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Antimicrobial Activities Of Pure Honey Against Some Bacteria In Wound Infection

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Abstract: ***Antibacterial Activity of pure honey on some Medically important bacteria including staphylococcus aureus, Escherical Coli, Bacillus subtilis and pseudomonas aeruginosa was defined using the agar well diffusion method, the result shows that pure honey exhibit strong antibacterial activity producing Zones of inhibition against the tested bacteria. Also, honey sample used in this study showed antibacterial activity than the commercially available antibiotic (Ciprofloxacin). The research shows that honey apart from their roles as food and supplements, could be suitable for the treatment of various infection caused by bacteria hence, should be used as a preventive and curative measure to common disease related to the test organism.***

Keywords: Antibacteria activity, honey, disease, resistance

I. INTRODUCTION

Honey is a grant divine from God, used since ancient times in the treatment of all diseases such as wound ulcers and burns. Honey is an extra ordinary healthy, high nutritious, yellowish brown, sweet viscid, supersaturated fluid product of honey bee of the general *Apis and meliponinae* produced from the nectar of flower (Eman *et al.*, 2013).

Honey is a traditional topical treatment for infected wounds. It can be effective on anti biotic resistance strains of bacteria. Honey is produced from many floral sources and its antibacterial activity varies with origin and processing. Honey was used to treat infected wound long ago as far back as 2000 years before bacterial were discover to be the cause of infection. Honey was describe as being "good for all rotten and hollow ulcers" (Atrooz. *et al;* 2008). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacterial including aerobes and anaerobe, gram positive and gram negative. The current relevance of antibiotic-resistance microbial species has led to a re-evaluation of the therapeutics use of ancient remedies including honey. Honey has been known to possess anti – microbial properties, as well as wound –healing activity (Zainol *et al*; 2013).

Many people are continually searching for alternative and more natural cures to alleviate the diseases conditions and ailment of man. The natural remedies employed are biological in origin and are often more in form of food that therefore play activities part in the physiological functions and balanced as nature intends. Prominent among those natural remedies is honey (Bedekar *et al;* 2009).

Pure honey has been shown to be bactericidal to many pathogenic micro organism including *salmonella spp*; shigella spp, other enteropathogenic like *Escherichia coli*, *vibro cholerae* and other gram negative and gram positive organism. The ability of honey to kill micro organisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its chemical nature, which include its contents of fetracycline derivatives, peroxides, amyiose, fatty acids, phenols, ascorbic acid, terpenes, benzyl alcohol and benezoic acid (Con-way *et al.*, 2010)

Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidize, which oxidize glucose to gluconic acid and hydrogen

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peroxide .hydrogen peroxide is the major contributor to the anti micro bacterial activity of honey, and the different concentrations of this compound is different honeys result in their varying anti microbial effect. It has further been reported that physical property along with geographical distribution and floral source may play an important role in the anti microbial activity of honey. The antibacterial activity screening and phytochemical analysis of essential plant has been of great interest in the discovery of drug effective in the treatment of several disease (Boom, 2004).

II. MATERIALS AND METHODS

SOURCE OF SAMPLE COLLECTION AND MODE OF IDENTIFICATION OF PURE/ORIGINAL HONEY

The honey sample use in this study was purchase from SBV pure honey deport, Shop 7&8 Tinuola shopping complex, Tipper garage Offa Kwara, State. Several experiments were conduct to ascertain that the honney sample were pure and original.

The sample was then collected in sterilized screw-caped containers and was kept in a dark, cool and dry place (at room temperature) overnight before they were finally transported to the Department of Science laboratory technology, Federal Polytechnic Offa for processing.

PROCESSING OF HONEY SAMPLES

The Sample was first filtered with a sterile mesh to remove debris, viscosity was reduced by heating honey at 30^{o}C for 30minutes. The sample was checked for purity by inoculating on blood agar plates and incubated overnight. Uncontaminated sample was stored at refrigeration temperature of about 4^{o}C until used.

PREPARATION OF CULTURED MEDIA

The cultured media used for this project was prepared according to The manufacturer's instruction. Mueller Hinton agar was prepared by weighing and dissolved 3.8g of the powder in 100ml of distilled water in a conical flask, then covered with cotton wool wrapped with aluminum foil and heated to dissolved on a hot plate after which it was auto craving, The media was allow to cool down to about room temperature (45^{o}C) before dispensed into sterile petri-dishes.

