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ANTIBACTERIAL ACTIVITY OF NIGERIAN HONEY AGAINST WOUND-BORNE PATHOGENS

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Abstract

The challenge of the continuous development of antibiotic resistance by pathogenic bacteria is a major health concern worldwide. The efforts at finding solutions to this challenge have led to the development of interest and investigation on the use of natural honey in the treatment of bacterial infections. This study was therefore carried out to investigate the efficacy of the Nigerian natural honey in the treatment of wound infections. Samples of honey purchased from Ijebu-Ode, Nigeria were investigated for antimicrobial effect, using bacterial isolates from infected wound swabs, that were cultured and examined for morphological and biochemical characteristics by standard methods. The antibacterial activity, Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the honey samples were determined by doubling dilution and agar diffusion methods. The bacterial isolates reacted differently to the various concentrations of honey. The MIC ranged between 7.5 mg/ml to 1.88 mg/ml for Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes; while Salmonella Typhi and Shigella dysenteriae

both had MIC of 3.75 mg/ml. The MBC values ranged between 7.5 mg/ml to 1.88 mg/ml for all the bacterial isolates. This study confirms the suitability of the Nigerian honey as therapeutic agent against infection by the tested bacteria pathogens. The need for international standardization of the quality of honey products for therapeutic and prophylactic application was also discussed.

Keywords: Nigerian honey, Antibiotic resistance, Wound infection, Bacterial pathogens, Effective chemotherapy.

Introduction

Natural honey is a supersaturated solution of sugars produced by bees of different Apis species from nectar of flowers or other plant secretions [1]. In Indian sub-continent, at least four Apis species are found which include Apis florae, Apis dorsata, Apis cerana and Apis andreniformis. However, Apis mellifera bees imported from Europe are widely used in honey farms for large scale natural honey production. Natural honey has been used effectively as medicine from ancient times as dressing for wounds and inflammations, as well as treatment of diseases of the gut [2]. In addition to its use as food, honey is used as a therapeutic substance, especially in the antibacterial treatment of gastroenteritis ulcers, bed sores, other surface infections and surgical conditions [2,3].

Some interesting bioactivities of natural honey include ant nociceptive, immune-modulatory and nematicidal activities [4,5]. Honey was successfully applied in the postoperative management of patients who had undergone radical vulvectomy for vulva carcinoma [6]. Accelerated healing and less bacteria colonization by the application of honey in patients who developed postoperative wound breakdown has also been reported [7]. Skin grafting surgical debridement and even amputation were avoided by local application of honey when conventional treatment failed [8].

Application of honey is an effective treatment of wounds because it is non-irritant, non-toxic, self-sterile, nutritive, bactericidal, easily applied and more comfortable than other dressings of wounds.
It has been shown that natural unheated honey has some broad spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria and even food spoilage bacteria [9]. Honey also has broad-spectrum activity against pathogenic fungi [10].

The antimicrobial activity of honey has been reported to be due to osmotic effects, acidity, hydrogen peroxide and phytochemical factors [11]. The continuous development of antibiotic resistance by pathogenic bacteria is a major health concern worldwide with economic, social and political implications [12]. The antimicrobial activity of honey is of therapeutic importance especially where the body's immune response is insufficient to clear infection [13].Studies on naturally synthesized plant substances like honey, for antibacterial activity represent an important and valuable means of sourcing for effective chemotherapy. This study was therefore undertaken to investigate the antibacterial effect of local honey on some bacterial pathogens recovered from wound samples from patients in some hospitals in Lagos, Nigeria. Bacteria were isolated from wounds and the minimum inhibitory and bactericidal concentrations of honey against the bacterial pathogens were determined in order to determine and assess the in vitro therapeutic efficacy of the Nigerian honey.

Materials and Methods

Collection of Honey Samples

The sample of honey purchased from Ijebu-Ode, Ogun State of Nigeria, was put in sterile sample bottles and then transported to the laboratory. The honey sample was filtered with sterile Seitz filter connected to an electronically operated vacuum pump. The honey filtrate was aseptically streaked on Nutrient Agar plates at 37^0C for 24 hr for sterility check. When found to be sterile, the honey sample was dispensed into sterile Pyrex sample bottles and kept in the refrigerator at 4^0C, prior to use [12].

