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Research Paper

A Pasture Honey Trial for Antibacterial Potency on Some Selected Pathogenic Bacteria

Omoya, F. O. and *Akharaiyi, F.C.

Microbiology Department, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria.

*Corresponding author

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ABSTRACT

The honey purchased from farmer was evaluated for its antimicrobial potency both in its crude vicious state and diluted form. The test clinical bacterial organisms *(Salmonella typhi, Shigella dysenteriea, Pseudomonas aeruginosa* and *Staphylococcus aureus*) were obtained from the state specialist hospital Akure, Nigeria, and tested for purity. The honey sample was filtered and sterile checked prior use for antibacterial assay. The crude honey showed high antibacterial activity over the test bacteria organisms with halo ranging from 4-20mm. The honey samples were sampled for minimum inhibitory concentration (MIC) values, which however showed higher antibacterial potency on the test organisms (8-45mm) than the crude. The control bacterial strains were more susceptible to both the honey samples and the conventional antibiotics than the clinical isolates comparing the effective dose of the honey to the conventional antibiotics concentrations. The MIC of the honey samples ranged from 4-5% (V/V) on the test bacterial organisms, and MBC between 4-6% (V/V) signifying its high potency. Meanwhile, the test bacteria organisms that were resistant to some of the commercial sensitivity disc were inhibited by the honey in both its crude and diluted forms. This suggests that the in vitro test of the honey samples is highly effective and the honey can be employed as a preventive and curative measure to the community diseases that could be caused by the tested bacterial organisms.

Keywords: Antibacterial; Crude; Pasture; Trial; Honey.

INTRODUCTION

The use of traditional medicine to treat infection has been practiced since the origin of man kind, and in past it was the only method available. Currently, due to the absence of sufficient modern health care system particularly in rural areas, people prefer to visit traditional healers and herbal medicines. The integration traditional and modern medicine is gaining increased recognition globally (WHO, 2000).

Honey produced by honeybees (*Apis melifera*) is one of the oldest traditional medicines considered to be important in the treatment of respiratory ailment,

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gastrointestinal infection and various other diseases. It is been used effectively as a dressing for wound, (including surgical wounds), burns and skin ulcers to reduce pain and odor quickly (Andargarchew, et al., 2004).

Honey is the substance made from the gathering of nectar, sugary deposits from plants and animals by honeybees (*Apis mellifera*) which in their natural scientific model, synthesized, purified and stored in comb in a vicious or gelly liquid. Most people think of honey as excellent food, but some others consider it an elixir and still others as medicine (Zaghloul, et al., 2001). Honey has been discovered for the treatments of bacterial infections by medical profession, particularly, where conventional modern therapeutic agents are failing (Molan, 2001). Honey like other saturated sugar syrups and sugar pastes, has an osmolarity sufficient to inhibit microbial growth (Chirife, et al., 1983). It has been reported that honey stimulates monocytes in cell cultures to release the cytokines TNF – alpha, IL – 1 and IL - 6, the cell messengers' that activates the many facets of the immune response to infection (Tonks, et al., 2001). In addition, to stimulate leucocytes honey provides a supply of glucose, which is essential for the respiratory burst in macrophages that produces hydrogen peroxide, the dominant component of their bacterial destroying activity (Molan, 2001). The acidity of honey may also assist in the bacteria destroying action of macrophages as pH inside the phagocytes value is involved in killing ingested bacteria (Molan, 2001).

The continuous development of antibiotic resistance of pathogenic bacteria and particularly of *Streptococcus pneumoniae* to Penicilin (PRSP) *Staphylococcus aureus* to methicilin (MRSA) and *Enterococcus* to Vancomycin (VRE) is a major health concern worldwide with economic, social and political implications. The minimum inhibitory concentration (MIC) for 82 epidemic strains of' methicilin-resistant *Staphylococcus aureus* (MRSA) was found to range from 3% to 8% (v/v) (Allen, et al., 2000). The antimicrobial activity of honey is very important therapeutically, especially in situations where the body's immune response is insufficient to clear infection (Al-Jabri, 2005). The screening of naturally synthesized plants substances, like honey for new antimicrobial compounds represent an important and valuable source for new effective chemotherapy. Therefore, this study is undertaken to examine the antibacterial effect of a pasture honey on some selected clinical pathogenic bacterial organisms.