COLLECTION AND MAINTENANCE OF MICROORGANISMS

The micro organisms used in this study which are also known to be potentially pathogenic to humans were obtained as clinical isolate from teaching Hospital in Ilorin, Kwara State, Nigeria. They include *Bacillus Subtiles*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomous aeruginosa*. The organisms were collected on sterile Mccartney. Courtney bottles, and incubated at 37^{o}C. They were kept at stock cultures in the refrigerator until when needed for the analysis. Biochemical Test such as catalyses test, lacto phenol test, indole test, oxidase test and gram stain were carried out to confirm the identity of the organism. These organism were maintained in agar slants at 4^{o}C until used.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antibacterial activity of pure honey in comparison with standard antibiotic ciprofloxacin (100mg/ml) in vitro on the isolates was determined by the agar well diffusion method as described by (Osho and Bello, 2010). This was done using pour plate method in which small colonies from each clinical isolates of the test organisms were made into suspension with 1ml of sterile distilled water in test tubes. 0.1ml of each suspension was dispensed into sterile Petri dishes after which melted and sterilized Mueller Hilton agar maintained at 45^{o}C was poured into the respective plates. The plates were allowed to set, four equidistant wells of 6mm in diameter were punched in each plate using a sterile cork borer. To each of the wells, 0.1ml of pure undiluted honey was introduced. A well filled with sterile antibiotic severed as control and the plates were allowed to stay for 15minutes for pre- diffusion to take place followed by incubation for 24-48hrs at 37^{o}C. The zones of inhibition were measured with the use of a metric rule.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF HONEY SAMPLE

The MIC was determined on plates of Mueller Hinton agar already inoculated with the different bacterial isolates. After setting holes (6mm in diameter) were bored on each agar plate using a sterile cork borer. The holes were labeled accordingly with numbers. The holes were sufficiently spaced to avoid the zone of inhibition from over-lapping.

0.1ml of each of the honey samples were introduced into the holes accordingly using sterile pipettes. The inoculated plates were allowed to stand for 15minutes to ensure proper diffusion of the honey into the medium and incubated at 37C for 24hours. After incubation, the plate were observed for inhibition zones around the holes. The plates were then examined and the diameter of the zone of inhibition was measured.

MAXIMUM BACTERICIDAL CONCENTRATION (MBC)

The MBC was done by picking an inoculation from the cleared zone of inhibition of each isolates and a streak was made on the fresh prepared media and incubated at 37C for 24- 48hours at inverted position.

III. RESULT

The results of the pure honey sample have shown antimicrobial activity against some selected bacteria with different zone of inhibition as stated in table 1 below.

Test Organisms	Dose (Ml)	Zone of Inhibition (MM)
Bacillus subtilis	0.1ml	10mm
Escherichia coli	0.1ml	14mm
Staphylococcus aureus	0.1ml	13mm

Pseudomonas aeruginosa	0.1ml	7mm

Table 1: Antimicrobial Activity of pure honey sample against the bacterial Isolates

The standard antibiotic used which served as control (ciprofloxacin 100mg/ml), has shown antimicrobial activity against some selected bacteria with different zones of inhibition as stated in table 2 below.

Test Organisms	Dose (ml)	Zone of Inhibition (MM)
Bacillus subtilis	0.1ml	8mm
Escherichia coli	0.1ml	10mm
Staphylococcus aureus	0.1ml	7mm
Pseudomonas aeruginosa	0.1ml	8mm

Table 2: Antimicrobial Activity of a Standard Drug Ciprofloxacin (100mg/ml) Against Some Bacteria

The result of the pure honey diluted at different concentration, has shown the minimum inhibitory concentration against some selected bacteria isolate as stated in table 3 below.

Test organisms	Concentration (MIC)					
	100%	90%	80%	70%	60%	50%
Bacillus subtilis						
Escherichia coli	+10	4*	-	-	-	-
Staphylococcus aureus	+14	+11	+8*	-	-	-
Pseudomonas aeruginosa	+13	+12	+9*	-	-	-
	+7	+5*	-	-	-	-

*NB Key: *= MIC (Minimum Inhibitory Concentration)*
+ = Activities (Zone of Inhibition)
- = No activities (No Zone of Inhibition)

Table 3: Minimum Inhibitory Concentration (MIC)

The pure honey diluted at different concentration has revealed that undiluted honey (100%) is bactericidal, because it shows no growth on the freshly prepared media while diluted one is bacteria static as stated in table 4 below.

