarajue@gmail.com

Abstract: In the past decades, honey has been subjected to various laboratory and clinical investigations. The antimicrobial properties of honey have been attributed to both the hydrogen peroxide as well as non-peroxide components. In the present study, three honey samples were collected and four bacterial strains were isolated from ear discharge samples. For the isolation of bacterial strains, the samples were collected from out-patient Department at a Multispecialty hospital in Bangalore, India. The collected ear discharge samples were processed to isolate the bacterial agents by using standardized protocols of isolation and identified by cultural characteristics , Gram staining and biochemical tests. Four strains of pathogenic bacteria identified were *Staphylococcus, Pseudomonas, Streptococcus* and *E.coli*. All the isolated bacteria were tested for their susceptibility against different honey samples. To check the antibacterial activity of honey on above bacteria, three different types of honey were procured: two raw honey samples and one commercial honey sample. Different honey samples showed different antibacterial activity on different bacteria. Our results showed that honey samples were significantly active against all bacteria tested. It was found that raw honey samples showed more antibacterial activity than commercial honey. Karnataka honey showed maximum sensitivity against all the isolated bacteria. Microorganisms have developed resistance to many antibiotics and this has created serious clinical problem in the treatment of infectious diseases. Therefore, current study will help in preparation of novel antibacterial drugs using natural products.
Keywords : Honey, Antibacterial activity, Pathogenic organisms, Ear discharge, Multispecialty hospital.

INTRODUCTION

Honey has functional properties in human health promotion which could be associated to its high osmolarity and antibacterial properties [1]. Honey is a mixture of sugars prepared by honey bees from the natural sugar solutions called nectar obtained from flowers or other plant secretions. By inverting the sucrose in the nectar, the bee increases the attainable density of the final product, and thus, raises the efficiency of the process in terms of caloric density. By the addition of enzymes and the evaporation of water contained in it, honey bees transform it into a sweet liquid [2]. Honey is known as a food, there is growing interest in the medicinal properties of honey and its role in the treatment of many different health problems . Honey has many therapeutic properties. The major antimicrobial properties are correlated to the hydrogen peroxide level which is determined by relative levels of glucose oxidase and catalase [3] whereas the non-peroxide factors that contribute to honey antibacterial and antioxidant activity are lysozyme, phenolic acids and flavonoids [4]. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes gram positive and gram-negative [5].

Apart from antibacterial properties, honey also plays a therapeutic role in wound healing and the treatment of eye and gastric ailments. This is partly due to its antioxidant activity [6] since some of these diseases have been recognized as being a consequence of free radical damage [7]. Besides the presence of hydrogen peroxide, some minerals particularly copper and iron present in honey may lead to the generation of highly reactive hydroxyl radicals as part of the antibacterial system[8]. Therefore, there must be mechanisms involved in honey to control the formation and removal of these reactive oxygen species.

Antibacterial action of honey on the principle of minimum inhibitory concentration (MIC) and its synergism with antibiotics are due to hydrogen peroxide which is produced enzymatically in honey, phenolic compounds and flavonoids .these compounds are the most important groups of compounds occurring in plants, which are found to exhibit anti carcinogenic, anti-inflammatory, antiatherogenic, antithrombotic, immune-modulating and analgesic activities and which may exert these functions as antioxidants [9]. They are also present in honey and have been reported to have some chemoprotective effects in humans [10]. Honey inhibits the growth of pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus,Salmonella, Shigella*, and *Vibrio cholera* and is superior to several well-known antibiotics.

The objective of the present study was to find out the antibacterial potential of honey against various pathogenic bacteria.

MATERIAL AND METHODS:

Sterilization of Materials

Glass wares which include conical flasks, beakers, test tubes, pipettes, McCartney bottles were washed with detergent after which they were rinsed and sterilized in the oven at 160°C for 1 hour. Inoculating loops and forceps were heated to redness in a Bunsen burner. The spatula, scalpel, mortar and pestle were disinfected with 70% alcohol.

