

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.905.106>

A Comparative Study on the Antimicrobial Activity of Natural and Artificial (Adulterated) Honey Produced in Some Localities in Ghana

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A B S T R A C T

Keywords

antimicrobial,
adulterated,
antioxidant,
moisture content,
susceptibility

Article Info

Accepted:
05 April 2020
Available Online:
10 May 2020

The study measured and compared quality parameters such as pH, moisture content, ash content and hydrogen peroxide concentration of three types of honey from different localities in Ghana. Using the agar well diffusion, antimicrobial potential of natural honey from two different sources as well as an adulterated honey were determined against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli*(ATCC 35218)and *Klebsiella pneumonia* (ATCC 27736). The results of the physico-chemical properties showed the following range of values; pH of (3.68– 4.49), moisture content (17.20 – 22.42%) and ash content (0.09 – 0.48%). The moisture content of the adulterated samples at 25% and 50% concentration in all cases exceeded the recommended 21%of good quality honey. The findings also indicated that, natural honey (Koforidua and Berekum) has a higher antimicrobial activity against all the bacteria compared to the adulterated honey. The susceptibility of *Escherichia coli*, to the honey treatment, was significantly different ($p=0.0383$) from *S. aureus* and *K. pneumoniae*. However, when the susceptibility of *S. aureus* and *K. pneumoniae*, when compared was insignificant ($p=0.6292$).The adulteration of the honey could have caused some of the antibacterial factors in the honey to be lost or reduced to non-lethal amounts, hence losing it antimicrobial properties.

Introduction

Honey has been recognized and used as a form of antimicrobial agent for centuries (Hegazi, 2011).It has been discovered to be used as therapeutic agent for wound healing potential (Jalali *et al.*, 2007),treatment of gastroenteritis in infants (Brady *et al.*, 2004) and liver disease (Frankel *et al.*, 1998) due to

its antioxidant activity (Atrooz, *et al.*, 2008). These therapeutic potentials are partly attributed to the enzymatic production of hydrogen peroxide, the low pH and high sugar content (Molan, 1992). These physiochemical properties are known to obstruct the growth of most clinical and environmental microorganism (Mullai and Menon, 2007).

In recent times, the potent activity of honey against certain antibiotic-resistant bacteria has increased the awareness for the application of honey as a therapeutic product (Kwakman *et al.*, 2011).

The quality of honey determines its acceptability which can be assessed by among other things, its physicochemical characteristics (Muruke, 2014). Shahnawaz (2013) and other studies have shown that the quality of honey is based on its physicochemical characteristics. Normally, natural honey is sticky and viscous with water content of 15-20%, pH of 3-5, sugar content of 65% and above, high viscosity, hydroxymethyl furfural (HMF) levels not exceeding 40 mg/kg and ash content of up to 0.6% (Codex Alimentarius Commission, 2001).

However, there are reports of the decline in quality of the honey in the open market due to the possible adulteration of these honeys being sold commercially as genuine products. A careful preliminary observation of the open market honey reveals off colors and sometimes a different taste and thickness compared to natural honey. Since pure honey is known to have antimicrobial properties, consumers of such products may rely on the questionable product in the market as antibacterial agents in the treatment of wounds and infections which may prove futile. This is because adulterated honey may have a relatively low antimicrobial and other healing properties as compared to natural or pure honey (Al-Waili *et al.*, 2012). There is scanty research in Ghana to assess the quality of honey produced for domestic consumption based on its physicochemical properties. As a result, it is very necessary to investigate and compare the antimicrobial activity of the honey in the open market to the pure or natural honey. This study therefore, seeks to assess the physicochemical properties of both

the natural and artificial (adulterated) honeys in relation to their antimicrobial activity by comparing the antimicrobial activity of the natural and adulterated honey from different localities in Ghana.

