In-vitro Antibacterial Effect of Honey against Selected Clinical Isolates from Wound of Patients Attending Dutse General Hospital, Jigawa State – Nigeria

[1]Sani, N.M*., [1]Abdulganiyu, F., [2]Mujahid, N.S., [3]Ado, A.,[4]Okoroiwu, I.A.G. and [4]Uchendu, F.N.

[1]Department of Microbiology and Biotechnology, Federal University Dutse Jigawa State – Nigeria.

[2]Department of Microbiology Kano University of Science and Technology Wudil

[3]Department of Microbiology, Federal University, Dutsin-Ma, Katsina State - Nigeria

[4]Department of Public Health Science, National Open University of Nigeria Jabi, Abuja

*Corresponding Author: E-Mail: nuramuhammadsani@gmail.com; Mobile:+2348065270565

Abstract: In an era of multidrug resistant bacteria, evaluation of more natural therapeutic options becomes necessary. This study was conducted to determine the antibacterial activity of honey on some clinical bacterial isolates from wound of patients attending Dutse General Hospital. Forty (40) wound swab samples were collected and analyzed out of which 33 demonstrated the growth of organisms. These were identified using morphologic characteristics on selective and differential media, as well as Gram's and biochemical reaction. Several organisms were isolated including*; Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Proteus spp, Klebsiella spp and Pseudomonas spp. S. aureus* is the most predominant organism isolated (34%). The antibacterial assay was determined using disk diffusion method with four different concentrations of two honey samples viz: 100% (v/v), 70% (v/v), 50% (v/v) and 30% (v/v). The tested organisms were sensitive to the different concentrations of honey used; however the highest activity was observed in 100% (v/v), the zone of inhibition ranges from 15-19mm in diameter. The highest zone of inhibition was observed in *S. aureus* (19mm) while *Pseudomonas* spp had the least (15mm). The antibacterial activity increased with increase in concentration. The MIC of the honey sample 1 and 2 were 1.25v/v for *S. aureus, E. coli, Klebsiella* spp and 2.5v/v for *pseudomonas* spp respectively. The MBC of the honey samples (1&2) were 2.5v/v for S. aureus, E. coli, *Klebsiella* spp and 5.0v/v for Pseudomonas spp respectively. The results of the study revealed that honey has a good antibacterial activity and as such can be used to treat wound infections.

Key Words: Pathogens, Assay**,** Honey, Isolate. Wound,

INTRODUCTION

Over the years, the use of natural preparations from plants for the treatment of various ailments has been practiced by people of diverse cultures (Van den-berg *et al.,* 2008). Many of these natural preparations have been described as natural "God –given" foods for the good health of the body (Boom, 2004). Treatment with chemicals (chemotherapy) seems to be inadequate and insensitive to many potential disease-causing micro-organisms. Anti-infective drugs (antimicrobial agents) are critically important in reducing the global burden of infectious diseases (Shears, 2000; Mulu *et al.,* 2005). The occurrence of drug-resistant microorganisms diminished the development of antibiotics, and few pharmaceutical companies remain active in this area, thereby posing a big challenge in this world of medicine (Bansal, 2005). Hence, the failure of these antibiotics has resulted to search for more effective sources of natural products from plants and other products including honey (Omoya *et al.,* 2011; Dixon, 2003). The antibacterial activity of honey was first recognized in 1892; however, it has a limited use in modern medicine due to lack of scientific support (Mohapatra *et al.,* 2011).The use of traditional medicine to treat infection has been practiced since the origin of mankind, and honey produced by *Apis mellifera (A. mellifera)* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments (Mundo *et al.,* 2004; Lubsy *et al.,* 2005).