Source of Sample:

Bacteria were isolated from ear discharge samples. 4 Ear discharge samples were collected from out-patient Department at a Multispecialty hospital in Bangalore, India.

Table -1:Tabulation for Samples

Samples	No. of Isolates	Sex
1	G1	Female
2	G2	Male
3	G3	Female
4	G4	Male
Total	**04**	

Sample Collection:

A designed sterile swab stick was used to collect specimen from the external auditory canal from the ear with acute otitis media. A sterile aural speculum was used to visualized the canal property while taking swab in case of children and adults with a narrow canal. Swab was taken from the site of any visible discharge in the canal, while collecting specimen from external auditory canal of ear with acute otitis external.

Honey Used:

Three type of honey was used in antimicrobial susceptibility testing. Raw honey used in the study was taken from different areas (Karnataka(Sringeri) and Kerala(Cochin)). Commercial honey used in study was purchased from Dabur India Ltd.

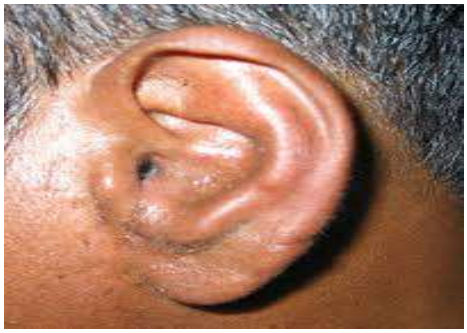


Figure- 1: Ear Discharge

Figure- 2: Karnataka(Sringeri) Honey

Figure- 3: Kerala(Cochin) Honey

Figure- 4: Dabur India Ltd Honey

Characterization of Bacterial Isolates

Wound samples were collected using sterile cotton swabs (fresh pus). The pus specimen was inoculated on Mannitol salt agar, cetrimide agar, eosin methylene blue, Macconkey agar, blood agar plates. The streaked plates were incubated at 37°C for 24 hr. Identification of isolates were done based on cultural characteristics, Gram staining, Catalase, Oxidase, Indole, MR-VP, Citrate, Nitrate reduction, Urease[11].

Table -2:Tabulation for results of colony characteristics

STRAIN NO.	COLONY SURFACE	COLONY COLOUR	VISUAL CHARACTERISTICS	SHAPE OF THE COLONY	HEIGHT OF THE COLONY
G-1	Smooth	Brown	Opaque	Circular	Raised
G-2	Smooth	Brown	Opaque	Circular	Raised
G-3	Smooth	Off white	Translucent	Irregular	Flat
G-4	Smooth	Off white	Translucent	Irregular	Flat

Table -3: Tabulation for results of Staining Techniques.

STRAIN NO.	GRAM STAINING	MORPHOLOGY (BACILUS/COCCI)
G-1	Positive	Cocci
G-2	Positive	Cocci
G-3	Negative	Rods
G-4	Negative	Rods

Table -4: Tabulation for results of Various Biochemical tests

S.No.	SAMPLES	INDOLE	MR	VP	NITRATE	OXIDASE	CATALASE	UREASE	CITRATE
1	**G-1**	**-Ve**	**+Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
2	**G-2**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**-Ve**
3	**G-3**	**+Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**+Ve**	**-Ve**	**-Ve**
4	**G-4**	**-Ve**	**-Ve**	**-Ve**	**+Ve**	**+Ve**	**+Ve**	**-Ve**	**+Ve**

Antibacterial Susceptible Testing:

Agar Well Diffusion Method:

The agar well diffusion [12] technique was employed to determine the antimicrobial activity. The honey samples were first inoculated separately on standard nutrient media with no test organisms so as to evaluate their possible contamination. Viscosity was reduced by heating honey at 30°C for 30 minutes. Thereafter, solidified Muller Hinton agar plates were flooded with the liquid inoculums of the different test organisms separately, using the spread plate method. The plates were drained and allowed to dry for 30 minutes after which four equidistant wells of 6 mm in diameter were punched using a sterile cork borer. Fifty µl of the honey samples were separately placed in the different punched wells and the plates were allowed to stay for 15 minutes for prediffusion to take place followed by incubation for 24-48 hrs at 37°C. The zones of inhibition were measured with the use of a calliper/ruler.