Materials and Methods

Collection of honey samples

Three different honey samples were used for this experiment comprising of two pure/natural honey samples sourced from different geographic regions namely the Berekum beehive in Brong Ahafo and Koforidua beehive in the Eastern region. The other honey was purchased in the open market at Ayeduase, a suburb of Kumasi. The vegetation of the various sample sites was noted and recorded. All the samples were stored in an opaque black polythene bag and under room temperature during the period of the investigation.

Measurement of pH

The pH of honey samples was done in triplicates and determined using a digital portable pH meter - Thermo Scientific RUSSEL RL 060P in accordance with AOAC (2,000). In between the readings of different samples, the electrode was washed with distilled water and dried with tissue paper. All honey samples were diluted to 75%, 50% and 20%.

Moisture content determination

Moisture content was determined using a standard method described by AOAC (2000). About 2 ml of honey samples in triplicates were put in pre-weighed dried crucibles, kept overnight in an oven at 110 °C and weighed. The loss in weight was taken as a measure of moisture content (Shahnawaz *et al.*, 2013) calculated by the following formula

$$\text{Moisture (\%)} = \frac{(\text{Weight of Fresh Sample} - \text{Weight of Dry Sample})}{\text{Weight of Fresh Sample}} \times 100$$

Determination of ash content

The standard method by AOAC (2000) was followed in the determination of honey ash content. 3 grams of honey were put in dried pre-weighed crucibles and were heated in a furnace at 500 °C for 5 hours, until ash samples produced became white or greyish white. The ashed samples were placed in desiccators and allowed to cool and then weighed. The percentage ash content was calculated as:

$$\text{Ash Content (\%)} = \frac{(\text{Weight of Sample after Ashing})}{\text{Weight of Fresh Sample}} * 100$$

Measurement of Hydrogen peroxide concentration

The hydrogen peroxide content of the three honey samples were determined by adding 0.1g of potassium iodide (KI) to acetic acid (CH_3COOH) to form a mixture. To this mixture, 1ml of the honey was added and observed. Yellowish or very light brown foam indicates a low peroxide concentration in the sample and brown foam shows a high concentration of hydrogen peroxide in the sample.

Bacterial isolates

The Bacteria used in this study were obtained from the Microbiology laboratory of the Pharmacy Department of the Kwame Nkrumah University of Science and Technology. Three plates of each bacteria cultures were prepared to test against each of the three honey samples. *Escherichia coli* (ATCC 35218) was cultured in MacConkey agar and incubated at 37° C for 24 hours.

Staphylococcus aureus (ATCC 25923) was cultured in Mannitol Salt agar and incubated at 37° C for 24 hours. *Klebsiella pneumonia* (ATCC 27736) was cultured in MacConkey agar and incubated at 37° C for 24 hours.

Antimicrobial activity

Antibacterial activities of the different honeys were determined by direct assay procedure (Gulfraz *et al.*, 2011). Cultures of each bacteria were prepared to test for their susceptibility to the three different honey samples. In each petri dish, wells of 14 mm diameter were created in the agar with a cork borer to accommodate the four different concentrations of each honey sample. Each of the wells was labeled to avoid mixing up the positions of the different honey concentrations.

The various concentrations of each honey sample were then placed in their respective wells and the entire set up was incubated at 37° C for 24 hours and then observed for any inhibition zone. The zone of inhibition was obtained by measuring the distance from the circumference of the well to the circumference of the clear zone formed around the well.

Statistical analysis

All analyses were performed in triplicates and data was presented as mean standard deviation. Differences in performance between honey samples were analyzed using analysis of variance (ANOVA) determined by IBM SPSS Statistics version 22.

The mean zones of inhibition of honey were also compared with that of the various antibiotics using Analysis of Variance (ANOVA) to determine the significant differences. Differences at $P<0.05$ were considered statistically significant.

