Original article:

Antibacterial Effect of Nigerian Honey on *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Background: Honey exhibits antimicrobial activities against a wide range of bacteria in different milieu. ***Objective***: To determine the antibacterial effect of Nigerian honey types on selected bacterial isolates; *Staphylococcus aureus* and *Escherichia coli*. For this reason raw and commercial honey samples were investigated. ***Methods:*** Two honey samples were used in this study sourced from two different institutions: raw (sunshine) and commercial (shoprite; Labsan) honey. They were tested on clinically selected Gram-positive and Gram-negative bacterial isolates: *Staphylococcus aureus* and *Escherichia coli* respectively. ***Results***: The honey samples examined were effective in inhibiting the growth of the test bacteria and the inhibitory effects were found to be superior from sunshine honey on *Staphylococcus aureus*. Among the selected bacteria used in this assay, *Staphylococcus aureus* was the most susceptible to the honey samples used and sunshine (raw) honey was found more effective. ***Conclusion***: The Nigerian honey types used in this study were found to be alternative natural antibiotic to conventional synthetic antibiotics.

Keywords: Sunshine honey; Labsan honey; antibacterial effect; susceptibility; *Staphylococcus aureus; Escherichia coli.*

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Introduction

Infections and other health related problems have been of great concern to human beings and chemotherapy is the main approach in the treatment of such conditions. Investigation into the microbial flora of human began in the late 19th century and since then; improvements in techniques have facilitated the recovery, identification and enumeration of a wide variety of microbial species. Most wounds support relatively stable polymicrobial communities[1] often without signs of clinical infection. However, potential pathogens may be present and the delicate balance between colonized wound and an infected wound depends on the interplay of complex host and microbial influences. The development of wound and other bacterial infections has deleterious effect on patients by causing increased pain, discomfort, inconveniences and can lead to life threatening conditions or even death. Major challenges encountered with antibiotics in clinical use are resistance to antibiotics which leads eventually to failure of the treatment. Infectious diseases are known to be treated with herbal remedies throughout the history of mankind; even today, natural substances continue to play a major role in primary health care as therapeutic remedies in many developing countries[1]. Over the years, there have been reports of the production of more potent antibiotics e.g. third and fourth generation of cephalosporin by pharmaceutical companies which are not readily available and expensive. Problems of various antibiotics include low efficacy, side effect which has led investigations into natural and potent antibacterial seeming to be the right step to take. The invasion of pathogenic organism is on the rise as a result, effects are been made to develop antibacterial agent from natural sources for better therapeutic effect.

The therapies have drawn the interest of both public and medicinal communities. Current research has been focused on herbal and aromatherapy product. However, a number of their product such as honey has shown therapeutic promise. Honey was used to treat infected wound as long as 2000 years ago before bacteria were discovered to be the cause of infection. More recently, honey has been reported to have an inhibitory effect to around 60 species of

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bacterial including aerobes and anaerobes, Gram positive and Gram negative. The current prevalence of the therapeutic use of ancient remedies, include honey committee on science and technology. The healing effect of honey could be due to various physical and chemical properties[2] and the floral source has been reported to play a major role in its biological properties[3].

Methods

Source of honey samples: The honey samples used were sourced from two (2) different sources; Sunshine honey obtained from Ministry of Agriculture, Akure, Ondo State and Labsan honey bought from Shoprite Mall Akure, Ondo State (Table 1).

Table 1: Sources of honey samples used in the study

Sl. No.	LOCATION	FLORAL SOURCE
1.	Sunshine honey from Ministry of Agriculture, Akure, Ondo State	Multifloral
2.	Labsan honey from Ogun State, bought in Shoprite, Akure, Ondo State	Multifloral

Bacteria Isolates used and their sources: The test bacteria used were clinical *Staphylococcus aureus* and *Escherichia coli*. They were obtained from Microbiology Laboratory of Federal University of Technology, Akure Ondo State.

Antibiotic sensitivity of the selected bacteria isolates: This was done using disc diffusion method according to[4]. Standard inoculum of 24 hours broth was spread on Mueller – Hinton agar using sterile swab in triplicate. The plates were dried before placing the antibiotic disc at equidistance. The plates were incubated for 24 hrs at 37°C and diameter of zone of inhibition was measured and recorded.

