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Antimicrobial Activities of Different Honeys Sold in Ado-Ekiti on Bacteria Associated with Upper Respiratory Tract Infections

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A B S T R A C T

Honey has been used for various purposes including treatment of some diseases locally especially in wounds and upper respiratory tract infections (URTIs). There is paucity of information on the scientific basis for the use of honey in the treatment of upper respiratory tract infections caused by bacteria. This study was carried out to determine antibacterial activities of different brands of honey marketed in Ado-Ekiti and relate with those of some orthodox antibiotics used in the treatment of URTIs. The antimicrobial activities of the honeys were assessed against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using agar well diffusion technique. Broth dilution method was used to determine the minimum inhibitory concentration (MIC) of the honey against the bacteria. Screening concentrations of neat honey and 80% honey inhibited *Staphylococcus aureus* and *Klebsiella pneumoniae* with zone of inhibition ranging between 2-10 mm. *Pseudomonas aeruginosa* was resistant to all concentrations of honeys except for one honey (Dr Bee) that inhibited the organism at 100% concentration. Orthodox antibiotics showed significantly higher mean zone of inhibition in relation to honey ($P < 0.001$). The sensitive organisms were inhibited in dose related manner. The mean MIC of the different honey brands was generally at 31.25% against *Staphylococcus aureus* while concentration of 50% showed bactericidal effect on *S. aureus*. *Klebsiella pneumoniae* was inhibited at mean concentration of 31.5% and bactericidal effect was observed against the organism at 40% mean concentration.

Introduction

Honey is made from nectar and sweet substances in plants by honeybees. It is desirable as sweetener and as a natural food product. In ages past, honey was regarded as a medicinal substance for the management of various medical problems (Zaghloul *et al.*, 2001).

Upper respiratory tract infections (URTIs) have been recognized as a nonspecific term that is used to describe acute infections in connection with the nose, paranasal sinuses, pharynx, larynx, trachea, and bronchi (Kho *et al.*, 2013). They are mild and self limiting in some cases but are life threatening in other

cases (Poole *et al.*, 2005). Causes of URTIs have been attributed mainly to some viral infections, but researches have also suggested the cause to be of bacterial origin (Poole *et al.*, 2005).

Honey is used locally in treatment of wounds. It is taken orally or in combination with some herbal liquids for treatment of mouth, throat and stomach diseases. There is indiscriminate use of honey in local treatment of upper respiratory tract infections. Children of ages 1-5 years are often given honey to treat cough and to decongest the respiratory passage (Personal communication). Use of honey in treatment of upper respiratory tract infection has not been well documented. Honey is taken as the best remedy for soothening sore throat and treatment of stomach ulcers. Honey has been rated most favorably for symptomatic relief of children's nocturnal cough and sleep difficulty due to upper respiratory tract infections. Honey may be a preferable treatment for the cough and sleep difficulty associated with childhood upper respiratory tract infection (Paul *et al*., 2007). In some resource limited areas today, honey is still being used to treat burns and wounds by covering wounds with clean gauze after each application of honey (Subrahmanyam, 1991).

The potency of honey as an antibacterial agent is credited to its strong osmotic effect, generally low pH (Kwakman and Zaat, 2012), the ability to produce hydrogen peroxide (Kacaniova *et al*., 2011) and some phytochemical factors. These factors include its content of tetracycline derivatives, peroxides, amylase, fatty acids, phenols, ascorbic acid, terpenes, benzyi alcohol and benzoic acid (Bogdanov, 1989; Molan, 1992). Antibacterial agent, hydrogen peroxide, produced by honey's glucose oxidase is broken down by catalase (Juraj *et al*., 2014). Honeys having high catalase activity has low antibacterial peroxide activity (Bogdanov *et al*., 2007, Bogdanov *et al*., 2008). Brudzynski (2006) established a good correlation between the peroxidase accumulation ability and antibacterial action expression by honeys. The high sugar concentration of honey is also considered responsible for the antibacterial activity (Mundo *et al.*, 2004). The higher the concentration of honey the longer is the period of growth inhibition. Total inhibition of growth is important for controlling infections (Molan, 1992).

Are there scientific explanations to justify the traditional usage of honey in the treatment of some bacterial upper respiratory tract (URT) infection? Do the different brands of honey used in Ado-Ekiti have similar antimicrobial properties? How do these brands of honey compare with orthodox antimicrobial agents in antimicrobial properties? This study was aimed at evaluating the antimicrobial activity of honey from different sources on *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* associated with upper respiratory tract infection, determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the different brands of honey on the test organisms and at comparing antimicrobial activity of the honey from different sources with those of antibiotics used in the treatment of upper respiratory tract infections.

