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In-vitro Antimicrobial Effects and Phytochemical Contents of Stingless Bee *Meliponula beccarii* Honey and Pollen from Baringo County, Kenya

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ABSTRACT

Background: Afro-tropical stingless bees produce several products including honey, propolis, cerumen and pollen, which are widely used as traditional medicine and food. In Baringo County, Kenya stingless bee honey in particular is widely used as a traditional remedy for respiratory disorders, stomach disorders and oral thrush, commonly associated with bacterial and fungal infections. However, scientific data on the antimicrobial activities and phytochemical content of stingless bee products from Baringo is scarce. **Objectives:** The objective of this study was to investigate the *in vitro* antimicrobial activities and phytochemical content of *Meliponula beccarii* stingless bee honey and pollen from Baringo County. **Materials and methods:** Eleven honey and pollen samples were conveniently sampled from eleven wild occurring stingless bee nests in three ecologically distinct areas. Increasing concentrations of honey and pollen samples were then prepared and tested against *H. influenzae, E. coli, MRSA and C. albicans* using agar well diffusion assay. The broth microdilution test was further performed to determine the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs). Standard qualitative methods were used to analyse the phytochemical contents of the honey and pollen samples. Data was analysed by two-way ANOVA and Tukey's post-hoc tests. **Results:** in comparison to distilled water (negative control), the honey samples had a significantly higher mean zones of inhibition at concentration of 100% v/v against *E. coli* (9.0±4.7 mm vs 0 mm, $p<0.0001$, N=11), *H. influenzae* (11.1±5.0 mm vs 0 mm, $p<0.0001$, N=11), and *MRSA* (10.8±5.9 mm vs 0 mm, p>0.0001, N=11). However, compared to ciprofloxacin standard (30ug/ml), all the concentrations of honey samples had significantly lower mean zones of inhibition against *H. influenzae (8.8*±2.4 mm vs 25±0.58 mm, p<0.0001, N=11), *E. coli (5.7*±3.4 mm vs 31.7±1.5 mm, p<0.0001, N=11) and *MRSA (*8.1±2.8 mm vs *27*±0 mm). All the honey samples did not exhibit activity against *C. albicans* even at 100% (v/v). The mean MICs against *E. coli, H. influenzae* and *MRSA* were 9.38% (v/v), 18.75% (v/v) and 18.75% (v/v), respectively. Notably, the honey samples exhibited bactericidal activity, only against *MRSA* with a mean MBC of 60.94% (v/v). All the pollen samples showed no antibacterial and antifungal activities against the tested micro-organisms. Qualitative analyses revealed that the honey and pollen samples of *Meliponula beccarii* contain alkaloids, phenolics, triterpenoids, flavonoids, saponins, tannins, glycosides and steroids, but not terpenoids. **Conclusion**: Some but not all *Meliponula beccarii* honey samples from Baringo County has antibacterial activities. The honey and pollen are rich in various phytochemical compounds. Our findings validate the use of *Meliponula beccarii* honey in traditional treatment of bacterial infections and its further investigation as a potential source of novel agents against drug resistant pathogenic bacteria.

Keywords: *Meliponula beccarii*, Honey, Pollen, Antimicrobial activities, Phytochemical content.

INTRODUCTION

Stingless bees (*Apidae, Meliponini*) species, which are found in the tropical and subtropical regions of the world [1] produce honey, cerumen, bee bread, propolis and pollen[2]. These products can be harvested either from the wild-occurring nests or specially designed artificial hives and have long been used traditionally for medicinal purposes. The ancient Mayan traditional doctors, for example, used stingless bee honey to cure poisonous stings and treat high fever, wounds, burns, cold and respiratory diseases [2, 3]. Several traditional medicinal uses of honey from various stingless bee species have also been documented among different native communities in other tropical and sub-tropical countries [2-4]. In Baringo County Kenya, recent ethnomedicinal and therapeutic uses studies documented that honey from *Meliponula beccarii* stingless bees is widely used in the treatment of various disorders with respiratory disorders, stomach disorders, throat ailments and wounds being the major ones [5, 6]. As such, there has been great interest in scientific studies of chemical content and pharmacological properties, particularly antimicrobial properties of stingless bee honey from different species and geographical regions. In this

regard, the honey of *Melipona beecheii species* of Mexico and Central America have been reported to have in vitro activity against *C. albicans* [7].

Trigona carbonaria honey from Australia has been studied and shown to have broad-spectrum activities against gram positive and gram-negative bacteria and limited activity against *Candida* [8]. *Tetragonula species* of South East Australia reportedly showed high bactericidal effects against *S. aureus* and *K. pneumoniae* [9]. Different Brazilian stingless bee honeys have also been shown to exhibit high antimicrobial activity against several bacterial strains and *C. albicans* [10]. The Costa Rican *Tetragonisca angustula* honey has also been documented to inhibit *S. aureus* biofilms with *Tetragonisca angustula* biofilm destruction factors (TABDFs) being reported to be responsible for the antibiofilm action [11]. The Malaysian *Trigona honey* has been found to exhibit high antibacterial and antibiofilm activities against *P. aeruginosa* and *S. pyogenes in vitro* [12]. In Africa, honey of *Meliponula species* found in Ghana has been investigated and found to have activity against *S. aureus* and *P. aeruginosa* [13]. More recently, honey of six stingless be species found in Tanzanian were reported to have antimicrobial activities against *S. aureus, B. subtilis, S. typhi* and *C. albicans* with honey of *Dactylurina schmidti* showing the strongest antimicrobial activity [14]. In Kenya, the antimicrobial activities of *Dactylurina schimidti* from Tana River, *Meliponula bocandei* and *Plebenia hylderbrandii* from Kakamega county, Western Kenya has been reported so far [15,16]. However, the antimicrobial activities of stingless bee products from other parts of Kenya including Baringo County remain largely unexplored. Moreover, there is limited data on the phytochemical content of most Kenyan stingless bee products. Therefore, the purpose of this study was to determine the antifungal and antibacterial activities as well as phytochemical composition of honey and pollen of *Meliponula beccarii,* which is a ground nesting stingless bee species found in various parts of Baringo County.

