

## **ANTIBACTERIAL ACTIVITIES OF HONEY FROM DIFFERENT LOCATIONS IN THE TROPICAL RAIN FOREST BELT OF NIGERIA, ON SELECTED BACTERIAL ISOLATES**

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### **ABSTRACT**

Honey has been used for various purposes including treatment of diseases. This study was carried out to determine antibacterial activities of honey samples obtained from different locations in the tropical Rainforest Belt of Nigeria (Abeokuta, Aiyetoro, Ajebo, and Saki) against reference bacterial isolates: *Escherichia coli* (*E.coli*) (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), using agar well diffusion technique. *Staphylococcus aureus* showed sensitivity to all honey samples used with the zone of inhibition ranging between 1.9 and 2.6mm. *Escherichia coli* was resistant to all concentrations of honey samples used. However, the present study did not explore the possible causative agent(s) involved in the antibacterial activity of the honey used.

**Keywords-** Agar, Antibacterial activity, *E. coli*, Honey, *Staphylococcus aureus*

### **INTRODUCTION**

Honey (a by-product of honey bees, *Apis mellifera*) is widely produced and used in Africa. It has been used by humans for various purposes (sweetening of food, treatment of diseases and preservation of food) for decades. However, only in the past few decades have the antiseptic and antibacterial properties of honey been discovered and scientifically explained (Majno, 1975).

Honey is known in the medical profession as an agent for the treatment of some diseases and infections resulting from wounds and burns (Jeddar *et al.*, 1985). In most

cases, it is used in combating infections defying standard antibiotics and antiseptic therapy (Molan, 1992).

Evidences of the ability of honey to induce healing due to its antibacterial and antifungal properties have been well documented (Molan, 2006). Honey has proven very valuable in the treatment of topical infections resulting from surgical wounds and burns (Efem, 1988; Subrahmanyam, 1991; Molan and Betts, 2000), peptic ulcers, stomatitis, conjunctivitis, and gastroenteritis (Armon, 1980; Haffejee and Moosa, 1985).

The healing and antibacterial ability of honey can be largely attributed to physical factors e.g osmolarity, chemical factors e.g hydrogen peroxide, cecropin-A and mellitin, 3, 5-dimethoxy-4-hydrobenzoic acid (syringic acid), volatiles and certain unknown substances from certain flora sources (Bogdanov, 1984; Al-somal *et al.*, 1994). Honey also maintains a moist wound environment that promotes healing and has a high viscosity which helps to provide a protective barrier to prevent infection (Lusby *et al.*, 2005).

It has been shown that natural un-denatured honey has some broad-spectrum antibacterial activity when tested against some pathogenic as well as food spoilage bacteria (Mundo *et al.*, 2004; Lusby *et al.*, 2005, Olawuyi *et al.*, 2010). Antibacterial and antifungal properties of honey have been well documented against a number of Gram positive and Gram negative bacteria and vary with sources and processing (Allen *et al.*, 1991). Studies have also shown variability of antibacterial effects of honey on these bacteria. Furthermore, it has been shown that different honeys vary substantially in their antibacterial activity, which varies with the plant source (Allen *et al.*, 1991, Mundo *et al.*, 2004; Lusby *et al.*, 2005; Wilkinson and Cavanagh, 2005).

The aim of this study was to compare the antimicrobial activities of honey samples that were obtained from different locations in the tropical Rainforest Belt of Nigeria, against reference isolates of pathogenic *Escherichia coli* and *Staphylococcus aureus*.

## MATERIALS AND METHODS

### Sampling

Four unprocessed honey samples (100ml)

each were randomly collected from four different locations of the tropical Rain Forest Belt; one per study location between April and June, 2010). The locations included Abeokuta(AB){7°9' 39"N, 3°20'54"E } and Ajebo (AJ) {6° 32' 0"N, 3° 32' 0" E} (Ogun State), Saki(SA) {9°05' N, 3°51' E } (Oyo State) and Aiyetoro (AY) {7° 59' 0" North, 6° 0' 0" East} (Kogi State). The samples were collected directly from the bee keepers into air-tight, clean sterile bottles. Foreign matters such as dead bees and particles of combs were removed by straining the samples through cheesecloth. They were protected from light and stored at room temperature (25°C), until used. While part of the samples were kept undiluted (100%) other parts were prepared into different dilutions (20, 25, 33.3 and 50 % v/v) with sterile distilled water in sterile flasks as follows; 1:1 (50%), 1:2 (33.3%), 1:3 (25%) and 1:4(20%).

### Measurement of pH

The pH of honey samples was measured using a digital pH meter (Model HI, Hannah Instrument, US).

### Test organisms

Test organisms used for antibacterial susceptibility test were reference isolates of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) obtained from the culture bank of the Department of Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta.

