

CONCENTRATION-DEPENDENT ANTIMICROBIAL ACTIVITY OF HONEY AGAINST STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, AND STREPTOCOCCUS PYOGENES FROM WOUND INFECTIONS: IMPLICATIONS FOR ALTERNATIVE WOUND-HEALING MANAGEMENT

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ABSTRACT

*The rise of antimicrobial resistance among wound pathogens has led to the need for affordable and effective alternative treatments. Honey has received renewed scientific interest because of its wide-ranging antimicrobial, anti-tumour, anti-inflammatory, antioxidant and wound-healing effects. This study investigated the antibacterial activity of natural honey obtained from Nsukka town, Enugu state Nigeria against common wound clinical isolates: Staphylococcus aureus, Escherichia coli, and Streptococcus pyogenes. Clinical isolates from wound samples were collected and identified using standard microbiological and biochemical methods. Serial dilution of the natural honey sample was made to concentrations of 25%, 50%, 75%, and 100% (v/v). Antibacterial activity was evaluated using the agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined through broth dilution and sub-culture techniques, respectively. Data was analyzed using one-way analysis of variance (ANOVA) at $p < 0.05$. The natural Honey sample showed concentration-dependent antibacterial activity against all test isolates. At 100% concentration, the average inhibition zones were 21.3 ± 0.5 mm for *S. aureus*, 19.8 ± 0.4 mm for *E. coli*, and 22.0 ± 0.5 mm for *S. pyogenes*. The MIC values were 25%, 50%, and 25% for *S. aureus*, *E. coli*, and *S. pyogenes*, respectively; the MBC values were 50%, 75%, and 50%, respectively. Statistical analysis of the results showed that there is no significant difference in the activity of honey against the isolates at $p < 0.05$. Gram-positive organisms (*S. pyogenes* and *S. aureus*) were significantly more susceptible than the Gram-negative isolate (*E. coli*). Natural honey showed substantial antibacterial activity against key wound pathogens, with effectiveness increasing with concentration. These results suggest that honey could serve as an affordable supplementary antimicrobial agent in wound care.*

KEYWORDS: Honey, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*, antimicrobial resistance, antibacterial activity, wound-healing effects.

Introduction

The global rise of antimicrobial resistance (AMR) has significantly reduced the effectiveness of conventional antibiotics. Consequently, there is increasing interest in alternative antimicrobial agents derived from natural products. Honey, is a natural sweet substance produced by Bees of the *Apis mellifera species* (Crane, 2015). It is a concentrated aqueous solution composed principally of a mixture of glucose and fructose, and at least 22 other complex carbohydrates, various amino and organic acids, proteins, antibiotic rich inhibine, enzymes, phenol antioxidants, aroma compounds, vitamins, minerals, pigments, waxes and pollen grains. It is viscous and acidic in nature with a pH range of 3.2- 4.5 and has been used therapeutically for centuries (Bogdanov *et al.*, 2017; Okeke *et al.*, 2018). Studies have revealed its antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties (Bogdanov, 2017). Honey exerts antibacterial effects through multiple mechanisms, including high osmolarity, acidic pH, hydrogen peroxide generation, and antimicrobial peptides. These multifactorial mechanisms reduce the likelihood of bacterial resistance compared with conventional antibiotics (Jenkins *et al.*, 2015; Da Silva *et al.*, 2016). Studies have highlighted the role of the phytochemical constituents of Honey- bioactive phenolic compounds (phloretin, p-coumaric acid, and 3-phenyllactic acid) and flavonoids. Results showed that these compounds enhanced the inhibition of bacterial growth even at sub-inhibitory concentrations (Nowak *et al.*, 2024).