Determination of Minimum Inhibitory Concentration (MIC):

The determination of minimum inhibitory concentration of the three honeys were carried out using the agar dilution method described [13]. Different concentrations of the honeys were prepared to give a final concentration in the range of 1.96 to 19.5mg/ml.2 ml of each dilution was mixed with Muller Hinton agar, poured into petridishes and allowed to set. The agar was spreaded with overnight broth culture of the test organisms and incubated overnight. The lowest concentration inhibiting growth was regarded as the minimum inhibitory concentration of the honey.

Determination of Minimum Bacterial concentration (MBC):

The minimum bacterial concentration of the three honeys were determined by taking 10µl of the culture medium from the broth MIC assay that showed no apparent growth and sub culturing it on a fresh BA. After incubation at 35^{o}C for 24 hrs, the MBC was read as the least concentration showing no growth on the BA plates.

RESULTS AND DISCUSSION:

Ear discharge in particular acute otitis external is relatively serious and unpleasant bacterial infection of the ear. It is one of the most common diseases encountered by an otolaryngologist. It is defined as redness or swelling of the external auditory canal or debris within the canal, accompanied by pain, itchiness discharge (otorrhoea), loss of hearing or pain stuffy feeling for more than three weeks duration found in a population. Ear infections are mainly caused by *Streptococcus, Pseudomonas*, *Staphylococcus* and *E.coli*. Normally, these bacteria are present on our skin as a normal microflora. However, in case of any injury they can act as secondary pathogens and causes infection. Honey was used to treat infected wounds as long ago as 2000 years before bacteria were discovered to be the cause of infection [14]. Honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing. In the present study, 4 ear discharge samples were taken from infected males and females. The commonly bacteria isolated from these samples were *S. aureus , Streptococcus, E.coli* and *Pseudomonas*. All these bacteria commonly inhabit the wounds and *Staphylococcus, Streptococci* are mainly present in ear infection. To check the antibacterial activity of honey on above bacteria, three different types of honey were taken; two raw honey samples (Karnataka and Kerala) and one commercial honey sample (Dabur honey). Different honey samples showed different antibacterial activity on different bacteria. Honey has several well known properties responsible for its antibacterial activity. These include a high concentration of sugars (approximately 80% w/v), a low pH (3.2-4.5 for undiluted honey), and the production of hydrogen peroxide, which upon dilution of honey is produced by glucose oxidase originating from the bees. In addition, unknown floral or bee components contribute to the activity. Large variation in antibacterial activity exists between honeys collected from different environment. Variation is due to the variation in the source of nectar. Even honey collected from a single location can have variation in antibacterial activity [15].

All honey samples showed antibacterial activity on isolated bacteria. Karnataka honey showed maximum sensitivity against all the isolated bacteria. The zone of inhibition showed by Karnataka raw honey was in between 15 mm to 17 mm (Table-5). The least sensitivity was shown by Dabur honey. The zone of inhibition was in between 8 mm to 13 mm (Table -7). The minimum zone of inbition showed by Dabur honey was 8mm. The Kerala raw honey also showed antibacterial activity, the zone of inhibition was in between 14mm to 15 mm (Table -6). Hence, maximum antibacterial activity was shown by Karnataka raw honey then Kerala raw honey and least by commercial honey (Dabur honey). The maximum zone of inhibition showed by Dabur honey was 13mm and minimum zone of inhibition was 8mm. Whereas Rahman *et al.* used disc diffusion method, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and gradient-plate techniques to evaluate the antibacterial activity of honey and propolis against *Staphylococcus aureus* and *Escherichia coli*. The growth of *S. aureus* was inhibited by application of propolis and honey at concentrations of 2.74 to 5.48 mg/ml respectively at both MIC and MBC. The greater inhibition zones (12.0 to 13.0 mm) were observed from honey at concentrations of 2.74 to 5.48 mg/ ml .

