

# The Effect of Local and Processed Honey on the Inhibition of Bacterial Growth

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**Abstract:-** The effect of honey on the inhibition of bacterial growth was investigated. This was done to simulate the possible impact of honey on bacteria in living tissues and the possible use of this naturally occurring food substance as a substitute for antibiotic use as a result of antibiotic resistance. Three (3) bacterial organisms *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were exposed to three varieties of honey; local honey (LH), Pure honey® (PH) and pure blossom honey ® (PBH) respectively in a culture media. The antibiotic tablet Ampiclox was used as control in the culture media. The bacteria were stained with the different honey types and Ampiclox antibiotic and the zones of inhibition were measured after 24 hours of staining. Result from the study indicate that apart from the control, the local honey achieved the greatest zone of inhibition followed by the pure honey ® and lastly by the pure blossom honey. Mean values for *Escherichia coli* inhibition were 10.33, 9.0, 8.3 and 12.33 for LH, PH, PBH and control (Ampiclox) respectively. While *Staphylococcus aureus* inhibition are 13.66, 10.0, 9.0 and 14.0 respectively for LH, PH, PBH and control. *Pseudomonas aeruginosa* showed inhibition rates of 11.33, 9.33, 8.66 and 13.33 for LH, PH, PBH and control (Ampiclox) respectively. There was a significant difference  $P < 0.05$  ( $P = 0.005$ ) in inhibition between PBH and Control (Ampiclox) in *Escherichia coli* but no significant difference between the others ( $P > 0.05$ ). There were significant differences ( $P < 0.05$ ) between LH and PH ( $P = 0.005$ ); LH and PBH ( $P = 0.001$ ) in *Staphylococcus aureus* inhibition. There was significant difference between LH and control ( $P > 0.05$ ). There were significant differences between the control (Ampiclox) and PH ( $P = 0.007$ ) and between the control (Ampiclox) and PBH ( $P = 0.003$ ) in *Pseudomonas aeruginosa* growth inhibition. There was no significant difference between control and LH. Based on the result of this finding LH can be considered as useful alternative for the treatment of infections of these bacterial origins in cases where Ampiclox resistance is established.

**Key words:** Honey, Bacteria, Inhibition, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

## I. INTRODUCTION

The 1950's represented a unique era in the fight against infectious diseases and the survival of mankind using antibiotics. Until recently, antibiotics have been used with almost flawless success with little or no impediment or resistance. As antibiotics became so extensively used and easy to afford, abuse of antibiotics was inevitable. The abuse of these drugs is so wide spread that the resistance of pathogenic bacteria to these drugs is growing. This resistance has been traceable to drug overdose, under dose of drugs through the

counter, ability of microorganisms to undergo genetic variability (mutation) and general misuse of drugs [1]. At present, the world is face with a mighty "bug", bacteria resistant to common antibiotics.

Fortunately, in Nigeria the use of naturally occurring products such as honey as a substitute for treating bacterial infection is beginning to provide a source of new hope. Honey is plentiful and abundantly found in major forest and farms in Nigeria. Hence there is an acute societal need to develop effective and alternative measure of treating and controlling of microbial infections using it. This study therefore seeks to ascertain the efficacy of the much touted use of honey as antibacterial therapy. The findings of this study will provide useful information for public health providers, biologist and the entire scientific community. After all, the divide between medical science and alternative medicine needs urgent bridge building.

## II. MATERIALS and METHODS

### A. Sterilization of Glassware

The glassware such as conical flasks, Petri-dishes, pipette, McCartney bottles were sterilized in the hot air oven at 180°C for 1 hour. Wire loop and Cork borer were heat flamed to reduce contamination. All glassware used for this work were thoroughly washed and rinsed with sterilized water.

### B. Sample Collections

Branded honeys were bought from a supermarket in Yenagoa metropolis, while the local processed honey was obtained from a local farmer in Amassoma town, Southern Ijaw Local Government Area of Bayelsa state, whose hobby is to process local honey from bees swam on trees. The local honey was tested to be sure it was obtained locally.

### C. Microorganisms Used

Clinically isolated pure culture of human pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus* and *pseudomonas aeruginosa* were obtained from a preserved culture obtained from a recent work carried out in the Biological laboratory, faculty of sciences, Niger Delta University, Bayelsa State. The organisms were inoculated into nutrient broth and incubated at 37°C, followed by refrigerator storage at 4°C until required for use.