MATERIALS AND METHODS

Collection of Honey and pollen samples

A total of 11 honey and pollen samples were collected from Baringo County, Kenya (Figure 1). Notably, Baringo County has a very diverse topography ranging from flat lands to steep hilly terrains and covers humid, sub-humid, semi-humid, semi-arid arid, arid and very arid climatic zones [17,18]. The mean annual rainfall in these climatic zones varies greatly ranging from as low as 450 mm in the semiarid to a high of 1,100-2,700 mm in the humid zones. The county experiences a dry season from January to mid-March, a rainy season from mid-March to mid-July, a second dry season between July and September, which is then followed by a short rainy season up to December. Ecologically, Baringo is divided into three major zones; namely the highlands, midlands and lowlands, with up to eleven distinct sub-ecological zones [17]. Specifically, four and two honey and pollen samples were collected from Lembus forest and Sabatia forest, respectively, both in Koibatek sub-county. One sample was collected from Katimok forest in Baringo North Sub-county. Three samples were from Morop-Tambaras conservancy forest in Baringo Central and one sample from Kipngochoch forest also in Baringo Central (Figure 1). All these sites are located in the midland and highland ecological zones. The sample collection was done between January and April 2022, which was the dry season in Baringo County. Briefly, wild occurring stingless bee nests were located with the aid of experienced honey gatherers. Once located, the nests were carefully excavated to expose the storage areas. The honey pots were then identified and 5 ml sterile syringes were used to suck the honey and transfer into sterile 50 ml well labelled bottle/tube for each nest (with identification of the sub counties and date of collection). Pollen pots were then identified and the pollen which was not mixed with honey was carefully collected using a pair of forceps and placed in sterile pre-labelled ziplock bags. The samples were then transported to the laboratory and stored in the fridge at 4°C until needed for experimental analysis.

Test microorganisms

Three bacterial strains; *E. coli* (ATCC 25922), *H. influenzae (*ATCC 49766*)* and *MRSA* (ATCC 12393) and one fungus; *C. albicans* (ATCC 10231) were used in this study to test for microbial activities of both the honey and pollen extracts. Prior to their use, they were sub-cultured on nutrient agar for bacteria and savoured dextrose agar for the fungi to check their viability and to obtain fresh colonies. This was done by aseptically picking a loopful culture from the respective original stock and streak-plating on the respective freshly prepared media in to obtain fresh and viable cultures.

Experimental design

The antimicrobial activity of *Meliponula beccarii* species honey and pollen against *H. influenzae*, *MRSA*, *E. coli* and *C. albicans* was determined using a (3 (1) ×3 (1) ×11 (11)) factorial experimental study design arranged in a completely randomized design. These factors were 3 increasing concentrations of *Meliponula beccarii* honey (50% v/v, 75% v/v and 100% v/v and 1 concentration of pollen (10% w/v); 3 bacterial strains (*E. coli, H. influenzae* and *MRSA*) and 1 fungi (*C. albicans*), the 11 non-pooled honey samples and 11 pollen samples.

Preparation of honey dilutions

Collected pure honey (100%) was diluted with sterile distilled water to obtain lower honey concentrations of 75% (v/v) and 50 % (v/v). This was done by measuring 0.75 ml, and 0.5 ml of pure honey and toping up with sterile distilled to exactly 1 ml to obtain 75% (v/v) and 50% (v/v), concentrations respectively. All the working honey concentrations were prepared in triplicates [19].

Preparation of pollen solution

The pollen was carefully removed from the pot after which 100 mg was weighed using an analytical weighing balance (ATY 224, Shimadzu) and put into a well labelled 2 ml cryovial tube.1 ml of sterile distilled water was added into the cryovial tube containing the pollen sample then mixed thoroughly to obtain homogenous pollen solutions of 10% (w/v) concentration for each of the eleven pollen samples, which were then kept in a refrigerator at 4-8 ^{0}C until needed for experiments.

Culture media preparation

Nutrient agar (HiMedia), Nutrient Broth (HiMedia) and Sabouraud Dextrose Agar (HiMedia) were used as culture media for bacteria and fungi, respectively. The preparation of these media was done as per the manufacturer's instructions. Briefly, Nutrient Agar (28 g), Nutrient Broth (13g) and Sabouraud Dextrose Agar (65 g) were weighed using electronic balance (ATY224; Shimadzu) then suspended in 1000 mls of distilled water and heated to boiling point to

ensure complete dissolution of the culture media powders. The well dissolved media was then sterilized by autoclaving at 121°C and 15 psi for 15 minutes using vertical pressure steam sterilizer (Jibimed LS-50 LD). Approximately, 20 ml of the sterile media was then dispensed aseptically into sterile disposable petri dishes (90×15 mm).

Preparation of the culture inoculum

The bacteria and fungi inoculums were prepared as described by Matuschek *et al*., [20] with some modifications. The 24-hour old bacteria and fungi colonies were suspended in sterile distilled water and the resultant turbidity compared to 0.5 McFarland standard. The turbidity of the inoculum was adjusted either by addition of more colonies or sterile distilled water to achieve inoculum that matched the McFarland standards for bacteria and fungi.