MATERIALS AND METHODS

The pasture honey sample purchased from a peasant farmer at Jegele farm settlement, in Akure, Nigeria, was taken to the laboratory and filtered with sterile Seitz filter connected to an electronically operated vacuum pump. The honey filtrate was aseptically streaked on nutrient agar plates and incubated at 37^0C for 24 h for sterility check and after the test which was negative the honey sample was dispensed into sterile pyrex sample bottles and kept at room temperature prior use.

***Test organisms*:** The test organisms used were clinical pure isolates of *Salmonella typhi*, *Shigella dysenteriae, Pseudomonas aeniginosa* and *Staphylococcus aureus*, collected from the state specialist hospital, Akure, Nigeria, while the control bacterial strains were collected from Bacteriology division of National Veterinary Research Institute (NVRI), Vom-Jos, Plateau State, Nigeria.

***Antibacterial screening of honey*:** Using the agar diffusion method, the test organisms approximately (10^7 - 10^8 cells in their log phase) were spread plated with a sterile glass spreader, previously flamed with ethanol. The nutrient agar plates were left for 2h to allow full embedment of the test organisms. With a sterile cork borer,

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(5mm) well were dug on the seeded plates. The filtered honey was aseptically pipetted into the bored wells, avoiding splashes and run over. The plates were incubated at 37°C for 24h un-inverted. The sensitivity of the test organisms is indicated by a halo around the wells and the diameter of the halo were taken as an index of the degree of sensitivity by measuring with a transparent plastic ruler at two different angles.

The minimum inhibitory concentration of the honey potency was evaluated by broth dilution method. Decreased concentration of the honey sample was prepared (1-7%) (v/v). In test tubes containing 8ml of sterile nutrient broth, 1 ml each of the honey concentration and 1 ml broth culture of test organisms approximately (10^7 -10^8 cells in their log phase) were introduced separately. The test tubes were rolled between two palms for even mix up and were incubated at 37°C for 24h. Turbid tubes after incubation, indicated negative and the least honey concentrations where clearity in medium starts, determines the Minimum Inhibitory Concentration (MIC). The Minimal Bactericidal Concentration (MBC) was determined by plating, 1 ml of the MIC positive tubes for 24h on nutrient agar to evaluate the bacteriostatic and bactericidal activities of the honey.

***Conventional antibiotic disc assay*:** The oxoid laboratory multor sensitivity discs (Gram +ve and Gram -ve), was used to assay the sensitivity pattern. The antibiotics and concentrations impregnated on the disc arms are: Augmentin (AUG) 30µg, Amoxylin (AMX) 25 µg, Ceftriazone (CRO) 30µg. Contrimoxazole (COT) 25µg, Ciprofloxacin (CPX) 10µg, Gentamycin (GFN) 10µg, Nitrofurantoin (NIT) 200µg, Ofloxacin (OFL) 54µg, Pentloxacin (PFN) 5µg. The same method used for the honey assay was also adopted for this test, except that the discs were picked with a sterile forcep and positioned at the centre of the seeded nutrient agar plates.

RESULTS AND DISCUSSION

Both the crude and diluted honey samples, exhibited various degrees of inhibitory halo in vitro with the agar diffusion method. However, the honey in diluted form demonstrated more antibacterial potency (8-45mm) than the crude honey (4-20mm). The control bacterial strains responded to the honey samples than the clinical isolates. The zone of inhibition of the control strains was between 20 – 46mm. *S. aureus* ATCC 25923. *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were the most susceptible to the honey sample among the control strains. Among the test organisms, *Staphylococcus aureus* was the most inhibited with both the crude honey (20mm) and diluted (12 - 45mm) (Table- 1).

Table-1: Sensitivity assay of honey (mm) on local isolates and control bacterial strains.

