ANTIBACTERIAL ACTIVITY OF HONEY ON *Staphylococcus aureusEscherichia coli and Streptococcus pyogen* ISOLATED FROM WOUND

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MB/2008/406

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CERTIFICATION PAGE

I certify that this research project was carried out by Onyeji Cynthia Onyinye (MB/2008/406) in the department of Microbiology and Biotechnology, Faculty of Natural Sciences, Caritas University, Amorji-Nike Enugu. The department recognizes that Onyeji Cynthia Onyinye (MB/2008/406) bear full responsibility for this work.

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DEDICATION

This work is dedicated to the most Sacred Heart of Jesus Christ and our mother Mary for an awesome protection, guidance and intercession throughout my stay in this school. To my parents Mr. & Mrs. D.N Onyeji for considering my education a priority.

ACKNOLEDGEMENT

My gratified thanks go to God almighty for his immeasurable blessings and wisdom upon me. To my wonderful Parents, Supervisor Dr. M.U Orji for his incomparable support and time. I wish to express my gratitude to my friends Joseph N. Ekwelonu, Chidera, Stella, Sopuluchukwu, Room Mates and finally my wonderful Course Mates, I say thank you all and God bless you.

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ABSTRACT

Antibacterial activity of honey obtained from two different locations in Enugu State (Nsukka & Ugwuaji) Nigeria on *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogens* isolated from wound was studied. Agar well diffusion method was used to determine the antibacterial activity of the honey on the test microorganisms. The result revealed that the two honey samples have heavy antibacterial activities against the test organisms and zones of inhibition were obtained showing high antibacterial activity. The antibacterial activity increased with increase in the concentrations and honey from Nsukka produced a high antibacterial activity (clearer zone) on *staphylococcus* aureus and *Escherichia coli* at all concentration and moderately for *streptococcus pyogens*. The use of honey as a therapeutic substance has been rediscovered by the medical profession on more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of ulcers and bed sores, and other infections resulting from burns and wounds.

CHAPTER ONE

1.0 Introduction

Infections and other health related problems have been of great concern to human beings and chemotherapy is the main approach in the treatment of such conditions. Investigation into the microbial flora of wound began in the late 19th century and since then; improvements in techniques have facilitated the recovery, identification and enumeration of a wide variety of microbial species. Most wounds support relatively stable polymicrobial communities (Bowkler, *et.al;* 2001) often without signs of clinical infection (Hansson,*et al;* 1993).

However, potential pathogens may be present and the delicate balance between colonized wound and an infected wound depends on the interplay of complex host and microbial influences (Emmerson, 1998). The development of wound infection has deleterious effect on

patients by causing increased pain, discomfort, inconveniences and can lead to life threatening conditions or even death.

Major challenges encountered with antibiotics in clinical use are resistance to antibiotics which leads eventually to failure of the treatment (Blair 2004). Infectious diseases are known to be treated with herbal remedies throughout the history of mankind; even today, natural substances continue to play a major role in primary health care as therapeutic remedies in many developing countries (Jonathan, *et.al*; 2007). Over the years, there have been reports of the production of more potent antibiotics e.g. third and fourth generation of cephalosporin by pharmaceutical companies which are not readily available and expensive. Problems of various antibiotics include low efficacy, side effect which has lead investigations into natural and potent antibacterial seeming to be the right step to take. The invasion of pathogenic organism is on the rise as a result, effects are been made to develop antibacterial agent from natural sources for better

therapeutic effect (Gills, 1992). The therapies have drawn the interest of both public and medicinal communities. Current research has been focused on herbal and aromatherapy product. However, a number of their product such as honey has shown therapeutic promise.

The presence in honey of various inhibins as described by (Doid and Dzaio, 1937) has been reported by several investigators. Honey was used to treat infected wound as long as 2000 years ago before bacterial were discovered to be the cause of infection in c.50 AD, Dioscorides described honey as been "good for all rotten and hollow ulcers" (Gunther, 1959). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacterial including aerobes and anaerobes, Gram positive and Gram negative (Molan, 1992). The current prevalence of the therapeutic use of ancient remedies, include honey committee on science and technology.

