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Sterility Examination and Comparative Analyses of Inhibitory Effect of Honey on Some Gram Negative and Gram Positive Food Borne Pathogens in South West Nigeria

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***Abstract*—**Food borne illnesses have been reported to be a global health challenge. Annual incidences of food–related diseases involve 76 million cases, of which only 14 million can be traced to known pathogens. Poor hygienic practices have contributed greatly to this. It has been reported that in the year 2000 about 2.1 million people died from diarrheal diseases, hence, there is a need to ensure food safety at all level. This study focused on the sterility examination and inhibitory effect of honey samples on selected gram negative and gram positive food borne pathogen from South West Nigeria. The laboratory examinations revealed the presence of some bacterial and fungal contaminations of honey samples and that inhibitory activity of the honey sample was more pronounced on the gram negative bacteria than the gram positive bacterial isolates. Antibiotic sensitivity test conducted on the different bacterial isolates also showed that honey was able to inhibit the proliferation of the tested bacteria than the employed antibiotics.

***Keywords*—**Food borne illness, gram positive and gram negative bacteria, honey, and inhibitory activity.

I. Introduction

FOOD borne pathogens are the leading cause of illness and death in less developed countries killing approximately 1.8 million people annually. In developed countries, food borne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, leading to great economic loss yearly. New food borne pathogens and food borne diseases are likely to emerge due to factors such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status. According to Center for disease control and prevention, [1], roughly 1 in 6 Americans (or 48 million people) get sick, 128,000 are hospitalized and 3,000 die of food borne diseases each year. All the food-borne diseases are associated with poor hygienic practices. There are growing concerns that terrorists could use pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth. This development has therefore facilitated research into the most important food borne pathogens and ways to combat their spread.

Honey is created by bees as a source of food. It has been used as a medicine since ancient times in many cultures. Honey has been discovered for the treatment of bacterial infections by medical profession, particularly, where convectional modern therapeutic agents are failing [2]. In the ancient times, the Egyptians and Greeks used natural unprocessed honey as a topical application to prevent microbial infections and aid wound healing. Since early times, common uses of honey included treatment of ulcers, bedsores and infections resulting from burns and wounds [3]. Honey has also been found to be effective against some microorganisms isolated from urinary tract infections [4] and in the treatment of infantile gastro-enteritis [5]. The first study on the antimicrobial effects of honey was conducted by Dustmann [6]. Since then, various other studies have been published on this subject [7] – [8]. As a result of the public health burden posed by the food borne pathogens globally, it is imperative that effective control measures are developed. This research focused on the use of honey as inhibitor of some selected gram negative and gram positive organisms in

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II. Methodology

A. Collection of Samples

Fifty honey samples were randomly purchased from peasant farmers at different locations in South Western Nigeria namely: Ondo State (Akungba Akoko, Owo and Akure), Ekiti State (Ifaki Ekiti, Iyin Ekiti, Ado Ekiti and Ikere Ekiti), Lagos State (Oshodi, Agege, Mushin), Oyo State (Ibadan, Ogbomoso, Oyo town) and Ogun State (Abeokuta, Sango-Ota, Ifo). These honey samples were taken to the laboratory and screened for antibacterial activity on selected pathogenic bacteria.

B. Sterility Assay on Honey

Exactly 1 ml from all the honey samples each were aseptically streaked on both nutrient agar and potato dextrose agar plates and incubated at 37° C for 24 hours and 28° C ± 1 for 72 hours respectively. They were then observed for growth. Standard microbiological analysis was carried out to identify microorganisms from the honey samples.

C. Test Organisms

The test organisms used for this research work were *Bacillus anthracis, Clostridium botulinum, Shigella dysentariae and Vibrio cholera*e.

D. Bio-Assay of Inhibitory Potency of Honey on the Bacterial Isolates

The agar well diffusion technique was employed in this bio-assay. Molten Mueller Hinton agar (Oxoid) was prepared by suspending 3.8 g of the powder in 100 ml of sterile distilled water and brought to boiling to dissolve the medium before sterilizing with autoclave at 121° C for 15 minutes. Inocula of the bacterial test organisms were prepared from 24 hour old cultures. The absorbance was read at 530 nm and adjusted with sterile distilled water to match that of a 0.5 Mac Farland standard solution. From this prepared bacterial solution, serial dilutions with sterile distilled water were prepared to give a final concentration of about 10^7 colony forming unit per milliliter (cfu/ml). 1 ml each of the prepared bacterial solutions were pour plated with sterile agar cooled to about 45° C. The plates were allowed to set. With a previously sterilized cork borer (4 mm size), wells of equal distance were bored. The honey samples were aseptically dispensed into the wells which were appropriately distinguished with codes. The plates were incubated at 37° C for 24 hours. Inhibition indicated by clear halo around the wells were measured and taken as degree of susceptibility of the organisms to the samples.

