ORIGINAL ARTICLE

# Variation in The Antimicrobial Potency of Honey Samples from Different Sources

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ABSTRACT

**Background:** Honey has been used for different purposes including management of wounds for centuries. Reports of considerable variations in the antimicrobial potency of honey samples from different sources exists but we found none from our sub-region. This comparative study tested the antibacterial activities of honey from five different sources in South-East Nigeria.

**Methodology:** The study involved 23 isolates from surgical wounds. Honey samples from five different sources were procured from the farmers. In-vitro antibacterial activity using dilution technique was done with the five honey samples and standard antibiotic susceptibility tests as control. The results were analysed by simple statistical methods and compared.

**Results:** All the honey samples inhibited the growth of isolates at neat concentration (without dilution) but their antimicrobial activities diminished as the samples were diluted. Honey samples from *Chorophora excels* (Iroko tree) and *Pentachlethra macrophyla* (oil bean tree) inhibited Proteus species at neat concentration only. Honey from rock inhibited methicillin resistant staphylococcus aureus (MRSA) at neat concentration only but honey from *Anarechadium occidentale* (Cashew tree) did same from a dilution of 1:2 and below. *Escherichia coli, Pseudomonas* species, *Klebsiella* species and *Proteus* were susceptible to Ciprofloxacin (used as quality control).

**Conclusion:** This work shows that antibacterial activity of honey differs according to sources. Honeys from *Anarchadium occidentale* (cashew) and *Vitex doniana* ("uchakiri or eli-eli") have higher efficacy in wound management than honeys from other sources in South-East Nigeria

***Key words**: Honey, Comparative studies, Anti-microbial activity, Wound healing*

## INTRODUCTION

The use of honey as a wound dressing agent dates from antiquity and the belief that the efficacy of honey is dependent on its source is common. Manuka honey is one such honey of high value but it is not available or affordable in all parts of the world. In many parts of the world including South-East Nigeria, there is no scientific evidence to justify the high premium

placed on honey from certain sources against others, hence the need for this study.

Several years before bacteria were discovered to be the cause of infections, honey was used for treatment of wounds and other diseases by people of various cultures.[1-8] Topically applied honey has been shown to accelerate healing more than the conventional agents used in dressing wounds like Edinburgh University solution of lime (EUSOL).[9,10]

Honey has anti-inflammatory, anti-oxidant, anti-bacterial, and anti-fungal actions which reduces the damage caused by free radicals from inflammation, thus preventing further necrosis.[5,6,7,8] It is effective in the treatment of burn wounds, surgical wounds, diabetic, chronic non healing ulcers, decubitus ulcers, Fournier's gangrene etc.[4-16] It is recommended for wound management in developing countries where better alternatives are hardly available nor affordable.[17]

It has been speculated that antibacterial activity of honey varies greatly with the source and processing. Most of the researches done with honey were based on honey in general, its use in the treatment of various ailments and comparison with other wound dressing agents. A few comparative studies with honey from different sources have been done in other climes,[6,7,8,18,19,20] but we are not aware of any such comparative work done in South-East Nigeria, providing justification for this work comparing the antibacterial activities of honey samples from five different sources locally available to us.

## METHODOLOGY

Bacterial isolates used were obtained from culture of wound specimens from patients in a tertiary health facility. These were isolated using Standard Microbiological procedures.

Isolates include: *Escherichia coli, Pseudomonas species, Klebsiella species, Proteus species, Methicillin Sensitive Staphylococcus aureus (MSSA)* and *Methicillin Resistant Staphylococcus aureus (MRSA).*

The control strains were: *Staphylococcus aureus* ATCC 29213, 43300 and *Escherichia coli* ATCC 13422.

**Sources of Honey Used**

The five different sources of honey samples used were:

(i) Rock
(ii) *Chorophora excelsa* (Iroko or "Oji")
(iii) *Anarehadium occidentale* (Cashew)
(iv) *Pentachlethra macrophyla* (Oil bean or "Ugba")
(v) *Vitex doniana* ("Uchakiri" or ("Eli-eli")

Undiluted and unsweetened honey samples were identified and labeled. They were preserved in a well corked dark bottles and stored in a refrigerator at temperature of +4$^{O}$C to +10$^{O}$C.

They were collected from Rocks and Trees in the town of Isuochi, Umunneochi L.G.A, Abia state, Nigeria.