1.1 Aims and objectives.

1. To determine antibacterial potential of honey.

2. To investigate the mechanism of antibacterial action of honey.
3. To determine the minimum inhibitory concentration of honey on bacterial isolates from wounds of human beings.
4. To yield additional knowledge such as the possible dilution of honey sample and activity of the honey sample in bacterial infection.

CHAPTER TWO

2.0 Literature Review

The medicinal properties of honey have been reported and documented by beekeepers and medical practitioners (Bankova, *et.al*; 2002). As a result of over use and abuse of antibiotics, there have been increases in the number of diseases, which seem to evolve to become more virulent with each generation. Investigations into natural and potent antimicrobials seemed to be the right step to take. The invasion of pathogenic organism is on the rise and as a result, effects are being made to develop antimicrobial agents from natural sources for better therapeutic effect (Gills, 1992). The therapies have drawn the interest of both public and medical communities. Current research has been focused on herbal and aromatherapy products.

Antimicrobial agents have been applied to wound for thousands of years (Moellering, 1995) but many remedies have been discontinued because the evidence to support their efficacy was anecdotal.

Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has lead to the emergence of antibiotics-resistance strains which, in turn, has driven the continued search for new agents. Unfortunately, the increased costs of searching for such agents and the decreasing rate of their discovery (Moellering, 1995) has made the situation increasing urgent and the prevalence of antibiotics-resistant microbial species now justifies the re-evaluation of former treatment (Anon, 1998). Honey has been used as a medicine since ancient times in many cultures and is still used in 'folk medicine'. The use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of ulcers and bed sores, and other infections resulting from burns and wound. In many of the cases in the cited reports, honey was used on infections not responding to standard effective in rapidly clearing up infection and promoting healing. Honey has also been found to be effective in treating bacterial gastroenteritis in infants.

The medicinal use of honey in wound treatment is derived from diverse ancient civilizations (Jones, 2001). The antibacterial properties of honey were recognized more than a century ago and have subsequently been extensively studied (Molan, 1992a, 1992b). A wide range of microbial species has been shown to be inhibited by honey but reported susceptibilities are not consistent. Failure to identify the botanical sources of honeys used in many of those studies, or to determine their antibacterial potency, makes comparison of reported sensitivities unreliable. It is remarkable that ancient physicians were selective in the honeys that they utilized in their remedies (Jones, 2001), although the underlying principles would have been obscure. Now it is possible to determine quantitatively the antimicrobial activity of a honey (Allen, *et. al;* 1991) and also to discriminate between honeys whose mode of action involves factors beyond their osmolarity in limiting bacterial growth (Allen, *et.al;* 1991). In most honeys this depends on the enzymic generation of hydrogen peroxide to varying degrees (Molan, 1992a)

but, in some honeys, there are additional phytochemical antibacterial factors (Molan, 1992a). In recent studies, the susceptibility of wound pathogens (Willix, *et. al;* 1992) and bacterial isolated from wound (Cooper and Molan 1999; Cooper *et. al*; 1999) to honeys of known floral source and defined antibacterial activity has been reported. However, the inhibition f antibiotic-resistant bacteria by honey has not been fully explored .using characterized honey, this study aims to extend the range of wound pathogens whose susceptibility to honey has been determined and to compare the susceptibility of antibiotic-sensitivity strains with those of antibiotics-resistant strains.

2.1 Wound infection

The moist environment of chronic wounds is an ideal growth medium for bacteria (O'Meara *et. al;* 2010) and infection is the prominent cause of delayed healing. This has become an increasing problem with the recent expansion of antibiotic-resistant bacteria (Leaper, 2006). Burns and chronic wound are particularly prone to infection

with 075% of dirty following burns involving infection (Thorn *et. al;* 2006). There is increasing interest in the use of topical antimicrobial to wound cure. Compounds such as honey, iodine and silver have been incorporated into dressings are simply the addition of an antimicrobial to a pre-existing product (Thomas, 2003). Many in vitro investigations into the efficacy of such product have been performed: however there is a confusing mixture of evidence and differences in research methodologies making interpretation and comparison of results difficult. In addition, they are very few randomized controlled trials comparing wound care product in clinical practice. A wound may be defined as a breech in the epidermis or dermis due to trauma or physiological change, activating the repair process (Benbow, 2005) wounds can be classified as either acute or chronic.

