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Antimicrobial Activity of Honey on *Staphylococcus Aureus*, *Escherichia Coli* and *Streptococcus Pyogenes* Isolated from Wound in Wukari, North East, Nigeria

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Abstract:

Honey is a sweet, viscous food substance produced by bees and some related insect which has anti-inflammatory, antimicrobial, phytochemical and antioxidant use in surgical wounds, ulcers, bed sores administered orally and by rubbing on the surface of the wound infection. The present was aimed to isolate and identify bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*) and subsequently determine the susceptibility of these organisms to honey. Twenty (20) clinical samples (wound swab) were collected from two (2) different hospitals in Wukari (Waritoma hospital and Ajiduku area hospital) and taking to Department of Microbiology Laboratory at the federal University Wukari for investigation using different concentration (20%, 40%, 60%, 80%, 100%) of honey using standard microbiological techniques. The results shows that at all concentration of honey *Streptococcus pyogenes* shows no zone inhibition for 20%, 40%, 60% and at 80% and 100% it showed zone of inhibition of 0.6mm and 1.6mm respectively to the honey when compared with the control (2.0mm). *Staphylococcus aureus* highly sensitivity at 80% honey with zone of inhibition at 2.1mm and 100% honey with zone of inhibition of 2.1mm when compared to control (2.0mm) and *Escherichia coli* shows more sensitivity to honey at 100% concentration with zone of inhibition 2.0mm when compared to control which has zone of inhibition of 1.9mm. The results therefore, indicate that the use of honey as antibacterial therapeutic substance is effective against bacterial at high concentration and effort should be made by medical professions to incorporate honey to the treatment of various types of wounds.

Keywords: Antimicrobial, Honey, Isolates, wound, Wukari

1. Introduction

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. 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 color, 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, 2007). 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 its 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 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 its simple sugar is absorbed into the blood stream with digestion. The optimum storage temperature for honey is below 520F (11°C) or in the 70-300 range (27°C) in an air tight container. The emergence in recent years of numerous resistant strains of pathogenic bacteria to a range of formerly efficient antibiotics constitutes a serious threat to public health (Raymond *et al.*, 2011). The antibacterial activity of honey refers to some bee products, presence of 'inhibin' which acts as an antibacterial factor other than H₂O₂, several factors such as osmotic properties of honey which is saturated or super saturated solution of sugars, 84% being a mixture of fructose and glucose, so inhibition by osmotic effect of dilute solutions of honey obviously depends on the species of bacteria (Saadia *et al.*, 2010). Recently, many researchers have reported the antibacterial activity of honey against *Staphylococcus aureus* (*S. aureus*), and *Streptococcus pyogenes* (*S. pyogenes*) (Hassanain *et al.*, 2010; Rajneeta *et al.*, 2009). The potential antibacterial of diluted honey originating in several countries was already studied (Nur-Azida *et al.*, 2010; Voidarou *et al.*, 2001; Sherlock *et al.*, 2010; Hayam and Dalia, 2011). According to the EUCD, (2001), honey is the natural sweet substance produced by Apismellifera bees from the nectar of plants or from the secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants which bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honey combs to ripen and mature. (Bogdanov *et al.*, 2004), stated that honey is the only food sweetener that can be used industrially without processing. Honey can be classified according to its origin (such as nectar or honey dews), mode of production and preservation. Honey is a concentrated aqueous solution composed of a mixture of glucose and fructose but also contains at least 22 other complex carbohydrates, various amino and organic acids, proteins, antibiotic rich inhibine, enzymes, phenol antioxidants, aroma compounds, vitamins, minerals, pigments, waxes and pollen grains (Bogdanov *et al.*, 2007). It is viscous and acidic in nature with a pH ranging between 3.2 and 4.5. Natural honey has been used as an effective medicine around the world since ancient time. It has had a valued use in traditional remedy for centuries. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut (Bogdanov *et al.*, 2008). 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 and oral bacteria (Maurice *et al.*, 2009; Adams *et al.*, 2009). Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds (Cooper *et al.*, 2002). According the Lusby *et al.*, (2005), the healing properties of honey can be Cooper ascribed to the fact that it offers antibacterial activity, maintains a moist wound environment that promotes healing and has a viscosity which helps to provide a protective barrier to prevent infection. They further, stated that its immune modulatory properties are relevant to wound repair. Many investigations reported that the antimicrobial activity of honey is due to phytochemical properties such as high content of reducing sugar, high viscosity, high osmotic pressure, low pH, low water activity, low protein content and presence of hydrogen peroxide (Molan and Cooper, 2002). Alnimat *et al.*, (2012), stated that the main antibacterial activity in honey is hydrogen peroxide, which is produced by glucose-oxidase action. The level of peroxide in honey is determined also by the presence of catalase, which originates from the pollen of plants (Weston, 2000). The amount of hydrogen peroxide is affected by light, temperature and oxygen which vary according to the processing and storage conditions of the honey. Research has revealed a positive correlation between the endogenous hydrogen peroxide concentration and the inhibitory activity of bacteria growth by honey (Bizerra *et al.*, 2002). Indeed, honeys with a high concentration of hydrogen peroxide have higher antibacterial activity. In some cases, according to Libonattiet *et al.*, (2014), other antibacterial activity of honey is due entirely to the non-peroxide components such as acidity, osmolality, flavonoids, phenolic compounds and lysozyme. Different studies have claimed that honey contains bioactive components such as lysozyme, a well-known antibacterial agent (Estrada *et al.*, 2005). Abd-El Aalet *et al.*, (2007), in their researched showed that honey had a pronounced inhibitory effect (85.7%) on gram-negative bacteria (*Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella*) in comparison to commonly used antimicrobial agents. A 100% inhibition was observed in the case of gram-positive methicillin resistant *Staphylococcus aureus* in comparison to the use of antibiotics alone. (Kwakman and Zaat, 2012) reported that the sugar content of honey is sufficient to retain antibacterial activity when diluted to approximately 20-40%. Based on extensive research on the medicinal uses of honey, its antimicrobial action on *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* was investigated using honey samples from Baissa, Kurumi Local Government Area, Taraba State, Nigeria