Results and Discussion

Physical examination of honey samples

The different honey samples were physically examined and the results summarized in Table 1. The parameters that were observed included the colour, viscosity and the presence of suspended particles. The purity of the samples was also indicated.

pH, moisture and ash content of honey samples

The pH of the honey samples was determined before they were used in the experiment. The general pH was acidic with a range of 3.69 - 4.49. The results of the pH measurements for each sample are represented in Table 2. There were varied moisture content of honey samples from 17.20 to 22.42%. On the average, the moisture content of all the samples was below 21%. The values obtained for the ash content varied from 0.10 to 0.48%, with the artificial honey having the highest ash content (0.48%) while sample from Berekum had the least (0.09 %) all at 25% concentration (Table 2).

Concentration of hydrogen peroxide in the honey samples

There was no hydrogen peroxide in the adulterated honey. The honey collected from Koforidua and Berekum had some concentration of hydrogen peroxide. Koforidua honey recorded the highest concentration of hydrogen peroxide whilst that from Berekum had a lower concentration of hydrogen peroxide (results not shown).

Susceptibility of the microorganisms to the different concentrations of honey used in the experiment

Antimicrobial sensitivity of the

microorganisms to the honey concentrations was determined using the agar well diffusion method. *S. aureus* (ATCC 25923) recorded the highest zones of inhibition in all concentrations against Koforidua pure honey. Artificial honey had the least potency against organisms tested, with honey from Berekum being the intermediate.

The zones of inhibition declined with a decrease in concentration of the honey sample. The only exception was seen with Berekum pure honey at 25% and 50%. Figure 1 shows the decline of the zone of inhibition upon dilution of honey and also the potency of the different types of honey against *S. aureus*.

Koforidua honey and Berekum honey recorded similar zones of inhibition (2mm at 100% and 1mm at 75%) against *E. coli* (ATCC 35218), as shown in Figure 2, with adulterated honey showing the least activity. 25% honey of all the samples did not give any zone of inhibition (0mm). With *E. coli* as well, activity can be seen to decline with decrease in concentration of honey. Figure 3 shows the sensitivity of *K. pneumoniae* to the different types of honey.

At 100%, the activity of Koforidua honey against organism was the highest among the three honey samples with a zone of inhibition of 5mm. However, there was a sudden decline in its activity upon dilution with no activity at all at 50% and 25%. Berekum honey showed a steady decline in the inhibition with dilution. Adulterated honey had the least antibacterial activity at 100% compared to the other honeys but inhibited *K. pneumoniae* at 50% whereas Koforidua did not at the same concentration.

The least sensitive bacteria to the honey treatment was *E. coli* as seen in Figure 4. The inhibition of *S. aureus* and *K. pneumoniae*

was of slight difference with Koforidua honey being the most potent honey against them, followed by Berekum honey. Figure 4 shows the sensitivity of each microorganism to 100% honey treatment.

Discussion

Honey has been recognized to have antibacterial activity, nevertheless there is a huge discrepancy in the potencies of different honeys and also the potential to inhibit bacterial growth. Physical examination revealed noticeable differences in colour between the honey samples from Koforidua, Berekum and the adulterated honey used in the study. Koforidua honey which was the darkest in colour recorded higher pH values and had a stronger flavor relative to the lighter coloured Berekum honey.

This trend was consistent with a number of studies which elaborate on the importance of honey colour in qualitative grading of different honeys. Reports have indicated that, dark honeys are characterized by having higher pH values, antioxidant activity and phenolic content than lighter honeys which are known to contain higher amount of sugars (Maeda *et al.*, 2005; Eleazu, 2012).

Dark honeys are also found to be richer in minerals, especially iron, manganese and copper, making them more suitable for medicinal purposes (Bouldini *et al.*, 2001). Furthermore, darker honeys tend to have stronger flavours compared to lighter honeys (Muruke, 2014). An assessment of the thickness of the honey samples revealed the three honey samples to be relatively viscous.

