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Antibacterial Activity of Honey from Wild Species of Stingless Bees; *Plebenia hylderbrandii* and *Meliponula bocandei*

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Abstract

One of the serious problems the world is facing today is the antimicrobial resistance on available antibiotics by most bacterial pathogens and the rising cost of finding effective antimicrobial agents. In recent years, efforts to find new drugs especially from natural sources have been boosted by the demand for an effective cure for infectious diseases. Only the antibacterial activity of *apis mellifera* honey and not stingless bee honey from western Kenya has been reported. This study was therefore carried out to determine the effect of *Plebenia hylderbrandii* and *Meliponula bocandei* honey samples on the growth of control; sensitive cases of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). Different honey concentrations (1.18% - 17.65% v/v) of the two samples were tested against the two micro-organisms. The samples were screened for their antibacterial potential against *Escherichia coli* and *Staphylococcus aureus* by agar well dilution method. The Partial inhibitory concentration (PIC), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined by *in vitro* method. The inhibitory effect of *Plebenia hylderbrandii* honey on *E. coli* and *S. aureus* growth was apparent at concentrations 3.53% and 1.76% (v/v) respectively. On the other hand, the inhibitory effect of *Meliponula bocandei* honey on *S. aureus* growth was at concentration 16.47% (v/v). *Plebe-*

nia hylderbrandii honey had bactericidal effect on both *E. coli* and *S. aureus* at concentrations 4.71% and 2.35% (v/v) respectively. However, *Meliponula bocandei* honey exerted bactericidal effect on *S. aureus* only at 16.47% (v/v) concentration. *Plebenia hylderbrandii* honey had higher antibacterial potency and can be a potential source of antibacterial substances. Moreover, the honeys tested in this study showed great antibacterial potential for *S. aureus*.

Keywords

Honey, Antibacterial Activity, Partial Inhibition Concentration (PIC), Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)

1. Introduction

Honey is one of the natural products whose usage has become a popular approach in both food preservation and medical treatments as a result of its low toxicity and potent activity. This has been attributed to the ineffectiveness of antibiotic drugs in curing diseases which have triggered the need for alternative antimicrobial control strategies in re-evaluation of therapeutic usage of ancient therapies including honey [1]. World Health Organization (WHO) statistics indicate that approximately 80% of the total population in various countries has used natural products in their principal health care [2]. For instance, in Ethiopia, China and India, nearly 80 percent of the total population depend on traditional therapies as a prime health care source [3] [4]. These natural products can be applied in the discovery and development of novel antimicrobial drugs which can be used in treating infectious diseases. Researchers have shown that these natural materials are normally more suitable and effective to consumers, hence leading to reduction on reliance to synthetic substances [4]. Moreover, the study of the natural compounds may result to active components' discovery that could prevent environmental hazards or ameliorate disease process in human cells [5]. Natural products as opposed to synthetic drugs experience less resistance from the target infectious agents [6].

These include medicinal plants of non-antibiotic drugs with antibacterial potential which have been discovered by scientists having the ability to combat such bacterial resistances towards antibiotics [7] and acting as alternative natural remedies for disease management. In addition to the medicinal plants, honey, a naturally available product, has been proven to possess antibacterial activity against numerous life-threatening bacteria [8], being effective against a variety of inflammatory cases and bacterial infections [9]. It has been used traditionally in treatment of gastrointestinal infections, respiratory diseases, and other various infections such as skin ulcers, burns and wounds [1]. Previous studies on its physicochemical parameters, chemical composition and antimicrobial activity has demonstrated its therapeutic potential [10] [11]. Its high viscosity provides

barrier to inhibit infection while its low pH level and high sugar content prevents microbes' growth [12]. Its moisture content determines its ability to persist stability and to resist decay by yeast fermentation [13]. Besides, its electrical conductivity is related to organic acids, mineral content, some complex sugars, etc. [14]. Honey's color intensity shows presence of pigments such as flavonoids and carotenoids, which are well-known to contribute to its antioxidant activity [15]. This in return contributes to its antibacterial activity. Honey possesses glucose oxidase and osmotic properties particularly presence of polyphenols and defensin-1, which contributes to its bactericidal effect [16] [17]. Honey also exhibits both bactericidal and bacteriostatic effects against gram-negative as well as gram-positive bacteria and shows antifungal activity [18].