2.2 honey

Honey is the sweet substance made by bees using nectar from flowers. Honey is made when the nectar and deposit from plant are

gathered, modified and stored in the honey comb by honey bees (National Honey Board, 1996). The definition of honey stipulates a pure product that does not allow for the addition of any other substance such as water or other sweeteners. The flower from which bees gather nectar largely determines the colour, flavor and aroma of honey (Caron, 2004). Honey is also said to be highly variable like must plant derived product and the chemical composition of honey also depends on the flower from which it is made. Antibacterial effect may therefore vary between different types of honey (Ovington, 1999). Honey is classified by the flower source and therefore is divided according to their package and processing. Generally honey is classified by the flora source of the nectar from which it was made. Honey can be from specific types of flower nectar indeterminate origin or can be blended after collection.

Honey originally used by the ancient Egyptians and Greeks, is a viscous, saturated sugar solution now widely used in wound care

(Simon *et. al*; 2009). High osmolarity prevents the growth of bacteria and encourages healing. This can be utilized for wound management through the application of sugar paste or honey. In addition, honey is believed to have specific antimicrobial properties, for example, preventing the growth of *staphylococcus aureus* even when diluted beyond the point at which osmolarity is no longer inhibitory (Moore *et. al*; 2001). Studies have reported that it may modestly decrease wound healing time, act as an anti-inflammatory, deodorize wounds, and enhance cell proliferation and expansion in in-vitro (Du Tort, 2009). Studies in support of its use indicate that honey-based treatment is preferential to silver or iodine, due to its comparative lack of toxicity.

The medicinal properties of honey have been known since ancient times. Indian medicines described honey as the nectar of life and recommend it in various ailments. There is a renewed interest in honey treatment as evidenced by the number of reputes appearing in

the scientific literature. Honey has useful on the treatment of surgical wounds, burns; decubitus ulcer and the antibacterial and antifungal properties of honey have been well documented. In burn in particular, honey has been found to control wound infection and accelerate wound healing. Honey bees transform nectar into honey by a process of regurgitation and store it as a primary food source in wax honeycombs inside the beehive. Beekeeping practices encourage over production of honey so the excess can be taken from the colony.

Honey gets it sweetness from the monosaccharide fructose and glucose and has approximately the same relative sweetness as that of granulated sugar. It has attractive chemical properties for baking, and a distinctive flavor that lead some people to prefer it over the sugar and other sweetness. Most microorganisms do not grow in honey because of its low water activities of 0.6. However, honey sometimes contained dormant endospores of the bacterium *clostridium botulinum* which can be dangerous to infants as the

endospores can transform into toxin-producing bacteria in the infant's immature intestinal tract, leading to illness and even death. Honey is also used in various medicinal traditions to treat ailments.

Honey is often eaten as an energy food (Caron, 2004) and yield about 64 centuries of energy which is high compared to other sweeteners (National Honey Board, 1996) its simple sugar is absorbed into the blood stream with digestion. The optimum storage temperature for honey is below 520F (110C) or in the 70-300 range (21-270C) in an air tight container (National Honey Board, 1996).

2.3 local test for real honeys

Honey can be adulterated by adding sugarcane syrup and other ingredient which can be processed to give the colour and near texture of honey. The aroma of the pure honey is not the same with that of the adulterated one and the pure honey does not leave sediments when diluted in water unless it was not properly filtered, although the originality of honey can be tested using honey stained match stick

which brings light immediately when struck while the match stick stained with adulterated honey does not bring light when struck.

2.4 Classification of honey

Crystallized honey: Is honey in which some of the glucose content has spontaneously crystallized from solution as the monohydration. Also called granulated honey" or candied honey" honey that has crystallized can be returned to a liquid state by warming.

Pasteurized Honey: Is honey that has been heated in a pasteurized process (161^{o}f(71.7^{o}c) or higher). Pasteurization destroys yeast cells. It also liquefies any microcrystals in the honey, which delays the onset of visible crystallization. However, excessive heat exposure also results in product deterioration as it increases the level of hydroxymethylfurfur and reduces enzyme (e.g diastase) activity. Heat also affects appearance.