Antibacterial assay of the honey types used: The antibacterial activities of the honey samples used on the selected test bacteria was done using agar well diffusion method according to[5]. A 0.1ml of each of the standard inocula was withdrawn with micropipette into a prepared plate of Mueller – Hinton agar and spread on the agar plate using a spreader. The plates were allowed to dry for 30mins after which a sterile cork borer (4 mm in diameter) was used to bore 7 wells in each plate, 0. 1ml of the local honey was introduced each into three of the wells on one side and 0.1ml of the control antibiotics was introduced into another set of three wells on the other side, sterile distilled water (0.1ml) was introduced in the 7th well in the middle. Another assay was set up using Labsan honey and the corresponding antibiotic as control. The plates were then incubated at 37°C for 24h. Clear zones of inhibition were measured showing antibacterial activity of the different honeys on the test bacteria.

Statistical Analysis: All experiments were done in triplicates. Mean, Standard deviation were calculated for all data using Descriptive Statistics, all data obtained were subjected to one way analysis of variance (ANOVA) using Minitab software and Difference between means was determined by Least Significance Difference (LSD) Test at $p \leq 0.05$.

Results

Antibiotic Sensitivity of Selected Bacteria Isolates: The results of antibiotic sensitivity of the selected bacteria showed that they displayed multi- antibiotic resistance to common antibiotics. *Staphylococcus aureus* was resistant to Norfloxacin, Amoxil, Streptomycin, Erythromycin and Ampiclox while the most sensitive antibiotic is Gentamycin (21.50mm) and the least are Ciproflox and Rifampicin (17.00mm) each (Table 2a). The second test isolate was *Escherichia coli*, resistant to all the antibiotics except Chloramphenicol (28.50mm), Ciprofloxacin (19.50mm), Gentamicin (17.50mm), Sparfloxacin (16.00mm) and Tarivid (4.50mm) (Table 2b).

Antibacterial activities of honey samples on selected bacteria isolate: All the two honey samples used in this study exerted varying degrees of growth inhibition of the selected test bacteria. The two honey samples; Sunshine and Labsan exerted the highest growth inhibitory activity on *Staphylococcus aureus* (26.50mm) and (20.83mm) respectively, (Figure 1a) compared to *Escherichia coli;* Sunshine and Labsan honey inhibitory activity (23.50mm) and (19.50mm) respectively (Figure 1b). On comparing the growth inhibition mediated by individual honey samples with the control antibiotics; Chloramphenicol and Gentamycin, it was observed that the antibiotics showed a little higher growth inhibition on the selected test bacteria than the honey samples (Fig. 1a and 1b).

Table 2a: Antibiotic Sensitivity Test for *Staphylococcus aureus*

Sl. No.	Antibiotics	Code (mcg)	Zone of inhibition (mm)
1.	Ciprofloxacin	CPX (10)	17.00
2.	Norfloxacin	NB (10)	0.00
3.	Gentamycin	CN (10)	21.50
4.	Amoxil	AML (20)	0.00
5.	Streptomycin	S (30)	0.00
6.	Rifampicin	RD (20)	17.00
7.	Erythromycin	E (30)	0.00
8.	Chloramphenicol	CH (30)	18.00
9.	Ampicloxin	APX (20)	0.00
10.	Levofloxacin	LEV (20)	19.50

Source: Optun Laboratories Nigeria Ltd.