Agar well diffusion antibacterial assays

The antibacterial activity of honey and pollen was tested by agar well diffusion method as previously described for natural products by Valgas *et al*., [21] and pollen by Carneiro *et al*.,**[22]**. Briefly, 1 ml of the inoculum of each test bacterial strain was pipetted into each petri dish. After which 20 ml of sterile nutrient agar media was added, mixed well and spread uniformly using a sterile cotton wool swab then allowed to solidify. Five wells measuring 6 mm in diameter were then punched on the solidified nutrient agar using a sterile cork borer. 30 µl of each of the three increasing concentrations per honey sample (50% v/v, 75% v/v and 100% v/v) were loaded onto three different wells, respectively.15 µl of Ciprofloxacin (30 µg/ml) standard and 15 µl of sterile distilled water was loaded to the remaining two wells in each petri dish as positive and negative controls, respectively, using a 5-50 µl micropipette. For each of the pollen sample, 30 µl of 10% w/v pollen solution was loaded in triplicates per petri dish, with 15 µl of Ciprofloxacin (30 µg/ml) standard and 15 µl of sterile distilled water (negative control). The inoculated petri dishes were then left to set for 15 minutes at room temperature to allow for diffusion of both honey and pollen extracts into the media prior to incubation at 37 °C for 18 hours in an incubator (D-91126 Schwabach FRG; Memmert GmbH). The zones of inhibition diameters were then measured in mm using a ruler and recorded. All samples were tested in triplicate on the same day.

Agar well diffusion antifungal assays

The antifungal activities of honey and pollen was tested by the agar well diffusion technique as previously described for natural products by Valgas et al., [21] and pollen by Carneiro *et al*., [22]. 1 ml of *C. albicans* inoculum was pipetted and added into each of the sterile petri dishes. Thereafter, 20 ml of Sabouraud Dextrose Agar was added and mixed well before spreading it uniformly using a sterile cotton wool swab and allowed to solidify. Five wells measuring 6 mm in diameter were then punched on the solidified Sabouraud Dextrose Agar using a sterile cork borer. 30 µl of each of the three increasing concentrations per honey sample (50% v/v, 75% v/v and 100% v/v) were loaded onto three different wells, respectively. 15 µl of nystatin standard (0.4 mg/ml) and 15 µl of sterile distilled water was loaded into the remaining two wells in each petri dish as positive and negative controls, respectively using a micropipette. For each of the pollen sample, 30 µl of 10% w/v pollen solution was loaded in triplicates per petri dish, with 15 µl of Nystatin (0.4 mg/ml) standard and 15 µl of sterile distilled water (negative control). All the inoculated petri dishes were left to set for 15 minutes at room temperature to allow for diffusion of both honey and pollen extracts into the media prior to incubation at 35°C for 24 hours in an incubator. The diameters of the inhibition zones were measured in mm using a ruler and recorded. The experiments were done in duplicates.

Minimum bacterial Inhibitory concentration (MIC) assay

The broth microdilution technique was used to measure MIC of honey as previously described for natural products by Valgas et al., [21] and for honey by Mama *et al*.,[19]. MIC was determined only for honey samples that had zones of inhibition >13 mm in the agar well diffusion assay. For *E. coli and* MRSA the selected one and four samples, respectively exhibited antimicrobial activity at concentration of 75% v/v. Therefore, 100 µl of honey sample (75% v/v) was subjected to four stepwise two-fold serial dilutions in 96-well microtitre plate (Thermo Scientific-nunc # 167008) and the MIC determined. For *H. influenzae* two *of* selected samples exhibited antimicrobial activity at concentration of 50% v/v and one at 75% v/v. Therefore, 100 µl of honey samples (50% and 75% v/v) were subjected to four stepwise two-fold serial dilutions in 96-well microtitre plates (Thermo Scientific-nunc # 167008). The MIC was determined as the lowest concentration of honey sample that inhibited the growth of the respective test microorganisms. This was done in triplicates for each honey sample.

Minimum bactericidal concentration (MBC)

The MBC of the honey was determined according to NCCLS criteria (2011). The contents of the nutrient broth used for MIC tests were picked from the respective wells on the microtitre plates using a sterile wireloop and individually sub-cultured by streak-plating on freshly prepared sterile nutrient agar plates and incubated at 37 °C for 18 hours in an incubator. The inoculated plates were then observed for bacterial growth such that the least concentration of honey that did not have the growth of the test bacteria was recorded as the MBC of the respective honey samples.

Qualitative analysis of phytochemicals

The phytochemical composition of each of the eleven honey and eleven pollen was analysed by standard qualitative tests as previously described [23]. Presence or absence was denoted by (+) and (-), respectively.

Data analysis

Data obtained from antimicrobial activity, MIC and MBC experiments was imported into the Microsoft excel 365 spread sheet. Data analysis was conducted using Graphpad prism software (version 7). Descriptive statistics was done and data expressed as mean values with standard deviation (± SD). Comparisons of different means were done by two-way Analysis of Variance (ANOVA) and Tukey's post-hoc test.

RESULTS

***In vitro* Antimicrobial activities of *Meliponula beccarii* honey and pollen**

The antimicrobial activity of *Meliponula beccarii* honey and pollen was determined by agar well diffusion and results expressed as mean diameter of growth-inhibition zones. In comparison to distilled water (negative control), the honey samples had a significantly higher mean zone of inhibition against *E. coli* at concentrations of 75% v/v (6.1±4.9 mm vs 0 mm, $p<0.0001$, N=11) and 100% v/v (9.0±4.7 mm

vs 0 mm, *p<0.0001*, N=11), but not at 50% v/v (2.1±3.7 mm vs 0 mm, p>0.05 *Figure 2A*). Compared to ciprofloxacin standard (30 ug/ml), the honey samples had a significantly lower mean zones of inhibition at all concentrations (5.7±3.4 mm vs 31.7±1.5 mm, *p<0.0001*, N=11, *Figure 2A*). Notably, however, only 9 out of the 11 honey samples had activity against *E. coli* at a concentration of 100% v/v with inhibition zones ranging from 8.67±0.58 mm to 14.33±0.58 mm. At a concentration of 75% v/v, 7 honey samples exhibited antibacterial activity against *E. coli*, albeit with smaller zones of inhibition compared to 100% v/v and only 3 honey samples had mild activity against *E. coli* at 50% v/v (Table 1). All pollen samples did not exhibit significant activity against *E. coli* (*Figure 3A*). When tested against *H. influenzae*, the honey samples exhibited significantly higher mean zone of inhibition compared to distilled water (negative control) at concentrations of 50% v/v (6.3± 5.5 mm vs 0 mm, *p<0.001*,N=11), 75% v/v (9.1±5.4 mm vs 0 mm, *p<0.0001*, N=11) and 100% v/v (11.1±5.0 mm vs 0 mm, *p<0.0001*, N=11) (*Figure 2B*), but which was lower than ciprofloxacin standard (8.8±2.4 mm vs 25±0.58 mm, *p<0.0001*, N=11), *Figure 2B*). Importantly, the individual honey samples exhibited varying activity at the different concentrations. At concentration of 100% v/v, *H. influenzae* was susceptible to 10 out of the 11 honey samples. At a concentration of 75% v/v 9 honey samples exhibited activity against *H. influenzae*, albeit with smaller zones of inhibition compared to 100% v/v and 7 honey samples had mild activity against *H. influenzae* at 50% v/v (Table 1). All pollen samples did not exhibit activity against *H. influenzae* (*Figure 3B*).

