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The antibacterial activity of honey on isolates from wounds of patients attending Hospital in two LGAs of Kwara State, Nigeria

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Abstract: The study conducts opinion survey on the profile of patients with wounds attending three hospitals. It investigates their knowledge of the use of honey for wound healing, establish the common types of wounds and determine the effect of honey on activity of wound microorganisms. 120 patients attending and undergoing treatment for various forms of wounds were surveyed. Also inhibitory effects of honey on microorganisms were determined for various honey concentrations. The result of the study revealed the respondents' profile as (51.67%) for 15 to 25 years and (73.33%) for the male sex. Most respondents (50.83%) were self-employed and (72.5%) lettered. Majority of the respondents (44.16%) patronized the General hospital Ilorin, while (21.67%) Kwara State University Medical Centre Malete. Burns constituted the highest (70.83%) of wound types. Eighty-five percent (85%) had previously used honey for wound treatment and was found to be most effective for burns treatment. *Streptococcus pneumonia* was the dominant bacteria species isolated from burns wound and *Staphylococcus aureus* from abscess. All isolated microorganisms were inhibited by honey concentrations. The clinical evaluation, application and use of Nigerian honey to prevent the growth of a wide range of human pathogens particularly for wound infection were among suggestions proffered.
Keywords: Wound, microorganisms, honey, inhibition, antibacterial.

INTRODUCTION

A wound is a type of injury which happens relatively quickly in which skin is torn, cut, or punctured. This type is termed open wound or where blunt force trauma causes a contusion and is called a closed wound [1, 2].

Wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions and more severe traumatic wounds like lacerations and those caused by crush or gunshot injuries [3]. Irrespective of the nature of injury, wounds are expected to heal within a predictable period. However, the treatment required to facilitate healing process will vary according to the type, site and the depth of a wound.

To enable healing process to progress through a natural series such as inflammation and granulation, to final re-epithelialization and remodeling require professional handling to avoid microbial invasion as exposed subcutaneous tissue provides a favourable substratum for a wide variety of microorganisms to contaminate and colonize. More so if the tissue is devitalized and the host immune response is compromised, the conditions become most favorable for microbial growth [4].

Wound contamination are likely to occur from sources including: the environment from microorganisms in the air, or those introduced by the injury, the surrounding skin (involving skin microflora) and endogenous sources such as found in mucous membranes [5, 6].

There are numerous reports in literature on the microbial, bacteriocidal and bacteriostatic activities of honey [7]. Opined that the bactericidal and fungicidal activities of pure honey against the wounds isolates; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans*, *Blastomyces dermatitidis* occur in a number of ways in vivo such as boosting the immune system and acting as antioxidant.

Scanty local antimicrobial susceptibility data of honey on wound microbes are however available for the country[7], scarcer are those for the study area (Kwara

State-North-Central, Nigeria). So, this study was designed to investigate the antibacterial activity of honey on isolates from wounds of patients attending hospital in two LGAs of Kwara State, Nigeria [7].

Opined that Bacterial wound infections are critical problems to the successful treatment of the wounds and often resulting in complications which sometimes lead to fatal sepsis. Over 95% of all injury-associated deaths occur in low-income and middle-income countries [8]. Burns are disturbing injuries with lots of consequences ranging from physical, functional, occupational and psychosocial damage [9, 10].

The common bacterial pathogens responsible for wound infections among others include: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and bacteria belonging to family Enterobacteriaceae [11].

Honey is a viscous concentrated solution of sugars produced by bees (*Apis mellifera*) through the collection and process of the nectar or sweet juices on certain plant species (honeydew). Honey is one of the most complex and precious natural biological products used since ancient times. Honey is rich, in both enzymatic antioxidants and non-enzymatic antioxidant like catalase, ascorbic acids, flavonoids and alkaloids [12, 13].

