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Comparative Evaluation of the Antibacterial Effects of Honey with Standard Antibiotic on Bacterial Isolates from Wound Infection

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NMB and ASK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BOA and FN managed the analyses of the study. Author AAI managed the literature searches. All the authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The aim of this study was to compare the antibacterial activities of the Honey against Ciprofloxacin on four bacterial isolates from a wound.
Study Design: It is a cross sectional comparative and observational study.
Place and Duration of Study: The study was conducted in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto State, Nigeria between July 2017 and October 2017.

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Methodology: One hundred and one (101) bacterial wound isolates were collected and identified using the standard microbiological methods of Gram staining and biochemical test. The activity patterns of the Honey concentrations and the standard antibiotic were determined using Kirby-Bauer disc diffusion and Punched Holes techniques. Similarly, minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the Honey were determined using Macrobroth dilution technique.

Results: Out of 101 isolates collected and identified, 33(32.7%) were *Staphylococcus aureus,* 29(28.7%) *Pseudomonas aeruginosa,* 21(20.8%) *Escherichia coli* and 18(17.8%) *Proteus mirabilis*. Antibacterial activity of honey was observed at 100% and 50% concentrations for *S. aureus* (10.7±0.13 mm and 8.4±0.16 mm), *P. aeruginosa,* (11.0±0.45 mm and 7.6±0.26 mm) and *E. coli,* (11.1±0.61 mm and 7.5±0.55 mm) respectively. Comparison of the inhibitory zone diameters showed that Ciprofloxacin (30.65±0.37 mm) had higher antibacterial activity than the raw honey (10.45±0.51 mm).

The Minimum inhibitory concentration (MICs) of crude honey on *S. aureus* was 5%, *P. aeruginosa* 50%, *E. coli* 20%, and *P. mirabilis* 100%, while the minimum bactericidal concentration (MBC) of crude honey on *S. aureus* was 50%, *P. aeruginosa* 100%, *E. coli* 100%, and *P. mirabilis* was resistant.

Conclusion: The result obtained from this study established that honey possessed antibacterial activity at 50% and 100% concentrations against *S. aureus, P. aeruginosa* and *E. coli*, which indicates that development of inhibition zones, depends on the concentration of the honey used as well as the nature of the tested pathogen. The findings also revealed that ciprofloxacin has higher antimicrobial activity than the type of honey used in this study.

Keywords: Honey; ciprofloxacin; bacteria; wound; MIC; MBC.

1. INTRODUCTION

A wound is an interruption or breaks in the continuity of the external surface of the body or of the surface of an internal organ, caused by surgical or other forms of injury or trauma. Small numbers of bacteria usually gain access even to clean surgical wounds; a larger number of bacteria invariably contaminate open wound incurred by accident [1]. Wound infections have however become a leading cause of frequent hospital visits and the use of antimicrobial agents is crucial in their management [2]. Regrettably, the conventional antimicrobial therapy has been seen posing problem in that the most incriminating bacteria are largely resistant to the readily available antibiotics. They developed resistance and this accounted for why naturopathic movements of the ancient time have blossomed from the 1990s [3].

Many of these natural preparations have been described as natural God-given foods for the good health of the body [3]. As such, honey (from *Apis mellifera)* have been identified among other natural substances, to have antimicrobial effects on some bacteria isolates from wound infections [2,4]. The increasing prevalence of chronic wounds together with the emergence of antibiotic resistant bacteria warrants further to improve wounds management practices and prevent complicated wound infection [5].

In his work, Manisha [6] emphasized that indeed, medicinal importance of honey has been documented in the world's oldest medical literatures, and since the ancient times, it has been known to possess antimicrobial property as well as wound-healing activity. He stressed further that the antimicrobial activity in most honeys is due to the enzymatic production of hydrogen peroxide. He, however, pointed out that another kind of honey, called non-peroxide honey (viz., manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked [6].

Honey was described as a thick sweet liquid made by honey bees (*Apis mellifera*) gotten from the nectar of flowers. It is a popular sweetener, nontoxic, nonirritant and a common household product [2]. Honey is rich in both enzymatic antioxidants and non-enzymatic antioxidants including catalase, ascorbic acid, flavonoids and alkaloids [7]. However, all honeys are not chemically equal and new bioactive components are still being discovered. This view is supported in the work of Kwakman [7].

The antibacterial activity of honey was first recognized in 1892, by Dustmann [8]. Honey is produced from many sources and its antimicrobial activity varies greatly with origin and processing [9]. Honey has been used as a medicine in many cultures for a long time [10]. It

has been rediscovered by the medical profession and it is gaining acceptance as an antibacterial treatment of topical infections resulting from burns and wounds [11]. Ibrahim and Aliyu [12] following their work on honey, they concluded that honey is a potential source of alternative antimicrobial agent with a broad spectrum activity.

The major antibacterial activity in honey has been found to be due to hydrogen peroxide (H_2O_2) produced enzymatically in the honey [13]. Its pH being between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens and thus the acidity is a significant antibacterial factor [11,14].

2. MATERIALS AND METHODS

2.1 Study Design

It is a cross sectional comparative and observational study.

