Evaluation of the Quality and Antimicrobial Activities of Honey Obtained From Different Sources in Southern Kaduna, Nigeria

Gosomji Andrew Dewan[1], Bwede Davou Danbok[2], Benson Matthew Lassa[3], Gola Shipi Sokdima[4] and Guyit Kevin Kyolbok[5]

[1] *Department of Chemistry, FCE Pankshin, Plateau State, Nigeria*
[2] *Department of Basic Sciences, Plateau State College of Health Technology Zawan, Nigeria*
[3] *Department of Biochemistry, Plateau State University Bokkos, Nigeria*
[4] *Department of Pharmacy, Plateau State College of Health Technology Zawan, Nigeria*
[5] *Department of Chemistry, University of Jos, Nigeria*

Abstract: **An evaluation of the quality and antimicrobial activities of honeys samples obtained from different sources in southern Kaduna was investigated. Parameters such as proximate analysis, antimicrobial activity, microbial inhibitory concentration (MIC) and minimum Bactericidal concentration (MBC) of the honey from three different locations were evaluated. While the fibre (1.30 – 2.30%), crude fat (1.29-2.30%), Ash content (0.25-0.30%), Nitrogen Free Extract (NFE) (77.65-79.37%), Calcium (0.01 mg/100mg) and phosphorus (0.004-0.006 mg/100mg) were the dominant mineral in the honey samples. The honey samples showed similar activity against the six (Staphylococcus aureus, Escheria coli, Streptococcus Pneumonea, Bacillus subtilis and Salmonella typhi) tested bacterial strains as shown by the different zones of inhibition. The maximum zone of bacterial inhibition of 11.0mm and 12.0mm were recorded for Bacillus subtilis for honey samples A, and B, the least zones of inhibition of 9.0mm each were observed for E.coli and Staphylococus aureus by honey samples from A and B respectively. Minimum inhibition concentration (MIC) of honey samples showed similar growth inhibition pattern. The minimum inhibition concentration was recorded at 3.125% for all the honey samples and bacteria with the exception of Pseud. aeruginosa and E. coli at 6.25% each. Minimum bactericidal concentration (MBC) of the honey samples indicated that all the microbes showed negative signs (no growth) in all the honey samples at various concentrations of the honey samples ranging between 3.125 to 100%. These results suggest that the honey samples are of good quality and the potency of these honeys against certain microorganisms suggests their potential to be used as an alternative therapeutic agent.**

***Keywords*: Honey, Antimicrobial Activity, microbes, MIC, MBC**

I. INTRODUCTION

Honey is a delicious viscous sweetener made naturally by bees out of their own nourishment from the nectar or secretion of flowering plants. It has a long history of human consumption as a natural food source and is also used as an ingredient in various food preparations (Durrani, Srivastava & Verma, 2011).

The composition and quality of honey vary, depending on the climatic region, whether wet or dry, the environmental temperature, the type of botanical plant used to produce it, the honey bees species, the treatment of honey during extraction, processing and subsequent storage conditions (Alvarez, 2011; Amri, & Ladjama, 2013). It comes in a range of colors including white, amber, red, brown, and almost black (Eleazu *et al*., 2012) depending on region and type of plant nectar (Alvarez, 2011). Its flavor and texture also vary with the flower nectar from which it is made (Alvarez, 2011).

In recent times attention has been turned to orthodox medicine. However, some people are skeptical about the use of synthetic drugs because of increase in resistance of pathogens and side effects. It is the quest for an alternative treatment for these pathogens that prompted the research work on honey. The use of honey as an alternative antimicrobial therapy could be attributed to the fact that it is easily available, cheap, non-toxic and moreover bacterial resistance to it is yet to be reported (Zainol *et al*., 2013). Although much is documented on the biological activity of honey, only scanty scientific reports could be seen on the antibacterial activity of honey from Southern Kaduna areas of Kaduna State, Nigeria. The research, therefore aimed at evaluating the composition and antimicrobial activities of the honey from different sources in Southern Kaduna.

II. METHODS

Three composite samples of honey were obtained from Kaura, Kachia, and Jama'a local areas (one from each of the three areas) of southern Kaduna in Nigeria. The sample were labeled A, B and C respectively. All honey samples were stored at ambient temperature, in plastic bottles with tight fitting lids, during the period of analytical investigation.

Determination of Nutritional Composition

Proximate Analysis carried out on the honey samples determined protein composition, dietary fiber, carbohydrate, moisture and ash. All samples were analyzed in triplicate

using standard analytical methods described by Association of official Analytical chemist (AOAC, 2009).