There are numerous reports in literature of the microbial, bacteriocidal and bacteriostatic activities of honey [6]. Opined that the bactericidal and fungicidal activities of pure honey against the wounds isolates; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans*, *Blastomyces dermatitidis* occur in a number of ways in vivo such as boosting the immune system and acting as antioxidant. Most of the isolated microorganism showed a considerable clear zone of inhibition, this implies that they are susceptible to pure honey compared to antibiotic sensitivity test, where no single antibiotic shows inhibitory effect against at least four isolates, including those organisms which are detrimental to wound healing (e.g; *S. aureus, P. aeruginosa*, and coliform bacteria) as all these were cleared off by pure honey [6, 14-20].

More recently, honey has been reported to possess antimicrobial properties which were realized for more than a century. The antimicrobial effect of honey is due to its high osmotic effect/ low water activity. The low water activity of honey is inhibitory to the growth of the majority of bacteria and many fungi. When applied topically to wounds, osmosis draws water from the wound into the honey thereby helping to dry the infected tissue and reduce bacterial growth. Even when diluted with water absorbed from wound it retains a water activity sufficiently low to inhibit growth of most bacteria [21].

Honey is mildly acidic, with a pH between 3.2 and 4.5; gluconic acid is formed in honey when enzyme glucose oxidase, catalyses the oxidation of glucose to gluconic acid, the low pH alone is inhibitory to many pathogenic bacteria [22].

It has been established that honey has an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives; it inhibits the growth of some pathogenic vegetative micro-organisms [23-26]. Some of the microorganism such as *E. Coli, S. epidemidis, S. aureus* and especially *P. aeruginosa* which were resistant to many types of antibiotics were excellently cleared off by the action of pure honey. This is due to the disruptive ability of the cell membrane potentials by honey and its ability to block adenosine triphosphate [27].

Research Objectives

The present study is set with the following objectives:

a. To conduct opinion survey on the profile of patients with wounds attending OPD at hospitals at study area
b. Investigate their knowledge on the use of honey for wound healing
c. Establish the common types of wounds at the study area and
d. Determine through laboratory analysis the effect of honey on activity of wound microorganisms.

MATERIALS AND METHODS

Study area

The study was conducted at two General Hospitals, Ilorin(Ilorin West) and the Kwara State University Medical Centre, Malete (Moro), Kwara State, North-Central, Nigeria.

Study design

Opinion survey

The sampling procedure was a multistage purposive sampling consisting of questionnaire administration and laboratory testing of the effect of honey on wound microorganisms. The data on opinion survey were collected from patients attending GOPD of General hospital, Civil Service Clinic and KWASU Medical Centre during 2016/2017 through a well-structured questionnaire and interview schedule. The designed questionnaire sought for patient's age, location, education, occupational status, social profile, nature of wound, use of honey for wound treatment and its effectiveness on wound types among others. One hundred and twenty questionnaires were administered to the patients that attend hospitals for wound treatment.

Laboratory analysis of effect of honey on activity of wound microorganisms

Collection of samples

A cross-sectional study was conducted using total of 120 pus/wound swab samples collected from Patients attending OPD sections of General and a University based Hospital at Ilorin and Moro LGAs, Kwara State undergoing treatment for various forms of wounds from September 2016 to March 2017. The samples were collected using a sterile cotton swab under an aseptic condition. Then specimens were transferred into the laboratory within 1 hour of collection for analysis.

Culture and identification of microorganisms

The wound swabs were inoculated and plated directly on Nutrient agar and Mac Conkey agar. After 24hours Pure Cultures of the microorganisms which were isolated and identified accordingly. All positive cultures were identified by their characteristic appearance on media, gram staining reactions and the pattern of biochemical profiles using standard procedures.

Identification of wound types

Wounds are identified by their various characteristics such as shape, depth and nature of their surface area. Interviewing the patient respondents was also a main criterion in wound identification.

