Hosted by

Antimicrobial, Physicochemical, Proximate and Heavy Metal Profiles of Selected Honey Samples from two Southwestern States in Nigeria

Titilayo O. Adesetan[a*], Olusegun A. Lawal[b], Funmilayo M. Oyeyipo[a], Omolara D. Popoola[a], Odunayo L. Orisasami[a] and Beatrice O. Oyekan[a]

a Department of Microbiology, Olabisi Onabanjo University, Ago – Iwoye, Nigeria.
b Department of Zoology and Environment Biology, Olabisi Onabanjo University, Ago – Iwoye, Nigeria.
***Corresponding author, e-mail: omolara.popoola@oouagoiwoye.edu.ng**
Co-authors' e-mail: titilayo.adesetan@oouagoiwoye.edu.ng, lawal.olusegun@oouagoiwoye.edu.ng, funmilayo.oyeyipo@oouagoiwoye.edu.ng, omolara.popoola@oouagoiwoye.edu.ng, oriodun1@gmail.com, oluadebeatrice@gmail.com

DOI 10.53974/unza.jabs.7.3.1215

ABSTRACT

Honey consists of a variety of sugars, minerals, vitamins, enzymes, proteins, phenolic mixtures, and organic components, which define if it is genuine or contaminated. As a result, this work was undertaken to examine the nutritional and heavy metal properties of honeys sold in two Nigerian states in the South west. Samples of honey were taken from twenty hives in two southwestern states and tested for antimicrobial, physicochemical, proximate, and heavy metal characteristics using standard recommended procedures, while heavy metals were detected with an Atomic Absorption Spectrophotometer. Zones of inhibition at 100% w/v for the test organisms are 10 – 28 mm for *E. coli*, 13 – 26 mm for *Salmonella*, 17 – 27 mm for *Staphylococcus aureus*, and 11 – 25 mm for *Klebsiella* with *S. aureus* being more sensitive to the honey samples. The range of pH for physicochemical is 2.51 – 3.87, Total Titratable Acidity 0.07 – 0.53 %, Acidity 1.20 – 6.77%, and the ranges for proximate analyses are 1.09 – 1.59% protein, 7.10 – 11.37% moisture, 0.33 – 0.63% Ash, 0.20 – 0.30% fat, 83.71 – 91.11% carbohydrate, and 342.95 – 371.20 kcal/g energy. There was a significant correlation recorded between protein, moisture, ash, fat, carbohydrate, and energy contents of the honey samples. All the honey samples were negative for lead, chromium, and cadmium. Copper was the only metal found, having concentration ranges of 5.0 to 25.0 mg/kg. Because the majority of the samples fulfilled the Codex Alimentarius Standard for honey, they can be taken or used therapeutically.

Keywords: *Antibacterial activities, Heavy metal, Honey, Physicochemical analysis, Proximate composition*

INTRODUCTION

Honey is a natural liquid sweetener whose composition changes based on geographic and environmental location, weather circumstances, plant species, and collecting and storage methods (Laleh *et al*., 2013). Honey has contributed chiefly to human well-being and living through food and medicinal compositions. Honey is often used as a traditional cure for a variety of illnesses as well as to help develop and maintain bodily structure (Adeniyi *et al*., 2014; Rao *et al*., 2016). Antioxidant, anti-inflammatory, antifungal, antimicrobial, and antibacterial properties are also present (Datti *et al*., 2020). However, the value and quantity of the honey can be impacted by the bee's species, activity, and the geographical and phytochemical of the flora (Ndife *et al*., 2014a). Trace elements such as Sodium (Na), Aluminium (Al), Potasssium (K), Magnesium (Mg), Manganese (Mn), Iron (Fe) Copper (Cu), and Calcium (Ca) were detected in honey in the studies on the investigation carried out on the proximate and mineral contents study of honey from various nations (Bogdanov *et al*., 2007; Cantarelli *et al*., 2008; Kambai *et al*., 2015). These are useful as a natural dietary supplement for people since they exist in some natural combinations (Oyeyemi *et al*., 2015). Despite the nutritional worth and composition of honey bees, there are few comparative data available in Nigeria about the nutritional elements and biochemical makeup of the many different types

of honey (Adeniyi *et al*., 2014). The kind and quantity of ingredients in honey might be used to determine where the honey comes from. Overuse of pesticides and harms on the environment by agriculturalists can both lead to the pollution of the different ecologies. When bees feed on the nectar of toxic plants, the honey produced will contain the pesticide residue, affecting its quality (Barganska *et al*., 2014). According to reports, honey bee workers travel 10 times on average each day to forage an area approximately 7 km2 away from their hives, which might expose them to various poisons (Rissato *et al*., 2007). Pesticides and heavy metals contamination from agricultural sources is a difficult issue that needs immediate response. Food contamination is as a result of the large-scale use of agrochemicals like pesticides to check disease and pests. In the majority of African nations, those who live in rural areas in extreme poverty have no choice but to use honey as sustainable measures to improve their health. As a result, this research examined the antimicrobial, nutritional and heavy metal properties of honey sold in two southwestern Nigerian states.

MATERIALS AND METHODS

Collection of Samples

The honey samples from the selected hives in Ogun and Oyo States were aseptically collected in sterile universal bottles and promptly transferred to the Microbiology Laboratory for immediate analysis.

Dilution of Honey Samples to Different Concentration

For 25% honey concentration – 25 ml of honey + 100 ml of sterile water, for 50% honey concentration – 50% of honey + 100 ml of sterile water, for 75 % of honey concentration -75 ml of honey + 100 ml of sterile water while for 100 per cent honey concentration - the honey concentrate itself.

Preparation of Inoculum

Using sterile inoculating loop, a single colony from the stock bottle was picked and inoculated directly onto the prepared Nutrient agar and incubated overnight at 37 ºC. Each of the test organism was prepared for antimicrobial assay by inoculating each into 3 ml saline water in test tube and then standardised with McFarland solution.

Antimicrobial Assay

The honey samples were determined for antibacterial activity *in vitro* against selected pathogens, viz: *Staphylococcus aureus, Escherichia coli, Klebsiella* sp., and *Salmonella* sp. using agar well diffusion method as described by Valgas *et al*. (2007).

