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ANTIBACTERIAL EFFECTS OF HONEY FROM DIFFERENT PART OF NIGERIA ON *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Traditionally, honey is used in the application of wound to reduce the possibility of infection. The study conducted here was to analyze the antimicrobial activities of local honey against *Staphylococcusaureus* and *Pseudomonasaeruginosa*. Also attempt was made to find out whether there are correlations between the antimicrobial activity of honey and their physicochemical, proximate and trace elemental parameters. This activity is mainly due to its low pH, osmolality and hydrogen peroxide accumulation. Honey samples were diluted at different concentrations: 20%, 40%, 60%, 80% and Net honey (100% of pure honey). The result indicated that most of honey samples exhibited zone of inhibition against both organisms. The honey samples showed decreasing activities upon dilution with water. The pH of the honey samples is from 3.5-4.3, showing that they are predominantly acidic in nature. The protein content of honey has been used for a long time as an index of quality. The result from the table below shows protein concentration of the honey samples ranges from 0.256 to 3.125%. The carbohydrate concentration of the honey samples ranges from 74.33 to 82.33%. Also, that of vitamins (A and C) shows highest concentration with 0.032%, 0.232% and lowest concentration with 0.011%, 0.021% respectively. The water content of these honey samples ranges from 16.2 to 24.6%. The density of honey is another reflection of intrinsic quality of the sweetener. Therefore from the results, the density of the honey samples ranges from 1380.2kgm^{-3} to 1439.0kgm^{-3}. It shows that our samples are rich in minerals. Heavy carcinogenic metals, Lead and Cadmium were undetected in all the samples which make the samples safe for consumption.

INTRODUCTION

Honey is a sweet food made by bees using nectar from flowers. The variety produced by honey beesgenus *Apis* is the one commonly referred to. It is the type of honey collected by beekeepers and consumed by humans (Aggad and Guemour, 2014). Honey produced by other bees and insects has distinctly different properties. Honey bees transform nectar into honey by a process of regurgitation and evaporation. They store it as a primary food source in wax honeycombs inside the beehive. Honey gets its sweetness from the monosaccharide fructose and glucose and has approximately the same relative sweetness as that of granulated sugar (Oregon State University, 2012). It has attractive chemical properties for baking and a distinctive flavor that leads some people to prefer it over sugar and other sweeteners. Most microorganisms do not grow in honey because of its low water activity (a_w) of 0.6 (Aggad and Guemour, 2014). However, honey sometimes contains dormant endospores of the bacterium *Clostridium botulinum* (Prescott *et al.,* 1999), which can be dangerous to infants, as the endospores can transform into toxin-producing bacteria in infants' immature intestinal tracts, leading to illness and even death (Shapiro *et al.,* 1998). Honey exhibits antibacterial properties; the growth of bacteria is inhibited due to the low water activity, high acidity, and the hydrogen peroxide activity (PA). This activity has been reviewed extensively by Molan (Molan, 1992), and is usually measured by diffusion assays (Patton *et al.,* 2006). In this technique, sample material is placed into wells bored into inoculated agar growth media; this diffuses into the agar around the well, and produces a zone of inhibited bacterial growth. Inhibition zones are used to determine the potency of the sample tested. Alternatively, discs prepared from sterile absorbent material are soaked in the sample solution and placed on inoculated growth media. The samples diffuse into the agar around the disc and are assayed for the ability to produce a zone of inhibition also, Patton *et al.* (Patton *et al.,* 2006) developed another method, a spectrophotometric assay, with greater sensitivity to also assess antibacterial activity in honey. Hydrogen peroxide is the principal component responsible for the antibacterial action of honey, although phenolic acids, lysozyme and flavonoids also contribute to a lesser degree (Weston *et al.,* 1999). Hydrogen peroxide is synthesized through the oxidation of glucose; a reaction catalyzed by the enzyme glucose oxidase. This enzyme originates from the hypo pharyngeal glands of the bees, and as with many enzymes, it is inactivated by light and

