# Baringo apis Bee Honey: Nutritional, Physicochemical, Phytochemical and Antibacterial Properties Validation Against Wound Bacterial Isolates

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**Abstract**

Skin wounds are a global public health concern demanding significant resources from the healthcare system. Their consequences include pain, social, physical or psychological impact. Hence the right approach to its management should be considered. This is towards reducing the economic burden while lowering morbidity and mortality through developing new preventive and therapeutic technologies.Bee (*Apis*) honey samples were collected from their beehives in Marigat Sub County, Baringo County, Kenya, followed by quantitative analysis of physicochemical, nutritive, phytochemical and antioxidant properties contributing to its antibacterial capacity. Different concentrations of honey ($10 \times 10^4$, $20 \times 10^4$, $50 \times 10^4$ and $75 \times 10^4$ µg/ml)) in impregnated discs were tested against each type of clinical isolates obtained from wound swabs collected from Nakuru County Referral Hospital Nakuru, as indicated in the previous study on Stingless bee honey analysis. The bacterial isolates obtained included; *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. Their individual antibacterial inhibition was then compared to cartridges containing antibiotics (Levofloxacin *5µg,* Ampicillin *10µg,* Tazobactam *110 µg,* Meropenem *10µg,* Gentamicin *10µg and* Chloramphenicol *30µg) t*hrough disc diffusion (Kirby-Bauer) technique.According to this study, quantitative analysis of the honey samples yielded $90.13 \pm 5.76$g/100g, $4.07 \pm 0.08$ and $114.28 \pm 26.66$ mg/g in sugar, pH and moisture, respectively. The phenolic compounds that act as antioxidants were in the mean value of total phenolic compounds ($80.81 \pm 36.25$mgGAE/100g), total flavonoids ($21.83 \pm 6.16$ mg RE/100g) and total carotenoids ($4.41 \pm 2.07$ mgβ – carotene/kg). These and other components contributed to the honey's antibacterial inhibition with a mean range of $14.54 \pm 2.0$mm to $17.58 \pm 3$mm, which was relatively higher than the antibiotics used (Gentamycin, Levofloxacin, Ampicillin, Tazobactum, Meropenem and Chloramphenicol). Control bacterial isolates ATCC 25923, ATCC 25922, ATCC 27736 and ATCC 27858 for *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* respectively, enhanced the standard in the analysis. The potency of honey from different botanical sources reveals important antimicrobial differences comparable to local antibiotics.Over and indiscriminate use of antibiotics has led to the emergence of multidrug-resistant bacterial strains, a global public health problem. Alternative antimicrobial strategies like plants and plant-based products such as honey need to be given more attention to solving this challenge. Hence the present study demonstrates that the composition of honey from honey bees (*Apis*) enables it to be proposed for prophylaxis and treatment of surface infections, which has traditionally been practiced in the management of wounds and burns.

**Keywords**: Bee Honey, Properties Validation

## 1.0 Introduction

According to Larsen et al. (2019), the honey bee is a social, flying insect within the genus *Apis* known for constructing perennial and colonial nests from secreted wax to store surplus honey. Among the strategies, humans employed in managing wounds was the application of humans that tracked back to about 8000 years ago and its antibacterial potency is influenced by floral sources and honey bee species (Pattamayutanon et al., 2017). Their precise chemical composition and physical properties in natural honey differ according to the plant species on which the bee foraged, the variety of vegetation available and the differences in climatic conditions (Pauliuc et al., 2020).

Wounds represent a simple or severe disorder to an organ or a tissue that spreads to other anatomical structures, in the presence of multiplying bacteria results in spreading cellular injury, eliciting an inflammatory response that needs to be reduced to aid the healing process (Norbury et al., 2016). In the United States, chronic wounds affect between 1.5% and 3% of the general population, 15% to 20.3% in China, and 14.8% overall incidence in Sub-Saharan Africa. In contrast, in Kenya, most injuries are implicated during drought seasons,

intertribal crushes, cattle rustling as well as attacks by wild animals, with the incidence rate of the surgical site and post-cesarean wound infections of 7.0% and 19%, respectively (Victor et al., 2013). The high treatment cost and the high poverty levels in Kenya make some wounds persist for months or years, placing a significant burden on the individual's health system. At the same time, other medications like corticosteroids and chemotherapeutic drugs are limited due to impaired wound healing (Ongarora, 2022). Antibiotics have been documented to possess various side effects like toxicities, which, combined with their over and misuse, is quite worrisome (Negut et al., 2018). Hence the local populations have been prompted to use various traditional methods like the medicinal plant, honey e.t.c to manage wounds. There is a need for scientific validation of such wound management strategies because they can possess lead compounds that can offer alternative Medicare that is cheaper and readily available.

## 2.0 Materials and Methods
### 2.1 Study Area
The study sampled the honey bee (*Apis mellifera*) honey samples from the semi-arid rangeland in Marigat sub-County in Baringo County, Kenya (Figure 1). Marigat sub-county is a home for most acacia, low trees and shrubs (latitude 00º26' to 00º32'N, longitude 36º00' to 36º09E and altitude 900 to 1,200 M above the sea level, agro-climatic zones IV &V, annual temp. & rainfall of 24.6º C & 671mm), according to Ednah et al. (2018).
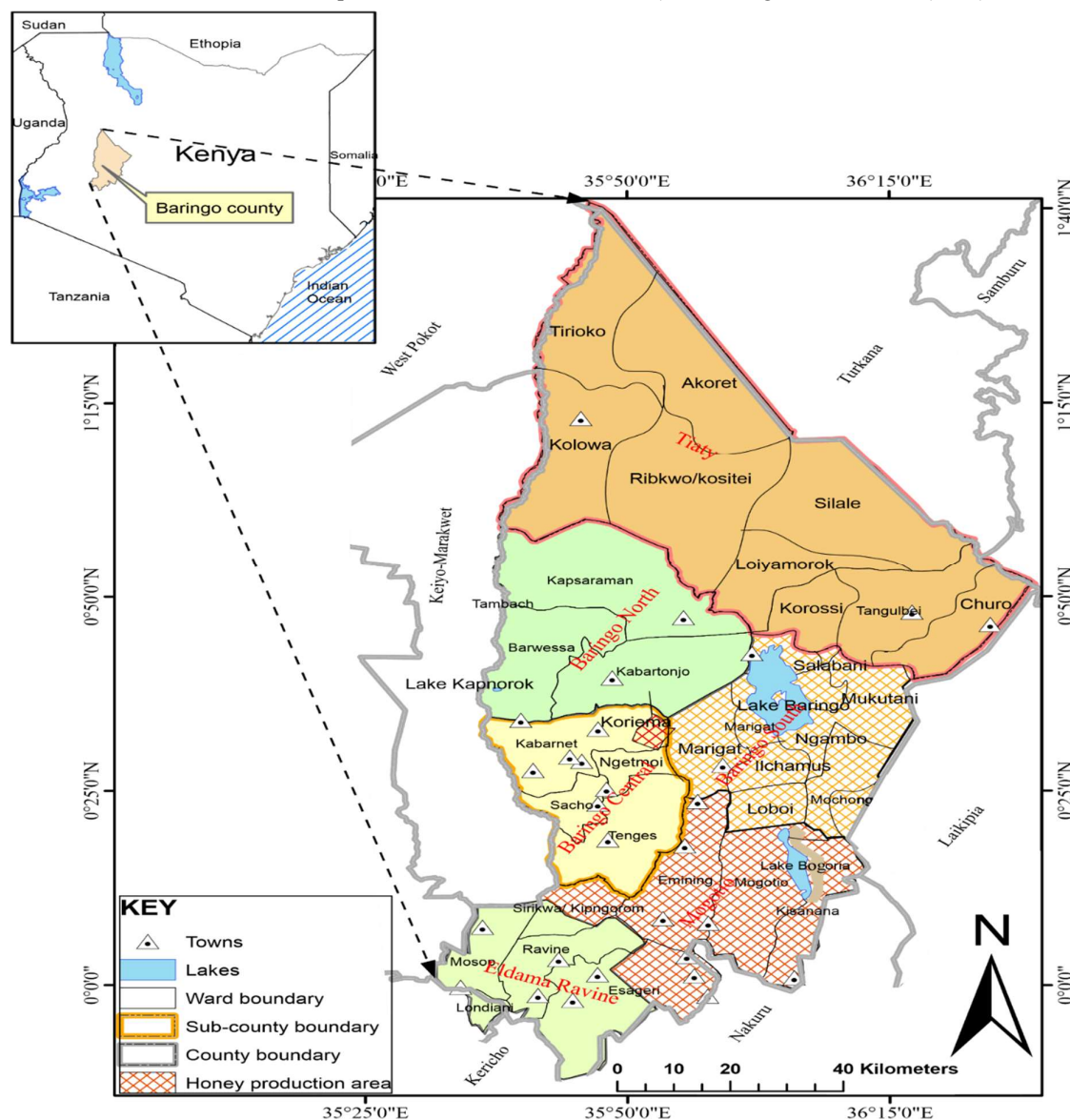