Raw honey: Is honey as it exists in the beehive or as obtained by extraction, settling or straining without adding heat. Raw honey contains some pollen and may contain small particles of wax, local raw honey is sought after by allergy suffers as the pollen impurities are thought to lessen the sensitively to fever.

Ultrasonicated honey: Has been processed by ultrasonication, a nonthermal processing alternative to honey when honey is exposed to ultrasonication, most of the yeasts cells are destroyed. Those cells that survive sonication generally lose their ability to grow, which reduces the rate of honey fermentations substantially.

Extraction

Honey is collected from wild bee colonies or from domesticated bee hives. Wild bee nests are sometime located by following a honey guild board. Collecting honey is typically achieved by using smoke from a bee smoker to pacify the bees, this causes the bee to attempt to save the resources of the hive from a possible forest of fire and make

them far less aggressive. The honey comb is removed from the hive and the honey extracted after which it is filtered.

2.5 Preservation

Because of its unique composition and chemical properties, honey is suitable for long –term storage and as easily assimilated even after long preservation. Honey and objects immersed in honey, have been preserved for decides and even centuries. The key to preservation is limiting access to humidity. In its cured state. Honey has sufficiently high sugar content to inhibit fermentation. If exposed to moist air, its hydrophilic properties will pull moisture into the honey, eventually diluting it to the point that fermentation can begin.

Honey should also be protected from oxidation and temperature degradation. It should not be preserved in metal containers. However, glass and plastic are now the favoured materials.

2.6 Properties and active ingredients of honey.

The good control of infection by honey is said to be attributed to the high osmolarity while its hydrogen peroxide contents, lysozyme and other unidentified substances from certain flora sources are responsible for its responsible for its antibacterial properties (khali *et.al*; 2001). The antibacterial activity of honey was also said to be mainly due to enzymes, glucose oxidase in honey which subrahamanyam said includes hydrogen-peroxide, flavoids and phenolic acids plus many other unidentified properties (subrahmanyam *et. al*; 2001). Also the chemical composition of honey is said to comprise of seven tetracycline, fatty acids, lipids, amylase, ascorbic acid, peroxidase and fructose all of which are attributed to its antimicrobial activity together with high osmolarity, low pH (3.6-3.7), content of phenol (inhibine), peroxidase glucose and fructose in honey and the presence of tetracycline derivatives of fatty acids (Al-jabril *et. al;* 2002).

A number of reasons have been suggested to include the shrinkage and disruption of the bacterial cell wall due to the osmotic effect of the sugar content, induction of an unfavorable environment with low water activity thereby inhibiting bacterial growth and a low pH of 3.6 as well as the fermentation of honey producing alcohol inside (subrahmanyam *et. al;* 2001). However the relative importance of these factors is said to depend on the sensitivity of the bacterial species and any additional substance in the honey (Al-jibril *et al* 2002).

Table1. **Composition of honey**

Parameter	average	range	standard deviation (SD)
Fructose (glucose)	1.23	0.76-1.86	0.126
Fructose (%)	38.38	30.91-44.26	1.77
Glucose (%)	30.31	22.89-40.75	3.04
Mineral (ash) %	0.169	0.020-1.028	0.15
Moisture (%)	17.2	13.4-22.9	1.46
Reducing sugar (%)	76.75	61.39-38.72	2.76
Sucrose	1.31	0.25-7.57	0.87
pH	3.91	3.42-6.10	-
Total activity (Meg /100g)	29.12	8.61-56.49	10.33
True protein (mg/100g)	168	57.5-67.5	70.9

Source: National Honey Board, 1996

2.7 Mode of action of some of the antibacterial substances in honey.

Honey contains various substances/properties which are responsible for the antibacterial properties observed with its use. Some of the modes of action of these agents include the following.

High osmotic pressure

Honey due to its high osmotic pressure is said to draw water from other sources such as tissue or bacterial cells. When it draws the water from bacteria, it kills them (Ovington, 1999).

Low water activity

Honey is a super saturated sugar solution with low water activity (Aw), which means that there is little available to grow if the water activity is below 0.94-0.99 and the water activity of ripened honey (0.56-0.62) does not support the growth of yeast. Diluted honey with high water activity will not be effective against those species of

bacteria that grow most rapidly at water activity of 0.99 (Molan, 2001).