Wound infections remain a major healthcare challenge worldwide. They are associated with delayed healing, prolonged hospitalization, increased healthcare expenditure, and elevated mortality rates. Common bacterial agents implicated in wound infections include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*. These are organisms known to be capable of causing severe tissue destruction and systemic complications (Gambo *et al.*, 2018). Traditionally, Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds. However, scientific investigations continue to demonstrate the broad-spectrum antibacterial activity of honey against wound pathogens (Akinmoladun *et al.*, 2023; Mustafa *et al.*, 2025), and the safety and synergistic activity of honey with other natural products such as *Moringa oleifera* and *Lactobacillus spp* probiotics (Onyeka *et al.*, 2022; El-Housseiny *et al.*, 2023).

Although several studies have reported the antibacterial activity of honey against clinically important pathogens, information on its effectiveness against wound-derived bacterial isolates remains limited in many developing countries, such as Nigeria. This study therefore investigated the antibacterial activity of natural honey from Nsukka, Enugu state, Nigeria against clinical isolates of *S. aureus*, *E. coli*, and *S. pyogenes* obtained from infected wounds.

Materials and Methods

Study Design

An experimental laboratory-based study was conducted to evaluate the antibacterial activity of natural honey against wound-associated bacterial pathogens.

Collection of Honey Samples

Raw, unprocessed honey was purchased from Ogige Market, Nsukka, Enugu State, Nigeria. Samples were aseptically transferred into sterile containers and stored at 4°C until analysis.

Isolation and Identification of Test Organisms

Clinical wound specimens were obtained from patients attending Enugu State University Teaching Hospital (ESUTH-Parklane Hospital), Enugu. Isolation was carried out using selective culture media including Mannitol Salt Agar, MacConkey Agar, and Blood Agar. Identification was based on colony morphology, Gram staining, and biochemical characterization.

Preparation of Honey Concentrations

Serial dilutions of Honey were prepared using sterile distilled water: 25%, 50%, 75%, and 100% (v/v).

Preparation of Bacterial Inoculum

Pure cultures were standardized to 0.5 McFarland turbidity equivalent to approximately 1.5×10^8 CFU/mL.

Antibacterial Assay

Antibacterial activity was determined using the agar well diffusion method on Mueller–Hinton agar. Wells of 6 mm diameter were filled with different honey concentrations. Standard antibiotic discs served as positive controls while sterile distilled water served as the negative control.

Determination of Minimum inhibitory concentration (MIC)

MIC was determined using the broth dilution method. The lowest concentration showing no visible growth after incubation was recorded as the MIC.

Determination of Minimum bactericidal concentration (MBC)

Aliquots from MIC tubes showing no visible growth were cultured on Mueller–Hinton agar. The lowest concentration producing no bacterial growth was recorded as the MBC.

Statistical Analysis

Experiments were conducted in triplicate. Results were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA, and differences were considered significant at $p < 0.05$.

Results

Table 1. Antibacterial Activity of Honey against Wound Isolates

Test Organisms	25%	50%	75%	100%	Positive Control (Antibiotic disc)	Negative control (Distilled water)
<i>S. aureus</i>	8.3 ± 0.6	12.7 ± 0.5	17.1 ± 0.8	21.3 ± 0.5	24.0 ± 0.3	0.0
<i>E. coli</i>	7.1 ± 0.5	11.4 ± 0.7	15.6 ± 0.9	19.8 ± 0.4	22.5 ± 0.6	0.0
<i>S. pyogenes</i>	9.0 ± 0.7	13.5 ± 0.6	18.2 ± 0.4	22.0 ± 0.5	25.2 ± 0.4	0.0

Table 2. MIC and MBC Values of Honey against Wound Isolates

Test Organisms	MIC (%)	MBC (%)
<i>S. aureus</i>	25	50
<i>E. coli</i>	50	75
<i>S. pyogenes</i>	25	50

Discussion

This study investigated the antibacterial activity of Natural Honey from Nsukka town, Enugu State, Nigeria against some common wound pathogens (*Staphylococcus aureus*, *Staphylococcus pyogenes* and *Escherichia coli*). Previous studies have demonstrated antibacterial effect of honey on some pathogenic bacteria of the urinary tract, gastrointestinal tract and wound infections (Moloney, 2016).