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heat (The more intense the level of light or heat, the faster the rate of inactivation). Room temperature and/or light, given enough time, are sufficient to reduce glucose oxidase activity, meaning that antibacterial activity in honeys can vary. *Staphylococcus aureus* has been reported to induce a diverse spectrum of diseases associated with considerable morbidity and mortality. Presentations of infections with *S. aureus* range from cutaneous infections, such as impetigo, boils, infections of wounds originating from prosthetic devices, to severe life-threatening infections, such as osteomyelitis, endocarditis and bacteraemia with metastatic complications (David and Daum, 2010). Those at risk of *S.aureus* infections include those with underlying disease, newborns, trauma victims, burn patients, drug abusers and neuropenic individuals (Chambers, 2001). Also, *Pseudomonas aeruginosa*is an opportunistic pathogen that can induce infections that often result in hospitalization and are very often life-threatening. If the gravity of its infectiousness was not enough to cause alarm, the behavior of this microbe will. This microbe exhibits innate resistance to many antibiotics and can adapt when exposed to antimicrobial agents and develop new resistance. This allows *Pseudomonas aeruginosa* to thrive and further grow even when treatment is being attempted. With such survivability, this bacterium is not only carefully monitored in hospitals, but its genome is constantly being updated into a database and is globally monitored due to its potential as a biological weapon. The objective of this study is to compare the antibacterial effect of different honey samples on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Nigeria.

MATERIALS AND METHODS

Collection of Samples

Five samples of honey produced by honey bee and commercial honey were collected from different source from Southern, Eastern, Western and Northern part of Nigeria. The local honey samples were obtained by draining the honey after manually uncapping the comb frames. These honey samples were aseptically collected in sterile screwed cap bottles and kept in a cool and dry place (at room temperature) overnight before they were finally transported to the laboratory according to Chessbrough (1984).

Preparation of Test Organism

Stocked cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* used in this study were obtained from the Microbiology Laboratory Unit, University Teaching Hospital, Ado-Ekiti, Ekiti-State. The isolates were identified based on standard microbiological techniques and sub-cultured in nutrient agar slants at 37^0C for 24hrs.

Protein Analysis

The following test describes a step by step procedure for Kjeldahl analysis outlined by AOAC (1990) which can be successfully applied to a wide range of samples. A representative sample was prepared and weighed; 1g of it was weighed with an accuracy of 0.1mg into a digestion tube. Two Kjeltabs (3.5g K_2SO_4 and 3mg Selenium) was added. 12ml of concentrated H_2SO_4 was carefully added and gently shaked to "wet" the sample with the acid. Sample containing high – fat (>10%fat) or carbohydrate levels use 15ml H_2SO_4. The exhaust system was attached to the digestion tubes in the rack and the water aspirator was set to full effect. The rack was loaded with exhaust into a preheated digestion block (42^0C). The samples were contained within the exhaust head after about 5 minutes the water aspirator was turned down until the acid fumes continue to digest until all samples are clear with a blue/green solution. This was normally between 30-60 minutes depending on the sample type. The rack of tubes was removed with exhaust still in place and put in the stand to cool for 10-20 minutes. Using a commercial air blower to increase cooling. Carefully, 80ml deionized water was added to the tubes. This can be done automatically at the start of the distillation cycle. 25-30 ml of receiver solution was added to a conical flask and placed into the distillation unit and the platform rose so that the distillate outlet is submerged in the receiver solution. The digestion tube was placed in the distillation unit and the safety door closed. Dilution water was automatically added on the Kjeltec 2300. 50ml of 40% NaOH was dispensed into the tube. On all Kjeltec's a delay time can be used between the addition of alkali and the start of the steam generator. The strong reaction between added alkali and the acid in the tube was reduced. The steam valve of Kjeltec 1002 was opened and distilled for approximately 4 minutes. On the Kjeltec, 2300 the complete distillation, titration and calculation of result is performed automatically. Titration of the distillate with standardized HCI (usually 0.1000N or 0.2000 N) was done until the blue/grey end point is achieved. The volume of acid consumed in the titration was noted.