Preparation of varying concentrations of honey and Amplicox

Varying concentrations of honey were prepared in the laboratory using test tubes, beaker and pipette. 100% honey was regarded as pure and was used undiluted; 70% honey was prepared by adding 30mls of sterile distilled water to 70mls of honey; while 50% honey was prepared by adding 50mls of sterile distilled water to 50mls of honey. Also, 500mg tablet of Amplicox (Beechaams product) was diluted in a measured quantity of distilled water in a test tube. This was thoroughly shaken to ensure proper dissolution in water.

Determination of minimum inhibitory concentration (MIC) of honey samples

The minimum inhibitory concentration of honey samples was determined by modification of the agar well dilution method as described by [28, 29]. The varying honey concentrations between 100% and 50% were used to determine the MIC of honey on the bacterial isolates.

The MIC was determined on plates of nutrient agar (Oxoid) already inoculated with the different bacterial isolates. After drying, holes (each 10mm deep and 8mm in diameter) were bored on each agar plate using a sterile cork borer. The holes were labeled accordingly with numbers. The holes were sufficiently spaced to avoid the zone of inhibition from over-lapping.

A 0.1ml of each of the honey samples were introduced into the holes accordingly using sterile Pasteur pipettes. The inoculated plates were allowed to stand for one hour to ensure proper diffusion of the honey into the medium and incubated at 37^0C for 24 hours. After incubation, the plates were observed for inhibition zones around the holes. The experiment was repeated three times for each bacterial isolate. The plates were then examined and the diameter of the zone of inhibition was measured. The lowest concentration of the different sample that produced no zone of inhibition of growth of the test organism was taken as the minimum inhibitory concentration. This experiment was duplicated where 0.1ml of prepared Amplicox was applied accordingly instead of honey to serve as the positive control.

Method of Data Analysis

Data obtained were subjected to descriptive statistics using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, 2011). One-way Analysis of Variance (ANOVA) was used to compare the bacterial inhibition zones between the control and the different concentrations of honey. Means were separated using Student-Newman-Keuls (SNK). Means were presented as Mean±Standard deviation. P value was set at 0.05.

RESULTS

Demography of the respondents

The age and gender of the respondents are represented in Figure-1. A greater percentage of the respondents (51.67%) were within the age of 15 to 25 years. However, the percentage of the respondents was observed to reduce with increase in age. Similarly, the respondents comprised of more of males (73.33%) than the females (26.67%). More of the respondents were self-employed (50.83%) and literate (72.5%) (Figure-2).