Figure 1: Geographical origin of the honey samples
Source: Baringo County Integrated Development Plan (BCIDP) 2018-2022

## 2.2 Theory / Calculation

### 2.2.1 Wound Bacterial Isolates

Bacterial isolates responsible for wound infections were obtained according to the established protocols by Mohammed et al. (2017). The sample size determination method for wound swabs employed for the target population was used by Smith (2015).

### 2.2.2 Honey Sampling Techniques

The honey samples were obtained from beehives using a smoker, which produced enough smoke to dull the bees' receptors to prevent the release of pheromones, making them less aggressive. The hive door was then removed and the thin bee wax covering the honey frames was scrubbed to expose the honey, which was then removed by draining into the straining cloth on top of a sieve into sterile containers.

The study adopted Kothari's (2004) sample size determination formulae to obtain the 26 honey samples for the study, as indicated in equation one.

$$N = \frac{Z^2 X P X Q}{D^2}, \quad N = \frac{(1.96)^2 X \left(\frac{1500}{19895}\right) X 1 - P)}{(0.05)^2} = \frac{103}{4} = 26 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots1$$

## 2.3 Analysis of the Physicochemical Properties of the Honey Samples

Analysis was done in accordance with the procedures described in the harmonized methods of the International Honey Commission (2009).

### 2.3.1 Determination of Sugar Content

The spectrophotometric method was used where 2.0g of the honey sample was mixed with 10ml dimethyl sulfoxide (DMSO) (25% v/v) followed by incubation in a water bath at 100ºc for 20 minutes. 0.5ml of the mixture was diluted with 9.5ml distilled water and after mixing, 0.5ml of phenol (5%) was added, followed by 2ml of 75% sulphuric acid ($H_2So_4$) slowly from the side of the test tube. The mixture was then transferred into a cuvette and the absorbance read at 492nm against a standard glucose solution.

### 2.3.2 Determination of Moisture in Honey Samples

The determination of moisture was done using a method described by Bogdanov et al. (2008). 2.0g of honey samples were weighed out and dried to a constant weight in a hot air oven at 70ºc and then cooled down and weighed again (oven-dry moisture content). The moisture content was then calculated by calculating the final dry weight difference from the initial weight.

### 2.3.3 Determination of Ph in Honey Samples

Lomiso et al. (2021) stated that a digital pH meter calibrated at pH 4 and 10 is normally used to determine honey's pH. Thus to determine the pH of honey, a digital pH meter calibrated at pH 4 and 10 was used in this study. A working honey solution was made by mixing 10g of honey in 75 ml of distilled water. The calibrated pH electrode and a stir bar were immersed into the solution and the pH reading on the meter was recorded.

### 2.3.4 Determination of Titratable Acidity in Honey Samples

The titratable acidity of the honey was determined using well-established protocols as used before by Makhloufi et al. (2020), where 10g of honey was dissolved in 75ml of distilled water, the calibrated reference electrode and a stir bar were put in the honey solution and titrated to pH 8.5 with 0.05M Sodium Hydroxide (NaOH) at a rate of 5ml/minute and recorded. 10ml of 0.05M NaOH was added into the solution and back titrated to pH 8.3 using 0.05 Molar (M) Hydrochloric Acid (MHCL) from a second burette and recorded. A blank determination procedure of 75ml distilled water was run to pH 8.5. Free acidity is titrable with sodium hydroxide up to the equivalent point.

### 2.3.5 Determination of Hydroxymethyl Furfural (HMF)

The spectrophotometric method of determination involving measurement of U.V. absorbance of honey solutions employed in this analysis was as used before by Escuredo et al. (2021). 5g of honey was dissolved in 25ml distilled water into a 50ml volumetric flask. 0. l5ml of Carrez solution I (15g potassium ferricyanide in 100 ml distilled water) and 0.5ml Carrez solution II (30g Zinc acetate in 100 ml distilled water) was added to precipitate proteins. The content was made up to 50ml with distilled water and filtered through a filter paper. After rejecting the first 10ml of the filtrate, the aliquots of 5ml were put in two test tubes. Then 5ml of distilled water was added to one tube (sample solution), 5ml of fresh 0.2% sodium metabisulphite solution (reference solution) was added to the second solution and the absorbance of the solutions at 284nm and 336nm was determined using a UV-Visible mini ± 1240 Shimadzu spectrophotometer.

### 2.3.6 Determination of Hydrogen Peroxide in Honey

Hydrogen peroxide in the honey samples was determined using a screening method by Kerkvliet (1996). 30% (w/v) concentration of honey at pH8 was prepared by weighing 3g of honey and mixing in 10 ml distilled water and adjusting the pH metre, followed by incubation in a water bath at 37ºc for 30 minutes. Hydrogen peroxide-specific test strips were immersed into the solution for one second, the excess solution was shaken off the strip and the colour developed read against a colour code to obtain the concentration of hydrogen peroxide in the honey samples.

