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Research Article

Antibacterial Activity of Organic Honey

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Abstract: In the present study the antibacterial effects and minimum inhibitory concentration (MIC) of organic honey was evaluated. Four bacterial species viz., *Escherichia coli, Bacillus subtilis, Pseudomonas fluoresces* and *Staphylococcus epidermidis* which we isolated from pure cultures were used in this study. This study was conducted using the disc diffusion assay method. The antibacterial activity of organic honey was compared with Manuka honey. The most inhibition was obtained with Manuka honey against *Escherichia coli* and *Staphylococcus epidermidis* with a peak inhibition against *Staphylococcus epidermidis* at 30 % concentration.

Keywords: Manuka Honey, organic honey, antibacterial activity, minimum inhibitory concentration

INTRODUCTION

Honey is a sweet, viscous fluid produced by bees from the collection of nectar, primarily from flowers. It is considered to be natural syrup. The Nectar is gathered by the bees and is slowly transformed into honey, through a long process involving the addition of enzymes and the gradual reduction of moisture.

Honey is a rich source of carbohydrates mainly Fructose and Glucose. The chemical composition of honey varies depending on the plant source, season and production methods. Therefore the Colour, Concentration and Compounds vary depending on the floral sources. Other compounds which can be found in Honey include Proteins and acids such as Gluconic Acid ($C_6H_{11}O_7$, also known as 2, 3, 4, 5, 6-pentahydroxyhexanoic Acid), Minerals and Anti-Oxidants such as Hydrogen Peroxide (H_2O_2) and Vitamins (B6 and B12) [1].

In this study antibacterial activity of organic honey which is readily available in health food stores was evaluated. Honey has been shown to have antibacterial properties, in particular Manuka honey. Manuka Honey has had extensive research done on it. It has been shown in many studies that Manuka Honey has antimicrobial effects [3-5]. In this study the antibacterial activity of the organic honey is compared with the Manuka honey (positive control). This is because it is known to have antibacterial properties.

MATERIALS AND METHODS

Chemicals

All the reagents and chemicals used in this study were of analytical grade.

Bacteria

Four different species of bacteria will be used in this study to explore the effectiveness of organic honey on the inhibition of growth; the bacteria chosen for this study are both Gram-Positive and Gram-Negative Bacteria, Aerobic and all four bacteria Genera have significance with interaction with humans (Homo sapiens). The four bacterial species, which would be used in this study are: *Escherichia coli, Bacillus subtilis, Pseudomonas flourescens and Staphylococcus epidermidis*.

Media

In this investigation Nutrient agar and Nutrient Broth were used to culture four different bacteria species. The nutrient agar was used to isolate colonies and to observe the zone of inhibition around sterile absorbent discs. The nutrient broth was used in making liquid cultures from isolated colonies from the agar plates. The liquid cultures were then used in the disc diffusion assay, the maximum recovery diluents was used to dilute the honeys to make up the serial dilutions.

Culture Preparation

Four Universal bottles containing 9ml each of nutrient broth were inoculated separately with *Escherichia coli, Bacillus subtilis, Pseudomonas flourescens* and *Staphylococcus epidermidis* using an inoculum loop. The nutrient broth solutions which were inoculated with *Escherichia coli, Bacillus subtillis and Staphylococcus epidermidis* were then incubated at 37˚C for up to 48 hours. The nutrient broth solution which was inoculated with *Pseudomonas flourescens* was incubated at 25˚C for up to 48 hours.

Antibacterial activity

The experiment method employed for this investigation was the Disc Diffusion Assay method, it was chosen because it was the easiest and the simplest method to use.

All the organisms used in the investigation were of level 1 classification, the inoculated culture plates were incubated at the temperatures which are stated in table 1, for up to 48 hours.

Table 1: Incubation Temperatures

S.No	Organism	Incubation Temperature (°C)
1	*Escherichia coli*	37
2	*Bacillus subtilis*	37
3	*Staphylococcus epidermidis*	37
4	*Pseudomonas flourescens*	25

These bacteria cultures were then stored at 4˚C.