**D. Preparation of Media**

The primary media employed were nutrient agar (NA) and nutrient broth (NB). All media were prepared according to manufacturer’s specification.

**E. Preparation of Disc**

Discs of 6mm in diameter were punched out using Whatman’s No 1 filter paper with the aid of Cork borer and placed in petri dish. The disc was then sterilized in hot air Oven at 180°C for 1 hour, after which they were allowed to cool with slight modification.

**F. Sample Preparation**

1) **Preparation of Antibiotic (Positive Control) and Disc:** The antibiotic used as control was Ampiclox (500mg). The discs were also gotten from whatman No.1 filter paper with size 6mm in diameter borer. 500 mg of Ampiclox capsule was poured out and dissolved in 100 ml of distilled and deionized water in a conical flask to give a 5:1 dilution (i.e. 5 mg/ml:1 concentration). From this, 10.00 ml was used to impregnate the sterilized paper discs (which were cut with a 6mm borer) in triplicates.

2) **Preparation of Sample Honey and Disc:**Also 10.00 ml of each of the sample honey (Local honey (LH), pure honey (PH) and Pure Blossom honey (PBH)) was impregnated on filter paper discs in triplicates. All the discs were sun-dried and kept dry in separate Petri dishes in desiccators. The dishes were transferred aseptically to already set media containing the pathogenic microorganism. The essence of the control is to determine the degree at which the different extracts can inhibit the growth of the clinically isolated organisms [2].

**G. Data Analysis**

Means and standard deviations were calculated for the various inhibitions observed. A Three way analysis of variance was carried out to compare the mean zones of inhibitions of bacteria using the different honey types and control. Turkey HSD Post Hoc test was employed to separate means. This was done using the SPSS® version 21.0 Statistical Package [3].

**III. RESULT AND DISCUSSION**

**A. Result**

Table 1: Mean Inhibition Values of Honey and Control

	Escherichia coli (mm)	Staphylococcus aureus (mm)	Pseudomonas aeruginosa (mm)
Local Honey (LH)	10.33±1.52 <sup>a</sup>	13.66± 0.57 <sup>a</sup>	11.33±0.57 <sup>a</sup>
Pure Honey (PH)	9.0±1.0 <sup>a</sup>	10.0±1.0 <sup>b</sup>	9.33±1.15 <sup>ab</sup>
Pure Blossom Honey (PBH)	8.33±0.577 <sup>ab</sup>	9.0±1.0 <sup>b</sup>	8.66±0.57 <sup>ab</sup>
Control (Ampiclox)	12.33±0.57 <sup>ac</sup>	14.0±1.0 <sup>a</sup>	13.33±1.52 <sup>ac</sup>

\*Means ± Standard deviation. Means with the same letter superscript on the same column are not significantly different. (P=0.05).