## 2.4 Nutritive Analysis of Honey

### 2.4.1 Determination of Sugar Content

The sugar content determined among the honey samples was carried out using standard procedures where 2.0g of the honey sample was mixed with 10ml dimethyl sulfoxide (DMSO) (25% v/v) followed by incubation in a water bath at 100oc for 20 minutes. 0.5ml of the mixture was diluted with 9.5ml distilled water and after mixing, 0.5ml of phenol (5%) was added, followed by 2ml of 75% sulphuric acid ($H_2So_4$) slowly from the side of the test tube. The mixture was then transferred into a cuvette and the absorbance read at 492nm against a standard glucose solution.

### 2.4.2 Determination of Honey Protein Concentration

The determination of crude proteins in honey was done according to the Kjeldahl method as used before by Ferreira et al. (2009). 0.5grams of honey was transferred to the Kjeldahl tubes, 2.5gram of the catalytic mixture was added, followed by 7ml sulfuric acid, mixed and labelled. The labelled tubes were then placed in a block digester and the temperature was gradually increased from $50°c – 400°c$ for 5 hours. Then the water tap was opened to let the water flow above the sensor, which was heated to boil using the steam generator. The samples in the digestion tubes were then dissolved in 10ml distilled water and the heat was turned off. To a 125ml Erlenmeyer flask, 15ml of boric acid (5%) was put, followed by 5drops of the mixed indicator (Methyl red and Bromocresol green), which is red for acidic and green for basic, 20ml of sodium hydroxide (50%) was then added to the digestion tubes until the samples were neutralized to a dark blue colour.50 ml of standard hydrochloric acid (0.01M) was put in a burette and titrated directly into the Erlenmeyer flask containing the mixture, where the end-point of the titration was indicated by the solution changing colour to pink and the volume of HCL used was recorded as a means of calculating the protein content of the honey solution.

### 2.4.3 Determination of Vitamin C Content

Vitamin C content among the honey samples was carried out using standard procedures by Ciulu et al. (2011). 100mg of honey sample was put into a flask, then 10ml of metaphosphoric acid 1% was added and mixed with agitation for 45minutes at room temperature. The homogenous solution formed was then filtered through Whatman filter paper No. 4. 1 ml of the filtrate was mixed with 9ml 2, 6- dichlorophenolindophenol (DCPIP) 0.005% and the absorbance was measured within 30 minutes at 515nm wavelength using a spectrophotometer.

### 2.4.4 Determination of Water-Soluble Vitamins in Honey ($B_2$, $B_3$, $B_5$ and $B_9$)

The determination among the samples was carried out using the standard procedures by Farag et al. (2018). 10 grams of the honey sample was dissolved by stirring in 10ml distilled water then 1ml of sodium hydroxide (2M) was added and mixed well. 12.5ml of phosphate buffer 1M (pH5.5) was added and the solution topped up to the 50ml mark in a volumetric flask with distilled water. The sample solutions were injected through the filter in a spectrophotometer at the selected wavelengths, i.e., Vi tB2- 210nm, VitB3 – 254nm, VitB5 – 210nm and VitB9 – 210nm and the triplicate readings were recorded.

### 2.4.5 Determination of Calcium, Magnesium, Iron and Zinc in Honey

This analysis was done using Atomic Absorption Spectrometer (AAS) as used before by Mbiri et al. (2011). 10gram of honey was heated to 500°c to obtain a constant dry weight with an infrared lamp to prevent foaming. The ash samples formed were then dissolved in 10ml perchloric acid (60%) and 10ml nitric acid (65%). The sample solutions were then filtered by Whatman filter paper No. 4. The individual minerals were determined directly in the ash solution using an air–acetylene flame and a hollow cathode lamp for all four samples. The instrument's response was periodically checked with known standards for each mineral. Different wavelengths were used for individual studied metals, including copper- 324nm, Iron – 248nm, magnesium- 285nm and Zinc – 213nm.

### 2.4.6 Determination of Sodium and Potassium in Honey Samples

This analysis was carried out following the standard procedures by Horwitz (2000). 5 grams of honey was weighed out into a 15ml polypropylene flask, then 0.4ml of caesium chloride solution (5% m/v) and 10ul of hydrochloric acid was added and mixed well. N – Propanol was added to the solution up to the 10ml final volume with constant mixing to produce a visually homogenous solution. Internal standards were prepared by diluting ethanol and yttrium in the ratio 1: 10 m/v and were then measured at 371nm wavelength. 100ul of each sample was introduced into the flame with a pipette into a micro funnel connected to the pneumatic nebulizer, and the measurements of the samples were carried out in the flame emission mode under different wavelengths specific for the minerals under test. This included; 766nm and 589nm for potassium and sodium, respectively.

### 2.4.7 Determination of Phosphorous in Honey Samples

The analysis of phosphorous content among the honey samples was done according to the described method by Socha et al. (2009). 10gram of honey were put into a porcelain crucible and dissolved in 5ml nitric acid (1N). The solution was then placed in well-labelled tubes and heated in a water bath for 3 minutes to ensure proper dissolving and produce a homogenous solution. Distilled water was added up to the 100ml mark and the solution was filtered using Whatman filter paper. Then 5ml of the solution was put into 100ml volumetric flask, followed by the addition of 10ml of phosphate standard (0.1mg/ml), 10ml of nitric acid (6N), 10ml of ammonium molybdate (0.2%), and

10ml of ammonium molybdate (5%) and finally dilution to the 100ml mark with distilled water. The solution was allowed to stand for 15 minutes at room temperature to allow complete colour development then absorbance was measured at 400nm wavelength against a reagent blank for auto-zero and recorded.

## 2.5 Determination of Phytochemical and Antioxidant Properties of Honey
### 2.5.1 Total Content of Phenolic Compounds (TCPC)
Phenolic compounds in the honey samples were determined by a method by Isla et al. (2011). 5 grams of each honey sample was diluted to 50ml with distilled water and mixed through continuous agitation, then filtered through Whatman filter paper. 2.5ml of 0.2N folin-ciocalteu reagent was added and incubated at room temperature for 5 minutes, followed by 2ml of 75g/l Sodium Carbonate ($NaCo_3$) and incubated again at room temperature for two hours in the dark. Finally, the absorbance was measured at 760nm against a methanol blank spectrophotometer. Gallic acid was used as a reference standard and expressed as mg gallic acid equivalent (mgGAE).

Flavonoids' presence in honey samples was determined using a method by Hudzicki (2009). 0.5 grams of honey was mixed with 5ml of methanol 50% and filtered through Whatman No.1 filter paper. 5ml of the honey solution was mixed with 5ml of 2% aluminium chloride ($AlCl_3$) and incubated for 30 minutes at room temperature, after which the aluminium complex formed was measured at 420nm using U.V. Visible spectrometer and expressed in milligram of rutin equivalent (RE) in grams of honey

The total content of carotenoids was determined by use of a well-established protocol as used by Ciulu et al. (2011). 1gram of the honey sample was mixed with 10ml of n-hexane acetate mixture and homogenized thoroughly for 10 minutes at room temperature and then allowed to stand in the dark for 30minutes. Filtration of the solution through No.1 Whatman filter paper was done and the absorbance of the filtrate was measured at 450nm using a spectrophotometer. The total carotenoid content was expressed as mg of $\beta$ – carotene equivalents (mg $\beta$- carotene /kg of honey).