Organisms	Crude honey	1%	2%	3%	4%	5%	6%	7%
Clinical Strians								
S. typhi	4	8	12	16	8	11	15	32
S. dysenteriae	10	10	13	19	22	25	29	42
S. aureus	20	12	15	20	28	30	30	45
P. aeruginosa	9	9	15	17	20	23	30	37
Control Strains								
K.aerogenes NCTC 418	20	20	25	25	28	30	33	36
E. coli ATCC 25922	26	30	33	36	37	38	38	45
S. aureus ATCC 25923	25	28	30	34	39	40	43	46
P. aeruginosa ATC 27853	22	24	28	31	35	38	40	44

- Values are means of determination on triplicate sets.

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Table-2 shows the sensitivity pattern of the tested clinical isolates, and control bacterial strains to conventional antibiotics. *Salmonella typhi* and *P. aeruginosa* had low responses to the antibiotics (1 - 10mm). *Shigella dysenteriae* responded to some of the antibiotics with inhibitory halo between 13-26 mm while *Staphylococcus aureus*, responded well to the antibiotics with inhibitory halo ranging from 15-29 mm. The various inhibitory halo displayed by the antibiotic discs on the test organisms, were of lower values compared to the halo observed with the honey on the agar diffusion method. The response of *Salmonella typhi* to the antibiotics showed sign of resistance, of which the honey was effective. However, the control bacterial strains also responded to the conventional antibiotics than the clinical isolates. Though two of the control bacterial strains were not the same bacterial species with the clinical isolates, great differences in high sensitivity to the conventional antibiotics than the clinical isolates was noted. In most cases where the tested clinical isolates were not responding to some of the antibiotics, the control bacterial strains were inhibited even with diameters between 3-18mm (Table-2). This signifies that the honey used in this study is of more therapeutic effect comparing the concentration (% v/v) of the effective dose of the honey to that of the used conventional antibiotics (µg/ml).

Table-2: Conventional antibiotic disc assay (mm) in sensitivity comparison between the clinical isolates and the control bacteria strains.

Organisms	AUG	NIT	AMX	CRO	COT	CPX	GEN	OFL	PFX
Clinical strains									
S. typhi	-	-	-	-	2	1	-	2	3
S. dysenteriae	-	15	18	26	26	22	-	13	-
S. aureus	-	29	20	15	-	28	10	12	15
P. aeruginosa	5	-	-	3	-	5	5	-	10
Control strains									
K .aerogenes NCTC 418	3	6	18	-	15	18	15	16	-
E. coli ATCC 25922	10	10	13	14	30	7	15	20	8
S. aureus ATCC 25923	5	32	35	10	40	41	31	18	20
P. aeruginosa ATCC 27853	8	12	9	18	12	20	9	10	18

- AUG-Augmentin, NIT-Nitrofurantoin, AMX-Amoxacillin, CRO-Ceftriazone, COT-Contrimoxazole, CPX-Ciprofloxacin, GEN-Gentamycin, OFL-Ofloxacin, PFX-Pefloxacin.
- Values are means of determination of triplicate sets.

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The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the honey sample is shown in (Table- 3). The MIC and MBC of the honey was effective on *Staphylococcus aureus* at 4%, on *Shigella dysenteriae* at 5%, on *Salmonella typhi* the MlC of the honey was effective at 4% and bactericidal at 6% while on *Pseudomonas aeruginosa* MIC of the honey sample was effective at 5% and MBC at 6%.

Table-3: Concentration of honey at which MIC and MBC % (v/v) were valuable on test organisms.

Organisms	MIC	MBC	Action
Clinical strains			
S. typhi	4%	6%	S
S. dysenteriae	5%	5%	C
S. aureus	4%	4%	C
P. aeruginosa	5%	6%	S
Control strains			
K. aerogenes NCTCC 418	3%	3%	C
E. coli ATCC 25922	4%	4%	C
S. aureus ATCC 25923	3%	3%	C
P. aeruginosa ATCC 27853	5%	6%	S

- S-Bacteriostatic, C-Bactericidal
- Values are means of determination on triplicate sets.

