Science World Journal Vol. 20(No 2) 2025
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University
https://dx.doi.org/10.4314/swj.v20i2.51

Full Length Research Article

PHYSICOCHEMICAL AND ANTIBACTERIAL EFFICACY OF HONEY FROM VARIOUS LOCATIONS IN NIGERIA AGAINST CLINICAL BACTERIAL SPECIES

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ABSTRACT
Honey has been recognised for its effectiveness in treating several human pathologies, possessing a broad-spectrum antimicrobial potential against various organisms, particularly bacteria. The antibacterial activity is primarily due to its low pH, high osmolality, and accumulation of hydrogen peroxide. The study was conducted to analyse the physicochemical and antibacterial activity of local honey against clinical bacterial species. Physicochemical analyses were carried out to determine the pH, water content and density of the honey samples. Honey samples were diluted at different concentrations: 20%, 40%, 60%, 80% and Net honey (100% of pure honey). The result indicated a pH of 3.5 to 4.5, which is predominantly acidic. The water content ranges from 16.4% to 24.4 %, and the density ranges from 1380.2kgm^{-3} to 1425.1kgm^{-3}.The density of honey is another reflection of the intrinsic quality of the sweetener. Honey samples used in this study exhibited a zone of inhibition on 100%, 80%, 60% and 40% dilution, but showed no zone of inhibition on 20% dilution to *E. coli* and *K. pneumoniae*. The honey samples showed decreasing activities upon dilution with water. The study confirmed that local honey has antibacterial activity against clinical bacterial species and, therefore, can be used as an alternative natural source of medicine to treat infections caused by bacteria.

Keywords: Antibacterial Efficacy, Honey, Nigeria, Clinical Bacterial spp.

INTRODUCTION

Honey, a sweet and viscous substance produced by *Apis mellifera* from floral nectar, is widely recognised for its dual role as both a food and a medicinal agent (Kambai *et al.,* 2015). It possesses notable therapeutic properties, including antioxidant activity that helps protect the body from inflammation (Romeio-Silva *et al.,* 2022), and antibacterial effects linked to DNA-degrading mechanisms mediated by hydrogen peroxide, low pH, high osmolality, and the accumulation of flavonoids and phenolic compounds (Mudenda *et al.,* 2023).

The antibacterial potency of honey is influenced by several factors such as its botanical origin, geographical source, and processing methods (Raweh *et al.,* 2023). In recent years, the evaluation of honey quality has focused on physicochemical characteristics, which include pH, sugar content, moisture, ash content, and acidity (Nair *et al.,* 2020). These parameters are standardized by international bodies such as Codex Alimentarius (2001) and adopted by national regulators like NAFDAC (2018), providing benchmarks for assessing honey purity and functionality.

Despite Nigeria's growing demand for honey—estimated at 440,000 tonnes annually—local production meets only about 10% of this need, leading to heavy reliance on imports valued at over ₦838 billion annually (NBS, 2018). Much of the locally produced honey is processed and sold without proper certification, raising concerns about its quality and safety.

Beyond its nutritional value, honey exhibits broad-spectrum antimicrobial activity, inhibiting over 60 species of bacteria, including both Gram-positive and Gram-negative strains, as well as fungi such as *Aspergillus*, *Penicillium*, and various dermatophytes (Agbabiaka *et al.,* 2020). Given these attributes, the present study investigates the physicochemical and antibacterial properties of honey samples against two clinically relevant Gram-negative bacteria: *Escherichia coli* and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Honey sample collection

Ten honey samples were collected from local markets in Gombe, Ogun, Edo, Ekiti, Delta, Oyo, Osun, Kogi, Abuja, and Ondo, Nigeria.

The samples were purchased in sterile screw-cap bottles, stored overnight at ambient room temperature (20–25°C) in a cool, dry place, and transported to the laboratory for analysis following the guidelines of Chessbrough (2010).

Water Content Determination

The water content of the honey samples was determined using their refractive index values, following the formula proposed by AbuJdayil et al. (2002):

% Water = 608.277 – 395.743 nD,

where *nD* represents the refractive index.

Determination of Density

Honey density was measured following the procedure described by AOAC (2010). A weight of 50 mL empty density bottle and honey samples were determined using a digital Metler balance. Afterward, the honey was then filled into the density bottle and reweighed. The densities of the samples were then determined.