### 2.5.2 Determination of Vitamin C (Ascorbic Acid) Content
Ascorbic acid in the honey samples was determined using a well-established protocol by Ciulu et al. (2011). One hundred grams of the honey sample, 10ml 1% metaphosphoric acid at room temperature, was added and filtered through Whatman filter paper. 1ml of the filtrate was mixed with 9ml 2,6, dichlorophenolindophenol (DCPIP) 0.005% and the absorbance was measured within 30 minutes at 515nm.

## 2.6 Quality Assurance
Quality Control (Q.C.) measures were implemented in all the laboratory analyses to ascertain the study findings' reproducibility and reliability. The International Control Bacterial strains from American Type Culture Collection (ATCC), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 27736), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were included in the study according to the Clinical and Laboratory Standards Institute (CLSI).

## 2.7 Antimicrobial Activity Determination of Honey Compared to Conventional Antibiotics
### 2.7.1 Preparation of Honey Discs
50µl of various dilutions of honey bee samples ($10 \times 10^4$, $20 \times 10^4$, $50 \times 10^4$ and $75 \times 10^4$ µg/disc) were impregnated on Whatman No. 1 filter paper discs (6mm) and allowed to dry under sterile conditions.

### 2.7.2 Disc Diffusion Method
The disc diffusion (Kirby-Bauer) technique, as used before by Mama et al. (2019), was employed using Muller-Hinton agar (Hi-Media, India), which was prepared according to the manufacturer's instructions. An inoculum of each isolate was prepared by emulsifying two colonies of the isolates in 10ml distilled water and adjusting the turbidity to $1.5 \times 10^8$ CFU/ml. (According to the McFarland Standards).

As done before, a sterile cotton swab was used to inoculate the Muller-Hinton plates by spreading evenly over the agar surface Wasihun and Kasa (2016). The culture plates were allowed to dry for 15 minutes. After that, the discs with different concentrations ($10 \times 10^4$, $20 \times 10^4$, $50 \times 10^4$ and $75 \times 10^4$ µg/disc) of honey test samples and discs impregnated with the artificial honey samples (negative control) were placed on the plates using sterile forceps and pressed gently to ensure complete contact with agar. A distance of 15mm was maintained from the edges to prevent overlapping inhibition zones.

The inoculated plates were incubated at 4oC for 2 hours to allow pre-diffusion of honey into the media. They were then incubated at 37oC for 24 hours and the diameter of each zone of inhibition was measured. Cartridges containing commercially available antibiotics, including Levofloxacin (5µg), Ampicillin (10µg), Tazobactum (110µg), Meropenem (10µg), Gentamycin (10µg) and Chloramphenicol (30µg) were also used. Sterile Whatman No. 1 filter paper discs were also on the plates as the negative control. The inhibition zones around each of the discs were measured to the nearest millimetre (mm) using a ruler across three directions perpendicular to each other ($90^0$). The mean of the readings was calculated.

## 2.8 Evaluation of the Bacteriostatic and Bactericidal Activity of Honey

Bacteriostatic and bactericidal properties of the honey samples were determined according to the micro-dilution method as used by Kassaye et al. (2006). 5ml of nutrient agar was put in 8tubes, 5mg (5000µg) of honey was added to tube one and mixed well by vortexing to make a concentration of 1000µg/ml. This was serially diluted twofold using nutrient agar up to tube number 6 to obtain various ranges of concentration between 250µg/ml and 31.25µg/ml. Tube 7 was labelled G.C. and no honey was added. A volume of 100µg/ml of the bacterial inoculum was added into all tubes except tube 9 (H.C.), followed by incubation at 37°C for 24 hours, followed by physical examination for turbidity. The tubes containing the least concentration showing no visible growth were considered Minimum Inhibition Concentration (MIC) and the previous lower dilution tube were inoculated onto sterile nutrient agar plates by the streak plate method and incubated aerobically at 37°C for 24 hours. The smallest concentration that did not show growth was considered the Minimum Bacteriostatic Concentration (MBC).

## 2.9 Data Analysis

The data in this study was first processed using IBM SPSS (Statistical Package for Social Sciences) statistics and Two-way ANOVA through the Graph Pad Prism (Version 8.0.2-263) statistical software was used to compare mean values among the various experiments and to analyze their significant difference (P values < 0.005). Variables were considered significant by * and highly significant when P<0.01(**), P < 0.001 (***), P< 0.0001 (****).

## 2.10 Approvals

To ensure the required quality assurance, this study was approved by IREC (Ethics clearance for research proposal) and NACOSTI (National commission for science, technology and innovation), as attached in supplementary data S1 and S2, respectively.

## 3.0 Results

## 3.2 Physicochemical and Nutritive Properties of Honey

Honey bee samples were analyzed for various physicochemical and nutritive properties (pH, free acidity, moisture, sugar, water-soluble vitamins, minerals, vitamin C and crude proteins) expressed as mean range ± S.D. (mean average± S.D. The findings were compared to the Standards of Joint FAO/WHO Codex Alimentarius Commission (2001). This collection of written codes forms the current and main determinant of honey standards advocated for use by all nations.

*3.2.1 Hydromethyl Furfural (HMF)*

The honey samples indicated a mean HMF concentration of 15.65 ± 15.2 mg/kg. According to the International Regulations Joint FAO/WHO Codex Alimentarius Commission (2001), these samples were within the required range of 5mg/kg to 80mg/kg for honey from tropical temperatures. The results indicated that there were significant differences between the examined samples (P<0.05) for Hydromethylfurfural (HMF) contents, as shown in figure three.

*3.2.2 Sugar Concentration*

The honey samples recorded a mean sugar content of 90.13 ± 5.8g/100g. Honey samples from the arid – semi-arid regions (Maoi and Mukutani) recorded higher mean sugar levels (94.0 ± 1.26 g/100g) compared to samples from the medium altitude regions' (Koriema and Kibingor) (86.0 ± 5.02 g/100g). All the samples analyzed were within the values recommended by the Joint FAO/WHO Codex Alimentarius Commission (2001) of 60g/100g to 800g/100g. The findings indicated a significant difference between them (P<0.05), as shown in figure three.

*3.2.3 Moisture*

The honey samples indicated a moisture mean content of 114.28 ± 26.6 mg/g, within the range of 6.5 mg/g to 210mg/g as recommended by the joint FAO/WHO Codex Alimentarius Commission (2001). There was also a significant difference in the moisture content of the honey samples across the different regions (P<0.05), as illustrated in figure three.

*3.2.4 Ph and Free Acidity*

The honey samples from honey bees had a mean pH value of 3.70 ± 0.10, which was within the recommended limit of 3.2 to 4.5 according to the Joint FAO/WHO Codex Alimentarius Commission (2001). The mean free acidity for the honey bee samples was 0.064 ± 0.005meq/kg, within the recommended levels of less than 50meq/kg according to Joint FAO/WHO Codex Alimentarius Commission (2001). The samples indicated a mean free acidity level and a control mean level of 0.03 ± 0.01 meq/kg, indicating low water content. Thus the absence of fermentation and no significant differences in free acidity and pH between them (P>0.05), as shown in figure three.