Overall, *Meliponula beccarii* honey samples also showed antibacterial activity against *MRSA.* As shown in *Figure 2C* the mean zone of inhibition of honey samples was significantly higher compared to distilled water (negative control) at the concentrations of 75% v/v (8.4±6.3 mm vs 0 mm, *p<0.001*, N=11) and 100% v/v (10.8±5.9 mm vs 0 mm, *p<0.0001*, N=11), but not at concentration of 50% v/v (5.2± 5.3 mm vs 0 mm). Similarly to *E. coli and H. influenzae*, the zone of inhibition of ciprofloxacin against *MRSA* was significantly higher than those of all the three honey sample concentrations (27±0 mm vs 8.1±2.8 mm, *Figure 2)*. Notably, individual honey samples exhibited varying zone of inhibiting with 2 honey samples showing no activity against *MRSA* at concentration of 100 v/v. At concentrations of 50% v/v and 75% v/v, 5 honey samples and 3 honey samples, respectively, also did not show any activity against *MRSA.* All pollen samples did not exhibit activity against *MRSA* (*Figure 3C*). Our result shows that *C. albicans* was neither susceptible to the honey at all concentrations nor the pollen samples (*Figure 2D and Figure 3D*)

Minimum Inhibitory Concentration (MIC) of *Meliponula beccarii* honey

The MIC of *Meliponula beccarii* honey against the three test bacteria are shown in Table 2. *E. coli* had the lowest MIC value of 9.38 % v/v, while the MIC value for both *H influenzae* and *MRSA* was18.75% v/v. The mean MIC of ciprofloxacin (positive control) was 0.075 µg /ml against *H. influenzae* while the tested concentrations against *E. coli* & *MRSA* did not result in detectable MIC.

Minimum Bactericidal Concentration (MBC) of *Meliponula beccarii* honey

The MBC of *Meliponula beccarii* honey against the three test bacteria are shown in Table 3. The results show that the MBC of *Meliponula beccarii* honey against *MRSA* was 60.94% (v/v). However, the tested concentrations did not result in detectable bactericidal effect against *E. coli and H. influenzae*. At the tested concentrations, ciprofloxacin (positive control) exhibited mean MBC values of 0.3 µg /ml for *H. influenza,* but no detectable bactericidal effect against *E. coli* and for *MRSA.* These results indicate that *Meliponula beccarii* honey is bactericidal only against *MRSA,* but not against *E. coli and H. influenza*e.

Phytochemical content of *Meliponula beccarii* honey and pollen

Preliminary qualitative screening of *Meliponula beccarii* honey samples revealed the presence of phenols, tanins, saponins, alkaloids, glycosides, steroids, flavonoids and triterpenoids. Notably, terpenoids were absent in all the analysed 11 honey samples (Table 4). Similarly, all the pollen samples contained phenols, tanins, saponins, alkaloids, glycosides, triterpenoids, steroids and flavonoids, but not terpenoids (Table 5).

DISCUSSION

Honey from *Meliponula beccarii* stingless bees is widely used by the natives of Baringo county, Kenya to treat various disorders associated with bacterial and fungal infections [5, 6]. *Meliponula beccarii* also produce pollen, whose antimicrobial activity remains unexplored. In this study, we investigated whether these *Meliponula beccarii* nest products have *in vitro* antimicrobial activities.

We show that indeed, all except two of the *Meliponula beccarii* honey samples tested had significant antibacterial activity against *E. coli, H. influenzae* and *MRSA* at a concentration of 100% (v/v) (*Figure 2A-C*). These findings agree with previous studies that have reported antimicrobial activities of honey from other stingless bee species including the Kenyan *Plebenia hylderbrandii* and *Meliponula bocandei* species [16], Thai *Tetragonula laeviceps* [24], Malaysian *Heterotrigona itama* Geniotrigona *thoracica* and *Heterotrigona erythrogastra* [25]. Previously, the antibacterial activity of stingless bee honey has been linked to the presence of bioactive phenols and flavonoids [25]. Therefore, the antibacterial activities of the *Meliponula beccarii* honey samples in our study may also be generally ascribed to the presence of phenols and flavonoids (Table 4). However, the role of physicochemical properties of the honey including pH and osmolality, cannot be ruled and should be investigated in future studies. We hypothesize that the antibacterial activity of the putative phytochemical agents present in our honey samples is concentration-dependent and or agent-specific. In support of this possibility, is the observed decrease in the zones of inhibition when the honey samples were diluted to lower concentrations of 75% v/v and 50% v/v (Figure 2A-C and Table 1). In fact most of the tested honey samples at 50% exhibited no significant difference in the zones of inhibition against *E. coli and MRSA* compared to distilled water (negative control) (Figure 2A and C), with up to 8 samples exhibiting no activity against *E. coli.* This could explain the lack of activity against the test bacteria by some samples even at 100% v/v, that is, such samples lacked the specific antibacterial phytochemicals or such phytochemical agents were present in amounts below the observed minimum inhibitory concentrations (Table 2). Contrary to our findings, a study on honey from the Kenyan *Dactylurina schimidti* stingless bee, reported lack of inhibitory activity against both *E. coli* and *S. aureus* at all the tested concentrations [15]. This difference could be due to species differences and different floral sources, which has been shown to be key determinant of the bioactive compounds present in stingless bee honey [10, 26].