III. Results And Discussion

The cultural characteristics (such as colony colour, edge, surface, elevation), physiological (indole, motility, catalase, methyl red, voges proskauer tests) and biochemical tests (involving the organisms ability to utilize carbohydrates) carried out identified the isolated organisms as *Streptomyces* species, *Escherichia coli, Bacillus cereus, Staphylococcus aureus* while *Penicillum notatum* and *Aspergillus flavus* were characterized using both the inspection of colonial features, fruiting body, mycelia features, cellular characteristics at (X100 and X 400) microscope magnification according to the criteria of Barnett and Hunter [9]. Among the isolated organisms *Staphylococcus aureus* and *Escherichia coli* occurred most as shown on the frequency tables (Table I and Table II).

The honey samples was able to inhibit all the test bacterial isolates with the highest zone of inhibition recorded in *Shigella dysentariae* (26 mm) followed by *Vibrio cholerae* (24 mm) *Clostridium botulinum* (20 mm) while the least zone was observed in *Bacillus anthracis* (14mm). Standard Antibiotic such as tetracyclin, ampicillin and streptomycin showed resistance to all the test bacterial isolates. Tetracycline showed the highest zone of inhibition of 25 mm on *Vibrio cholerae*.

The result of the isolated microorganisms from honey sample showed that bacteria are most common than fungi isolates in the samples. These organisms could be as a result of contamination from humans through handling, larva of the bees and harvesting containers. *Escherichia coli* is known to be among the micro flora of the intestine which could be introduced through improper handling during harvesting. According to Root [10], pollen may be the original source of microbes in the intestine of honeybees and it has been suggested that flowers and hives are more important sources of microbes than the soil. Sackelt [11] also reported that *Saccharomyces, Micrococcus* and *Bacillus* species could be readily isolated from honeycombs and adult bees. Peter *et al*., [12] reported that possible route of transmission into extracted honey will include air (in the house or while the honey was packed), honey handlers (from skin infection, sneezing or faecal contamination). Those findings are in agreement with this study that some honey samples could be contaminated with microorganisms.

This study emphasized that honey has the ability to inhibit bacteria which are resistant to some antibiotics. The resistance displayed by ampicilin, tetracycline and streptomycin could be as a result of drug abuse. Honey was able to compete favorably with the commercial antibiotics by displaying high zone of inhibition against the test organisms. The high activity of honey according to Radwan *et al.,* [13] might be due to some physico–chemical properties such as high content of reducing sugar, high viscosity, high osmotic pressure, low pH, low water activity (a_w), low protein content and hydrogen peroxide.

IV. Conclusion

The results in this study confirmed honey to possess antibacterial potency by displaying high zones of inhibition that are higher than the standard antibiotics employed in this study. It can also be concluded that gram negative bacteria were inhibited more than the gram positive bacteria, which is a good development as gram negative organisms have been known to be more resistant to antibiotics because of the lipopolysaccharide and thick peptidoglycan layer they possess in their cell wall. Honey can be used to control the public heath challenges posed by food borne diseases caused by these bacteria.

TABLE I
FREQUENCY OF OCCURRENCE OF MICROORGANISMS ISOLATED FROM HONEY

Isolated microorganisms	**Frequency of occurrence of the isolates**				
BACTERIA ISOLATES	**ONDO**	**OYO**	**LAGOS**	**EKITI**	**OGUN**
Streptomyces species	-	+	-	-	-
Escherichia coli	+	-	+	+	-
Bacillus cereus	-	+	-	-	-
Staphylococcus aureus	+	-	-	+	+
FUNGI ISOLATES					
Aspergillus flavus	-	-	-	-	-
Penicillium notatum	-	+	-	-	+

TABLE II
ANTIMICROBIAL ACTIVITY OF HONEY AND STANDARD ANTIBIOTIC DISC

Test Bacteria	**Zone of inhibition (mm)**							
Gram Positive bacteria	Honey	GEN	NAL	COT	NIT	STR	TET	AMP
Bacillus anthracis	14.67^{c}±0.45	14	15	10	14	10	R	R
Clostridium botulinum	20.67^{b}±0.22	17	12	10	12	7	R	R
Gram Negative bacteria	Honey	GEN	PEN	CHL	ERY	STR	TET	AMP
Shigella dysentariae	26.23^{a}±0.57	10	7	12	15	R	19	15
Vibrio cholerae	24.67^{a}±1.15	15	9	10	12	R	25	17

There were four replicates per treatment with 20 honey samples per replicate. Mean with columns with the same letters are not significantly different $P \leq 0.05$.
Key: R=Resistance (no zone of inhibition), GEN=gentamycin, NAL=Nalicilline, COT= Cotrimoxazole, NIT=Nitrofuratin, STR=Streptomycin, TET=Tetracyclin, AMP=Ampicillin.

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