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Full Length Research Paper

Antibacterial efficacy of crude and diluted honey on four wound isolates

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The antimicrobials activities of crude and diluted honey were determined against four clinical isolates from surface and deep wounds. *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* were isolated from wounds by routine microbiological methods. Kirby-Bauer's disc diffusion method was employed in determining the susceptibility of bacterial isolates to different concentrations of honey. The results from the zone of inhibition obtained (in mm) showed that the growth of all isolates was completely inhibited by 20-100% honey concentrations. The minimum inhibitory concentrations (MIC) of honey for *P. aeruginosa* and *S. aureus* were at 10 % (v/v) while that of *E.coli* and *K. pneumoniae* were 20% (v/v). The degree of susceptibility of the wound isolates to honey was compared with that of ten commercially available antibiotic discs. The result obtained revealed that the susceptibility pattern of honey at 40% (v/v) compared favourably and better than amoxicillin, streptomycin, ceftriazone and erythromycin.

Keywords: Honey, antibacterial activities, wound bacteria

INTRODUCTION

Honey is a thick, sweet liquid made by bees from the nectar of flowers. It contains water, glucose, fructose, proteins, vitamins and minerals (Al-Waili, 2004). Honey is rich, in both enzymatic antioxidants and non-enzymatic antioxidant including catalase, ascorbic acids, flavonoids and alkaloids (Bogdnov, 1989). That honey has antibacterial properties, has been known for more than a century (Dustman, 1979). The antimicrobial effect of honey is due to its high osmotic effect, high acidity, presence of hydrogen peroxide and phytochemical factors (Molan, 1992). There are so many reports on bacteriocidal and bacteriostatic activities of honey (Molan, 1992; Molan, 1998; Chinakwe, 2006; Al-Waili, 2004; Abd-Elaal *et al*., 2007; Al-Somail *et al*., 1994; Molan *et al*., 2000; Ndaisaba *et al*., 1993; Subrahmanyam *et al*., 2001; Taormina *et al*., 2001).

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The development of antibiotic resistance in bacteria is becoming a major problem. This has lead to an increasing interest in the use of alternative therapies including honey. This in essence justifies this study on the antibacterial efficacy of different concentrations of honey obtained from natural sources. The study also compares the antibacterial effects of honey with commercial antibiotics.

MATERIALS AND METHODS

Isolation and characterization of Test organisms

Sterile swab sticks was used to collect surface and deep samples from patients with wound infections. Samples obtained with swab sticks were suspended in sterile peptone waste to resuscitate infective microorganisms according to the modified Kirby- Bauers sensitivity testing technique (Carter and Chengappa, 1991; Cheesbrough, 2000). Ten-fold dilution of the suspension was made in

Table 1. Colonial and cell morphology of bacteria isolated from wounds

Colonial characteristics	Grams morphology	Capsule	Spore	Motility	Flagellum	Probable identity
Smooth circular and golden yellow colonies	gram positive oval cells in clusters	-	-	-	-	*Staphylococcus* sp
Smooth and shiny colonies with green pigments	gram negative small short single rods	-	-	+	+	*Pseudomonas* sp
Smooth moist shiny colonies	gram negative small short single rods	-	-	+	+	*Escherichia coli*
Moist and mucoid raised creamy colonies	gram negative large rods in short chains	+	-	-	_	*Klebsiella* sp

peptone water and aliquot portion (0.1ml) inoculated onto surface dried fresh nutrient and MacConkey agar media. Inocula were spread evenly and incubated at 37^0C for 24-48h (Cheesbrough, 2000; Beishir, 1987; Sharma, 2009).

Isolates were characterized (Cheesbrough, 2000; Harrigan and McCance, 1990; Pelczar and Chan, 1977; Sharma, 2009) and pure cultures identified (Buchannan and Gibbon, 1974; Carter and Chengappa, 1991) and preserved on slant at 4^0C.

Collection of Crude Honey Samples

A pure crude honey sample was obtained in a botanical garden at *Elu-orie* in Aku village in Igbo Etiti Local Government area of Enugu State, Nigeria. Sample was kept in the dark until used.

Preparation of paper discs

High potency discs (6.25mm) made from Whatman No.1 grade of filter paper were sterilized in a glass Petri dish at 121^0C for 15 mins (Carter and Chengappa, 1991).