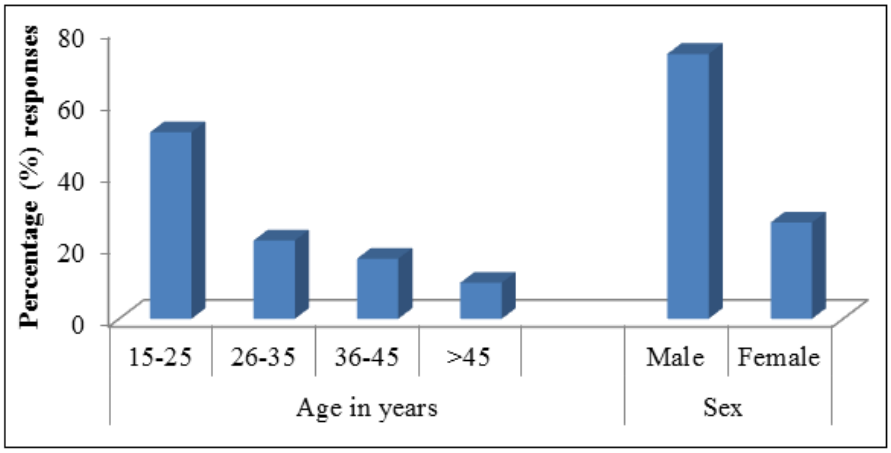

Fig-1: Age and gender of the respondents

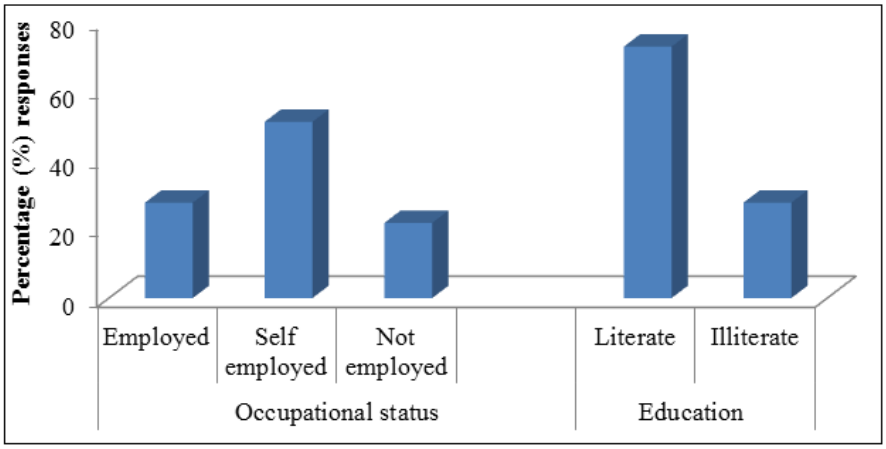

Fig-2: Occupational status and level of education of the respondents

Hospitals patronized and the nature of wound of the respondents

Most of the respondents (44.16%) patronized the General hospital (Table-1). This was followed by the Civil Service Clinic (34.17%) and the Kwara state university medical centre (21.67%) respectively. Out of the six types of wound identified from the respondents, wound sustained from burns (70.83%) had the highest occurrence, 62.5% of the wounds were fresh while 37.5% were stale wounds.

Table-1: Responses of patients on hospitals patronized, types and the nature of wound

		Frequency	Percentage (%)
Location	General Hospital	53	44.16
	Civil Service Clinic	41	34.17
	KWASU Medical Centre	26	21.67
	Total	120	100
Types of wound	Avulsion	10	8.33
	Abscess	3	2.5
	Incision (Cuts)	11	9.17
	Burns	85	70.83
	Scads	5	4.17
	Laceration (Tear)	2	1.67
	Others	4	3.33
	Total	120	100
Nature of wound	Fresh	75	62.5
	Stale	45	37.5
	Total	120	100

KWASU = Kwara State University, Malete
Survey 2017

Respondents' perspective on the usage of honey in wound treatment

Most of the respondents (85%) have used honey for wound treatment in the past (Figure-3). On the other hand, 15% of the respondents have not used honey for wound treatment. Considering the effectiveness of honey against the different types of wound, most of the respondents affirmed that the use of honey was effective in the treatment of wound sustained as a result of avulsion, abscess, burns and scads (Table 2). However, of all the wound type, wound sustained from burns (70%) and scads (12.5%) were identified to be more effectively treated using honey.

Three main sources of information on the use of honey in the treatment of wounds were identified by the respondents (Table 2). These include Health professionals, Relatives/friends and the Media. Also, a greater percentage of the respondents (63.33%) obtained this information from the Health professionals. On the other hand, the Media was the least source of information (15.84%) on the use of honey in wound treatment to the respondents.

