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Antibacterial activity of raw honey of three bee species collected from Kannad Taluka of Aurangabad district

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Abstract

The aim of present investigation was to study the antibacterial activity of raw honey of three bee species collected from Kannad taluka of Aurangabad district. Honey samples were collected and assayed against four bacterial species by using Agar well diffusion method. All honey samples showed sensitivity against four bacterial species. Zone of inhibition clearly showed that honey obtained from *Apis florea* and *Apis cerana indica* was highest antibacterial activity, while honey of *Apis dorsata* was minimum antibacterial activity. Antibacterial activity showed that *Bacillus subtilis* was more sensitive than *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*.

Keywords: Honey, *Apis dorsata*, *Apis florea*, zone of inhibition, antibacterial activity, Sensitivity, etc.

Introduction

Honey, one of the major bee products, is a sweet viscous natural fluid made from the nectar of plants. Honey was defined as “the sweet substances produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs” [1]. Honey comprises hydrogen-peroxide, flavonoids and phenolic acids and many other unidentified properties. The chemical composition of honey is said to comprise of seven tetracycline, fatty acids, lipids, amylase, ascorbic acid, peroxidase and fructose all of which are attributed to its antimicrobial activity together with high osmolarity, low pH (3.6-3.7), content of phenol (inhibine), peroxidase, glucose and fructose in honey and the presence of tetracycline derivatives of fatty acids [2, 3]. Honey is an outstanding source of flavonoids, a natural antioxidant which have the ability to protect against allergens, viruses and carcinogens [4].

The antibacterial activity of honey is highly complicated due to the participation of multiple compounds and due to the large variation in the concentrations of these compounds among honeys (Kwakman and Zaat, 2012) [5]. Acidity is also said to be one of the component that contributes to the antibacterial property of honey [6].

The use of honey as a traditional medicine for microbial infections dates back to ancient times. The capability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature [7]. Honey has formerly been shown to have wound healing and antimicrobial properties, but this is dependent on the type of honey, geographical location and flower from which the final product is obtained [8]. It is well established that honey prevents a broad spectrum of bacterial species. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, Gram positives, and Gram negatives [9]. There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be especially useful against bacteria, which have developed resistance to many antibiotics [10]. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is decreased. This type of bacterial resistance to the antimicrobial agents constitutes a very serious threat to public health and all kinds of antibiotics, including the major last-resort drugs, as the frequencies of resistance are increased worldwide [11, 12]. Honey inhibits the growth of pathogenic bacteria such as *E. coli*, *S. aureus*, *Salmonella spp.*, *Shigella spp.*, *B. subtilis* and *V. cholera* and is superior to several well-known antibiotics.

The present study deals with antibacterial activity of raw honey collected from three different bee species of Kannad taluka of Aurangabad district (M. S.) India, against four bacterial species.

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Material and Methods

Study area: Geographically, Kannad taluka of Aurangabad district is located at 20° 27' N 75° 13' E. The average altitude of this area is 633 meter above sea level. The total area of Aurangabad district is about 10.07 lakh hector is out of which 8.12 lakh hector is under agriculture and 0.12 lakh is forest area. Farmers are engaged in cultivation of traditional crops like sunflowers, mustard, maize, cotton, jowar, pearl millet, pulses, onion, drumstick as well as other seasonable vegetables and also cultivating fruit plants like pomegranate, sweet orange etc. in the area provide and independent system in which the bees assist in carrying out cross pollination.

Sample Collection: Honey samples were collected from three different bee species namely *Apis dorsata*, *Apis cerana indica* and *Apis florea* from Kannad taluka of Aurangabad district. These samples were assayed against four different bacterial species viz. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus subtilis*.