However, Koforidua and Berekum honey were thicker than the artificial honey. Moisture content of natural honey has been proposed to be less than 21% by the Codex Alimentarius (2001) which would render

honey highly viscous. The low moisture content of the honey has a role in its own shelf-life and its antibacterial potential. The artificial honey was less thick than the two natural honey samples which implied that water had been added during its processing. Nyau *et al.*, reported in 2013 that, honey with a high moisture content indicated adulteration and this supports the claim that the adulterated honey used in this study had a higher water activity than the natural honeys.

Studies have also shown that moisture content of honey is one of the criteria that determine the shelf stability of honey (Azenedo *et al.*, 2003). The values obtained for the ash content varied from 0.10 to 0.48%, with the artificial honey having the highest ash content (0.48%) while sample from Koforidua had the least (0.10 %) at 25% concentration. Vanhanen *et al.*, 2011 reports that ash content is a reflection of the total inorganic minerals that are present in a sample after incineration.

Generally, all the types of honey used had some activity against all the selected bacteria with varying degrees of sensitivity. However, there was a decrease in antibacterial activity with a decrease in the concentration of honey used. Koforidua honey generally recorded higher zones of inhibition against the test bacteria than the Berekum honey and the adulterated honey.

This could be attributed to a higher phenolic content in darker honeys as compared to lighter honeys (Eleazu, 2012). The study demonstrated that honey indeed has antimicrobial properties, with natural honey showing more activity against bacteria than the artificial honey.

The main drivers responsible for the differences in antimicrobial potential of the pure honey from different sources and between the pure and impure honey used in

this experiment could be the different chemical composition of the honeys resulting from differences in floral sources as well as the species of bees involved in their production. (Sohaimy *et al.*, 2015).

In almost all the studies where two or more types of honey were used, differences were observed in the antimicrobial activities. The amount of variation seen in some cases has been large and in others, small. In this study, where different sources of honey were used, the vegetation in which the bees foraged was noted in order to establish a connection between antibacterial activity and phytochemical factors present as a result of the bees' feeding activities. These natural factors would be evidently absent or in minute concentrations in processed or adulterated honey.

The susceptibility of *Escherichia coli*, to the honey treatment, was significantly different ($p=0.0383$) from *S. aureus* and *K. pneumoniae*. However, when the susceptibility of *S. aureus* and *K. pneumoniae*, was compared, the difference in sensitivities to the honey treatment was insignificant ($p=0.6292$). Consistent with Maciorowski *et al.*, (2007), *E. coli* was observed to have a relatively higher resistance to all the types and concentrations of honey used in this experiment.

This might be as a result of certain mechanisms employed by *E.coli* to survive amidst unfavourable conditions such as high osmolarity and low pH of the surrounding. It is able to cause an alteration of the composition of its cell wall in order to actively prevent the diffusion of ions into the cell. For this reason, *E. coli* is known to have a relatively high acid tolerance and can withstand pH ranges as low as 3.3 - 4.2 because it prevents H⁺ ions from penetrating its cell wall and disrupting its cellular

processes. A study conducted by Mandal and Mandal in (2011) on the medicinal property and antibacterial activity of honey also revealed *E. coli* to be the least susceptible microorganism when it was used in an experiment together with Methicillin-resistant *Staphylococcus aureus* (MRSA) and *P. aeruginosa*. *Staphylococcus aureus* had very low resistance to the honey treatment compared to *E. coli*, although it is Gram-positive and therefore has a thick peptidoglycan cell wall as reinforcement.

This could be partly due to the high osmolarity, hydrogen peroxide content and low pH of the honey samples. Its highest sensitivity which was also the highest recorded in the experiment, was against Koforidua honey which also had a high concentration of hydrogen peroxide.

This combination of factors may have caused the penetration of the peptidoglycan cell wall which resulted in the high susceptibility. The pattern observed on *S. aureus* was consistent with a study by Kwakman *et al.*, (2011). *K. pneumoniae* had a similar mean zone of inhibition to *S. aureus* and their sensitivities to honey do not vary significantly.

It is known to be a Gram-negative bacterium hence there is absence of a thick peptidoglycan cell wall which makes it more vulnerable to stress in its environment. This could explain why it showed a relatively low resistance to honey.

