Original Research Article

Antibacterial Effect of Raw Honey on Clinical Bacterial Isolates of *Pseudomonas aeruginosa, Staphylococcus aureus,* And *Escherichia coli*

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Abstract: The development of multiple drug resistant bacteria, causing human infection in clinical practice unfold the use of natural sources like honey, medicinal plants of non-antibiotic drugs, having antibacterial potentiality. Beside the medicinal plants, the antibacterial effects of raw honey on clinical bacterial isolates of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* were studied. The raw honey samples were collected from the beekeepers at Umugwu Ezedike in Udi Local Government Area Enugu State Nigeria. The honey samples were prepared by diluting the honey with distilled water to produce honey of various concentrations, i.e. 20% v/v, 40% v/v, 60% v/v, 80% v/v and 100% v/v. The clinical isolates from Accident and Emergency Department University of Nigeria Teaching Hospital Ituku-Ozalla Enugu were cultured in Nutrient agar, Cetrimide agar, Macconkey agar, and Methylene Blue agar (EMB) for identification and confirmation. The antibacterial effects of raw honey on the clinical bacterial isolates were evaluated in-vitro using agar well diffusion methods with Gentamicin as control. The results showed that raw honey demonstrated antibacterial effect against the test isolates with higher effect in *Staphylococcus aureus* (with zone of inhibition of 21mm) followed by *Pseudomonas aeruginosa* (19mm) *and Escherichia coli* (18mm). The minimum inhibitory concentration (MIC) of the extract from this study ranges from 12.5-50mg/ml while the minimum bactericidal concentration (MBC) of the extracts ranges from 25-50mg/ ml. Hence, raw honey has antibacterial properties and can also serve as a therapeutic substance for the treatment of bed sores, and other infections resulting from burns.
Keywords: Raw honey, antibacterial effects, *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* infections.

INTRODUCTION

Honey is a carbohydrate rich syrup produced by bees or sweet material made from floral nectars by certain bees, primarily the honey bee (Apis mellifera) (Mundo *et al.,* 2004). Fructose and glucose are the major components of honey but a large number of other chemical compounds are present in small quantities. The medicinal uses of

honey have been reported among the Egyptians, Chinese, Greeks and the Romans, where it is used for the treatment of wounds and disease (Bogdanov *et al.*, 2007). Honey is best known for its inhibitory effect on around 60 species of bacteria including aerobes and anaerobes, gram positive and gram negative (Mundo *et al.*, 2004). Honey can be expected to have a direct nutrient effect on regenerating tissue because it contains a wide range of amino-acids, vitamins and trace elements in large quantities. Wound infection is the cause of increased duration of hospital stays, profound discomfort and significant increase in healthcare cost (Rubin, 2006). Infection in a wound delay healing and may cause wound break down and complete wound dehiscence. Therefore, it is important to have the ground knowledge of the causative agents of wound infection to enable easy control of wound infection and selection of antimicrobial therapy as a good measure for infection control. Aerobic organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and beta haemolytic *Streptococci* have been most currently reported as the cause of delay wound healing. Honey quickly rendered wounds infected with *Staphylococcus aureus* sterile (Rubin, 2006). Various skin conditions such as Fournier's gangrene, burns, topical leer, bed sores and diabetic ulcers, that were not responding to antibiotic treatment responded well to the application of raw honey. The mechanisms involved in antibacterial activity of honey is linked to high osmotic nature and naturally low pH (3.2-4.5), presence of phytochemical factors such as tetracycline derivatives, peroxides, amylase, fatty acids, phenols, ascorbic acid, terpenes, benzyl alcohol and benzoic acid and also ability to produce hydrogen peroxide (Bogdanov *et al.*, 2007). The easily availability, cheap, non-toxic could be attributed to the use of honey as an alternative antimicrobial therapy and moreover bacterial resistance to it is yet to be reported. It is remarkable that ancient physicians were selective in the honeys that they utilized in their remedies, although the underlying principles would have been obscure. Now it is possible to determine the antibacterial effects of raw honey on some clinical bacterial isolates.

MATERIALS AND METHODS
Study Area
The study was carried out at in Enugu metropolis, clinical stock culture of *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* was collected from Accident and Emergency Department University of Nigeria Teaching Hospital Ituku-Ozalla Enugu Nigeria. It is sited along Enugu-Port Harcourt Express Way; it is about 21 kilometers drive from Enugu capital city. The site of the hospital covers an area of 200 acres while the entire parcel of land is about 306 hectares. The space has solved the accommodation needs of the hospital. Federal Government assisted Equipment Program has also equipped the new hospital complex which has elevated it to an international standard.

