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IN VITRO ANTIBACTERIAL ACTIVITY OF HONEY ON SOME BACTERIA ISOLATED FROM WOUND

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ABSTRACT

The moist environment of chronic wounds is an ideal growth medium for bacteria thereby causing delay in healing. Honey is commonly used as a topical antibacterial agent in most cases to complement the conventional antibiotics treatment. This work was carried out to determine the invitro antibacterial activity of honey on some wound isolates. One hundred (100) swab samples were obtained from wounds of patients who consented to participate in the study. The specimens were streak inoculated onto Chocolate agar and Cysteine Lactose Electrolyte Deficiency agar (CLED). Cultures were incubated at 37^0C for 18 to 24hours. After incubation bacteria isolates were identified by cultural characteristic, Gram staining technique and biochemical tests. Results from the study revealed that *Staphylococcus aureus* was the most prevalence bacteria isolates 35(41.7%), followed by coagulase negative staphylococcus (CoNS) 19(22.6%), *Escherichia coli* 6(7.1%), *Klebsiella spp.* 2(0.4%), *Proteus mirabilis 3*(3.6%), *Proteus vulgaris* 11(13.1%), *Streptococcus spp* 7(8.3%) and *Pseudomonas aeruginosa* 1(1.2%). The Minimum inhibitory concentration of honey against the wound isolates shows *Pseudomonas aeruginosa* with the highest MIC (50 mg/ml). The present study has demonstrated the antibacterial activity of honey on wound isolates. However, findings from this study are expected to add to the body of knowledge regarding wound infections.

KEYWORDS: Honey, antibacterial activity, *Staphylococcus aureus.*

INTRODUCTION

The medicinal properties of honey have been known since ancient times.[15] Honey has been useful in the treatment of surgical wounds and burns and the antibacterial and antifungal properties of honey have been well documented.[16] In burns in particular, honey has been

found to control wound infection and accelerate wound healing.[16] Antimicrobial agents have been applied to wound for thousands of years.[12] Major challenges encountered with antibiotics in clinical use are resistance to antibiotics which leads eventually to failure of the treatment.[4] Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has lead to the emergence of antibiotics-resistance strains which, in turn, has driven the continued search for new agents.[12]

Honey is commonly used as a topical antibacterial agent to treat wound infection.[6] Several studies have also shown that difficult-to-heal wounds respond well to honey dressing.[18] In addition honey reverses the inflammation, swelling, pain, attracts macrophages, and accelerates sloughing of devitalized tissue and formation of a healthy granulation bed.[9]

Honey is highly variable like most plant derived products and the chemical composition of honey also depends on the flower from which it is made therefore antibacterial effect may vary between different types of honey.[2] Several honey such as Medihoney, Manuka honey (New Zealand), Jellybush honey (Australia) and Raw honey has been reported to inhibit more than 80 species of bacteria.[10] According to Maddock, honey can disrupt the interaction between *Streptococcus pyogenes* and the human protein fibronectin on the surface of damaged cells.[10]

However, the antibacterial activity of honey is mainly attributed to osmolarity and enzymic generation of hydrogen peroxide.[9] The generation of low level H_2O_2 has been shown to improve oxygen delivery to tissues needed for tissues regeneration. Other substances in honey that enhance the antibacterial activity include flavonoids, lysosomes, phenolics and many other unidentified properties.[16] The high osmolarity (17% water and 83% sugar primarily glucose and fructose) and low pH (3.6-3.7) enhances antibacterial activity by prevents the growth of bacteria and encourages healing.[1]

However, there is increasing interest in the use of topical antimicrobial in the treatment of wound infection. Compounds such as honey have been incorporated into wound dressings.[17] Having enumerated the medical importance of honey especially in this era of multi drug resistant species of bacteria. The present study focuses on the *in vitro* antibacterial activity of wild honey against some bacteria isolates from wound.

MATERIALS AND METHODS

Study area and population

The study population includes patients who attended Bingham University Teaching Hospital and Daisyland Orthopedic Hospital located within Jos metropolis in Plateau State, Nigeria.

Ethical consideration

Prior to sample collection ethical clearances were sought and obtained from the aforementioned hospitals.

Source of Honey

The honey used for the was sourced locally from commercial a honey shop (D shy Honey shop) located in Jos metropolis.

Sample collection and processing

One hundred wound swabs were collected from both in and out patients receiving care at the Bingham University Teaching Hospital and Daisyland Orthopedic Hospital in Jos. Samples were streak inoculated onto Chocolate agar and Cysteine Lactose Electrolyte Deficient Agar (CLED). The culture plates were incubated aerobically at 37^{O}C for 24hours. Identification of bacterial isolates was done by cultural characteristics, Gram staining technique and biochemical tests (Cheesbrough,).

Standardization of inocula

The test organisms were inoculated by transferring representative organisms from fresh culture plate into sterile saline bottle. The mixture was shaken to achieve homogenous suspension. The homogenous suspension was later adjusted to 0.5 McFarland's standard.[11]

Antibacterial activity of Honey

The antibacterial efficacy of honey was tested by agar well diffusion method.[3] The cultures from the standardized broth were aseptically swabbed on sterile dried Mueller Hinton agar plate using sterile cotton swabs. Wells of 6 mm diameter were bored on the inoculated culture plates using a sterile borer, the base of each hole was filled with molten nutrient agar to seal the bottom of the plate. The test honey was prepared by diluting in sterile distilled water at different dilution (concentration) Net, 50%, 25%, 12.5% also net honey (100%). Aliquots of 100 µl volume of different concentrations were transferred into labeled wells. The wells were also filled with 100 µl positive control (ciprofloxacin10µg) and distilled water was used as

negative control. The plates were incubated at 37^0C for 24 hrs and the zones of inhibition were recorded.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) is defined as the lowest concentration of honey that inhibits the growth of the test organisms as indicated by the absence of visible turbidity in the tube compared with the control tubes. Briefly, dilutions of honey were made using nutrient broth in two fold serial dilutions in test tubes. An overnight broth culture of the test organism was adjusted to McFarland turbidity standard and 50 µl of the cell suspension was added to each of the tubes. The tubes were incubated aerobically at 37°C for 18-24hours. The MIC was indicated by the highest dilution of honey that showed no visible growth of the test organism.