The natural honey samples in this study exhibited increasing antibacterial activity with increasing concentration against the clinically important wound pathogens (Table 1); thus, indicating a concentration-dependent response. At 100% honey, *S. pyogenes* showed highest activity (22.0 ± 0.5), followed by *S. aureus* (21.3 ± 0.5) while *E. coli* showed least activity (19.8 ± 0.4). At 20% honey, *S. pyogenes* showed highest activity (9.0 ± 0.7), followed by *S. aureus* (8.3 ± 0.6) while *E. coli* showed least activity (7.1 ± 0.5). This study is supported by Usanga *et al.*, (2020), who studied antibacterial effect of honey from Ishielu, Ebonyi State, Nigeria. Also, studies by Okeke *et al.*, (2018) who compared the antibacterial efficacy of honey from Nsukka and Ugwuaji towns of Enugu State, Nigeria on wound pathogens. Greater susceptibility was observed among Gram-positive bacteria (*S. pyogenes* and *S. aureus*) when

compared with Gram-negative bacteria (*E. coli*). This may be attributed to structural differences in bacterial cell envelopes. Gram-negative organisms possess an outer lipopolysaccharide membrane that limits penetration of antimicrobial compounds (Usanga *et al.*, 2020).

The MIC and MBC findings further demonstrated the stronger susceptibility of *S. aureus* and *S. pyogenes* when compared with *E. coli* (Table 2). These observations corroborate previous reports and similar findings reported by Akinmoladun *et al.*, 2023, showing that Gram-positive wound pathogens are more readily inhibited by honey than Gram-negative organisms.

The antibacterial activity observed in honey may be attributed to the high osmotic pressure exerted by the sugar component of honey (Molan and Cooper, 2000; Gambo *et al.*, 2018), and to the synergistic action of its physicochemical properties and bioactive components, including hydrogen peroxide (Ali, 2004; Brudzynski, 2006; Gambo *et al.*, 2018), acidic pH, osmotic stress, flavonoids, phenolic acids, and bee defensin-1 (Wahdan, 1998; Gambo *et al.*, 2018) and enzyme content including glucose oxidase (White *et al.*, 1963; Gambo *et al.*, 2018). Unlike conventional antibiotics that target specific bacterial pathways, honey acts through multiple mechanisms, reducing the likelihood of resistance development (Usanga *et al.*, 2020).

Conclusion

Natural honey from Nsukka, Enugu State, Nigeria has demonstrated significant antibacterial activity against wound-derived isolates: *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*. Antibacterial efficacy increased with concentration, with Gram-positive bacteria exhibiting greater susceptibility than Gram-negative bacteria. Thus, crude honey locally sourced within Nigerian locality may be used as an effective supplementary antibacterial agent for wound management. Also, the findings support the potential integration of honey into wound management protocols in National primary healthcare as an affordable and accessible complementary antimicrobial agent, particularly in low-resource settings where access to advanced antimicrobial therapies may be limited.

Recommendations

Clinical trials involving Honey-based wound dressings and topical formulations could be developed and evaluated for clinical applications, to validate the therapeutic efficacy of honey in infected wound treatment. Future studies to characterize the physicochemical and phytochemical properties of Nigerian honey samples from Nsukka and molecular investigations should be undertaken to elucidate the mechanisms underlying honey-mediated bacterial inhibition with comparative studies involving medical-grade honey and conventional antibiotics.

Declarations

Conflict of Interest: The Authors have declared that no conflict of interests exist

Author Contributions: Nnamani, N- Lead/Principal researcher

Ugwuozor L, Eze L, Nnamani V-Co-researchers

Ikeyi, A-Admin and Coordinator

Consent and Funding: It is not applicable

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