Determination of pH

The pH of the honey samples was measured using a Hanna pH meter (model HI 9025C, Sigma Aldrich) following the AOAC (2010) method. The pH meter was calibrated with buffer solutions at pH 4 and 9. For each measurement, 10 g of honey was dissolved in 75 mL of carbon dioxide-free water in a 250 mL beaker. The solution was stirred with a magnetic stirrer, the pH electrode was immersed, and the pH was recorded after stabilization.

Bacteria Cultures Collection
Stock cultures of *E. coli* and *K. pneumoniae* were collected from the Microbiology Laboratory, at the University Teaching Hospital in Ado-Ekiti, Ekiti State Nigeria. The bacteria species were previously characterized using standard microbiological methods described by Chessbrough, (2010). Pure bacterial cultures were stored at 4 0C until used.

Antimicrobial efficacy Test
Antimicrobial efficacy of honey against *E. coli* and *K. pneumoniae* was determined using Kirby Bauer's agar well diffusion method as adopted by Guruvu *et al*., (2021). Stock solution of honey was prepared by diluting in sterile distilled water to obtain concentrations of 20, 40, 60, and 80 and 100 % and ofloxacin (5µg) as a control drug. A 100 µl 0.5 McFarland standardized bacterial inoculum suspension was streaked inoculated on a nutrient agar plate, using a sterile 6.00 mm cork borer, wells are developed and filled with the honey stock concentrations. The plates were incubated at 37 ^{o}C for 24 hours, and the diameter of zones of inhibition developed was measured with the use of a ruler.

Data Analysis
Data analysis results were expressed as means ± standard deviation and differences between means were analysed statistically using an analysis of variance (ANOVA) according to Fisher's PLSD test. Differences were considered significant when $p \leq 0.05$.

RESULTS
The sensory and physicochemical evaluation of ten natural samples from different Nigerian locations is presented in Table 1. The result showed notable variation in taste, pH, density, moisture content, and color. Mild hot sensations were reported in samples A, C, and D, while sample B had a warm, bitter taste. More intense sensations were observed in samples E (strong hot), I (deep warm), and H (hot on the tongue), indicating higher pungency. In contrast, samples F, G, and J showed no hot sensation, suggesting minimal pungent content.
The pH of the honey samples ranged from 3.4 to 4.5. Lower pH values, found in samples E, A, and D, correlated with stronger taste, while higher pH values in samples C, H, G, and F indicated milder acidity. Density ranged from 1380.2 to 1425.1 kg/m³, with sample I showing the highest and sample H the lowest, reflecting differences in solute concentration. Moisture content varied between 16.4% and 24.4%; sample H had the highest moisture level, and sample J the lowest, suggesting differences in concentration and potential shelf life.
The color of the honey ranged from light amber to dark brown. Darker samples, such as F and G, lacked hot sensations and may have undergone oxidation or aging. Lighter and golden-amber samples were more likely to show heat-related sensory responses, indicating a possible association between color and chemical activity.

Table 2 presents the antibacterial efficacy of honey from different sources against clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. The results show that efficacy varied across concentrations (20%–100%), with no inhibition observed at 20% for any sample.

Against *K. pneumoniae*, sample E showed the highest inhibition (27 mm at 100%), followed by D (22 mm), I (21 mm), A (20 mm), and J (16 mm). Samples G, F, C, and H exhibited moderate inhibition (5–16 mm) at higher concentrations. Sample B showed weak activity, with inhibition beginning only at 80% concentration (10 mm).

For *E. coli*, sample E again displayed the strongest activity (19 mm at 100%), followed by I (15 mm), D (14 mm), and A (12 mm). Moderate inhibition was recorded in F (up to 9 mm) and B (8 mm), while C, G, and H showed no inhibition at any concentration.