A study conducted by Adeshina *et al.*, in 2013 also found *K. pneumoniae* to be readily susceptible to honey treatment due to its inability to prevent the movement of disruptive ions and compound across its cell wall and also to facilitate resistant mechanisms to evade the attack on its cellular processes.

Table.1 Physical properties of Koforidua honey, Berekum honey and adulterated honey used in the study

Properties	Koforidua Honey	Berekum Honey	Adulterated Honey
Colour	Dark brown	Gold	Reddish brown
Viscosity	Highly viscous	Highly viscous	Highly viscous but considerably less viscous than Koforidua and Berekum honey
Suspended particles	Many suspended particles were present in the honey sample	Few suspended particles were present in the honey sample	No suspended particles were present in the sample
Purity	100% raw and organic	100% organic	Purity unknown due to adulteration

Table.2 pH, percentage moisture and ash content of honey samples from Koforidua, Berekum and Artificial (Ayeduase) as means of triplicate determinants

Source of Honey				
	Concentration (%)	Koforidua	Berekum	Artificial (Ayeduase)
pH	25	4.16	3.89	4.49
	50	4.15	3.78	4.43
	75	4.13	3.72	4.42
	100	4.08	3.68	4.33
Moisture content	25	17.89	18.22	22.42
	50	17.80	18.20	21.50
	75	17.32	17.81	20.52
	100	17.20	17.80	20.00
Ash content	25	0.18	0.24	0.48
	50	0.10	0.09	0.16
	75	0.16	0.21	0.18
	100	0.20	0.19	0.12

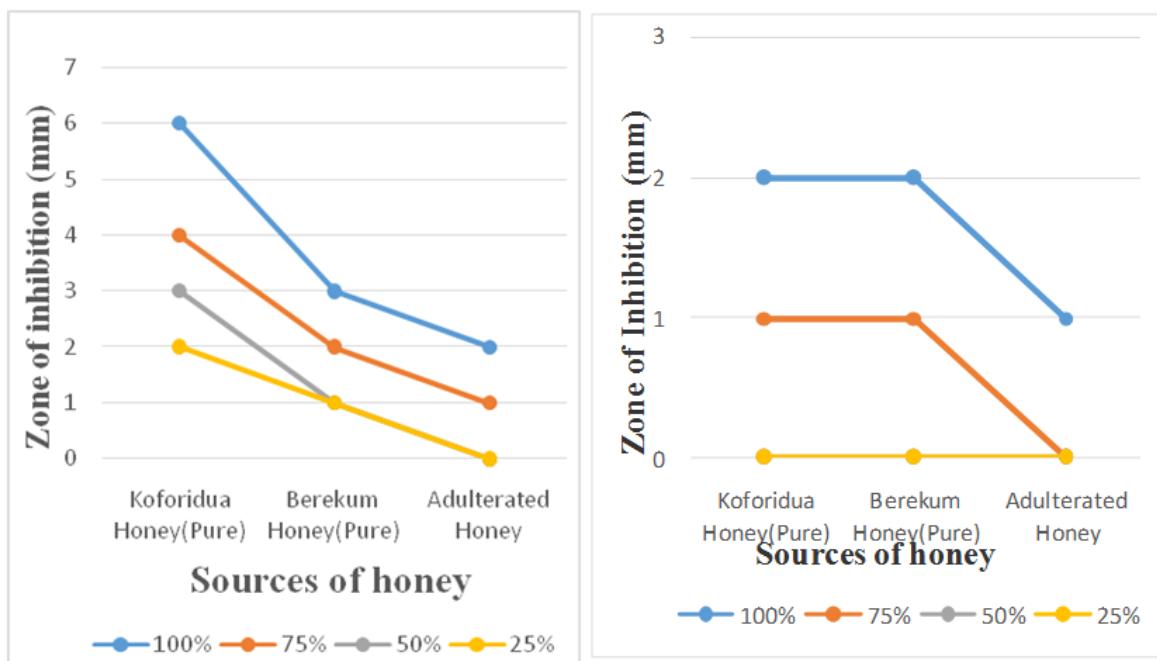


Figure.1 A comparison of the susceptibility of *S. aureus* to different concentrations of two pure honey samples and one artificial honey sample