Antibacterial activities: The screening of antimicrobial activities of each honey sample (1:1) on the tested pathogens used in this investigation was determined on Mueller Hinton agar media (all tested organism grow on Mueller Hinton agar media), by the using agar well diffusion method [13, 14]. Wells of 6 mm diameter and 5 mm depth were made on the solid

agar using a sterile glass borer. Approximately 20µl of each extract was inoculated onto wells were made in the spread plate culture of each microbial isolates. The plates were performed in triplicates. All plate of the tested organisms was then allowed to incubate at 37 °C for overnight. After 24 hours of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm) and represented as, +++ = Maximum antibacterial activity, ++ = Average antibacterial activity and + = Minimum antibacterial activity. In this study Penicillin was used as a standard antibiotic.

Results and Discussion

In the present study the comparative study of antibacterial activity of raw honey harvested from three bee species found in Kannad taluka shows significant variations against test organisms (Table no. 1 and Plate - I). Samples of each species of raw honey harvested from Kannad taluka of Aurangabad district and their antibacterial activity assayed against four different bacterial species viz. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. Different honey samples showed different antibacterial activity against tested pathogens.

Table 1: Antibacterial activity of honey samples of three honey bee species

Sr. No.	Bacterial strains	<i>Apis florea</i>	<i>Apis dorsata</i>	<i>Apis cerana indica</i>
1	<i>Bacillus subtilis</i>	+++	+++	+++
2	<i>Staphylococcus aureus</i>	+	+	++
3	<i>Escherichia coli</i>	++	++	++
4	<i>Salmonella typhi</i>	+++	++	+++

+++ = Maximum antibacterial activity, ++ = Average antibacterial activity and + = Minimum antibacterial activity

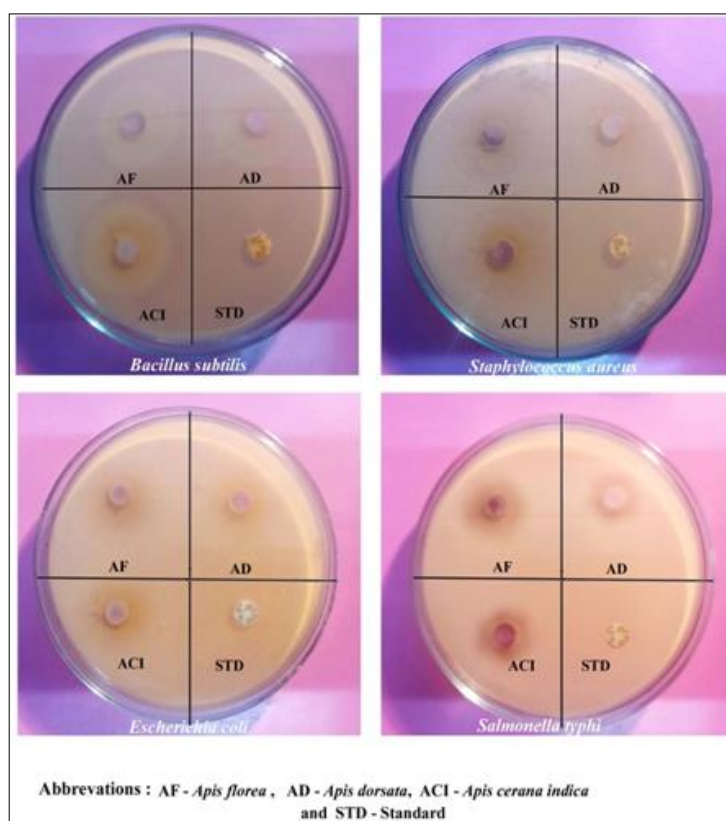


Plate 1: Antibacterial activity of honey of three honeybee species from Kannad taluka

The results of this study are line with a number of previous studies that have been reported by different researchers in the past. The honey harvested from *Apis florea* showing the maximum antibacterial activity (+++) against the bacterial strain like, *Bacillus subtilis* and *Salmonella typhi*, average antibacterial activity (++) showing against *Escherichia coli* and the minimum antibacterial activity (+) against and *Staphylococcus aureus*.