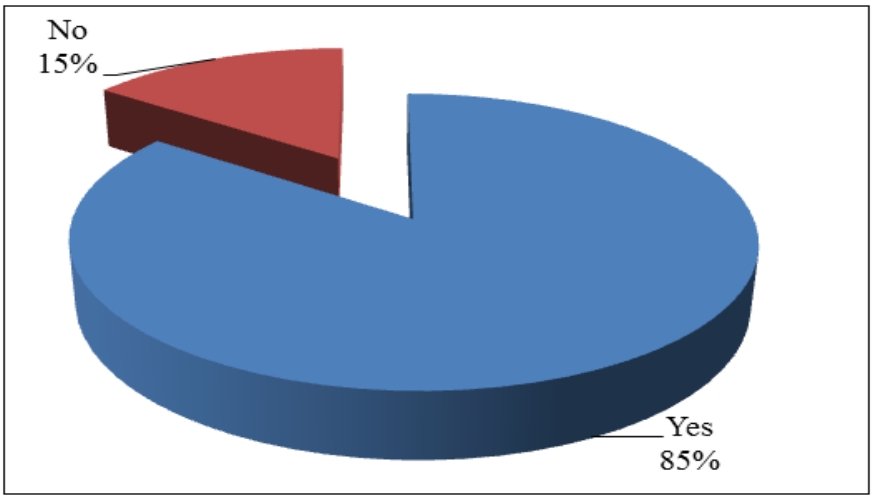

Fig-3: Percentage of respondents using honey for wound treatment

Table-2: Respondents' perspective on the usage of honey in wound treatment

If yes, on what type of wound/effectiveness?		Responses (%)	Overall (%)
Avulsion	Effective	75.0	
	Not effective	25.0	
	Total	**100.0**	**3.33**
Abscess	Effective	62.5	
	Not effective	37.5	
	Total	**100.0**	**6.67**
Incision(Cuts)	Effective	50.0	
	Not effective	50.0	
	Total	**100.0**	**5.00**
Burns	Effective	92.9	
	Not effective	7.1	
	Total	**100.0**	**70.00**
Scads	Effective	86.7	
	Not effective	13.3	
	Total	**100.0**	**12.50**
Laceration(Tear)	Effective	50.0	
	Not effective	50.0	
	Total	**100.0**	**1.67**
Others		**1**	**0.83**
TOTAL			**100.00**
Source of information on honey usage			
	Health professional	76	63.33
	Relatives/friends	25	20.83
	Media	19	15.84
	Total	**120**	**100**

Survey 2017

Nature of wound and prevalent bacterial isolate

A total of one hundred and twenty (120) wounds from wound patients using the General hospital (Ilorin), Civil Service Clinic (Ilorin) and the Kwara State University Medical Centre (Malete) were encountered during the study period (Table 3). More of the wound patients were recorded to visit the General hospital (Ilorin) than the other health centres used. The types of wounds identified from the patients include Abscess, Cuts, Burns, Scads, Laceration and Incision. Wound sustained from cuts had the highest relative wound occurrence (20.83%). This was lowest in the wound sustained from scads (10%).

Table-3: Distribution of wound patients from each of the Health centres used

Type of Wound	General Hospital (Ilorin)	Civil Service Clinic (Ilorin)	KWASU Medical Centre (Malete)	Total	RWO (%)
Abscess	8	6	8	22	18.33
Cuts	18	5	2	25	20.83
Burns	17	4	0	21	17.50
Scads	7	2	3	12	10.00
Laceration	11	6	1	18	15.00
Incision	9	6	7	22	18.33
Total	**70**	**29**	**21**	**120**	**100.00**

RWO = Relative wound occurrence

Survey 2017

The characteristics of each of the wound types identified and used for this study were presented in Table 4. The dominant bacteria species (present on all the wound samples collected) present on each of the wound types was also presented in Table 4. *Staphylococcus aureus* was the dominant bacteria species on Abscess wound type. Similarly, *Pseudomonas aurigenosa, Streptococcus pneumonia, Escherichia coli, Clostridium perfringens* and *Streptococcus pyogenes* were the identified dominant bacteria species on wound sustained from Cuts, Burns, Scads, Laceration and Incision respectively.