Physicochemical Analysis of the Honey Samples

pH was determined by AOAC (2005) procedure, acidity as described by Jacobs (1999), while total titratable acidity was determined by titrating 25 mL of diluted honey sample against 0.1 N NaOH using the indicator phenolphthalein. The comparative quantity of lactic acid was calculated as follows:

$$\text{lactic acid (\%)} = \frac{\text{Titre value x Normality x 9}}{\text{volume of sample.}}$$

Proximate Analysis of Honey Samples

Crude protein, ash, moisture, fat, crude protein and dietary fiber were determined according to AOAC (2005). Carbohydrate was calculated using equation 1 while Energy was determined by calculation based on the contents of carbohydrate, protein and fat multiplied by 4,4 and 9 respectively and the result added together as shown in the equation below (Charrondiere *et al*., 2004)

Total carbohydrate (g/100g) = 100 – (water + protein + ash + fat + dietary fibre) 1

Energy (kcal/g) = (fat x 9) + (protein x 4) + (carbohydrate x 4) 2

Determination of Heavy Metals

0.2 ml of the honey sample was placed into a Kjeldahl digestion tube, 6ml of Aqua Regia reagent was added and allowed to stay overnight (Aqua Regia is a mixture of concentrated nitric acid and concentrated hydrochloric acid in a ratio 1:2). On the second day it was digested in a digestor for 20 minutes. It was then taken out of the digestor and washed into a volumetric flask of 100 ml capacity, it was completed up to 100 ml with distilled water. The heavy metals [Lead (Pb), Zinc (Zn), Chromium (Cr) and Cadmium (Cd)] were determined by aspirating directly from the 100 ml volumetric flask into the Atomic Absorption Spectrophotometer (AAS).

RESULTS

Antimicrobial Assay

The antimicrobial properties (zones of inhibition) of the various honey samples at different concentrations against four pathogenic bacterial species are shown in Tables 1a and 1b. All test organisms were susceptible to the honey samples at 50, 75 and 100% w/v concentration. Results also showed that antimicrobial activities of all the honey samples increased significantly ($p = 0.01$) against the organism with increase in the concentrations used. Hence, antimicrobial activities were highest at 100 per cent w/v concentration of the honey samples. At *100%* w/v concentration, zone of inhibition against *E. coli* was significantly high in the honey sample F

(28.0 ± 0.0 mm), followed by sample E which exhibited a bactericidal effect on *S. aureus* with an inhibition zone of 27.0 ± 0.7 mm at the same concentration.

Also, zones of inhibition recorded at 100% w/v concentration against *Klebsiella* was highest with honey sample F (25.0 ± 0.0 mm), this was however not significantly different from those of honey samples E, H, K and N at the same concentration.

Physicochemical analysis of honey samples

The highest pH, TTA, Acidity and TSS were recorded in samples D, V, V and Q with values 3.87 ± 0.01, 0.52 ± 0.02, 6.77 ± 0.09, and 10.77 ± 0.03 respectively. At $p < 0.05$, there is no substantial difference in the pH of samples A, G, H, I, J, K, M, O, P, the TTA of Z, S, P, M, O, P, and the Acidity of A, H, N, O (table 2).

Proximate composition of honey samples

For proximate composition, samples V, O, V, V, L and I have the highest values of 1.59 % ± 0.04, 14.17% ± 1.30, 0.63% ± 0.02, 0.30% ± 0.01, 91.11% ± 0.3 and 371.20 kcal/g for protein, moisture content, ash, fat, carbohydrate and energy respectively. No dietary fiber was found in all the samples. At $p < 0.05$, there is no significant difference in the ash and protein contents of all the honey samples (table 3).

Relationship between honey nutritional parameters

There was significant correlation (Pearson correlation) recorded between protein, moisture, carbohydrate, energy, ash and fat contents of the honey samples (Table 4). However, the correlations between carbohydrate content, protein, moisture, ash, fat and carbohydrate contents were negative. Similarly, the correlations between energy content, protein, moisture, carbohydrate, ash and fat, contents were negative. On the other hand, significantly positive correlation exists between acidity and total titratable acidity ($r = 0.986$, $p = 0.001$). Also, the correlation between protein, total titratable acidity and acidity and those recorded between fat content, total titratable acidity, and acidity were significantly positive.

Heavy Metals in Honey Samples

Zinc was the only metal discovered in all the twenty samples (Fig 1), however, cadmium, lead and chromium were not noticed in any of the samples. Honey sample from Iperu (U) had the highest zinc concentration, while samples from Apata (E), Provost (F), Imosan 2 (L), and Odo Epo (S) had the least zinc concentration.

Table 1a: Zones of inhibition in mm of different honey samples against *E. coli* and *Salmonella* sp.