Susceptibility Test

One-tenth milliliter (0.1ml) of different concentrations (10%, 20%, 30%, 40%, 60% and 80%) of honey was inoculated into a Petri dish containing 30 sterile paper discs each. The same volume of undiluted honey (100%) was inoculated into another petri dish containing sterile paper discs. The impregnated discs were dried in an oven maintained at 40^0C for 60 mins.

Molten Mueller Hilton agar was poured into a sterile Petri dish and allowed to set. One milliliter of the broth cultures of the wound isolates was transferred using a sterile 1ml pipette onto the surface of the molten agar plate. Excess fluid was discarded after swirling the dish sufficiently to ensure even distribution on the surface of the agar plate. With the Petri dish lid in place, the surface of the agar was allowed to dry for 5 mins (Cruickshank et *al*., 1975; Carter and Chengappa, 1991).

A sterile forceps was used to place firmly four discs impregnated with the different concentrations of the honey into the different medium containing the test organisms. This was repeated for the control. This process was repeated with a commercially prepared antibiotic disc. Plates were allowed to stand for 30 mins before incubating aerobically at 37^0C for 3-5 days (Cruickshank *et al*., 1975; Cheesbrough, 2000).

The diameter of the zone of inhibition near the respective discs was measured to the nearest milliliter (Cruickshank *et al*., 1975; Cheesbrough, 2000; Carter and Chengappa, 1991). The standard commercial antibiotic discs used were amoxicillin, ofloxacin, citrimazole, streptomycin, chloramphenicol, ceftriazone, gentamycin, pefloxacin, cotrimazole, ciprofloxacin and erythromycin.

RESULTS

The colonial and cell morphologies of the bacteria isolated from the wound samples is shown in Table1. Table 2 shows the biochemical characteristics of the isolates. The identities of the isolates were cross matched with those present in a standard manual (Buchannan and Gibbon, 1974; Carter and Chengappa, 1991). Three gram negative bacteria, namely, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* and one gram positive bacterium, *Staphylococcus aureus* were isolated from the wound samples.

The data in Table 3 shows that honey has effective antibacterial activities on the wound isolates as indicated by the diameter of their zone of inhibition. The minimum inhibitory concentration (MIC) of honey on *P. aeruginosa* and *S. aureus* was at 10% (v/v) while that of *Kl. pneumoniae* and *E. coli* was at 20% (v/v). For *P. aeruginosa* and *S. aureus,* the maximum activity was recorded at 100% honey concentration while *Kl. Pneumoniae*, the maximum activity was observed at 80%

Table 2. Biochemical characteristics of bacteria isolated from wounds

										Sugar Fermentation					
Cat	**Oxi**	**Coag**	**In**	**MR**	**VP**	**Cit**	**Urease**	**NO_3**	**H_2S**	**Glu**	**Suc**	**Lac**	**Mn**	**F**	**Identity of isolate**
+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	*Staphylococcus aureus*
+	+	+	-	+	-	+	+	+	+	+	-	-	+	-	*Pseudomonas aeruginosa*
+	_	-	+	+	-	-	-	+	-	+	-	+	+	+	*Escherichia coli*
+	-	-	-	-	+	+	+^{s}	-	-	+	+	+	+	-	*Klebsiella pneumoniae*

Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, methyl red; VP, voges proskaeur; Cit, citrate; NO_3, nitrate reduction; H_2S, hydrogen sulphide; Glu, glucose; Suc, sucrose; Lac, lactose; Mn, mannitol; F, fructose; s, slow reaction

Table 3. Antimicrobial activities of honey concentrations on bacteria isolated from wounds

Bacterial isolates	**Zone of inhibition (mm)**						
	10%	**20%**	**30%**	**40%**	**60%**	**80%**	**100%**
Pseudomonas aeruginosa	6.5	6.5	11.0	12.0	9.0	7.0	12.0
Staphylococcus aureus	6.5	8.0	8.0	28.0	12.0	9.0	11.0
Escherichia coli	0.0	6.5	7.5	7.0	8.0	10.0	12.0
Klebsiella pneumoniae	0.0	6.5	8.0	10.0	7.0	15.0	6.5

Table 4. Antimicrobial activities of commercially available antibiotics on bacterial isolates

