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Evaluation of the Physicochemical, Antimicrobial and Anti-Oxidant Activities of Overseas and Nigerian Honeys

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Abstract

Natural honey is frequently used as a food sweetener and in conventional medicine to treat a variety of human ailments and disorders. This study evaluates the physicochemical, antimicrobial and antioxidant properties of four Nigerian honey samples from North-West, North-Central, South-Eastand a 15-year old honey (obtained from South-South), alongside four honey samples from the UK; which are a blend of European Honeysso as to determine the justification for the high demand of overseas honey as against Nigerian honey. The physicochemical parameters analyzed include; moisture content, ash content, pH, sugar content, density, and viscosity. The antioxidant capacities were evaluated using 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical scavenging activity. Agar diffusion method was used to measure the antimicrobial activities of the honey samples against some selected microorganisms (Escherichia coli, Klebsiellasp, Staphylococcusaureus, Aspergilussp, Penicilliumsp, Fusariumsp, Candidasp). GC-MS was carried out to identify the compounds in the samples. The results of the physicochemical analysis showed that the characteristics of the honey samples fall within international standards. This study has demonstrated that North-West honey exhibited optimum antioxidant and antimicrobial activities with an IC_{50} of 0.5mg/ml. The MIC values of North-West honey are similar to the control streptomycin and the difference considered insignificant (P value is > 0.05). Therefore, honey produced in this region in Nigeria is good enough for export if properly packaged. Furthermore, this study illustrates that age has an effect on the moisture, antimicrobial and antioxidant activity, as well as the overall state of the honey.

Keywords: Honey, physicochemical parameters, Antimicrobial activities, Antioxidant activities

Introduction

Honey is a naturally sweet and viscous liquid made by different honey bee species (Apismellifera, Apisceranaindica, and Apismellipodae) from the nectar of blossoms or from the secretions of living sections of plants. The technique by which these bees create honey is the same around the world, but the variations in honey's physical and chemical characteristics are mostly caused by its botanical and geographic origins. Honey is formed from a variety of flora components, primarily from plants, as evidenced by the variation in taste, flavor, aroma, and color (Elbanna et al., 2014). This significant variation is also clearly visible in the content and nutritional benefits of honey.

According to Meda et al. (2004), both the wider populace and practitioners of traditional medicine are starting to acknowledge honey as a reliable and effective therapeutic agent. Its antibacterial, anti-inflammatory, and antioxidant capabilities have been endorsed for their positive effects.

Traditional and herbal medicine practitioners have utilized honey as an antimicrobial agent. The antibacterial properties of honey were originally proven by the Dutch scientist BernardusAdrianus van Ketel in 1892.J. H. Dustmann (1979) Since then, a great deal of research has demonstrated that honey possesses broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria, although effectiveness varies greatly amongst different honeys.The spread of bacteria that are resistant to antibiotics in recent decades has reignited interest in studying honey's antibacterial properties. Methylglyoxal, hydrogen peroxide, and royalisin are components of honey that are being investigated in the early stages for potential antibiotic usage. Additionally, honey is a supersaturated sugar solution, which means that there is little to no water left behind to enable the growth of germs. Sugars have a high affinity for water molecules (bacteria and yeast). As a result, the microorganisms dry out and eventually die. However, it has been discovered that hydrogen peroxide, which is formed when the enzyme glucose-oxidase oxidizes glucose when honey is diluted, is responsible for the majority of the antibacterial action (Temaru et al., 2007). (Lurlina and Fritz, 2005) Most of the time, heat or the presence of catalase can quickly destroy the peroxide activity in honey.

It has been shown that honey has a considerable antioxidant content, which is quantified as its capacity to scavenge free radicals. Honey's antioxidant capacity is defined as its ability to reduce oxidative reactions in the human body. An atom, molecule, or compound that is very unstable due to its atomic or molecular structure is known as a free radical. As they try to combine with other molecules, atoms, or even single electrons to form a stable complex, free radicals are exceedingly reactive. As a result, in many different kinds of organisms, reactive oxygen species (ROS) and free radicals lead to molecular changes and gene alterations. It is generally recognized that many diseases are brought on by oxidative stress (Küçük et al., 2007).

