THE INBIBITION POTENTIALS OF DIFFERENT HONEY SAMPLES AGAINST *STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI* AND *BACILLUS* SPECIES ISOLATED FROM CLINICAL SOURCE

Abstract

As a result of the increased prevalence of antibiotic resistance among different bacteria, different plants and other natural products have been studied and found to be highly effective against pathogenic bacteria, especially with the increased prevalence of antibiotic resistance among different bacteria. Honey, over the years has been used as an antibacterial agent to treat certain infections caused by bacteria and is believed to be effective especially in rural areas. This study was thus aimed at comparing the effect of different honey samples against some pathogenic bacteria (*Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus*) isolated from clinical source. The antibacterial sensitivity test was carried out using agar well diffusion method while the Minimum inhibitory concentration and Minimum bactericidal concentration were determined using broth tube micro dilution technique in two fold dilution. The inhibition efficiency of the honey samples on the test organisms increased with increase in concentration from 20 to 100% as 100% concentration had the highest zone of inhibition. *Staphylococcus aureus* (6.33mm – 26.33mm) was the most sensitive to the honey samples while *Bacillus cereus* (0.00 – 19.67mm) was less sensitive. At concentrations of 20 – 80%, raw and Rowse honey were more effective on *E. coli* compared to PG honey, while at 100%, PG honey was more effective on *Staphylococcus aureus*. Raw and Rowse honey were more effective at 20 -60% concentrations followed by PG honey; whereas at 80 -100% concentrations, Raw and PG honey were more effective. *Bacillus cereus* was resistancet to the honey samples at 20 – 60% but sensitive at 80 – 100% concentrations to Rowse, Raw and PG honey. The inhibition efficiency of the honey samples on the growth of the tested organisms was found to be dependent on the concentration and type of honey used, as well as they type of organism tested. The result of the minimum inhibitory and minimum bactericidal concentration showed that *Staphylococcus aureus* was inhibited most at a lower concentration of 25% compared to other bacterial isolates. All honey samples tested did not show any bactericidal effect but was bacteriostatic to some of the tested organisms. Ppharmacological standardization and clinical evaluation on the effect of honey is essential before honey can be used as a preventive and curative measure to common diseases related to the tested bacterial species.

Comment [MM1]: Ppharmacological = Pharmacological

Keywords: Honey, antibiotic resistance, antimicrobial agents, minimum inhibitory concentration,

Article Outline

INTRODUCTION

Comment [MM2]: Most part of the reference is more than 10 years old I suggest you change the introduction. I propose you to read a few articles such as
- Honey: Its medicinal property and antibacterial activity (April 2011Asian Pacific Journal of Tropical Biomedicine 1(2):154-60)
- Antibacterial activity of Manuka honey and its components: An overview (November 2018AIMS Microbiology 4(4):655-664)
- Antioxidant properties and antimicrobial activity of manuka honey versus Polish honeys (J Food Sci Technol
. 2020 Apr;57(4):1269-1277)

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. It 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, 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 times. It has valued uses as 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 broad-spectrum antibacterial activity when tested against some pathogenic and oral bacteria (Mauric *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).

Lusby *et al.* (2005), stated that the healing properties of honey can be 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 investigators 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 agent 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 bacterial growth by honey (Bizerra *et al.*, 2002). Indeed honey with a high concentration of hydrogen peroxide has s higher antibacterial activity.

Libonatti *et al.* (2014), reported that the 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 some gram-negative bacteria (*Pseudomonas aeruginosa, Enterobacter spp.*, and *Klebisella spp.*) 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, comparison of the activities of sterilized and non-sterilized honey and its antimicrobial action on *Stapylococcus aureus, Eschericha coli and Bacillus cereus,* the antimicrobial effect of different honey samples against these organisms obtained from clinical sources were investigated.

MATERIALS AND METHODS

MATERIALS

Collection and preparation of samples.

A total of three honey samples were used in this study. Two were bought from a supermarket in Port- Harcourt metropolis and one was bought from local bee keepers in Etche local Government, Rivers state, Nigeria. The samples were stored in sterile bottles at a temperature of 20 – 21^{o}C in a dark place before analyses.