This study further shows that honey of *Meliponula beccarii* from Baringo do not have any antifungal activity against *C. albicans* (Figure 2D and Table 1). This is contrary to the ethnomedicinal studies in Baringo, which reported widespread traditional use of *Meliponula beccarii* honey in the management of oral thrush, which is commonly caused by *C. albicans* [5].The result of our study is also in contrast to previous studies that reported inhibitory activity of *Melipona beechei* honey [7] and Kelelut (*Trigona)* honey [27] against *C. albicans* at as low as honey concentration of 10% v/v. Honey produced by Tanzanian stingless bees[14] and Brazilian *Scaptotrigona bipunctata*, *Melipona quadrifasciata*, *Melipona bicolor* and *Melipona marginata*[10] have also been reported to have antifungal activity against *C. albicans*. This could be due to species and region-specific differences in the phytochemical contents of the honey [10, 28].

Pollen is usually collected by the stingless bees during the collection of the nectar, the main raw material for making the honey. Surprisingly, however, all the pollen samples of *Meliponula beccarii* tested in this study neither exhibited neither antibacterial activity nor antifungal activity against *C. albicans* (Figure 3A-D), despite having the same phytochemical profile compared to the honey. Pollen extract of *Melipona compressipes Manaosensis* from Maues, Brazil similarly did not inhibit the growth of *E. coli* and *S. aureus*, which is consistent with the findings of this study [22]. However, some previous studies have reported the antimicrobial activities of stingless bee pollen contrary to our study [22, 29, 30]. The lack of antimicrobial activity in pollen despite of the similar phytochemical profiles with honey samples (Tables 4 and 5), further reinforces the possibility that the antimicrobial activity is phytochemical-specific and/or concentration-dependent. Therefore, pollen might lack the specific antimicrobial compounds present in honey or if present might be in very low concentrations that cannot effectively inhibit the growth of the tested micro-organisms. It is also important to note that once collected, the honey and pollen undergo differential processing and storage, which might lead to differential bioactivity [10].

Meliponula beccarii honey exhibited the lowest MIC of 9.38 % (v/v) against *E. coli* compared to 18.75 % (v/v) for *H. influenzae* and *MRSA* (Table 2), indicating a higher susceptibility of Gram-negative bacteria to the honey. These findings contradict those reported by Boorn *et al*.,[8], which reported higher sensitivity of Gram-positive bacteria particularly *S. aureus* to Australian *Trigona carbonaria* honey than the Gram-negative bacteria. Garedew *et al*., [31] also reported that Gram-negative bacteria are less sensitive to honey activity compared to Gram-positive bacteria, contrary to our findings. Compared to our findings, Nishio *et al*., [32] also reported lower MIC of *Scaptotrigona bipunctata* and *Scaptotrigona postica* honeys with values ranging from 0.63 to 10% among Gram-positive bacteria and from 2.5 to 10% among Gram-negative bacteria. Wavinya *et al*., [16] reported that *Plebenia hylderbrandii* honey from western Kenya had bacteriostatic effect against *S. aureus* and *E. coli* while *Meliponula bocandei* honey had bacteriostatic effect on *S. aureus*, but not on *E. coli.* Altogether, these findings highlight great variability in the antibacterial properties of honey from different stingless species and geographical regions, which should be considered when formulating them for therapeutic uses.

At a much higher concentration of 60.94 % (v/v), *Meliponula beccarii* honey is bactericidal against *MRSA*, but not against *E. coli* and *H. influenza (*Table 3*)*. This agrees with a study by Wavinya *et al*., [16] which also reported a higher bactericidal activity of *Plebenia hylderbrandii* and *Meliponula bocandei* honey on gram positive bacteria particularly *S. aureus* than on *E. coli*. In recent times, MRSA has become a global public threat, as it is resistant to most antibiotics except the carbapenems and vancomycin [33, 34]. Our results shows that *Meliponula beccarii* honey can be a source of novel bactericidal compounds against *MRSA*, which warrants further studies to isolate and characterize such potential compounds.

To the best of our knowledge this is the first study to profile the phytochemical content of *Meliponula beccarii* honey from Kenya. Our findings showing presence of flavonoid and phenols in *Meliponula beccarii* honey are consistent with the findings of other studies of honey from different stingless bee species and regions around the world. Phenolics and flavonoids, for example, were reported to be present in honey of *Scaptotrigona bipunctata*, *Melipona marginata*, *Tetragonisca angustula*, *Trigona hypogea*, *Melipona quadrifasciata*, and *Tetragona clavipes* stingless bees [28]. The *Melipona bicolor*, *Melipona quadrifasciata*, *Melipona marginata*, and *Scaptotrigona bipunctata* Brazilian stingless bee honey also contained phenolics, flavonoids and tannins [10]. In addition, honey of *Heterotrigona itama* and *Geniotrigona thoracica* Malaysian stingless bees, were found to contain flavonoids and phenolic compounds [35] The results of this study are in agreement with the reported phytochemical content of honey of several species of stingless bees in East and Northern Kalimantan, Indonesia which similarly had tannins, alkaloids, flavonoids, triterpenoids and saponins [36]. However, the honey lacked steroids which were present in this study. Similar to this study also, alkaloids, steroids, triterpenoids, phenolics, saponins, tannins were reported in the honey of *Tetragonula laeviceps* of Indonesia [37]. Rubber honey from *Trigona itama* bees was reported to contain saponins, flavonoids and phenols which were also detected in this study, but alkaloids, tannins, triterpenoids, and steroids were not found [38]. Wavinya *et al*., [16] similarly reported the presence of tannins, flavonoids and phenolic glycosides out of the 35 other organic compounds that were identified in a phytochemical analysis of honey from the Kenyan *Plebenia hylderbrandii* and *Meliponula bocandei* stingless bee species. Our results on the pollen phytochemistry are also consistent with several other studies in Brazil [22, 29] and Philippines [39].