		E. coli					*Salmonella* sp.				
Sample code	Location	**25**	**50**	**75**	**100**	**p - value**	**25**	**50**	**75**	**100**	**p - value**
		(%w/v)					**(%w/v)**				
A	Ibadan	0.0±0.0^{c}	8.0±0.7^{c}	10.0±0.0^{d}	10.0±1.4^{f}	0.01*	0.0±0.0^{c}	11.0±0.0^{c}	13.0±0.0^{c}	15.0±0.7^{d}	0.01*
D	Ayetoro	10.0±1.4^{b}	10.0±0.0^{c}	15.0±0.7^{c}	15.0±0.7^{e}	0.01*	0.0±0.0^{c}	12.0±0.7^{c}	15.0±0.7^{c}	17.0±0.7^{d}	0.01*
E	Apata Ibadan	11.0±0.0^{b}	15.0±1.4^{b}	19.0±0.0^{b}	20.0±1.4^{c}	0.01*	12.0±0.7^{a}	15.0±0.0^{b}	18.0±1.4^{b}	23.0±0.7^{b}	0.01*
F	Provost	14.0±0.7^{a}	14.0±0.7^{b}	20.0±0.0^{a}	28.0±0.0^{a}	0.01*	13.0±0.7^{a}	16.0±1.4^{b}	21.0±0.0^{a}	26.0±0.7^{a}	0.01*
G	Ijanran	13.0±0.0^{a}	15.0±0.0^{b}	18.0±0.7^{b}	22.0±0.0^{b}	0.01*	10.0±1.4^{b}	13.0±1.4^{c}	17.0±0.0^{b}	22.0±0.0^{b}	0.01*
H	Imodi	0.0±0.0^{c}	12.0±1.4^{c}	15.0±1.4^{c}	17.0±1.4^{d}	0.01*	11.0±0.7^{a}	10.0±0.0^{c}	15.0±0.0^{c}	18.0±0.7^{c}	0.01*
I	Ikangba Farm	0.0±0.0^{c}	11.0±0.7^{c}	16.0±0.0^{c}	18.0±1.4^{d}	0.01*	8.0±0.0^{b}	12.0±1.4^{c}	13.0±0.7^{c}	15.0±0.0^{d}	0.01*
J	Ijebu Igbo 1	0.0±0.0^{c}	10.0±0.0^{c}	11.0±0.7^{d}	12.0±1.4^{e}	0.01*	0.0±0.0^{c}	12.0±1.4^{c}	13.0±1.4^{c}	16.0±0.0^{d}	0.01*
K	Ijebu Igbo 2	11.0±1.4^{b}	13.0±0.0^{b}	17.0±1.4^{b}	24.0±0.0^{b}	0.01*	10.0±1.4^{b}	16.0±0.7^{b}	19.0±1.4^{b}	23.0±0.7^{b}	0.01*
L	Imosan 2	13.0±0.7^{a}	18.0±0.7^{a}	19.0±0.0^{a}	20.0±0.7^{c}	0.01*	11.0±0.7^{a}	19.0±0.0^{a}	21.0±1.4^{a}	24.0±0.0^{b}	0.01*
M	Imodi 2	0.0±0.0^{c}	10.8±0.4^{c}	14.0±0.7^{c}	14.0±0.0^{e}	0.01*	10.0±0.7^{b}	12.0±1.4^{c}	14.0±0.0^{c}	15.0±0.7^{d}	0.01*
N	Idofo Farm	8.0±0.0^{b}	15.0±0.0^{b}	17.5±0.7^{b}	18.0±0.7^{d}	0.01*	10.0±0.0^{b}	13.0±0.7^{c}	17.0±1.4^{b}	19.0±0.0^{c}	0.01*
O	Ago-Iwoye	9.0±0.0^{b}	14.0±0.7^{b}	15.0±0.7^{c}	17.0±0.7^{d}	0.01*	9.0±0.0^{b}	12.0±0.0^{c}	15.0±0.7^{c}	18.0±1.4^{c}	0.01*
P	Odogbolu Farm	0.0±0.0^{c}	13.0±0.0^{b}	13.0±0.0^{d}	13.0±0.7^{e}	0.01*	0.0±0.0^{c}	10.0±0.0^{c}	13.0±1.4^{c}	13.8±0.4^{e}	0.01*
Q	Hausa	0.0±0.0^{c}	11.0±1.4^{c}	10.0±0.7^{d}	18.0±1.4^{d}	0.01*	0.0±0.0^{c}	11.0±0.7^{c}	16.0±0.0^{b}	19.0±0.7^{c}	0.01*
S	Odo Epo	0.0±0.0^{c}	12.0±0.0^{c}	12.0±0.0^{d}	17.0±0.7^{d}	0.01*	0.0±0.0^{c}	11.0±0.7^{c}	13.0±0.7^{c}	15.0±0.7^{d}	0.01*
U	Iperu	0.0±0.0^{c}	11.0±0.7^{c}	14.0±0.7^{c}	21.0±1.4^{c}	0.01*	0.0±0.0^{c}	13.0±0.0^{c}	15.0±0.0^{c}	17.0±0.0^{d}	0.01*
V	Slowbay	8.0±0.0^{b}	10.0±0.7^{c}	11.0±0.7^{d}	23.0±0.7^{b}	0.01*	10.0±1.4^{b}	15.0±0.7^{b}	17.0±1.4^{b}	20.0±0.7^{c}	0.01*
Z	Provost 1	0.0±0.0^{c}	10.0±0.7^{c}	10.0±0.0^{d}	13.0±0.0^{e}	0.01*	8.0±0.0^{b}	10.0±0.0^{c}	14.0±1.4^{c}	16.0±0.0^{d}	0.01*
AD	Provost 2	9.0±0.0^{b}	11.0±0.7^{c}	12.0±0.7^{d}	15.0±0.7^{e}	0.01*	9.0±0.7^{b}	11.0±0.0^{c}	12.0±0.0^{c}	13.0±0.0^{e}	0.01*

$^{a\,b\,c}$ Means (± Standard deviation) in the same column having similar superscript are not significantly different ($p > 0.05$), *Mean significantly different between concentrations

Table 1b: Zones of inhibition in mm of different honey samples against *Staphylococcus aureus* and *Klebsiella*