Disc Diffusion Assay

Five sets of four Nutrient Agar plates were set out; each agar plate in every set was inoculated separately with the bacteria *Escherichia coli, Bacillus subtilis, Pseudomonas flourescens and Staphylococcus epidermidis*, by pipetting 100Cl of each bacterium directly onto the agar surface of each plate of every set.

Using the spread plate technique, the bacteria samples were then spread across the surface using a glass spreader. The plates were left to dry for 15 minutes, whilst sterile absorbent discs were placed into each honey flask. The absorbent discs were left in the honey for 10 minutes to absorb the honey. An absorbent disc from honey was placed on every agar plate in each set.

The plates which were inoculated with *Escherichia coli, Bacillus subtilis and Staphylococcus epidermidis* with then incubated at 37˚C for up to 48 hours. The plates which were inoculated with *Pseudomonas flourescens* was then incubated at 25˚C for up to 48 hours.

Detection of Antibacterial Activity

After the plates had been incubated the inhibition of the bacteria was determined by the visual confirmation of a zone of inhibition. A zone of inhibition is a clear area surrounding the absorbent disc.

Detection of the Minimum Inhibitory Concentration

Honey Preparation

Organic honey and Manuka honey were diluted using maximum recovery diluent (MRD), in which six dilutions were prepared. The concentration of each dilution was measured using weight in grams of honey against the volume in cm^3 of MRD, grams/volume (g/vol.). Using universal bottles, the honey concentrations were prepared using the following measurements of honey and MRD as seen in table 2.

Table 2: Honey Dilutions

Percentage (%) Concentration	Weight in grams, of honey	Volume in cm^3 of MRD
0	0	10
10	1	9
20	2	8
30	3	7
40	4	6
50	5	5

Each honey dilution was kept at room temperature out of direct sunlight.

Disc Diffusion Assay

In this method, for each honey, four sets of six nutrient agar plates were set out, each set was then inoculated with one species of bacteria. In each set of nutrient agar plates, each agar plate was inoculated with bacteria by pipetting 100Cl of nutrient broth bacterial culture, directly onto the agar surface. Using the spread plate technique, the bacteria samples were then spread across the surface of the agar using a glass spreader.

The plates were left to dry for 15 minutes, whilst sterile absorbent discs were placed into each honey concentration of the two honeys. The absorbent discs were left in the honey dilutions for 10 minutes to absorb the honey. An absorbent disc from each honey dilution series was placed directly onto the surface of every agar plate in each set; this was done for each honey.

The plates which were inoculated with *Escherichia coli, Bacillus subtilis and Staphylococcus epidermidis* were then incubated at 37˚C for up to 48 hours. The plates which were inoculated with *Pseudomonas flourescens* was then incubated at 25˚C for up to 48 hours.

The 0 percent honey dilution for each honey, which contained only MRD as stated in table 1 is a negative control for inhibition. The amount of inhibition was recorded by measuring the diameter of the zone of inhibition, in millimetres (mm), this was measured using a ruler. The measurement included the diameter of the absorbant disc.

RESULTS

Each experiment was done in triplicate to get an average result; the two statistical methods were used in analysing the results, were the mean (x) and Standard Deviation (SD).

The mean was used to determine the average inhibition of each honey at each concentration; the Standard Deviation was used to determine the amount of error at each concentration of inhibition.

Where there was positive (+) inhibition on the disc diffusion assays, there was a visible clear ring around the absorbent disc, where there was no growth of bacteria colonies. Where there was negative (-) inhibition on the disc diffusion assays, there was no visible clear ring, there would be the appearance of growth around the absorbent disc.

Table 3: Bacterial Inhibition at 100% concentration of honey

S.No	Organism	Organic Honey	Manuka Honey
1	*Bacillus subtilis*	_	+
2	*Staphylococcus epidermidis*	_	+
3	*Pseudomonas flourescens*	+	+
4	*Escherichia coli*	+	+

'+' Positive Inhibition; '-'Negative Inhibition

The results in table 3 show that at 100% concentration of honey, both the honeys have antibacterial activity. The Manuka honey inhibits all four bacteria species; this shows that this honey acts on both gram positive and gram negative bacteria.