Currently, many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria (Mundo *et al.,* 2004; Lubsy *et al.,* 2005). In most ancient cultures honey has been used for both nutritional and medical purposes. The belief that honey is a nutrient, a drug and an ointment has been carried into our days, and thus, an alternative medicine branch, called Apitherapy, has been developed in recent years, offering treatments based on honey and other bee products against many diseases including bacterial infections (Mundo *et al.,* 2004; Lubsy *et al.,* 2005).The current prevalence of microbial resistant to antibiotic has led to a re-evaluation of the therapeutic use of ancient remedies like honey. Many people in the developing countries like Nigeria depend on local medicinal plants as remedy for their diseases and illnesses probably either because of the absence of modernized functional health care facilities or due to traditional and ancestral beliefs. This study was conducted to determine the antibacterial activity of honey on some clinical bacterial isolates from wound of patients attending Dutse General Hospital, Jigawa State - Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out at Dutse General Hospital, Dutse local government area of Jigawa state situated in the northwestern part of the country between latitudes 11.00^{o} N to 13.00^{o} N and longitudes of 8.00^{o} E to 10.15^{o} E. Dutse is one of the 27 local government areas in Jigawa State. It is located between latitudes of 11^{o} 32' 42 "N and longitude of 9^{o} 8' 20 "E – 9^{o}27' 21 "E. It has a projected population of 394,631 and has vegetation which lies between the Sudan savannah with element of Guinea savannah in the southern part (Dutse Emirate Council, 2018).

Sample Collection

Forty (40) swab samples were collected from wounds of both in and out patients attending Dutse General Hospital using sterile swab sticks. Samples were aseptically collected on a sterile cotton swab by rotating on lesion surface with sufficient pressure as described by (Mohammed aman *et al.*, 2015) and were immediately transported to the Microbiology Laboratory at Federal University Dutse for further analysis to avoid drying of the smears which could lead to wrong results.

Honey Samples

The honey samples were collected from Dutse modern market in sterile screw cap bottles. The honey samples were filtered with a sterile mesh to remove debris and the honey was protected from bright light to prevent photo-degradation of glucose oxidase enzyme (Mohammedaman *et al.*, 2015). The authenticity of the honey was identified using a local method as described by the honey farmers, in which a matchstick is coated with the honey and lit. If fire is produced it indicates a good quality honey and inability to light indicates an adulterated honey.

Isolation, Identification and Characterization of Organisms

Swabs collected were cultured by streaking them on Eosine methylene blue agar, Blood agar and Mannitol salt agar plates and the inoculated plates were then incubated at 37^{o}C for 24hours. The plates were observed morphologically and the suspected colonies were subjected to Gram's reaction and appropriate biochemical tests (Cheesbrough, 2004).

Preparation of Honey Sample

Hundred percent pure honey (100%v/v) was obtained after the filtration using sterile gauze. To get 70% honey solution (v/v) 7.0ml of honey was diluted in 3.0ml of sterilized distilled water. To obtain 30% and 50% honey solution, 3.0ml and 5.0ml of honey were diluted in 7.0ml and 5.0ml of sterile distilled water respectively as described by Mohammed aman *et al.* (2015).

Antibacterial Assay

The antibacterial activity of honey was assayed using disk diffusion technique (Cheesbrough, 2004). Well isolated colonies from each bacterial isolate culture were separately put into 4ml sterile nutrient broth and aseptically incubated at 37^{o}C overnight. The overnight broth cultures were adjusted to 0.5 McFarland standard. These were used as inocula for the antimicrobial assay.

A representative colony of each isolated bacteria was used for the antimicrobial assay. Using a sterile wire loop, the organism were picked and radially streaked on the nutrient agar plates. Using a sterile forcep already sterile disks impregnated with each concentration of honey at 100%, 70%, 50% and 30% were placed onto appropriately labeled plates. A standard ciprofloxacin antibiotics disk was used as positive control. The plates were incubated at 37^{o}C for 24 hours. The diameter of the zones of inhibition around the disks were measured in mm and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using broth dilution method as described by Mohammed aman *et al.* (2015) with slight modification. Thus, seven sterile test tubes were placed in the rack and labeled. Broth control and growth control tubes were used as quality control tubes. Five milliliter of freshly prepared nutrient broth was added to each tube, sterilized and cooled. Then five milliliter of undiluted honey (100%) was added to test tube number one and honey control (positive control) with a sterile micropipette. A two fold serial dilution was performed by transferring five ml from the first tube into the second test tube with a separate sterile micropipette and vortex for homogenization. After thorough mixing, 5ml transferred from tube 2 to tube 3. This procedure continued until the fifth tube with a dilution of 3.125 % v/v is reached, and finally 5ml was taken from the tube 5 and discarded. The broth control tube which received no inocula served as negative control. Each tube except the negative control tube was inoculated with the standardized inocula. The tubes were incubated at 37^{o}C for 24 hours and observed by visual inspection for the presence or absence of growth (i.e presence or absence of turbidity). The MIC was recorded as lowest concentration of honey that inhibited bacterial growth (no visible growth or turbidity) (Cheesbrough, 2004).