**Antimicrobial Susceptibility Testing**

This was carried out using Mueller Hinton Agar (Oxoid) plates for both Test and Control organisms. The agar plates were inoculated with overnight culture of organisms standardized using 0.5 Mc Farland Standard and antibiotic susceptibility disks were placed onto the plate and incubated at 37$^{o}$C overnight. The antimicrobial susceptibility disks used include: Amoxycillin (AMX) 25ug, Cotrimoxazole (COT) 25ug, Chloranphenicol (CHL) 30ug, Amoxicillin clavulanic acid (AUG) 30ug, Erythromycin (ERY) 5ug, Tetracycline (TET) 30ug, Cloxacillin (CXC) 5ug, Gentamicin (GEN) 25ug, Ciprofloxacin (CIP) 25ug, Ofloxacin (OFL) 30ug, all from Oxoid. After over-night incubation, the plates were examined for zones of inhibition and the zone diameter measured and reported as: Sensitive (S), Resistant(R) or Intermediate (I) according to Standard Interpretation Chart.[21]

**Dilution of Honey Samples for The Study**

Antibacterial activity of honey samples towards the isolates and control organisms were analyzed using Brain Heart Infusion broth (Oxoid). The broth was prepared and dispensed in 2mls amount into tubes corked and sterilized at 121$^{o}$C for 15 minutes. Then two-fold serial dilutions of honey samples were made and with the controls as shown in the protocol below, 0.02ml of the broth was used to inoculate the honey that were doubly diluted.

**Determination of Minimum Bactericidal Concentration (MBC)**

With the aid of a sterile standard wire loop which measures 0.02ml, 0.02ml from the standardized inoculum for organism 'Y' was transferred in to each of the dilutions except the controls.

The tubes were incubated overnight and were sub cultured on to MacConkey agar plates including the controls. These were re-incubated overnight and the plates examined for presence or absence of growth.

The lowest concentration in the last tube that had no growth MacConkey agar is considered the minimum bactericidal concentration (MBC) for the particular honey sample.

**Figure 1.** Protocol for honey sample 'X'

| Tubes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Dilutions of honey in broth | Neat | 1/2 | 1/4 | 1/8 | 1/16 | 1/32 | Honey 'X' control | BHI broth control |

Honey sample 'X' = One of the honey samples from the 5 floral sources
Tube 6 = Control for dilution (No microorganism added)
Tube 7 = Control for honey sample 'X' (No microorganism added)
Tube 8 = Control for BHI broth sterility test

**Identification of Methicillin Resistant Staphylococcus Aureus (MRSA)**

Methicillin resistant *Staph aureus* (MRSA) was tested for using Cefoxitin disk diffusion method on Mueller-Hinton agar.[21] Direct colony suspension to obtain 0.5 McFarland turbidity using sterile swab sticks dipped in the suspension and streaked on the agar plate to make a lawn wait for some minutes and 30ug Cefoxitin disk was applied and incubated for 24 hours. Less than/equals 21 mm zone diameter was taken as MRSA positive and zone diameter greater than or equals 22 mm was considered MRSA negative *S. aureus* ATCC 29213 (sensitive) and *S. aureus* ATCC 43300 (resistant) were used as quality controls.[21]

**Ethical Issues**

Ethical approval for the study was obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi

RESULTS

The antibacterial activities of honey specimens from five sources were studied against some common aerobic organisms associated with wound infection. The overall susceptibility pattern of Gram negative isolates to the antibiotic disc showed that the highest inhibition to the bacterial isolates was seen with Ciprofloxacin as shown in the Table 2.

The susceptibility testing done on the Gram positive organisms *Methicillin Resistant Staphylococcus aureus* (MRSA) and *Methicillin Sensitive Staphylococcus aureus (MSSA)* revealed that out of the 9 antibiotic discs tested, only Ciprofloxacin (100%),

Augmentin (81.8%)and Gentamicin (72.7%)were susceptible; Tetracycline (60%) and Chloraphenicol (63.6%) were intermediate; others were resistant (This is as seen in Table 3).

The minimum inhibitory concentration of the five honey samples against the isolates showed that samples from *Chorophora excelsa* (Iroko tree) and *Pentachlethra macrophyla* (Oil bean) could only inhibit Proteus species at neat concentrations (without dilution), while honey from *Vitex doniana* was most active with minimum bactericidal concentration (mic) as low as 1:8 in some isolates. This is shown in Table 4.