Determination of Vitamin A

Two grams of sample was weighed into a beaker.10ml of distill water was added. It was shaked carefully to form paste. 25ml of alcoholic KOH was added to the solution. It was heated in a water bath for 1 hour with frequent shaking. The mixture was cooled rapidly and 30ml of water was added. The mixture was transferred into a separating funnel. 250ml of chloroform was used for the extraction three times. 2g of anhydrous Na_2SO_4 was added to the extract to remove any trace of water. The mixture was then filtered into 100ml of volumetric flask and made up to mark with chloroform. The absorbance was read at 328nm.

Determination of Vitamin C (Ascorbic acid)

One millilitre of sample was pipetted into 500ml flask (when the vitamin C is low) 100ml flask (when the vitamin C is high). From solution in step (Shapiro *et al.,* 1998), pipette 1ml into a 50ml flask and add 0.8ml of (10ppm K_2CR_2O7) solution into it. Add 1ml of 1M H_2SO_4. After 10mins, Add 1ml of 0.25% DPC Make up with distill H_2O.

Vitamin C (Ug/100g) = Absorbance of sample x dilution Factor/ Weight of sample.

Determination of Refractive Index

The hand refractometer (Atago R5000 with MD1333-1520 series) was used in this process. A drop of sample was placed on the sample space of the refract meter and covered with the

lid. Through the eye piece it was observed to read the calibration and adjusted to read. After adjusting to a clear vision, the reading was noted.

Determination of Water Content

The water content of the honey samples were calculated from the respective refractive index values. Hence, the water content was determined by adopting the expression below from (Abu-Jdayil *et al.,* 2002)

% water = 608.277 – 395.743 nD

Where, nD = refractive index.

Determination of Density

The 50ml density bottles were used in this process. The density bottles were weighed and weight noted. The bottles was filled with samples and reweighed. The weight of only honey was obtained from where the densities were obtained AOAC (1990).

Determination of pH

The Hanna pH meter (model HI 9025C Sigma-Aldrich) was calibrated using buffer 4 and buffer 9 before taking the readings of the samples.10g of the honey sample was dissolved into 75ml carbon dioxide-free water in a 250ml beaker. It was stirred with magnetic stirrer, the pH electrodes was immersed and recorded the pH AOAC (1990).

Antimicrobial Assay

The antimicrobial activity of different samples of honey against *Staphylococcus aureus* and *Pseudomonas aeruginosa* tested using agar diffusion technique (Kirby Bauer's well diffusion method). Test materials were prepared by diluting each honey in sterilized, double distilled water at different dilution (concentration) 20%, 40%, 60%, and 80% and Net honey i.e. 100%. The plates were prepared using 20ml of sterile nutrient agar. The surface of the plates was inoculated using a 100µl of 0.5 Mafarland standardized inoculum suspension of bacteria and allowed to dry. Wells, 6.0mm in diameter, were cut from the culture media using sterile metal cylinder and then filled with the test honey. The plates were incubated at 37^0C and observed after 24 hours for clear, circular inhibition zones around the wells were measured with the use of ruler (Rubinstein *et al.,* 1986).

RESULTS AND DISCUSSION

The result of this study described the physicochemical properties, proximate analyses, trace elements and antibacterial effect of honey samples on *Staphylococcus aureus* and *Pseudomonasaeruginosa*. The protein content of honey has been used for a long time as an index of quality (Nazarian *et al.,* 2010). This trace quantity of protein is introduced into the honey during the regurgitation of the nectar in the stomach of the bees during processing in the comb. Bognadov *et al.* (2008) recommended a protein content benchmark of 1.25-2.5% for pure honey. Looking at the results above, five of our honey samples namely sample A, B, F, H and J which has the value of 1.956%, 1.563%, 1.563%, 1.956 and 1.944 respectively suggests that they are pure. These results also suggest that they are pure judging only by their protein content. These results however conform to that of Odeyemi *et al.* (2013) that gave the protein concentration of the Nigerian honeys from 1.625 to 3.375%. Likewise, Agunbiade and Banjo *et al.* (2011) gave the protein concentration of the Nigerian honeys as 1.43-2.72%. Our above results for the protein content of these samples which coincidentally were 1.956% for the two samples fall within the standard recommended by Bognadov *et al.* (2008). The results above show that samples C, D, G and I have protein content of 1.237, 1.094, 1.169 and 0.256% respectively.