Test organisms	Concentration (MBC)					
	100%	90%	80%	70%	60%	50%
Bacillus subtilis						
Escherichia coli	+	-	-	-	-	-
Staphylococcus aureus	+	-	-	-	-	-
Pseudomonas aeruginosa	+	-	-	-	-	-
	+	-	-	-	-	-

Key:- + = Activities (No Growth observed)
- = No Activities (Present of Growth)

Table 4: Minimum Bactrocidal Concentration (MBC)

IV. DISCUSSION

The result have shown that pure honey contains antimicrobial activity against both gram negative and gram positive bacteria as indicated by their zones of inhibition. *Escherichia coli and Staphylococcus aureus,* shows greater level of susceptibility (15mm and 14mm respectively) and *pseudomonas aureuginosa* was the most resistant isolate tested in this study as it shown the least zone of inhibition of 7mm as stated in (Table 1).

The antibacterial activity at a standard drug used (Ciprofloxacin) which served as control has shown maximum zone of inhibition of (10mm) against *Escherichia coli and Staphylococcus aureus* posses lowest zone of inhibition of (7mm), as stated in (table2).

The result of pure honey diluted at different concentration, has revealed the minimum inhibitory concentration against some selected bacteria with different zones of inhibition. It was observed that the antibacterial activity of pure honey depend on the concentration used as stated in (tables 3).

The pure honey diluted at different concentration has revealed that undiluted honey (100%) is bactericidal while diluted honey at different concentration was bacteria static as stated in (table 4). The observation agreed with (Conway *et al.,* 2010), which stated that pure honey has been shown to be bactericidal to many pathogenic micro organisms.

The antibacterial action of honey may be due to the H_2O_2 (hydrogen peroxide) and non peroxide antibacterial factors. The activity of H_2O_2 works when honey is diluted moreover, the hygroscopic feature of honey causes withdrawal of moisture from the surroundings by osmosis leading to microbial death (Eman, *et al.,* 2013).

The large variance in antibacterial potency of different honeys may be due to their floral source and geographical origin expressed as large discrepancy in results reported between authors and hospitals using honey in similar ways. Some have reported rapid clearance of infection in a range of different types of wound, with all wounds becoming sterile in 3-6 days 7 days or 7-10 days (Eman *et al.,* 2013). Others have reported bacteria still present in wounds after 2 weeks (Zainol *et al.,* 2013).

MIC of selected honeys as antibacterial against isolated bacterial strains from wounds and burns, investigation displayed that, the total MIC of honeys in Saudi Arabia ranged from 3.04% to 26.66%, the lowest total MIC was for Katad honey (3.04%) and the largest MIC was for Wadi-Rk honey (26.33%). The isolated bacterial strains *P. aeruginosa* (12.44%), *Staphylococcus aureus* (13.36%), *Escherichia coli* (14.50%). The results indicated that *P. aeruginosa* isolated from wounds and burns was more susceptible for inhibition with (12.44%) MIC by different types of honeys in Saudi Arabia in honey markets followed by *Staphylococcus aureus* (13.36 %), Escherichia coli (14.50%). The MICs of various types of honeys for various pathogenic bacterial strains have been determined by many authors (Cooper, et al., 2002). The results of the present study nearly coincide with (Zainol, *et al.,* 2013), who reported that there is correlations between MIC and Equivalent Phenol Concentration EPC value of Malaysian honey were proven to be dependent on bacteria species and honey origin.

V. CONCLUSIONS

Honey obtained from SBV Original honey Deport Tipper garage Offa Kwara, State possesses antimicrobial activity. It is a potential source of alternative antimicrobial agents with a

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broad spectrum activity. The results of the study also support the traditional application of honey. The antimicrobial activity of honey even at lower strength (minimum inhibitory concentration) of 90%, 80% and 70% justified their efficacy in the treatment of burns/wounds especially those treatment associated with *Staphylococcus aureus, Escherichia coli* and *pseudomonas aeruginosa*.

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