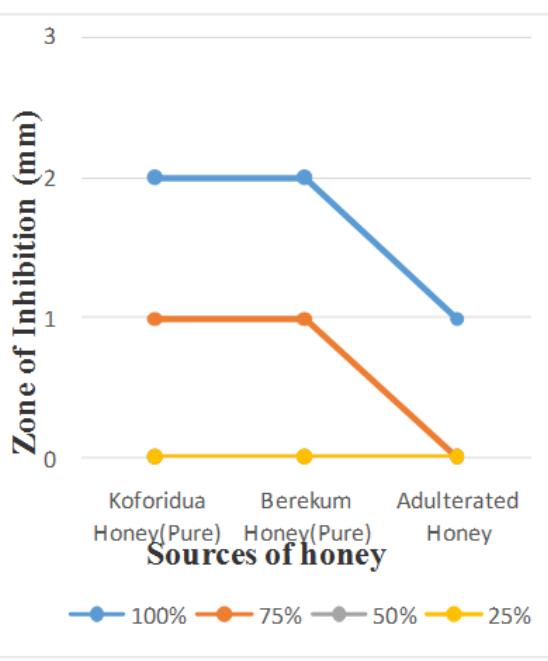


Figure.2 A comparison of the susceptibility of *E. coli* to different concentrations of two pure honey samples and one adulterated honey sample

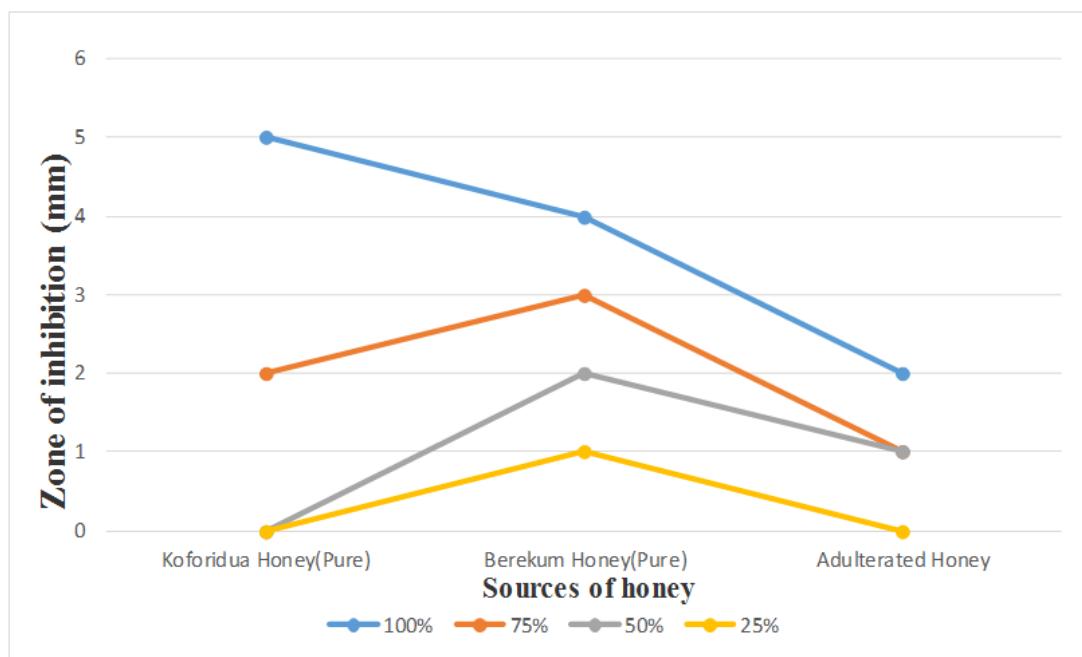


Figure.3 A comparison of the susceptibility of *K. pneumoniae* to different concentrations of two pure honey samples and one adulterated honey sample

