CHARACTERIZATION OF A LOCAL RAW HONEY SAMPLE AND ASSESSMENT OF ITS ANTIMICROBIAL ACTIVITY

Soumi Guha Polley*, Khusboo Jhunjhunwala and Mou Saha

Department of Microbiology,

Lady Brabourne College,

P1/2 Suhrawardy Avenue, Kolkata 700017, India

*Corresponding author E-mail: soumi.guha@gmail.com

Abstract:

Antimicrobial agents, specially antibiotics, are the first and the most important medicine to fight the infectious diseases worldwide. The emergence of antibiotic resistant pathogens is greatly reducing the efficacy of this wonder drug. With rampant and widespread misuse of antibiotics, antibiotic resistance nowadays is a global threat to human health. Hence, the search for newer strategies to combat antibiotic resistance and infectious diseases is gaining impetus with each passing day. The use of natural products is an attractive alternative due to its low toxicity, easy availability and potent activities. Honey, the concentrated sweetener of nature, has long been known for its medicinal values. In traditional Indian medicine honey had been used as an external wound healer as well as an internal medication. In recent years various reports have been published about the antimicrobial activity of different raw, unheated honey samples against both broad range of bacterial and fungal species. Here we report the antimicrobial activity of a raw, untreated honey sample procured from a local beehive. The honey showed significant inhibitory effect against *Pseudomonas* sp. and *Vibrio* sp. at 50% and 25% (v/v) concentrations. Antifungal activity against *Candida* sp. and *Aspergillus* sp. was also observed. The physical and chemical parameters of the honey were also characterized.

Keywords: Honey, traditional medicine, antimicrobial agents

Introduction:

Antibiotics, the wonder drug of modern medicine, are fast losing its efficacy due to emergence of antibiotic resistance. Antibiotic resistance is becoming a real threat to global health as well as food safety (Levy & Marshall, 2004; Ventola, 2015). Dissemination of antibiotic resistance genes through environmental bacterial sample by horizontal gene transfer is giving rise to new resistant population (Wintersdorff, *et al.*, 2016). Misuse and overuse of antibiotics is

further aggravating the situation with time. Thus the search for alternative antimicrobials is urgently needed and the repertoire of natural products can be used to find such as an alternative (Mahady, Huang, Doyle, & Locklea, 2008). The added advantage of the natural products is their low toxicity and easy availability. Various herbs, plant extracts, essential oils and honey are often used for their antimicrobial properties. (Slover, Danziger, Adeniyi, & Mahady, 2009)

Honey is probably the oldest known wound dressing used from ancient times (Molan P. , 2006; Molan P. , 2009). In Ayurveda honey has been used both as a topical medicine as well as an internal medicine (Ediriweera & Premarathna, 2012). Honey, the natural sweetener is produced by honey bees and can be classified into different categories according to their floral source. Manuka honey, the most -studied honey for its antimicrobial activity, has broad spectrum antibacterial activity against sixty different species including some multi-drug resistant bacteria (Molan P. , 1992). Tualang honey, a Malaysian multifloral jungle honey, has been reported to have variable activities against wound and enteric bacteria (Tan, *et al.,* 2009). Medihoney is a licensed medical wound dressing product in Europe and Australia (Simon, Traynor, Santos, Blaser, Bode, & Molan, 2009). Nowadays a lot of medical grade honey is sold with standardised level of antibacterial activity (Deb Mandal & Mandal, 2011). Raw, untreated, natural honey often shows good antimicrobial activity.

In this report we have characterized a locally available raw untreated honey sample procured from a local beehive. We have analysed the physical properties and sugar composition of the honey sample. Further studies were carried out to ascertain its antimicrobial activity,

Materials and Methods:

Sample Collection:

Raw untreated honey sample from blossoms of blackberry plant were procured from a local beehive. The sample was collected in a clean container.