But the Organic honey expressed antibacterial activity *against Pseudomonas flourescens* and *Escherichia coli*. This shows that this honey act on these gram negative bacteria only. It did not appear to express any activity against *Bacillus subtilis* and *Staphylococcus epidermidis*.

Identification of Minimum Inhibitory Concentration

Table 4: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Escherichia coli*

Concentration of Honey	Manuka Honey	Organic Honey
0 %	0±0	0±0
10 %	22.6±0.715	0±0
20 %	24±0.144	7.33±0.0787
30 %	25.66±0.044	6±0.8816
40 %	27±0.0416	7.66±0.577
50 %	27.6±0.0753	8.66±0.176

According to table 4, these results show that with concentrations of honey up to 50%, the Manuka and Organic Honey's have antibacterial activity against the gram negative bacteria *Escherichia coli* with a minimum inhibitory concentration of 10% for the Manuka and 20% for the Organic honey. The Manuka showed the largest zones of Inhibition.

Table 5: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Bacillus Subtilis*

Concentration of Honey	Manuka Honey	Organic Honey
0 %	0±0	0±0
10 %	0±0	21±0.087
20 %	3±0.173	25±0.041
30 %	6±0.086	29.66±0.08
40 %	9.66±0.516	31.33±0.102
50 %	9.33±0.068	32.33±0.094

These results (Table 5) show that with concentrations of honey up to 50%, the Manuka and Organic Honey have antibacterial activity against the gram positive bacteria *Bacillus Subtilis* with a minimum inhibitory concentration of 20% for the Manuka and 10 for the Organic Honey. The organic flower honey had the largest zones of inhibition out of all the honeys.

Table 6: Disc Diffusion Assay for the Minimum Inhibitory Concentration on Staphylococcus epidermidis

Concentration of Honey	Manuka Honey	Organic Honey
0 %	0±0	0±0
10 %	20.3±0.015	0±0
20 %	21.3±0.037	0±0
30 %	26±0.134	0±0
40 %	10.66±0.0514	0±0
50 %	11±0.0175	0±0

According to table 6 above, these results show that the Manuka honey had antibacterial activity against the gram positive bacteria *Staphylococcus epidermidis*. The Manuka honey had the largest zones of inhibition and had a minimum inhibitory concentration of 10%. Organic honey showed no antibacterial inhibition of the gram positive *Staphylococcus epidermidis* up to concentration of 50%.

Table 7: Disc Diffusion Assay for the Minimum Inhibitory Concentration on *Pseudomonas fluorescens*

Concentration of Honey	Manuka Honey	Organic Honey
0 %	0±0	0±0
10 %	0±0	0±0
20 %	2.33±0.174	9±0.111
30 %	2.66±0.142	8.6±0.066
40 %	8.33±0.061	8±0.125
50 %	8.33±0.057	7.66±0.0619

According to table 7, both the honeys have shown antibacterial activity against the gram negative bacterium *Pseudomonas fluorescens*. The minimum inhibitory concentration was found to be 20%.

DISCUSSION

The results obtained shows that as expected both the honeys, exhibited a level of antibacterial activity which generally increased with increasing concentration. The degree of antibacterial activity varied according to the type of bacteria and type of honey. The minimum inhibitory concentration (MIC) has been observed to lie between 10% and 20% for both the honeys against all the bacteria used in this investigation. These results are in agreement with Ali ATM *et. al;* [5] who found that the honey concentration 20 % was sufficient to inhibit the growth of a range of isolates. The expected range of the minimum inhibitory concentration was between 10-50 %as shown by the research done by Barret J *et. al;* [2] who observed an MIC of 5-10 % and by Al-Waili NS [6] who observed an MIC of 30-50 %.

Conclusion

It is clear from this study that different honeys act differently on the same microorganism. This is to be expected since the composition of each honey is different. The composition would be different for each honey according to the different floral sources and the species of bee.

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