RESULTS

Table 1 shows the percentage distribution of bacteria isolated from wound samples, *Staphylococcus aureus* was the predominant bacterial isolates 35 (41.7%), followed by coagulase negative staphylococcus (CoNS) 19(22.6%), *Escherichia coli* 6(7.1%), *Klebsiella spp.* 2(0.4%), *Proteus mirabilis 3*(3.6%), *Proteus vulgaris* 11(13.1%), *Streptococcus spp* 7(8.3%) and *Pseudomonas aeruginosa* 1(1.2%).

All concentrations of honey were active against test organisms. The undiluted honey (18.1±3.7) and positive control (22.3± 5.2) showed a wider zone diameter compared to other dilutions 50% (15.9±3.5), 25% (12.4±3.5) and 12.5% (8.6± 3.6) as recorded in table 2.

The minimum inhibitory concentration (MIC) of honey against bacteria isolates tested was recorded in table 3. The least MIC (6.25mg/ml) value was noted in *Staphylococcus aureus*, CoNS and *Streptococcus spp*., whereas *Pseudomonas aeruginosa* had the highest MIC (50 mg/ml).

Table1: Percentage distribution of Bacteria isolated from wound specimens

Bacterial isolates	No. of occurrence	Percentage
Escherichia coli	6	7.1
Klebsiella spp.	3	3.6
Proteus mirabilis	11	13.1
Proteus vulgaris	1	1.2
Pseudomonas aeruginosa	35	41.7
Staphylococcus aureus	7	8.3

Coagulase negative staphylococcus	19	22.6
Total	84	100.0

Table 2: Antimicrobial activity of Honey on Bacterial isolated from wound specimens (Zone diameter in mm)

Test organisms	Net(mm)	50%(mm) (ciprofloxacin)	25% (mm)	12.5%(mm)	control
Escherichia coli	15.6±3.8	15.3±2.7	13.0±2.4	9.1± 1.8	21.3±7.2
Klebsiella spp	24.0±5.6	21.0±1.4	17.0±1.4	12.0±2.8	33.0± 7.0
Proteus mirabilis	17.3±2.3	15.0±1.7	12.6±1.1	8.6± 1.1	22.0± 2.0
Proteus vulgaris	18.0±3.7	16.1±3.5	13.9±3.1	8.5± 2.9	21.4± 5.8
Pseudomonas aeruginosa	26.0±0.0	24.0±0.0	20.0±0.0	16.0±0.0	31.0± 0.0
Staphylococcus aureus	18.6±3.3	16.7±3.5	12.2±4.1	8.6± 4.4	22.2± 4.1
Streptococcus spp.	16.0±4.1	13.4±2.7	11.0±2.5	8.5± 2.9	20.5± 6.1
CoNS	17.7±3.5	14.7±3.2	11.5±2.8	16.0±0.0	22.7± 5.2
Total	18.1±3.7	15.9±3.5	12.4±3.5	8.6± 3.6	22.3± 5.2

Key: CoNS – Coagulase negative staphylococcus

Table 3: Minimum inhibitory concentration (MIC) of Honey on Bacterial isolates from wound specimens

Test organism	MIC (mg/ml)
Escherichia coli	12.5
Klebsiella spp	25
Proteus mirabilis	12.5
Proteus vulgaris	12.5
Pseudomonas aeruginosa	50.0
Staphylococcus aureus	6.25
Streptococcus spp.	6.25
CoNS	6.25

CoNS – Coagulase negative staphylococcus

DISCUSSION

A wide range of bacterial species has been shown to be inhibited by honey but reported susceptibilities from different studies are not consistent. Failure to identify the sources of honeys used in many of those studies, or determination of their antibacterial potency often makes comparison of reported sensitivity unreliable. However, the inhibition of antibiotic resistant bacteria by honey has not been fully documented.

This present study has revealed *Staphylococcus aureus* as the most prevalent wound isolates 33 (41.7%) which is in agreement with the work of [20] that *Staphylococcus aureus* is the most common wound pathogen.

The antibacterial activity of honey indicated that the Neat (undiluted) honey produced the widest zones of inhibition and this is supported by various studies.[13] that honey becomes ineffective when there is a high dilution by wound exudates. On the contrary.[2] reported that maximum antibacterial activity can be achieved when diluted to concentration between 50% and 30%. The antibacterial activity was demonstrated on both Gram positive and Gram negative bacteria, Gram negative bacteria were more susceptible to honey. This agrees with a study by.[13] that honey has more activity against both Gram positive than Gram negative organisms.

The highest MIC 50 mg/ml (the concentration to which honey can be diluted by wound exudate and still prevent bacterial growth) was recorded in *Pseudomonas aeruginosa* while the least MIC 6.25mg/ml was obtained for *Staphylococcus aureus*, CoNS and *Streptococcus* spp. This correlates with the studies conducted by.[7] and[5] that *Pseudomonas aeruginosa* was more resistant to honey than other bacterial isolates. In a similar study, a low MIC was observed for *Staphylococcus aureus*.[19]

CONCLUSION

In conclusion honey was found to possess high antibacterial activity against bacteria causing wound infections. Though the source of the honey is a very important, it is more affordable, available and safer than systemic antibiotics and may well complement conventional therapies in the future.

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