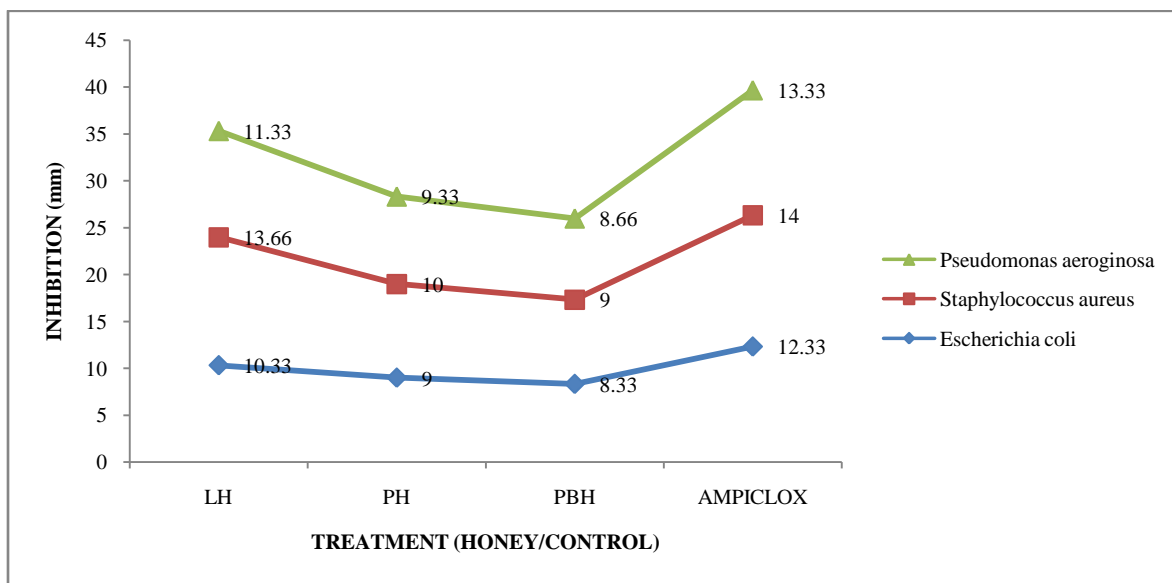


Fig. 1: Mean Inhibition Values of Honey and Control

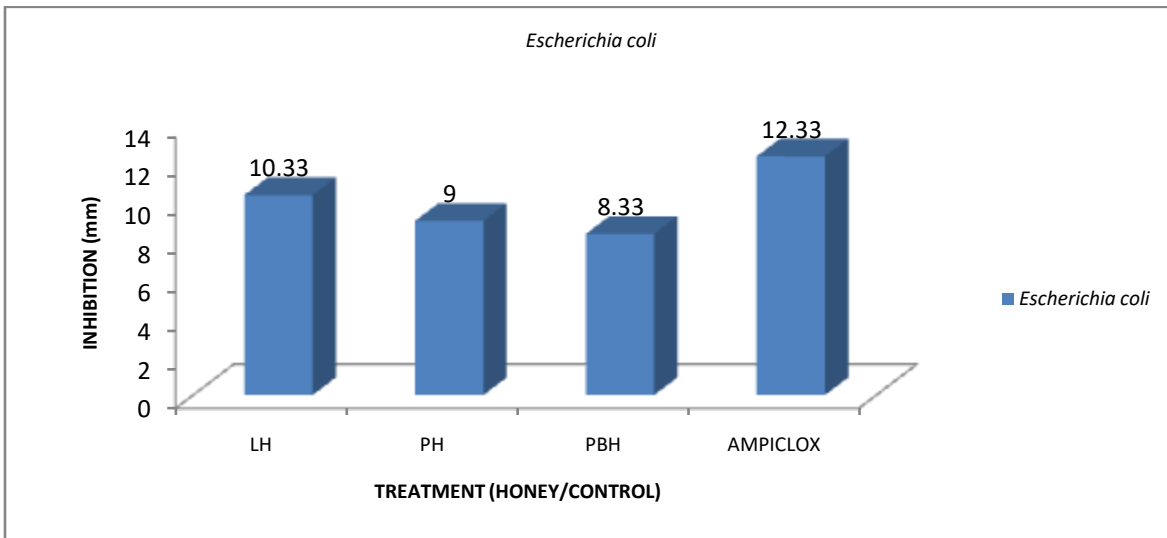


Fig. 2: Mean Inhibition Values of Honey and Control.