The antioxidants in honey are predicted to scavenge free radicals to lessen the degree of damage that could otherwise occur, even if they do not directly decrease the inflammatory process. Honey's anti-oxidant properties work by preventing the growth of free radicals, which are ions of metals like iron and copper that stimulate their creation. Also, honey's anti-inflammatory capabilities have a strong clinical foundation and don't have any negative side effects. Common components of honey, such as flavonoids and other polyphenols, may impound these metal ions in complexes, limiting the initial production of free radicals. The phenols, including

quercetin, hesperetin, and chyrsin, aswell as the Maillard products known as melanoidins, are the primary antioxidants in honey.

Additionally, studies have demonstrated that honey enhances wound healing, lessens scar size, and promotes tissue regeneration (Molan, 2001).

While much studies have focused on European and Asian honeys, lesser emphasis has been accorded Nigerian Honeys, hence this work on the Evaluation of the Physicochemical, Antimicrobial and Antioxidant activities of Overseas and Nigerian honey namely;four Nigerian honey samples from North-West, North-Central, South-Eastand a 15-year old honey (obtained from South-South), alongsidefour honey samples from the UK; which are a blend of European Honeyswas undertaken.

Methods and Materials

Collection of Samples: The three European honey samples were purchased from departmental stores and their labels bear their source. The South-East honey was obtained from a farm in Opi-Nsukka, in Enugu state, while the North-West honey from Kwoi in Kaduna state. The North-Central honey was obtained from Abuja- it is a blend of several honey, whereas the South-South15-year old honey was purchased from Ewu-Esan in Edo state Nigeria. The honey samples were coded as follows: European Honeys- EU1, EU2, EU3, EU4.

The Nigerian honeys were Named: S-E for the South-East Honey; N-W for the North-West Honey; N-C for the North-Central Honey and S-S for the 15-year old South-South Honey. In total eight honey samples were analyzed.

Equipment and reagents used

Equipment and Materials used: Analytical weighing balance, ph meter, thermometer, UV spectrophotometer, desiccator, hot air oven, water bath, whatman filter paper no. 1, sterile pipette, ostwald viscometer, 25ml pycnometer bottles, erlenmeyer flasks, volumetric flasks, glass funnel (pyrex) muffle furnace, conical flasks, beakers, crucibles, sterile cork borer, petri dishes, sterile pasteur's pipette, incubator, meter rule, universal bottles, stop watch, masking tape, cotton wool.

Reagents and chemicals used

Methanol, ethanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), distilled water, phenol crystals (5%), concentrated sulphuric acid (Conc. H_2SO_4), buffer solutions (pH 4,7,9)

Methods

Physicochemical analysis of samples: The physicochemical analyses of the eight honey samples were carried out to obtain quantitatively the following: pH, moisture content, viscosity, density, ash content, and sugar content.

Determination of Viscosity

The capillary viscometric measurements were performed using an Ostwald viscometer using the (AOAC, 1990) techniques with a little modification.

Determination of pH

Briefly, 2g of each honey samples were dissolved in 15ml of distilled water to form a solution. The solution was stirred until an even mixture was obtained. The pH meter was standardized with buffer solutions (pH 4,7 and 9). The pH meter was inserted into each of the honey sample solution and retained for a short while until the reading stabilized. The pH was measured using a pH meter (Jenway pH meter, model 3510).The reading was then recorded from the display (A.O.A.C, 1990).

Determination of Density

The pycnometry method was used to determine the density of the honey samples and their solutions. The densities of the various samples were calculated, obtained and recorded.

