

Antibacterial Activities of Selected Samples of Honey in South-Western Nigeria against Bacteria in Wound Infections

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Abstract: The global challenges resulting from the emergence of numerous resistant pathogenic bacteria have led to a search for alternative therapy ethnomedically. As a result, honey is increasingly valued for its antibacterial activity. In this study, the antibacterial activity of different honey samples was assessed *in vitro* using agar dilution at different concentration of 5%, 10%, 20%, 30%, 40%, 50%, 75% and 100% (v/v) dilution. The antibacterial activity was also determined by spectrophotometric assay at 620 nm. The result showed that KG honey sample inhibited the different bacterial isolates at a concentration of 5% and LB honey sample inhibited the isolates at 20% while other honey samples inhibited the bacterial isolates at concentrations ranging between 10% and 30% (v/v) dilution of each honey samples. Thus, the minimum inhibitory concentrations ranged between a concentration of 5% and 30% (v/v) dilution even though antibacterial activities concentration ranged from 5% - 100% (v/v). *Ps. aeruginosa* ATCC 15442 was considered the most susceptible of all the test isolates. The increases in the absorbances showed that the bacterial populations in each of the honey samples were reduced as the exposure time increases. The study shows that honey being sold in Nigeria may be used as a therapeutic agent to treat infections as they may be an excellent alternative to curtail the further spreading of multi-drug-resistant microorganisms in Nigeria.

Running Title: Antibacterial activities of honey against bacteria

Keywords: Antibacterial, Honey, Multidrug Resistance, Wound Infections

INTRODUCTION

Honey produced by *Apis mellifera* is one of the oldest traditional medicines considered important in the treatment of several human ailments (Mondo *et al.*, 2004). The ancient Greeks believed that consuming honey could result in living longer (Kwakman *et al.*, 2002). While many pathogenic organisms have developed resistance to many antibiotics (Patton *et al.*, 2006) and multidrug resistance is on the increase, efforts are being intensified to develop antimicrobial agents from natural sources for better therapeutic effects (Gills *et al.*, 1992). Consequently, despite the fact that researchers have focused on the bactericidal potential of honey against many pathogenic aerobic and anaerobic, Gram-negative and Gram-positive bacteria (Haffejee *et al.*, 1985; Hannan *et al.*, 2004), Basson *et al.* (1994) and Lusby *et al.* (2005) indicated that natural unheated honey has some broad-spectrum antibacterial activity against pathogenic, oral and food spoilage bacteria.

Although the chemical component of honey is dependent on the type of honey, geographical location, season, flower source from which the final product is derived and production methods ((Molan and Cooper, 2000; Nisbet *et al.*, 2010), honey contains enzymatic and non-enzymatic antioxidants (Bogdnov, 1989), numerous types of sugar, acids, vitamins, protein and minerals (Al-Waili *et al.*, 2004) as well as alkaloids, antaquinone, glycosides, cardiac glycosides, flavonoids and reducing compounds (Chute *et al.*, 2010). High sugar concentration, low pH, hydrogen peroxide generation, proteinaceous compounds or other unidentified components present in the honey may provide antimicrobial activity (Mundo *et al.*, 2004). Besides its antimicrobial properties, honey can clear infections in a number of ways *in vivo*, boost the immune system, act as anti-inflammatory and antioxidant agents and stimulate cell growth (Al-jabri *et al.*, 2005). While raw honey contains copious amounts of flavonoids and other polyphenols exhibiting different biological activities and act as antioxidants (Blassa *et al.*, 2006;

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Pyrzyńska and Biesaga, 2009), methyl syringate and methylglyoxal contribute to the non-peroxide activities of honey (Marvic, 2008; Adams et al., 2008).

Since honey has been used from ancient times as a method of accelerating wound healing (Van der berg et al., 2008), its possibility to heal wound has constantly been demonstrated (Molan, 2006; Simon et al., 2008). The antibacterial properties of honey increase the growth of new tissue to heal the wound (Lusby et al., 2002). When applied topically, it rapidly clears wound infection to facilitate healing of deep surgical wounds with infection (Ahmed et al., 2003). Its application can promote the healing of peptic ulcers and gastritis (Molan et al., 2001) and methicillin-resistant *S. aureus* infected wounds (Natarajan et al., 2003). In cognizance of its therapeutic potential, the antimicrobial activities of diluted honey from different countries have been reported (Voidarou et al., 2011; Moussa et al., 2012). In Nigeria, there is a need to validate the therapeutic potentials and the antibacterial activities of different honey samples commonly sold for consumption. The aim of this study, therefore, was to investigate the antibacterial activities of five different honey samples against bacteria in wound infections.

