J. Soils and Crops 33 (1) 120-124, June 2023 ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

DETERMINATION OF ANTIBACTERIAL POTENTIAL OF MELGHAT HONEY SAMPLES

Kshitij N. Shah[1]

ABSTRACT

Honey is a natural product used for the treatment of many diseases due to its anti-microbial, anti-oxidantal, and anti-inflammatory potential. Melghat, a tribal region of Maharashtra, is full of a variety of forests and honey is a prime jungle product, basically collected by the tribal people from the hives of wild bees. In the present study, the antibacterial potential of the Melghat honey samples from four different locations was evaluated by using the disc diffusion technique. The samples were collected and examined in 2019. The antibacterial potential of the Melghat honey samples was detected against six species of bacteria, three from each gram-positive (*Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus)* and gram-negative (*Escherichia coli*, *Salmonella typhi,* and *Pseudomonas fluorescens)*. Except for bacteria *E. coli,* all of the honey samples tested positive against all of the bacterial species tested. Surprisingly, no honey sample showed its activity against the bacteria *E. coli*. The results showed that the Melghat honey has good potential against bacteria and therefore must have tremendous therapeutic properties. It is concluded that there is a greater need for research to explore the antimicrobial properties of Melghat honey.

(Key words: Melghat, Honey, Antibacterial, Chikhaldara, Amravati)

INTRODUCTION

Due to the development of resistance of microorganisms to recent drugs, there is a need to use natural materials against microbes. Honey is one of these materials, that has the potential to work against the pathogens in various diseases. The ancient Indian medicinal therapy “Ayurveda” has been using honey as a prime ingredient in medicines against many diseases (Boussaid *et al*., 2018). It was reported very firstly in 1982 that the honey shows antimicrobial properties. To date, there are numerous research papers published showing the antimicrobial capacity of honey. Manuka honey showed its activity against more than 60 species of bacteria. Malaysian honey and Egyptian honey, as well as other varieties of honey throughout the world, including India, have shown their potential against a variety of microbial as well as viral species. Many mechanisms have been proposed to justify the reason for the antibacterial activities demonstrated by honey, though it is not entirely unspoken to this day. Some of the factors which are supposed to be accountable for the antimicrobial potential of honey are; generation of hydrogen peroxide; high concentration of sugars; presence of phenolic compounds; presence of compounds that are proteinaceous over and above some physical characteristics such as low pH of honey, its low water activity due to less moisture content, the osmolarity of honey, the presence of the enzymes, as well as the presence of some other unidentified compounds (Khan *et al*., 2018; Kalidasan *et al*., 2017; Ismail, 2017; Mandal and Mandal, 2011).

As reported by Bhoyar *et al*. (2018), Melghat is a region in Amravati district and spread over Dharni and Chikhaldara tehsils. It is a forest area predominantly inhabited by tribal people. Melghat honey is extensively used by the tribal people as food and medicine and is produced from the different nectaries of an assortment of flowers. It is assumed that the honey from Melghat must possess antimicrobial potential. The potential of honey against some selected bacterial species was determined here in this study by applying the standard methods.

MATERIALS AND METHODS

Honey samples were collected from four different locations in Melghat forest and designated as H_1, H_2, H_3, and H_4. The antibacterial potential of these samples was detected against six species of bacteria, three from each gram-positive (*Staphylococcus aureus, Bacillus subtilis, and Bacillus cereus)* and gram-negative (*Escherichia coli*, *Salmonella typhi,* and *Pseudomonas fluorescens)*. The samples were examined for the detection of antibacterial potential by the disc diffusion method (Anonymous, 2011; Tendencia, 2004). The CLAIRO COMBI, i.e. the combined microbial sensitivity discs, were made by using the sterile and special grade filter paper, were used. The paper possessed fourteen projected arms. The tip of each projection arm was carefully soaked into the stipulated amount of chemotherapeutic sample. The discs are found to be very useful for the evaluation of the *in vitro* potential of the materials, which may show their activities as agents

1. Assoc. Professor, Dept. of Food Science, Arts, Science and Commerce College, Chikhaldara

for some fast-growing bacteria. The antibacterial activities of several difficult species can also be determined by using these discs by diffusing them in agar media.

The method is based on the simple truth that, for any antibiotic substance, the range of the inhibition zone created by the substance is inversely related to the Minimum Inhibitory Concentration (MIC) of the strain, which is estimated by the dilution method provided that the test conditions must be the same. The following procedure was followed for the determination of the antibacterial potential of honey against some bacterial species.