Determination of Minimum Bactericidal Concentration (MBC)

The incubated tubes showing no visible growth/turbidity in MIC were sub-cultured onto sterile nutrient agar plates and were incubated at 37^{o}C for 24 hours aerobically. The least concentration of honey that did not show growth was considered as the MBC. The inoculated plates were scored as bactericidal if no growth, bacteriostatic if there is light to moderate growth and no antibacterial activity if there is heavy growth (Mohammed aman *et al.,* 2015).

RESULTS

A total of 40 wound samples were analyzed in this study. Bacterial isolates were obtained from 33 wound samples, while 7 samples had an insignificant growth. Seven bacterial species were identified namely: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Klebsiella* spp*, Pseudomonas* spp, *Escherichia coli* and *Proteus* spp.

Table 1: Cultural, Microscopic and Biochemical Characteristics of the Isolates

Agar	Colony appearance	Grams reaction	Ca.	CO.	IN.	MR.	VP.	CI.	OX.	Inference
A	Flat colourless - pale pink	-ve rod	+		-	-	-	+	+	*Pseudomonas* spp
A	Pinkish-purple mucoid growth	-ve rod	+		-	-	+	+		*Klebsiella* spp
A	Green metallic sheen	-ve rod	+		+	+	-	-		*Escherichia coli*
A	Purple with swamming	-ve rod	+		-	+	-	+		*Proteus* spp
B	Yellowish with mannitol fermentation	+ve cocci (clusters)	+	+						*Staphylococcus aureus*
B	Whitish without mannitol fermentation	+ve occi(clusters)	+	-						*Staphylococcus epidermidis*
C	Beta heamolysis	+ve cocci (chain)	-	-						*Streptococcus pyogenes*

Key**:** a = Eosine Methylene Blue Agar, b = Mannitol Salt Agar, c = Blood Agar, + =Positive, - = Negative, Ca. = Catalase, CI. = Citrate, CO. = Coagulase, IN. = Indole, MR. = Methyl-Red, VP. = Voges-Proskauer, OX. = Oxidase.