MATERIALS AND METHODS

Honey Samples

Five different honey samples locally produced honey from apiary were bought from different pharmaceutical outlets in the Southwest part of Nigeria. The honey samples were stored at 23-25°C in the dark. For antibacterial assays, each of the honey samples was used undiluted and at 5%, 10%, 20%, 30%, 40%, 50% and 75% v/v dilution by weighing a required amount of each of the honey and diluting to a final volume of 100 ml with sterile deionized distilled water (Cooper et al., 2002a). Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive (White et al., 1963).

Test microorganisms

The test organisms including *Pseudomonas aeruginosa* ATCC 15442, *Shigella flexneri* KZN, *Enterococcus faecalis* KZN, *Escherichia coli* ATCC 25922, *Serratia mercerscens* ATCC 9986, *Klebsiella pneumoniae* ATCC4352, *Proteus vulgaris* ATCC 6830, *Enterococcus faecalis* ATCC 29212, *Enterococcus cloacae* ATCC 13047 and *Shigella sonnei* ATCC 29930 were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa. Colonies from fresh overnight growth cultures of the different bacterial strains were suspended in 3 - 4 ml

sterile nutrient broth and incubated for 2 - 3 h at 37°C. These isolates were diluted with distilled water and adjusted to 0.5 McFarland's standard yielding approximately 1.0×10^6 CFU/ml before being used in this study.

Antibacterial testing by agar dilution method

The antibacterial activity of the different honey samples was carried out by agar dilution and spectrophotometric assays (Patton et al., 2006; Tan et al., 2009). For the agar dilution assay, double strength Mueller Hinton Agar (MHA) medium was prepared and incorporated with the different honey samples at concentrations (v/v Honey + MAH) of 5%, 10%, 20%, 30%, 40%, 50% and 75%. Since honey is a very viscous liquid, it was kept at 50°C before mixing with the agar to achieve uniform homogenization. On mixing the honey with the Mueller Hinton agar maintained at 50°C, the mixture was vigorously vortexed and dispensed into sterile petri dishes. After being allowed to solidify, each of the test organisms was streaked on the different concentrations of the different honey samples before being incubated at 37°C for 24 h and observed for growth. The experiment was performed in duplicate to ensure reproducibility of the results. Three agar plates were set up as controls. One agar plate was inoculated with all test strains to confirm their viability. Second control plate contained medium only and the third plate contained different honey samples to check for the sterility of the medium and the honey. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of honey at which no visible bacterial growths were observed.

Spectrophotometric testing

The sample was prepared by weighing 30%, mix well and poured into 10 test tubes, after which diluted 100 µl of the test organisms were introduced into the 10 test tubes. Spectrophotometric reading was taken at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h at 625 nm. These are aimed at determining the level of turbidity after hours of inoculation.

RESULT

In this study, the different honey samples exhibited a varied degree of inhibitory effects against the different bacterial isolates. The minimum inhibitory concentrations of the honey samples varied from each other against the bacterial isolates. While KG honey sample inhibited the different bacterial isolates at a concentration of 5% and LB honey sample inhibited these isolates at 20%, other honey samples inhibited the bacterial isolates at concentrations ranging between 10% and 30% (v/v) dilution of each honey samples. While six of the isolates were inhibited by

20% dilution and four of the isolates were inhibited at 30% of the UB honey dilution and this antibacterial activity was a reverse in NF honey samples, eight of the isolates were inhibited by 10% and two isolates were inhibited by the 20% of the DB honey samples as shown in Table 1. No microbial growths were observed on the agar plates containing honey samples used as sterility test for each of the honey samples. While KG honey was the most effective honey inhibiting bacterial isolates at 5%, *Ps. aeruginosa*

ATCC 15442 was considered the most susceptible of all the test isolates. From Figures 1-5, the spectrophotometric reading of the antibacterial activities of the different honey samples showed that there were increases in the reading of the absorbances at 625 nm over a period of 6 h. The increases in the absorbances showed that the bacterial populations in each of the honey samples were reduced to allow more penetration of light as the exposure time increases.