A Mueller-Hinton agar solution, maintained at pH 7.3, was used for the experiment. Petri plates with a depth of approximately 4 millimeters were prepared.

Pure cultures were used for the detection of antibacterial activity in honey samples. Before the preparation of inoculums, the gram staining was carried out. The sterilized wire loop was used for the transfer of colonies. The broth culture was kept in an incubator for five hours at a temperature of 35-37°C. The turbidity was adjusted to the McFarland standard level of 0.5 by adding a solution of $BaCl_2 2H_2O$ and H_2SO_4 as well as sterile broth. The plates were inoculated immediately with the prepared dilute solution. Excess inoculums were removed using a sterile cotton swab.

The combi discs were placed over the surface of the medium in the Petri dish with the help of flame sterile forceps. The disc was pressed by applying a little pressure in order to have complete contact with the medium. The zones of inhibition appeared, showing the extent of inhibition. The diameters of the zones developed at the end of the incubation period were measured.

RESULTS AND DISCUSSION

Discs of size 10 mm were used and the results obtained after 24 hours of incubation at 37°C are given in the following Tables 1 and 2. The zones are obtained as shown in figures 1 to 3.

The zones of inhibition were at their maximum value for the bacterial species *S. typhy* in three honey samples *viz*. H_2, H_3, and H_4 while it is on the second position for sample H_1. It was found from the results that all the honey samples showed their activities against almost all the bacterial species. The Melghat honeys showed their highest activity against the gram negative bacteria *S. typhy*. On the contrary there was no evidence obtained to show the activities of the Melghat honey samples against the gram negative species *Escherichia coli.* Among the gram-positive species, three out of the four honey samples, i.e.. samples H_1, H_2, and H_3, have shown their maximum potential against the species *B. cereus*. While for the remaining honey sample, i.e., sample four H_4, it is in the second position. Thus, the Melghat honey samples have shown their highest potential against *B. cereus* among the gram negative bacterial species selected for the examination.

Table 1. Antibacterial activities against gram-positive bacteria

Sr. No.	honey samples	zone of inhibition		
		Staphylococcus aureus	***Bacillus subtilis***	***Bacillus cereus***
1	**Sample 1 (H_1)**	11 mm	13 mm	16 mm
2	**Sample 2 (H_2)**	14 mm	12 mm	15 mm
3	**Sample 3 (H_3)**	12 mm	12 mm	14 mm
4	**Sample 4 (H_4)**	15 mm	12 mm	12 mm

Table 2. Antibacterial activities against gram-negative bacteria

Sr. No.	honey samples	zone of inhibition		
		Escherichia coli	***Salmonella typhi***	***Pseudomonas fluorescence***
1	**Sample 1 (H_1)**	—	14 mm	12 mm
2	**Sample 2 (H_2)**	—	16 mm	12 mm
3	**Sample 3 (H_3)**	—	17 mm	14 mm
4	**Sample 4 (H_4)**	—	16 mm	13 mm