Determination of Ash Content

Twenty milliliter ofeach honey samples were weighed in crucible. The honey samples were charred on a Bunsen flame until samples turned black, dried and with no trace of foam. It was then ashed in a furnace at 600°C and cooled in a desiccator until a constant weight was obtained (AOAC, 2000).

$$\% \text{ Ash} = \frac{(\text{Weight of crucible + ash}) - (\text{Weight of empty crucible}) \times 100}{\text{Sample weight}}$$

Determination of Sugar Content

The sugar content of honey samples were determined by means of spectrophotometer according to AOAC (2000) but with little modifications. 0.5ml of samples were weighed in a beaker and 1ml of ethanol in 2ml of sterile distilled water was added. The mixture was shaken and 10ml of ethanol boiled to 100°C was added and agitated for even mix. 10mls of this solution was centrifuged for 10mins to obtain a clear supernatant of free sugar for the analysis. The supernatant was decanted in a volumetric flask and made up to 100ml with sterile distilled water. 1ml of solution was obtained in a test tube where 0.5ml of 5% phenol and 2.5ml concentrated sulphuric acid were added for color development. The spectrophotometer was calibrated with sterile distilled water at a wavelength of 490nm and the absorbance of each samples were observed and recorded.

Determination of Moisture Content

The moisture content of the honey samples was determined by hot oven method (A.O.A.C, 1990). The crucibles used for the study were washed, dried and their weight obtained. 10 g of each of the honey samples was obtained. Each sample in a crucible was dried in an oven at 70 °C for 2 hours and at 100 °C for the next 4 hours until the weight was constant. The samples were cooled in the desiccators and the dry weight of the sample plus crucible was taken. The percentage moisture content was calculated as:

Percentage (%) moisture content = $B - C/A \times 100$

Where A = Sample weight before drying

B = Weight of crucible + sample prior to drying

C = Weight of crucible + sample after drying

B-C = Loss in weight of sample after drying.

Antimicrobial analysis of honey samples

Organisms used: The organisms used include two Gram-negative Bacteria; Escherichia coli, Klebsiellasp, a Gram-positivebacteria; Staphylococcus aureus, and four Fungi; Aspergilussp, penicilliumsp,Fusariumsp, Candida sp. Clinical isolates of these organisms were obtained from the University of Port-Harcourt Teaching Hospital (UPTH).

Standardization of the test Microorganisms:

Clinical isolates of the test organisms; Staphylococcus aureus, Escherichia coli, Klebsiellasp, Candida sp, Aspergilussp, Penicilliumsp, Fusariumspwere collected from University of Port Harcourt Teaching hospital and standardized to $1x10^6$ cells/ml using McFarland standard.

Antimicrobial Susceptibility Test of Honey Using Agar Diffusion Method

Bauer-Kirby agar diffusion method with slight modification was used to carry out this experiment. Sterile pertri dishes were labelled in triplicates for the test organisms. A 0.1ml of each microorganism was aseptically added to the prepared Mueller Hinton Agar pour in the universal bottle and properly mixed. The mixture was then poured aseptically in to the corresponding petri dish and allowed to solidify on the workbench. After the agar had solidified on the petri dish, a sterile cork borer was used to remove a disc of agar from the agar layer in order to produce a well in each agar plate. The well was properly labeled with 100 percent stock concentrations of EU1, EU2, EU3, EU4, N-

W, N-C, S-E, and S-S honeys. Using a sterile Pasteur's pipette, 0.1ml of the stock concentrations was carefully dropped in to the well and then left on the bench for 15 minutes for proper diffusion. The plates (petri dishes) were incubated at 37°C for 24 hours. The diameter of the resulting zones of inhibition were measured in millimeter (mm) through the base of the plates using a meter rule.

Determination of Minimum Inhibitory Concentration (MIC) of Honey Extracts on Clinical Isolates.

