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Short communication

Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria

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The antibacterial effect of eight unprocessed commercial honey sold in some local markets in Nigeria were investigated. These samples were selected to examine and compare their ability to inhibit the growth of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis.* The test organisms were isolated from wound swabs of patients at the University of Port Harcourt Teaching Hospital (UPTH), Nigeria. Agar diffusion method was employed to ascertain degree of sensitivity of the isolates to different honey samples. Different honey samples showed varying degrees of antibacterial activity. Excellent antibacterial activity was observed with respect to honey from northern Nigeria with zones of inhibition of 17.0 mm. Next to it was honey from southern, eastern and western Nigeria with zones of inhibition of 15.4, 13.5 and 11.6 mm, respectively. *P. aeruginosa* and *P. mirabilis* showed less antibacterial activity and can be regarded as being resistant to the inhibitory effects of honey. Investigated honey samples began to significantly inhibit microorganisms at 80 and 100% concentrations, demonstrating that commercial honey sold locally in Nigerian markets has lesser antibacterial effects on microorganisms.

Key words: Nigeria, antibacterial activity, honey, zone of inhibition.

INTRODUCTION

Honey is the substance made when nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honeybees *Apis melifera* (Martins et al., 2001). In 1999, Al Waili et al. reported that honey has been used topically for medical purposes. Honey is composed mainly of sugars (70 - 80%) such as fructose, sucrose, glucose, etc a low level of water, proteins, hydrogen peroxide, and gluconic acid. As a topical agent, honey has a debriding and cleansing action and acts as a barrier to prevent infection. Its antimicrobial properties as a topical agent has been described and documented both in *in vitro* and *in vivo* studies and evidence supports its usefulness in wound healing (Davis, 2005; Cooper et al., 2002). Recent studies have reported the benefit of honey in the treatment of burns, skin grafts, Fournier's gangrene, radiation induced mucositis and dermatologic conditions such as seborrhea and dermatitis (Willix et al., 1992). The antibacterial property of honey is derived from the osmotic effect of its high sugar content, its low moisture content, its acidic properties of gluconic acid and the antiseptic properties of its hydrogen peroxide (Khan et al., 2007). In 2002, Cooper et al. demonstrated that wounds infected with *Staphylococcus aureus* are quickly rendered sterile by honey.

Different types of honey are available depending on the source of nectar used in its production. The source of the nectar determines the degree of antibacterial activity of the honey (Efem, 1988, 1993). High demand for honey coupled with poverty often lead to product scarcity and adulteration and consequently, reduction in product quality (Omode and Ademukola, 2008). People tend to visit the local market than buy honey directly from the apiary. The present study is aimed at investigating the antibacterial activity of unprocessed commercial honey sold in the local markets from different parts of Nigeria (north, south east and west) on the following wound isolates: *S. aureus, Pseudomonas aeruginosa, Escherichia*

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Table 1. Antibacterial activity of Western Nigerian honey against selected microorganisms.

Organisms	% concentrations (w/v) /diameter zone of inhibition (mm)				
	100	80	60	40	20
S. aureus	11.60 ± 0.40	11.40 ± 0.02	9.90 ± 0.01	7.60 ± 0.15	2.80 ± 0.05
P. aeruginosa	5.80 ± 0.15	5.30 ± 0.14	4.10 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
E. coli	11.20 ± 0.30	10.00 ± 0.50	8.30 ± 0.06	5.90 ± 0.30	2.80 ± 0.08
P. mirabilis	8.60 ± 0.10	7.90 ± 0.35	5.40 ± 0.18	1.20 ± 0.10	1.10 ± 0.02

Table 2. Antibacterial activity of Southern Nigerian honey against selected microorganisms.

Organisms	% concentration (w/v) /diameter of zone of inhibition (mm)				
	100	80	60	40	20
S. aureus	15.40 ± 0.15	13.20 ± 0.10	10.50 ± 0.40	7.20 ± 0.08	4.60 ± 0.30
P. aeruginosa	3.30 ± 0.03	1.40 ± 0.03	0.60 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
E. coli	14.80 ± 0.60	12.30 ± 0.06	9.70 ± 0.07	6.90 ± 0.01	2.20 ± 0.02
P. mirabilis	7.30 ± 0.07	6.30 ± 0.07	4.30 ± 0.05	0.90 ± 0.04	0.00 ± 0.00

coli and *Proteus mirabilis.*

MATERIALS AND METHODS

Sample collection and preparation

A total of eight unprocessed commercial honey samples were collected from four different locations in Nigeria, they include north (Taraba), south (Rivers), East (Enugu) and West (Oyo). Two samples were obtained from each location in August, 2008 and stored at room temperature (24 – 26°C). Honey samples were prepared into different concentrations (20, 40, 60, 80 and 100% v/v) with sterile distilled water in volumetric flasks.

Test organisms

Test organisms were isolated from the wound swabs of patients at the University of Port Harcourt Teaching Hospital. Standard methods (Cheesbrough, 2000) were used for isolation and identification of bacterial isolates.