This findings, showed antibacterial potency of the pasture honey in its crude and diluted forms. Since antibiotics are sometimes associated with adverse effects on hosts which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosupression and allergic reaction (Al-Jabri, 2005) the increase in antibiotic resistant microbial strains particularly to first-line inexpensive and broad-spectrum antibiotics, the fast spread of resistant organisms in developing countries due to complex socioeconomic and behavioral antecedents (Okeke, et al., 1999), the need for the development of alternative antimicrobial agents most especially of natural forms for the treatment of infectious diseases, is an important innovation to be embraced. Molan (2001), reported that, honey selected for clinical use should be evaluated on the basis of antibacterial activity levels determined by laboratory testing. However, the honey used in this research, showed very high antibacterial potency on some of the test organisms even at diluted level, as low as 1% (V/V).

The low level performance of the crude honey than the diluted form could be as a result of the acidic nature, honey is characteristically quite acidic (Molan, 2001). in view of this, the pH might be low to make the honey effect the antimicrobial potency high enough for much inhibitory activities, most especially of man and animal pathogens whose optimum pH for growth is normally between 7.2 - 7.4 . However, the higher antibacterial potency displayed by the diluted honey on the test organisms could be as a result of rising level in hydrogen peroxide: a tendency of hydrogen peroxide in diluted honey which increases its inhibitory potency, giving a kind of slow release of antiseptic at a level which is antibacterial.

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A lot of plants parts have been discovered in Nigeria to have antimicrobial potency (Akharaiyi and Omoya, 2005), this could be the high antibacterial potency observed in this study hence bees majorly obtained their materials from plant parts.

The dilution of the honey for the *in-vitro* test is to evaluate its inhibitory potentials, a proposal on *in—vitro* test, where body fluid is higher than the conditions present in artificial growth medium. A wide range of minimum inhibitory concentration (MIC) values of honey necessary for complete inhibition of bacterial growth, have been reported in studies comparing different honeys tested against single species of bacterial, from 25%-0.25% (V/V); >50%-1.5% (V/V); 20%-0.6% (V/V); 50%-1.5% (V/V). Also a survey of many honey samples have been found with their activity near or below the level of detection in an agar diffusion assay (Allen, et al., 1991), but a test with this honey, displayed MIC at 4%-5% (V/V) and wide range of inhibitory halo in an agar diffusion assay on the four test organisms used in this study. This indicates that, the honey has sufficient antibacterial potency to effect ailment of diseases that could be characterized by the tested bacterial strains.

The comparison result of the honey to antibiotics in this research is in agreement with Ibrahim (1981). Karayil et al., (1998) who found honey to be superior to cephaloridine, ampicilin, gentamicin, nitrofurantoin, nalidixic acid and contrimoxazole in inhibiting growth of nine types of pathogenic organisms.

The result obtained in this study conforms that of Kingsley (2001) who reported the antimicrobial activity of honey from honeybees against *P. aeruginosa, S. aureus, E. coli P. mirabilis, C. freundii, S. feacalis, S. flexinari* and *S. typhi*.

Report on the popular Manuka honey to completely prevent the growth of *S. aureus* is 1.8%, *S. pyogenes* (3.6%), *E. coli* (3.7%), *S. typhimurium* (6.0%), *P. mirabilis* (6.3%) and *P. aenuginosa* (7.3%) (Molan and Betts, 2000). However in this study, the percentage by volume of honey that completely prevent*s S. aureus* is 4%, *P. aeruginosa* 6%. This finding suggests that there is variation in antimicrobial activities of honey in different geographical locations and could be attributed to the materials available for honey bees in preparing their valuable honey.

CONCLUSION

The crude honey antibacterial potency on both the clinical and control bacterial strains was not as effective as in its diluted form. The control bacterial strains were found more susceptible than the clinical isolates to the honey samples tested for its trial for possible antibacterial potency. Sign of drug resistance shown by most of the clinical isolates to some of the conventional antibiotics was observed, whereas, the least susceptible of the tested bacterial strains to the honey samples was far above (2.5 mm) as the least standard for acceptable antimicrobial susceptibility rating.

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