*3.2.5 Hydrogen Peroxide*

The honey samples indicated a hydrogen peroxide concentration of 0.62 ± 0.25mM. Honey samples from the Mukutani region recorded the highest mean concentration of 1.25 ± 0.35mM compared to the other areas. They were within the recommended range of 0.5 and 2.5mM, according to the Joint FAO/WHO Codex Alimentarius

Commission (2001). According to the analysis, there was no significant difference in hydrogen peroxide concentration in honey samples from the different sampling regions, as indicated in figure three.
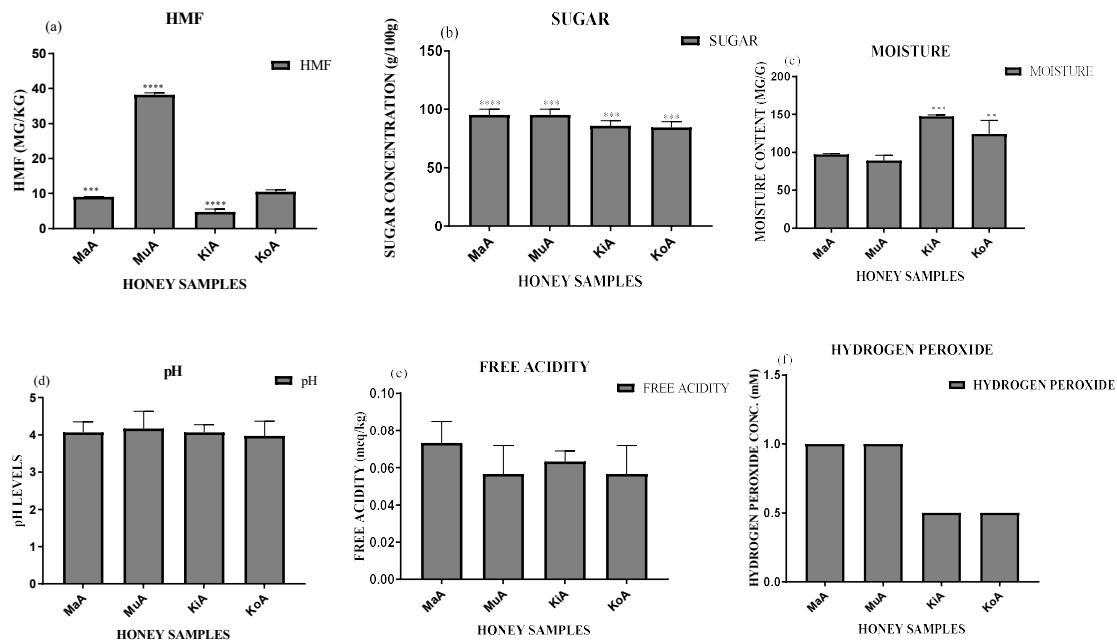


Figure 3: Physicochemical and nutritive properties of honey samples from honeybee (*Apis*); Hydromethylfurfural – HMF (a), Sugar (b), Moisture (c), pH (d), Free acidity (e) and Hydrogen peroxide (f). The values are represented in mean ± S.D. as error bars represent Standard deviation (S.D.). Stars represent significant values (P<0.05) on the bars (*P< 0.05, **P<0.01, ***P<0.001 and **** P<0.0001). (KEY: MaA – Maoi Apis, MuA – Mukutani Apis, KiA – Kibigor Apis, KoA- Koriema Apis).

### 3.2.6 Water-Soluble Vitamins

According to the study's findings in this study, honey contains water-soluble vitamins such as Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5 and Vitamin B9 in the following respective mean range concentrations; $0.21 \pm 0.03$ to $0.64 \pm 0.02$mg/l (mean average $0.40 \pm 0.16$mg/l), $0.72 \pm 0.03$ to $2.39 \pm 0.07$ mg/l (mean average $1.32 \pm 0.62$ mg/l)), $0.65 \pm 0.5$ to $4.19 \pm 0.02$ mg/l (mean average $2.65 \pm 1.07$mg/l ), $0.01 \pm 0.0$ to $1.22 \pm 0.03$mg/l (mean average $0.50 \pm 0.52$mg/l) and $0.11 \pm 0.00$ to $1.64 \pm 0.10$ mg/l (mean average $1.16 \pm 0.52$mg/l ). The highest vitamin concentration was vitamin B3, followed by vitamin B2, then vitamin B9, Vitamin B5, and finally Vitamin B1, with mean vitamin concentrations of $1.32 \pm 0.92$mg/l. All the honey samples under investigation in the study contained detectable vitamin levels, with high Vitamin $B_1$ and $B_3$ levels recorded in samples from Maoi. In contrast, high Vitamin $B_1$, $B_2$ and $B_9$ levels were recorded from Kibingor, as shown in figure four. These findings were within the Limit of Detection (LOD) approved by the International Union of Pure and Applied Chemistry of 1.10 mg/kg to 1.75mg/kg. Still, though it is not vitamin-rich food, honey can replace the water-soluble vitamins continuously removed from the human body through urine. This analysis also indicated that Vitamin $B_1$ and $B_2$ had no significant differences (P>0.05), while Vitamin $B_2$, $B_3$ and $B_9$ were significantly different (P<0.05).
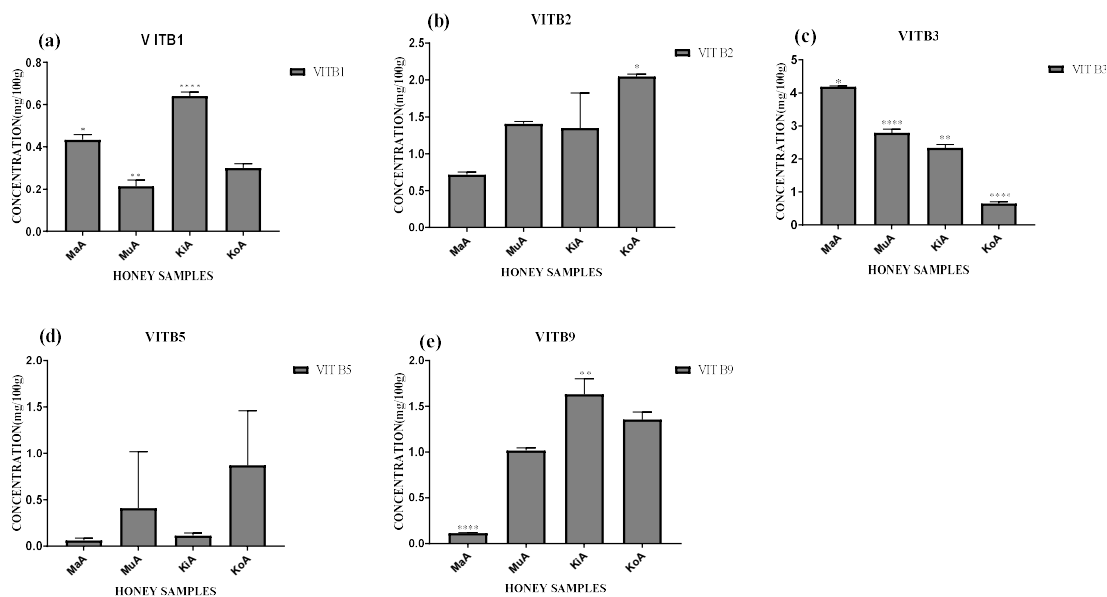
Figure 4: Physicochemical and nutritive properties of honey samples from honeybee (*Apis*); Vitamin $B_1$ (A), Vitamin $B_2$ (B), Vitamin $B_3$ (C), Vitamin $B_5$ (D) and Vitamin $B_9$ (E). The values are represented in mean ± S.D. as error bars represent Standard deviation (S.D.). Stars represent significant values ($P<0.05$) on the bars (*$P<0.05$, **$P<0.01$, ***$P<0.001$ and **** $P<0.0001$) (KEY: MaA– Maoi Apis, MuA – Mukutani Apis, KiA– Kibigor Apis, KoA - Koriema Apis).

