**In vitro antimicrobial properties of apis mellifera L. and Meliponulla beccarii L. honeys from Kellel and West Wollega Zones, Western Ethiopia**

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**ABSTRACT**

Honey is a sweet natural product synthesized from nectars of flowers by different types of honey bees. It has been used as medicine in different cultures. The aim of this study was to investigate the antimicrobial activities of honey samples from different sources against selected clinical and standard microorganisms. Antimicrobial activities of the honey samples against selected standard and clinical microorganisms were done using agar disc and agar well diffusion methods. The highest diameter of inhibition zone against clinical and standard strains was recorded for Meliponula beccarii honey (MBH) (16 ± 3 mm) followed by Apis mellifera fresh honey (AMFH) (15 ± 2 mm) and Apis mellifera market honey (AMMH) (11 ± 3 mm) by well diffusion method. The mean inhibition zone recorded by the disc diffusion method were generally lower than that of the well diffusion method. However, none of the honey samples inhibited Candida albicans. The mean Minimum Inhibitory Concentration (%) and Minimum Bactericidal Concentration (%) against all the tested bacteria were 9.9 and 53.6 by MBH, 11.0 and 56.2 by AMFH, and 15.4 and 64.3 by AMMH, respectively. The fresh honey samples directly collected from the hives and honey samples from stingless bees showed higher antimicrobial effects against the tested pathogenic bacteria. This implies that honey could be used as an alternative therapy against diseases caused by the bacterial pathogens.

**Introduction**

Infectious diseases are the leading cause of death in children and young adults in low-income countries accounting for 45% deaths.\textsuperscript{[1]} The introduction of antibiotics is considered a breakthrough in the field of medical microbiology as it dramatically reduced human and livestock morbidity and mortality. However, soon after the introduction of the antibiotics, an increase in the frequency of resistant pathogens was observed.\textsuperscript{[2]} The antibiotic resistance has been attributed to the overuse and misuse of medications, as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements.\textsuperscript{[3,4]} Morbidity and mortality associated with bacterial infections remain high despite antimicrobial therapy.\textsuperscript{[5]} Thus, an alternative therapy is required for the treatment of diseases.

Among the natural products used for treatment of various ailments, honey has been used for treatment of wounds, ulcers, bed sores, and other skin infections resulting from burns.\textsuperscript{[6–8]} Honey is a sweet and flavorful natural product produced by different types of honey bees from nectars of various...
bee floras. It is a supersaturated sugar solution composed mainly of fructose (38%) and glucose (31%).

Honey has antioxidant, antibacterial, antifungal, anti-viral and anti-inflammatory properties. The antimicrobial activity of honey is attributed to and affected by several factors such as its chemical compositions which include sugars, proteins, minerals and phenolic compounds, honey sources/floral types, entomological and geographical origin, climate, harvesting time, processing and storage conditions. Honey quality is also affected by processes starting form harvesting to consumptions. Storage conditions such as materials used for storage and the length of storage have the potential to change the flavor and microbial load of honey. Among the microorganisms, yeasts, and sporeforming bacteria are commonly found in honey due to post – harvest handling. Ethiopia is the leading honey producer in Africa and the 10th in the world. The high potential for honey production in Ethiopia is due to its diversified agro-climatic conditions and biodiversity. Ethiopia is also known to have over 10 million bee colonies and over 800 identified honey-source plants, among the bee colonies 5 to 7.5 million are hived. Despite the unique agro-climatic conditions, biodiversity, and large numbers of bee colonies, few researches have been conducted regarding the antimicrobial activities of honey in Ethiopia. Most of these studies were restricted to the Northern part of the county, and no research has been done on the antimicrobial activities of Meliponilla beccarii honeys. The objective of the current study is therefore to assess the antimicrobial activities of honey samples collected from western parts of the country which are known for their rich bees flora and biodiversity.

Materials and Methods

Honey sample collection

This study was conducted in West and Kellam Wollega Zones of Western Oromia, Ethiopia. The honey samples were collected from two Zones (Kellel Wollega and West Wollega Zones), of the Western Oromia, Ethiopia. The honey types collected from the study areas were grouped into three, namely, A. mellifera fresh honey samples (AMFH) directly collected from beehives, A. mellifera honey samples purchased from local markets (AMMH), and stingless bee Meliponilla beccarii (M. beccarii) honeys purchased from farmers in the study areas. A total of 39 honey samples (13 from each honey type) were randomly collected.

Preparation of honey samples

Each of the honey samples was filtered using sterile gauze to remove impurities or debris in the crude honey such as bee wax, brood, and dead bees. From the strained table honey 75% v/v honey solution was prepared to test the susceptibility of test microorganisms to the different honey samples. Three mL of pure honey was transferred into 1 mL of sterile distilled water during the preparation of honey samples for antimicrobial test.