**Table 2.** Overall Susceptibility pattern of Gram negative isolates to the antibiotics

| **Isolates** | **NO** | **AMX** | **COT** | **GEN** | **OFL** | **AUG** | **TET** | **CIP** |
|---|---|---|---|---|---|---|---|---|
| *E. coli*(control strain) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| *E. coli* | 5 | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| *Pseudomonas spp* | 6 | 0 | 0 | 1 | 4 | 1 | 0 | 6 |
| *Klebsiella spp* | 6 | 0 | 0 | 0 | 0 | 0 | 1 | 4 |
| *Proteus spp* | 5 | 0 | 0 | 1 | 3 | 1 | 0 | 4 |
| **Total** | **23** | **1** | **1** | **4** | **9** | **3** | **2** | **16** |
| % | **100** | **4.3** | **4.3** | **17.3** | **39.1** | **13** | **8.69** | **69.5** |

The overall susceptibility pattern of Gram negative isolates to the antibiotic disc tested showed that the highest inhibition to the organisms was seen with Ciprofloxacin.

The susceptibility testing done on the Gram positive organisms, *Methicillin Resistant Staphylococcus aureus* and *Methicillin Sensitive Staphylococcus aureus (MRSA* and *MSSA)* revealed that out of the 9 antibiotic discs tested, only Ciprofloxacin (100%), Amoxicillin clavulanic acid (81.8%), Gentamicin (72.7%), Tetracycline and Chloramphenicol (63.6%) inhibited these isolates above 60%. Others inhibited the isolates below 55%.

## DISCUSSION

The determination of antibacterial activities of honey samples from five different sources showed that honey has in-vitro antibacterial activities against these isolates commonly incriminated in human diseases and wound infections - *E. coli*, *Pseudomonas spp, Klebsiella spp, Proteus spp,* Methicillin sensitive and Methicillin Resistant *Staphylococcus aureus* (MSSA and MRSA).

**Table 3.** Susceptibility pattern of Staphylococcus (both *MRSA* and *MSSA*) isolates to the antibiotics

| **Isolates** | **NO** | **AMX** | **COT** | **CHL** | **AUG** | **ERYTH** | **TET** | **CXC** | **GEN** | **CIP** |
|---|---|---|---|---|---|---|---|---|---|---|
| MSSA (Control) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| MSSA | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| MRSA | 5 | 0 | 0 | 1 | 3 | 0 | 1 | 0 | 2 | 5 |
| **TOTAL** | **11** | **6** | **6** | **7** | **9** | **6** | **7** | **6** | **8** | **11** |
| % | **100** | **54.5** | **54.5** | **63.6** | **81.8** | **54.5** | **63.6** | **54.5** | **72.7** | **100** |

**Table 4.** Minimum Bactericidal Concentration of the five honey samples against bacterial isolates

| **Tested isolates** | | **1** ***Rock*** | **2** ***Chlorophora excelsa*** | **3** ***Anarehedium occidentale*** | **4** ***Pentachlethra macrophyla*** | **5** ***Vitex doniana*** |
|---|---|---|---|---|---|---|
| ***E.coli*** | Control | 1/4 | 1/4 | 1/2 | 1/2 | 1/8 |
| | 1 | 1/4 | 1/4 | 1/2 | 1/2 | Neat |
| | 2 | Neat | Neat | Neat | 1/2 | Neat |
| | 3 | Neat | Neat | Neat | Neat | 1/4 |
| | 4 | 1/2 | Neat | Neat | Neat | 1/8 |
| | 5 | 1/2 | Neat | Neat | Neat | 1/2 |
| ***Pseudomonas spp*** | | | | | | |
| | 1 | 1/2 | 1/4 | 1/2 | Neat | 1/2 |
| | 2 | 1/2 | 1/4 | 1/4 | Neat | 1/4 |
| | 3 | 1/4 | 1/4 | 1/4 | 1/2 | 1/2 |
| | 4 | 1/2 | 1/4 | 1/4 | Neat | 1/8 |
| | 5 | 1/2 | 1/4 | 1/4 | 1/2 | 1/2 |
| | 6 | 1/4 | 1/8 | 1/4 | Neat | 1/4 |
| ***Klebsiella spp*** | | | | | | |
| | 1 | Neat | 1/4 | 1/4 | 1/2 | 1/4 |
| | 2 | Neat | 1/4 | 1/4 | Neat | 1/4 |
| | 3 | 1/2 | Neat | 1/4 | Neat | 1/4 |
| | 4 | Neat | 1/4 | 1/4 | Neat | 1/2 |
| | 5 | 1/2 | Neat | 1/4 | Neat | 1/2 |
| | 6 | 1/2 | Neat | 1/4 | Neat | 1/2 |
| ***Proteus spp*** | | | | | | |
| | 1 | 1/2 | Neat | 1/2 | Neat | 1/8 |
| | 2 | 1/4 | Neat | 1/2 | Neat | 1/4 |
| | 3 | Neat | Neat | 1/4 | Neat | 1/2 |
| | 4 | 1/2 | Neat | 1/2 | Neat | 1/2 |
| | 5 | 1/2 | Neat | 1/4 | Neat | 1/8 |
| ***MSSA*** | Control | 1/4 | 1/8 | 1/4 | 1/4 | 1/4 |
| | 1 | 1/2 | 1/4 | 1/4 | 1/2 | 1/8 |
| | 2 | 1/2 | 1/4 | 1/4 | 1/4 | 1/8 |
| | 3 | 1/2 | 1/4 | 1/4 | 1/4 | 1/8 |
| | 4 | 1/2 | 1/4 | 1/4 | 1/2 | 1/8 |
| | 5 | 1/2 | 1/8 | 1/4 | 1/4 | 1/8 |
| ***MRSA*** | | | | | | |
| | 1 | Neat | 1/8 | 1/2 | Neat | Neat |
| | 2 | Neat | 1/4 | 1/2 | 1/4 | 1/8 |
| | 3 | 1/2 | 1/4 | 1/4 | 1/4 | 1/8 |
| | 4 | Neat | 1/4 | 1/4 | Neat | 1/2 |
| | 5 | 1/2 | 1/4 | 1/2 | 1/4 | 1/2 |