Determination of Mineral Compositions

The mineral compositions presented in the honey samples include: Calcium and Phosphorus. Calcium was determined using atomic absorption spectrophotometer (Model: Buck VGP 210) and Phosphorus was determined calorimetrically (Gallenkamp UK Model).

Antimicrobial Activities

Antimicrobial activities of the different honey were determined by direct Assay procedure. Nutrient agar plates were swabbed with the respective overnight culture of six clinically important microbial strains (*Staphylococcus aureus, Escheria coli, Streptococcus Pneumonea, Bacillus subtilis and Salmonella typhi*) obtained from the University of Jos Laboratory. Sterile 6mm diameter filter paper disc impregnated with honey samples, were placed on the pre-seeded agar and incubated at room temperature for 24 hours.

The antimicrobial activity was observed as increased diameter (mm) of clear zone of growth inhibition. The standard drug used is Gentamycin injection (10mg/ml). For each honey, duplicate trials were conducted against each organism.

MIC determination

The minimum inhibitory concentration of the honeys was determined using broth tube dilution method according to (Kacaniova *et al.,* 2011). Nine sterile test tubes were placed in rack, labeled each 1 through 7. Honey control tube (HC) and growth control tube (GC) were used as a quality control. One ml of freshly prepared nutrient broth was added to each tube, sterilized and cooled. Then one ml of undiluted honey solution 100 % was added to test tube number 1 and HC with a sterile micropipette and tips. Two-fold serial dilutions of the honey were prepared by adding 2ml of 100v/v of the honey into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50v/v of the extract. The process continues serially up to test tube No. 7, hence producing the following concentrations; 50(1/2), 25(1/4), 12.5(1/8), 6.25(1/16), 3.125(1/32) and 1.563(1/64)v/v. The last test tube (GC) does not contain extracts and serve as negative control. The GC tube received no honey was served as a growth control while the HC tube received no bacterial inoculums was served as a honey control. Exactly 0.5ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37 °C for 24 hours. After incubation the test tubes were observed for growth by checking for growth or turbidity.

MBC determination

The MBC was determined by incubating tubes showing no visible sign of growth or turbidity in MIC. They were sub-cultured onto sterile nutrient agar plates by streak plate method and incubated at 37 °C for 24 h aerobically. The least concentration of honey that did not show growth of test organisms was considered as the MBC (Kacaniova *et al.,* 2011).

III. RESULTS AND DISCUSSIONS

Table 1: Proximate Analysis of Honey

Weight (g) per 100grams of sample

Sample	Moisture (%)	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Ash (%)	NFE (%)	Ca (%)	P (%)
Sample A	15.43	0.83	2.30	1.82	0.25	79.37	0.01	0.004
Sample B	17.85	0.50	1.40	2.30	0.30	77.65	0.01	0.006
Sample C	16.69	1.23	1.30	1.29	0.25	79.24	0.01	0.006

Proximate composition of the different honey samples as shown in table 1 above indicates the moisture content of honey samples varied from 15.43 to 17.85%. The average moisture content of all the samples were below 21% (16.66%) which is regarded as good according to Codex Alimentarius (2001) specifications.

The moisture content of honey is one of the criteria that determine the shelf stability of honey (Ezema, 2013). Thus the higher the moisture, the higher the probability that honey will ferment upon storage. A high moisture content of honey is also an indicator of adulteration (Nyau, Mwanza & Moonga, 2013).

Very low values were recorded for the protein and fat contents of the different honey samples, which ranged from 0.50 to 1.23% and 1.29 to 2-30% respectively. The low values recorded are in agreement with that reported by (Alvarez, 2011). The range 0.50-1.23 indicates that the honey is not an adequate source of dietary protein.

The values obtained from the ash content varied from 0.25 to 0-30% with honey from kachia (B) having the highest (0.30%) while honey samples from Kaura (A) and Jama'a (C) had the least (0.25%). Ash content indicates the total inorganic minerals present in a sample after incineration (Vanhanen, Emmertz & Savage, 2011). The low ash values recorded fell within the range typical of natural nectar honeys (Nyau *et al*., 2013) and not of honey dew honeys, which have been reported to have high ash content (Viuda-Martos *et al*., 2010). The Codex Alimentarius standard specified an ash content of not more than 0.6% for normal honey.