Commercial antibiotics	***P. aeruginosa***	***S. aureus***	***E. coli***	***Kl. pneumoniae***
Amoxicillin (AMX)	10.0	22.0	11.0	9.5
Ofloxacillin (OFL)	29.0	32.0	12.0	26.0
Streptomycin (STR)	10.0	22.0	0.0	16.0
Chloramphenicol (CHL)	11.0	29.0	0.0	14.0
Ceftriazone (CEF)	9.0	10.0	0.0	0.0
Gentamycin (GEN)	12.0	24.0	9.0	15.0
Pefloxacin (PEF)	19.0	24.0	12.0	28.0
Cotrimazole (COT)	0.0	26.0	11.0	19.0
Ciprofloxacin (CPX)	28.0	30.0	10.0	24.0
Erythromycin (ERX)	9.0	28.0	10.0	10.0

Table 5. Antibacterial activities of six commercial antibiotics compared with 40%v/v concentration of honey

Bacterial isolates	**Zone of inhibition of commercial antibiotics (mm)**						
	40%v/v	**AMX**	**OFL**	**GEN**	**PEF**	**CPX**	**ERX**
Pseudomonas aeruginosa	12.0	10.0	29.0	12.0	19.0	28.0	9.0
Staphylococcus aureus	28.0	22.0	32.0	24.0	24.0	30.0	28.0
Klebsiella pneumoniae	10.0	9.5	26.0	15.0	28.0	24.0	10.0
Escherichia coli	*7.0*	*11.0*	*12.0*	*9.0*	*12.0*	*10.0*	*10.0*

honey concentration. At 10% (v/v) honey concentration, *E. coli* and *Kl. Pneumoniae* were both resistant.

Table 4 shows the antimicrobial activities of commercially prepared antibiotics on the bacterial isolates. *E. coli* was resistant to ceftriazone, chloramphenicol and streptomycin; *Kl. Pneumoniae* was resistant to ceftriazone while *P. aeruginosa* was resistant to cotromazole. *Staphylococcus aureus* showed high level of susceptibility to the entire antibiotic tested. All the test isolates were susceptible to amoxicillin, ofloxacillin, gentamycin, pefloxicin, ciprofloxacin and erythromycin. Table 5 compares the activity of 40%v/v honey concentration and six most effective commercial antibiotics on test organisms.

DISCUSSION

Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus and *Escherichia coli* were

isolated from patients with various degree of wound infection. Similar organisms have been reported (Subrahmanyam *et al*., 2001; Carter and Chengappa, 1991; Stepp and Woods, 1998; Nester *et al*., 1998; Baron, 1996; Mahon and Manuselis, 1995; Talaro and Talaro, 1993; Willey *et al*., 2008). The results obtained from this study shows that honey has antimicrobial actions on some bacteria isolated from wounds. Table 3 shows that all the isolates were sensitive to honey even at concentration as low as 20% (v/v).

The antimicrobial activity of honey has been attributed to several properties of honey including its osmotic effect; naturally low pH and the presence of inhibine which consists of hydrogen peroxide as well as phelonic acids, flavonoids and lysozyme (Molan, 1992). The susceptibility pattern of *P. aeruginosa* and *S. aureus* showed that there was an increase in antimicrobial activity with increased honey concentration up to 40% (v/v) concentration. Above 40% (v/v), the zone of inhibition decreased until at 100% when it increased again. This pattern was explained in the study conducted by Chinakwe (2006) that the antibacterial properties of honey formulation increased when water was added. Hydrogen peroxide, an inhibine component of honey is produced only when honey is diluted with water (Taormina *et al*., 2001). The noticeable increase in activity of undiluted honey (100%) for both *P. aeruginosa* and *S. aureus* may be due to the role played by other antimicrobial properties, such as low pH and high osmotic pressure of honey besides hydrogen peroxide.

The sensitivity pattern of *E. coli* indicated an increased zone of inhibition with an increased honey concentration, thus the maximum activity was noticed at honey 100%. The explanation may be that the main antibacterial factors responsible were acidity and osmotic pressure. As the dilution decreased, acidity and osmotic pressure increased, resulting in a corresponding increase in the zone of inhibition. *Kl. Pneumonia* presents an irregular pattern in the response to the different concentrations of honey. Although the reason(s) for this fluctuation is not clear, the capsule and other determinant factors such as enzyme may influence this erratic behaviour.

The antibacterial potentials of honey, especially at 40%v/v on the tested gram negative bacteria were better than amoxicillin, streptomycin, chloramphenicol, ceftriazone and erythromycin (Table 4). Abd-Elaal (2007) reported that honey had a more inhibitory effect on gram negative bacteria than amoxicillin and ceftriazone. Forty percent (40%v/v) honey concentration was adjudged the most effective for almost all the test organisms and compares favourably with the six most effective commercial antibiotics (Table 5).