Table 2b: Antibiotic Sensitivity Test for *Escherichia coli*

Sl. No.	Antibiotics	Code (µg)	Zone of inhibition (mm)
1.	Septrin	SXT (30)	0.00
2.	Chloramphenicol	CH (30)	28.50
3.	Sparfloxacin	SP (30)	16.00
4.	Ciprofloxacin	CPX (30)	19.50
5.	Amoxacillin	AM (30)	0.00
6.	Augmentin	AU (10)	0.00
7.	Gentamycin	CN (30)	17.50
8.	Pefloxacin	PEF (30)	0.00
9.	Tarivid	OFX (10)	4.50
10.	Streptomycin	S (30)	0.00

Source: Maxicare Medical Laboratory Nigeria

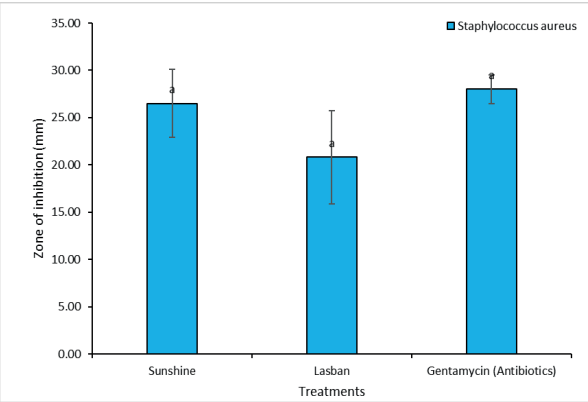

Figure 1a: Antibacterial activity of Sunshine and Labsan honey on *Staphylococcus aureus*.

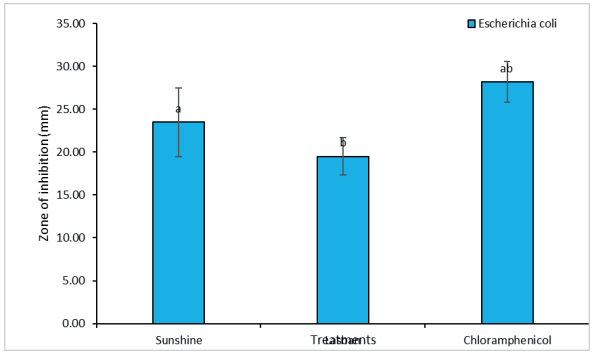

Figure 1b: Antibacterial activity of Sunshine and Labsan honey on *Escherichia coli.*

Discussion

The two honey samples used in this study inhibited the growth of the selected bacteria isolates. They showed varied antibacterial activities on the test bacteria. This result is an indication that honey can be a potential treatment for diseases caused by *Staphylococcus aureus* and *Escherichia coli*[6]. The antibacterial activity exerted by honey on the selected test bacteria in this study is in agreement with the findings of Adebolu[7] that pure honey has bactericidal activity against *Escherichia coli*. In this study, only undiluted or raw (Sunshine) honey (100% concentration) displayed greater antibacterial activities. This is in agreement with Sharma et al.[8] and Ahmadi-Motamayel et al.[9]. The reduction in antibacterial activity recorded with commercial (Labsan) honey in this study is in agreement with the work of Chen et al.[10]. The observation that Gram-positive bacteria like *Staphylococcus aureus* are more susceptible to honey than their Gram-negative counterpart disagrees with report of Al Naama[11] that Gram-negative bacteria showed increased susceptibility to honey than Gram positive bacteria in their study. The high susceptibility of Gram positive bacteria might be as a result of the high percentage of the peptidoglycan layer of the cell wall as compared to the low percentage found in the cell wall of Gram-negative bacteria. The result of this study agrees with previous report of honey completely inhibited major wound infectious pathogens such as *Staphylococcus aureus*[12,13]. Our results, however, agree with the report of Sohaimy et al.[14] and Almasaudi et al.[15] that *S. aureus* is the most susceptible bacterial species to the honey they collected from Iraq and Egypt respectively. Mohapatra et al.[16] also reported that *Staph. aureus* was the most sensitive to all the honey samples they worked on among the test bacterial strains they used.

Conclusion

This study has shown that the antibacterial activity of the two honey samples used in this study vary on the selected test bacterial isolates. For example, honey sample from Sunshine, Akure was the most effective in inhibiting the growth of clinical *Staphylococcus aureus* and *Escherichia coli*. These results clearly show that local honey samples like Sunshine honey in Nigeria are furnished with a broad spectrum antibacterial activity on the selected test bacteria. These findings therefore could be exploited in the treatment of wounds and other infections caused by these bacteria as an alternative to conventional antibiotics especially to which the test bacteria have developed resistance.

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