Table -5: Antibacterial activity of raw honey collected from Karnataka(Sringeri)

S.No	Name of Bacteria	Well No.	Volume used (µl)	Diameter of Zone(mm)
G1	*Staphylococcus*	1	50	16.5
		2	50	16.5
		3	50	16.5
G2	*Streptococcus*	1	50	15.0
		2	50	15.0
		3	50	15.0
G3	*E.coli*	1	50	17.0
		2	50	17.0
		3	50	17.0
G4	*Pseudomonas*	1	50	15.5
		2	50	15.5
		3	50	15.5

Table -6: Antibacterial activity of raw honey collected from Kerala(Cochin)

S.No	Name of Bacteria	Well No.	Volume used(µl)	Diameter of Zone(mm)
G1	*Staphylococcus*	1	50	14.0
		2	50	14.0
		3	50	14.0
G2	*Streptococcus*	1	50	15.0
		2	50	15.0
		3	50	15.0
G3	*E.coli*	1	50	14.0
		2	50	14.0
		3	50	14.0
G4	*Pseudomonas*	1	50	15.0
		2	50	15.0
		3	50	15.0

Table -7: Antibacterial activity of the commercial honey (Dabur honey)

S.No	Name of Bacteria	Well No.	Volume used(µl)	Diameter of Zone(mm)
G1	*Staphylococcus*	1	50	10.0
		2	50	10.0
		3	50	10.0
G2	*Streptococcus*	1	50	13.0
		2	50	13.0
		3	50	13.0
G3	*E.coli*	1	50	10.0
		2	50	10.0
		3	50	10.0
G4	*Pseudomonas*	1	50	8.0
		2	50	8.0
		3	50	8.0

Figure- 5: Antibacterial activity of raw honey collected from Karnataka(Sringeri)

Figure- 6: Antibacterial activity of raw honey collected from Kerala(Cochin)

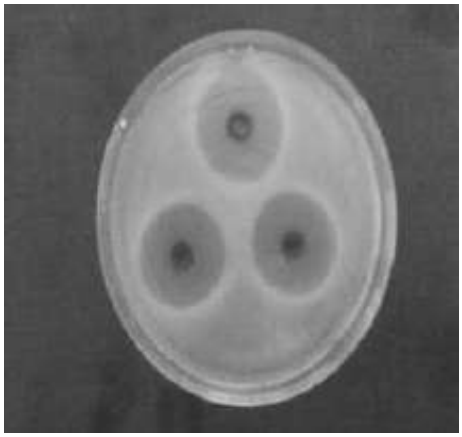


Figure- 7: Antibacterial activity of the commercial honey (Dabur honey)

CONCLUSIONS:

The aim of present study was to check the antibacterial activity of honey samples against the bacteria isolated from ear discharge samples. This study evaluated the antibacterial activity of honey samples collected from the different locations. Samples were collected from Karnataka and Kerala and one commercial honey was "Dabur Honey". The honey samples were tested for antibacterial activity against four bacteria which were isolated from ear discharge samples. Antibacterial activity was determined as an equivalent of the inhibition zones diameters (in millimeters) after incubation of cultures at 37° C for 24 hours. The zone diameters were shown in the table - 5, 6 and 7. All honey samples showed sensitivity against bacteria. Clear zones of inhibition were present around the wells. Both samples of raw honey i.e. Karnataka raw honey and Kerala raw honey showed very satisfactory antibacterial activity against bacteria than the commercial honey sample i.e; Dabur honey. Hence, it is concluded that Karnataka raw honey shows maximum antibacterial activity as compared to other samples of honey i.e; Kerala raw honey and Dabur honey. Commercial honey (Dabur honey) shows minimum antibacterial activity against bacteria isolated from ear discharge samples.