Table 1: Mean diameter of inhibition zones (mm) of individual 11 honey samples evaluated by agar well diffusion method

		Zones of inhibition (mm) of test micro-organisms			
Honey sample	Concentrations	*E. coli* Mean±SD	*H. influenzae* Mean ±SD	*MRSA* Mean ±SD	*C. albicans* Mean ±SD
1	100 %	14.33 (±0.58)	20.333 (±0.58)	16.33 (±0.58)	-
	75%	11.00	18.33 (±0.58)	19.00 (± 1.00)	-
	50%	9.00 (±0)	15.00 (±1.00)	11.67 (± 0.58)	-
2	100 %	10.33(± 0.58)	16.33(±0.57)	14.67 (± 2.89)	-
	75%	9.00 (±0)	14.67(±0.58)	11.33 (± 0.58)	-
	50%	-	13.00(±1.00)	9.00 (±0)	-
3	100 %	10.67 (±0.58)	13.33 (±1.16)	-	-
	75%	10.00 (±0)	11.333 (±1.16)	-	-
	50%	-	-	-	-
4	100%	11.33 (±0.58)	11.67(±0.58)	10.67 (± 0.58)	-
	75%	9.67(±0.58)	10.33(±0.58)	8.67 (± 0.58)	-
	50%	7.33 (±0.58)	9.00 (±0)	-	-
5	100%	9.00 (±0)	10.67(±0.58)	11.67 (± 0.58)	-
	75%	-	8.67(±0.58)	9.33 (± 0.58)	-
	50%	-	7.33(±0.58)	7.00 (± 0)	-
6	100%	11.33 (±0.58)	10.67(± 0.58)	13.67(± 1.16)	-
	75%	9.67 (±0.58)	9.67(±0.58)	11.00(±1.00)	-
	50%	7.33 (±0.58)	8.67(±0.58)	9.33 (± 0.58)	-
7	100%	-	-	-	-
	75%	-	-	-	-
	50%	-	-	-	-
8	100%	11.00 (±0)	11.33(±0.58)	11.67 (±0.58)	-
	75%	8.67 (±0.58)	10.00(±0)	-	-
	50%	-	9.00(±0)	-	-
9	100%	12.00 (±0)	10.33(±0.58)	18.33(±5.77)	-
	75%	9.33 (±0.58)	9.00(±0)	15.67(± 5.51)	-
	50%	-	7.33(±0.58)	13.67)±5.51)	-
10	100%	-	8.67 (± 0.58)	11.67(± 0.58)	-
	75%	-	7.67(±0.58)	9.33(± 0.58)	-
	50%	-	-	7.00(± 0)	-
11	100%	8.67(±0.58)	9.33 (± 0.58)	9.67 (± 0.58)	-
	75%	-	-	7.67 (± 0.58)	-
	50%	-	-	-	-
Positive controls	Ciprofloxacin (30µg/ml)/Nystatin (0.4 mg/ml)	31.67 (±1.53)	24.67 (±0.58)	27.00	15(± 0)
Negative control	Distilled water	-	-	-	-

NB: Mean zone of inhibition (mm) includes the well's diameter (6 mm), N=3; (–) = no clear zone of inhibition. Samples 1 and 2 were from Chemusu forest; samples 3 and 4 from Sabatia forest, sample 5 was from Narasha Forest, sample 6 from Sigoro, all in Koibatek sub-ounty. Samples 7, 8, 9, 10 and 11 were collected from Katimok, Kipngochoch, Kapkomoi, Sacho Kaplel and Kituro, respectively.

Table 2: Mean MIC of *Meliponula beccarii* honey and ciprofloxacin (positive control)

Micro-organism	*Meliponula beccarii* honey (% v/v)	Ciprofloxacin (µg /ml)
E. coli	9.38	ND
H. influenzae	18.75	0.075 µg /ml
MRSA	18.75	ND

ND-not detected

Table 3: Mean MBC of *Meliponula beccarii* honey and ciprofloxacin (positive control)

Micro-organism	*Meliponula beccarii* honey (% v/v)	Ciprofloxacin (µg /ml)
E. coli	N/D	ND
H. influenzae	N/D	0.3 µg /ml
MRSA	60.94	ND

N/D: bactericidal activity not detected

Table 4: Phytochemical content of *Meliponula beccarii* honey

Phytochemical	Test	Honey samples and Inferences										
		1	2	3	4	5	6	7	8	9	10	11
Phenols	Ferric chloride	+	+	+	+	+	+	+	+	+	+	+
Tanins	Ferric chloride	+	+	+	+	+	+	+	+	+	+	+
Saponins	Foaming	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	Dragendoff's	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Modified Borntrager	+	+	+	+	+	+	+	+	+	+	+
Steroids	Salkowski	+	+	+	+	+	+	+	+	+	+	+
Flavanoids	NaOH test	+	+	+	+	+	+	+	+	+	+	+
Triterpenoids	Liebermann-burchard	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski	-	-	-	-	-	-	-	-	-	-	-

(+) presence; (-) absence; 1-11 represent individual honey samples. Samples 1 and 2 were from Chemusu forest; samples 3 and 4 from Sabatia forest, sample 5 was from Narasha Forest, sample 6 from Sigoro, all in Koibatek sub-ounty. Samples 7, 8, 9, 10 and 11 were collected from Katimok, Kipngochoch, Kapkomoi, Sacho Kaplel and Kituro, respectively.