### Source and dilution of standard drug

A concentration of 0.2% pharmaceutical grade ciprofloxacin, 2 mg/ml (ampoule) marketed as Cifran® (Fidson Drugs Nig. Ltd) was obtained for use as positive control.

***In-vitro antibacterial susceptibility tests***

Antibacterial activity of the different honey samples were evaluated using the agar well diffusion assay as previously described (Allen *et al.*, 1991). Pure cultures of test bacterial organisms were grown on nutrient agar. Three to five (3-5) colonies of each test organism was carefully picked from the nutrient agar and inoculated into 4-5ml of Mueller Hinton broth (Oxoid, UK) and incubated for 4-6 h at 35°C, until a turbidity of 0.5 McFarland standards was achieved. Where the turbidity exceeded the 0.5 McFarland standard, turbidity was adjusted with sterile saline. These result in suspension containing approximately  $1-2 \times 10^8$  CFU/ml of test bacteria (Woods and Washington, 1995). The suspension for each test bacterium (*Staphylococcus aureus* and *E.coli*) was then seeded evenly onto the surface of Mueller Hinton agar plates (Oxoid,UK) in quadruplicates with a sterile swab. A pair of Mueller Hinton agar plate (each inoculated with a different test organism) was used for each honey sample. Using a sterile 6 mm diameter cork borer, 7 wells were cut in the agar to which 50µl of each appropriate dilutions (20, 25, 33.3, 50 and 100% w/v) of honey were added. Also, added to the wells were standard drug-ciprofloxacin (2 mg/ml) and sterile distilled water which served as positive and negative control respectively. The plates were incubated at 37°C for 24-48 h under aerobic condition and

were thereafter examined at 24 h and 48h for zones of inhibition respectively.

**RESULTS**

The pH values of the four honey samples evaluated are as shown in (Table 1). The pH ranged from 3.4 to 4.0 with a mean of 3.7. The results of antibacterial activity of the honey samples evaluated are shown in Table 2. The data showed inhibitory effects of graded dilutions of honey samples on the test bacterial organisms as being dose-dependent. *Staphylococcus aureus* showed sensitivity to all of the four honey samples tested with the highest zone of inhibition of 2.6mm at 100% concentration recorded in honeys from Ogun state. The least zone of inhibition was 1.9mm at 20% recorded in Saki and Aiyetoro in Oyo and Kogi States respectively. *Escherichia coli* was observed to be resistant to all preparations of honey samples used in this study (Table 2). Although both test organisms (*E.coli* and *Staphylococcus aureus*) showed sensitivity to the standard antibiotic (Ciprofloxacin) which was used as positive control, Gram-negative *E.coli* was found to be more susceptible than Gram-positive *Staphylococcus aureus* with average diameter of zones of inhibition been 2.6mm and 1.3mm respectively (Table 2).

**Table 1: pH values of honey samples**

Honey samples	pH values
1. Abeokuta	3.4
2. Ajebo	3.5
3. Saki	3.8
4. Aiyetoro	4.0
Mean	3.7

**Table 2: Antibacterial activity of different honey samples**

Plate	Well1 (100%)	Well2 (50%)	Well3 (33.3%)	Well4 (25%)	Well5 (20%)	Well6 (0.2% Cip)	Well7 (D/W)
<b><i>E. coli</i></b>							
Diameter of zone of inhibition (mm)							
ABE	R	R	R	R	R	2.4	0
AJE	R	R	R	R	R	2.7	0
AYE	R	R	R	R	R	2.4	0
SAE	R	R	R	R	R	2.7	0
MEAN						2.6	0
<b><i>Staphylococcus aureus</i></b>							
ABS	2.6	2.5	2.5	2.4	2.3	1.2	0
AJS	2.6	2.4	2.4	2.3	2.1	1.2	0
AYS	2.5	2.4	2.4	2.3	1.9	1.4	0
SAS	2.4	2.4	2.1	1.9	1.9	1.4	0

**ABE**- Abeokuta honey + *E. coli*; **ABS**- Abeokuta honey + *Staphylococcus aureus*; **AJE**-Ajebo honey + *E. coli*; **AJS**- Ajebo honey + *Staphylococcus aureus*; **AYE**- Aiyetoro honey + *E. coli*; **AYS**- Aiyetoro honey + *Staphylococcus aureus*; **SAE**- Saki honey + *E. coli*; **SAS**- Saki honey + *Staphylococcus aureus*; **Cip**- Ciprofloxacin; **D/W**- Sterile Distilled Water; **R**-Resistant

## DISCUSSION

This study was carried out to determine the variation in the antibacterial activities of honey samples obtained from different locations in the same ecological zone. The pH values of the honey samples ranged from 3.4 to 4.0. The values correlate with pH range of 3.61 and 4.05 reported in some parts of Nigeria by Omafuvbe and Akanbi (2009). However, the pH range obtained in this study was lower than the range (4.31 - 6.0) reported for Nigerian honey from other locations (Adebisi et al., 2004). The acidic pH values of honey in this study is of note since healing which is caused by oxygen release from haemoglobin has been attributed to acidification (Leveen et al., 1973).