Sources of Bacterial Isolates

The bacterial isolates which included Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus and Pseudomonas aeruginosa were recovered from wound swabs from Lagos State University Teaching Hospital (LASUTH), Crystal Hospital, Egbeda and General Hospital, Ifako-Ijaiye in Lagos State of Nigeria. The isolates were identified using a range of cultural, morphological and biochemical characteristics as earlier described [14].

Determination of antibacterial property of honey

The antibacterial activity of honey was carried out using the cork borer method in which a 4mm sterile cork borer was sterilized by flame and used to bore four equidistant wells of 6mm diameter in the solidified nutrient agar plate, aseptically. The nutrient agar plates were then inoculated with 3-hour old suspension of bacterial isolates, with approximately 10^7-10^8 cells in their log phase, using a sterile swab stick to seed the surface of the agar plate evenly. Each well was filled with the honey samples of different percentage i.e. 100%, 75%, 50% and 25% respectively. A 100mg/ml of Ampiclox was used as control which was repeated for each test organism. The agar plates were then incubated at 37^0C for 24 hours and the zone of inhibition around each well was measured with a transparent plastic ruler at two different angles, and recorded accordingly.

The Minimum Inhibitory Concentration of the honey's potency was evaluated by agar diffusion method [15]. Three hundred milligrams of honey was added to 10ml of nutrient broth to give a honey concentration of 30mg/ml. SSterile test tubes, numbered 1 to 5 were set up containing 1.5ml of sterile nutrient broth in the 1st test tube and 1ml of the broth in each of test tubes 2 to 5. A doubling dilution of the 30mg/ml honey was then performed by adding 0.5ml of the honey into the 1st test tube and 1ml of the mixture was transferred to the 2nd tube. The transfer of 1ml of mixture was continued from the 2nd to the 5th tube where the final 1ml of mixture taken was discarded. This gives the concentration of honey in the 1st to the 5th test tube as 75mg/ml, 3.75mg/ml, 1.88mg/ml, 0.94 mg/ml and 0.47mg/ml respectively. The text tubes were serially seeded with a loopful of test organisms and were incubated at 37^oC for 24 hours.

A control (Ampiclox) containing the same honey concentration in sterile nutrient broth were seeded with the test organisms and were incubated at 37^0C for 24 hours. Turbid tubes after incubation indicated negative reaction and the least honey concentrations where clarity (i.e. no growth of organisms) of medium begins determined the minimum inhibitory concentration (MIC). The

Minimum Bactericidal Concentration (MBC) was carried out by culturing 1ml of the positive tubes on nutrient agar plates for 24 hours at 37^0C and the plates were examined for bacterial growth.

Results

Table 1 shows that the bacterial isolates tested reacted differently to different concentrations of honey. Escherichia coli had the highest susceptibility while Staphylococcus aureus had the least susceptibility. Pseudomonas aeruginosa was 100% resistant at 25% concentration of honey.

The MIC ranged between 7.5mg/ml to 1.88 mg/ml for E.coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes while sSalmonella Typhi and Shigella dysenteriae had MIC of 3.75mg/ml (Table 2).From table 3, the MBC values ranged between 7.5mg/ml to 1.88mg/ml for the entire bacterial test, as there was no growth at 0.94mg/ml.

Table 1: Antibacterial activity of honey

Test organism	100%	50%	25%	Ampiclox (control)
Escherichia coli	22mm	19mm	10mm	20mm
Pseudomonas aeruginosa	21mm	15mm	-	21mm
Shigella dysenteriae	10mm	20mm	25mm	20mm
Salmonella Typhi	10mm	12mm	15mm	22mm
Staphylococcus aureus	18mm	15mm	8mm	19mm
Streptococcus pyogenes	20mm	16mm	8mm	16mm

Table 2: Minimum Inhibitory concentration (MIC) of honey against wound-borne bacteria pathogens