Table 5: Phytochemical content of *Meliponula beccarii* pollen

Phytochemical	Test	Pollen samples and Inferences										
		1	2	3	4	5	6	7	8	9	10	11
Phenols	Ferric chloride	+	+	+	+	+	+	+	+	+	+	+
Tanins	Ferric chloride	+	+	+	+	+	+	+	+	+	+	+
Saponins	Foaming	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	Dragendoff's	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Modified borntrager	+	+	+	+	+	+	+	+	+	+	+
Steroids	Salkowski	+	+	+	+	+	+	+	+	+	+	+
Flavanoids	Ammonia	+	+	+	+	+	+	+	+	+	+	+
Triterpenoids	Liebermann burchard	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski	-	-	-	-	-	-	-	-	-	-	-

(+) presence; (-) absence; 1-11 represent individual pollen samples. Samples 1 and 2 were from Chemusu forest; samples 3 and 4 from Sabatia forest, sample 5 was from Narasha Forest, sample 6 from Sigoro, all in Koibatek sub-ounty. Samples 7, 8, 9, 10 and 11 were collected from Katimok, Kipngochoch, Kapkomoi, Sacho Kaplel and Kituro, respectively.

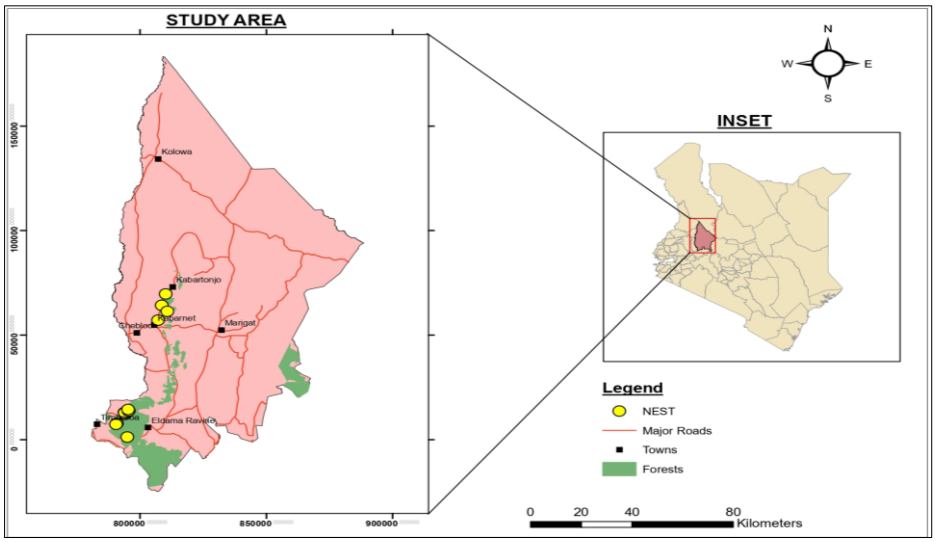

Figure 1: Map of Kenya showing the location of Baringo County (inset), and the geo-locations of the eleven nests that were sampled (shown on the left side as yellow-coloured circles). Some geolocations are superimposed on top of others