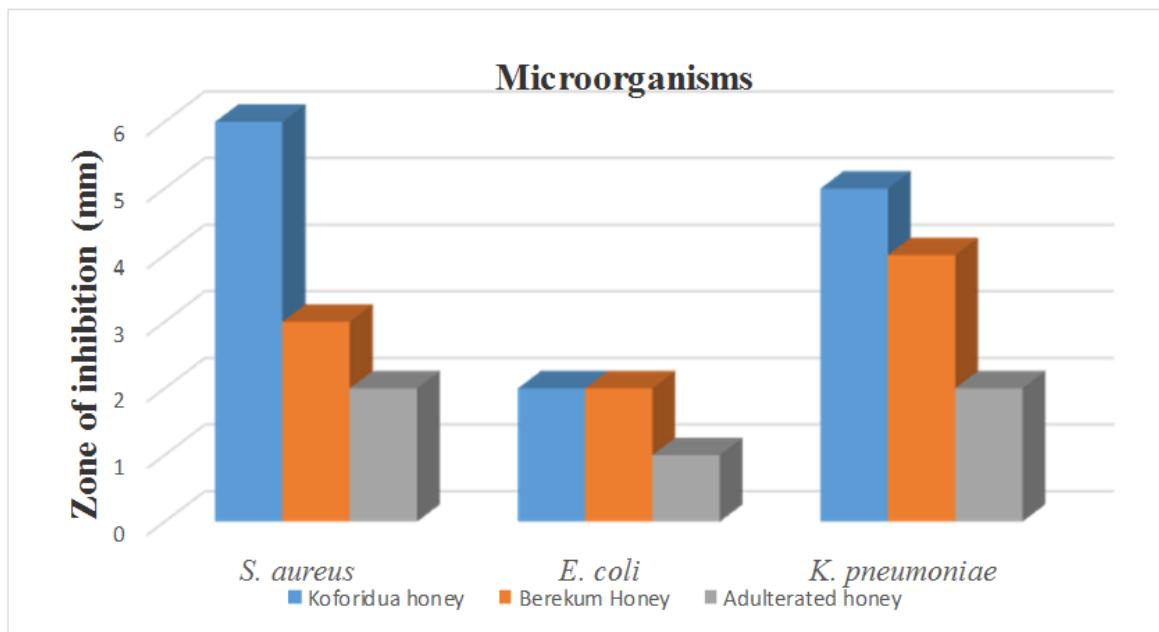


Figure.4 A comparison of the degree of sensitivity of each of the microorganisms to undiluted (100%) honey

The study showed the variability of some quality characteristics of honey samples from the different locations in Ghana. In this work, the principal physicochemical properties such as pH, ash content and moisture content of the three honey samples have been determined. Honey colour and moisture content are two important physicochemical parameters that may be used to assess quality of honey.

The findings also indicated that, natural honey has a higher antimicrobial activity against all the bacteria compared to the artificial or adulterated honey. The adulteration of the honey could have caused some of the antibacterial factors in the honey to be lost or reduced to non-lethal amounts, hence losing its antimicrobial properties.

Acknowledgement

The authors would like to gratefully acknowledge everyone involved in this research especially to the Technicians at the Microbiology Laboratory at the Department

of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana for their guidance and support.

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How to cite this article:

Matthew Glover Addo, Abdul Hakim Mutala and Kingsley Badu. 2020. A Comparative Study on the Antimicrobial Activity of Natural and Artificial (Adulterated) Honey Produced in Some Localities in Ghana. *Int.J.Curr.Microbiol.App.Sci*. 9(05): 962-972.
doi: <https://doi.org/10.20546/ijcmas.2020.905.106>