Test organism	7.5mg/ml	3.75mg/ml	1.88mg/ml	0.94mg/ml	0.47mg/ml
E. coli	-	-	-	+	+
*Pseudom*onas a*eruginos*a	-	-	-	+	+
Salmonella Typhi	-	-	+	+	+
Shigella dysenteriae	-	-	+	+	+
*Staphylococc*us a*ureus*	-	-	-	+	+
Streptococcus pyogenes	-	-	-	+	+

Keys: + = Turbidity - = No turbidity

Keys: + = Turbidity - = No turbidity

Tables 3: Minimum bactericidal concentration (MBC) of honey against wound borne bacterial pathogens

Test organism	7.5mg/ml	3.75mg/ml	1.88mg/ml	0.94mg/ml	0.47mg/ml
E. coli	-	-	-	+	+
Pseudomonas aeruginosa	-	-	-	+	+
Salmonella Typhi	-	-	-	+	+
Shigella dysenteriae	-	-	+	+	+
Staphylococcus aureus	-	-	-	+	+
Streptococcus pyogenes	-	-	-	+	+

Discussion

This study revealed that honey is characteristically quite acidic with the pH value range from 3.2 to 4.5, which are low enough to inhibit the growth of bacterial pathogens. The optimum pH for growth of bacterial species normally falls between 7.2 and 7.4. The minimum pH values for the growth of common wound infecting species had been reported as follows; E.coli, 4.3 pH, Pseudomonas aeruginosa, 4.4 pH and Streptococcus pyogenes, 4.5 pH [16]. These pH values show that undiluted honey has significant antibacterial effect. This further supports an earlier study [1] which confirmed that the antibacterial properties of honey may be due to its acidic nature and the hydrogen peroxide produced. The antibacterial activity of undiluted honey against pathogenic wound bacteria such as E.coli, Pseudomonas aeruginosa, Streptococcus pyogenes, and Staphylococcus aureus agrees with othe results [17,18] who showed that undiluted honey was also able to inhibit the growth of Proteus mirabilis, Pseudomanas aeruginosa, E. coli, Streptcoccus faecalis, Clostridium spp. and S.aureus that were cultured from wounds. A 50% concentration of honey has a bactericidal effect on all the test bacteria but was relatively weak on the same of organisms at 25%. This agrees with the findings that if honey is diluted, especially by body fluids which are well buffered, the pH will be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria [19]. This may be responsible for the resistance demonstrated by Pseudomonas aeruginosa.

The minimum inhibitory concentration and the minimum bactericidal concentration of honey observed in this study are in contrast with earlier findings [15].This could be due to the environmental factors where the honey was purchased, or to the nectars from which the bees produced the honey, which might have led to the retardation of the antimicrobial potential property of the honey used in this study compared to previous studies. This study also shows that honey can be used as a therapeutic agent against the tested pathogens. Honey would therefore be suitably active for both therapeutic and prophylactic application. This study therefore further confirms the findings of an earlier study [7,13].
The practice of consuming honey as food cannot be stopped as it has both nutritional and medicinal (antibacterial) values. Government agencies should therefore educate people on the importance of

honey as a therapeutic agent to several kinds of ailments including wound infection, coupled with its nutritive value. Government should also educate the public on the handling volatile equipment to avoid serious injury. In order to have a respectable world market for honey, the honey produced in the world today must fulfill international quality standards in terms of colour range, flavour and density so as to avoid product rejection. The various kinds of honey which have antimicrobial properties should be licensed for clinical use, as antiseptics. Honey can be developed into rubbery gel that can be molded to conform to any shape to increase the practicability of use with medical devices, beyond the use of honey-impregnated dressing currently available.

Conclusion

This study reveals that honey produced by Apis mellifera and other types of honey possess antibacterial activities and are effective against both Gram positive and Gram negative bacteria. This is a demonstration of the broad-spectrum activities of honey. However, pharmacological standardization and clinical evaluation on the effect of honey are essential so as to make honey one of the standard and official remedies to effectively combat pathogens that have developed resistance to many conventional antibiotics that are abused. In the light of the enormous potential for the application of honey within clinical environment, a continuing need exists for industrial production of potent natural honey and emphasizes its incorporation into the health care system worldwide.

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