Sterile petri dishes were labelled in triplicates for the various test organisms. A 0.1ml of each of the, microorganisms were added aseptically to the prepared Mueller Hinton Agar pour in the universal bottle and properly mixed. The mixture was aseptically poured in to the corresponding petri dish and allowed to solidify on the workbench. After the agar had solidified on the petri dish, a sterile cork borer was used to remove five (5) discs of agar from the agar layer in order to produce five (5) wells in each agar plate. The wells were labeled for the five (5) concentrations of each of the honey that showed activity during the preliminary study. The concentration included 50%, 25%, 12.5%, 6.25%, and 3.125%. Using a separate sterile Pasteur's pipette 0.1ml of each concentration of honey was carefully added to each of the wells and allowed to stand on the workbench for 15 minutes for proper diffusion of the extracts. All the plates were incubated at 37°C for 24 hours. The diameter of the resulting zones of inhibition were measured in Millimeter (mm) through the base of the plates using a meter rule.

Determination of antioxidant activities

The honey samples' capacity to scavenge DPPH radicals was assessed. In a nutshell, after dilution in distilled water, different quantities of the evaluated honey kinds were combined with a 1 ml freshly made methanolic solution of the DPPH radical (100 M). The mixture was thoroughly mixed, then the absorbance was measured at 517 nm after incubating at room temperature in the dark for 20 minutes. The percentage of radical scavenging capacity (RSC) of the tested samples was calculated according to the following equation: RSC (%) $=[(Acontrol - Asample)/Acontrol] \times 100$, where $A_{control}$ and A_{sample} are the absorbance values of the control and the tested samples, respectively, the tested sample alone in methanol was used as a blank in each experiment and the DPPH radical alone in methanol was used as the control. The reference standard utilized was ascorbic acid. The concentration that resulted in a 50% DPPH radical scavenging rate is known as the IC_{50} value.

GC-MS Analysis of Honey Samples

The identities of the compounds present in the honey samples (N-W, EU1 and EU3 honey samples) were determined using a GC-MS model Agilent HP-5ms fitted with a restek column measuring 30m in length, 0.25 mm in internal diameter, and 0.25μmthickness. As the GC was run in splitless mode with a 1 ml/min flow rate, helium gas was used as the carrier. Sample injection volume was 1 microliter, with injection temperature kept at 50°C. The programmed column temperatures were 180°C for 2 minutes, 270°C for 5 min, and the maximum temperature was 325°C.

Statistical Analysis

The means were obtained from mean calculations using three determinations. The standard deviation of the mean (SD) was also determined. Statistical significance of results was determined by one-way ANOVA and paired t-test using the Graph pad PRISM VERSION 8.

Results

Physico - chemical properties of honey samples.

Table 1: Physico-chemical properties of Nigerian and foreign honey samples.

Honey samples	pH	Moisture Content (%)	Ash content (%)	Viscosity	Density (g/ml)	Sugar content
N-C	3.61	18.70	0.10	1.238	1.0744	0.567
N-W	4.50	15.20	0.20	2.722	1.5808	0.564
S-E	3.79	14.80	0.25	2.464	1.5720	0.564
EU1	2.74	9.70	0.10	2.736	1.5948	0.566
EU2	3.56	14.10	0.30	1.671	1.5888	0.567
EU3	4.02	16.20	0.00	1.757	1.5840	0.563
EU4	4.15	12.30	0.10	2.890	1.5764	0.565
S-S	3.54	18.20	0.30	2.449	1.5056	0.567

The pH values of the honey samples from different sources investigated revealed that all samples were within the acidic range of pH of 3.54 and 4.50. Honey samples from N-W, S-E and N-C had pH values of 4.50, 3.79, and 3.61 respectively, whilst the honey samples from overseas (EU) had similar values; EU1 had a value of 4.28, EU2 honey had a value of 3.56, EU3 honey had a value of 4.02, and EU4 honey had a value of 4.15. The 15-year old S-S honey had a pH of 3.54. The pH range (2.74 - 4.50) obtained in this study was however lower than the range (4.31 - 6.0) reported for Nigerian honey from other locations (Adebiyi et al., 2004).