The total carbohydrate content of samples did not follow any definite pattern and cannot be said on its own to be an index of quality. However Codex Alimentarius recommended that reducing sugars in honey should be more than 60%. This cannot be used as an index of quality here since all the samples passed this test. Likewise the vitamin content of samples did not follow a definite order. According to Codex Alimentarius, the moisture content of honey should not be above 21%. Almost all our samples conform to this specification with the exception of samples H and I. The value of 22.6% and 24.6% recorded for samples H and I respectively suggested that they has more water than it should have. It is good to point out that every case like this may not be that of water adulteration but the season of harvest can have impact on the water content of honey.

The density of honey is another reflection of intrinsic quality of the sweetener. The density of our honey samples were from 1380.2-1439.kg/m^3. A similar result was obtained for the Pakistani honey by Rehman *et al.* (2008). When the density profile of honey in a particular region is noted, it could be easy to tell by aid of this instrument whether honey is adulterated or not. However one limitation of this method is water content which equally influences the density of samples.

The pH of our samples ranges from 3.5 to 3.9. This similar to standard recommended by Codex Alimentarius which ranges from 3.2 to 5.5. Rehmann *et al.* (2008) also obtained similar results which is from 3 to 5 for the Pakistani honey. It can be seen that all our samples falls within the range thereby passing the test. Theresult of elemental analyses of our honey samples shows that our samples are rich in minerals. Heavy carcinogenic metals, Lead and Cadmium were undetected in all the samples which make the samples safe for consumption. The Zinc content of our samples ranges from 0.7823 (sample H from Nsukka, Enugu-state) to 7.6072 (sample G from Blessed Ekiti-state). In line with the works of Pisani *et al.* (2008), Downey *et al.* (2005), Fernandez-Torres *et al.* (2005), which gave the range to be from 1.3 to 7.8, it clearly shows that some of our samples are off the specification which suggests that they could be adulterated or stored with Zinc. In Similar, studies from Pisani *et al.* (2008), Downey *et al.,* (2005), Fernandez-Torres *et al.* (2005), the Manganese content is from 13.3 to 136. Most of our samples conform to this standard.

Table 1. Origin of the Samples and their details

S.No.	Sample codes	Sample Names	Locations	Collection Date	Appearance	Colour	Taste
1.	A	Gauraka	Niger	June, 2012	Clear	Deep golden amber	Hot sensation in tongue
2.	B	Yola	Adamawa	May, 2012	Tinny pollens observed	Deep brownish amber	Absence of hot sensation
3.	C	Zuru	Sokoto	May, 2012	Contains dirty brown patches	Light amber	Absence of hot sensation
4.	D	Keffi	Nasarawa	May, 2012	Clear	Golden amber	Absence of hot sensation
5.	E	Sunshine	Ondo	March, 2012	Clear	Dark brown	Mild hot sensation
6.	F	Real Oasis	Ekiti	March, 2013	Clear	Brown	Burning sensation
7.	G	Blessed	Ekiti	March, 2013	Visible presence of pollen	Light golden amber	Mild hot sensation
8.	H	Nsukka	Enugu	November, 2012	Wax deposits present	Dark brown	Burning sensation
9.	I	Shaki	Oyo	May, 2012	Contains white foam at the surface	Dirty brown	This sample taste bitter like dogonyaro
10.	J	Ijebu Mushin	Ogun	November, 2012	Clear	Golden amber	Burning sensation