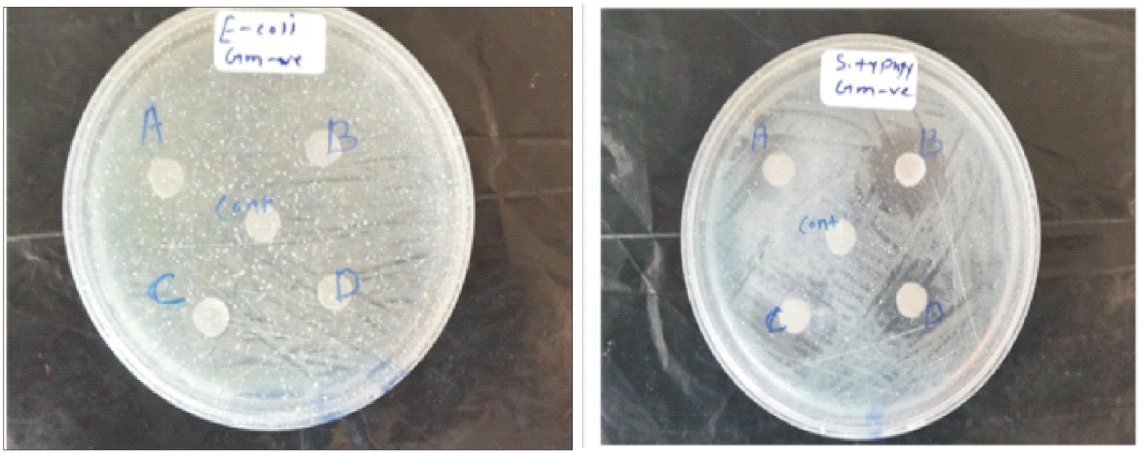

Figure 1. Zones of inhibition against *E. coli* and *S. typhi*

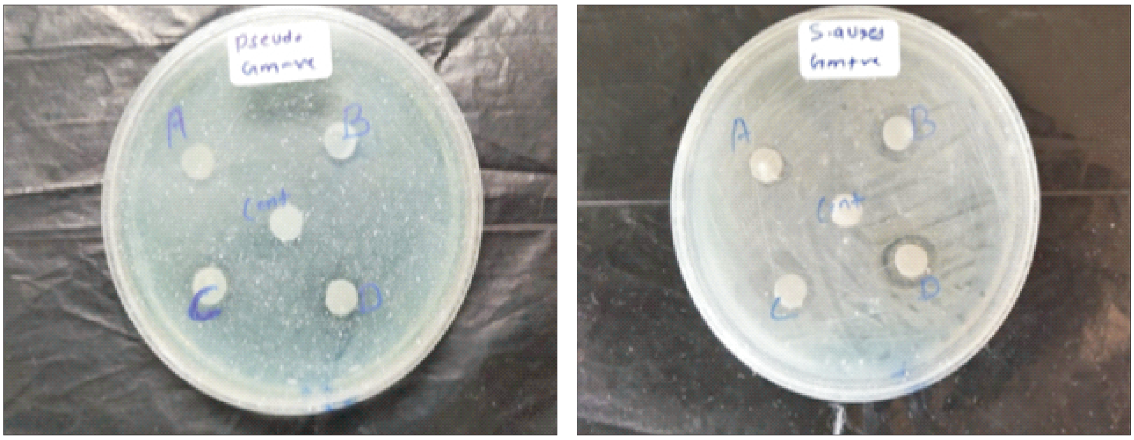

Figure 2. Zones of inhibition against *P. fluorescence* and *S. aureus*

Thus, the Melghat honey samples showed their prospective against five bacterial strains out of the six species that were selected for the experimental work. The zones of inhibition were of significant diameter, which might make this honey a spectacular natural antibiotic with no side effects. It was revealed that natural and unheated honey have comparably excellent potency against pathogenic bacteria (Mandal and Mandal, 2011). Tambekar and Rathod (2007) studied the antibacterial potential of Melghat and other branded honeys against the human pathogens *S. aureus, S. typhy, S. epidermidis, E. aeruginous, P. vulgaris, E. coli, Ps. aeruginosa,* and *K. pneumoniae*. It was reported that Melghat honey was found more effective against all the pathogens than the branded honey. The Melghat honeys, being wild, are the most natural and might be useful for therapeutic applications.