The honey harvested from *Apis dorsata* showing the maximum antibacterial activity (+++) against the bacterial strain *Bacillus subtilis*, average antibacterial activity (++) showing against *Escherichia coli* and *Salmonella typhi* and the minimum antibacterial activity (+) showing against *Staphylococcus aureus*.

The honey harvested from *Apis cerana indica* showing the maximum antibacterial activity (+++) against the bacterial strain *Bacillus subtilis* and *Salmonella typhi* as well as average antibacterial activity showing against *Staphylococcus aureus* and *Escherichia coli*.

Overall results indicated that honey samples of three bee species showing the antibacterial activity against four bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*). It was observed that *Apis florea* and *Apis cerana indica* showing the maximum antibacterial activity on four studied bacterial strains in comparison with the honey samples obtained from *Apis dorsata*.

Results also demonstrate that *Bacillus subtilis* and *Salmonella typhi* were the most sensitive, while *Staphylococcus aureus* and *Escherichia coli* were less sensitive to the inhibitory action of honey.

This variation is due to the variation in the source of nectar. Even honey collected from a single site can have variation in antibacterial activity [15]. However pharmacological standardization and clinical evaluation on the effect of honey are essential so as to make honey one of the standard and official medicines that could be used to effectively combat some of the mutated pathogens that have developed resistance against the abused conventional antibiotics [16]. The zone of inhibition of antibacterial activity was showed in the following manner.

Bacillus subtilis > *Salmonella typhi* > *Escherichia coli* > *Staphylococcus aureus*.

Antimicrobial activity of honey is thought to be due to some physicochemical parameters such as high content of reducing sugars, high viscosity, high osmotic pressure, low pH, low water activity (aw), low protein content and hydrogen peroxide [17, 18]. These results suggest that the honey samples used contained some bio components whose antimicrobial activities against all four tested organisms are highly comparable with penicillin antibiotic. Here it could be explained that the sources of nectar is a contributory part to the difference in the antimicrobial potential of the honeys used in this study as compared with previous studies [16]. The origin of flora determines attributes of natural products such as honey making the composition highly variable [8]. Honey is produced from several sources, and its antimicrobial activity varies greatly with origin and processing. Osho and Bello [16] studied the antimicrobial activity of honey produced by *Apis mellifera* on some common human pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Escherichia coli*) and reported that honey samples used were effective in inhibiting the growth of the test organisms at 25-100% concentrations (w/v). Raju and Goli [19] determined the In-vitro comparative

antimicrobial activity of commercial and raw honey against various bacteria isolated from ear discharge and reported that zone of inhibition (mm) against different bacterial strains namely *Staphylococcus* 10-16mm, *Streptococcus* 13-15mm, *E.coli* 10-17mm and *Pseudomonas* 8-15mm. Ndife *et al.*, [20] studied the antibacterial activity of Nigerian honey sourced from different floral locations and reported that zone of inhibition (mm) against different bacterial strains namely *S. aureus* 1.70-5.85, *E.coli* 2.10-6.10 and *B. subtilis* 2.05-3.20 mm. Goncalves *et al.*, [21], studied the antimicrobial properties of selected Portuguese commercial monofloral honey samples and their MIC values ranges between 6.25-25% as well as also reported that *E. faecalis* has most sensitive and *S. aureus* has least sensitive one.

Honey has many properties and these are 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 bees. In addition, unknown floral or bee components contribute to the activity. Large variation in antibacterial activity exists between honeys collected from different environment [15].

Conclusion

Honey produced by three different bee species such as *Apis florea*, *Apis dorsata*, and *Apis cerana indica* has antibacterial activity and is effective against both gram positive as well as gram negative bacteria. Hence, it was conclude that raw honey can be used in treating human and plant diseases especially those caused by tested microorganisms and it is a potential source of novel substances for future drug discovery.

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