According to Lusby *et al.* [12], antimicrobial activity of honey varies depending on hydrogen peroxide present. The variation also results from the bee species, concentration and type of honey, floral and entomological origin in different seasons, pathogen tested and external factors such as type of soil, climate, and the maturation period [19]. In western part of Kenya, only antibacterial activity of *Apis mellifera* honey has been documented and nothing on stingless bee honey has been reported therefore the study was set to scientifically evaluate the *in vitro* antibacterial activity (bactericidal & bacteriostatic effects) of honeys produced by stingless bees *Plebenia hylderbrandii* and *Meliponula bocandei* against *E. coli* and *S. aureus*. These bacteria are commonly involved in causing urinary tract infections, diarrhea, septicemia, wound infections, and community acquired and nosocomial infections in humans. Besides, this is the first time these two honeys are being investigated for antibacterial activity.

2. Materials and Methods

2.1. Study Design

An experimental study was conducted to determine the antibacterial activity of honey obtained from two stingless bee species *Plebenia hylderbrandii* and *Meliponula bocandei* against *E. coli* and *S. aureus*. (12 × 2 × 2) factorial experiment was organized in completely randomized design (CRD). The factors include; 12 concentrations; 1.18%, 1.76%, 2.35%, 2.94%, 3.53%, 4.71%, 5.88%, 7.06%, 8.24%, 9.41%, 10.59% and 11.76% (v/v) for *Plebenia hylderbrandii* honey and 4.71%, 5.88%, 7.06%, 8.24%, 9.41%, 10.59%, 11.76%, 12.94%, 14.12%, 15.29%, 16.47% and 17.65% (v/v) for *Meliponula bocandei* honey; 2 bacteria (*E. coli* & *S. aureus*) and 2 honey samples (*Plebenia hylderbrandii* & *Meliponula bocandei*). The varying concentrations between the two samples were due to differences in inhibitions of the samples against the microorganisms tested.

2.2. Study Area and Period

The experimental study was carried out at Masinde Muliro University of Science and Technology (MMUST) Microbiology Laboratories from December 2019 to January 2020.

2.3. Sampling

The two honey samples (*Plebenia hylderbrandii* and *Meliponula bocandei*; 500 ml each) were obtained from the Centre for African Medicinal and Nutritional Flora and Fauna (CAMNFF) in MMUST in Kakamega County, Kenya. Honey was collected in sterile bottles and taken to the Microbiology laboratory, MMUST for analysis.

2.4. Sample Preparation

Twelve concentrations of each honey sample were prepared using DMSO with adequate mixing to determine the antibacterial activity. A stock solution of 11.76% (v/v) was prepared for *Plebenia hylderbrandii* honey from which the lower concentrations (1.18%, 1.76%, 2.35%, 2.94%, 3.53%, 4.71%, 5.88%, 7.06%, 8.24%, 9.41%, 10.59%) were made. Similarly, a stock solution of 17.65% (v/v) was prepared for *Meliponula bocandei* honey and the lower concentrations (4.71%, 5.88%, 7.06%, 8.24%, 9.41%, 10.59%, 11.76%, 12.94%, 14.12%, 15.29%, and 16.47%) prepared from it. The honey samples were prepared in triplicate for all the concentrations.

2.5. Bacterial Strains and Media

The bacterial strains (sensitive standard culture collections) used in this study were acquired from the Department of Medical Microbiology Laboratory, University of Nairobi. They included Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 25923). These bacteria were identified by standard bacteriological techniques following Harley [20]. The Nutrient Broth and Nutrient Agar (Himedia) used in the experiments were prepared according to the manufacturer's instructions.

2.6. Inoculum Preparation

The bacterial cultures were been maintained on nutrient agar slants stored at 4˚C prior to subculture. The bacterial strains (*E. coli* & *S. aureus*) were sub-cultured into Tryptone-Soy Broth (Himedia) and incubated at 37˚C for 6 hours. The culture age was at the exponential (logarithmic) phase. The bacterial growth was harvested using saline sterile water, its absorbance adjusted at 600 nm to viable cell count of 10^4 CFU/spot using a spectrophotometer (UV-1100 Spectrophotometer, China).