The carbohydrate and energy content (Nitrogen Free Extract, NFE) of the honey samples were in the range of 77.65 to 79.37%. Glucose and fructose are the major component of carbohydrate found in honey and the ratio of their preponderance is a factor in determining adulteration levels and honeys suitability for diabetes management (Doner, 1997; Nombré *et al*., 2010 & Escuredo *et al*., 2014).

The mineral content of the honey samples analyzed as presented in Table 1 indicated a total mineral content of the honey samples ranging from 0.005-0.007% (Ca and P) with the sample B from Kachia and sample C (from Jama'a) having the highest in the total content, while sample A (Kaura) have the least total mineral content. The samples have an equal low percentage of calcium (0.01%) but vary in the percentage of phosphorus which range between 0.004 to 0.006, with samples B and C having an equal amount 0.006 each and sample A 0.004. The results for the amount of phosphorus with range 0.004 to 0.006 were in agreement with the works of (Agunbiade *et al*., 2012). The results show that these Nigerian honey samples are quite rich.

Mineral content is considered as a quality criterion indicating the possible botanical origin of honey (Vanhanen *et al*., 2011) the differences in mineral content majorly depend on the type of soil in which the original nectar bearing plants were located (Alvarez, 2011; Amri, & Ladjama, 2013).

Table 2: Results for Antimicrobial Activities

	Zone of inhibition (mm diameter)					
Parameter	*Staphy aureus*	*Pseudomonas* aeruginos	*Escherichia coli*	*Streptococcus pneumonia*	*Bacillus subtilis*	*Salmonella typhi*
Honey A	9.5	10	9	11	11.5	10
Honey B	9	10.5	9.5	10	12	11
Honey C	9.5	11.5	10	9.5	10	10
Gentamicin	21	18	16	16.5	19.5	20

The inhibitory activity of the different honey extracts are presented in Table 2. The results indicated that the different honey extracts exhibited a wide range and a broad- spectrum antimicrobial activity against all the tested bacteria from 9.0-12.0 mm, and this was compared well with Gentamicin. The highest inhibitory effect of the honey B was observed against *Bacillus subtilis* (12.0mm) whereas the lowest was observed against *staphy. Aureus* (9.0mm) and *E. coli* (9.0mm) for honey A and B respectively. The antimicrobial activity may involve complex mechanisms like the inhibition of the synthesis of cell wall, cell membrane, nucleic acids and protein as well as the inhibition of the metabolism of nucleic acids. The factors responsible for the antimicrobial activity of honey are high osmolarity (Snowdon & Cliver, 1996), acidity, the enzymatic formation of hydrogen peroxide, bee origin, floral source, storage conditions and possible contribution of phytochemicals (Baltrušaitytė *et al*, 2007; Alvarez-Suarez *et al.*, 2010; Al-Habsi, & Niranjan, 2012). It is possible that honeys with high antimicrobial activities could contain high quantities of Polyphenols or glucose oxidase or both as these have also been reported to possess antibacterial properties (Khalil, Sulaiman & Boukraa, 2010). Thus for optimum antibacterial activity, honey should be stored in cool, dark place and be consumed or used when fresh.

Table 3: Minimum Inhibition Concentration (MIC% v/v)

A (KAURA)

Isolates	(1)	½	¼	1/8	1/16	1/32	1/64
Staph.Aureus	-	-	-	-	-	-	+
*P.*aeruginosa	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	+	+
Strep. pneu	-	-	-	-	-	-	+
Bacillus sub.	-	-	-	-	-	-	+
Salm. typhi	-	-	-	-	-	-	+

B (KACHIA)

Isolates	(1)	½	¼	1/8	1/16	1/32	1/64
Staph.Aureus	-	-	-	-	-	-	+
P. aeruginosa	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	+	+
Strep. pneu	-	-	-	-	-	-	+
Bacillus sub.	-	-	-	-	-	-	+
Salm. typhi	-	-	-	-	-	-	+

C (JAMA'A)

Isolates	(1)	½	¼	1/8	1/16	1/32	1/64
Staph.Aureus	-	-	-	-	-	-	+
P. aeruginosa	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	+	+
Strep. Pneu	-	-	-	-	-	-	+
Bacillus sub.	-	-	-	-	-	-	+
Salm. Typhi	-	-	-	-	-	-	+

Table 4: Minimum Bactericidal Concentration (MBC% v/v) of the Honey Samples

A

Isolates	(1)	½	¼	1/8	1/16	1/32	1/64 MBC%v/v	
Staph.Aureus	-	-	-	+	+	+	+	25
Pseud aeruginosa	-	-	-	+	+	+	+	25
E.coli	-	-	+	+	+	+	+	50
Strept. pneum	-	+	+	+	+	+	+	100
Bacillus.sub	-	-	-	-	-	+	+	6.25
Salmonella typhi	-	-	-	+	+	+	+	25