TABLE 1: Antibacterial activity of five different pure natural undiluted honey (v/v)

Organisms used	Minimum inhibitory concentrations of different hone samples (v/v)				
	KG honey	UB honey	LB honey	NF honey	DB honey
<i>E. cloacae</i> KZN	5%	20%	20%	30%	10%
<i>P. vulgaris</i> ATCC 6830	5%	30%	20%	10%	10%
<i>E. faecalis</i> KZN	5%	20%	20%	30%	20%
<i>E. coli</i> ATCC 25922	5%	30%	20%	30%	10%
<i>S. flexineri</i> KZN	5%	20%	20%	30%	10%
<i>Ps. aeruginosa</i> ATCC 15442	5%	20%	20%	10%	10%
<i>S. sonnei</i> ATCC 29930	5%	30%	20%	10%	10%
<i>K. pneumoniae</i> ATCC 10031	5%	30%	20%	20%	20%
<i>S. mercescens</i> ATCC 9986	5%	20%	20%	30%	10%
<i>Ps. aeruginosa</i> ATCC 19582	5%	20%	20%	30%	10%

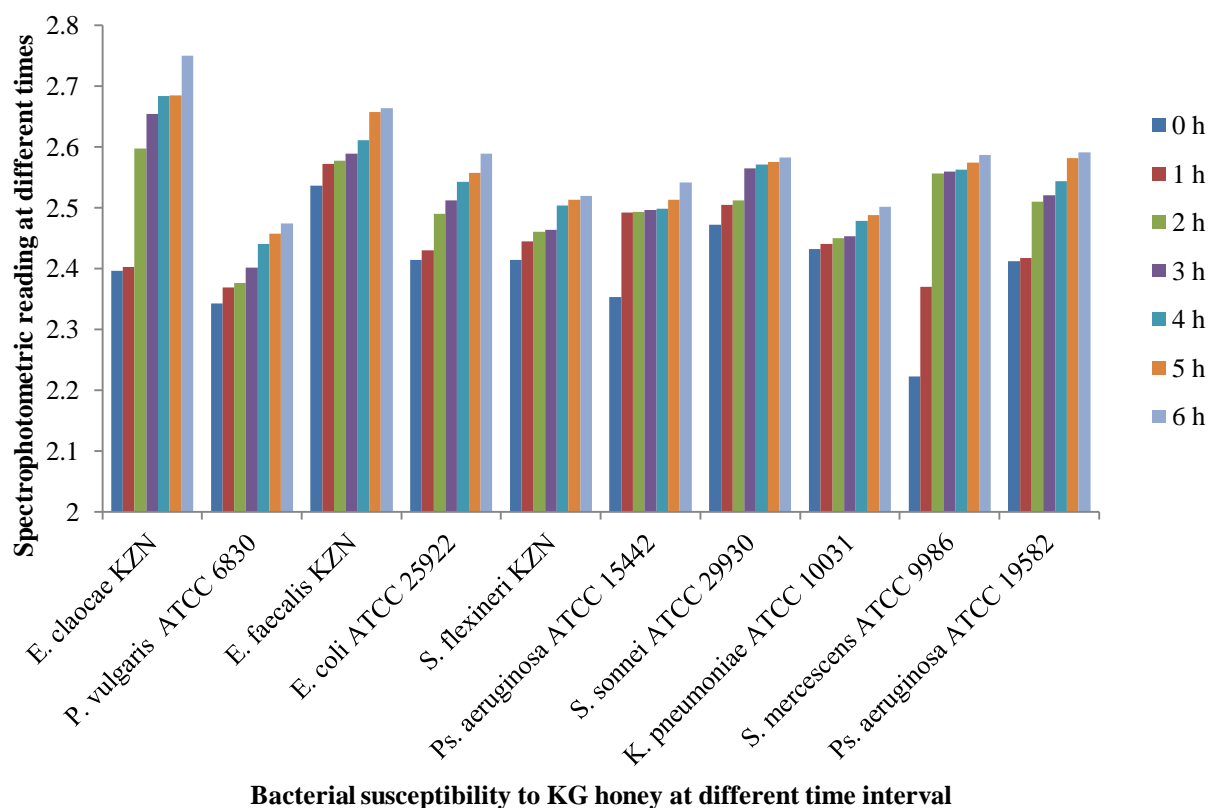


Figure 1: Spectrophotometric reading (kinetics) of bacterial susceptibility in KG honey 30% (v/v)