		Staphylococcus aureus					*Klebsiella* sp.				
Samples code	Location	**25**	**50**	**75**	**100**	**p - value**	**25**	**50**	**75**	**100**	**p - value**
			(%w/v)					(%w/v)			
A	Ibadan	0.0±0.0^{c}	11.0±0.7^{c}	15.0±0.0^{b}	21.0±0.7^{c}	0.01*	0.0±0.0^{c}	11.0±0.0^{c}	13.0±0.7^{c}	15.0±1.4^{c}	0.01*
D	Ayetoro	0.0±0.0^{c}	10.0±0.0^{c}	16.8±0.4^{b}	25.0±0.0^{b}	0.01*	9.0±0.0^{b}	15.0±0.0^{b}	17.0±0.0^{b}	20.0±1.4^{b}	0.01*
E	Apata Ibadan	13.0±0.0^{a}	16.0±1.4^{a}	20.0±1.4^{a}	27.0±0.7^{a}	0.01*	12.0±0.0^{a}	16.0±0.7^{b}	22.0±0.0^{a}	24.0±1.4^{a}	0.01*
F	Provost	14.0±1.4^{a}	17.0±1.4^{a}	21.0±0.0^{a}	23.0±0.7^{b}	0.01*	13.0±0.0^{a}	18.0±0.0^{a}	22.0±0.7^{a}	25.0±0.0^{a}	0.01*
G	Ijanran	9.5±0.7^{b}	13.0±1.4^{b}	15.0±0.7^{b}	22.0±0.7^{c}	0.01*	10.0±0.0^{b}	15.0±0.0^{b}	18.0±0.7^{b}	11.0±0.0^{c}	0.01*
H	Imodi	9.0±0.0^{b}	15.0±1.4^{b}	18.0±0.0^{b}	20.0±1.4^{c}	0.01*	0.0±0.0^{c}	15.0±0.7^{b}	19.0±0.0^{b}	23.0±1.4^{a}	0.01*
I	Ikangba Farm	8.0±0.0^{b}	11.0±0.0^{c}	15.0±1.4^{b}	20.0±0.0^{c}	0.01*	0.0±0.0^{c}	11.0±0.0^{c}	13.0±1.4^{c}	15.0±0.7^{c}	0.01*
J	Ijebu Igbo 1	9.0±0.0^{b}	14.0±0.0^{b}	16.0±0.0^{b}	21.0±0.0^{c}	0.01*	0.0±0.0^{c}	13.0±0.7^{c}	16.0±0.0^{b}	20.0±1.4^{b}	0.01*
K	Ijebu Igbo 2	10.0±1.4^{b}	15.0±0.7^{b}	19.0±0.0^{a}	22.0±1.4^{c}	0.01*	10.0±0.0^{b}	14.0±0.7^{b}	19.0±1.4^{b}	22.0±0.0^{a}	0.01*
L	Imosan 2	10.0±1.4^{b}	18.0±0.0^{a}	22.0±0.7^{a}	24.0±1.4^{b}	0.01*	9.0±0.0^{b}	14.0±0.0^{b}	18.0±0.7^{b}	20.0±0.0^{b}	0.01*
M	Imodi 2	8.0±0.0^{b}	10.0±1.4^{c}	12.0±1.4^{c}	23.0±0.0^{b}	0.01*	8.0±0.0^{b}	13.0±0.7^{c}	16.0±0.0^{b}	19.0±0.0^{b}	0.01*
N	Idofo Farm	10.0±1.4^{b}	18.0±1.4^{a}	22.0±1.4^{a}	25.0±1.4^{b}	0.01*	11.0±0.7^{a}	15.0±0.7^{b}	18.0±0.0^{b}	22.0±1.4^{a}	0.01*
O	Ago-Iwoye	10.0±1.4^{b}	16.0±0.0^{a}	19.0±1.4^{a}	21.0±0.7^{c}	0.01*	8.0±0.0^{b}	12.0±0.0^{c}	15.0±0.0^{b}	17.0±0.7^{b}	0.01*
P	Odogbolu Farm	9.0±0.7^{b}	15.0±0.0^{b}	18.0±1.4^{b}	22.0±0.0^{c}	0.01*	0.0±0.0^{c}	10.0±0.7^{c}	12.0±1.4^{c}	14.0±0.0^{c}	0.01*
Q	Hausa	10.0±0.0^{b}	15.0±0.7^{b}	17.0±0.0^{b}	23.0±0.0^{b}	0.01*	10.0±0.7^{b}	15.0±0.0^{b}	17.0±0.0^{b}	21.0±0.7^{b}	0.01*
S	Odo Epo	0.0±0.0^{c}	13.0±0.7^{b}	17.0±1.4^{b}	23.0±0.7^{b}	0.01*	0.0±0.0^{c}	10.0±0.0^{c}	12.0±0.7^{c}	14.0±1.4^{c}	0.01*
U	Iperu	9.0±0.0^{b}	15.0±0.0^{c}	19.0±0.0^{b}	24.0±0.7^{b}	0.01*	10.0±0.0^{b}	14.0±0.7^{b}	16.0±0.7^{b}	19.0±1.4^{b}	0.01*
V	Slowbay	12.0±1.4^{a}	16.0±1.4^{a}	20.0±0.0^{a}	23.0±0.0^{b}	0.01*	9.0±0.0^{b}	12.0±0.7^{c}	17.0±0.0^{b}	21.0±0.0^{b}	0.01*
Z	Provost 1	10.0±0.7^{b}	18.0±1.4^{a}	20.0±0.7^{a}	24.0±0.0^{b}	0.01*	0.0±0.0^{c}	11.0±0.0^{c}	13.0±0.7^{c}	15.0±0.7^{c}	0.01*
AD	Provost 2	0.0±0.0^{c}	13.0±0.0^{b}	15.0±0.0^{b}	17.0±1.4^{d}	0.01*	0.0±0.0^{c}	10.0±0.0^{c}	12.0±0.7^{c}	14.0±0.0^{c}	0.01*

$^{a\,b\,c}$ Means (±Standard deviation) in the same column having similar superscript are not significantly different ($p > 0.05$), *Mean significantly different between concentrations

Table 2: Physicochemical analysis of honey samples from the different hives.

Sample Code	Location	Colour	Total Titratable Acid	pH	Acidity
A	Ibadan	Amber	0.21±0.01^{e}	3.01±0.01^{c}	2.97±0.13^{e}
D	Ayetoro	Dark Amber	0.09±0.01^{g}	3.87±0.01^{a}	1.33±0.13^{h}
E	Apata	Dark Amber	0.44±0.01^{b}	3.81±0.01^{a}	4.77±1.43^{b}
F	Provost	Light Amber	0.12±0.01^{f}	3.35±0.01^{b}	1.73±0.13^{g}
G	Ijanran	Amber	0.27±0.01^{d}	3.03±0.01^{c}	3.77±0.13^{c}
H	Imodi 1	Dark Amber	0.19±0.01^{e}	3.21±0.01^{c}	2.60±0.10^{e}
I	Ikangba Farm	Dark Amber	0.14±0.00^{f}	3.21±0.01^{c}	1.67±0.33^{g}
J	Ijebu Igbo 1	Amber	0.28±0.00^{d}	3.01±0.01^{c}	3.90±0.00^{c}
K	Ijebu Igbo 2	Amber	0.32±0.01^{c}	3.03±0.01^{c}	4.23±0.07^{b}
L	Imosan 2	Dark Amber	0.23±0.01^{e}	3.35±0.15^{b}	3.23±0.13^{d}
M	Imodi 2	Dark brown	0.16±0.01^{f}	3.05±0.01^{c}	2.23±0.12^{f}
N	Idofo Farm	Dark Amber	0.20±0.00^{e}	3.42±0.01^{b}	2.70±0.00^{e}
O	Ago-Iwoye	Dark Amber	0.21±0.01^{e}	3.16±0.01^{c}	2.83±0.13^{e}
P	Odogbolu Farm	Dark Amber	0.11±0.00^{f}	2.91±0.01^{c}	1.60±0.00^{g}
Q	Hausa	Amber	0.08±0.00^{gc}	2.51±0.01^{d}	1.20±0.00^{h}
S	Odo Epo	Amber	0.12±0.01^{f}	48±0.01^{b}	1.73±0.13^{g}
U	Iperu	Amber	0.10±0.01^{g}	3.66±0.01^{a}	1.47±0.13^{h}
V	Slowbay	Dark Amber	0.52±0.02^{a}	3.64±0.01^{a}	6.77±0.09^{a}
Z	Provost 1	Light Amber	0.15±0.01^{f}	3.43±0.02^{b}	2.13±0.13^{f}
AD	Provost 2	Light Amber	0.07±0.01^{g}	3.41±0.01^{b}	1.40±0.20^{h}