2.7. Antibacterial Activity of Honey Samples

2.7.1. Determination of PIC

The antibacterial activities for the honeys were determined by agar dilution method as described by [21]. The honey samples were prepared as described by Mandal *et al.* [1]. Honey was stored in the dark at room temperature until used for antibacterial assays. Under aseptic conditions, different honey concentrations were prepared for the two honey samples as described above. Molten nutrient agar was distributed in 15 ml each in sterile culture tubes and autoclaved

at 121˚C for 15 minutes. Honey at different concentrations was put in sterile culture plates and the sterile molten agar at 45˚C was dispensed into the respective culture plates and swirled for uniform mixing. The mixture was left to set, followed by inoculation of the microorganisms (10^4 CFU/spot) on the culture plates by use of a sterile transfer loop. The plates were then incubated for 24 hours at 37˚C. A nutrient agar plate with DMSO (without honey) was similarly inoculated as negative control, whereas a nutrient agar plate with ampicillin was used as positive control. Ampicillin was a preferred a control in this study because it covers a variety of infections, including those of the respiratory and urinary tracts, septicaemia and enteric infections. The microorganisms used were sensitive standard microorganisms /controls of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The experiments were done in triplicate, and results reported in minimum growth (+), moderate growth (++), heavy growth (+++) and no growth (-) of bacteria on the agar plates to determine the partial inhibitory concentrations (PIC).

2.7.2. Determination of MIC and MBC

Minimum Inhibitory Concentration (MIC)

MIC of the antibacterial agents for the isolates was determined by tube dilution method as described by Mama *et al.* [17]. Concisely, 11 sterile test tubes were positioned in the rack, labelled from test tube 1 to 8. Growth control tube (GC), honey control tube (HC) and broth control tube (BC) was used as quality controls. One (1) ml of the freshly prepared nutrient broth was put in each tube, sterilized and cooled. Then 1 ml of neat honey solution was put in test tube 1 and HC using sterile micropipette and tips. Twofold serial dilution was accomplished by transferring 1 ml from test tube 1 into test tube 2 with discrete sterile micropipette and tips and then vortexed for homogenization. After comprehensive mixing, one milliliter was transferred by use of other sterile micropipette and tips from test tube 2 into test tube 3. This procedure was continually performed until the dilution of 1:128 was reached in the 8^{th} test tube. Finally, 1ml was obtained from test tube 8 and discarded. The growth control tube that lacked honey and broth control tube that lacked bacterial inoculums acted as growth control while the honey control tube that had no bacterial inoculums acted as honey control. All the tubes except the HC tube were inoculated with 1ml of the respective prepared organisms' culture. The procedures for all tested organisms to each honey were done in triplicate. Incubation of the tubes was done at 37˚C for 24 hours and observation done by visual inspections for growth absence and presence. MIC was documented as the lowermost honey concentration that inhibited bacterial growth with no visible growth.

2.7.3. Minimum Bactericidal Concentration (MBC)

In determination of MBC, the incubated tubes with no visible growth sign in MIC above were sub-cultured onto sterile nutrient agar plates without honey and antibiotics by Streak plate method and incubated at 37˚C for 24 hours aerobically [17]. The least concentration of honey that didn't show test organisms'

growth was considered as the MBC. The inoculated plates were recorded as bactericidal if no growth was observed even after extended incubations, bacteriostatic if light to moderate growth was observed and no antibacterial activity if heavy growth was observed [17].

2.8. Determination of Physicochemical Parameters

The pH of the honey was determined by preparing a 10% (w/v) solution of honey in deionized water and the pH measured by use of a pH meter [22]. The moisture content of the honey samples was determined by use of Karl Fischer method. The electrical conductivity was determined according to Bogdanov *et al.* [23]. A 20% (w/v) solution of honey was suspended in deionized water and the EC determined using a conductivity meter. The honey samples' color intensity was determined using the method of Beretta *et al.* [24]. Concisely, dilution of the honey samples was done using warm deionized water (45˚C - 50˚C) to 50% (w/v). Filtration of the resulting solution was done using a 0.45 µm filter in order to remove large particles. Absorbance was measured at 450 and 720 nm by use of a spectrophotometer and the difference of the absorbance expressed as mAU.

3. Results

The results of the *in vitro* susceptibility of the test microorganisms to the honey samples were varying as shown in **Tables 1-4**. *Plebenia hylderbrandii* honey had a bactericidal activity at a concentration of 4.71% (v/v) and 2.35% (v/v) for *E. coli* and *S. aureus,* respectively. *Meliponula bocandei* honey, on the other hand, had a bactericidal activity at a concentration of 16.47% (v/v) for *S. aureus* but had no bactericidal activity for *E. coli* since none of the concentrations in this study showed inhibition. From the results in **Tables 1-4**, it is evident that both honeys had a higher antimicrobial activity against *S. aureus* than *E. coli* (**Tables 1-4**).