### 3.2.7 Minerals

All the honey samples analyzed contained different quantities of minerals (sodium, Iron, Zinc, magnesium, calcium and phosphorous), with potassium being the most abundant mineral component across all regions with a mean concentration of $16.17 \pm 2.6$ to $20.37 \pm 1.7$mg/l (mean average $17.78 \pm 1.29$mg/l). Calcium was deduced to be the 2nd in abundance among the honey samples with a range mean value of $1.33 \pm 0.02$ to $2.32 \pm 0.08$mg/l (mean average $1.86 \pm 0.33$mg/l). This was followed by sodium, Iron, Zinc, magnesium and phosphorous in the following concentrations; $0.37 \pm 0.23$ to $2.47 \pm 1.02$mg/l (mean average $1.43 \pm 0.96$mg/l), $0.36 \pm 0.11$ to $2.16 \pm 0.40$mg/l (mean average $1.33 \pm 0.67$mg/l), $0.27 \pm 0.06$ to $0.44 \pm 0.07$ mg/l (mean average $0.41 \pm 0.06$mg/l), $0.09 \pm 0.02$ to $0.74 \pm 1.36$mg/l (mean average $0.34 \pm 0.19$ mg/l) and $0.056 \pm 0.02$ to $0.21 \pm 0.02$mg/l (mean average $0.12 \pm 0.05$ mg/l) respectively as shown in figure five.

### 3.2.8 Crude proteins

The bees mostly secrete proteins in honey through the salivary and hypopharyngeal glands. Their composition may result from different bee origins and the flower nectar used by them. The honey samples contained detectable amounts of proteins giving an average value of $0.72 \pm 0.23$g/100g, as shown in figure five, which was slightly higher than the recommended range of 0.9 to 0.5g/100g according to Watson and Zibadi (2013). Very high levels in wounds can either slow or stop healing by destroying the growth factors and the fibronectin in the wound necessary for the activation of fibroblast and migration of epithelial cells Mandal and Mandal (2011). The protein content of the honey samples was significantly different ($p<0.05$).
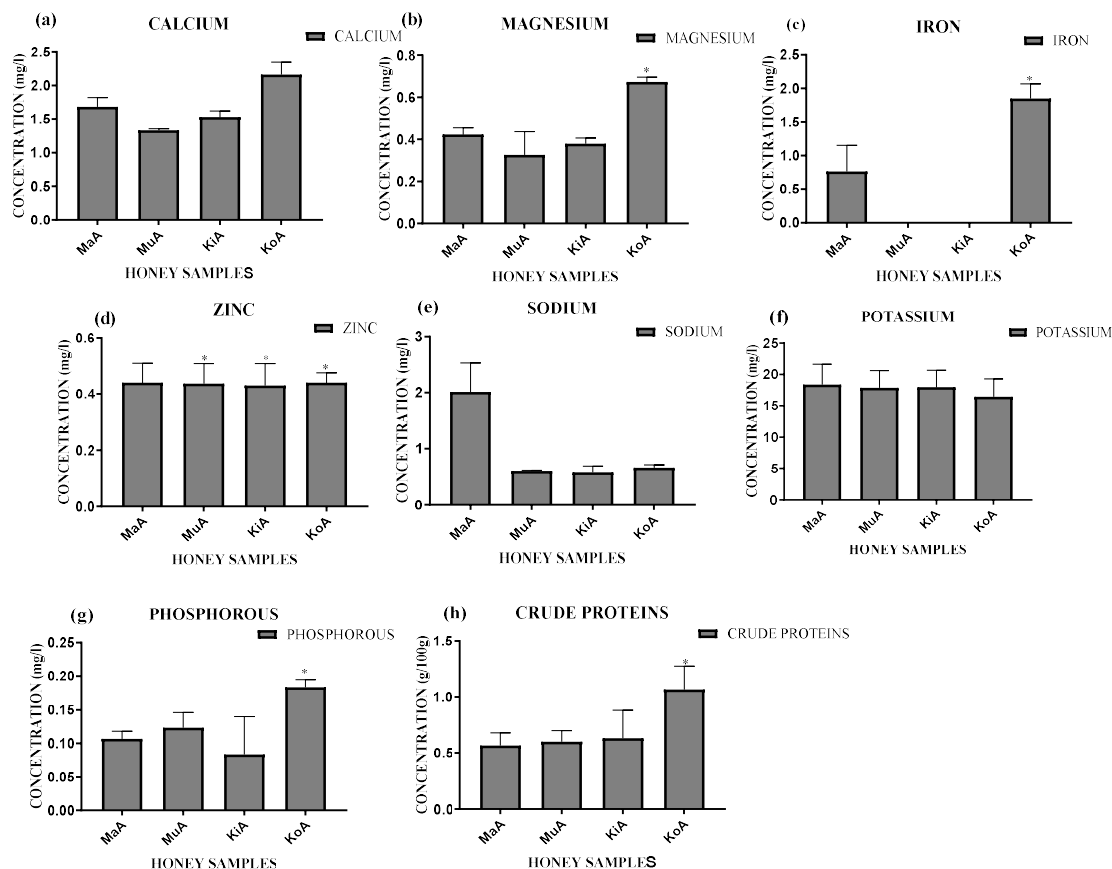
Figure 5: Physicochemical and nutritive properties of honey samples from honeybee (*Apis*); Calcium – ca (a), Magnesium – mg (b), Iron – fe (c), Zinc – zn (d) Sodium – Na (e), Potassium - K (f), Phosphorous - P (g) and Crude proteins (h). The values are represented in mean ± S.D. as error bars represent Standard deviation (S.D.). Stars represent significant values ($P<0.05$) on the bars (*$P< 0.05$, **$P<0.01$, ***$P<0.001$ and **** $P<0.0001$) (KEY: MaA – Maoi Apis, MuA – Mukutani Apis, KiA – Kibigor Apis, KoA- Koriema Apis).