Materials and Methods

Research site

The study was conducted in Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. Ado Ekiti is situated in southwest Nigeria. The city is the State Capital and Headquarters of the Ekiti State. The inhabitants are mainly of the Ekiti sub-ethnic group of the Yoruba. The city lies between latitude 7^{O} 34' and 7^{O} 44' north of the equator and longitude 5^{O} 11'

and 5^{O} 18' east of the Greenwich Meridian and 36.7 Km^2 (Olusegun, 2013). Ado Ekiti has a State owned University - Ekiti State University, Ado-Ekiti; a privately owned University - Afe Babalola University, Ado-Ekiti; and a Polytechnic - the Federal Polytechnic, Ado-Ekiti. The city is situated at elevation 439 meters above sea level and has a population of 424,340 making it the biggest in Ekiti State (Worldatlas, 2016).

Sample collection

Ten (10) samples each for *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* were investigated. These clinical isolates of bacterial species involved in upper respiratory tract infections were obtained from the bacteriology department of Ekiti State University Teaching Hospital Ekiti-State Nigeria and Federal Teaching Hospital Ido-Ekiti, Ekiti-State.

Honey from six different sources sold in Ado-Ekiti was used for this study. The brands were: Dr Bee honey from Kutunku Farm, Gwagalada, Abuja; Rowse Honey from Rowse Honey Ltd, United Kingdom; ABUAD Honey from Afe Babalola University Ado-Ekiti (ABUAD) Farm, Benectar Honey from Orita Challenge, Ibadan; Ido Honey from Ido-Osi Farm, Ido-Ekiti, Ekiti State; Jummy Honey from Ikere, Ekiti-State. The different brands of honey were purchased at supermarkets in Ado- Ekiti, and stored in the laboratory away from direct light (cupboard). These brands of honey were checked for sterility by streaking them on blood agar and incubated at 37^{O}C for 24 hours (Mohammed *et al*., 2014). None of the brands showed any growth and were thus selected for this study.

Identification of test bacteria

All the isolates were characterized using standard microbiology and biochemical tests as described by Barrow and Feltham (2004) and Cheesbrough (2006). Bacterial isolates were identified according to Barrow and Feltham (1993) and (Garrity *et al*., 2005). The various tests employed were: Gram staining reaction, catalase, coagulase, oxidase, motility, Indole, Urease and Citrate tests and sugar reactions.

Preparation of bacteria inoculums

Five (5) colonies of fresh isolates of the morphologically identical pure culture of each test organism were picked from MacConkey agar (Oxoid Ltd, UK) and suspended in 5ml of peptone water and incubated at 37^{O}C for 24 hours, to reactivate the organisms (Mohammed *et al*., 2014). The bacteria suspension obtained were compared with the 0.5 Mc Farland Standard (10^5-10^6 cfu/ml) by either diluting the bacteria suspension with sterile distilled water or incubated further for bacteria multiplication until a turbidity that matched that of 0.5Mc Farland Standard was reached (Mohammed *et al*., 2014). The resulting bacterial suspensions were further diluted 1: 100 in sterile nutrient broth (Oxoid Ltd, UK) to set inoculums density of 1 x 10^4 cfu/ml for use (Wood and Washington, 1995; Miles and Amyes, 1996)). The process was repeated in the preparation of all the selected bacteria isolates and stored at 4-8^{O}C until used same day.

Antibiotic sensitivity test of Honey brands

Antibacterial activities of the different honey brands were evaluated using agar well diffusion method of in-vitro antimicrobial sensitivity testing (Mounyr *et al*., 2016). A neat (100%) concentration (Roland *et al* 2007) and 80% concentrations of the honey brands were tested against the test organisms on Mueller-Hinton agar plate (Oxoid Ltd, UK). Sterile cotton swabs dipped in the inoculums were used to streak the entire surfaces of Mueller Hinton agar plate evenly.

The plates were allowed to dry for 5 minutes. Holes with diameter of 6 mm were punched aseptically with sterile cork borer. Fifty microlitre (50 µl) of each concentration of honey was separately used to fill the holes to the brim and incubated at 37^{O}C for 24 hours. The zones of inhibition were measured.

Determination of MIC and MBC of honey brands

Following the initial antimicrobial screening tests using honey brands at 100 and 80% concentrations, the minimum inhibitory concentration tests of each honey brand was determined using the broth tube dilution method as described by Barrow and Feltham (2004). After reading the minimum inhibitory concentration (MIC) tests, all the tubes that showed no turbidity before the MIC tube were sub-cultured on MacConkey agar (Oxoid Ltd, UK), chocolate and blood agar (Nutrient agar from Oxoid Ltd., UK) to determine the minimum bactericidal concentration (Kacaniova *et al*., 2011).