Test microorganisms used for the current study

For this work, the standard bacterial strains (S. aureus-ATCC-25923, E. coli- ATCC-25922 and P. aeruginosa-ATCC-27853) and clinical microbes (S. aureus from pus, E. coli from urine, P. aeruginosa from fluid, and Salmonella typhi from blood and Candida albicans from urine) were obtained from Ethiopian Public Health Institute (EPHI).
**Inoculums preparation**

0.5 McFarland standard was prepared by mixing 0.5 mL of 0.048 M BaCl₂(1.175% W/V BaCl₂ 2H₂O) with 99.5 mL of 0.18 M H₂SO₄ (1% V/V) and thoroughly mixed to ensure that it is evenly suspended (Andrews, 2006). Then, the standard was distributed in to a capped test tube of the same size and volume as those used to prepare the test inoculums.

Inoculums was prepared by picking 2–3 colony from 24-hour old culture that was grown on their selective media (E. coli on eosin methylene blue, S. aureus on mannitol salt agar, S. typhi on selenite agar, P. aeruginosa on pseudomonas agar and C. albicans on Potato dextrose agar) and suspended in 5 mL saline solution (0.85%). The suspended inoculums were vortexed for 15 seconds and their turbidity was adjusted by adding colony or saline solution into microbial stock solution and compared visually with standardized 0.5 McFarland (10⁸ CFU/ml) against a white paper background with contrasting black lines.

**Determination of antimicrobial activity of honey**

**Agar well diffusion assay**

Agar well diffusion test was carried out according to Moussa et al. [38] Mueller Hinton Agar and Potato dextrose agar plates were uniformly seeded by means of sterile cotton swab from inoculated saline solution containing bacteria and fungus strains, respectively. The plates were left on the bench for excess fluid to be absorbed. Using a sterile cork borer, 6 mm diameter, and 4 mm deep wells were made in the seeded agar medium. Using a micropipette, 60 µL of honey samples with a concentration of 75% each sample was added into the wells in the plates. Positive controls (chloramphenicol (30 µg/60 µL) and griseofulvin (25 µg/60 µL) as well as sterile distilled water negative control was equally filled in the well. The plates were incubated at 37°C for 24 h. The diameters of inhibition zones of each sample including the wells were measured using caliper in mm and the results were recorded accordingly. All the assays were done in triplicate.

**Agar Disc diffusion assay**

Agar disc diffusion test was carried out according to Moussa et al. [38] The solidified Mueller Hinton agar plate for bacteria and Potato Dextrose Agar plate for fungi were uniformly seeded using sterile cotton swab from inoculated saline solution containing the respective test strains. The plates were left on the bench for excess fluid to be absorbed. Using a puncher, 6 mm discs were made from Whatman no 1 and sterilized to be placed on the seeded plates. A 10 µL of the prepared honey solution was pipetted on the surface of the disc. Chloramphenicol (30 µg was dissolved in 25 µL sterile distilled water per a disc) and griseofulvin (25 µg was dissolved in 25 µL sterile distilled water for a single disc) were used as a positive control for bacterial and fungal strains, respectively, while 25 µL sterile distilled water was used as a negative control. All the assays were replicated three times.

**Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) was determined by broth dilution method (Boateng and Diunase, 2015). To determine the lowest concentration of antimicrobial activity, the stock honey solution (75%) which was prepared by diluting 3 ml or g/ml of Mueller Hinton Broth that showed high inhibition zone against the test microbes was used. A two-fold serial dilution was prepared from the stock solution. An 18–24 h bacterial culture was used and standardized by comparing its turbidity with the 0.5 McFarland standard. Nine sterile capped test tubes were prepared and labeled. Then, 4 ml (75%), 2 ml (37.5%), 1 ml (18.75%), 0.5 ml (9.375%), 0.25 ml (4.68%), 0.125 ml (2.35%), and 0.0625 ml (1.17%) were pipetted from the stock honey solution (75%) in a series of seven tubes. Then, to each test tube, 1 ml of the standardized inoculum suspensions were pipetted while the negative control tube (eighth test tube) contained only broth and the positive control tube (ninth test tube) contained one
milliliter microbes + broth for comparison. The tubes were vortexed and incubated for 24 h at 37°C. Finally, by comparing with the control tubes clear tubes were used as the least honey concentration that inhibited bacterial growth.