Table 2. Proximate Analyses and Physicochemical Properties Results

S.No.	Sample codes	Sample Names	%Protein	%CHO	%Vit A	%Vit C	Refractive Index	$\%H_2O$	Density(kg m^{-3})	pH
1.	A	Gauraka	1.956	81.44	0.011	0.148	1.492	17.8	1403.8	3.9
2.	B	Yola	1.563	82.33	0.018	0.063	1.496	16.2	1410.4	3.9
3.	C	Zuru	1.237	81.21	0.014	0.106	1.491	18.2	1410.8	3.9
4.	D	Keffi	1.094	79.34	0.022	0.084	1.488	19.4	1425.4	4.3
5.	E	Sunshine	3.125	79.33	0.018	0.253	1.487	19.8	1396.4	3.9
6.	F	Real Oasis	1.563	79.78	0.022	0.106	1.488	19.4	1396.4	3.7
7.	G	Blessed	1.169	79.88	0.031	0.232	1.492	17.8	1439.0	3.6
8.	H	Nsukka	1.956	77.32	0.028	0.042	1.480	22.6	1408.7	3.7
9.	I	Shaki	0.256	74.33	0.027	0.021	1.475	24.6	1380.2	3.5
10.	J	Ijebu Mushin	1.944	81.31	0.032	0.096	1.486	18.2	1403.8	3.5

Keys : CHO- Carbohdrate, Vit A- Vitamin A, Vit C- Vitamin C, H_2O- Water content.

Table 3. Elemental analyses results (mg/kg)

S.NO.	Sample Code	Sample Name	Zn	Cu	Pb	Cd	Ca	Mg	Fe
1.	A	Gauraka	1.8418	4.6654	ND	ND	435.4856	61.4600	5.1311
2.	B	Yola	2.0925	1.9836	ND	ND	366.8750	70.6339	4.8478
3.	C	Zuru	7.5146	2.6313	ND	ND	494.3823	62.3200	5.7685
4.	D	Keffi	3.5030	4.7889	ND	ND	610.2775	64.8702	4.9591
5.	E	Sunshine	2.8497	0.7568	ND	ND	242.0281	41.5222	1.0249
6.	F	Real Oasis	4.3305	4.6349	ND	ND	543.3992	27.2263	8.2451
7.	G	Blessed	7.6072	4.1306	ND	ND	536.9218	65.0660	15.6319
8.	H	Nsukka	0.7823	1.6579	ND	ND	546.8797	50.2845	4.8849
9.	I	Shaki	0.8392	1.3506	ND	ND	273.2171	46.2378	5.5031
10.	J	Ijebu Mushin	2.2302	4.7178	ND	ND	18.0160	18.5091	3.5962

Keys: Zn- Zinc, Cu- Copper, Pb- Lead, Cd-, Cadmium, Ca- Calcium, Mg- Manganese, Fe- Iron and ND-Not Detected.

Table 4. The Antibacterial Effect of Honey on *Staphylococcus aureus*

	Honey Concentration, Diameter of clear zone Inhibition(mm)						
S.No.	Sample codes	Sample Names	20%	40%	60%	80%	Net Honey
1.	A	Gauraka	0	0	0	0	15
2.	B	Yola	0	0	0	14	20
3.	C	Zuru	0	0	12	16	17
4.	D	Keffi	0	0	0	0	15
5.	E	Sunshine	0	0	0	8	10
6.	F	Real Oasis	0	0	0	0	0
7.	G	Blessed	14	15	18	18	23
8.	H	Nsukka	0	0	0	0	12
9.	I	Shaki	0	0	0	0	0
10.	J	Ijebu Mushin	0	10	16	20	25

Table 5. The Antibacterial Effect of Honey on *Pseudomonas aeruginosa*

	Honey Concentration, Diameter of clear zone Inhibition(mm)						
S.No.	Sample codes	Sample Names	20%	40%	60%	80%	Net Honey
1.	A	Gauraka	0	0	8	9	15
2.	B	Yola	0	0	0	15	20
3.	C	Zuru	0	0	8	9	10
4.	D	Keffi	0	0	10	12	18
5.	E	Sunshine	0	0	10	15	20
6.	F	Real Oasis	0	0	0	0	15
7.	G	Blessed	0	0	0	0	10
8.	H	Nsukka	0	0	13	10	12
9.	I	Shaki	0	0	0	0	0
10.	J	Ijebu Mushin	0	9	16	18	26