Fig 1: Entrance View of University of Nigeria Teaching Hospital Ituku-Ozalla Enugu   Nigeria

Collection and Transportation of the Clinical Stock Culture
The clinical stock culture of *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* was collected from Accident and Emergency Department University of Nigeria Teaching Hospital Ituku-Ozalla Enugu Nigeria and was immediately transported to Microbiology Laboratory Enugu State University of Science and Technology for identification and confirmation.

Isolation of the Organisms from the Clinical Stock Culture
This was carried out as described by (Cheesbrough, 2010). The organisms from clinical stock culture were serial diluted in 10-fold and

cultured on Nutrient agar and incubated for 24 hours at 37°C. After incubation, discrete colonies were sub cultured on Nutrient ager, Cetrimide agar, MacConkey agar, Mannitol salt agar and Eosin Methylene Blue agar (EMB) to obtain pure culture. The sub-culturing of the isolates was done by placing each colony in the middle of the agar plate and streaked up and down and across the plate and incubated at 37°C for 24 hours.

Identification of the Isolates from the Clinical Stock Culture

The identification was done using a method described by (Cheesbrough, 2000). The isolates were identified and characterized based on the morphological and biochemical test which include; Sub-culturing appearance on selective and differential media Cetrimide agar, Mannitol Salt Agar and Eosin Methylene Blue agar (EMB). Gram staining, Indole test, Catalase, test and Oxidase test, Urease test, Citrate Utilization test, Methyl red test, Glucose fermentation test, Lactose fermentation test and Voges-proskaur test.

Collection and Transportation of the Raw Honey Samples

The raw honey samples were collected without additives from the beekeepers at Umugwu Ezedike in Udi Local Government Area Enugu State Nigeria. The honey samples was transferred into a sterile stainless ice parked flask and immediately transported to Microbiology Laboratory Enugu State University of Science and Technology for identification.

Identification Test of the Raw Honey

This was carried out by (Molan, 2011). A spoonful of raw honey was added to a glass of warm water, and was stirred slowly. This was carried out to know whether it dissolved in the water. Raw honey is known naturally to remains stuck as a lump on the spoon or sticks together and sunk as a solid lump in water. Candle wick dipped in honey was set fire to check for presence of water in the honey which might stop the honey from burning.

Preparation of Raw Honey Concentrations

According to (Molan, 2011). Different concentrations of raw honey contributing 20% v/v, 40% v/v, 60% v/v, 80% v/v and 100% v/v were made in sterile distilled water. This was carried out by dissolving the individual volumes of honey into respective volumes of sterile distilled water.

Antibacterial Effects of Raw Honey on the Clinical Bacterial Isolates

The antibacterial effect of raw honey against the clinical bacterial isolates was tested using agar well diffusion method as described by (Ali *et al.*, 2017). The culture media used for the assay was the Mueller Hinton Agar. One hundred microlitres (10^6 CFU /ml) of fresh microbial culture of the clinical isolates was spread on plates containing Muller Hilton agar using a sterile glass spreader. Six plates in total were used to carry out this assay. Five agar wells of 6mm diameter each was punched off into each of the plates using a sterile cork borer. Each well was filled with different concentrations of the raw honey using a sterile dropper. The plates were incubated at 37°C for 24 hrs and zones of inhibition were observed. This in-vitro experiment was compared with the use of a sensitivity disc Gentamicin which served as a control.

Determination of Minimum Inhibitory Concentration (MIC) of the Honey Extracts

The MIC of the honey was determined as described by Ahmed and Beg (2001), using broth dilution technique. Two-fold serial dilutions of the honey were prepared by adding 2ml of 100v/v of the honey into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50v/v of the extract. The process continues serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25v/v and 3.125v/v. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37 °C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity.

Determination of Minimum Bactericidal Concentration (MBC) of the Honey Extracts

From the result of MIC, the test tubes that did not show visible growth were used for MBC determination. About 0.1 ml was aseptically transferred onto the surface of Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hours. The MBC of the extracts was recorded as the lowest concentration of the extract that had less than 99% growth on Mueller Hinton agar plates (Weston, 2000).

RESULTS

The morphological characteristics of the clinical isolates; *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are shown in Table 1.