Table 3: Proximate composition of honey samples from the different hives

A	Ibadan	1.34±0.01^{a}	10.93±0.09^{e}	0.41±0.00^{b}	0.25±0.00^{a}	87.07±0.10^{c}	0.00	355.89
D	Ayetoro	1.20±0.03^{a}	12.33±0.60^{c}	0.37±0.01^{c}	0.23±0.01^{a}	85.87±0.64^{d}	0.00	350.35
E	Apata Ibadan	1.38±0.01^{a}	11.37±0.09^{d}	0.42±0.00^{b}	0.26±0.00^{a}	86.57±0.10^{c}	0.00	354.14
F	Provost	1.36±0.01^{a}	11.22±0.08^{d}	0.54±0.00^{a}	0.26±0.00^{a}	86.62±0.10^{c}	0.00	361.06
G	Ijanran	1.37±0.01^{a}	11.20±0.10^{d}	0.42±0.00^{b}	0.26±0.00^{a}	86.76±0.12^{c}	0.00	354.86
H	Imodi 1	1.37±0.02^{a}	10.33±0.93^{e}	0.42±0.01^{b}	0.25±0.00^{a}	87.62±0.95^{c}	0.00	358.21
I	Ikangba Farm	1.31±0.01^{a}	7.10±0.40^{h}	0.40±0.00^{b}	0.24±0.00^{a}	90.95±0.41^{a}	0.00	371.20
J	Ijebu Igbo 1	1.33±0.01^{a}	10.90±0.06^{e}	0.41±0.00^{b}	0.24±0.00^{a}	87.12±0.07^{c}	0.00	355.96
K	Ijebu Igbo 2	1.38±0.01^{a}	11.33±0.03^{d}	0.42±0.00^{b}	0.26±0.00^{a}	86.61±0.04^{c}	0.00	354.30
L	Imosan 2	1.09±0.01^{a}	7.27±0.38^{h}	0.33±0.00^{c}	0.20±0.00^{a}	91.11±0.39^{a}	0.00	370.60
M	Imodi 2	1.26±0.02^{a}	8.67±1.01^{g}	0.39±0.01^{c}	0.23±0.00^{a}	89.45±1.04^{b}	0.00	364.91
N	Idofo Farm	1.15±0.01^{a}	9.47±0.09^{f}	0.35±0.00^{c}	0.21±0.00^{a}	88.82±0.10^{b}	0.00	361.77
O	Ago-Iwoye	1.42±0.01^{a}	14.17±1.30^{a}	0.44±0.00^{b}	0.27±0.00^{a}	83.71±1.28^{e}	0.00	342.95
P	Odogbolu Farm	1.31±0.03^{a}	10.70±0.20^{e}	0.52±0.01^{a}	0.25±0.01^{a}	87.23±0.24^{c}	0.00	356.41
Q	Hausa	1.37±0.05^{a}	11.27±0.42^{d}	0.54±0.02^{a}	0.26±0.01^{a}	86.56±0.49^{c}	0.00	354.06
S	Odo Epo	1.16±0.04^{a}	9.57±0.32^{f}	0.46±0.01^{b}	0.22±0.01^{a}	88.59±0.38^{b}	0.00	360.98
U	Iperu	1.19±0.02^{a}	9.70±0.15^{f}	0.47±0.01^{b}	0.22±0.00^{a}	88.41±0.18^{b}	0.00	360.38
V	Slowbay	1.59±0.04^{a}	13.04±0.33^{b}	0.63±0.02^{a}	0.30±0.01^{a}	84.44±0.39^{d}	0.00	346.82
Z	Provost 1	1.28±0.01^{a}	10.53±0.12^{e}	0.51±0.01^{a}	0.24±0.00^{a}	87.43±0.14^{c}	0.00	357.00
AD	Provost 2	1.31±0.03^{a}	10.80±0.26^{e}	0.52±0.01^{a}	0.25±0.01^{a}	87.12±0.32^{c}	0.00	355.97

$^{a\,b\,c\,d\,e\,f\,g\,h}$ Means (±Standard error of mean) in the same column having similar superscripts are not significantly different at $p < 0.05$

Table 4: Pearson correlation between honey nutritional parameters

	Total Titratable Acid	pH	Acidity	Protein	Moisture	Ash	Fat	Carbohydrate	Energy
Total Titratable Acid	1								
pH	0.217	1							
Acidity	0.986**	0.167	1						
Protein	0.562**	-0.197	0.569**	1					
Moisture	0.305	0.061	0.324	0.654**	1				
Ash	0.075	-0.053	0.101	0.611**	0.454*	1			
Fat	0.499*	-0.161	0.504*	0.981**	0.725**	0.671**	1		
Carbohydrate	-0.327	-0.041	-0.346	-0.706**	-0.997**	-0.509*	-0.774**	1	
Energy	-0.332	-0.05	-0.352	-0.633**	-0.977**	-0.418	-0.695**	0.972**	1