Partial inhibition for *Plebenia hylderbrandii* honey was observed from 1.18% - 2.94% (v/v) for *E. coli* and 1.18% (v/v) for *S. aureus* while complete inhibition was stronger for *S. aureus* than *E. coli* at Minimum Inhibitory Concentrations (MIC) of 1.76% (v/v) and 3.53% (v/v) respectively as presented in **Table 5**. On the other hand, for *Meliponula bocandei* honey, partial inhibition was observed at a concentration of 10.59% - 17.65% and 9.41% - 15.29% (v/v) for *E. coli* and *S. aureus*, respectively; while complete inhibition was observed at a Minimum Inhibitory Concentration (MIC) of 16.47% (v/v) for *S. aureus* while for *E. coli* no complete inhibition was detected since none of the concentrations from the study showed inhibition (**Table 5**). The Minimum Bactericidal Concentration (MBC) value for *Plebenia hylderbrandii* honey was 4.71% (v/v) and 2.35% (v/v) for *E. coli* and *S. aureus* respectively (**Figure 1(b)** and **Figure 1(c)**) while for *Meliponula bocandei* honey the MBC value was 16.47% (v/v) for *S. aureus* (**Figure 1(a)**) but it wasn't detected for *E. coli* since none of the tested concentrations showed inhibition as presented in **Table 5**.

Table 1. PIC & MIC (% v/v) of *Plebenia hylderbrandii* honey against bacterial pathogens.

Bacterial strain	Honey concentrations (% v/v) of *Plebenia hylderbrandii* honey against bacterial pathogens												MIC Value (% v/v)
	11.76	10.59	9.41	8.24	7.06	5.88	4.71	3.53	2.94	2.35	1.76	1.18	
E. coli (25922)	–	–	–	–	–	–	–	–	+	+	+	+	3.53
S. aureus (25923)	–	–	–	–	–	–	–	–	–	–	–	+	1.76

+ means minimum growth; _ means no growth; _ indicates MIC values.

Table 2. PIC & MIC (% v/v) of *Meliponula bocandei* honey against bacterial pathogens.

Bacterial strain	Honey concentrations (% v/v) of *Meliponula bocandei* honey against bacterial pathogens												MIC Value (% v/v)
	17.65	16.47	15.29	14.12	12.94	11.76	10.59	9.41	8.24	7.06	5.88	4.71	
E. coli (25922)	+	+	+	+	+	+	+	+	+	+	+	+	N/A
S. aureus (25923)	–	_	+	+	+	+	+	+	++	++	++	++	16.47

+ means minimum growth; ++ means moderate growth; _ means no growth; _ indicates MIC values.

Table 3. MBC (% v/v) of *Plebenia hylderbrandii* honey against bacterial pathogens.

Bacterial strain	Honey concentrations (% v/v) of *Plebenia hylderbrandii* honey against bacterial pathogens							MBC Value (% v/v)
	5.88	4.71	3.53	2.94	2.35	1.76	1.18	
E. coli (25922)	_	_	+	+	N/D	N/D	N/D	4.71
S. aureus (25923)	_	_	_	_	_	+	+	2.35

N/D means not detected; + means minimum growth; _ means no growth; _ indicates MBC values.

Table 4. MBC (% v/v) of *Meliponula bocandei* honey against bacterial pathogens.

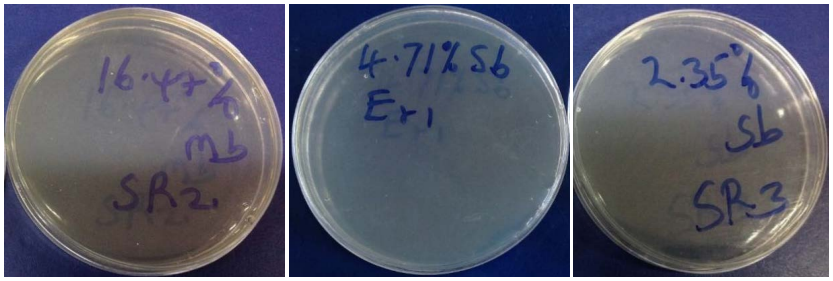
Bacterial strain	Honey concentrations (% v/v) of *Meliponula bocandei* honey against bacterial pathogens			MBC Value (% v/v)
	17.65	16.47	15.29	
E. coli (25922)	N/D	N/D	N/D	N/D
S. aureus (25923)	_	_	+	16.47

N/D means not detected; + means minimum growth; _ means no growth; _ indicates MBC value.

