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Antimicrobial Activity of Honeys from Nsukka and Ugwuaji in Enugu State, on Selected Pathogenic Bacteria Isolated from Wound

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Abstract Studies were carried out to determine the anti-microbial activity of honeys from Nsukka and Ugwuaji in Enugu State, on three selected pathogenic bacteria (*Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes*) isolated from wound. The antibacterial sensitivity test was carried out using well diffusion method. The inhibitory efficiency of the honey samples on the growth of the tested organisms increased with increase in concentration from 20 to 100%. *Staphylococcus aureus* (3.88 – 22.61mm) was the most sensitive to the honey samples while *Streptococcus pyogenes* (1.26 – 13.26mm) was the less sensitive. The zone diameters of inhibitions of the organisms at different concentrations of the honey samples were found to be statistically significant. The inhibition efficiency of the honey samples on the growth of the tested organisms was found to be dependent on concentration and type of honey used and the nature of the tested organisms.

Keywords Bacteria, Antibacterial, Activity, Honey and inhibition efficiency

1. Introduction

According to the EUCD, (2001), honey is the natural sweet substance produced by *Apis mellifera* 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 uses 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 (Mauric *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

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from the pollen of plants (Weston, 2000). The amount of hydrogen peroxide is affected by light, temperature and oxygen which varies 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 Libonatti *et al.*, (2014), other antibacterial activity of honey is due entirely to the non-peroxide components such as acidity, osmolarity, 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 Aal *et al.*, (2007) showed that honey had a pronounced inhibitory effect (85.7%) on gram-negative bacteria (*Pseudomonas aerugunosa, Enterobacter spp*., *Klebisella*) 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 *Stapylococcus aureus, Eschericha coli and Streptococcus pyogenes* was investigated using honey samples from Nsukka and Ugwuaji in Enugu State, Nigeria.

2. Materials and Methods

Sample collection: Twenty (20) honey samples each were purchased from the local beekeepers in Nsukka and Ugwuaji, Enugu state, Nigeria. The samples were stored in clean and closed polyethylene flasks at 20 – 21°C in a lightless place until analysis.

Bacterial strains

Strains of *Staphylococcus aureus, Eschericha coli* and *Streptococcus pyogenes* were obtained from the Medical Department of University of Nigeria Teaching Hospital and Enugu State University Teaching Hospital, Parklane, Enugu State.

Antibacterial Sensitivity Test

The antibacterial activity of honey samples from the two different locations (Nsukka and Ugwuaji) in Enugu state was tested invitro against three pathogenic bacteria (*Staphylococcus aureus, Eschericha coli* and *Streptococcus pyogenes*) using well diffusion method (Cooper *et al.*, 2002). The test samples were prepared by diluting each in sterilized water at different dilutions (concentration), 20%, 40%, 60%, 80% and 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.

A distance was maintained from the edges of the plates to prevent overlapping of the inhibition zones.

The plates were incubated for 24hrs at 37°C. This invitro experiment was compared with the use of a sensitivity disc (Clindamycin), which served as a control.

After incubation the plates were examined and the diameter of the inhibition zones was measured in triplicate for each isolate.

Statistical Analysis

The results obtained were expressed as mean ± standard deviations and differences between means were analyzed statistically using an analysis of variance (ANOVA), SPSS version 18.0 for windows. Differences were considered significantly when $p \leq 0.05$.

3. Results and Discussion

Table 1 shows that honey samples from Nsukka at concentrations of 20, 40, 60, 80 and 100% gave 5.81, 10.17, 16.48, 19.86 and 22.61mm zone of inhibitions respectively on *Staphylococcus aureus*.

The honey samples at concentrations of 20, 40, 60, 80 and 100% inhibited the growth of *Escherichia coli* to 2.51, 5.62, 10.43, 14.73 and 17.56mm respectively. The growth of *Streptococcus pyogenes* was inhibited to 1.26, 4.34, 6.60, 8.45 and 13.26mm at 20, 40, 60, 80 and 100% honey concentrations respectively. It was observed that the inhibition efficiency of the honey samples on the growth of the test organisms increased with increase in concentration from 20 to 100%.

Table 1. Antibacterial activity of honey samples from Nsukka against the isolated pathogenic bacteria

Test organism	Zone of inhibition (mm) at different honey concentrations						F test P value
	20%	40%	60%	80%	100%	Control	
Staphylococcus aureus	5.81 ± 0.32	10.17 ± 0.14	16.48 ± 0.53	19.86 ± 0.94	22.61 ± 0.11	24.08 ± 0.5	0.01
Eschericha coli	2.51 ± 0.13	5.62 ± 0.22	10.43 ± 0.60	14.73 ± 0.81	17.56 ±0.49	26.35 ± 0.28	0.00
Streptococcus pyogenes	1.26 ± 0.08	4.34 ± 0.12	6.60 ± 0.20	8.45 ± 0.52	13.26 ± 0.40	22.67 ± 0.19	0.00