The moisture content of the honey samples varied between 9.70 % and 18.70 % (Table 3.1). The moisture content of Nigerian honey samples; N-C honey, N-W honey, and S-E honey are 18.70, 13.20, and 14.80 respectively. The foreign honey samples have moisture content as follows; EU1; 9.70, EU2; 14.10, EU3; 16.20, EU4; 12.30 and the 15-year old S-S honey has moisture content of 18.20. The moisture content of the samples falls within the range reported for floral honeys (Mincione and leuzzi, 1993; Anupama et al., 2003; Malika et al., 2005).

The ash content of the various honey samples ranged from 0.10 % to 0.30 %. The ash content for Nigerian honey samples in percentage; N-C honey, N-W honey, and S-E honey were 0.10, 0.10, and 0.25, respectively. The 15-year old S-S honey gave an ash content of 0.30 % whereas the foreign honey samples EU1, EU2, EU3, and EU4 yielded ash content values in percentage as follows; 0.1, 0.30, 0.00, 0.10 respectively.

The viscosity values for the Nigerian honey samples; N-C honey, N-W honey, and S-E honey were 1.238, 2.722, and 2.464. While the viscosity for foreign honey samples EU1, EU2, EU3 and EU4 were 2.736, 1.671, 1.757, and 2.890. The 15-year old S-S honey had a viscosity of 2.449.

The density of the honey samples ranged from 1.0744 to 1.5948, Nigerian honey samples; N-C honey, N-W honey, and S-E honey have densities of 1.0744, 1.5808, 1.5720 respectively, while the foreign honey samples EU1, EU2, EU3 and EU4; have densities of 1.5948, 1.5888, 1.5840 and 1.5764 respectively. The 15-year old honey has a density of 1.5056.

The honey samples have sugar content ranging from 0.563 to 0.567. The Nigerian honey samples; N-C honey, N-W honey, and S-E honey have sugar content values of 0.567, 0.564, and 0.564 respectively. The 15-year old S-S honey had a sugar content value of 0.567 while the foreign honey samples EU1, EU2, EU3, and EU4; had sugar content values of 0.566, 0.567, 0.563 and 0.565 respectively.

Antimicrobial analysis

The antimicrobial assay of Nigerian and foreign honey showing the Inhibition zone diameter in mm is presented in Table 2 and Figure 1 below.

Table 2: Results of antimicrobial assay of the Honey samples showing the IZD (mm)

Test organisms	N-C	N-W	S-E	EU1	EU2	EU3	EU4	S-S	Streptomycin (+ control)	Negative control
S. aureus	-	9.5 ± 1.4	5.5±0.7	8±1.4	1	6.5±1.0	-	-	14±2.8	-
E. coli	-	17.5 ±0.7	2±0.1	4.5±0.3	-	2.5±0.2	-	-	16±0.7	-
Candida sp.	-	-	-	-		-	-	-	17±2.2	-
Klebsiella sp.	9±1	8±1.4	12±0.6	-	4	-	-	-	10±1.4	-
Fusarium sp.	-	-	-	-	-	-	-	-	14±1.7	-
Penicillium sp.	-	-	-	-	-	-	-	-	9±1.0	-
Aspergilus sp.	-	-	-	-	-	-	-	-	22±3.3	-

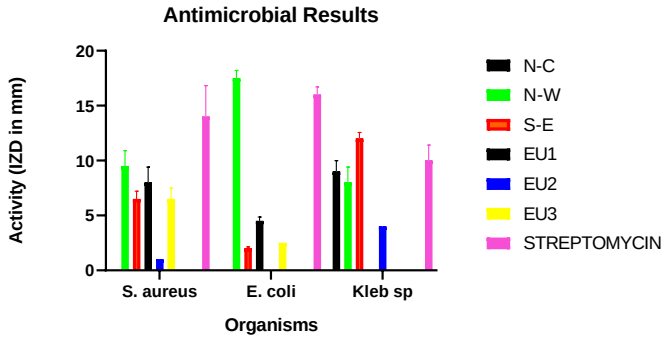

Figure 1: A chart showing the zones of inhibition of the samples and control against the test organisms.