Table 1: Morphological Characteristics of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*

Bacterial Isolates	Macroscopic Characteristics	Microscopic Characteristics
Pseudomonas aeruginosa	Consisted of bright green colour colonies on Cetrimide agar and also appeared opaque on MacConkey agar	Consisted of Gram negative slim rods which appeared pink.
Staphylococcus aureus	Colonies appeared yellow on Manitol salt agar and also consisted of smooth, golden yellow colonies on Nutrient agar	Consisted of Gram positive cocci in clusters which appeared purple.
Escherichia coli	Appeared mucoid bright pink colonies on MacConkey agar and also consisted of thick, metallic green sheen on Eosin Methylene Blue agar	Consisted of Gram negative single short rods which appeared pink.

The biochemical characteristics of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are shown in Table 2.

Table 2: Biochemical Characteristics of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*

Bacterial isolate	Gram Stain	Indole test	Catalase test	Oxidase test	Urease test	Citrate Utilization test	Methyl red test	Glucose fermentation test	Lactose fermentation test	Voges-p roskaur test
Pseudomonas aeruginosa	-	-	+	+	-	+	-	-	-	-
Staphylococcus aureus	+	-	+	-	+	+	+	+	+	+
Escherichia coli	-	+	+	-	-	-	+	+	+	-

KEYS
- Negative
+ Postive

The antibacterial effects of raw honey on the clinical bacterial isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are shown in Table 3.

Table 3: Antibacterial Effects of Raw Honey on the Clinical Bacterial Isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*

Conc. (v/v)	20%	40%	60%	80%	100%	Control
Bacterial Isolates	IZD (mm)	1ZD (mm)	IZD (mm)	IZD (mm)	IZD (mm)	
Pseudomonas aeruginosa	11	13	15	17	19	20
Staphylococcus aureus	10	11	14	17	21	23
Escherichia coli	10	12	13	16	18	21

KEYS:
Inhibition Zone Diameter (IZD mm)
Sensitive: (≥12mm)
Moderately Sensitive: (10-11mm)
Resistant: (≤9mm)

The minimum inhibitory concentration and minimum bactericidal concentration of raw honey on the test organisms are shown in Table 4.

Table 4: MIC and MBC of raw honey on the clinical bacterial isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*

Bacterial isolate	Different concentrations of raw honey extracts tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* with reference to MIC and MBC					MIC	MBC
	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml		
Pseudomonas aeruginosa	-	-	-	+	+		
Staphylococcus aureus	-	-	-	+	+		
Escherichia coli	-	-	-	+	+	12.5mg/ml	25mg/ml

KEYS
MIC: minimum inhibition concentration
MBC: minimum bactericidal concentration
(+) : showing growth ie. (Showed turbidity)
(−) : no growth ie. (There was no turbidity)

DISCUSSION

Studies were conducted on the antibacterial effects of raw honey against clinical bacterial isolates from Accident and Emergency Department University of Nigeria Teaching Hospital Ituku-Ozalla Enugu Nigeria. The isolates were morphologically and biochemically identified as *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* using standard identification techniques (Tables 1 & 2). These are in line with the work of Cheesbrough, (2000). The antibacterial effects of raw honey on the clinical bacterial isolates of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* was evaluated using the agar well diffusion method and the results showed that all the clinical bacterial isolates were sensitive to raw honey exhibiting inhibition zones diameter of 19mm, 21mm and 18mm at 100% v/v concentration against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* respectively (Table 3). The result obtained was in accordance with the work of (Ali *et al.*, 2017) that carried out in-vitro antibacterial activity and phytochemical screening of *Psidium guajava* on some enteric bacterial isolates of public health importance. Studies were also carried out to determine the Minimum inhibitory concentration and Minimum bactericidal concentration of the raw honey against *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli.* Results were obtained for the minimum inhibitory concentration which showed that at a concentration 12.5mg/ml the raw honey was able to inhibit the growth of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli.* The minimum bactericidal concentration (MBC) of the honey showed that the honey can kill the test isolates at concentration of 12.5– 50mg/ml (Table 4). The findings agree with work of (Mundo *et al.*, 2004).

CONCLUSION

This study showed that there was antibacterial effect of raw honey on the clinical bacterial isolates of *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* which also associated with wound infection. The antibacterial effect of raw honey was largely due to certain antibacterial properties it possessed, which include low water activity, low PH, high osmotic pressure, and possession of certain gluco-oxidase enzymes which produce aseptic substances such as hydrogen peroxide. Hence, raw honey can be used as a therapy for wound infection and also it ushered relief to the condition of antibiotic resistance encounter in clinics.

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