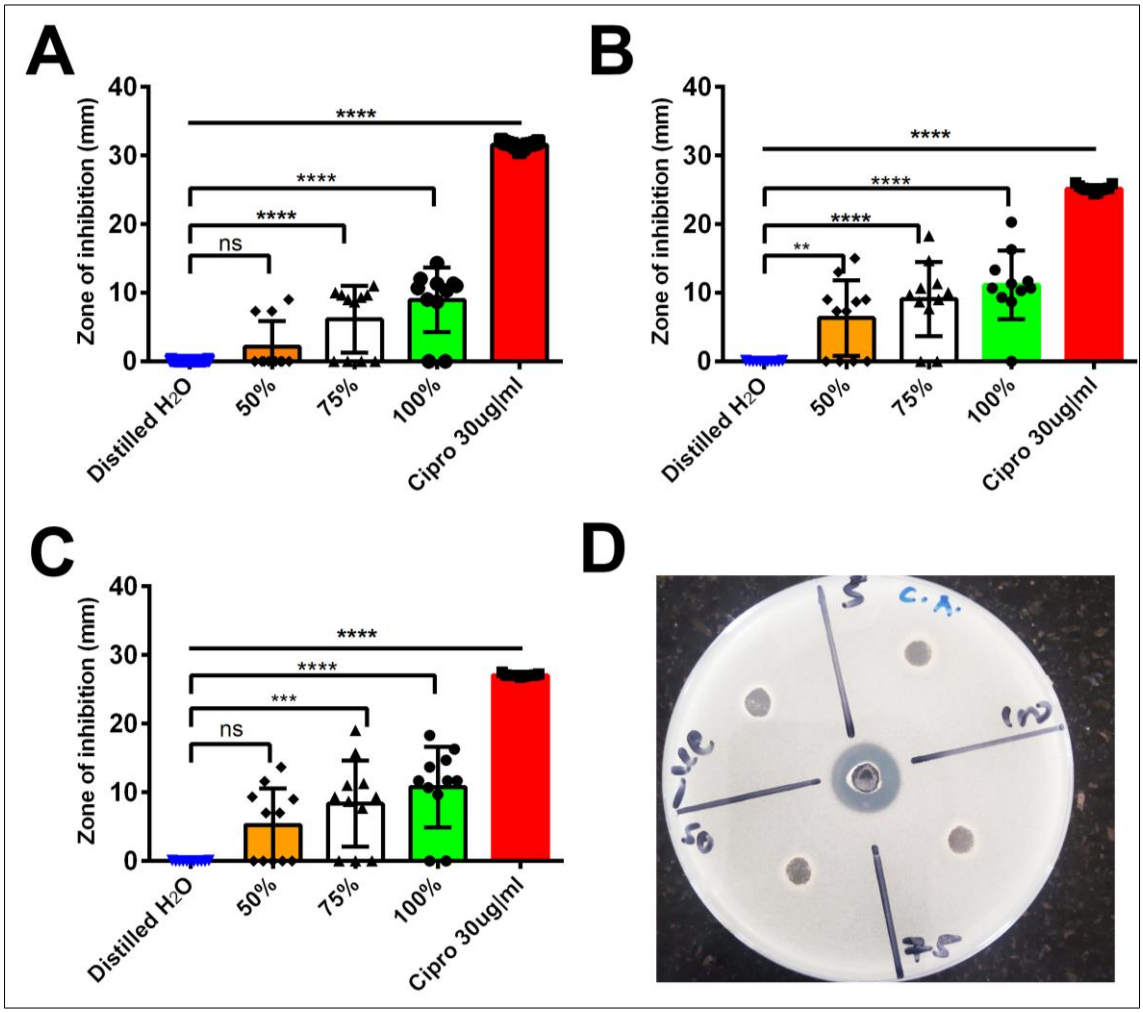

Figure 2: Increasing concentrations (50 to 100% v/v) of *Meliponula beccarii* honey exhibit varying antimicrobial activities in agar well diffusion assay. A) Show concentration dependent-increase in the mean zone of inhibition of honey against *E. coli* B) show concentration dependent-increase in the mean zone of inhibition of honey against *H. influenzae* C) show concentration dependent-increase in the mean zone of inhibition of honey against *MRSA* D) show a representative plate depicting no activity of honey against *C. albicans* in comparison with nystatin positive control. All data are expressed as mean + SD. Data was analysed by two-way ANOVA with Tukey´s post hoc test. n.s: non-significant, ***: p<0.001, ****: p<0.0001. CA: *Candida albicans*, -ve: negative control Cipro: ciprofloxacin

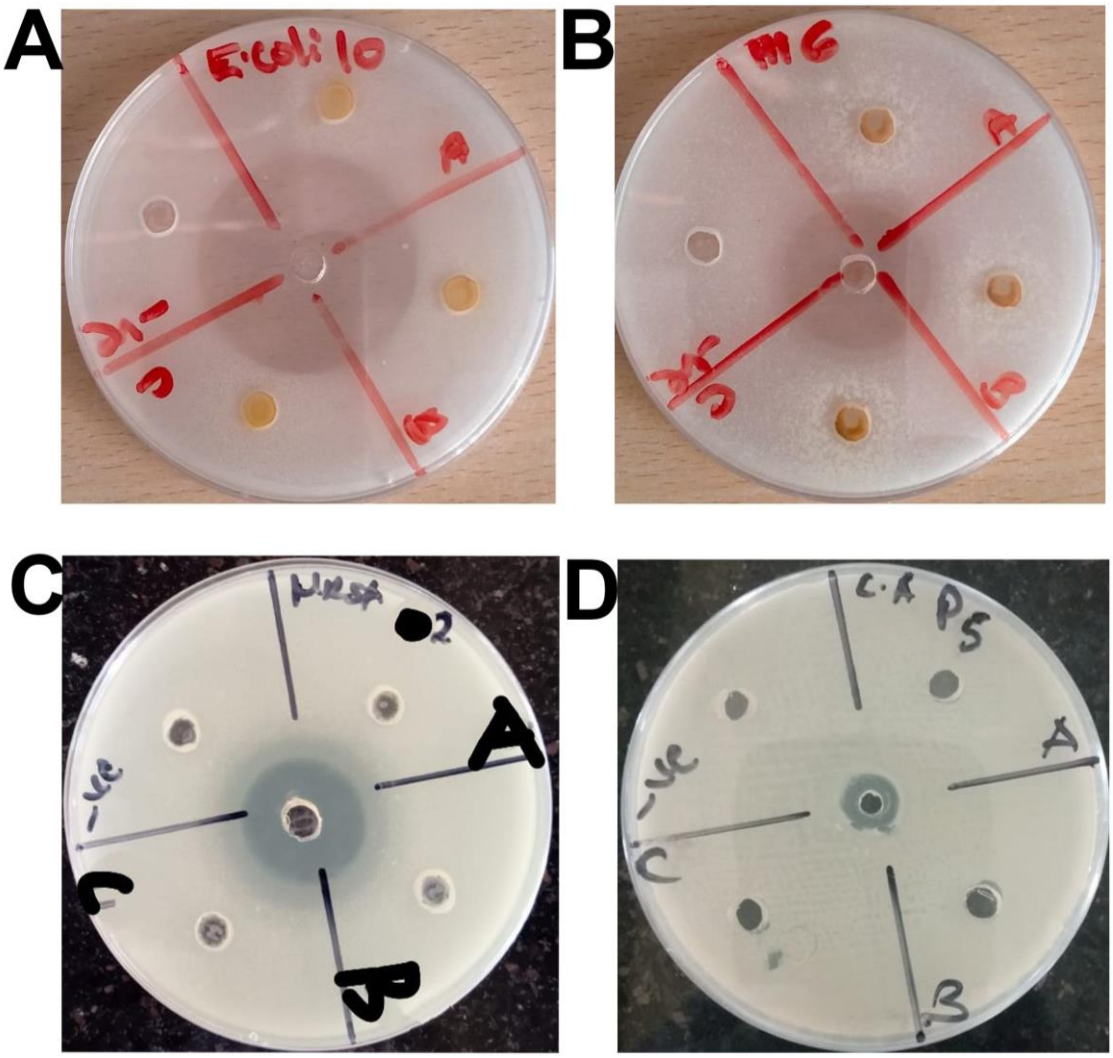

Figure 3: *Meliponula beccarii* pollen exhibits no antimicrobial activity in agar well diffusion assay against. A) *E. coli*, B) *H. influenza*, C) *Methicillin resistant staphylococcus aureus* (*MRSA*) and D) *C. albicans.* (–ve)-negative control. The clear zones of inhibition in the middle of the plates are for the positive controls.

CONCLUSION

In conclusion, honey of *Meliponula beccarii* from Baringo has potent *in vitro* antibacterial activity but no activity against *C. albicans*. The honey and pollen of *Meliponula beccarii* contain diverse phytochemical compounds, which can be further investigated for discovery of new antibiotics, including anti-MRSA.

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Declaration of Conflict of interest

The authors declare no conflict of interest

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