## 3.3 Phytochemical Properties of Honey

The phytochemical components of honey analyzed (Total flavonoid, Total phenolic, Vitamin C and Total carotenoid content) were expressed as mean range ± S.D. (mean average± S.D.). All the findings were compared to the Standards of the joint FAO/WHO Codex Alimentarius Commission (2001). The honey samples analyzed contained a mean content of Total Phenolic, Flavonoid and Carotenoid of 80.81 ± 36.25 mgGAE/100g, 21.83 ± 6.16mgRE/100g and 4.41 ± 0.27 mgβcarotene respectively. The mean values obtained indicated that the mean phytochemical components of the honey samples were within the recommended levels according to Joint FAO/WHO Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme and World Health Organization (2001) of total phenolic content (161mgGAE/100g to 186 mgGAE/100g), total flavonoid content (15.6 mgRE/100g to 54.23 mgRE/100g), total carotenoid content (1.29 mgβcarotene to 11.6 mgβcarotene) and Vitamin C (0 to 18mg/100g). These findings prove that all the investigated honey samples from Marigat are a good source of phenolic acids, flavonoids, Vitamin C and Carotenoids, which confer their good antioxidant potential and their concentrations indicated a significant difference across the different regions, as shown in figure six.
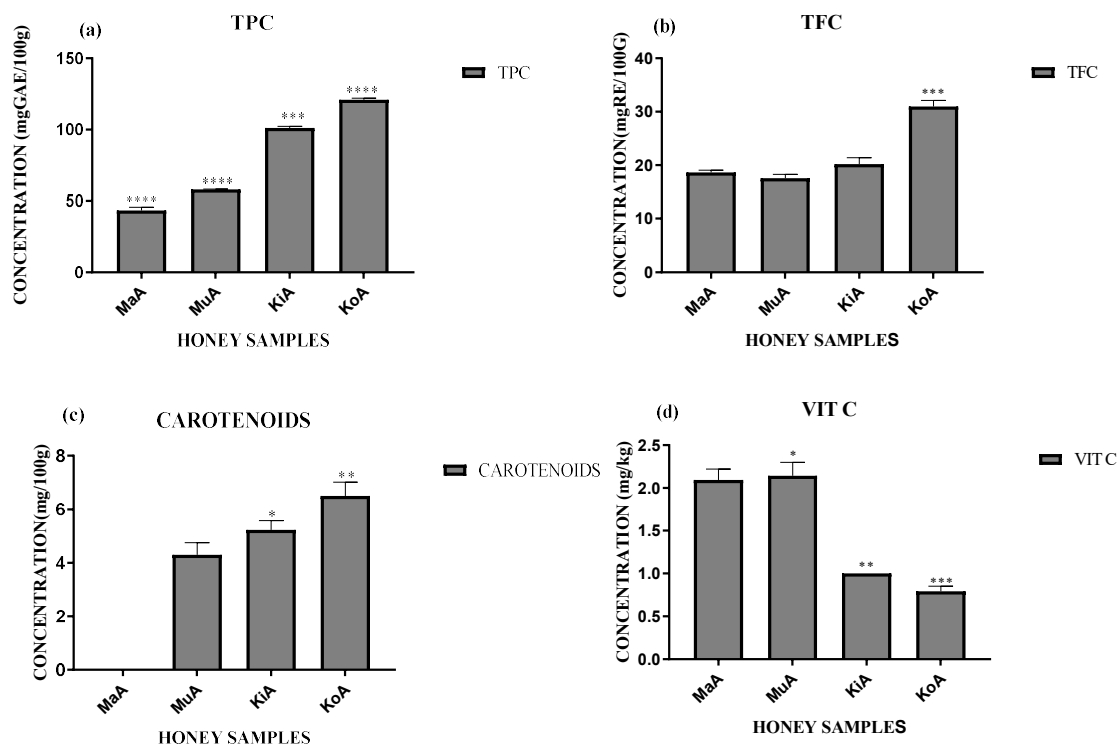
Figure 6: Phytochemical properties of honey samples from honeybee (*Apis*); Total flavonoid content – TFC (a), Total phenolic content – TPC (b), Carotenoids (c) and Vitamin C (d). The values are represented in mean ± S.D. as error bars represent Standard deviation (S.D.). Stars represent significant values (P<0.05) on the bars (*P< 0.05, **P<0.01, ***P<0.001 and **** P<0.0001) (KEY: MaA – Maoi Apis, MuA – Mukutani Apis, KiA – Kibigor Apis, KoA- Koriema Apis).

## 3.4 Antibacterial Efficacy of Honey Samples and Selected Antibiotics Against Isolates Obtained from Wounds and Burns

### 3.4.1 Disc Diffusion

The antibacterial efficacy of the honey samples was compared with those of commonly used antibiotics (Levofloxacin (5 µg), Ampicillin (10 µg), Tazobactam (110 µg), Meropenem (10 µg), Gentamicin (10 µg) and Chloramphenicol (30 µg)), compared to sterile discs impregnated with varying dilutions ($10x10^4$, $20x10^4$, $50x10^4$ and $75x10^4$ µg/disc) of the honey samples. The antibacterial potency of the honey samples was indicated by the production of clear zones in concentrations of $50x10^4$ and $75x10^4$ µg/disc, with minimal inhibition or non for the concentrations of $10x10^4$ and $20x10^4$ µg/disc. The honey samples produced great bacterial inhibitions against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli* shown in table one.

Table 1: Mean inhibition of the honey dilutions ($10\times10^4$, $20\times10^4$, $50\times10^4$ and $75\times10^4$ µg/disc) against the bacterial isolates

| | | | HONEY SAMPLES (*Apis*) | | |
|---|---|---|---|---|---|
| ISOLATES/CONC. $10\times10^4$ | | MuA | MaA | KiA | KoA |
| *S. aureus* | 10 | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.10 \pm 0.00$ |
| | 20 | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.10 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 50 | $15.28 \pm 0.01$ | $13.47 \pm 0.01$ | $12.62 \pm 0.19$ | $18.38 \pm 0.01$ |
| | 75 | $18.45 \pm 0.02$ | $18.58 \pm 0.60$ | $15.90 \pm 0.29$ | $23.60 \pm 0.54$ |
| *E.coli* | 10 | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 20 | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 50 | $6.49 \pm 1.09$ | $6.84 \pm 0.07$ | $9.35 \pm 0.01$ | $9.66 \pm 0.29$ |
| | 75 | $9.43 \pm 0.58$ | $9.24 \pm 0.00$ | $12.18 \pm 0.12$ | $12.47 \pm 0.23$ |
| *K. pneumoniae* | 10 | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 20 | $6.00 \pm 0.00$ | $6.10 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 50 | $16.74 \pm 0.31$ | $16.25 \pm 0.0$ | $12.58 \pm 0.48$ | $10.07 \pm 0.39$ |
| | 75 | $17.34 \pm 0.33$ | $17.35 \pm 0.51$ | $15.53 \pm 0.00$ | $14.19 \pm 0.19$ |
| *P. aeruginosa* | 10 | $6.20 \pm 0.00$ | $6.00 \pm 0.00$ | $6.10 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 20 | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ |
| | 50 | $11.80 \pm 2.18$ | $18.60 \pm 0.26$ | $19.42 \pm 0.07$ | $16.65 \pm 0.70$ |
| | 75 | $14.67 \pm 0.83$ | $21.46 \pm 0.39$ | $20.20 \pm 0.00$ | $18.63 \pm 0.17$ |

## 6.3 Bacteriostatic and Bactericidal Activity of Honey

Various dilutions of honey bee honey samples (Neat, 500, 250, 125, 62.5, and 31.25µg/ml ) were subjected against the bacterial isolates from infected cutaneous wounds and burns to determine both the bacteriostatic (MIC) and bactericidal (MBC) activities as shown in table two. The MIC activity of the honey samples against all the isolates was shown at 62.5 µg/ml, while the MBC activity was at 250 µg/ml. The findings did show that it has an optimum bacteriostatic activity on the test isolates that ranged from a mean of $140.6 \pm 7.86$µg/ml as MIC and $187.5 \pm 7.22$µ/ml as its MBC.