Collection and confirmation of bacteria isolates

Bacterial isolates used in this study were wound associated bacteria including *Staphylococcus aureus, Escherichia coli and Bacillus cereus.* The isolates were obtained from Optimal Diagnostic Center, Mgbuoba, Port-Harcourt. The isolates were collected in sterile Bijou bottles containing nutrient broth and immediately incubated at a temperature of 37^{o}C for 24 hours. The isolates were identified microscopically and biochemically using tests such as Grams Stain, catalase, simmon Citrate utilization, indole, motility, methyl Red-Voges proskauer, oxidase, sugar fermentation, starch hydrolysis, coagulated, hemolysis and spore stain as described by Cheesbrough (2005).

Methods

Antibiotics sensitivity test: This test was performed using disc diffusion method as described by the Clinical and Laboratory Standard Institute (2009). The following antibiotics used inclde; Ceftazidime, cefuroxime, gentamicin, ceftriaxone, Erythromycin, cloxacillin, Ofloxacin, Augmentin, Cefixime, Nitrotrantion and Ciprofloxacin.

Antibacterial Sensitivity test of honey on bacterial isolates: The sensitivity of honey samples was determined using agar-well diffusion method as described by Okeke *et al*. (2018).

Determination of minimum inhibitory concentrations (MICs) of honey on the bacterial isolates: The MIC of honey was determined using broth tube dilution method as described by Kacaniova *et al.* (2011).

Determination of minimum bactericidal concentrations (MBC) of honey on the bacterial isolates: The MBC of honey was determined according to the method of Kacaniova *et al*. (2011).

Statistical analysis: Results obtained were expressed as mean ± standard deviations and differences between means were analyzed statistically using analysis of variance (ANOVA) on the SPSS version 22.0; differences were considered significant when $p \leq 0.05$ and where differences occurred, Tukey method was used to separate the means.

RESULTS AND DISCUSSION

Figure 1 shows the effects of different concentrations of honey samples on the growth of *E. coli*. From this figure, PG honey at concentrations of 20, 40, 60, 80 and 100% gave 0.00, 3.67, 12.00, 20.33 and 31.00 mm zones of inhibition, respectively. Also raw honey at the same concentrations gave 0.00, 6.67, 17.67, 22.67 and 29.33 mm zones of inhibition, respectively while Rowse honey at similar concentration on *Escherichia coli* gave 1.33, 8.33, 15.33, 21.67 and 29.67 mm zones of inhibition, respectively.

Figure 2 shows the effect of different concentrations of honey samples on the growth of *S. aureus*. It showed that PG honey gave zones of inhibition of 6.33, 10.33, 15.67, 21.00 and 28.67 mm at concentrations of 20, 40, 60, 80 and 100%, respectively while on Raw honey at similar concentration gave zones of inhibition of 8.00, 13.00, 18.67, 23.67 and 29.67 mm, respectively. Furthermore, the effect of Rowse honey on the growth of *S. aureus* at concentrations of 20, 40, 60, 80 and 100% gave 7.67, 10.00, 15.33, 18.33 and 26.33 mm zones of inhibition, respectively.

Figure 3 showed that only 80 and 100% concentrations of the honey samples were effective on *B. cereus*. PG honey was effective on *B. cereus* at a concentration of 100% with zone of inhibition of 1.33 mm, while Raw honey inhibited its growth at 80 and 100% concentrations with zones of inhibition of 2.33 and 7.00 mm, respectively. Also, Rowse honey inhibited at 80 and 100% concentrations with inhibition zones of 9.67 and 14.67mm, respectively.