Table 4: Distribution, nature of wound and bacteria isolated

Wound type	Wound characteristics	Dominant bacteria Species	No*
Abscess	An abscess is a collection of pus that has built up within the tissue of the body with symptoms such as redness, pain, warmth and swelling.	*Staphylococcus aureus*	**22**
Cuts	A wound made by a cutting object such as knife blade, and other pointy object.	*Pseudomonas aurigenosa*	**25**
Burns	A burn is a type of injury, caused by heat or fire.	*Streptococcus pneumonia*	**21**
Scads	A scad is a type of injury, caused by heat from hot liquids such as water, oils etc.	*Escherichia coli*	**12**
Laceration	Laceration is an irregular tear-like wounds caused by some blunt trauma	*Clostridium perfringens*	**18**
Incision	Incision is caused by sharp edged object(knife, razor or glass sphincter)	*Streptococcus pyogenes*	**22**
Total			**120**

*No = Number of wound samples with the prevalent bacterial species

Survey 2017

Antibacterial inhibitory effect of honey concentrations

Table-5 shows the antibacterial inhibitory effect of honey concentration at 50%, 70% and 100% on the dominant bacteria species identified from the different wound types. At 100% honey concentration, the inhibition zones recorded against *Staphylococcus aureus* and *Streptococcus pyogenes* were not significantly different ($p > 0.05$) from those of the control. On the other hand, 100% honey concentration showed significantly higher ($p < 0.05$) zones of inhibition against *Pseudomonas aurigenosa, Streptococcus pneumonia, Escherichia coli* and *Clostridium perfringens* than the control antibiotics (Ampiclox) used. Also, at 70% honey concentration, there was no significant difference ($p > 0.05$) in the zones of inhibition recorded against *Streptococcus pneumonia, Escherichia coli* and *Clostridium perfringens* from those of the control antibiotics (Ampiclox-500mg) used. However, antibacterial effect was significantly lowest ($p < 0.05$) at 50% honey concentration against all the bacterial isolates (Table-5).

Table-5: Antibacterial inhibitory effect (mm) of honey concentrations on isolates

	Honey concentration			
Isolates	50%	70%	100%	Control
Staphylococcus aureus	7.0±0.5^{c}	16.0±0.5^{b}	22.0±0.5^{a}	22.0±0.3^{a}
Pseudomonas aurigenosa	0.0±0.0^{d}	14.0±0.3^{c}	24.0±0.3^{a}	22.0±0.7^{b}
Streptococcus pneumonia	8.0±0.2^{c}	19.0±1.0^{b}	23.0±0.2^{a}	20.0±0.5^{b}
Escherichia coli	6.0±0.5^{c}	17.0±1.0^{b}	22.0±0.5^{a}	18.0±0.6^{b}
Clostridium perfringens	9.0±0.7^{c}	20.0±1.0^{b}	26.0±0.5^{a}	21.0±0.2^{b}
Streptococcus pyogenes	8.0±0.6^{c}	17.0±0.5^{b}	21.0±1.0^{a}	21.0±0.9^{a}

^{abcd}Means±Standard deviation of zones of inhibition in the same row for each of the bacteria isolates having similar superscripts are not significantly different at $p < 0.05$

Survey 2017

DISCUSSION

The result of demographic status of the wound patients attending OPD of the three hospitals (General hospital-Ilorin, Civil Service Clinic-Ilorin and Kwara State University Medical Centre-Malete) showed that (51.67%) were within the age range of 15 to 25 years, (73.33%) males and (26.67%) females. Above fifty percent were self-employed (50.83%), (27.50%) employed and 21.67% not employed while (72.5%) were literate (Figure-1 & 2). The patients were within the agile age group with males dominating and with majority self-employed and employed in power driven tasks in the form of usage of electricity, chemical, mechanical and other energy sapping activity that may predisposes most of the wound patients to various wound sustaining sources such as cuts, laceration, burns, scads etc. This finding on nature and causes of common wounds is in line with the works of [9 and 10] who also reported that Burns are disturbing injuries with lots of consequences ranging from physical, functional, occupational and psychosocial damage. [3]also observed that wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions and more severe traumatic wounds like lacerations.