Table 1. Source and Physicochemical Properties of Honey from Some States in Nigeria

Sample code	Locations	Collection date	Colour	Taste	pH	Density (kg m^{-3})	% water
A	OritaObele,Ondo	February, 2024	Light Amber	Sweet	3.7±0.01	1401.4±7.47	19.2±0.35
B	OlowaborokaKogi	February, 2024	Golden Amber	Bitter	4.2±020	1390.2±3.65	19.5±0.24
C	Zuba, Abuja	March, 2024	Light Amber	Sweet	4.5±0.00	1402.6±2.98	19.5±0.67
D	Ibadan, Oyo	March, 2024	Light Amber	Sweet	3.6±0.01	1411.4±3.12	19.2±0.00
E	Inesi-ile, Osun	February, 2024	Golden Amber	Sweet	3.4±0.03	1410.5±6.56	17.8±0.18
F	YastindamGombe	March, 2024	Dark brown	Sour	4.2±0.21	1388.1±2.01	22.3±0.33
G	OlabisiOlabanjo University, Ogun	February, 2024	Dark brown	Sour	4.3±0.01	1406.8±2.19	19.8±0.03
H	Ekpoma, Edo	February, 2024	Golden Amber	Sweet	4.5±0.00	1380.2±0.00	24.4±0.23
I	ABUAD Ekiti	February, 2024	Yellow Amber	Bitter	3.5±0.04	1425.1±0.67	17.6±0.14
J	Agbor, Delta	March, 2024	Light Amber	Sour	3.5±0.03	1410.3±1.09	16.4±0.33

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DISCUSSION

The physicochemical characteristics and antibacterial activity of the honey samples against *K. pneumoniae* and *E. coli* were demonstrated by the results of this investigation. Samples of honey ranged in density from 1380.2±0.00 to 1425.1±0.67kg/m^3. When a region's honey density profile is recorded, it may be simple to determine whether or not honey has been tampered with the help of this tool. The water content of the samples, which also affects their density, is a drawback of this approach. High density of honey samples (I, D, E and J) could be due to its elevated sugar content, chemical composition of the flower nectar, or the temperature at which is stored. Additionally, the way these sugar molecules are arranged in honey contributes to its density.

The range of pH values of samples is 3.4±0.03 to 4.5±0.00. The Codex Alimentarius standard, which falls between 3.2 and 5.5, is comparable to this. Rajindran et al. (2022) also found comparable results, ranging from 3 to 4 for the Malaysian honey. Most microbes thrive at neutral pH levels, which fall between 6.5 and 7.5. A notable aspect of honey's antibacterial effectiveness is its acidity, which ranges from pH 3.2 to pH 4.5. The outcome of the result of this study is similar to a study published by Raweh et al. (2023). Low pH honey samples (E and I) are more active due to its acidity, which inhibits the growth of many bacteria.

Gluconic acid, which is present at a concentration of about 0.5% (w/v), is one of the major organic acids that cause the acidity of honey creating an unfavorable environment for most microorganisms (Bucekova *et al.,* 2023). Glycogenic acid is a very strong antibacterial agent that is produced when an endogenous glucose oxidase enzyme oxidises glucose. It is evident that every sample has a good antibacterial effect because it falls within the CODEX spectrum. The low pH of undiluted pure honey may be part of its antibacterial strength, but when diluted in other body fluids, pH is insufficient to stop the growth of many bacteria (Brudzynski, 2020).

A significant component of all living things, water can be found in a variety of foods as either free or bound molecules. A concentration of 0.56 to 0.62 is low enough to support the growth of bacteria or other microbes, which is in the range of water activity in honey, which measures the unbound water molecules (Almasaudi, 2021). The water content in this study ranges from 16.4±0.33% to 24.4±0.23%. With a water content of 24.4±0.23% and 22.3±0.33%, respectively, two samples from Yastindam Gombe and Ekpoma Edo surpassed the 20% threshold permitted by European Honey Commission rules (The Council of European Union, 2002).Similarly, the water content was within the ranges found in Malaysian honey (12.7% and 19.5%) (Rajindran *et al.,* 2022) and Moroccan honeys (except one, which was between 14.3% and 20.2%) (Amina *et al.,* 2016).The high moisture honeys (H and F) have reduced activity due to increased water availability, which promotes more favorable environment for microbial growth and extends shelf life. The excessive water content in some of the samples could be a result of the wet climatic conditions, harvest of unripe honey or adulteration of the sample with sugar-based materials (Chute *et al.,* 2010).