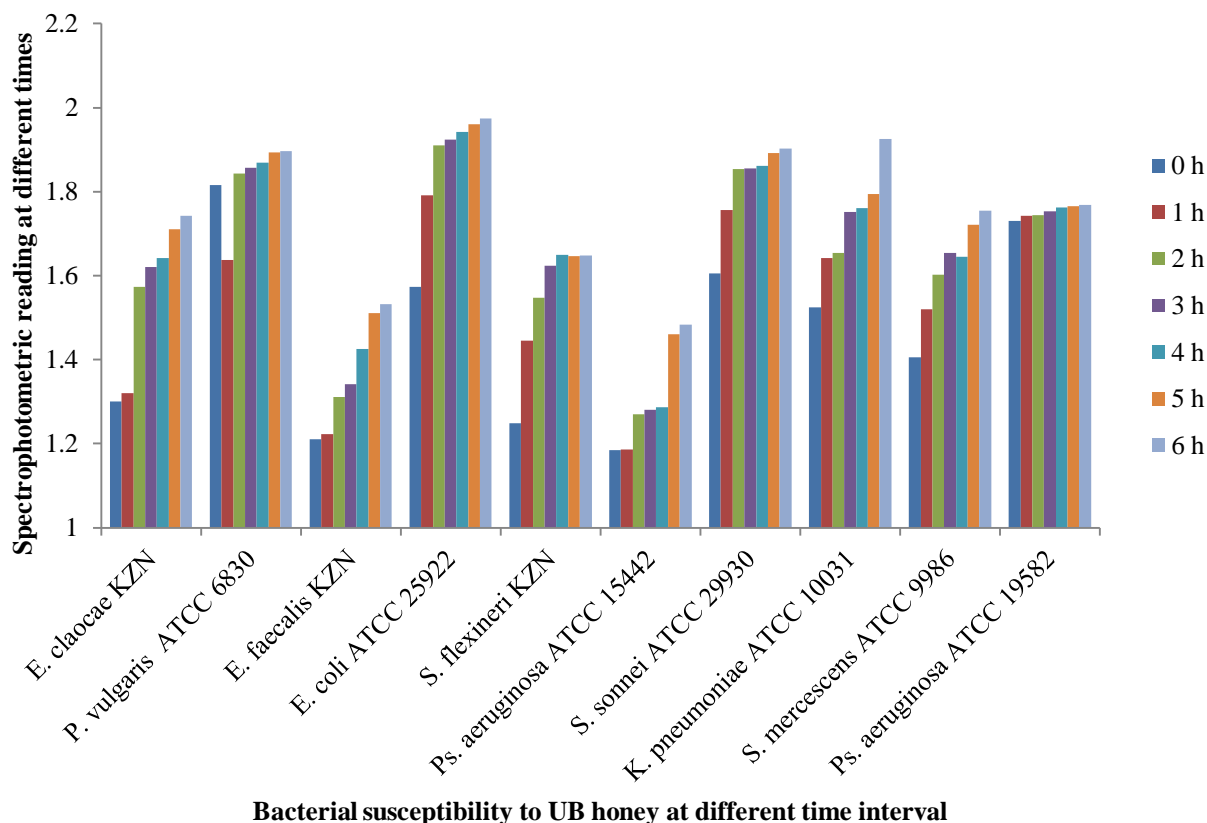


Figure 2: Spectrophotometric reading (kinetics) of bacterial susceptibility to UB honey 30% (v/v)