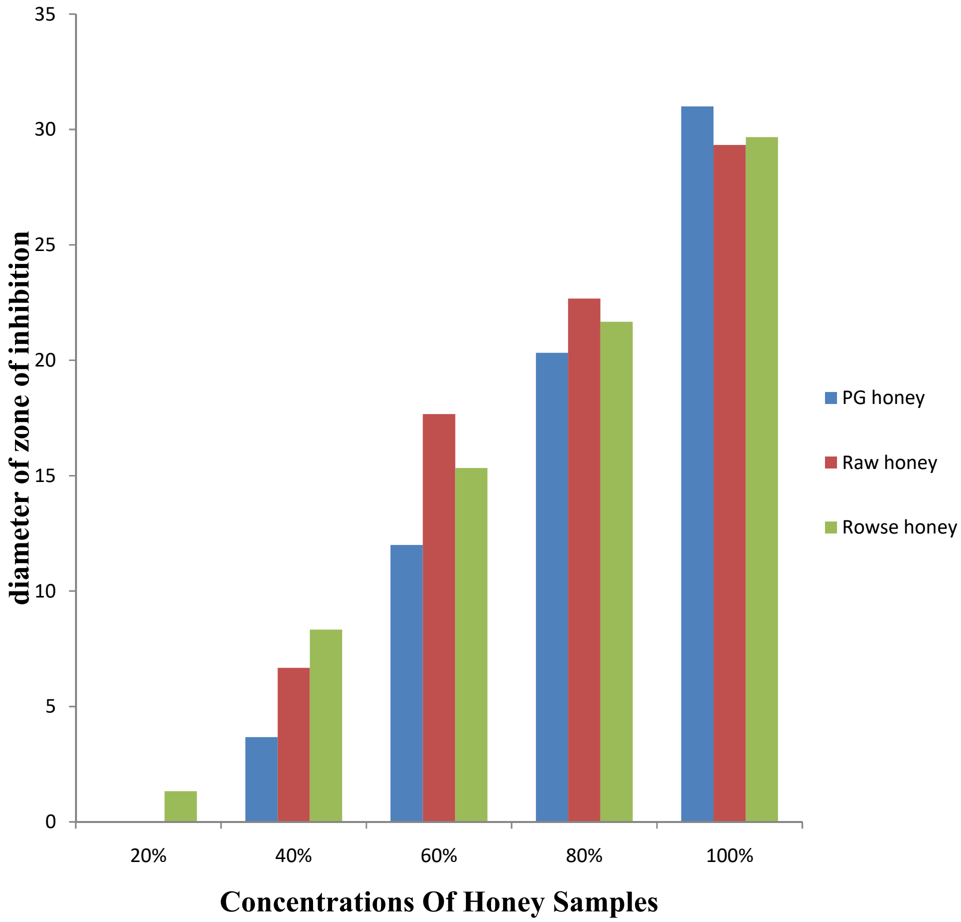

Fig 1: Effects of different concentrations of honey samples on the sensitivity pattern of *Escherichia coli* as shown by the diameter of the zones of inhibition.