Antibiotics sensitivity test of commercially prepared discs

Agar disk-diffusion method (Mounyr *et al*., 2016) was used to test the sensitivity of the commercially prepared antibiotic discs against the test organisms. One milliliter of the previously prepared isolates was used to flood the Muller Hinton agar and commercially prepared antibiotics discs were placed on the agar and incubated overnight at 37^{O}C for 24 hours and the inhibition zones were measured.

Statistical analysis

Data generated in this study was subjected to standard statistical test using statistical package for social sciences (SPSS) software version 17 with probability value set at 0.05.

Results and Discussion

All the honey brands passed sterility test. The mean zones of inhibition of test organisms against different honey brands are represented in table 1. The widest mean zone of inhibition was recorded in Dr Bees honey (8.4±1.5 mm) while the least mean zones of inhibition were recorded in honey from ABUAD farm (2.2±2.0 mm). The zones of inhibition at 100% honey concentrations were generally wider than those recorded against 80% concentrations of honey brands. Most of the honey tested did not inhibit *Pseudomonas aeruginosa*, only 100% concentration of Dr Bees honey inhibited *Pseudomonas aeruginosa* with mean zone inhibition of 1.3±0.4 mm (Table 1).

The mean minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBC) of honeys against *Staphylococcus aureus* and *Klebsiella pneumoniae* are represented in table 2. The lowest mean MIC against *Staphylococcus aureus* was recorded in Dr Bees honey (27.5% concentration) while the highest mean MIC (31.25% concentration) were recorded in four of the six honey brands investigated. The lowest mean MIC of honey brands against *Klebsiella pneumoniae* was recorded in Dr. Bee honey (32.25%) while the highest MIC was recorded in Benenectar honey (33.75%). The lowest MBC of the honey brands against *Klebsiella pneumoniae* was recorded in Ido honey (40.0%) while the highest MBC was observed in Benenectar honey (55.0%) – Table 2. The overall mean MIC and MBC of all the honeys tested against *Staphylococcus aureus* are 29.83% and 50.92% respectively. Similarly the overall mean MIC and MBC of all honeys tested against *Klebsiella pneumoniae* were 32.33% and 47.5% respectively.

Gentamicin and cefuroxime recorded significant inhibition zone ($p < 0.001$) against

Klebsiella pneumoniae in relation to the honey brands tested (Table 3). In table 3 the inhibition of Cefetaxidime and augmentin recorded against *Klebsiella pneumoniae* are significantly higher than results from the honey brands ($p < 0.05$). Similarly in Table 4, augmentin inhibited *Staphylococcus aureus* more significantly ($p < 0.001$) in relation to the honey brands tested. Gentamicin and cefetaxidime significantly inhibited *Staphylococcus aureus* significantly ($p < 0.05$, and $p < 0.001$) in relation to the honey brands tested. However, the inhibition zone of cefuroxime is not significantly higher than those of Dr Bee honey ($p > 0.05$) but for the rest of the honey brands tested it is significantly higher ($p < 0.05$).

The different honey brands showed inhibition against *Staphylococcus aureus* and *Klebsiella pneumoniae*. This is in agreement with the findings of Subrahmanyan (1991), Molar (1992) and Anyanwu (2011) who affirm that honey has inhibitory effect on *Klebsiella pneumoniae*. Olawuyi *et al* (2010) and Mohammed *et al* (2014) are in agreement that *Klebsiella pneumonia*e is not inhibited by honey. This work equally agrees with these earlier researchers who claimed that honey is inhibitory to *Staphylococcus aureus* (Cooper *et al*., 2002b, George and Cutting, 2007). Only Dr Bee honey showed inhibition against *Pseudomonas aeruginosa*. Efem (1988) reported that *Pseudomonas aeruginosa* is not inhibited by honey. The findings of this work are in contrast with this position. Cooper *et al* (2002a) and, George and Cutting (2007) also affirm that *Pseudomonas aeruginisa* is inhibited by honey. The variation in the zone of inhibition of the different honeys implied that the constituents of honey may differ from place to place. It has been reported that antibacterial activities of honey can be traced to the nectars and pollens (Allen *et al*., 1991). The flowering plants from which nectars are obtained by bees affect the antibacterial quality of the resultant honey produced. Not all plants have antibacterial quality. This study agrees with other earlier researchers who claimed that there is variation in the antimicrobial properties of honey from different sources (Allen *et al.*, 1991, Molan, 1992).