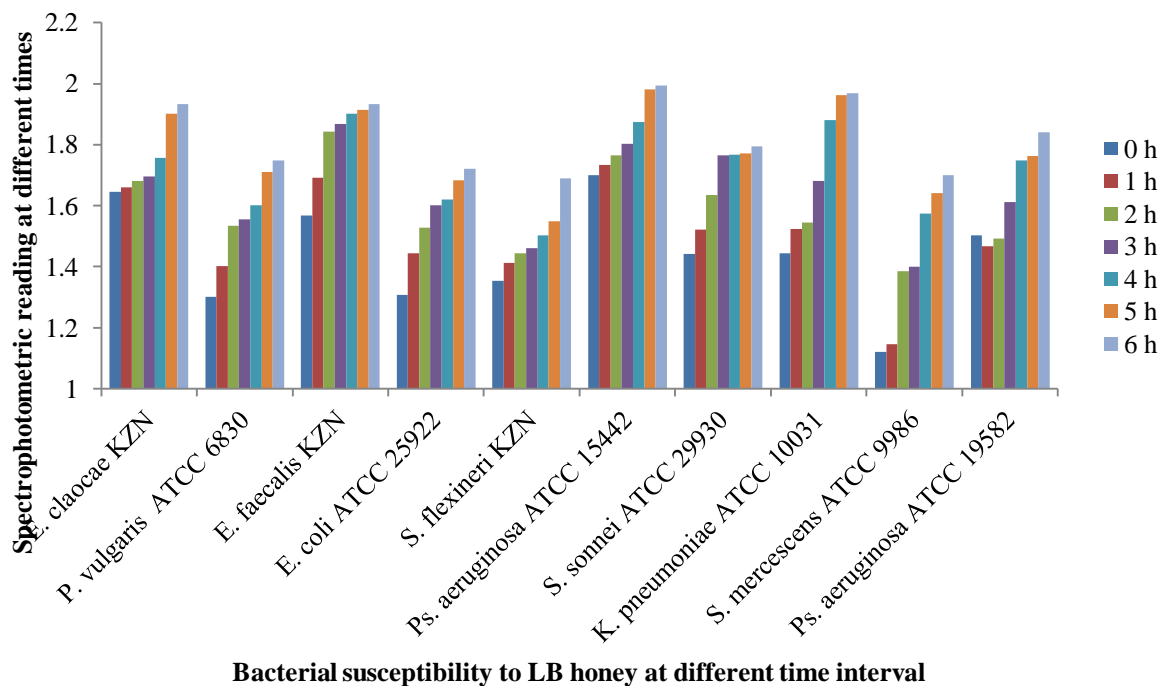


Figure 3: Spectrophotometric reading (kinetics) of bacterial susceptibility in LB honey 30% (v/v)