2.2 Source of Test Organisms

A total of 101 bacterial isolates from wound infections were collected from the Medical Microbiology Laboratory, Usmanu Danfodiyo University Teaching Hospital, UDUTH, Sokoto. They comprise of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis.* The organisms were clinical isolates collected from general bacteriology bench of microbiology laboratory of UDUTH, isolated from wound infections. Bacteria biochemical tests were performed to confirm the identity of all the isolates.

2.3 Preparation of Honey Concentrations

The honey used was obtained from a recognized pure honey vendor in Sokoto South local government, Sokoto metropolis. Thereafter, the honey sample was diluted to 5%, 10%, 20%, 50% (v/v) of its original concentration using sterile distilled water. The 100% honey was referred to as 'neat'.

2.4 Bacterial Isolation

The bacterial isolates used were clinical bacterial isolates from wound infections isolated in the Medical Microbiology Laboratory unit of Usmanu Danfodiyo University Teaching Hospital, Sokoto. The organisms of interest were *Staphylococci aureus, Pseudomonas aeruginosa, Proteus mirabilis, and Escherichia coli*. Following their isolations on the bench, they were subjected to biochemical confirmations. Following the confirmations, the isolates were each subcultured on nutrient agar, incubated at 37°c for 24 hours. This is done to produce discrete colonies of the isolates.

2.5 Preparation of Inoculum

Direct colony suspension method was the technique employed in the preparation of the inoculums in this study as recommended by CLSI [15]. After overnight subculture, selected colonies of the isolates were picked with a sterile inoculating loop and suspended in 5mL of sterile normal saline to make a suspension. The turbidity of the inoculum suspension was adjusted to that of 0.5 McFarland standard (10^5 CFU/ml) against a card with a white background and contrasting black lines under an illuminated surface.

2.6 Inoculation of Tests Plate

Mueller Hinton agar plates were prepared aseptically, allowed to set and dry. The carefully adjusted inoculum suspension was allowed to stand for 15 minutes and a sterile cotton swab dipped into the adjusted suspension, rotated several times and press firmly on the inside wall of the tube above the fluid to remove the excess fluid from the swab [15]. Thereafter, the swab was streaked over the entire sterile surface of the dried Mueller Hinton agar plate. This procedure was repeated twice by rotating the plate at approximately 60° each time to ensure an even distribution of the inoculums [15].

2.7 Agar Diffusion Test (Punched Hole Method)

This was done with the aid of the sterile standard cork borer. Five wells of 6mm in diameter were punched at different sites on the plates. The bottoms of the wells were sealed with a drop of the sterile Mueller Hinton agar to prevent diffusion of the honey under the agar. The first well was filled with 5%, second well 10%; third well with 20%; fourth well with 50% and the fifth well with 100% (well 1 to 5). A prepared ciprofloxacin disc (5 µg/disc) was used as positive control at the centre of the agar.

The plates were allowed on the bench for 40 minutes, for pre-diffusion and then incubated at 37°C overnight. The resulting zones of inhibition

were measured in millimeters. The diameters of the zones of inhibition of the bacterial isolates in question were taken at a particular concentration of the tested honey.

Assessment of the Antimicrobial Activities of Honey: The susceptibility of the test organism was identified by zones of inhibitions, which was indicated by a clear zone around the wells to which different concentrations of honey were added.

2.8 Minimum Inhibitory Concentration

The minimum inhibitory concentration gives the lowest concentration (highest dilution) of the honey that can inhibit the growth of the test bacteria. This was determined by using the broth tube dilution method as described by Ceyhan and Ugar [16]. The freshly prepared nutrient broth was used in sterile tubes. 1 ml of nutrient broth was put into test tubes number two (2) to test tube number twelve (12). 1 ml of the honey concentration was added to tubes 1 and 2. The honey in tube 2 was therefore diluted 1:2. It was properly mixed then 1ml was transferred to tube 3 giving 1:4 dilution. This was continued until the 11^{th} tube from which 1 ml was discarded. The tube 12 which contained only nutrient broth, served as control. 1ml of the standard inoculum of each of the organism was then added to all tubes. The entire procedure was repeated for all the test organisms that might be susceptible to honey. The tubes were thoroughly mixed and incubated at 37°C for 24 hrs. Thereafter, they were visually observed for turbidity after incubation by comparing with the control tube.

2.9 Minimum Bactericidal Concentrations of the Raw Honey

The MBC of the honey used was determined by sub-culturing (on solid media) 0.01 ml (10 µL) of the highest concentrations of the dilutions which show visible growth and all the tubes showing no visible sign of growth in the MIC tube dilution test [17].

2.10 Statistical Analysis

Data generated was presented in the form of mean ± SEM. The mean inhibitory zone diameters, MICs and MBCs of the individual crude honey were compared to that of the standard antibiotics by one way ANOVA. Mean differences were considered significant when $p < .05$. All the statistical analysis were carried out by using the Statistical Packages for Social Sciences (SPSS) version 20.0 (California Inc., USA).