Measurement of Physical Properties:

The pH of the honey sample was estimated by using pH paper strip of suitable range. The moisture content of honey was measured by incubating 1ml of honey sample in a dessicator and weighing the sample till no change in weight was observed in two successive days. The final weight was subtracted from the initial weight to determine the moisture content

Determination of Sugar Composition by Thin Layer Chromatography:

The sugar composition of the honey sample was determined by running different dilutions of honey samples, 5%, 10%, 25% and 50%, as well as raw honey sample on preformed silica TLC plates. n-butanol, acetic acid and water in the ratio (3:1:1) was used as a solvent and diphenylaniline, aniline and phosphoric acid as the detection reagent. Glucose, fructose, lactose and sucrose were used as standards.

Well Diffusion Assay:

Four dilutions of honey samples (5%, 10%, 25% and 50%) were prepared by using sterile LB-broth. 100 ul of overnight bacterial (*Pseudomonas aeruginosa., Vibrio cholerae., B. subtilis, E. coli, S aureus, Klebsiella sp. Salmonella sp.*) or fungal culture (*Aspergillus niger, Candida albicans, Penicillium notatum*) were plated on respective agar plates. Wells were prepared on the agar plates and 50 ul of each honey dilutions were added to the wells in duplicates. The plates were incubated at 37^{o}C for 24hrs and diameter of zone of inhibition (ZOI) was recorded in triplicate.

Spectrophotometric Assay of Growth Inhibition:

Four dilutions of honey samples (5%, 10%, 25% and 50%) were prepared by using sterile LB-broth. For each microbial culture a set of four different honey dilutions along with a suitable control where no honey sample was added were inoculated with 2% overnight culture. Samples were incubated at 37^{o}C overnight and O.D. was measured at 595 nm.

Results and Discussion:

Physical Properties of Honey:

The raw honey sample collected from blackberry blossoms was dark brown in colour. Other physical properties are listed in Table 1

Table 1: Physical Properties of Honey:

pH	Density (gm/cc)	Moisture Content
4.5	1.405	13.75 %

Moisture content is an important property of honey and determines its quality as higher moisture content (greater than 20%) facilitates fermentation of honey by yeasts (Binnie, 2018). This raw honey sample is quite good in quality as its moisture content is quite low. The dark colour of the honey indicates presence of pigments in it (Albaridi, 2019). The low pH of honey is often related to its antimicrobial property (Albaridi, 2019).

Sugar Composition of Honey:

The main component of honey is sugar. Different types of sugars in varied proportions are present in different types of honey samples. However the primary components are glucose and fructose (Ouchemoukh, Schweitzer, Bachir-Bey, Djoudad-Kadji, & Louaileche, 2010). The sugar composition of this honey sample was assessed by running a thin layer chromatography. Figure 1 shows the TLC plate profile. In case of the raw honey and 50% honey sample there was huge drag due to high viscosity.

Figure 1: TLC profile of honey sample

Lane1-Lane5 are dilutions 5%, 10%, 25%, 50% and undiluted honey respectively. The different sugars lactose (lane6), glucose (lane7) sucrose (lane8), fructose (lane9) were run as standards. Lower three dilutions migrated well and distinct spots were observed. From thin layer chromatography presence of glucose, fructose, sucrose and a slight amount of lactose can be confirmed in this honey sample.

Antimicrobial Activity of Honey:

Honey has long been reported to have wound healing activity (Molan P. , 2009). Many local honey samples have shown good antibacterial activity (Albaridi, 2019). Hence we have analyzed the antibacterial as well as the antifungal activity of this honey sample by two different methods. First well diffusion assay was performed with seven different bacterial strains such as *Pseudomonas aeruginosa., Vibrio cholerae., B. subtilis, E. coli, S aureus, Klebsiella sp. Salmonella sp.* and three different fungal strains *Aspergillus niger, Candida albicans, Penicillium notatum.* However zone of inhibition was observed for only two bacterial strains *Pseudomonas aeruginosa., Vibrio cholerae* and two fungal strains *Aspergillus niger, Candida albicans.* The diameters of zone of inhibition are given in Table 2.