*Correlation significant at $p < 0.05$, **Correlation significant at $p < 0.01$,

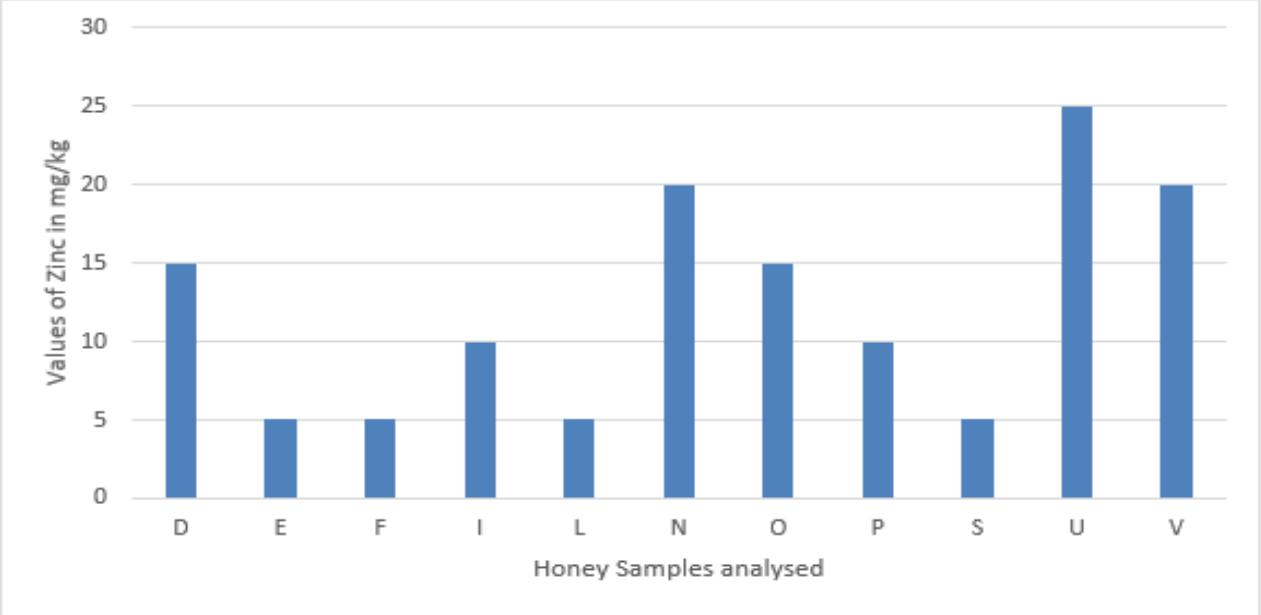

Fig 1: Concentration of Zinc in honey samples in mg/kg

DISCUSSION

The honey samples exhibited different activity against the test organisms. There was increase in inhibition zones as the concentration increases. This conforms to the study of Badawy *et al*. (2004), Odeyemi *et al*. (2013). Antimicrobial activity was highest at *100%* concentration for sample F against *E. coli*, *Salmonella* and *Klebsiella*, followed by Sample E against *S. aureus*. According to Odeyemi *et al*. (2013), the type of pathogen dictates the effectiveness of honey as an antimicrobial agent. *S. aureus* seems to be more susceptible to all the samples with an inhibition range of 17 – 27 mm. However, Omafuvbe and Akanbi (2009) reported that *S. aureus* was resistant to all the honey samples analysed but that *E. coli* was highly susceptible. Most of the test organisms displayed resistance to some of the honey samples at 25% concentration.

For physicochemical analysis, the pH range of 2.51 – 3.87 was obtained in this study, which is lower compared to the results of Omafuvbe and Akanbi (2009), Adenekan *et al*. (2010) and Osuagwu *et al*. (2020) that reported a pH range of 3.61-4.05, 2.8 - 4.5 and 4.0 - 4.3 respectively in honey samples analysed from different parts of Nigeria. Also, Chua and Adnan (2014), Azonwade *et al*. (2018), Bouhlali *et al*. (2019) and Nemo and Bacha (2021) reported a pH range of 3.21 – 3.45, 3.65 – 4.09, 3.59 – 4.62 and 3.73 -3.89 respectively in samples of honey from Malaysia, Benin, Morocco and Ethopia. The acceptable pH value by Codex Alimentarius (2001) is 3.2 – 4.5, in which eighteen samples fall within the limit except samples P and Q with pH < 3.00. The pH of some of the samples did not indicate any significant difference at $p < 0.05$. The TTA obtained in this finding was in a range of 0.08 to 0.52%. Adebowale *et al*. (2014) and Oyeyemi *et al*. (2015) reported a range of 0.62 – 1.63 mg formic acid/kg, 2.73 and 2.31% for beekeeper honey and street vendor honey respectively. There is substantial difference at $p < 0.05$ in the TTA values among the samples. The acidity in this finding is higher than range of 1.36 – 1.55% reported by Ndife *et al*. (2014b). There is also a significant difference at $p < 0.05$ for acidity among the samples. According to Azonwade *et al*. (2018), the acidity of honey is as a result of organic acids present in it. Acidic nature of honey is extremely important because it improves is stability and durability (Mahmoudi *et al.,* 2012).

The values for the ash content compared well with the result of Azonwande *et al*. (2018) and Osuagwu *et al*. (2020) who recorded the ranges for ash content as 0.42 – 0.53 and 0.67 – 0.68 respectively. Only one sample (V) exceeded the standard limit of 0.6 % recommended by Codex Alimentarius (2001). Other authors reported a lower pH content of 0.18 - 0.50 (Adenekan *et al*., 2010), 0.07 - 0.36 for multiflower honey, 0.0 – 0.41 for Acacia honey (Prica *et al*., 2015), 0.19-0.27 (Chua and Adnan, 2014) and 0.16-1.00 (Akharaiyi and Lawal, 2016). However, Odeyemi *et al*. (2013) reported 0.79 % ash for Oasis honey. At $p < 0.05$, there is no substantial difference in the ash contents of samples A, E, G, H, I, J, K, O and U. According to Rodriguez *et al*. (2004), ash content of honey is subject to the type of material gathered by the bees during the foraging on the vegetation.

The values for the moisture content aligns with the study of Prica *et al.* (2015) and Osuagwu *et al*. (2020) who reported moisture content in the range of 14.6 to 18.6 % for Meadow honey and 11.13 to 16.16% respectively. The value for the moisture content in this research falls within the Codex Alimentarius (2001) value of ≤ 20%. The values for moisture content are significantly different at $p < 0.05$ among the samples, and this variation can be elucidated by the constitution and floral source of honey samples (Malika *et al*., 2005). Moisture also depends on the season and geographic location (Prica *et al*., 2015). Fredes and Montenegro (2006) stated that honey with reduced moisture would have a long storage life, while high moisture volume can lead to granulation (Rodriquez *et al*., 2004).

The protein range was between 1.09 to 1.59% in our finding. This is lower to the result of Oyeyemi *et al*. (2015) who reported protein content of 5.65 % and 6.25% for honey samples from street vendor and bee keeper respectively, and higher than the findings of Chua and Adnan (2014) and Osuagwu *et al*. (2020) who recorded a range of 0.36 to 1.02% and 0.04 to 1.06% respectively. At $p < 0.05$, protein content of all the honey samples is not significantly different. Variation in protein content can be due to soil composition, location and floral origin (Osuagwu *et al.*, 2020).