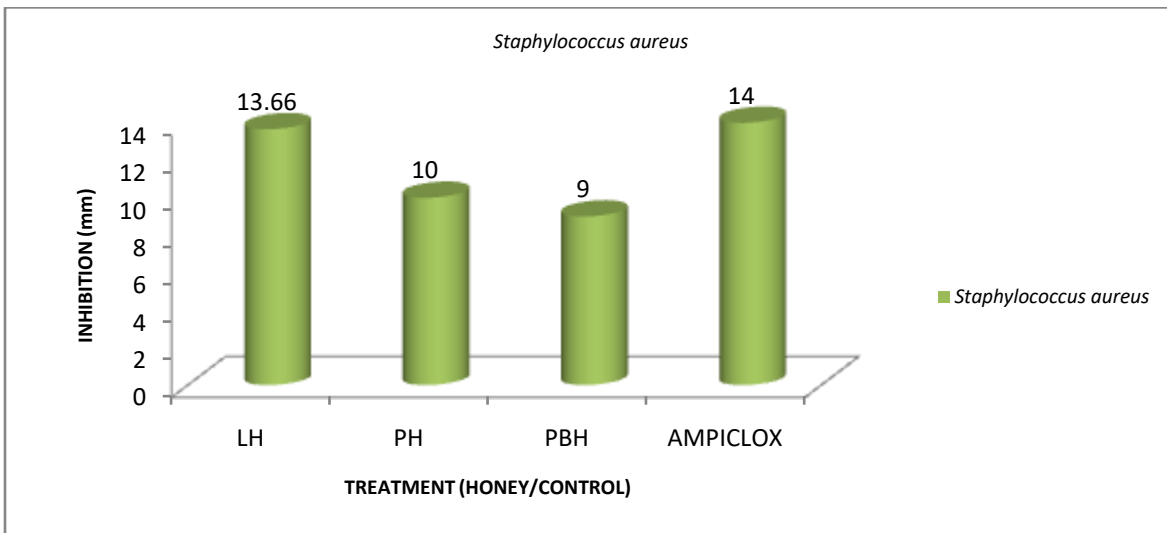


Fig. 3: Mean Inhibition Values of Honey and Control

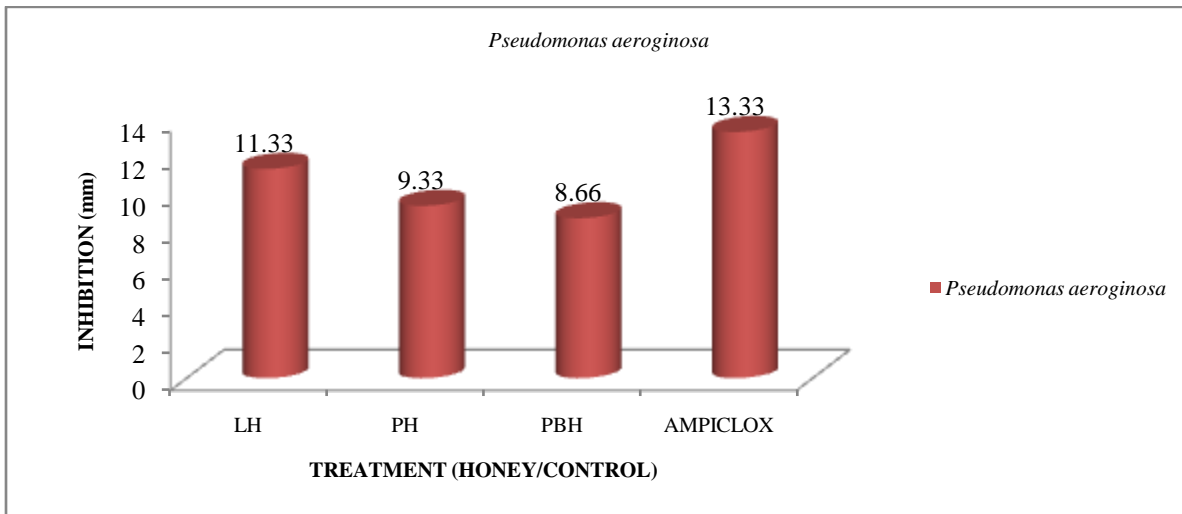


Fig.4: Mean Inhibition Values of Honey and Control

The result of the study is represented in Table 1 and Figures 1–4 above. Three (3) bacterial organisms *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were exposed to three varieties of honey; local honey (LH), Pure honey® (PH) and pure blossom honey ® (PBH) respectively in a culture medium. The antibiotic tablet Ampiclox was used as control in the culture media. The bacteria were stained with the different honey types and Ampiclox antibiotic and the zones of inhibition were measured after 24 hours of staining. Result from the study indicate that apart from the control, the local honey achieved the greatest zone of inhibition followed by the pure honey ® and lastly by the pure blossom honey. Mean values for *Escherichia coli* inhibition were 10.33, 9.0, 8.3 and 12.33 for LH, PH, PBH and control (Ampiclox) respectively. While *Staphylococcus aureus* inhibition are 13.66, 10.0, 9.0 and 14.0 respectively for LH, PH, PBH and control. *Pseudomonas aeruginosa* showed inhibition rates of 11.33, 9.33, 8.66 and 13.33 for LH, PH, PBH and control (Ampiclox) respectively. There was a significant difference  $P < 0.05$  ( $P = 0.005$ ) in inhibition between PBH and Control (Ampiclox) in *Escherichia coli* but no significant difference between the others ( $P > 0.05$ ). There were significant differences ( $P < 0.05$ ) between LH and PH ( $P = 0.005$ ); LH and PBH ( $P = 0.001$ ) in *Staphylococcus aureus* inhibition. There was significant difference between LH and control ( $P > 0.05$ ). There were significant differences between the control (Ampiclox) and PH ( $P = 0.007$ ) and between the control (Ampiclox) and PBH ( $P = 0.003$ ) in *Pseudomonas aeruginosa* growth inhibition. There was no significant difference between control and LH.