On test isolates, it was found that certain honey samples exhibited no activity at all, while others displayed varying levels of activity. Several samples (B, C, F, H, and J) only showed inhibition at very high concentrations (80-100%) and had no activity at 20-60%, which have weak efficacy against *K. pneumoniae*. The finding of this study suggest potential for topical application, pending further in vivo studies. Additionally, a study by Combarros-Fuertes et al. (2020) supported this finding by reporting that honey samples decreased when diluted with water, except for 100% net honey itself. Hydrogen peroxide generation by glucose oxidase in diluted honey contributes to its antibacterial activity (Nair *et al.,* 2020).Surprisingly, none of the samples exhibited any antibacterial action when 20 % water was adulterated. Inesi-ile Osun's results show a good zone of inhibition against *K. pneumoniae* when honey was diluted. This outcome concurred with that of Ayub et al. (2020).

Table 2 Efficacy of Honey from Different Sources against Clinical Isolates of *Klebsiella pneumoniae* and *Escherichia coli*

Code	Concentration										(5µg/disc)
	Klebsiella pneumonia					*Escherichia coli*					
	20 %	40 %	60 %	80 %	100 %	20 %	40 %	60 %	80 %	100 %	
A	0±0.00	2±0.11	9±0.58	14±0.17	20±1.15	0±	2±0.29	6±1.00	10±1.00	12±0.15	15±0.06
B	0±0.00	0±0.00	0±0.00	10±0.20	11±0.10	0±00	0±0.00	0±0.00	0±0.00	8±0.10	13±0.30
C	0±0.00	0±0.00	0±0.00	6±0.20	13±0.10	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	6±0.20
D	0±0.00	9±0.00	12±0.00	16±0.00	22±0.00	0±0.00	4±0.50	6±0.00	10±0.30	14±0.00	12±0.00
E	0±0.00	11±0.00	14±0.40	17±0.00	27±0.30	0±0.00	9±0.00	12±0.22	15±0.00	19±0.01	15±0.00
F	0±0.00	0±0.00	0±0.00	8±0.01	10±0.03	0±0.00	0±0.00	0±0.00	5±0.10	9±0.01	8±0.00
G	0±0.00	0±0.00	0±0.00	12±0.03	14±0.20	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	11±0.00
H	0±0.00	0±0.00	0±0.00	5±0.10	9±0.04	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	6±0.01
I	0±0.00	5±0.00	13±0.12	15±0.01	21±0.22	0±0.00	7±0.00	11±0.02	13±0.10	15±0.20	14±0.00
J	0±0.00	0±0.00	0±0.00	12±0.50	16±0.01	0±0.00	0±0.00	0±0.00	0±0.00	9±0.00	10±0.03

Table 3. Correlation Matrix between Physicochemical Properties and Efficacy of Honey Against *Klebsiella pneumoniae* and *Escherichia coli* at 100% Concentration

Property	pH	Density	% Water	*K. pneumoniae* (100%)	*E. coli* (100%)
pH	1.00	-0.73	0.75	**-0.86**	**-0.90**
Density	-0.73	1.00	-0.84	**0.76**	0.53
% Water	0.75	-0.84	1.00	-0.67	-0.55
K.pneumoniae	-0.86	0.76	-0.67	1.00	**0.82**
E. coli	-0.90	0.53	-0.55	0.82	1.00

Key Observations
pH has a strong negative correlation with antibacterial activity, especially against *E. coli* (r = -0.90) and *K. pneumoniae* (r = -0.86). Lower pH (more acidic) tends to enhance antibacterial activity. Density shows a moderate to strong positive correlation with antibacterial activity, especially against *K. pneumoniae* (r = 0.76). Water content (%H_2O) has a negative correlation with both antibacterial activities, indicating that lower water content may contribute to higher antibacterial effectiveness.