The honey samples in this study showed different levels of antibacterial activity against various bacteria strains and noantimycotic action. EU1 honey, EU2 honey, EU3 honey, N-W honey and S-E samples showed inhibitory activity against staphylococcus aureus, with the diameter of zones of inhibition 8 mm, 1 mm, 6.5 mm, 9.5 mm and 5.5 mm respectively with N-W having the highest inhibition, while N-C honey, 15 years old S-S honey and EU4 honey showed no activity against the organism. EU1 honey, EU2 honey, S-E, and N-W honey samples all exhibited inhibitory activity against E. coli with the corresponding diameter of zones of inhibition 4.5 mm; 2.5 mm; 17.5 mm and 2 mm respectively. Similarly, EU2, N-C, N-W and S-E honey samples showed inhibitory activity against klebsiellasp with diameter of zones of inhibition 4 mm, 9 mm, 8 mm, 12 mm respectively. None of the honey samples showed activity against candida sp, Aspergilussp, penicilliumsp, Fusarium sp.

The negative control showed no antimicrobial activity, whereas the positive control did. The positive control, streptomycin had diameter of zones of inhibition for the organisms; Staphylococcus aureus, Escherichia coli, klebsiellasp, Aspergilussp , penicilliumsp,Fusariumsp, and Candida sp. as follows; 14mm, 16mm, 10mm, 22mm, 9mm, 14mm and 17mm respectively.

The results of the Inhibitory activity (MIC) of Nigerian and overseas honey against some organisms are presented in Figure 2.

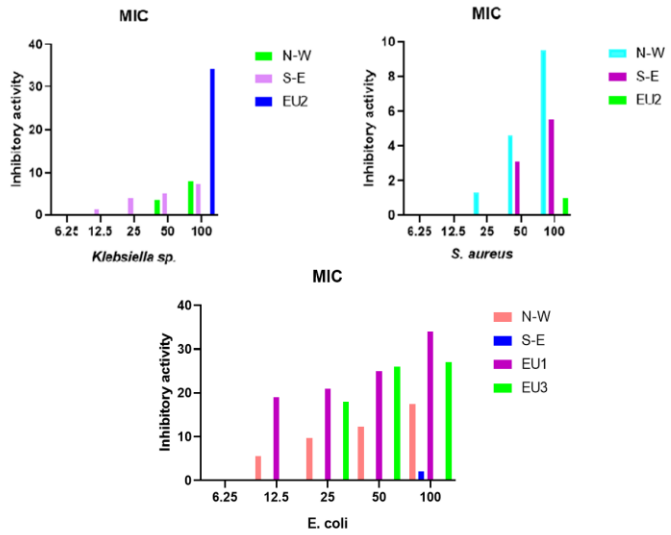

Figure 2: Showing MIC of the Honey samples

Table 3: Inhibitory activity (MIC) of positive and negative control

Organisms	Streptomycin (+ Control)	Negative Control
S. aureus	14mm	-
E. coli	16mm	-
Klebsiella sp.	10mm	-