On the dominant bacteria species (present on all the wound swab samples collected), *Staphylococcus aureus* was the dominant on Abscess wound and similarly, *Pseudomonas aurigenosa, Streptococcus pneumonia, Escherichia coli, Clostridium perfringens* and *Streptococcus pyogenes* on Cuts, Burns, Scads, Laceration and Incision respectively (Table-4). The result of this current study is corroborated by [11] who opined that the common bacterial pathogens responsible for wound infections among others include: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and bacteria belonging to family Enterobacteriaceae.

On the usage of honey in wound treatment, most of the respondents (85%) have previously used honey for wound treatment in the past (Figure-3). Majority of the respondents affirmed the effective use of honey in the treatment of avulsion, abscess, burns and scads (Table-2). However, of all the wounds type, burns (70%) and scads (12.5%) were identified to be more effectively treated using honey. The effect of honey in wound treatment is due to its high osmotic effect/ low water activity. The low water activity of honey is inhibitory to the growth of the majority of bacteria and many fungi. When applied topically to wounds, osmosis draws water from the wound into the honey thereby helping to dry the infected tissue and reduce bacterial growth. Sources of information on the use of honey in the treatment of wounds were identified by the respondents as (63.33%) health professionals and (15.84%) from media [21-26] (Table-2).

The result of the study on the antibacterial inhibitory effect of honey concentrations (50%, 70% and 100%) on the dominant bacteria species revealed that the inhibition zones recorded against *Staphylococcus aureus* and *Streptococcus pyogenes* were not significantly different ($p > 0.05$) from those of the control antibiotics (Ampiclox) used. On the other hand, undiluted (100%) honey showed significantly higher ($p < 0.05$) zones of inhibition against *Pseudomonas aurigenosa, Streptococcus pneumonia, Escherichia coli* and *Clostridium perfringens* than the control. Also, at 70% honey concentration, there was no significant difference ($p > 0.05$) in the zones of inhibition recorded against *Streptococcus pneumonia, Escherichia coli* and *Clostridium perfringens* from those of the control antibiotics (Ampiclox) used.

However, antibacterial effect was significantly lowest ($p < 0.05$) at 50% honey concentration against all the bacterial isolates (Table-5). This shows that pure (100%) honey even showed better inhibitory activities against *Pseudomonas aurigenosa* (of cut wound), *Streptococcus pneumonia* (for burn wound) *Escherichia coli* (of scad wound) and *Clostridium perfringens* (of lacerated wound) than the control antibiotics (Ampiclox) used. This is in line with previous findings where most of the wound isolated microorganism showed a considerable clear zone of inhibition, implying that they are susceptible to pure honey compared to antibiotic sensitivity test, where no single antibiotic shows inhibitory effect against at least four isolates, including those organisms which are detrimental to wound healing (e.g; *S. aureus, P. aeruginosa*, and coliform bacteria) as all these were cleared off by pure honey [6, 14-20].

CONCLUSION

Honey has been established as a very promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria and in the treatment of persistent wound infections that do not respond to antibiotic therapy. The potency of Nigerian honey potential to prevent the growth of a wide range of possible human pathogens suggests its potential for use as an alternative therapeutic agent, for clinical evaluation and application, particularly for wound infection.

RECOMMENDATIONS

The researchers hereby recommend that:

1. Further research is needed be conducted to assess the efficacy of honey as an inhibitor of fungal growths in clinical trials, especially in the treatment of patients with disease conditions related to fungi.
2. Further researches are required on the use of different sources of honey to check and compare their antimicrobial activities.
3. The research is recommended to be transposed to animals using in-vivo and in-vitro experiments to compare the effect of honey on microorganisms in both ways.

Ethical approval and consent to participate

The patients were included after understanding the study and had given an informed consent. All aspects of the study were approved by the Kwara State University Ethical Review Board.

Competing interests

The authors declare that they have no competing interests.

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