2. Materials and Methods

2.1. Sources of Sample and Honey

The wound sample used in this work was collected from the Laboratory Department of Waritoma hospital and Ajiduku area Wukari Taraba state. The honey used was obtained from local commercial producers in Baissa, Kurumi Local Government Area, Taraba State. It did not contain any diluents or additives and had been heated. Standard microbiological techniques were used.

2.2. Antimicrobial Sensitivity Test

The antimicrobial activity of the honey against the three pathogens was tested in - vitro using Agar Well Diffusion (Kirby Bauer's method). The test materials were prepared by diluting each honey in sterilized soluble distilled water at different dilution (concentration) 20%, 40%, 60% and 80% also net honey (100%). Nutrient agar plate was 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 plate was properly inoculated with each test organism and each was filled with different concentration of the honey. The plates were incubated at 37°C for 24hrs and observed for zone of inhibition. This in-vitro experiment was compared with the use of sensitivity disc (Augumentine) which served as a control

3. Results

The result of the study on the isolation and identification of bacteria from wounds and their susceptibility to honey are as shown in table 1, 2 and in figures 1 and 2.

S/N	Morphological Characteristics	Gram Reaction	CAT.	COA.	OXI.	CIT.	IND	MR.	GLU.	LACT.	SUC.	Bacterial Isolates
1	Medium, spherical, yellow, dried, and flat.	+ve Cocci	-	-	-		-	-	+	+	+	<i>Streptococcus</i> spp
2	Large, spherical, creamy, swampy, dried, and raised	+ve Cocci	+	+	-	-	-	+	+	-	+	<i>Staphylococcus aureus</i>
3	Small, spherical, pink, mucoid, and raised	-ve Rods	-	-	-	-	+	+	+	+	+	<i>Escherichiacoli</i>
4	Large, spherical, pink, mucoid and raised	-ve Rods	-	-	-	-	-	+	+	+	+	<i>Klebsiellaspp</i>
5	Large, spherical, pink, moist, and raised	-ve Rods	-	-	-		+	-	+	+	+	<i>Proteus</i> spp
6	Large, spherical, pink, moist and raised	-ve Rods	-	-	-	+	-	-	+	+	+	<i>Pseudomonasspp</i>

Table 1: Morphological, Microscopic and Biochemical Characteristic of Isolates

Organism/Concentration of honey (%)	20%	40%	60%	80%	100%	control
<i>Staphylococcus Aureus</i>	0.4	0.7	1.2	2.1	2.7	2.0
<i>Escherichia coli</i>	Nz	0.5	1.5	1.8	2.0	1.9
<i>Streptococcus pyogene</i>	Nz	Nz	Nz	0.6	1.6	2.0

Table 2: Zone of Inhibition on Various Concentration Hone

Figure 1: Pie Chart Showing Zone of Inhibition and Honey Concentration

Figure 2

4. Discussion

The aim of this study was to evaluate the antibacterial activity of honey on *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* isolated from wound. In order to achieve this clinical bacterial isolate were obtained from wound samples collected from people within Wukari area and identified using standard microbiological and biochemical techniques. The results showed great variation in the antimicrobial activity of honey on *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*. As shown in table 2 above, honey has less antimicrobial activity on *Streptococcus pyogenes*, but in the case of *Staphylococcus aureus* and *Escherichia coli*, it is more effective at higher concentration. It is observed that the zone of inhibition is increased as the honey concentration increase. This observation agrees with Al-jibril, (2002). The antibacterial activity of honey investigated by Al-jibril (2002) in which he looked at the effects of two honey on their collection of MRSA –strains of *Staphylococcus aureus* that could cause ward closure in hospitals because they are resistant to most or all of the commonly used antibiotics. All the strains, in the study were found to have their growth halted completely by honey. It has been shown that the potency of honey on the isolates vary very markedly. The number of variable factors involved makes it impossible to predict with any certainty that a particular honey will have high antibacterial activity. This project confirms the medicinal property of honey. Honey was used as natural antibiotic against the isolated organisms. Furthermore, honey should be emphasized for healing of various wounds. The result of this study suggest that honey poses components with antibacterial properties against pathogenic bacteria, therefore, offers of scientific bases for the traditional use of honey for the treatment of wound infection. It should be explored as a significant remedy regarding surgical wounds and ulcers. Considering the practical applications, it should be used without any dilution for the welfare and services of mankind.

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