The results from this study showed that ciprofloxacin which was used as positive control showed marked efficacy against *E.coli* in all honey samples used while it was less efficient against *Staphylococcus aureus* in comparison to the honey samples. This study showed that honey from the tropical Rain Forest Belt of Nigeria have exceptional efficacy against *Staphylococcus aureus* as all four honey samples inhibited the growth of this organism. This organism has been previously reported to be exceptionally sensitive to the bactericidal effect of honey (Molan, 1992a). Although Agbaje et al. (2006) and Olawuyi et al. (2010), reported the inhibitory effect of some Nigerian honey samples on *Staphylococcus aureus* at 50 and 100% concentration respectively, the current study showed the inhibitory effect of the honey samples to as low as 20%. Some other workers had reported the complete inhibition of Methicillin Resistant *Staphylococcus aureus* (MRSA) at honey concentration of as low as 10% (Willix et al., 1992; Molan and betts, 2000).

The exhibited antibacterial activities to *Staphylococcus aureus* may have been due to the osmotic effect, the effect of acidic pH and the sensitivity of this organism to hydrogen peroxide (Postmes et al., 1997). The undiluted form of the honey samples utilized osmotic effect and acidic pH to inhibit the bacterial growth, while on dilution; they produced hydrogen peroxide, which helped them to maintain their antibacterial activities.

Although Olawuyi et al. (2010) reported that *E.coli* was sensitive to 50 and 100% of some Nigeria honey samples; result from the current study contradicts this assertion as *E.coli* showed resistance to all concentrations of the honey samples evaluated. However, this study agrees with the findings of Radwan et al. (1984) who observed that honey stopped the growth of *Escherichia coli*. Also, in a similar study, Nuriza-Tumin et al. (2006), reported the sensitivity of *E.coli*, to all honey samples evaluated in Malaysia. In a study by Cortopassi-Laurino and Gell (1991), *Escherichia coli* was shown to have the least susceptibility to honey amongst six other bacterial isolates; this is in agreement with the current study in which case the reference strain of *Escherichia coli* was not sensitive to the tested honey samples.

Abeokuta, Ajebo and Saki are all settlements in South-Western Nigeria and fall in the tropical Rain forest vegetation zone. However, Aiyetoro which is a settlement in the North-Central geographic zone falls in the boundary between the Guinea savannah vegetation zone of the North-Central and Rain Forest vegetation of the South-West. This transitional status of Aiyetoro may explain its close association in terms of flora and fauna and hence, the similarities observed in its honey antibacterial activity and those of the honey samples obtained in the

South-Western locations.

In similar studies, White *et al.* (1960) reported the influence of climatic condition on the antibacterial activities of honey, while Allen *et al.* (1991) showed that honey samples of the same floral source have similar antibacterial activities. These findings are in agreement with that of the current study since the study locations fall under similar ecological zones with similar flora. Hence, the antibacterial activities of these honey samples might have been influenced by the similar vegetation and climatic condition prevalent in their locations of collection. The differences observed in the antibacterial activity of the honey samples to the two bacteria organisms can be attributed to a number of reasons. One possibility might be related to the differences in susceptibility of the used bacteria organisms to the antibacterial activity of honey used.

Other possible explanation for these observations could be the differences in the phytochemical components of the antibacterial agent(s) present in these honeys. These agents may utilize hydrogen peroxide and non-peroxide antioxidant components. In an earlier work, Melissa *et al.* (2004) reported that dilution of honey enhances hydrogen peroxide mediated antibacterial activity. This may partly explain some of the antibacterial activity of these honey samples. However, the present study was unable to clarify the possible causative agent(s) involved in the antibacterial activity of the honey used. Identification and characterization of the active principle(s) may provide valuable information on the quality and possible therapeutic potential of these Nigerian honeys.

## CONCLUSION

The results obtained in this study showed that Nigerian honey can be used as antibacterial agents for some bacteria. Although, Nigeria honey is natural, available and safe, it will need to be purified and standardized to be used for therapeutic purposes.

## ACKNOWLEDGEMENT

The authors wish to acknowledge Dr F.O. Fashina of Section of Swine Health, Production Animal Studies Department, University of Pretoria, Onderstepoort, South Africa for the review of the manuscript, the technical assistance of Mr P.A. Akinduti and secretarial assistance of Miss Esther Iwegbu both of College of Veterinary Medicine, Federal University of Agriculture, Abeokuta.

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(Manuscript received: 18th May, 2011; accepted: 24th June, 2011).