B

Isolates	(1)	1/2	1/4	1/8	1/16	1/32	1/64 MBC%v/v
Staph.Aureus	-	-	+	+	+	+	+ 50
Pseud. aeruginosa	-	-	-	+	+	+	+ 25
E.coli	-	-	+	+	+	+	+ 50
Stept. pneum.	-	+	+	+	+	+	+ 100
Bacillus sub.	-	-	-	-	-	-	+ 3.125
Salmonella typhi.	-	-	-	-	+	+	+ 12.5

C

Isolates	(1)	½	1/4	1/8	1/16	1/32	1/64	MBC%v/v
Staph.Aureus	-	-	+	+	+	+	+	50
Pseud. aeruginosa	-	-	-	+	+	+	+	25
E.coli	-	-	+	+	+	+	+	50
Stept. pneum.	-	+	+	+	+	+	+	100
Bacillus sub.	-	-	-	-	-	+	+	6.25
Salmonella typhi.	-	-	-	+	+	+	+	25

Key:
+ = positive (growth)
- = No growth

Table 3 above shows results of the minimum inhibition concentration of honey samples recorded as positive or negative. The results obtained reveal the varying levels of the antibacterial activities of honey extracts against bacterial isolates studied. This signs denote the effectiveness of the honey samples A, B and C on the tested bacteria.

The Minimum Inhibitory Concentration (MIC) of all the honey extracts, A (Kaura), B (Kachia) and C (Jama'a) showed similar growth inhibition patterns at 100%, 50%, 25%, 12.5%, 6.25% and 3.125%. The minimum inhibition concentration was recorded at 3.125% for all the honey samples on bacteria with the exception of *Pseud aeruginosa* and *E. coli* at 6.25% each. These results agree with the findings of (Ahmed *et al*., 2014).

Table 4, shows results of the minimum bactericidal concentration (MBC) of the honey samples A, B, and C. All microbes showed negative signs (no growth) in all the honey samples at various concentrations of the honey samples ranging between 3.125 to 100%. This is a good indication that honey can be use for curative purpose against bacteria. The level of the antibacterial activity increased generally with increase in honey concentration. *Bacillus.subtilis* showed the most sensitive at lower MIC and MBC of the honey samples, while *streptococcus pneumoniae* showed the highest of MBC at a concentration of 100% for all the honey samples. *Pseudomonas aeruginosa* was reported to be resistant to honey by Efem (1988), however, the bacteria was sensitive to all honeys tested in this study at a moderately low concentration. This result agrees with the works of (Ahmed *et al.*, 2014; Araya and Berhe, 2016). All collected honeys showed varied bacteriostatic and bactericidal activities, and none of the isolates was resistant to tested honeys.

IV. CONCLUSION

The results of this study show the variability of some quality characteristic of honey samples from different sources. The honey samples were mostly of good quality when compared to Codex Alimentarius honey specifications. The honey samples from the southern Kaduna region of northern Nigeria has a good overall acceptability ratings, however, the difference in composition and quality of honey samples may also be influenced by factors such as geographical and botanical origin of the flora, type and activity of the bee, the extraction technique employed and storage conditions (Al-Habsi & Niranjan, 2012; Escuredo *et al*., 2014). The honeys tested from the three different areas showed varied bacteriostatic and bactericidal activities against the tested bacteria species. The potency of these honeys against certain microorganisms suggests their potential to be used as an alternative therapeutic agent in the face of antibiotic resistance. However, pharmacological standardization and clinical evaluation on the effect of honey are essential before using honey as a preventive and curative measure to common diseases related to the tested bacterial species.

V. RECOMMENDATION

With respect to this research, the following recommendations are stipulated to any researcher whose area of interest is the microbial activity and composition of honey further work should be done on the difference in composition and sensory quality of the honey samples which may also be influenced by other factors. Secondly, further research should be conducted on the role of honey in the cure of diabetes and also other products of bee hive like propolis, brood, venom, pollen and royal Jelly should be looked into.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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REFERENCES

[1] Agunbiade, O. S., Ojezele, O. M., Ojezele, J. O., & Ajayi, A. Y. (2012). Hypoglycaemic activity of Commelina africana and Ageratum conyzoides in relation to their mineral composition. *African health sciences*, *12*(2), 198-203.