REFERENCES:

1. Effem E;Clinical observations on the wound healing properties of honey, British Journal of Surgery, 1988 ,75:679–681.
2. Aljadi AM and Kamaruddin MY; Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys, Food Chemistry,2004, 85:513–518.
3. Weston RJ and Broncklebank LK; Identification and quantitative levels of antibacterial components of some New Zealand honeys, Food Chemistry, 2000 , 70: 427-428.
4. Arreazroman D and Gomezcaravaca AM; Identification of phenolic compounds in rosemary honey using solid-phase extraction by capillary electrophoresis–electrospray ionization-mass spectrometry, Journal of Pharmaceutical and Biomedical Analysis, 2006,41:1648–1656.
5. Basualdo C,Sgroy V, Finola M S and Marioli JM; Comparison of the antibacterial activity of

honey from different provenance against bacteria usually isolated from skin wounds, Veterinary Microbiology, 2007, 124:375–381.
6. Bogdanov S; Nature and Origin of the Antibacterial Substances in Honey, Lebensmittel-Wissenschaft & Technologie, 1997, 30:748–753.
7. Carson M C; Ion-pair solid-phase extraction, Journal of Chromatography, 2000, 8: 343-350.
8. Chirife J, Herszage L, Joseph A and Kohn ES; In vitro Study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds, Antimicrobial Agents and Chemotherapy, 1983, 23 (5): 766-773.
9. Dibb WL; Microbial aetiology of otitis externa, Journal of Infection, 2003,22(3):233-239.
10. Estevinho L, Pereira AP and MoreiraO L;Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey, Food and Chemical Toxicology, 2008, 46(12):774-779.
11. Sneath HA, Peter MS,Nicolas H and Hold GL; Bergey's manual of systemic bacteriology, (vols. 1 & 2) (Williams and Wilkins Company, Baltmore, MS, USA), 1986.
12. Kwakman PH, Tevelde A and Deboer L;How honey kills bacteria,FASEB Journal 2010,24(7):2576-82.
13. Frankel S, Robinson GE and Berenbaum MR; Antioxidant capacity and correlated characteristics of fourteen unifloral honeys, Journal of Apicultural Research, 1998, 37:27–31.
14. Gheldof N, Wang XH and Engeseth NJ; Identification and quantification of antioxidant components of honeys from various floral sources, journal of Agricultural and Food Chemistry, 2002, 50:5870–5877.
15. Hawke M and Wong J; “Clinical and microbiological features of Otitis Externa”, J. Otolaryngol, 1984,13: 289-295.
16. Henriques A, Jackson S and Cooper R; Free radical production and quenching in honeys with wound healing potential, Journal of Antimicrobial Chemotherapy, 2006, 58(4):773-777.
17. Mccarthy J; The antibacterial effects of honey: Medical fact or fiction, American Bee Journal, 1995 , 135:341-342.
18. Molan PC; The antibacterial properties of honey, Chemistry of New Zealand, 1998,59:10-14.
19. Molan PC, Russel KM; Non-peroxide antibacterial activity in some New Zealand honeys, Journal of Apicultural Research, 1998, 27:62-67.
20. Sackett W G; Honey as a carrier of intestinal diseases, Bulletins of University State Colorado Agriculture and Experimental Station , 1919,252: 1-18.
21. White JW and Subers MH;The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system, Biochemical et Biophysical Acta, 1963, 73: 57-70.
22. Williamson I, Benge S, Mullee M, and Little P; Consultation for middle ear disease, antibiotics prescribing and risk factors for reattendance: a case linked cohort study. Br Journal Gen. Practice, 2006,56(24):170 – 175.