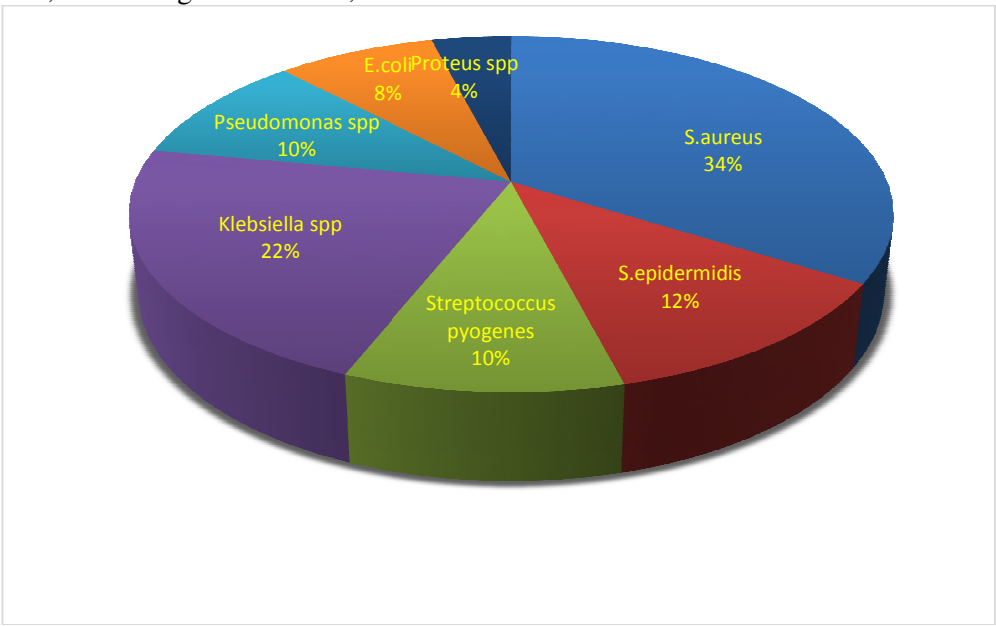

Figure 1: Percentage occurrence of isolates from wound.

Table 2 shows the zone of inhibition of the two honey samples against selected isolates at different concentrations. At concentrations of 30v/v, 50v/v, 70v/v, and 100% (v/v), the growth of *Klebsiella spp* was inhibited to 9mm, 12mm, 14mm and 16mm respectively. The growth of *S.aureus* was inhibited to 8mm, 12mm, 15mm and 19mm at 30v/v, 50v/v, 70v/v and 100% (v/v) honey concentrations respectively, and that of *E.coli* at 30v/v, 50v/v, 70v/v and 100% (v/v) was inhibited to 10mm, 13mm, 15mm, and 17mm respectively. Equally at concentration of 30v/v, 50v/v, 70v/v and 100% (v/v), the growth of pseudomonas was

inhibited to 8mm, 11mm, 13mm and 15mm respectively. Similarly, honey sample 2 had some level of antibacterial activity against all isolates at different concentrations.

Table 2: The antibacterial activity of Honey samples on selected isolates

Test Organisms	Zone of inhibition(mm) Honey concentration (v/v)								
	100%		70%		50%		30%		Ciprofloxacin
	S1	S2	S1	S2	S1	S2	S1	S2	
Klebsiella spp	16	18	14	15	12	13	9	10	27
S.aureus	19	16	15	13	12	12	8	8	29
E.coli	17	17	15	13	13	10	10	7	26
Pseudomonas spp	15	15	13	12	11	11	8	8	28

Key: Ciprofloxacin – Positive Control, S1= Honey Sample 1, S2= Honey Sample 2

Table 3 shows the MIC test result of the 2 honey samples. The lowest MIC value was observed at concentration of 12.5%v/v against *Klebsiella spp*, *S.aureus* and *E.coli* and the highest at concentration of 25%v/v against *Pseudomonas spp*. for honey sample 1. Similarly, the lowest MIC value for sample 2 was seen at a concentration of 12.5%v/v against *Klebsiella spp*, *S.aureus* and *E.coli* while the highest was seen at concentration of 25%v/v against and *Pseudomonas spp.*

Table 3: Minimum inhibitory concentration of Honey samples on the tested organisms

Test Organisms	Concentration of the Honey (v/v)									
	50%		25%		12.5%		6.25%		3.125%	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Klebsiella spp	-	-	-	-	MIC	MIC	+	+	+	+
S.aureus	-	-	-	-	MIC	MIC	+	+	+	+
E.coli	-	-	-	-	MIC	MIC	+	+	+	+
Pseudomonas spp	-	-	MIC	MIC	+	+	+	+	+	+

Key: - = no growth, + = presence of growth, MIC = minimum inhibitory concentration, S1= Honey Sample 1, S2= Honey Sample 2

Table 4 shows the MBC of honey samples against the tested organisms where *Pseudomonas spp* had the highest MBC of 50%v/v for sample 1. Sample 2 had a uniform MBC of 25%v/v for all the isolates.