The range for carbohydrate in this study is 83.71 to 91.11% which aligns with the Codex Alimentarius (2001) standard of > 83%. Our finding is higher than 61.89 to 78.67% reported by Chua and Adnan (2014) and lower than 97.94 to 98.98% reported by Osuagwu *et al*. (2020). For total energy, the range was 342.95-371.20 kcal/g which is lower to 507.16kcal/g in bee keeper honey and higher than 281.45 kcal/g in street vendor honey reported by Oyeyemi *et al*. (2015). However, this conforms to the finding of Ndife *et al*. (2014b) who reported 326.25- 337.04 kcal/g. Chua and Adnan (2014) and Osuagwu *et al*. (2020) reported 2.450 -2.932 kcal/g and 1668.60-1669.53 kJ respectively. Chua and Adnan (2014) stated that the high sugar in honey sample is mainly responsible for the energy value.

There is no dietary fibre in all the honey samples which aligns with the findings of Chua and Adnan (2014), therefore all the samples have available carbohydrates since unavailable carbohydrate are referred to as dietary fibre (Charrondiere *et al.*, 2004).

All the honey samples in this study are within the 0.21 to 0.30% prescribed by Codex Alimentarius (2001) for fat content. Oyeyemi *et al*. (2015) reported fat content of 0.80 and 1.23% for honey samples from street vendor and beekeeper respectively. Osuagwu *et al*. (2020) reported a range of 0.31 to 0.35% for their honey samples. However, Chua and Adnan (2014) did not detect any fat in their samples. There is no significant difference among the fat content of all the samples analysed.

Cadmium, chromium and lead were not detected in any of the samples analysed. This conforms to the finding of Laaroussi *et al*. (2020) who also did not detect lead in the honey samples analysed, but detected zinc (1.09 – 4.02 mg/kg) in all the samples and cadmium in only one. This contradicts the findings of Osuagwu *et al*. (2020) who reported the presence of cadmium and lead in samples of honey from the Guinea savannah zones of Nigeria. Only zinc was detected in all the samples analysed. Oyeyemi *et al*. (2015) detected zinc along with other mineral element in beekeeper and street vendor honey in concentration of 124.24 mg/100g and 89.92 mg/100g. However, a lower concentration of zinc in the range 1.64 – 2.06 mg/kg for honey samples in western states of Nigeria was reported by Akharaiyi and Lawal (2016). The presence of mineral elements in honey could indicate pollution of plant used by the honey, soil and topographical origin of honey (Franchini *et al*., 2007, Pohl, 2009) and the container used in collecting the honey. Zinc though a trace element, is a major player in the creation of DNA, cell growth, proteins building, healing of damaged tissues and support a healthy immune system. The Recommended Dietary Allowance (RDA) for adults is 11 mg and 8 mg for men and women while the acceptable maximum intake is 40 mg daily for adults (Institute of Medicine, 2001).

CONCLUSION

This research has showed that seventeen out of the twenty honey samples met with the laid down international standard regulation for honey, although, the protein contents for all exceeded the limit, this variation may be as a result of the soil composition, location and floral origin, therefore, these honeys can be taken as food and also for medicinal use.

Acknowledgement

The researchers are grateful to Dr. Bamidele Julius of the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta for the Statistical Analysis and Mr. Femi Oreyemi for assisting with the collection of the honey samples from the different Apiaries.

REFERENCES

Adebowale OJ, Salaam HA and Agboola O A 2014 Comparative physico-chemical composition of natural honey from traditional bee-breeders in Ogun State. *Journal of Biological Sciences and Bioconservation* 6(2): 31 - 40.

Adenekan MO, Amusa NA, Lawal AO and Okpeze VE 2010 Physico-chemical and microbiological properties of honey samples obtained from Ibadan. Scientific *Journal of food Science and Nutrition,* 2:104.

Adeniyi KA, Daudu OAY, Abubakar A, Ismail AD, Busari MB, Abdulsalami H and Oyibo-Usman KA 2014 Comparative analysis of the proximate and nutritional compositions of Nigerian bitter and sweet honey from Apis mellifera. International *Journal of Scientific and Research Publications* 4 (11): 1-4.

Akharaiyi FC and Lawal HA 2016 Physicochemical analysis and mineral contents of honey from farmers in Western States of Nigeria. *Journal of Natural Sciences Research* 6(19):78-84.

AOAC 2005 Official Methods of Analysis of AOAC. 18th Edition. Association of Analytical Chemists, Washington DC., USA. Azowande FE, Paraiso A, Dossa CPA, Dougnon VT, N'tcha C, Mousse W and Baba- Moussa L 2018 Physicochemical characteristics and microbiological quality of honey produced in Benin. *Journal of Food Quality* 2018: 13. https://doi.org/10.1155/2018/1896057

Badawy OFH, Shafii SSA, Tharwatt EE and Kamal AM 2004 Antibacterial activity of bee honey and is therapeutic usefulness against Escherichia coli 0157:H7 and *Salmonella* Typhimurium infection. Rev. Sci. Technol. Int., Epiz 23:10111-10122. https://doi.org/10.20506/rst.23.3.1543

Barganska Z, Slebioda M and Namiesnik J 2014 Pesticides Residues Level in Honey from Apiaries Located off Northern Poland. Food Control, 31: 196-201. https://doi.org/10.1016/j.foodcont.2012.09.049

Bogdanov S, Haldimann M, Luginbühl W and Gallmann P 2007 Minerals in honey: environmental, geographical and botanical aspects. Journal of Apicultural Research and Bee World 46 (4): 269–275. https://doi.org/10.1080/00218839.2007.11101407 Bouhlali EDT, Bammou M, Sellam K, El Midaoui A, Bourkhis B, Ennassir J, Alem C and Filali-Zegzouti Y 2019 Physicochemical properties of eleven monofloral honey samples produced in Morocco. *Arab Journal of Basic and Applied Sciences,* 26(1): 476-487. https://doi.org/10.1080/25765299.2019.1687119 Cantarelli MA, Pellerano RG, Marchevsky EJ and Camiña JM 2008 Quality of honey from Argentina: study of chemical composition and trace elements. The *Journal of the Argentine Chemical Society,* 96 (1-2): 33-41. Charrondiere UR, Chevassus-Agnes S, Marroni S and Burlingame B 2004 Impact of different macronutrient definitions and energy conversion factors on energy supply estimations. J. Food Compos. Anal. 17(3-4): 339-360. https://doi.org/10.1016/j.jfca.2004.03.011