Like zinc, it could be observed that manganese content increases with the purity of honey. Also the calcium content ranges from 47.7 to 341.0 and most of our samples conform to this standard. Likewise, the standard for iron content should be greater than 3.7 mg/kg. Sunshine honey (sample E) failed this test. This suggests that sunshine honey might have been adulterated. In a similar development, the standard for copper which is from 0.5 mg/kg saw all the samples passing the tests. The antimicrobial activity shows that eight honey samples (A, B, C, D, E, G, H, J) were effective against *Staphylococcus aureus*. The implication of this is that *S. aureus* infected wounds that are not treated properly with antibiotics can be treated with topical application of honey (Chute *et al.,* 2010). The honey samples showed decreasing activities upon dilution with water except for 5% and 10% water adulteration that gave high activities like pure honey itself (Rehman *et al.,* 2008). This could be attributed to hydrogen peroxide effect induced by the interaction of glucose oxidase in the presence of free water in honey (Molan, 2001). Surprisingly, 20% water adulteration showed no activity expect sample G (Blessed, Ekiti-state) meaning that the optimum concentration equilibrium for antibacterial property of honey sample was between 5% and 10%. Also worthy of note were results from Blessed honey Ado-Ekiti, which showed good zone of inhibition against *Staphylococcus aureus* at different dilutions of honey. This result is close to that of Chute *et al.* (2010).

The honey samples J from Ijebu Mushin, Ogun-State, and sample C Zuru, Sokoto-state also exhibited good antibacterial activities against *Staphylococcus aureus*. It was as well observed that honey samples from Shaki, Oyo-State and Real Oasis honey, Ado-Ekiti exhibited zero antibacterial properties against *Staphylococcus aureus*. We suspect the integrity of these samples as they might have been adulterated by their producers for maximization of profit. From the results sample J (i.e. sample from Ijebu Mushin, in Ogun-State) has the highest inhibition zone with 25mm at 100% concentration followed by sample G (i.e. sample from blessed in Ekiti-State) with inhibition zone of 23mm. At 80% concentration samples B, C, E, G, J (sample from Yola Adamawa-State, Zuru, Sokoto-State, Sunshine,Ondo-State, Blessed Ekiti-State and Ijebu Mushin, Ogun-State respectively) exhibited zone of inhibition to *S. aureus*. At 60% concentration samples C, G, J (i.e. sample from Zuru, Sokoto-state, blessed in Ekiti-State and Ijebu Mushin Ogun-State) shows clear zone to *S. aureus*with diameters of 12mm,18mm and 16mm respectively. At 40% concentration samples G (Blessed Ekiti-State) and J (Ijebu Mushin Ogun State) shows zone of inhibition to *S. aureus.* The bases of antibacterial activities of honey are quite controversial.

The demonstration of antibacterial properties of honey was first carried out by Dold *et al.* (1937) who first suggested the possibility of hydrogen peroxide as the principal factor for antibacterial activity in honey. There were also inferred that hydrogen peroxide concentration was activated by dilution of honey. If hydrogen peroxide is the only cause of antibacterial properties of honey, then it is expected that full strength honey would show no antibacterial properties which is not the case here suggesting that flavonoids (Havesteen, 1983) and Phenolic acids (Caffeic acid and ferulic acids) presence in honey could be contributing to the antibacterial potency in honey.