Glucose oxidase enzyme

Glucose oxidase is an enzyme contained in honey. This enzyme is said to produce hydrogen peroxide, which kills bacteria when it breaks down to form oxygen radicals (Ovington, 1999).

When honey is used topically, hydrogen peroxide is produced by dilution of the honey with body fluids. As a result, hydrogen peroxide is released slowly and acts as an antiseptic.

Low pH/ acidic environment

The natural acidity of honey will inhibit many pathogens. The minimum pH values for some species that commonly infect wound ranges from 4.0-4.5. Since honey characteristically has a pH range of

3.2-4.5, the acidity of honey is a significant factor in its antibacterial activity (Molan, 2001).

2.8 Clinical conditions that respond to treatment with honey

Honey not only possesses significant antibacterial activity, it has also been shown to actively promote healing (Blair, 2004). Some of the clinical conditions that respond to treatment with honey include the following:

The use of honey as regards to wounds- Honey acts as highly vicious barrier preventing bacteria penetration and colonization of wound surface (Subrahmanyam *et al*; 2001).

The use of honey to treat severe acute post operative wound infection- Topical application of crude undiluted honey have been used in the treatment severe acute post operative wound infection due to gram positive and gram negative bacteria following caesarean sections and hysterectomies. This was said to lead to lead to faster

eradication of bacterial infection, reduced period of antibiotic use and hospital stay, accelerated wound healing, prevent dehiscence and used for restructuring and results in minimal scar formation (Al-wali and Sallom, 1999).

Treatment with honey in chronic meningococcal skin lesions- chronic infected meningococcal skin lesions have also been said to have been successfully treated with honey (Dunford, *et al*; 2000).

Honey on gastritis/ diarrhoea- infantile gastroenteritis and diarrhea are usually treated with hydrating glucose and electrolyte solutions, when honey at 5%(v/v) concentration replace the glucose in dehydration fluid, the duration of diarrhea is shortened in patience with bacterial gastroenteritis compared to control group on standard therapy (National Honey Board, 1999). Besides the antibacterial activity of honey, the anti fungal activity has also been reported and honey has been used in treatment of vagina yeast (*Candida albican*)

and tinea e.g, ringworm and athlete foot caused by dermatophytes (National Honey Board, 1996)

Lastly, to assume the effectiveness of honey as an antimicrobial agent, its exposure to heat and light should be limited.

2.9 Honey as an antibacterial agent

Work on the antibacterial activity of honey has been going on since the 18th century and various researchers have shown that honey exerts antibacterial activity against various organism including gram negative and gram positive bacteria (subrahamanyam *et al*; 2001).

An in-vitro experiment to determine the antibacterial effect of Omani and Africa honey against *staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using standard antibacterial assay showed that both honey samples were active against these organism. Omani honey was reported to have anti-*staph*. *aureus* and *E.coli*

activity against *pseudomonas* which previously was reported not to be susceptible to honey (Al-jabri *et al*; 2002).

An in-vitro study to confirm the potential antimycobacteria failed to grow in plates containing honey with a conc. of 100% and 20% as a result, Avilenna recommended honey in the treatment of tuberculosis (Avilenna,1991).in one study to investigate the none peroxide antibacterial activity of honey against *staphylococcus* and *micrococcus lateus* using quantitative turbidimetric assay involving serial dilution of the honey sample. The non peroxide antibacterial activity in honey samples was found to correlate significantly with aid content of the honey but not with its pH (Bogdanod, 1997).

Honey is produced from many different floral sources and its antibacterial activity varies with origin and processing dioscorides stated that pale yellow honey from Africa was the best. Aristotle when discussing different honeys, referred to pale honey as being "good as a salve for sore eye and wounds" substantial amount of

honey need to be applied to a wound to achieve adequate potency although honey may be very viscous and even solid at room temperature. Honey becomes very fluid at body temperature and even more fluid when diluted with proportionally small volume of exudates. It is therefore very important that sufficient honey should be applied to a wound and kept in a place if good therapeutic effect is to be obtained. For the optimal minimal inhibitory concentration (MIC) of the antibacterial components of honey to be reached at the deepest sites of infection there need to the high conc. possible on the surface and a "reservoir" of sufficient quality that it is not substantially depleted by diffusion into the wound tissues.