Table.1 Mean zone of inhibition of test organisms against different honey brands at 100 and 80% concentration

Honey brands	Honey concentration (%)	Mean zone inhibition of test organisms (mm)		
		Staphylococcus aureus	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***
Benenectar	100	7.6±1.8	7.2±2.2	0.0
	80	3.8±2.8	4.0±3.0	0.0
Dr Bee	100	8.4±1.5	8.1±1.5	1.3±0.4
	80	5.5±3.1	1.5±1.3	0.0
ABUAD	100	2.3±2.0	2.7±2.5	0.0
	80	1.4±1.1	2.0±1.5	0.0
Ido	100	6.9±1.6	6.5±2.7	0.0
	80	3.7±2.3	4.4±2.2	0.0
Rowse	100	4.1±3.3	3.6±3.0	0.0
	80	2.6±2.2	2.4±2.2	0.0
Jummy	**100**	6.7±1.5	6.0±2.6	**0.0**
	80	2.6±2.3	3.7±3.4	**0.0**

Table.2 showing the Mean MIC and Mean MBC of different honey brands against Staphylococcus aureus and Klebsiella pneumonia

Test organisms	Mean MIC and MBC of different honey brands (%)					
	Benenectar	**Dr Bees**	**ABUAD**	**Ido**	**Rowse**	**Jummy**
	MIC					
Staphylococcus aureus	31.25	27.5	26.5	31.25	31.25	31.25
	MBC					
	40.5	65.0	50.0	50.0	50.0	50.0
	MIC					
***Klebsiella* pneumoniae**	33.75	31.25	32.5	32.5	32.5	31.5
	MBC					
	55.0	50.0	50.0	40.0	45.0	45.0

Note: Mean MIC of all the honey brands against *Staphylococcus aureus* = 29.83
Mean MIC of all the honey brands against *Klebsiella pneumoniae* = 32.33
Mean MBC of all the honey brands against *Staphylococcus aureus* = 50.92
Mean MBC of all the honey brands against *Klebsiella pneumoniae*. = 47.5

Table.3 Comparison of the mean zone of inhibition of Klebsiella pneumoniae by gentamicin, cefetazidime, augmentin, and cefuroxime against those of different honey brands

Antimicrobial agent/Honeys	**Mean zone of inhibition of *Klebsiella pneumoniae* (mm)**	**Student t**	**P value**
Gentamicin	18.2±7.22		
Benenectar	7.2 ±2.74	4.56	< 0.001*
Dr bees	8.1±1.48	4.29	< 0.001*
Abuad	2.7±2.54	6.36	< 0.001*
Ido	6.5±2.27	4.85	< 0.001*
Rowse	3.6±3.00	5.85	< 0.001*
Jummy	6.0±2.61	4.98	< 0.001*
Cefetazidime	16.0±9.64		
Benenectar	7.2±2.74	2.81	0.012*
Dr bees	8.1±1.48	2.56	0.020*
Abuad	2.7±2.54	4.22	0.001*
Ido	6.5±2.27	3.03	0.007*
Rowse	3.6±3.00	3.88	0.001*
Jummy	6.0±2.61	3.17	0.005*
Augmentin	19.0±14.27		
Benenectar	7.2±2.74	2.58	0.019*
Dr bees	8.1±1.48	2.40	0.027*
Abuad	2.7±2.54	3.56	0.002*
Ido	6.5±2.27	2.74	0.014*
Rowse	3.6±3.00	3.34	0.004*
Jummy	6.0±2.61	2.83	0.011*
Cefuroxime	19.8±4.02		
Benenectar	7.2±2.74	8.66	< 0.001*
Dr bees	8.1±1.48	7.83	< 0.001*
Abuad	2.7±2.54	11.37	< 0.001*
Ido	6.5±2.27	9.11	< 0.001*
Rowse	3.6±3.00	10.23	< 0.001*
Jummy	6.0±2.61	9.09	< 0.001*

Key: *P < 0.05, significant.