Table 5. The *in vitro* antibacterial activity: PIC, MIC and MBC % (v/v) of honey produced by stingless bees in nutrient agar by agar dilution method against *E. coli* and *S. aureus*.

Honey type/Control	Bacterial strain	Antibacterial activity of honey % (v/v)		
		PIC	MIC	MBC
Plebenia hylderbrandii honey	*E. coli* (25922)	1.18 - 2.94	3.53	4.71
	S. aureus (25923)	1.18	1.76	2.35
Meliponula bocandei honey	*E. coli* (25922)	10.59 - 17.65	N/D	N/D
	S. aureus (25923)	9.41 - 15.29	16.47	16.47

N/D means not detected.



(a) (b) (c)

Figure 1. (a) MBC for *M. bocandei* against *S. aureus* (b) MBC for *P. hylderbrandii* against *E. coli* (c) MBC for *P. hylderbrandii* against *S. aureus*.

The positive control had a wide PIC range between 0.118% - 0.5882% (w/v) against *E. coli* while for *S. aureus* was only at 0.05882% (w/v). It had a higher MIC value of 1.176% (w/v) against *E. coli* than for *S. aureus* which had a lower value of 0.088% (w/v). Similarly, its MBC value was 1.176% for *E. coli* compared to 0.118% (w/v) for *S. aureus* as presented in **Table 6**. This indicates that the positive control had a higher bactericidal activity for *S. aureus* than for *E. coli*. On the other hand the *in vitro* antibacterial activity of negative control indicated heavy growth (**Table 7**) compared to the positive control.

The physicochemical parameters are presented in **Table 8**. The analysed honey samples were acidic in character. *Meliponula bocandei* honey had higher pH (3.99 ± 0.006^a) than *Plebenia hylderbrandii* honey (3.54 ± 0.012^a). The moisture content of the honey samples ranged from 24.54% to 27.79%. *Plebenia hylderbrandii* honey had higher moisture content (27.79% ± 0.023%) than *Meliponula bocandei* honey (24.54% ± 0.042%). The honey samples' electrical conductivity (EC) ranged between 0.42 to 1.00 mS/cm. *Meliponula bocandei* honey had EC of 0.42 ± 0.002^a mS/cm while *Plebenia hylderbrandii* honey had EC of 1.00 ± 0.001^a mS/cm. The color intensity of the honey samples ranged between 1.11 ± 0.002^a to 1.2 ± 0.004^a mAU. *Meliponula bocandei* honey had a higher color intensity of 1.2 ± 0.004^a mAU while *Plebenia hylderbrandii* honey had 1.11 ± 0.002^a mAU. There was no significant difference between the two samples in all the physicochemical parameters.

The number of replicates (N) for all the physicochemical properties in each honey type was 3.

From the results in this table, similar letter "a" accompanying each mean indicate that there is no significant difference between the physicochemical properties in the two samples under study.

4. Discussion

Honey has various characteristics that are believed to contribute to its total antimicrobial activity. These comprise of pH (being 3.2 - 4.5), moisture content, electrical conductivity, color intensity, H_2O_2 concentration, phytochemical factors, and high osmotic effect [22]. The acceptable range for pH of honey is between 3.2 and 6.1, low enough to inhibit pathogens [25]. The acidity nature is

Table 6. The *in vitro* antibacterial activity (PIC, MIC & MBC) of positive control.

Positive Control	Bacterial strain	Antibacterial activity of honey % (w/v)		
		PIC	MIC	MBC
Ampicillin	*E. coli*	0.118 - 1.176	1.176	1.176
	S. aureus	0.05882	0.088	0.118

Table 7. The *in vitro* antibacterial activity of negative control.

	Bacterial Strain	**0 (% v/v) for 3 replicates**
Negative control	*E. coli*	**+++**
	S. aureus	**+++**

+++ means heavy growth.

Table 8. Physicochemical parameters.