Honey produced as a food often is not well filtered and may contain various particles in it. Also, although honey does not allow vegetative bacteria to survive, it does contain viable spores, including clostridia. This processing kills clostridia spores without loss of any of the antibacterial activity.

In medicine, historically, honey has been used by humans to treat a variety of ailments, from gastric disturbances to ulcers, wound and burn through ingestion or topics application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained. Different honeys have different properties which were known since ancient times.

2.10 Practical consideration for the clinical use of honey

The amount of honey required on the wound relates to the amount of fluid exuding from the wound diluting it. The frequency of dressing change required will depend on how rapidly the honey is being diluted by exudates. If there is no exudates, dressing needs to be changed thrice weekly to maintain a “reservoir” of antibacterial components as they diffuse into the wound tissue.

To achieve best results, the honey should be applied to an absorbent dressing prior to application. If applied directly to the wound, the

honey tends to run off before the secondary dressing is applied to hold it in place.

Honey will not soak readily into absorbent dressing, soaking is facilitated by warming the honey to body temperature and or adding 1 part water to 20 parts honey to make the honey more fluid. Any depression or cavity in the wound bed need to be filled with honey in addition to using a honey impregnated dressing, this is to ensure that the antibacterial component of the honey diffuse into the wound tissue. Since infection may lie in the tissue underlying the wound margins, honey dressings need to extend beyond the inflamed area surrounding the wound (Postmes *et al*; 1997).

2.11 Adverse reaction of honey

Allergic reactions to honey are rare and have been attributed in some cases to a reaction of some specific pollen in the honey (Molan, 1992). Honey processed for use in wound care is passed through fine

filters which removes most of the pollens. in more than 500 published reports on the clinical usage of honey in open wounds, there have been no adverse reactions noted other than a localized stinging sensation described by some patients. This may be due to the acidity of honey as it has not been reported when the acidity is neutralized (Brady *et al*; 1997). A number of histological studies examining wound tissues also support a safe use of honey.

2.12 Resent research on honey

Honey originally used by d ancient Egyptians and Greeks is a viscous, saturated sugar solution now widely used in wound care (Simon *et al*; 2009). High osmolarity prevents the growth of bacteria and encourages healing. This can be utilized for wound management through the application of sugar paste or honey. In addition, honey is believed to have specific antimicrobial properties for example, preventing of *staphylococcus aureus* even when diluted beyond the point at which osmolarity is no longer inhibitory (Moore *et al*; 2001).

Studies have reported that it may modestly decrease wound healing time, act as anti-inflammatory deodorize wounds, and enhance cell proliferation and expansion in vitro (Du toit, 2009). Studies in the support of the use honey indicate that honey-based treatment is preferential to silver or iodine due to its comparative lack of toxicity.

Recently, further research was carried out at Waikato hospital looking at the effect of honey on their collection of multiple resistance (MRSA) strains of *staphylococcus aureus* that cause ward closures in hospital because they are resistant to most or all of the commonly used antibiotics (Wilix, *et al*;2009). All of the strains were found to have their growth halted completely by the honeys diluted to 5-10%. In the last few years, it has been recognized that dyspepsia and stomach ulcers are frequently caused by infection of the stomach by specie of bacteria (*Helicobacter pylori*). The possibility that the healing infect of honey on the stomach may be through its action on this bacterium which was suggested by Niaz-Al-somai (1999) at the

University of Waikato. In collection with microbiologist at the Waikato hospital, he tested strains of *H. pylori* isolated from biopsy samples of stomach ulcers, using the same honey that had been tested on the wound-infected species of bacteria. It was found that the honey with hydrogen peroxide did not prevent the growth of cultures of *H.pylori* when added at concentration up to 50%, but the manuka honey completely halted growth of the bacteria at a concentration of 50% (Wilix *et al*;2009). The source of the nectar used in the production of the honey may have caused difference in the antimicrobial activities of honey from different source.