Table.4 Comparison of the mean zone of inhibition of Staphylococcus aureus by gentamicin, cefetazidime, augmentin, and cefuroxime against those of different honey brands

Antimicrobial agent/Honeys	Mean zone inhibition of *Staphylococcus aureus* (mm)	Student t	P value
Gentamicin	19.2±4.02		
Benenectar	7.6±1.82	2.98	< 0.001*
Dr bees	8.4±1.53	2.70	0.015*
Abuad	2.3±2.00	4.91	< 0.001*
Ido	6.9±1.55	3.26	0.004*
Rowse	4.1±3.30	4.06	< 0.001*
Jummy	6.7±1.49	3.33	0.004*
Cefetazidime	18.8±2.32		
Benenectar	7.6±1.82	4.07	< 0.001*
Dr bees	8.4±1.53	3.73	0.002*
Abuad	2.3±2.00	6.53	< 0.001*
Ido	6.9±1.55	4.45	< 0.001*
Rowse	4.1±3.30	4.95	< 0.001*
Jummy	6.7±1.49	4.55	< 0.001*
Augmentin	19.8±2.72		
Benenectar	7.6±1.82	10.82	< 0.001*
Dr bees	8.4±1.53	10.54	< 0.001*
Abuad	2.3±2.00	11.45	< 0.001*
Ido	6.9±1.55	13.45	< 0.001*
Rowse	4.1±3.30	13.93	< 0.001*
Jummy	6.7±1.49	14.41	< 0.001*
Cefuroxime	14.6±10.12		
Benenectar	7.6±1.82	2.11	0.049*
Dr bees	8.4±1.53	1.92	0.071#
Abuad	2.3±2.00	3.77	0.001*
Ido	6.9±1.55	2.38	0.029*
Rowse	4.1±3.30	3.12	0.005*
Jummy	6.7±1.49	2.44	0.025*

Key: *P < 0.05, significant; # P > 0.05, not significant

The multi-resistant nature of *Pseudomonas aeruginosa* was also demonstrated in this work. However, Dr Bee inhibited *Pseudomonas aeruginosa* though with narrow zone of inhibition. Inhibition of bacteria depends on the type of honey as all honeys do not have similar antibacterial potentials. The neat concentration of this honey taken orally may be of help in the management of URTIs caused by *Pseudomonas aeruginosa* especially when there is no sensitive antibiotic against the organism. Moreover, although *Pseudomonas aeruginosa* is a known multi-resistant bacterium, honeys that inhibited the organism are likely to have certain properties not found in other types of honey. Investigation of this property in honeys such as Dr Bee honey could lead to discovery of chemical substance that will combat the problematic bacteria.

Commercially prepared gentamicin,

cefuroxime, cefetaxidime and augmentin antibiotic discs showed significantly higher zone of inhibition in relation to the honeys tested. Among the honey brands however, Dr Bee honey had a comparable zone of inhibition in relation to the antibiotic cefuroxime. While some honeys may have moderate antibacterial properties, others may actually have antibacterial properties comparable with those of some antibiotics. The honey brands used in this study showed both bacteriostatic and bactericidal properties on both Gram negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*) bacteria. The different honey brands were mostly bacteriostatic at mean honey concentration above 31% (mean MIC) against *Klebsiella pneumoniae* while the bactericidal concentrations were above mean honey concentration of 40% against *Klebsiella pneumoniae.* Mean honey concentrations of 31 % were bacteriostatic against *Staphylococcus aureus* while honey concentrations of 50% were bactericidal against *Staphylococcus aureus*. These results were similar to those reported by Nzeako and Handi (2000) who recorded bactericidal properties of honey at 50-100% concentration. Other authors have however recorded bactericidal property of honey at less than 40% concentration (Willex *et al*, 1992).

The traditional use of honey in the treatment of URTIs especially those caused by *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* is supported by this study findings. Honey has been used to heal recalcitrant wounds infections whereby it was found to be effective *in vitro* against a wide range of multi-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and multi-resistant *Pseudomonas aeruginosa* (Cooper *et al*., 2002a,b, George and Cutting, 2007).

Combination of factors such as low permeability of its cell wall, genetic capacity to express resistant mechanisms, mutation chromosomal genes which regulate resistance genes and ability to acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages has been adjudged as being responsible for the multi-resistant nature of the *Pseudomonas* aeruginosa (Molan and Betts, 2000, Lambert, 2002).

This study concludes that honey has antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. These activities however vary from honey to honey. The traditional use of honey in the treatment of bacterial infection is justified by this study. The antimicrobial activities may vary from one environment to another. While some honeys may show strong antibacterial activity others may not inhibit bacteria. Orthodox antibiotics generally showed significantly higher inhibitory activity against the test organisms in relation to the honeys tested. All honeys may not have the same inhibitory strength against all bacteria. Honey may however be a useful remedy in the handling of multiresistant *Pseudomonas aeruginosa* but such usage may require sensitivity testing to ascertain the potency of the type of honey against the organism. Researches directed towards the discovery of substances in honey types that inhibit *Pseudomonas aeruginosa* are recommended further study.

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