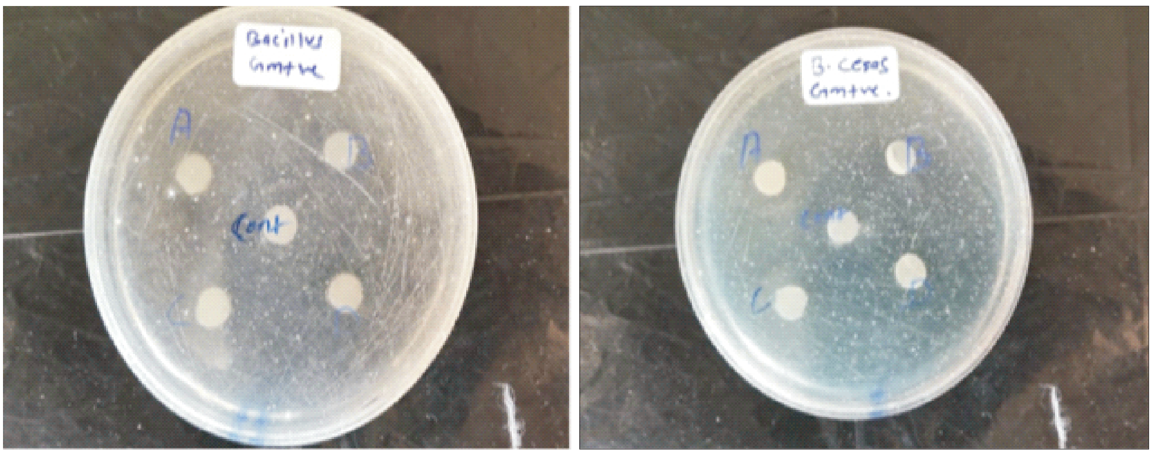

Figure 3. Zones of inhibition against *B. subtilis* and *B. cereus*

Kalidasan *et al.* (2017) evaluated the antibacterial potential of three varieties of honey, *viz*., Kombu honey, Malan honey, and commercial honey, against some bacterial species and obtained the zones of inhibition for *S. aureus* (34, 26, and 22 mm), *M. luteus* (30, 23, and 20 mm), *A. baumani* (22, 17, and 17 mm), *P. fluorescens* (23, 21, and 18 mm), *S. flexneri* (25, 22, and 19 mm), *P. mirabilis* (24, 20, and 15 mm), *E. coli* (28,19,and 17 mm), *K. pneumonia* (26, 18, and 16 mm), *S. typhi* (24, 21, and 19 mm), and *B. cereus* (32, 23, and 21 mm). In another study by Mandal and Mandal (2011), the honey samples under examination had shown their potential against *E. coli* (12-24 mm), *S. typhi* (0-20 mm), *S. aureus* (20-21 mm), and *P. fluorescence* (15-16 mm).

With the exception of *E. coli*, the results by Mohapatra *et al.* (2011) and Chauhan *et al.* (2010) were found to be analogous with the current work. Mohapatra studied the antibacterial activities of raw and processed honeys and got the zones against *E. coli* (16.14-28.49 mm), *S. typhy* (31.85-37.94 mm), *P. fluorescence* (13.09-35.95 mm), *S. aures* (8.58-11.54 mm), *B. subtilis* (7.25-11.19 mm), and *B. cereus* (6.94-23.7 mm). While Chauhan got the data against *E. coli* (14.0-29.4 mm), *S. typhy* (29.47-38.12), *P. aeruginosa* (13.09-35.95 mm), *S. aureus* (8.9-10.0 mm), *B. subtilis* (7.25-11.19 mm), and *B. cereus* (6.94-12.83 mm).

The application of honey as an antimicrobial agent for the infections found its roots in the prehistoric periods. The broad continuum of microbial species is restrained by honey. The differences in the activities of honey against the various bacteria may be the consequence of the effects of its heaps of physical and chemical properties. The mechanism of the antibacterial potential of honey was not understood clearly, but there were many factors that could be attributed to its splendid performance against a range of microorganisms. The physical and the above chemical factors are reported to be important for the properties of honey. The high osmotic pressure exerted due to the high sugar content disables the bacterial cells from growing. The water is squeezed out of their cells that brings the bacterial cells towards death. Due to the high amount of sugar, the viscosity of honey is elevated, causing the formation of a layer to protect the infected area from the septic bacteria. The high acidity (low pH) of honey is also responsible for its inhibitory effect against bacteria (Harun *et al*., 2017; Almasaudi *et al*., 2017).

Abeshu and Gelata (2016) reported that the formation of hydrogen peroxide due to the effect of glucose oxidase, an enzyme, is one of the most prominent parameters reported by most of the researchers behind the antibacterial property of honey. It is the byproduct of the synthesis of gluconic acid from glucose that acts as a destroying agent. The accumulation of H_2O_2 depends on the age of the honey, the effect of heating, and the floral origin. Another reason reported for the antibacterial properties of honey is the presence of 'inhibine', which may be sensitive to heat and light. They again added that the hydrogen peroxide in honey gets activated on dilution with body fluids and acts as an antibacterial agent.

According to Zainol *et al.* (2013), phenolic compounds and carbohydrates, the products of Maillard reactions, some proteins, and other compounds like peptides, methylglyoxal, etc., are also responsible for the antibacterial potential of honey. It is revealed that besides hydrogen peroxide, various non-peroxide components mentioned above, as well as methyl syringate and some unidentified compounds, showed antibacterial activities. Saranraj and Sivasakthi (2018) have supported the theory of defensins and the role of peptides in the antimicrobial mechanism. He elaborated that the peptides disrupt the membrane of microorganisms and carry out the depolarization of the inner membrane that leads to the reduction of cytoplasmic ATP. This hurdles the respiration of the bacterial cell, causing its death. Tambekar and Rathod (2007) suggested that the redox potential of vitamin C in honey plays a key role in its antibacterial activity.

Almost all the civilizations, traditions, and generations of the ancient as well as modern eras accepted the use of honey as a food and medicine due to its numerous beneficial properties, including antimicrobial potency. Honey not only shows its inhibitory effect against bacteria but also against fungus, viruses, and others. Many researchers supported this claim and revealed various possible mechanisms of honey's antimicrobial action (Israili, 2014; De-melo *et al*., 2017; Szweda, 2017).