CHAPTER THREE

Materials and Methods

3.1 Source of Sample

The organisms used in this work was collected /obtained from the medical department of UNTH and Parklane Hospital Enugu.

3.2 Source of Honey

The honey used was obtained from local commercial producers in Enugu North (Nsukka) and Enugu South (Ugwuaji). It did not contain any diluents or additives and had not been heated.

3.3 Identification of test organisms

The test organism which was collected from the medical department of UNTH and Parklane Hospital Enugu was purity

tested by sub culturing the test organisms on fresh agar plates and caring out biochemical tests such as Gram staining, indole test, catalase and coagulase test to identify the organisms.

3.4 **Gram staining**

This reaction is done to identity organisms that are Gram positive (+ve) and Gram negative (-ve)

Procedure – A smear of the isolate was made on a clean grease free slide, air dried and heat fixed. The slide was flooded with 0.5% solution of crystal violet and allowed for 30 seconds. The stain was washed off with water and flooded again with lodine solution (mordent) and allowed for 10 seconds after which it was washed off. The slide was counter stained with saffranin for 30 seconds, rinsed with water and air dried. The stained slide was viewed under the microscope using immersion oil under x100 objective lens (cheese bough, 2002)

3.5 **Indole Test**

Procedure – The test organism (isolate) was inoculated in a test tube containing 3ml of sterile tryptone water. Incubation was done at 37^0C for 24hrs. 0.5ml of kovac's reagent was added and shacked gently. The examination for a red ring like colour on the surface of the layer within 10 minute was done. Ochei et al (2001)

3.6 **Catalase Test**

Procedure – This was performed by dropping a loopful of hydrogen peroxide on a clean grease free slide followed by the mixing of the loopful of isolate with the hydrogen peroxide on the slide. The production of gas bubbles from the mixture which occurred almost immediately, is a positive reaction

$2H_20_2 \rightarrow 2H_2 + O_2$

3.7 Coagulase test

This test is used to differentiate *staphylococcus aureus* from *streptococcus sp*. It is known to cross-link the $\propto$ and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. A loopful of the test isolate is smeared on a slide, mixed with normal saline and treated with a drop of serum which is then mixed together. Agglutination or clumping occurs within 5-10 second which shows positive

3.8 Antibacterial Sensitivity Test

The antibacterial activity of honey collected from two different honey dealers at Enugu State against the three pathogens was tested in-vitro using well diffusion method (Kirby Bauer's method). The test materials were prepared by diluting each honey in sterilized double distilled water at different dilution (concentration) 20%, 40%, 60% and 80% also net honey (100%).

Nutrient agar plates were prepared and each plate was properly inoculated with each test organism using streaking method with the help of a sterile wire loop. Wells were made using a sterile cork borer and each well was filled with different concentrations of the honey. The plates were incubated at 37^0C for 24hrs and observed for zone of inhibitions.

This in-vitro experiment was compared with the use of a sensitivity disc (Augumentine) which served as a control.

CHAPTER FOUR

Result

The result of the biochemical test carried out in order to identify the organisms is stated in table 2 below

Table 2

Test organism	Gram stain	Catalase Test	Indole Test	Coagulase Test
1. *Staph. aureus*	+	+	NA	+
2. *E.coli*	-	-	+	-
3. *Strep. Pyogen*	+	-	NA	-

Key:

NA= not applicable.

\+ = positive

\- =negative

The result below shows that honey has antibacterial potential. Different concentration of the honey produced different zones of inhibition.

The zone of inhibition of honey from Nsukka against the organism is shown in table 3

Table 3

Test organism	Zone of inhibition (mm)&Honey concentration					
	20%	40%	60%	80%	100%	control
Staphylococcus aureus	5	10	16	18	20	24
Escherichia coli	2.5	5	9	13	15	26
Streptococcus pyogen	1	4	6	8	10	30

Table 4

Test organism	Zone of inhibition(mm) &Honey concentration					
	20%	40%	60%	80%	100%	control
Staphylococcus aureus	3	5	10	15	17	24
Escherichia coli	1	3.5	6	11	12	26
Streptococcus pyogen	Nz	2	5	7	9	30

Key;