Table 2. Antibacterial activity of honey samples from Ugwuaji against the isolated pathogenic bacteria

Test organism	Zone of inhibition (mm) at different honey concentrations						F test P value
	20%	40%	60%	80%	100%	Control	
Staphylococcus aureus	3.55 ±0.20	5.61 ± 0.15	10.89 ± 0.45	15.41 ± 0.11	17.08 ± 0.40	24.08 ± 0.05	0.00
Eschericha coli	1.30 ± 0.26	3.81 ± 0.32	6.24 ± 0.33	11.19 ± 0.80	13.90 ±0.55	26.35 ± 0.28	0.00
Streptococcus pyogenes	-	2.28 ± 0.30	5.77 ± 0.14	7.46 ± 0.56	10.05 ± 0.22	22.67 ± 0.19	0.00

For the three organisms, the zone of inhibitions differs significantly at different concentrations of the honey samples.

Table 2 shows that at concentrations of 20, 40, 60, 80 and 100%, the honey samples from Ugwuaji inhibited the growth of *Staphylococcus aureus* to 3.55, 5.61, 10.89, 15.41 and 17.08mm respectively.

Also, the growth of *Escherichia coli* on the agar plate was inhibited to 1.30, 3.81, 6.24, 11.9 and 13.90mm at 20, 40, 60, 80 and 100% honey concentration respectively.

Equally, there was inhibition efficiency on *Streptococcus pyogenes* to 2.28, 5.77, 7.46 and 10.05mm at 40 60, 80 and 100% honey concentrations respectively. Just as observed for the test organisms in Table 1, Table 2 equally indicated that increase in concentration of the honey samples increased its inhibition efficiency.

Results of Table 1 and 2 shows that among the three studied pathogenic bacteria, *Staphylococcus aureus* was the most inhibited (17.08 – 22.64mm) while *Streptococcus pyogenes* was the least inhibited (10.05 – 17.56mm).

Comparison of Tables 1 and 2 shows that honey samples from Nsukka was more efficient in inhibiting the growth of the studied pathogenic organisms than those from Ugwuaji. Literature has shown that different honey type possesses different efficacies against the same type of bacteria (Almasaudi *et al.*, 2017).

Hence, the antibacterial effects of honey are not only due to osmilarity, visicosity, presence of hydrogen peroxide and low protein contents but due to other important factors that affect the composition of honey. (Cooper *et al*., 2002).

According to Jing *et al.*, (2014), such factors depend to a great extent on the bees source, the location of the flowers and related weather conditions, the storage time and conditions and the method of preservative treatment. The results of this study was in agreement with the study performed by Al-Haj *et al.*, (2009) using Malaysian honey on both methicillin sensitive *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus*. They concluded that honey completely inhibited the growth of the two bacteria. Also, the reports of this study agrees with studies carried out by Taormina *et al.*, (2001), in which they tested antibacterial activity from six floral sources against *Escherichia Coli, Salmonella thyphimurium, Shigella sonnei*, *Staphylococcus aureus* and *Bacillus cereus* using disc diffusion method. Their results showed that the development of inhibition zones depends on the concentration of the honey used as well as the tested pathogen. Osho and Bello (2010) compared the antibacterial effect invitro of amoxicillin, tertracyclin and chloramphenicol antibiotics versus the antibacterial effect of two honey solutions of different concentrations (5, 25, 50 and 100%) against *S.aureus*, *Pseudomonas aeruginosa*, *E. coli, Klebisella pneumonia* and *Bacillus subtilis* by the agar diffusion method. They reported that both honeys tested were effective at 25 and 100% against all the micro-organisms evaluated. Agbagwa and Peterside, (2010), examined different honey samples: Western-Nigeria honey, Southern Nigerian Honey, Eastern Nigerian honey and Northern-Nigeria honey and compared their abilities to inhibit the growth of *S.aureus*, *P.aeruginosa*, *E.coli* and *P.mirabilis* and obtained an average zone diameter of inhibition ranging from 5.3 – 11.6mm, 14-15.4mm, 4.4 – 13.5mm and 9.1 – 17mm, respectively with honey concentrations between 80 – 100%.

4. Conclusions

The result of this study shows that the two different honey samples (Nsukka and Ugwuaji) inhibited the growth of the tested organisms at different concentrations. Honey samples from Nsukka gave higher inhibition efficiency on the growth of the tested bacteria than honey samples from Ugwuaji. The zone diameters of inhibitions of the organisms at different concentrations of the honey samples were found to be statistically significant.

The order of sensitivity of the tested organisms to the honey samples decreased in the following order:

Staphylococcus aureus > *Eschericha coli* > *Streptococcus pyogenes*

Finally the bactericidal effect of the honey samples were found to be dependent on the concentration and type of the honey used and the nature of tested bacteria.

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