It was noted that antibacterial activities of honey samples were not the same but varied according to the source. This is important in choosing honey to be used for various purposes and manufacturers of medical grade honey should state their floral sources to make this choice possible.

The use of honey from locally available floral sources for this study is important for practitioners in this sub region to be properly guided. Secondly, this provides a baseline for future studies in this field and adds to the body of literature.

This finding of difference in efficacy of honey samples based on sources agrees with work done with honey from different sources in other countries.[6,7,8,18,19,20] Ranzato *et al.* examined the wound healing properties of honey using three widely used monofloral honeys and found out that acacia (Black locust, *Robina pseudoacacia*) and Buckwheat (*Fagopyrum spp*) are better than Manuka (*Leptospermum scoparium*) in efficacy.[6] Carnwath and colleagues worked on equine wound bacterial isolates with 29 products from different sources found that of the 8 products effective against the 10 isolates of concentrations ranging from <2%-16% v/v, found that Scottish heather honey is better than others.[18] On the contrary, Suleiman and Al-Nahari found that the effect was concentration and the type of the honey dependent; and Manuka honey (UMF+20) had a better effect.[19,20]

We found that honey from *Anarchadium occidentale* (cashew) exhibited inhibitory action on the isolates even at a higher dilution (lower concentration) including control for *E. coli* (dilution 1:2). Honey from *Vitex doniana* ("Uchakiri" or "Eli –eli") exhibited inhibitory action on *E. coli* more at neat concentration than honey sample from *Anarchadium occidentale* (cashew). Honey from *Anarchadium occidentale* and Vitex *doniana* inhibited all the isolates at higher dilutions (lower concentrations) than at neat concentrations observed with honey from *Chorophora excelsa* (Iroko or "Oji") and *Pentachlethra macrophyla* (oil bean or "Ugba"). Honey from *Pentachlethra macrophyla* inhibited *Klebsiella* at neat concentration and honey from Rock inhibited *MRSA* at neat concentration. Staphylococcus species (both *MRSA* and *MSSA*) were susceptible to honey, especially from *Anarchadium occidentale* (Cashew), *Vitex doniana* ("Uchakiri" or "Eli-eli") and *Chorophora excelsa* (Iroko or "Oji"), giving support to the work done by Molan and Natarajan.[11,22] Generally, samples from *Anarchadium occidentale* and *Vitex doniana* showed higher susceptibility to most of the isolates than other honey samples and at a higher dilution. The work confirms that antibacterial activity of honey differs according to sources. It is recommended that uncontaminated pure honey be used or sterilized, laboratory- tested honey to get rid of bacteria (including spores of Clostridia) and fungi which may contaminate the honey samples.[3,12,18,23] Indeed, Carnwath found out that of the 29 products from different sources, culture revealed aerobic fungi and bacteria in 18 samples and Shapiro reported botulism in his study.[18,23]

The limitations of the study include the fact that this is a single centre study. Multi-centre studies may be needed for further validation.

## CONCLUSION

This work shows that antibacterial activity of honey differs according to sources and this should be considered in selecting honey for wound management.

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