[2] Ahmed M, Sahile S, Subramanian C. (2014). Evaluation of antibacterial potential of Honey against some common human pathogens in North Gondar zone of Ethiopia. Int J Pure Appl Zool; 2(4):286–295

[3] Al-Habsi, N. A., & Niranjan, K. (2012). Effect of high hydrostatic pressure on antimicrobial activity and quality of Manuka honey. *Food chemistry*, *135*(3), 1448-1454.
[4] Alvarez, L. M. (2011). Honey proteins and their interaction with polyphenols.
[5] Alvarez-Suarez, J. M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F. & Battino, M. (2010). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food and Chemical Toxicology*, *48*(8-9), 2490-2499.
[6] Amri, A., & Ladjama, A. (2013). Physicochemical characterization of some multifloral honeys from honeybees Apis mellifera collected in the Algerian northeast. *African Journal of Food Science*, *7*(7), 168-173.
[7] Association of Official Analytical Chemists. (2009). *Official methods of analysis* (Vol. 222). Washington, DC: Association of Official Analytical Chemists.
[8] Azevedo, A. M., Martins, V. C., Prazeres, D. M., Vojinovic, V., Cabral, J. M., & Fonseca, L. P. (2003). Horseradish peroxidase: a valuable tool in biotechnology. *Biotechnology annual review*, *9*(3), 1387-2656.
[9] Baltrušaitytė, V., Venskutonis, P. R., & Čeksterytė, V. (2007). Antibacterial activity of honey and beebread of different origin against S. aureus and S. epidermidis. *Food Technology and Biotechnology*, *45*(2), 201-208.
[10] Codex Alimentarius (2001). Draft revised standard for honey (at step 10 of the codex procedure). Codex Alimentarius commission, F.A.O, Rome, Alinorm, 25, 19-26.
[11] Doner, L. W. (1997). Rapid purification of commercial gellan gum to highly soluble and gellable monovalent cation salts. *Carbohydrate polymers*, *32*(3-4), 245-247.
[12] Durrani, A. M., Srivastava, P. K., & Verma, S. (2011). Development and quality evaluation of honey based carrot candy. *Journal of food science and technology*, *48*(4), 502-505.
[13] Eleazu, C. O., Oti, E., Eleazu, K. C., & Obi, V. (2012). Nutrient composition, polyphenolic contents and free radical scavenging activities of five honey samples from NRCRI, Umudike and different locations in south eastern Nigeria. *Afr J Food Sci Technol*, *3*(7), 160-167.
[14] Escuredo, O., Dobre, I., Fernández-González, M., & Seijo, M. C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food chemistry*, *149*, 84-90.
[15] Ezema, C. (2013). Probiotics in animal production: A review. *Journal of Veterinary Medicine and Animal Health*, *5*(11), 308-316.
[16] Kacaniova M, Vukovic N, Bobkova A, Fikselova M, Rovna K, Hascik P. (2011). Antimicrobial and antiradical activity of Slovakian honeydew honey samples. J Microbiol Biotechnol Food Sci; 1(3):354–360.
[17] Khalil, M. I., Sulaiman, S. A. & Boukraa, L. (2010). Antioxidant properties of honey and its role in preventing health disorder. *The Open Nutraceuticals Journal*, *3*(1).
[18] Nombré, I., Schweitzer, P., Boussim, J. I., & Rasolodimby, J. M. (2010). Impacts of storage conditions on physicochemical characteristics of honey samples from Burkina Faso. *African Journal of Food Science*, *4*(7), 458-463.
[19] Nyau, V., Mwanza, E. P., & Moonga, H. B. (2013). Physico-chemical qualities of honey harvested from different beehive types in Zambia. *African Journal of Food, Agriculture, nutrition and development*, *13*(2).
[20] Snowdon, J. A. & Cliver, D. O. (1996). Microorganisms in honey. *International journal of food microbiology*, *31*(1-3), 1-26.
[21] Vanhanen, L. P., Emmertz, A. & Savage, G. P. (2011). Mineral analysis of mono-floral New Zealand honey. *Food Chemistry*, *128*(1), 236-240.
[22] Viuda-Martos, M., Fernández-López, J. & Pérez-Álvarez, J. A. (2010). Pomegranate and its many functional components as related to human health: a review. *Comprehensive reviews in food science and food safety*, *9*(6), 635-654.
[23] Zainol, M. I, Yusoff, K. M, and Yusof, M. Y. M. (2013). Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine, 13: 129. DOI: 10.1186/1472-6882-13-129