For the Minimum Inhibitory Concentration (MIC) analysis, only N-W, EU2, and S-E honey samples showed inhibitory actions against staphylococcus aureus at several concentrations; N-W honey (25%, 50% and 100%) with the diameter of zones of inhibition as follows 1.3 mm, 4.6 mm, 9.5 mm, EU1 honey (only at 100%) with a diameter of zone of inhibition of 1 mm, S-E honey (50% and 100%) with diameter of zones of inhibition of 3.1 mm, 5.5 mm respectively. The other honey samples showed no activity against the organism. N-W honey, S-E, EU3 and EU1 honey samples exhibited inhibitory activity against E. coli ; N-W honey (12.5%, 25%, 50%, and 100%) with the following diameter of zones of inhibition 5.6 mm, 9.7 mm, 12.3 mm, and 17.5 mm respectively; S-E honey (50%, and 100%) with the following diameter of zone of inhibition 0.3 mm, and 2 mm accordingly ; EU3 honey (25%, 50%, and 100%) with diameter of zones of inhibition of 18 mm, 26 mm, and 27 mm respectively ; EU1 honey (12.5%, 25%, 50%, and 100%) with diameter of zones of inhibition as follows 19 mm, 21 mm, 25 mm, 34 mm accordingly. There was no significant difference observed between the MIC for N-W honey and that of the control Streptomyces against E. coli. EU2 honey sample showed no inhibitory action against the organism. Only N-W, EU2 and S-E honey samples showed activity against Klebsiellasp; N-W (50% and 100%) with diameter of zones of inhibition as follows 3.7 mm, and 8 mm respectively; EU2 (100%) with diameter of zone of inhibition of 4 mm; S-E (12.5%, 25%, 50% and 100%) with resulting diameter of zones of inhibition of 1.3 mm, 5.2 mm, 7.5 mm, and 12 mm respectively. The negative control still showed no activity, whereas the positive control showed activity against the organisms; Staphylococcus aureus, Escherichia coli, klebsiellasp, with diameter of zones of inhibition of 40mm, 52mm and 58mm.

Table 4: Antioxidant activity (using DPPH) of Nigerian and foreign honey.

Concentration (1%)	N-C honey	N-W honey	S-E honey	EU1 honey	EU2 honey	EU3 honey	EU4 honey	15 year-old S-S honey	Positive control
Percentage inhibition	0	47.5%	0	39.4%	0	34.8%	25.5%	0	72 %

The results of the antioxidant inhibition of the samples showed N-W honey having the highest inhibition (47.5%), followed by EU1 honey (39.4%), EU3 honey (34.8%) and then EU4 honey (25.5%). The other samples showed no inhibition, whereas the positive control ascorbic acid has an inhibition value of 72%.

Table 5: Showing the IC_{50} of honey samples and Ascorbic acid.

Samples	IC_{50} (mg/ml)
EU1	2.0
N-W	0.5
EU3	0.8
EU4	0.7
ASCORBIC ACID	10 µg/ml

Table 6: Compounds found in the GCMS of N-W, EU3 and
EU1

N-W honey	EU3 honey	EU1 honey
3-Eicosene	2,8 decadiyne	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
2-chloropropionic acid	Bicyclo [10.1.0] trideca-4,8-diene-1,3-carboxamide	.beta. –bisabolene
Octadecane		4-(1,5-Dimethylhex-4-enyl)cyclohex-2-enone
Heptadecane		
Octatria contylpenta fluoro propionate		
Carbonic acid		
Ethanol		
1-Octadecanesulphonyl chloride		
Tetracontane,		
Oxalic acid		
Carbonic acid,		
2-Piperidinone, N-[4-bromo-n-butyl]-		
Ethanol, 2-(octadecyloxy)-		
Carbonic acid,		