Table 2: Minimum bacteriostatic and bactericidal concentration –MIC/MBC (mg/ml) of the different concentrations of honey bee honey samples (500, 250, 125, 62.5, and 31.25µg/ml).

| | *Staphylococcus aureus* | | *Escherichia coli* | | *Pseudomonas aeruginosa* | | *Klebsiella pneumonia* | |
|---|---|---|---|---|---|---|---|---|
| | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) | MIC (µg/ml) | MBC (µg/ml) |
| **MuA** | 125 | 250 | 250 | 250 | 125 | 250 | 125 | 250 |
| **MaA** | 62.5 | 62.5 | 250 | 250 | 125 | 250 | 250 | 250 |
| **KiA** | 125 | 125 | 250 | 250 | 125 | 250 | 250 | 250 |
| **KoA** | 125 | 250 | 250 | 250 | 125 | 125 | 250 | 250 |

KEY: MIC – Minimum inhibition concentration, MBC – Minimum Bactericidal concentration, MaA – Maoi Apis, MuA - Mukutani Apis, KiA- Kibingor Apis, KoA - Koriema Apis).

## 4.0 Discussion

The use of traditional and herbal medicine to treat infections has been practiced since humanity's origin as the only treatment option. Various plants and their extracts have been used for treatments requiring antimicrobial activity and the most popular substance described in ancient medicine is honey (Yupanqui et al., 2022). This is the natural sweet substance obtained from the secretions of the living parts or excretions of plants which the honey bees (*Apis mellifera*) collect and store.

The honey samples' various physicochemical properties (sugar, pH, Moisture, HMF, Hydrogen peroxide and free acidity) were analyzed due to their role in maintaining a hostile environment unsuitable for bacteria to thrive in the wounds. Similar to the study's findings was a study done by Noori et al. (2013), who obtained a pH of 3.47 and 3.76 and an HMF concentration of 15.36 mg/kg on honey samples collected from Kerala, India, as well as Dhahian and Talah honey samples in NewYork. These components work synergistically to give honey its

antimicrobial properties, but contrary to these findings, the results by Alvarez-Suarez et al. (2010) on Saudi and Kashmiri honey samples that recorded a lower moisture content of $14.73 \pm 0.36$ to $18.32 \pm 0.67$mg/g and a lower sugar content of $72.36 \pm 0.32$ g/100g. These differences could be due to variations based on the botanical origins, handling and storage conditions.

Phytochemicals, mainly phenolics present in honey in small quantities, were also determined since they confer honey's flavour, appearance and bioactivities through antioxidant activity. The study's results differed from those of previously reported values of 0.32 and 3.70mgβ carotene /kg by other studies, including Ciulu et al. (2011). Contrary to the study's findings, lower flavonoids and phenolic contents were recorded by Cucu et al. (2021), with lower moisture levels recorded in the results of Deng et al. (2018). This indicates that honey's concentration of phenols which are the main contributors to its health-promoting properties varies depending on its botanical and geographical origin.

According to this study, the antimicrobial property of honey increased with increasing concentrations of the specific honey samples, as indicated in table one. Similar dose-dependent antibacterial behaviour of honey was also observed by Ghramh et al. (2019) while studying the antibacterial potential of honey from different nectars. The study results were in agreement with Wright et al. (2021), who showed that strains of Pseudomonas aeruginosa were naturally resistant to routinely used and higher antibiotics but were very sensitive to the antibacterial action of honey. Therefore, the variation in honey's antibacterial potential could depend on its botanical and geographical source, storage conditions and the metabolism of honey bees (Usanga et al., 2020).

Honey bee samples also act synergistically with some currently used antibiotics by showing comparable inhibitions against all the bacterial isolates, as Hegazi (2011) indicated. Similar to the study's findings was the study by Khalil et al. (2013). who reported the antibacterial activity of Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia by indicating high inhibition against tetracycline (50μg). Similarly, Maddocks and Jenkins (2013) evaluated the synergistic effect of honey against six Gram-negative and six Gram-positive bacterial isolates compared to Cefotaxime (30ug/disc), ciprofloxacin (5ug/disc) and tobramycin (30ug/disc). They deduced that honey samples had higher antibacterial activity indicated by significantly high growth inhibition. Honey has good antibacterial activity against numerous microorganisms of different genera and no honey-resistant phenotypes have yet emerged. Its unique property lies in the ability to fight infection on multiple levels making it more difficult for bacteria to develop resistance. Unlike conventional antibiotics, honey doesn't target the essential growth processes of bacteria, which mostly results in bacteria building up resistance to the drugs (Mohapatra et al., 2010).

The study also showed that honey samples from honey bees (Apis) have antibacterial activity (bacteriostatic and bactericidal effect), similar to antibiotics against test organisms. This was in tandem with the findings of other researchers, Wasihun and Kasa (2016), who indicated that honey provides an alternative therapy against certain bacteria. The mean MIC of the analyzed honey samples was at 6.25μg/ml, similar to the findings of Borges et al. (2013) on red and white honey obtained from the Eastern zone of Tigray as well as both raw and processed honey collected from rural areas of Western U.P., Indian. Contrary to this study were the findings of Zainol et al. (2013), who reported high MIC values of 500μg/ml, 1500μg/ml and 1750μg/ml for *P. aeroginosa*, *E. coli* and *S.aureus* with an MBC of 5250μg/ml. Lower MIC values of 5μg/ml and 25μg/ml and MBC values of 6.25μg/ml and 50μg/ml on gelam and pineapple honey against *S.aureus* and *E.coli* were also reported by Bussmann et al. (2010).

**5.0 Conclusion**

The present study adds another scientific clue to the large body of data confirming the ability of honey from honey bees (*Apis mellifera*) to inhibit human pathogens that are susceptible or resistant to antibiotics. In addition, the antimicrobial properties of honey tested in this study do not differ much from those of samples collected from other areas. Hence it is clear that regardless of the area of origin or plant sources, honey poses a considerable antimicrobial activity potent against multi-resistant pathogens. This will pave the way to isolate the most active constituents of honey to be synthesized as medicine for future use.

**Conflict of interests**

The authors have not declared any conflict of interest.

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