#### B. Discussion

The result of the study indicates that the different honey types possess therapeutic (inhibitory) potentials on the different bacteria. This is in agreement with the findings of [4] who also observed that honey has a great inhibitory effect on the gram-negative bacteria *S typhi*, *P aeruginosa* and *E. coli*.

Also, the reports of this study agrees with studies carried out by [5], in which they tested antibacterial activity from six floral sources against *Escherichia Coli*, *Salmonella thyphimurium*, *Shigellasonnei*, *Staphylococcus aureus* and *Bacillus cereus* using disc diffusion method. Their results showed that the development of inhibition zones depends on the concentration of the honey used as well as the tested pathogen.

In this study, it is observed that inhibition of bacteria growth is related to the type and source of the honey used. Notably, it is observed that the LH > PH > PBH in its inhibitory potentials. The antibacterial effects of honey are not only due to osmolality, viscosity, presence of hydrogen peroxide and low protein contents but due to other important factors that affect the composition of honey [6]. According to [7], such factors depend to a great extent on the bee's source, the location of the flowers and related weather conditions, the storage time and conditions and the method of preservative treatment.

Natural honey consists mainly of carbohydrates (about 82%), water and other minor components. Those minor ingredients include: proteins, minerals, phytochemicals and antioxidants. It has been reported that those minor ingredients are the ones that are responsible for medical and biological activities of honey in the treatment of infections, burns, wounds and ulcers [8].

Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [9]. It has further been reported that physical property along with geographical distribution and different floral sources may play important role in the antimicrobial activity of honey [5].

The order of sensitivity (inhibition) of the tested organisms to the honey samples decreased in the following order: *Staphylococcus aureus* > *Pseudomonas aeruginosa* > *Escherichia coli*. *Staphylococcus aureus* is the most inhibited of all the bacteria exposed to honey and the control (ampiclox). This may be as a result of the fact that *Staphylococcus aureus* is gram positive bacteria whereas *Pseudomonas aeruginosa* and *Escherichia coli* is gram negative. Although *Staphylococcus aureus* is the most pathogenic, gram positive bacteria are most susceptible to antibiotic and cleaning products easily because they contain thick peptidoglycan layer whereas gram negative bacteria contain a thin peptidoglycan layer containing lipopolysaccharide which acts as camouflage and prevents the antibodies from identifying the antigens.

Lastly, the LH had inhibitory values that are near and similar to that obtained with the administration of ampiclox (control) with no significant difference ( $P > 0.05$ ) in inhibitory rates in all the bacteria studied. This may be due to the fact that they both possess similar antimicrobial properties.

#### IV. CONCLUSION

The result of this study shows that the three different honey samples (LH, PH and PBH) inhibited the growth of the tested organisms. Local honey (LH) samples from Amassoma gave higher inhibition efficiency on the growth of the tested bacteria than the processed honey samples (PH and PBH). The zone diameters of inhibitions of the organisms for different honey samples were found to be statistically significant. However, there is no significant difference ( $P > 0.05$ ) between the LH and control. The level of Inhibition of the different honey types and control indicate that Ampiclox > LH > PH > PBH.

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Based on the findings from this study, local honey (LH) can be considered as a useful alternative for the treatment and

prevention of infections of these bacteria origins, in case Ampiclox resistance is established.

#### ACKNOWLEDGMENT

We are immensely grateful to the Authorities of the Niger Delta University for allowing us the use of their laboratory and other facilities.

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