NZ=No zone of inhibition

CHAPTER FIVE

5.1 Discussion

The study which was carried out to investigate the possible antibacterial activity of honey from different locations (Nsukka and Ugwuaji) in Enugu state on some pathogenic microorganism (*staphylococcus aureus*, *Escherichia coli*, & *streptococcus pyogen*) isolated from wound demonstrated antibacterial spectrum and efficiency against the test bacterials.Comparing the antibacterial activity of the two honey sample, it was observed that honey from Nsukka produced the highest zone of inhibition (effect) at the net (100%) concentration and their zone of inhibition was 20, 15 and 10 mm respectively for *staphylococcus aureus*, *Escherichia coli* and *streptococcus pyogen* (Table 3).

A distinct observation was made with the Ugwuaji honey which showed no antibacterial activity (No zone of inhibition) at the

concentration (dilution) of 20% on *streptococcus pyogen*, hence no result was recorded compared to the other honey sample. From the result represented on table 3 and 4, it was observed that the zone of inhibition was increased with the concentration of the honey i.e. an increase in the honey concentration increases the zone of inhibition. This observation agrees with Wilix *et al*; (2009)

The antibacterial activity of honey was recently investigated Wilix *et al*; (2009) by microbiologist looking at the effect of two honey on their collection of MRSA – strains of *Staphylococcus aureus* that cause ward closures in hospitals because they are resistant to most or all of the commonly used antibiotics. All the strains they studied were found to have their growth halted completely by the honeys at 5 – 10%. Also it has been demonstrated (Efem; 1993) that honey can accelerate wound healing and also possess bacterial properties

5.2 **Conclusion**

It has been shown that the potency of the antibacterial activity can vary very markedly. The number of variable factors involved makes it impossible to predict with any certainly that a particular honey will have a high antibacterial activity assurance.

Though both honey was from the same state, honey from Nsukka demonstrated more effective antibacterial activity than that of Ugwuaji. Based on the findings of this research honey possess antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Only *Streptococcus pyogen* was found to be moderately susceptible in the diluted honey at different concentration. The honey used for this reach showed antibacterial effect against Gram positive and Gram negative bacterial. Both topical antimicrobial agent (O'meare *et al*; 2001) and appropriately selected antibiotics (Bowler *et al*; 2001) are

valuable in the treatment of infected wounds but the routine use of systemic antibiotics for chronic wounds without signs of clinical infection is not recommended (O' meara *et al*; 2001)

5.3 **Recommendation**

It is recommended that honey should be used as substitute for antibiotic because of its effect on bacterial. Precaution against loss of antibacterial activity should be taken and honey with high activity should not be blended with honey of low activity because a honey with low activity could well have components present that destroy antibacterial activity. The intake of honey frequently is recommended because it will go a long way to reduce the incidence of common bacterial infections. Topical use of honey on wounds is advised to prevent bacterial infection thus accelerate wound healing. For retail sale it could well be packaged in brown glass containers like other medical products to prevent loss of antibacterial activity on exposure to light.

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APPENDIX 1

Equipments

Incubator

Refrigerator

Weighing balance

Applicator stick

Wire loop

Cotton wool

Aluminum foil

Petri dish

Latex gloves

Measuring cylinder

Autoclave

Conical flask

Pipette

Test tube rack

Needle and syringe etc.

Reagents

Hydrogen peroxide

Crystal violet

Saffranin

Iodine

Kovac’s reagent

Sterilization

All glass wears and Medias were sterilized in the autoclave at 121^0C for 15mins. While the wire loop was sterilized by flaming to red hot.

APPENDIX 2

Media

Nutrient agar (NA)

Procedure;

Nutrient agar powder of 5.6g was measured on a weighing balance following manufactures instruction. The nutrient agar powder was dissolved in 200ml of distilled water and total dissolution was ensured before autoclaving. The sterilized medium was allowed to cool a little after which it was dispensed aseptically into petri dishes and allowed to gel.

MacConkey Agar

Procedure;

MacConkey agar powder of 5g was weighed out and dissolved in 160ml of distilled water in a conical flask covered using cotton wool wrapped

in foil. The medium was properly mixed and then autoclaved at 121^0C for 15mins for sterilization before it was aseptically dispensed into petri dishes.