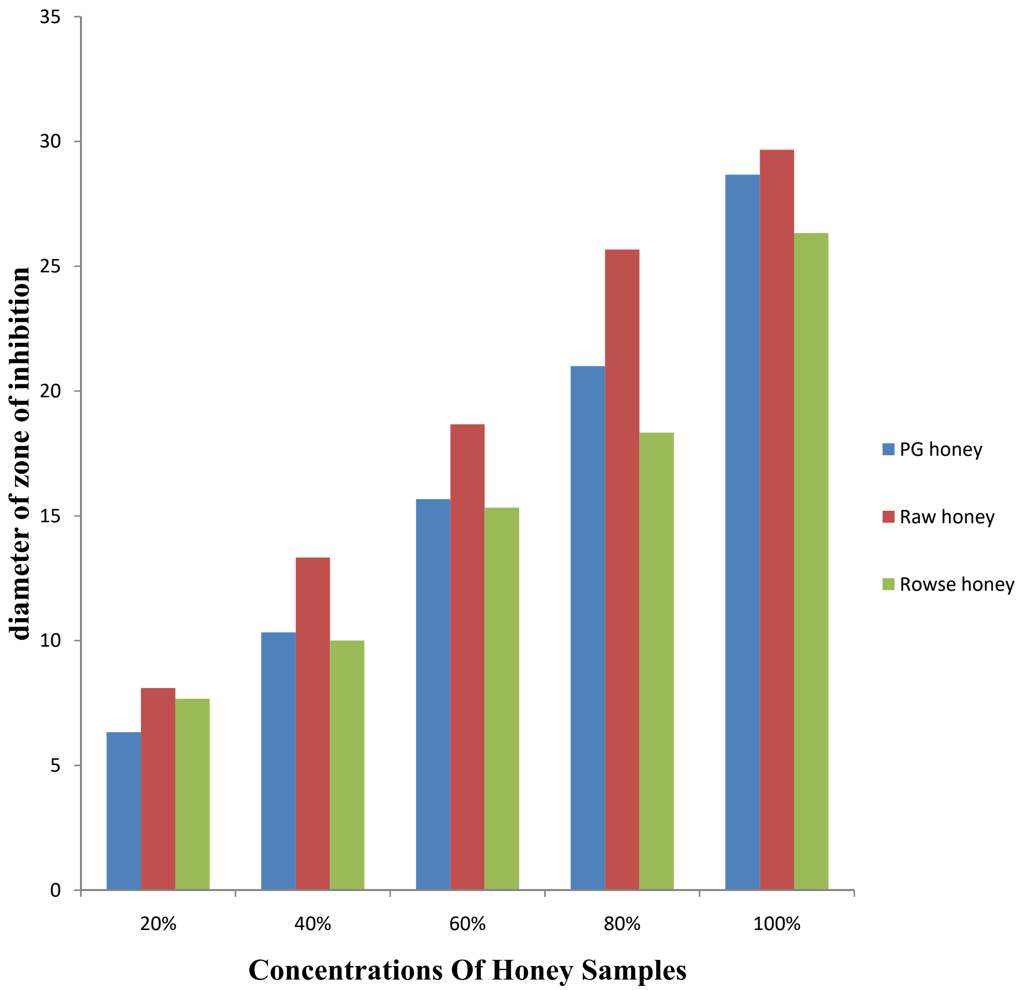

Fig 2: Effects of different concentrations of honey samples on the sensitivity pattern of *Staphylococcus aureus* as shown by the diameter of the zones of inhibition.