Type of Honey	pH	Moisture content (MC)	Color intensity	Electrical conductivity (EC)
P. hylderbrandii	3.54 ± 0.012^a	27.79 ± 0.023^a	1.11 ± 0.002^a	1.00 ± 0.001^a
M. bocandei	3.99 ± 0.006^a	24.54 ± 0.042^a	1.20 ± 0.004^a	0.42 ± 0.002^a

contributed by presence of organic acids in honey [26]. In this study, *Plebenia hylderbrandii* honey had a pH of 3.53667 ± 0.01155 while *Meliponula bocandei* honey had a pH of 3.98667 ± 0.00577. These values are within the acceptable range hence their contribution to their antibacterial activity. Honey samples from Spanish, Turkish and Brazilian had pH values ranging between 3.63 to 5.01, 3.67 to 4.57 and 3.10 to 4.05 respectively which were consistent with the ones from this study [27]. The Moisture content for honey may range between 13% to 29% [28]. *Plebenia hylderbrandii* honey (27.79% ± 0.023%) had higher moisture content than *Meliponula bocandei* honey (24.54% ± 0.042%) which were within that range. The results from this study also corresponded to the ones carried out for stingless bee honeys by Neupane *et al.* [29] and Jimenez *et al.* [30] which ranged between 20.12% to 29.1% and 20.61% to 28.04% respectively. However, results of this study were lower compared to the values (28.46%, 30.28%, 31.35% and 31.54%) obtained from a research carried out on Nigerian honey by Oyo, Osun, Ekiti, Lagos and Ogun [31]. The variation might be due to difference in geographical location, climate, bee species, among other factors. The honey samples' electrical conductivity ranged between 0.42 to 1.00 mS/cm. *Meliponula bocandei* honey had EC of 0.42 ± 0.002^a mS/cm, which was below the maximum value of 0.8 mS/cm as indicated in European Union and Codex directive while *Plebenia hylderbrandii* honey had EC of 1.00 ± 0.001^a mS/cm, which was above the maximum value of 0.8 mS/cm. Comparatively, the results from the investigated samples were in agreement with research carried out by Saxena *et al.* [32] whose honey EC ranged between 0.33 to 0.94 mS/cm. However, EC is closely related to mineral content, proteins, organic acids, polyols, and some complex sugars [32]. Higher acid and ash contents indicate higher con-

ductivity, and hence higher antibacterial activity [33] and this could be the reason for the high activity in *P. hylderbrandii*. Honey's color intensity is represented by the ABS_{450}. In this study, the honeys' ABS_{450} values ranged between 1.11 ± 0.002^{a} to 1.2 ± 0.004^{a} mAU. A similar research done by Ahmed *et al.* [34] obtained values ranging between 1.26 and 1.44 mAU. In comparison, the ABS_{450} values were also conveyed to be between 724 and 1188 mAU in Algerian honey samples [35]; 25 and 3413 mAU in Italian honey samples [24]; 70 and 495 mAU in Slovenian honey samples [36]; 524 and 1678 mAU in Indian honey samples [32] and between 254 and 2034 mAU in Bangladesh honey samples [37]. These differences may be due to variation in floral and geographical origin [34]. The color of honey is also dependent on various factors such as ash content, mineral content, storage time and heat [34] [38]. Color intensity correlates with antioxidant activity in that high color intensity implies high antioxidant activity [32].

This study demonstrates that honey from Kenyan stingless bee species *Plebenia hylderbrandii* and *Meliponula bocandei* has antibacterial activity. This activity varied between the two honeys and the discrete test organisms were also found to vary in their susceptibilities. On the contrary, a research carried out on honey from Kenyan stingless bee species, *Dactylurina schimidti*, from Coast did not show any inhibitory effect against both *E. coli* and *S. aureus* in all the tested concentrations which ranged between 25% - 100% (v/v) [39]. Another previous study conveyed MICs of honey from various Guatemalan stingless bees including *Melipona beecheii*, *Geotrigona acapulconis*, *Tetra. Angustula* and *Scaptotrigona spp.* ranging from 2.5% - >10% (v/v), with most of the MICs being 5% (v/v) [40]. For *Apis mellifera* honeys, the MICs published for medical honey or Manuka honey ranged between 2% - 8% (v/v) for Gram - positive cocci tested [41]. In addition, reports show that *Apis mellifera* honey inhibits most test organisms at concentrations 2.5% - 7.5% (v/v) [42]. In the current study, *Plebenia hylderbrandii* honey had a higher antibacterial effect than *Meliponula bocandei* honey (**Table 5**). The substantial differences between the antibacterial activities displayed by the two honeys could be because of discrepancies in the stingless bee honeys tested. These discrepancies may have also resulted from their feeding habits since they exploit plant-based resources [43] including pollen, nectar, latex, resin, scents, oil, seed and leaves during their foraging flight [44] which are found in diverse sites on broad diversity of crop plants.