Chua LS and Adnan NA 2014 Biochemical and Nutritional Components of Selected Honey Samples. Acta Scientiarum Polonorum Technologia Alimentaria 13(2): 169-179. https://doi.org/10.17306/j.afs.2014.2.6

Codex Alimentarius 2001 Revised Standard for Honey. Allinorm 25: 19-26. Datti Y, Ahmad UU and Hafsat N 2020 Comparative Analysis of the Mineral Compositions of Honey Samples Collected from the Three Senatorial Districts of Kano State, Nigeria. FUDMA *Journal of Sciences* (FJS), 4(3):170–177. https://doi.org/10.33003/fjs-2020-0403-267

Franchini RA, Ferraz de Souza A, Colombara C, Matos R and Matos RC 2007 Rapid determination of hydrogen peroxide using peroxidase immobilised on Amberlite IRA-743 and minerals in honey. *Journal of Agriculture and Food Chemistry* 55(17): 6885 – 6890. https://doi.org/10.1021/jf071062c

Fredes C and Montenegro G 2006 Heavy metal and other Trace elements in Honey Bee in Chile. Cien. Inv. Agric., 33:50-58. https://doi.org/10.7764/rcia.v33l1.328

Institute of Medicine 2001 Panel on Micronutrients. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Valadium, and Zinc. National Academic Press, Washington, DC. 12, Zinc. Jacobs BM 1999 The Chemical Analysis of Food and Food Products. 3rd ed. CBS Publishers and Distributors, New Delhi, India.

Kambai C, Popoola V, Ugbe CJ, Janfa N and Ukanyirioha C 2015 Proximate and Mineral Elements Composition of Honey from Selected Hives in Jos Metropolis, Nigeria. IOSR *Journal of Environmental Science, Toxicology and Food Technology,* 9 (9): 94-98. https://doi.org/10.9790/2402- 09919498

Laaroussi H, Bouddine T, Bakour M, Ousaaid D and Lyoussi, B 2020 Physicochemical properties, Mineral content, Antioxidant activities and Microbiological quality of Bupleurum spinosum Gouan honey from the Middle Atlas in Morocco. *Journal of Food Quality* 2020:12 pages. https://doi.org/10.1155/2020/7609454

Laleh M, Mohsen E and Ali H 2013 Evaluation of some Physicochemical and Rheological Properties of Iranian Honeys and the Effect of Temperature on its Viscosity. American-Eurasian *Journal of Agricultural and Environmental Sciences,* 13(6):807-819. https://doi.org/10.5829/idosi.aejaes.2013.13.06.1971

Mahmoudi R, Zare P, Tajik H, Shadfar S and Nyiazpour F 2012 Biochemical Properties and Microbial Analysis of Honey from North-Western Regions of Iran: Seasonal Effects on Physicochemical Properties of Honey. *African Journal of Biotechnology* 11(44): 10227-10231. https://doi.org/10.5897/ajb12.371

Malika N, Mohammed F and Chakib E 2005 Microbiological and Physio-chemical properties of Moroccan honey. International Journal of Agriculture and Biology 7(5):773-776. Ndife J, Kida F and Makarfi T 2014a Quality assessment of Nigerian honeys sourced from different floral locations. *Journal of Food and Nutrition Sciences,* 2 (4), 162-167. https://doi.org/10.11648/j.jfns.20140204.20

Ndife J, Abioye L and Dandago M 2014b Quality Assessment of Nigerian Honey sourced from different floral locations. *Nigerian Food Journal* 32(2): 48-55. https://doi.org/S0189-7241(15)30117-X

Nemo R and Bacha K 2021 Microbial quality, physicochemical characteristics, proximate analysis, and antimicrobial activities of honey from Anfilo district. Food Bioscience 42: 101132. https://doi.org/10.1016/j.fbio.2021.101132 Odeyemi AT, Adefemi SO and Adebayo AA 2013 Antimicrobial and Proximate Properties of some processed Honey in Ado-Ekiti. International. Journal of Aquatic Science 4(1): 36-43. Omafuvbe BO, Akanbi OO 2009 Microbiology and physico-chemical properties of some commercial Nigerian honey. *African Journal of Microbiology Research* 3(12): 891-896.

Osuagwu OS, Oyerinde AA, Onipede AS and Ombugadu A 2020 Comparative Studies of the Physicochemical Properties and Mineral Elements of Honey Produced in the Guinea savannah Zones of Nigeria. Biomedical *Journal of Scientific and Technical Research* 24(5): 18548-18561. https://doi.org/10.26717/bjstr.2020.24.004105 Oyeyemi SD, Kayode J and Owolabi MO 2015 Comparative Nutritional Studies on Honey Samples in Ado Ekiti, Ekiti State, Nigeria. Donnish Journal of Medicinal Plant Research 2(2): 016 -020. Prica N, Zivkov-Balos M, Jaksic S, Mihaljev Z, Ljubojevic D, Vidic B and Savic S 2015 Physicochemical Analysis as an Indicator of the Quality of Honey Originating from Vojvodina Region. First International Symposium of Veterinary Medicine 178 – 182. Pohl P 2009 Determination of mineral content in honey by atomic absorption and emission spectrometric. TrAC Trends in Analytical Chemistry 28(1): 117-125. https://doi.org/10.1016/j.trac.2008.09.015

Rao PV, Krishnan, KT, Salleh N and Gan SH 2016 Biological and therapeutic effects of honey produced by honey bees and stingless bees: A comparative review. Rev. Brasil. Farmacog., 26: 657-664. https://doi.org/10.1016/j.bjp.2016.01.012

Rissato SR, Galhiane MS, de Almeida MV, Gerenotti M and Apron BM 2007 Multiresidue Determination of Pesticides in Honey Samples by Gas Chromatography-Mass Spectrometry and Application in Environmental Contamination. Food Chemistry, 101: 1719-1726. https://doi.org/10.1016/j.foodchem.2005.10.034

Rodriguez GOde, Ferrer BSde, Ferrer and Rodriguez B 2004 Characterisation of honey produced in Venezuelan. Food Chemistry 84(4): 449-502. https://doi.org/10.1016/S0308-8146(02)00517-4

Valgas C, de Souza SM, Smania EFA and Smania (Jr.) A 2007 Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38 (2): 369-380. https://doi.org/10.1590/S1517-83822007000200034