Table 2: Zone of inhibition diameter

Honey Dilutions (v/v)	**Average diameter of zone of inhibition (cm)**			
	Pseudomonas aeruginosa	***Vibrio cholerae***	***Aspergillus niger***	***Candida albicans***
50%	**3.23**	**3.47**	**2.13**	**2.23**
25%	**2.9**	**2.8**	**1.83**	**1.73**
10%	**2.27**	**-**	**-**	

To further validate the observed results spectrophotometric assay for growth inhibition was performed for two bacterial and two fungal strains that have given zone of inhibition. Figure 2 shows the growth inhibition results for different strains at various dilutions of honey. For two bacterial species, *Pseudomonas aeruginosa.*, *Vibrio cholerae* good growth inhibition was observed at 50% and 25% dilutions of honey. For *Aspergillus niger* good inhibition was observed for all dilutions except 5%. Whereas for *Candida albicans* only 50% and 25% showed inhibition. Inhibition at 50% dilution may be due to high osmolarity. However growth inhibition at lower dilutions i.e. 25% and 10% proves that this honey has some additional component responsible for its antimicrobial activity.

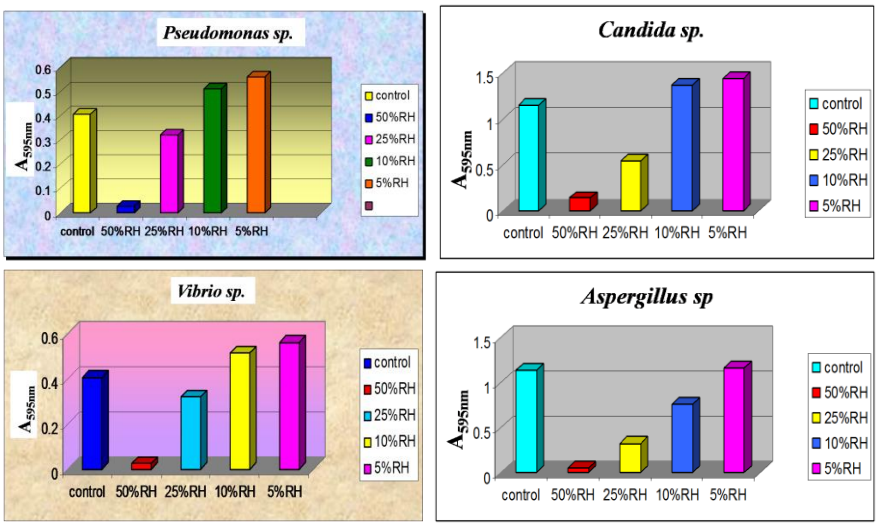

Figure 2: Graphical representation of zone of inhibition for two bacterial and two fungal cultures at different dilutions of honey

Conclusion:

The antimicrobial property of honey is often attributed to its high osmolarity, acidity, low water activity, hydrogen peroxide and non-peroxide phytochemical components like methylglyoxal (Deb Mandal & Mandal, 2011). Hydrogen peroxide in honey is produced by the enzyme glucose oxidase, which is naturally present in honey. However in undiluted honey it is inactive and gets activated only upon dilution. In diluted honey glucose oxidase acts on glucose of honey to produce hydrogen peroxide (Molan P. , 1992; White Jr., Subers, & Schepartz, 1963). Analyzed local raw honey sample showed significant antibacterial as well as antifungal activity at 50% and 25% (v/v) dilutions. To identify the origin of its antimicrobial activity well diffusion assay was performed with peroxidase treated honey that failed to show any zone of inhibition. Thus this particular honey sample when treated with peroxidase showed a drastic reduction in its

antimicrobial activity indicating hydrogen peroxide to be a major player in determining its antimicrobial activity.

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