Six types of bacteria were used for the experiment to detect the antibacterial potential of Melghat honey samples. It was concluded that all the honey samples showed their activities against almost all the bacterial species. The highest activity was observed against the gram-negative bacterial species *Salmonella typhi*. Surprisingly, it was observed that no honey sample showed its activity against the gram-negative bacteria *Escherichia coli*. Among the gram-positive species, three out of the four honey samples have shown their maximum potential against the species *Bacillus cereus*. The zones of inhibition were of significant diameter, which might make these honeys a spectacular natural antibiotic with no side effects.

REFERENCES

Abeshu, M. A. and B. Geleta, 2016. Medicinal uses of honey. Bio. Med. (Aligarh), **8**(2): 1-7. http://dx.doi.org/10.4172/0974-8369.1000279

Almasaudi, S.B., A. Al-Nahari, M. Sayed, A. El-Ghany, E. Barbour, S. Muhayawi, S. Al-Jaouni, Azhar, M. Qari, Y. Qari and S. Harakeh, 2017. Antimicrobial effect of different types of honey on *Staphylococcus aureus*. Saudi J. Bio. Sci. **24**: 1255–1261.

Anonymous, 2011. Guidelines. Establishment of national laboratory-based surveillance of antimicrobial resistance. World Health Organization. Regional Office for South-East Asia.

Bhoyar, S., H.K. Deshmukh, R.V. Mahajan, R. Ciannella, B. Bohra and S.G. Zalte, 2018. Assessment of socio economic status and agroforestry systems adopted by Indian tribal farmers in lower hills of Melghat region in Maharashtra state, J. Soils and Crops. **28**(1): 47-53.

Boussaid, A., M. Chouaibi, L. Rezig, R. Hellal, F. Donsi, G. Ferrari and S. Hamdi, 2018. Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. Arab. J. Chem. **11**: 265-274.

Chauhan, A., V. Pandey, K.M. Chacko and R. Khandal, 2010. Antibacterial activity of raw and processed honey. E. J. Bio. **5**(3): 58-66.

De-Melo, A.A., L. Muradian, M.R. Sancho and A.P. Mate, 2017. Composition and properties of *Apis mellifera* honey: a review. J. Api. Res. https://doi.org/10.1080/00218839.2017.1338444

Harun, U., A. Filis and D. Dilek, 2017. A research on the chemical and microbiological qualities of honeys sold in Istanbul. Food Sci. Technol. Campinas. **37**(Suppl. 1): 30-33.

Ismail, M.M. 2017. Honey prebiotic and antibiotic. EC Microbiology, Editorial (8.5): 243-245.

Israili, Z.H. 2014. Antimicrobial properties of honey. Am. J. Therapeu. **21**: 304-323.

Kalidasan, G., P. Saranraj, V. Ragul and S. Sivasakthi, 2017. Antibacterial activity of natural and commercial honey- a comparative study. Advan. Bio. Res. **11**(6): 365-372.

Khan, S.U., S.I. Anjum, K. Rahman, M. Ansari, W. Khan, S. Kamal, B. Khattak, Muhammad and H.U. Khan, 2018. Honey: single food stuff comprises many drugs. Sau. J. Bio. Sci. **25**: 320-325.

Mandal, M.D. and S. Mandal, 2011. Honey: its medicinal property and antibacterial activity. Asian Pac. J. Trop. Biomed. **1**(2): 154-160.

Mohapatra, D.P., V. Thakur and S.K. Brar, 2011. Antibacterial efficacy of raw and processed honey. Biotech. Res. Int. pp. 1-6. doi:10.4061/2011/917505.

Saranraj, P. and S. Sivasakthi, 2018. Comprehensive review on honey: biochemical and medicinal properties. J. Acad. Indus. Res. **6**(10): 165-181.

Szweda, P. 2017. Antimicrobial activity of honey. Chap 10, Honey Analysis, Intec (Publ.) pp. 215-232. http://dx.doi.org/10.5772/67117

Tambekar, D.H. and G.N. Rathod, 2007. Indian Melghat honey: a prospective antibiotic. J. Pharma. Toxi. **2**(1): 80-84.

Tendencia, E. A. 2004. Disk diffusion method. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment pp. 13-29.

Zainol, M.I., K.M. Yusoff and M.Y. Yusof, 2013. Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine, **13**(129): 1-10. http://www.biomedcentral.com/1472-6882/13/129

Rec. on 03.09.2022 & Acc. on 18.09.2022