The finding of this study show that efficacy varied across concentrations (20%–100%), with no inhibition observed at 20% for any sample. Differences in activity may result from variations in floral source, geographic origin, or possible adulteration, which warrants further investigation. At 100% concentration, all samples exhibited antibacterial activity, with a sample from Inesi-ile in Osun State having the highest inhibition zone with 27mm average and a sample from Ekpoma, Edo State giving the lowest inhibition zone of average 9mm. There are moderate to strong activity at 80% concentrations, for samples that exhibited a zone of inhibition against *K. pneumoniae*. At 60% and 40% concentration, sample E from Inesi-ile Osun State, sample I ABUAD Ekiti, sample D Ibadan Oyo State and sample A Orita-Obele, Ondo state showed clear zone to *K. pneumoniae* with diameters of (average of 14mm, 13mm, 12mm and 9mm) and (average of 11mm, 9mm, 5mm and 2 mm) respectively.
The summary of the antibacterial Effect of Honey against *E. coli*. Seven samples of honey used in this study exhibited good antibacterial properties against *E. coli*. It was observed that honey samples from Zuba, Abuja; Olabisi Onabanjo University, Ogun State; and Ekpoma, Edo State, exhibited zero antibacterial properties against *E. coli*. The lack of activity in these samples could be a result of variations in floral source, geographic origin, or possible adulteration, which warrants further investigation. From the results, a sample from Inesi-ile, Osun State has the highest inhibition zone with 19mm at 100% concentration, followed by a sample from ABUAD, Ekiti State with the zone of inhibition of 15mm. Inhibition zones of the samples taken from Ibadan, Oyo State, Orita Obele, Ondo State, Yatindam Gombe, Agbor Delta, and Olowaboroka Kogi State were 14 mm, 12 mm, 9 mm, and 8 mm, average respectively.
According to a study, honey had an inhibitory activity of >9.4+0.5mm against all of the pathogens under study, in contrast to control manufactured honey's <8mm (Obey *et al.,* 2022). Inesi-ile, Osun State, ABUAD Ekiti State, Ibadan, Oyo State, Orita-Obele, Ondo State, and Yastindam, Gombe State samples showed a zone of inhibition against *E. coli* at 80% concentration. Clear zones of inhibition to *E. coli* were seen in samples from Inesi-ile, Osun State, ABUAD, Ekiti State, Ibadan, Oyo State, and Orita-Obele, Ondo State. Samples from Inesi-ile, Osun State, ABUAD, Ekiti State, Ibadan, Oyo State, and Orita-Obele, Ondo State demonstrated distinct zones of inhibition against *E. coli* at 60% and 40% concentrations. Additionally, another study showed that several honey components greatly reduced *E. coli* growth (Kassym *et al.,* 2024). All 10 honey samples exhibited no zone of inhibition against *E. coli* at a 20% dosage. The primary component of honey's antibacterial activity is hydrogen peroxide, and the antimicrobial properties of various honeys vary depending on the proportion of this chemical (Almasaudi, 2021).
Samples from Inesi-ile, Osun State, ABUAD, Ekiti State, Ibadan, Oyo State, and Orita-Obele, Ondo State demonstrated distinct zones of inhibition against *E. coli* at 60% and 40% concentrations. Additionally, another study showed that several honey components greatly reduced *E. coli* growth (Kassym *et al.,* 2024). Honey samples exhibited no zone of inhibition against *E coli* at a 20% dosage. The primary component of honey's antibacterial activity is hydrogen peroxide, and the antimicrobial properties of various honeys vary depending on the proportion of this chemical (Almasaudi, 2021).*Escherichia coli*was not inhibited at all by some honey samples (C, G, and H) due to outer membrane impermeability and efflux mechanisms. Honey is a promising natural antibacterial agent due to its unique combination of physicochemical properties and its capacity to inhibit bacterial proliferation (Romario-Silva *et al.,* 2022).
The correlation matrix revealed that the pH of the honey samples showed a strong negative correlation with antibacterial activity against both *Klebsiella pneumoniae* (r = –0.86) and *Escherichia coli* (r = –0.90), indicating that lower pH (more acidic honey) enhances antibacterial effects. Honey density exhibited a moderate to strong positive correlation with antibacterial activity, particularly against *K. pneumoniae* (r = 0.76), suggesting that denser honeys—likely richer in sugars and bioactive compounds—are more effective. Conversely, water content (%H_2O) negatively correlated with antibacterial activity (r = –0.67 for *K. pneumoniae,* r = –0.55 for E. coli), implying that lower moisture content contributes to greater inhibition. Additionally, the antibacterial effects against *K.*

https://dx.doi.org/10.4314/swj.v20i2.51

pneumoniae and *E. coli* were strongly correlated (r = 0.82), suggesting that honeys effective against one pathogen tend to be similarly effective against the other. Overall, honeys with low pH, high density, and low water content demonstrated superior antibacterial activity.

Conclusion

The honey samples analysed in the study demonstrated considerable antibacterial activity attributed to their physicochemical qualities, positioning them as a viable natural alternative for treating specific infections. However, further studies of clinical evaluation on honey to determine the therapeutic measures for bacterial infections are essential. Additionally, specific honey types and their antibacterial mechanisms can help optimise their use in wound care and other applications..

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