Table 5 is the summary of the antibacterial Effect of Honey against *Pseudomonas aeruginosa*. Nine of our honey samples exhibited good antibacterial properties against *Pseudomonas aeruginosa.* It was as well observed that honey samples from Shaki, Oyo-State exhibited zero antibacterial properties against *Pseudomonas aeruginosa*. We suspect the integrity of these samples as they might have been adulterated by their producers for maximization of profit. From the results, sample J (i.e. sample from Ijebu Mushin in Ogun-State) has the highest inhibition zone with 26mm at 100% concentration followed by sample E (i.e. sample from sunshine in Ondo-State) and sample B (i.e. sample from Yola in Adamawa state) with inhibition zone of 20mm each. It collides with the study of Malika *et al.* (2004). At 80% concentration samples A, B, C, D, E, H, J (sample from Gauraka Niger-State, Yola Adamawa-State, Zuru Sokoto-State, KeffiNasarawa state, Sunshine Ondo-State, Nsukka Enugu state and Ijebu Mushin Ogun State respectively) exhibited zone of inhibition to *Pseudomonas aeruginosa*. At 60% concentration samples A, C, D, E, H, J (i.e. sample from Gauraka Niger-State, ZuruSokoto-State, Keffi Nasarawa-State, Sunshine Ondo state, Nsukka Enugu state and Ijebu Mushin Ogun state) shows clear zone to *Pseudomonas aeruginosa* with diameters of 8mm, 8mm,10mm,10mm,13mm and 16mm respectively. It is similar with Ndip *et al.* (2007) work research. At 40% concentration only samples J (Ijebu Mushin Ogun State) shows zone of inhibition to *Pseudomonas aeruginosa.* At 20% concentration all our ten honey samples shows no zone of inhibition to *Pseudomonas aeruginosa.* Hydrogen peroxide is the major contributor to the antimicrobial activity of honey and the different concentrations of this compound in different honeys result in their varying antimicrobial effects (Tahany *et al.,* 2011).

In comparison with local honey samples and commercial honey samples in this study show that both exhibited different antibacterial activity towards the two organisms used in this study. Remember that local honey samples from this study were the honey collected directly from bee farms. In other words, they are the honey in which the sources of origin were known. The local honey samples from this study are samples D, G, H, I and J. In addition, commercial honey samples from this study were collected and brought from open markets. Those of the commercial honey samples from this study were samples A, B, C, E and F. From the Table 4 of the result, local honey samples exhibited more antibacterial activity on *S. aureus* than commercial honey samples which shows few zone of inhibition on three samples. This implies that the commercial honey samples might have been adulterated by their producers for maximization of profit. Similarly, Table 5 result from this study show that local honey exhibit more zone of inhibition on *Pseudomonas aeruginosa* compared to his counterpart (the commercial honey samples) which exhibited little result from the antibacterial activity. From this study, it shows that local honey samples have proven high antibacterial effect.

Conclusion and Recommendation

The proximate analyses of samples results corroborated well with other research works used in this study. The protein content of honey is the major index of quality. This trace

quantity of protein was introduced into the honey during the regurgitation of the nectar in the stomach of the bees during processing in the comb. The different in time of harvest of the samples negatively affected the results obtained. The total carbohydrate content of samples did not follow any definite pattern and cannot be said on its own to be an index of quality. However Codex Alimentarius (2000) recommended that reducing sugars in honey should be more than 60%. This cannot be used as an index of quality here since all the samples passed this test. Likewise the vitamin content of samples did not follow a definite order. The elemental analyses showed that the samples are rich in minerals and zinc content. It has been shown that the potency of antibacterial activity can vary among this honey samples. This study has revealed that honey samples were effective against *Staphylococcus aureus* (gram positive) and *Pseudomonas aeruginosa* (gram negative). The zone of inhibition decreased with further dilution. Most of the Net honey and its various concentrations inhibited the growth of *S. aureus* and *P. aeruginosa.* This study provided a sight on antibacterial activity of local honey of Nigeria and proved that many honey samples have the potential for the therapeutic use as antibacterial agents.

Honey is used for wound treatment all over the world. Local honey samples used for this work showed high antibacterial effect. Introducing bulking center at honey area association's level to enable traders easily access viable economic quantities at once and introduce quality assurance mark as a marketing tool to increase domestic consumption levels leading to consumers gaining confidence in local honey.

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