The study further shows that microorganisms may differ in their susceptibilities to honeys; with *S. aureus* being more susceptible when compared to *E. coli*. This finding is evidenced by the concentrations of the honeys that inhibited *S. aureus*, which were much lower than those that inhibited *E. coli* (**Tables 1-4**). It is also evident that the positive control was more active against *S. aureus* than *E. coli* as evidenced by its higher MIC and MBC values against *E. coli*. This could have resulted from the fact that Gram-negative bacteria such as *E. coli* are less sensitive to honey activity compared to Gram-positive bacteria, for instance, *S. aureus* [45]. This is also in agreement with a study by Boorn *et al.* [46], which

also showed *S. aureus* as the most sensitive organism to honey having MIC ranges 4% to >10% (w/v) for Gram-positive bacteria and 6% to >16% (w/v) for Gram-negative bacteria.

Honey have been proved to have antibacterial effect against *E. coli*. A study by Tan *et al.* [47] reported that the MICs for Tualang honey and Manuka honey for *E. coli* ranged from 17.5% (v/v) to 22.5% (v/v). On the same study, the MBC values ranged from 17.5% to 25% (v/v). Another study by Mulu *et al.* [42] demonstrated that the concentration of *Apis mellifera* honey from Ethiopia which fully prevented *E. coli* growth was 6.5% (v/v). According to Mandal *et al.* [1], the bactericidal effect of the *Apis mellifera* honey tested against *E. coli* was achieved at concentration 3% (v/v). In addition, a study by [48] reported that honey has antibacterial effects against *E. coli* having a MIC of 20% (v/v), concentrations tested ranged 0% - 30% (v/v). A research on stingless bee honey from Ethiopia also demonstrates its antibacterial activity against *E. coli* [49]. *Trigona laeviceps* honey, a stingless bee honey from Thailand also demonstrated its antibacterial activity against *E. coli* [50]. Nevertheless, Cruz *et al.* [51] have displayed the antibacterial activity of stingless bee honey against *E. coli* with MIC ranging between 10% - 20% (v/v). However, there is scarce information for the antibacterial activity of stingless bee honey compared to *Apis mellifera* honey. In this study, *Plebenia hylderbrandii* honey had a bactericidal effect at a concentration of 4.71% (v/v) against *E. coli* (**Figure 1**). On the contrary, the bactericidal effect for *Meliponula bocandei* honey against *E. coli* wasn't obtained since growth was observed in the concentration range tested. This calls for further research for its determination.

Generally, honey also demonstrates high *in vitro* anti-Staphylococcal activity [52] although other studies confirms that this activity vary based on different samples obtained from similar botanical sources, with MIC ranges of 3.12% and 6.25% (v/v) [53]. A study done by Irish *et al.* [54] reported that honey from Australian stingless bee species *Trigona carbonaria* possessed antibacterial activity against *Staphylococcus aureus*. A study by Mama *et al.* [17] reported that the percentage by volume of honey to completely inhibit MRSA growth was in the range of 18.7% to 37.5% (v/v). The concentrations tested in the study ranged between 25% - 100% (v/v). On the contrary, a study carried out in Ethiopia, found out that the% v/v of *Apis mellifera* honey that prevented growth of *S. aureus* was 6.5, which is higher than our result obtained from *plebenia hylderbrandii* honey whose inhibitory concentration was 1.76% (v/v) and lower than that of *Meliponula bocandei* whose inhibitory concentration was 16.47% (v/v) for *S. aureus*. The concentrations from the study ranged between 10% - 100% (v/v) [55]. Another study by Willix also reported that the percentage by volume of Manuka honey to completely inhibit *S. aureus* growth was 1.8% (v/v) [56]. Similarly, Sherlock *et al.* [57] reported that inhibitory potential of Manuka honey from stinging bees on MRSA was only at concentrations > 12.5% (v/v) while Ulmo 90 honey was bactericidal on MRSA at concentrations of 3.1% (v/v) and 6.3% (v/v). French *et al.* [58] also reported that manuka honey inhibits *S. aureus*

growth at concentrations 2.7% - 5% (v/v). In this study, the % v/v of the two types of honey to completely inhibit *S. aureus* was 1.76% (v/v) and 16.47% (v/v) for *Plebenia hylderbrandii* and *Meliponula bocandei* honeys respectively (**Figure 1**).