Discussion

The acidic pH of honey is desirable since acidification has been shown to promote healing by causing oxygen release from hemoglobin (Leveen et al., 1973). The pH of honey is low enough to prevent the growth of many species of bacteria. From the experiment, the 15-year old honey has the least pH value of 3.54, whereas, the N-W honey has the highest pH value of 4.50. All the samples showed favourable pH values. The honey sample with the highest moisture content is 18.20, while the sample with the lowest moisture content is EU1 honey; 9.70. The variations in the moisture content of honey have been attributed to the composition and floral origins of honey (Malika et al., 2005). Moisture content is practically the most important quality parameter, since it affects storage life and processing characteristics. The low moisture content of honey also forms an important part of the system which protects honey from attack by microorganisms, hence, the lower the moisture content, better the honey. The ash content of the honey samples varied between 0.00 and 0.30% and it falls within the range reported for Nigerian honey samples from other locations (Adebiyi et al., 2004) and other countries (Jeffery and Echazarreta, 1996; Malika et al., 2005). The Codex Alimentarius Commission (1969) established the essential composition and quality factors for honey as: "Mineral content (ash): not more than 0.6%. Honeydew honey and blends of honeydew and floral honey: not more than 1.0%". The floral origin of honey has been reported responsible for the variability in ash content (Vit et al., 1998). The ash content of the honey samples produced from Nigeria, the UK, and the 15-year-old honey sample did not change noticeably. Different soil and atmospheric conditions, together with the type and physiology of each plant, have been implicated in the diversity in ash content among honey samples (Kamal, et al., 2002). The viscosity values for the honey samples ranged from 1.238 to 2.890. Viscosity is simply correlated to the easiness to flow, the higher the viscosity the more difficult it is for the honey to flow. The density of the honey samples ranged from 1.0744 to 1.5948. The honey samples have sugar content ranging from 0.563 to 0.567. High sugar content of undiluted honeys prevent the growth of many species of microorganisms.

The honey samples in this study showed different levels of antibacterial activity against various bacteria strains and no antimycotic action. All the honey samples excluding Abuja honey, 15-year old honey and EU4 honey showed no activity against the organisms. EU1 honey, EU2 honey, S-E, and N-W honey samples all exhibited inhibitory activity against E. coli. Similarly, EU2, N-C, N-W and S-E honey samples showed inhibitory activity against klebsiella sp. None of the honey samples showed activity against candida sp, Aspergilussp, penicilliumsp,Fusarium sp. For the Minimum Inhibitory Concentration (MIC) analysis, only N-W, EU2, and S-E, honey samples showed inhibitory actions against staphylococcus aureus at several the other honey samples showed no activity against the organism. N-W honey, S-E, EU3 and EU1 honey samples exhibited inhibitory activity against E. coli. EU2 honey sample showed no inhibitory action. Only N-W, EU2 and S-E honey samples showed activity against Klebsiella sp.there is no significant difference between streptomycin the control and N-W honey for the test organisms, therefore, it can be said that N-W honey has the best antimicrobial activities. The non-susceptibility of some of the test organisms to the honey samples could be due to the emergence of resistant strain. In addition, several factors may influence the antimicrobial activity of honey, these factors include its physico -chemical properties, botanical origin, entomological origin and symbioses

with beneficial bacteria. For example, DeMera and Angert (2004) reported that honey from different phytogeographic regions varied in their ability to inhibit the growth of bacteria and yeasts suggesting that botanical origin plays an important role in influencing the antimicrobial activity.

The results of the antioxidant inhibition of the samples showed N-W honey having the highest inhibition (47.5%), this could be due to the presence of high levels of polyphenols and antioxidant enzymes in the sample. Other samples with the absence of inhibition could be as result of the fact that they have very little or no capacity to scavenge free radicals, physicochemical characteristics of the samples, etc.

The variety in the results obtained from the samples may be as a result of different meteorological conditions encountered during production, the type or types of plants used as the source nectar, the circumstances for processing and storing, beekeeping practices for collecting and extracting honey, local vegetation and the experimental conditions.

Conclusion

In conclusion, this study has demonstrated that the Physicochemical, Antimicrobial and Antioxidant activities of Nigerian honeys; N-W, and S-E, compare favourably with the UK honeys; EU1 honey and EU3 honey. But altogether, N-W honey has the most outstanding Antimicrobial and Antioxidant activities. The physicochemical characteristics of these honey samples fall within the international standard limits by codex Alimentarius. Therefore, honey produced in this state in Nigeria is good enough for export if properly packaged. Furthermore, this study also illustrates that age has an effect on the moisture, antimicrobial activity, antioxidant activity, and the overall state of the honey, as honey wrongly stored for a long time can lose all of its therapeutic properties evident in the results obtained from the 15-year old honey.

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