3. RESULTS AND DISCUSSION

One hundred and one (101) bacteria wound isolates were collected and identified using the standard microbiological methods [15,17,18], out of which 33(32.7%) were *Staphylococcus aureus,* 29(28.7%) *Pseudomonas aeruginosa,* 21(20.8%) *Escherichia coli,* and 18(17.8%) *Proteus mirabilis* (Table 1). The raw honey obtained was prepared into different concentrations (v/v) of 100%, 50%, 20%, 10% and 5%.

Antibacterial activity of honey was observed at 100% and 50% concentrations for *S. aureus* (10.7±0.13 mm and 8.4±0.16 mm), *P. aeruginosa,* (11.0±0.45 mm and 7.6±0.26 mm) and *E. coli* (11.1±0.61 mm and 7.5±0.55 mm) respectively (Table 2).

The Minimum inhibitory concentration (MICs) of crude honey on *S. aureus* was 5%, *P. aeruginosa;* 50%, *E. coli;* 20%, and *P. mirabilis,* 100%, while the minimum bactericidal concentration (MBC) of crude honey on *S. aureus* was 50%, *P. aeruginosa;* 100%, *E. coli;* 100%, and *P. mirabilis* were resistant (Table 3).

The honey used has an established potential to prevent microbial growth. Besides this property, honey clears infection in a number of ways including boosting the immune system, inducing anti-inflammatory and antioxidant activities, and via stimulation of cell growth [19]. In this study, the antibacterial activities of the raw honey were tested against four wound associated bacteria viz; *S. aureus, P. aeruginosa, E. coli,* and *P. mirabilis.* The antibacterial activity of the extracts was recorded when the inhibition zone was greater than 6 mm.

The results of the *in vitro* susceptibility and minimum inhibitory concentration of diluted and raw honey had a varying degree of antibacterial activities against Gram-positive as well as Gram-negative bacteria in a dose-dependent gradient. The results are in consonance with previous studies [2,20,21,22]. They found that honey inhibited the growth of *S. aureus, Escherichia coli, and Pseudomonas aeruginosa* and 100% concentrated honey was more effective than other dilutions [23].

Table 1. The identified bacterial isolates and their source

Bacterial	No. Isolated	Source	Percentage (%)
Staphylococcus aureus	33	Wound swab/pus/aspirate	32.7
Pseudomonas aeruginosa	29	"	28.7
Escherichia coli	21	"	20.8
Proteus mirabilis	18	"	17.8
Total	101		100

Table 2. Comparison of the inhibitory zone diameters of raw honey with standard antibiotic against the clinical bacterial isolates

Isolate	Zone of inhibition (mm)							F	P
	Honey Conc. (%)					Neg. control	Std drug (µg/disc)		
	100	50	20	10	5	DW	Cipro (5)		
S. aureus	10.7±0.13	8.4±0.16	6.8±0.14	6.0±0.00	6.0±0.00	6.0±0.00	34.7±0.47	1.4	0.24
P. aeruginosa	11.0±0.45	7.6±0.26	6.0±0.00	6.0±0.00	6.0±0.00	6.0±0.00	23.2±0.34	1.3	0.31
E. coli	11.1±0.61	7.5±0.55	6.0±0.00	6.0±0.00	6.0±0.00	6.0±0.00	26.4±0.39	0.9	0.49
P. mirabilis	9.0±0.83	6.0±0.00	6.0±0.00	6.0±0.00	6.0±0.00	6.0±0.00	38.3±0.29	2.0	0.16

Data are presented as mean ± SEM by using ANOVA. Values greater than 6±SEM indicate activity.
Key: Std drug = Standard antibiotics, Neg.= Negative, Cipro.= Ciprofloxacin, S.= Staphylococcus, P.= Pseudomonas E.= Escherichia, Prot.= Proteus DW = Distilled water

Table 3. The MICs and MBCs of the raw honey against the bacterial isolates

Isolate	MIC	MBC
	Honey (%)	Honey (%)
Staphylococcus aureus	5	50
Pseudomonas aeruginosa	50	100
Escherichia coli	20	100
Proteus mirabilis	100	---

Key - = No concentration could affect the MBC

In the case of *Proteus mirabilis,* antimicrobial activity was achieved only by crude honey (100%); this observation was also reported in the study done by Yahaya et al. [2]. but differs from the results of other studies which showed that at low concentrations, the pathogens had cleared zones of growth [24,25]. The difference in sensitivity could be due to the different growth rate of bacteria, nutritional requirements, inoculum's size, temperature, and the test methods [26].

4. CONCLUSION

Findings from this study revealed that honey possessed antibacterial activity at 50% and 100% concentrations against three (*S. aureus, P. aeruginosa,* and *E. coli*) of the tested pathogens which indicates that development of inhibition zones depends on the concentration of the honey used as well as the nature of the tested pathogen. Comparison of the zone diameters of inhibition of the organisms with the standard antibiotic (Ciprofloxacin) were found not statistically significant at the different concentrations of the honey.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval to conduct this study was obtained from the ethics and Research committee of Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto in accordance with the university standard. This was sought to allow the use of some of the clinical bacterial isolates from wound swabs of patients having wound/burns isolated in medical microbiology laboratory unit of the hospital.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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