REFERENCES

Abd-Elaal AM, El-Hadidy MR, El-Mashad NB, El-Sabaie AH (2007).Antimicrobial effect of bee honey in comparism to antibiotics on organisms isolated from infected burns. *Annals of Burns and Fire Disaster*, 20(2): 91-94.

Al-Somail N, Coley KE, Molan PC, Hankock BM (1994). Susceptibility of *Helicobacter pylori* to the antibacterial activity of Manuka honey. *J. Royal Society Med*., 87: 9-12.

Al-Waili NS (2004). Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. *J. Med. Food*, 7(2): 210-222.

Bagdnov S (1989). Determination of pinocembrin I honey using HPLC. *J. Agric. Resources*, 23(1): 55-57.

Baron S (1996). *Medical Microbiology*. Fourth edition, The University of Texas Medical Branch, Galveston, TX, USA. pp 265-266, 270-271, 282, 351.

Beishir I (1987). *Microbiology in Practice. A* Self- *Instructions Laboratory Course*, 4th edition, Harper and Row Publishers, New York, pp 96-111, 120-130, 238-272.

Buchannan RE, Gibbon NE (1974). *Bergeys Manual of Determinative Bacteriology*, Williams and Wilkins Co. Baltimore, USA.

Carter GR, Chengappa MM (1991). *Essentials of Veterinary Bacteriology and Mycology*. Fourth edition, Lee and Febiger, Philadelphia, USA. Pp. 71-263.

Cheesbrough M (2000). *Laboratory Practice in Tropical Countries*. Cambridge University Press. Cambridge, USA. pp 38, 62-70,138.

Chinakwe EC (2006). Antibacterial effect of honey formulation on bacteria isolated from wounds. *Nigerian J. Microbiol.* 20(3): 1263-1267.

Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975). *Medical Microbiology*. Churchill, London. Pp. 199.

Dustman JH (1979). Antibacterial effect of honey. *Apiacta*, 14: 7-11. Harrigan WF, McCance ME (1990). *Laboratory Methods in Food and Dairy Microbiol*., 8th edition, Academic Press Inc., London. pp. 7-23, 286-303.

Mahon CR, Manuselis G (1995). *Text Book of Diagnostic Microbiology*. W.B. Saunders Co., Philadelphia, USA. Pp. 237-239, 280-281, 294-295, 875.

Molan PC (1992). The antibacterial activities of honey. *Bee World*, 73: 5-22.

Molan PC (1998). Honey as a dressing for wounds, burns and ulcers: A Brief Review of Clinical Reports and Experimental Studies. *Primary Infection*, 6(4): 148-158.

Molan PC, White RJ, Cooper R, Dunford C (2000). The use of honey in wound management. *Nurse Standard*, 15(11): 63-68

Ndaisaba G, Bazira L, Habonimana E, Moteganya D (1993). Clinical and Bacteriological results in wounds treated with honey. *J. Orthopedic Surgery*. 7(2): 202-204.

Nester EW, Robert CE, Pearsall WW, Anderson DG, Nester MT (1998). *Microbiology: A Human Perspective*. Second edition, WCB/ McGraw- Hill CO., Boston, USA. Pp. 260, 265, 651-671.

Pelczar MJ, Chan ECS (1977). *Laboratory Exercise in Microbiology*, Black Dot. Inc, New York, USA.

Sharma K (2009). *Manual of Microbiology: Tools and Techniques*. Second edition, Ane Books PVT. Ltd., New Delhi, India. Pp. 149-199.

Stepp CA, Woods M (1998). *Laboratory Procedures for Medical Office Personnel*. W.B. Saunders Co., Philadelphia, USA. Pp. 351, 366.

Subrahmanyam M, Archan H, Pawer SG (2001). Antibacterial activity of honey on bacteria isolated from wounds. *Annals of Burns and Fire Disaster*, 14(1): 124-128.

Talaro K, Talaro A (1993). *Foundations in Microbiology*. Wm.C Brown Publishers, Dubuque, IA., USA. pp 87, 341, 355-356, 769.

Taormina PJ, Neimira BA, Beuchat LR (2001). Inhibitory activity of honey against food-borne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *Int. J.Food Microbiol*., 69: 217-225.

Willey JM, Sherwood LM, Woolverton CJ (2008). P*rescott, Herley, and Klien's Microbiology*. Seventh edition, McGraw-Hill Higher Education, Boston, USA. Pp. 581.