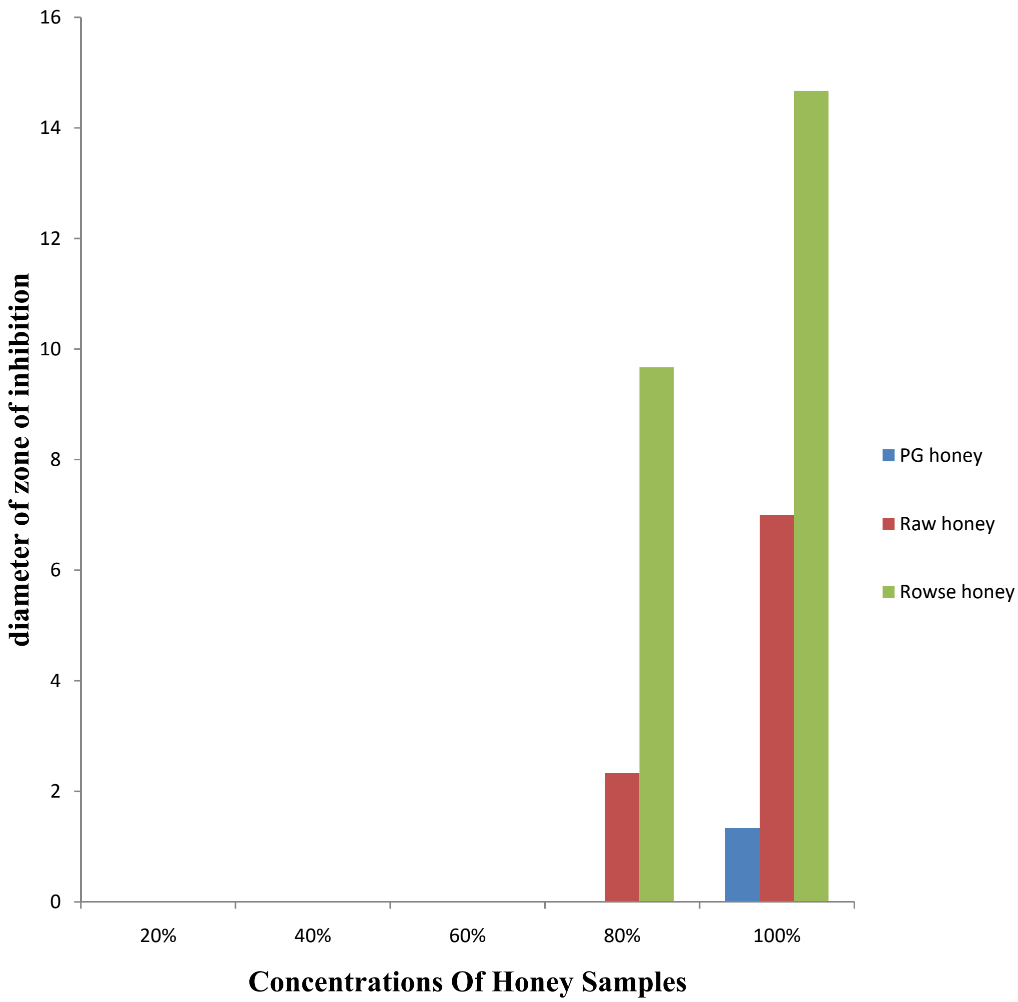

Fig 3: Effects of different concentrations of honey samples on the sensitivity pattern of *Bacillus cereus* as shown by the diameter of the zones of inhibition.

Discussion

The varied inhibition levels of the samples is due to the fact that different honey types possess different efficacies against the same type of bacterium and different bacteria (Almasaudi *et al.*, 2017). Hence, the antibacterial efficacy of honey is not only due to osmolality, viscosity, presence of hydrogen peroxide and low protein contents but due to other factors that affect the composition of honey. (Cooper *et al*., 2002). Such factors depend to a great extent on the bee's source, the location of the flowers and related weather conditions, the storage time and conditions and the method of preservative treatment according to Jing et al. (2014).

The results of this study was in agreement with the study performed by Al-Haj *et al.* (2009) who used 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 is in consonance with the study by Taormina *et al.* (2001), where they investigated the antibacterial activity of honey from six floral sources against *E. coli, Salmonella thyphimurium, Shigella sonnei*, *Staphylococcus aureus* and *Bacillus cereus* using disc diffusion method. Their results showed that the development of inhibition zones depended on the concentration of the honey used as well as the test pathogen; their result showes that *B. cereus* was least inhibited while *S. aureus* was most inhibited by the different honey samples.

Comment [MM3]: The discussion is very short, please try to have a good comparison with other articles such as
- Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece (May 2018International Journal of Molecular Medicine 42(2))
- Antimicrobial Activity of Honey (Am J Ther . Jul-Aug 2014;21(4):304-23)

Conclusion

The results of this study showed an increase in concentrations of honey samples increased their inhibitory effects on the test isolates. Also, among the three studied pathogenic bacteria, E. coli was the most inhibited with 29.33, 29.67 and 31.00 mm zones of inhibition by Raw, Rowse and PG honey samples, respectively while B. cereus was the least inhibited with 1.33, 7.00 and 14.67 mm zones of inhibition by PG, raw and Rowse honey samples, respectively. Comparison of the results of the figures showed that PG honey was most effective on E.coli with zone a inhibition of 31.00 mm while on S. aureus Raw honey was the most effective with a diameter of 29.67 mm. Also, Rowse honey showed higher efficiency on B. cereus with inhibition diameter of 14.67mm. Although, the three honey samples exhibited varied inhibitory effects on the same bacterium and the different bacteria, all three samples were found to have antibacterial effects against the isolates. This further proves that honey is a potent antibacterial agent and could be used in place of synthetic antibiotics if properly standardized especially with the rising occurrence of antibiotic resistance among synthetic drugs.

Reference

Comment [MM4]: More attention to references, it will be better to be the last 10 years.

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