Determination of antibacterial activity

Prepared concentration of honey samples were tested against test isolates using Agar well diffusion method (Allen et al., 1999). Test organisms were suspended in 3 - 4 ml of nutrient broth (NB, Oxoid). Following incubation at 37°C for 2 to 3 h, samples were diluted with normal saline to a turbidity that is equivalent to 0.5 Mc Farland standard (10^6 CFU)/ml) (Woods and Washington, 1995). A loopful (5 mm) of bacterial suspension was applied to the center of a sterile solidified Muller Hinton agar (Oxoid, UK) plates and spread with a sterile dry swab stick. Inoculated plates were stored at 4°C for 30 min to set and wells were dug with the aid of a sterilized cork-borer of 5 mm internal diameter. Dilutions (20, 40, 60, 80 and 100% w/v) of honey samples were made using sterile distilled water. 0.1 milliliter of each concentration was added to each well. Cultures were incubated at 37°C for 18 – 24 h. Antibacterial activity was assessed by measuring the size of the zone of inhibition surrounding wells.

RESULTS AND DISCUSSION

Results of antibacterial activity of honey samples from the four regions of Nigeria (western, southern, eastern, and northern) of Nigeria are shown in Tables 1 - 4. *S. aureus* was the most sensitive to honey samples tested with average zone of inhibition of 11.6 mm at 100% honey concentration. The average zone of inhibition for *E. coli* was 11.70 mm. *P. aeruginosa* and *P. mirabilis*, however, were more resistant to honey samples than *S. aureus* and *E. coli,* with average zone of inhibition ranging from 4.10 to 8.60 mm. The antibacterial effect of honey samples on microorganisms increased as honey concentration was increased. Honey samples obtained from southern part of Nigeria demonstrated improved antibacterial activity when compared to honey samples obtained from Western part of Nigeria (Table 2). Average inhibition of the honey samples (100%, w/w) on selected pathogenic microorganisms as measured by inhibitory zones were 15.4 and 14.8 mm for *S. aureus* and *E. coli*, respectively; resulting in about 32% improvement over honey samples from northern Nigeria (Tables 1 and 2).

Inhibitory effect of honey samples obtained from the eastern part of Nigeria on *E. coli* was similar to that obtained from the western part of Nigeria (Tables 1 and 3). Honey samples from the northern Nigeria as presented in Table 4 has the highest inhibitory effects with zones of inhibition for *S. aureus*, *P. aeruginosa*, *E. coli* and *P. mirabilis* ranging from 1.3 – 17.0 mm; 1.9 - 9.9 mm; 2.3 – 15.1 mm and 1 - 11.0 mm, respectively. Honey samples used in this study showed higher antibacterial activity for Gram positive than Gram negative microorganisms; the reason is not clear but is in accordance with other findings (Rahmanian et al., 1970). Jeddar et al. (1985) previously found that honey inhibited the growth of bacteria at 40% dilution and this finding is in conformity

Table 3. Antibacterial activity of Eastern Nigerian honey against selected microorganisms.

Organisms	% concentration (w/v) /diameter of zone of inhibition (mm)				
	100	80	60	40	20
S. aureus	13.50 ± 0.18	11.60 ± 0.03	8.90 ± 0.11	5.00 ± 0.25	2.6 ± 0.10
P. aeruginosa	9.30 ± 0.06	4.40 ± 0.14	1.40 ± 0.02	1.00 ± 0.10	0.00 ± 0.00
E. coli	11.10 ± 0.10	7.60 ± 0.02	5.10 ± 0.05	3.80 ± 0.03	1.50 ± 0.18
P. mirabilis	6.10 ± 0.05	4.50 ± 0.12	3.00 ± 0.23	0.00 ± 0.00	0.00 ± 0.00

Table 4. Antibacterial activity of Northern Nigerian honey against selected microorganisms.

Organisms	% concentration (w/v) /diameter of zone of inhibition (mm)				
	100	80	60	40	20
S. aureus	17.00 ± 0.45	15.80 ± 0.10	11.10 ± 0.13	8.30 ± 0.05	1.30 ± 0.02
P. aeruginosa	9.90 ± 0.20	9.10 ± 0.06	6.40 ± 0.02	4.60 ± 0.10	1.90 ± 0.01
E. coli	15.10 ± 0.03	12.10 ± 0.10	8.40 ± 0.30	4.90 ± 0.20	2.30 ± 0.09
P. mirabilis	11.10 ± 0.05	9.90 ± 0.15	6.60 ± 0.15	3.80 ± 0.02	1.00 ± 0.24

with our present findings on the effect of honey on microorganisms. Any zone diameter havening less than 7 mm shows that the organism is resistant to the honey sample but if the zone diameter is greater than 11 mm it suggests that the microorganism is sensitive to the honey sample. to the microorganisms involved. At 20% concentration none of the honey samples inhibited the microorganisms. At 40% honey concentration, only honey from the northern Nigeria inhibited *S. aureus* and achieved a zone diameter of 8.3 mm, this is not in conformity with the results of Molan et al. (1992) whose findings revealed higher degree of sensitivity. Differences in the level of sensitivity may be due to variation in the antibacterial potential of honey used in the present study and the source of honey samples. The source of the nectar used in the production of the honey may have caused the differences in the antimicrobial activities of honeys from different sources (NHB, 1994). The difference in sensitivity can also be due to different growth rate of microorganisms, nutritional requirements, inoculums' size, temperature and the test methods (Gail, 1995).

All the honeys used in this study exhibited a level of antibacterial activity and the same honey act differently on different microorganism. The results obtained from this study showed that unprocessed commercial honey sold locally in Nigerian market possesses some level of antibacterial activity which is not as high as those obtained directly from apiary. This suggests that adulteration or improper storage conditions may have played a role in reducing the inhibitory capacity of the honeys tested.

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