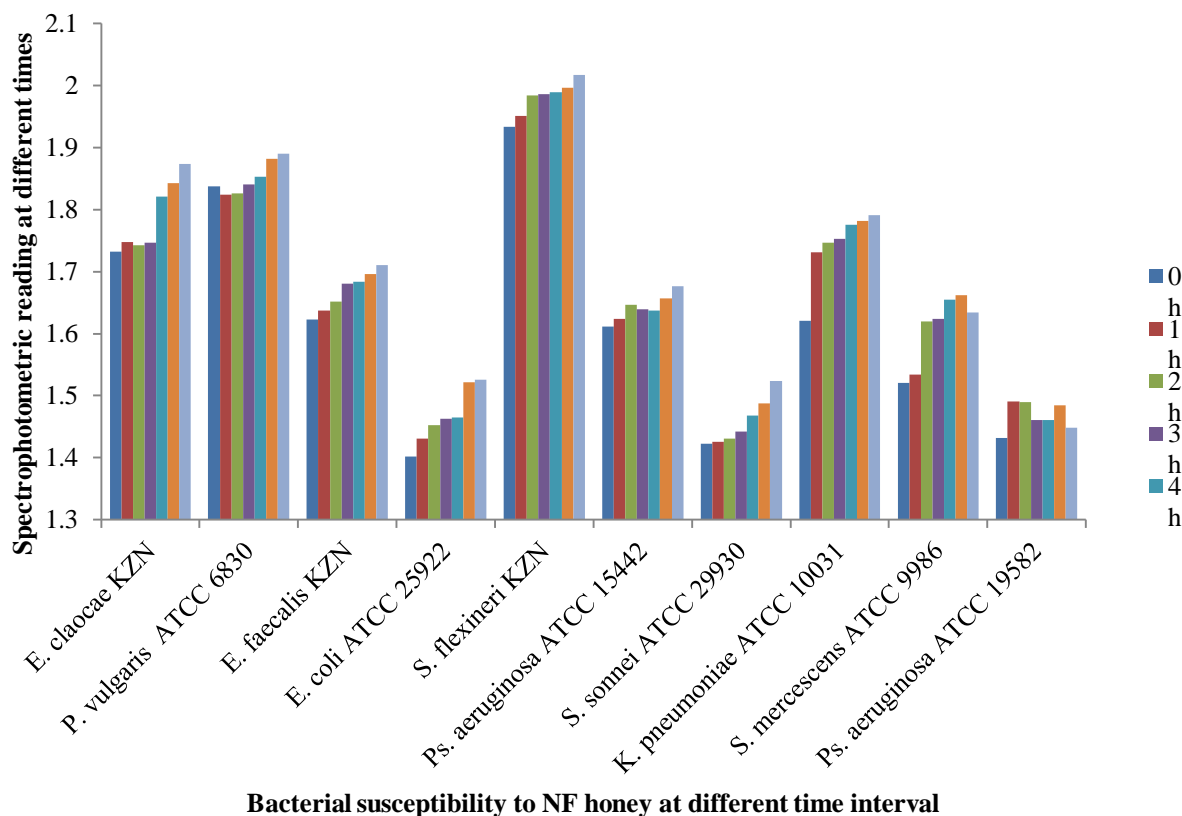


Figure 4: Spectrophotometric reading (kinetics) of bacterial susceptibility in LB honey 30% (v/v)

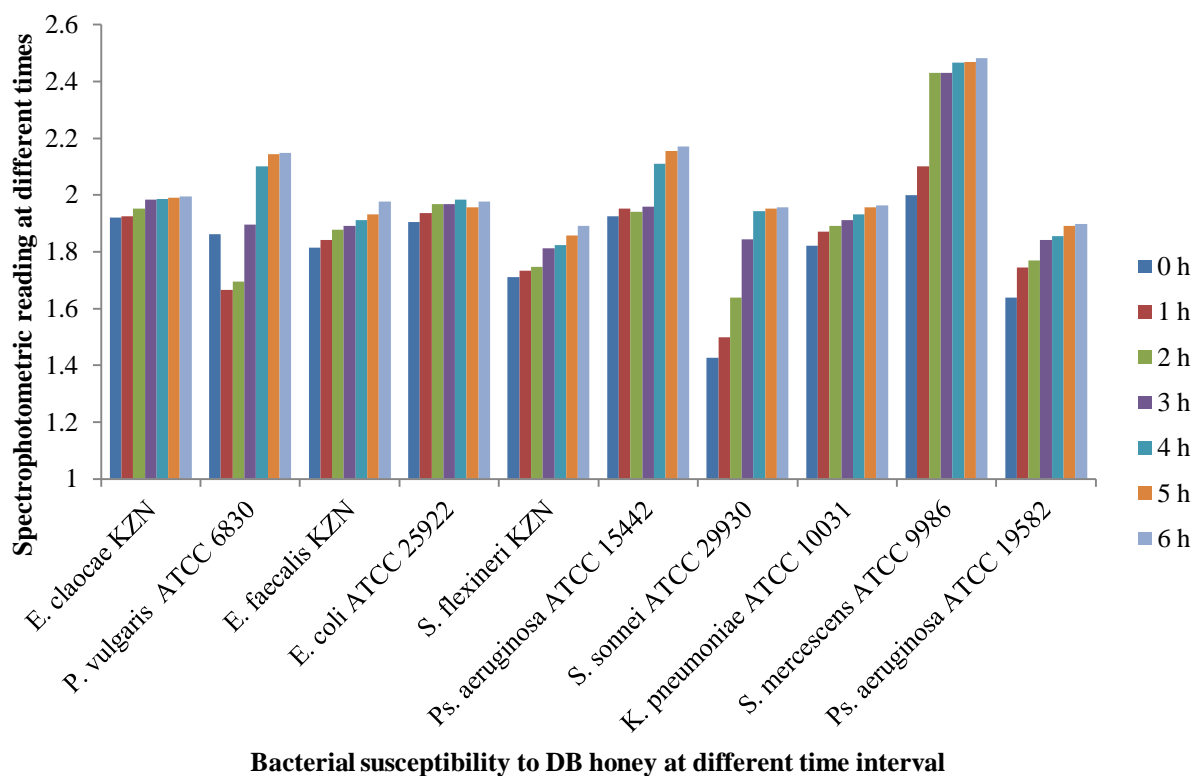


Figure 5: Spectrophotometric reading (kinetics) of bacterial susceptibility in LB honey 30% (v/v)