Table 4: Minimum Bactericidal concentration of Honey samples on the tested organisms

Test Organisms	Concentration of Honey (v/v)					
	50%		25%		12.5%	
	S1	S2	S1	S2	S1	S2
Klebsiella spp	-	-	MBC	MBC	+	+
S.aureus	-	-	MBC	MBC	+	+
E.coli	-	-	MBC	MBC	+	+
Pseudomonas spp	MBC	-	+	MBC	+	+

Key: - = no growth, + = presence of growth, MBC = minimum bactericidal concentration, S1= Honey Sample 1, S2= Honey Sample 2

DISCUSSION

Staphylococcus aureus (42.5%) was the most predominant organism isolated from wound infection. This finding is consistent with reports of similar studies conducted in various parts of Nigeria: Ibadan (Okesola and Kehinde, 2008), Benin-city (Egbe *et al.,* 2011), Ekpoma (Isibor *et al.,* 2008), Maiduguri (Gadzama *et al.,* 2007). This might be due to the fact that *S. aureus* is a resident flora of the skin and therefore easily colonizes wounds resulting to tissue injury due to the change in habitat. However this finding contradicts that of Pondei *et al.* (2013) where *Pseudomonas spp* was the most predominant organisms isolated from wound.

Of the two honey samples tested against the isolates, honey sample 1 at concentrations of 30%, 50%, 70%, and 100% (v/v) inhibited the growth of *Klebsiella* spp , *S. aureus*, *E. coli* and *Pseudomonas* spp. It was observed that the inhibition efficiency of the honey samples on the growth of the test organisms increased with increase in concentration from 30 to 100%. For the four organisms, the zone of inhibition differs significantly at different concentrations of the honey samples with 100% being the most effective. This might justify the reason why traditional healers prescribe honey to treat wounds and skin burns.

Concentrations of 30, 50, 70, and 100% (v/v) of honey sample 2 inhibited the growth of all the tested organisms. Table 3 indicated that increase in concentration of the honey samples increased its inhibition efficiency. The highest antibacterial activity was observed with 100% (v/v) where zones ranged between (15-19mm), these correlates with similar study by (Ibrahim and Aliyu, 2015). Results of Table 2 shows that among the four studied pathogenic bacteria, *Staphylococcus aureus* was the most inhibited (16-19mm), while *Pseudomonas* spp was least inhibited (15mm).

The lowest MIC at concentration of 1.25v/v against *Klebsiella* spp, *S. aureus* and *E.coli* and the highest was seen at concentration of 2.5v/v against and *Pseudomonas spp.* the lowest MIC at concentration of 1.25v/v against *Klebsiella* spp, *S. aureus* and *E. coli* and the highest was seen at concentration of 2.5v/v against *Pseudomonas* spp.

The MBC of honey 1 on the tested organisms showed that *Pseudomonas* spp had the lowest MBC at concentration 5.0v/v and the MBC for the *Klebsiella* spp, *S. aureus* and *E. coli* had the lowest at concentration of 2.5v/v. Honey sample 2 showed bactericidal effects on the tested organisms at a uniform concentration of 2.5v/v. The antibacterial potency differences among the studied honey samples could be attributed to the natural variations in floral sources of nectar and the different geographical locations since honey micro components possess physicochemical and phytochemical characteristics resulting in its potency that differs with botanical and geographical origins (Nahed *et al.,* 2011). The obtained results (the ranges of effective concentrations) are in agreement with the results of Nahed *et al.* (2011) who investigated honey sourced from Egypt, Malaysia and Ethiopia with MIC & MBC values of 12.5 & 50% v/v, 50% & 6.25% w/v and 6.25% w/v respectively.

This study however has demonstrated that honey might not adequately proffer a total solution to the current problems facing bacterial chemotherapy. Users therefore need to be enlightened that honey, being a natural product with very few side effects may not necessarily be superior to conventional therapies. The latter should be employed where necessary without skepticism.

CONCLUSION

Honey possesses antibacterial effects with a broad spectrum of activity. The results of the study also support the traditional application of honey. The antimicrobial activity of honey even at low concentration justifies its efficacy in the treatment of burns/wound especially those associated with *Staphylococcus* species, *Pseudomonas* sp., *Klebsiella* spp. and *Escherichia coli.*

RECOMMENDATIONS

Further work should be encouraged for the extraction of the crude components of honey and their anti-biogram. It is therefore recommended that pure natural honey should be stocked in hospitals/clinics in order to encourage its application in the treatment of various degrees of burns, scalds and other wound infections.

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