Although the tested honey showed antibacterial effect, other studies demonstrate that not all honeys have similar antibacterial activity degree. Numerous previous reports of antibacterial activities of stingless bee honeys are hard to compare with the current study due to differences in methods under studies [39]. In addition, most of these honeys were produced by different bee species other than *Plebenia hylderbrandii* and *Meliponula bocandei* and from floral sources and regions discrete from those found in Kenya which has further limited the comparisons.

Moreover, a study carried out by Mohapatra *et al.* [59] shows that honey is effective against both gram-negative (*P. aeruginosa*, *E. coli* and *Salmonella typhi*) and Gram-positive bacteria (*Bacillus subtilis*, *S. aureus*, *Bacillus cereus*, *Micrococcus luteus* and *Enterococcus faecalis*); this effect is either bactericidal or bacteriostatic. A study carried out in Ethiopia on red and white honeys indicate their antibacterial activity with the MIC and MBC of all the isolates (*Escherichia coli*, *proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia, Streptococcus pyogenes*) ranging between 6.25% - 50% and 12.5% - 100% (v/v) respectively [60]. A study by Ng *et al.* [60] also found out that stingless bee honeydew honey exhibit antibacterial property against *E. coli* and *S. aureus*. In this study, *Plebenia hylderbrandii* honey had both bacteriostatic and bactericidal effect on *S. aureus* at concentrations above 1.76% (v/v) and 2.35% (v/v) (**Figure 1**) respectively and on *E. coli* at concentrations above 3.53% (v/v) and 4.71% (v/v) respectively. However, *Meliponula bocandei* honey had both bacteriostatic effect at a concentration of 16.47% (v/v) and bactericidal effect at a concentration of 16.47% (v/v) on *S. aureus* but had neither bacteriostatic nor bactericidal effect on the growth of *E. coli* isolate because of the observed growth in all concentrations. In this study, the bactericidal effect was higher than that of Ulmo 90 honey from honeybee, which may have resulted from different geographical origin [57]. However, in this study the honeys had a lower bactericidal activity on *E. coli* than on *S. aureus*. This could be attributed by the lower susceptibility of *E. coli*.

Antibacterial activity of honey varies depending on various factors such as bee species, storage time, honey type and its concentration, type of microbe, test methods used, honey components/characteristics, geographical location, and source of nectar on which the reared bees were fed [61]. The difference may also be attributed to bees' foraging behavior and feeding habits which vary depending on the plant resources exploited. Tan *et al.* [47] also indicated that honey has many sources of production and its antimicrobial activity may vary depending on the processing and origin. The antibacterial activity of stingless bee honey differs from that of *Apis* honey which is attributed to the storage of the specific bees. According to Ewnetu *et al.* [62], stingless bee honey has higher antibacteri-

al effects than *Apis mellifera* honeys. In western Kenya, *Apis mellifera* honey has been reported to possess antibacterial effects against *E. coli* [63]. However, no stingless bee honey has been explored for comparison purposes hence the purpose for this study. In addition, the efficacy of honey against test microorganisms depends on the honey used, botanical origin variation, honey processing, geographical location, and bee health [57].

In comparison with the formerly published data, it is evident that *Plebenia hylderbrandii* and *Meliponula bocandei* honeys have similar activity to the other medical honeys and thus can be used as therapeutic agents.

5. Conclusion

Stingless bee honey possesses antibacterial activity with both bacteriostatic and bactericidal effects on *E. coli* and *S. aureus.* From this study, activity against *E. coli* on *Meliponula bocandei* honey was limited since it had neither bacteriostatic nor bactericidal effect on *E. coli*. Thus, it can be inferred that *Plebenia hylderbrandii* honey was highly effective than *Meliponula bocandei* honey. The stingless bee honey samples differed in activity although they are from similar botanical region and the basis for this observation need to be determined.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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