DISCUSSION

The control of infectious diseases due to antimicrobial resistance is a serious challenge to be addressed (WHO, 2010). In the recent time, the emergence of numerous resistant pathogenic bacterial strains to a wide range of formerly efficient antibiotics has constituted a serious threat to public health (Raymond et al., 2011) while this incidence has paved way to the occurrence of infections that are only treated by a limited number of antimicrobial agents (Roula et al., 2010). This global challenge, calling for the development of new antibiotics sufficient for the treatment of chronic infections (Choffnes et al., 2010), has led a great population of the world to rely on traditional medicines as primary health care, as an alternative and complementary medicines (WHO, 2008) because they are natural, less expensive, non-toxic and has no adverse effects on the healing process of tissues (Dunford et al., 2000; Lusby et al., 2002; Hamzaoglu et al., 2000). Consequently, efforts have been unleashed by researchers to unravel the unknown benefits of the inestimable attributes of honey and its applications (Irish et al., 2008; Cursons, 2010; Kumari et al., 2010; Zaid et al., 2010).

From this study, the absence of microbial growths on agar plates used as sterility control test may be due to the inhibitory effects of the chemical components of the different honey samples (Souza et al., 2007; Teixeira et al., 2008). If bacteria had been detected in the control samples, the presence of isolates may have indicated contamination from a secondary source. However, the antibacterial activities of the different honey samples showed that the minimum inhibitory concentrations ranged between a concentration of 5% and 30% (v/v) dilution even though antibacterial activities concentration ranged from 5% - 100% (v/v). While Nzeako and Hamdi (2000) indicated that honey had no activity at 40% (v/v) dilution, Chanchao et al. (2006) indicated that honey provides the same inhibition ability at 25% and 50% (v/v) dilution but not at a dilution of 75% (v/v). Lusby et al., (2005) reported that honey other than commercially available antibacterial honeys like manuka (*Leptospermum scoparium*) honey can have equivalent antibacterial activity on certain clinical isolates. Although the antibacterial activity of different types of honey can vary by a factor up to 100 (Willix et al., 1992), the great variety of antibacterial activity of honey may be attributed to differences in the botanical and geographical origin, and consequently in the chemical composition (Akkol et al., 2007).

In previous studies, the antimicrobial activities of honey have been reported. Zumla and Lulat (1989)

reported that honey is a very good inhibitor to *E. coli*, *Salmonella* and *Shigella*. French et al. (2005) reported the effectiveness of manuka honey against pathogenic bacteria such as *S. aureus* and *Helicobacter pylori*. Wilkinson (2005) investigated the susceptibility of *E. coli* and *Ps. aeruginosa* to the activity of 13 honey samples. Tan et al., (2009) reported that honey samples, in their study, showed activity against *E. coli*, *S. aureus*, *B. Subtilis* and *Ps. aeruginosa*. These reports are in agreement with the results of the present study. The antibacterial effect of the different honey that was used in this study showed a remarkable effect through the rate at which they inhibited the test bacterial isolates used. The bacterial growths were diversely inhibited at concentrations of 5%, 10%, 20%, 30%, 40% and 50% (v/v) dilution of the honey samples. Although the mechanism of antibacterial action of honey is most likely a combination of a number of different factors, the antibacterial activity in most honey samples is due to the enzymatic production of hydrogen peroxide in addition to unidentified phytochemical components (Cooper et al., 2002b). While its dilution enhances hydrogen peroxide mediated antibacterial activity (Melissa et al., 2004), honey has antioxidant properties and increases cytokine release which may explain its antimicrobial effects (Gheldof et al., 2002; Schramm et al., 2003; Tonks et al., 2003). Since hydrogen peroxide is a major contributor to the antimicrobial activity of honey, the different concentrations of this compound in the different honeys could have resulted in their varying antimicrobial effect recorded in this study.

In conclusion, this study confirms that honey possesses antimicrobial activity and holds great promise as antimicrobial agent against pathogenic microorganisms. It may be used as a therapeutic agent and an effective herbal medicine to treat infections as they may be an excellent alternative to curtail the further spreading of multi-drug-resistant microorganisms in Nigeria.

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