**Minimum Bactericidal Concentration (MBC)**

A dilution showing no visible growth during the MIC test was sub-cultured onto a fresh Mueller Hinton Agar plate by streaking using sterile inoculating loop and incubated at 37°C for 24 h. The lowest concentration of the extracts showing no growth on the Muller Hinton Agar plate was recorded as MBC.\(^{[35]}\)

**Data analysis**

SAS version 9.1.3 computer package was used for analyzing all the data. Means and standard deviations of the recorded data were calculated using SAS Software. Determination of the significant differences between honey samples was done using one-way ANOVA. Inhibition zone, MIC, MBC, and microbial qualities of the tested honey samples were used for mean separation. Multiple pairwise comparison between the mean values was done using the Least Significant Difference (LSD).

**Results**

The antimicrobial activity of honey from the well diffusion method ranged from 9.7 ± 2.0 mm by the A. mellifera honey against clinical E.coli to 18.3 ± 3.2 mm by M. beccari honey against clinical S. aureus. A. mellifera honey samples directly collected from the market were statistically \((p < .0001)\) less effective against all the test pathogens compared to both fresh A. mellifera directly collected from hives and stingless (M. beccari) honey samples collected from farmers. No activity of the honey samples collected from the three sources were observed against clinical C. albicans. The most susceptible test organism to all the honey types was clinical S. aureus (Table 1).

The antimicrobial activities of the honey samples from different sources done using the disc diffusion method is indicated in Table 2. Generally, similar patterns of susceptibility was observed where A. mellifera honey samples collected directly from the markets had lower activity against all the tested pathogens compared to fresh A. mellifera honey samples directly collected from the hives and honey samples from stingless bee (M. beccari) collected from the farmers. No inhibition of the honey samples from the different sources was observed against clinical C. albicans, and the antimicrobial

| Table 1. Mean Inhibition zone of the tested samples against pathogenic microorganisms by agar well diffusion method including the well (6 mm diameter) at 75% honey concentration. |
|---|---|---|---|---|---|---|---|
| Microbes | AMMH | AMFH | MBH | CONT | LSD | p-value |
| CEC | 9.7 ± 2 \(^{[b]}\) | 13.5 ± 2.4 \(^{[b]}\) | 13.9 ± 3.2 \(^{[b]}\) | 20.4 ± 1.2 \(^{[a]}\) | 1.19 | <.0001 |
| SEC (ATCC 25922) | 9.9 ± 2.3 \(^{[c]}\) | 13.7 ± 1.7 \(^{[b]}\) | 13.9 ± 3.4 \(^{[b]}\) | 23.1 ± 1.7 \(^{[a]}\) | 1.21 | <.0001 |
| CPA | 10.1 ± 1.9 \(^{[d]}\) | 14.7 ± 1.4 \(^{[c]}\) | 16.6 ± 2.3 \(^{[b]}\) | 25.3 ± 2.9 \(^{[a]}\) | 1.73 | <.0001 |
| SPA (ATCC 27853) | 11.1 ± 2.5 \(^{[c]}\) | 14.7 ± 2.6 \(^{[b]}\) | 15.7 ± 2.4 \(^{[b]}\) | 25.6 ± 2.4 \(^{[a]}\) | 1.26 | <.0001 |
| CSA | 12.8 ± 4.1 \(^{[c]}\) | 17.8 ± 2.3 \(^{[b]}\) | 18.3 ± 3.2 \(^{[b]}\) | 28.3 ± 4.2 \(^{[a]}\) | 1.81 | <.0001 |
| SSA (ATCC 25923) | 11.7 ± 3.6 \(^{[c]}\) | 16.0 ± 4.7 \(^{[b]}\) | 15.7 ± 3.2 \(^{[b]}\) | 26.2 ± 3.2 \(^{[a]}\) | 1.82 | <.0001 |
| CST | 10.5 ± 2.0 \(^{[d]}\) | 13.8 ± 2.1 \(^{[c]}\) | 15.8 ± 1.7 \(^{[b]}\) | 27.4 ± 2.8 \(^{[a]}\) | 1.13 | <.0001 |
| X | 10.8 ± 2.6 | 14.8 ± 2.4 | 15.7 ± 2.8 | 25.2 ± 2.5 | | |
| CCA | ND | ND | ND | 11 ± 2.1 | | |

Note: Means followed by different letters (a, b, c, d) within the row are statistically significant difference at \(p \leq 0.05\). AMMH: A. mellifera L. honey purchased from market, AMFH: A. mellifera L. honey harvested directly from hive, MBH: Honey sample from M. beccari L. CONT: Positive control, LSD: Least Significant Difference at alpha = 0.05, SD: Standard Deviation, X: Overall mean, CEC: Clinical Escherichia coli, SEC (ATCC 25922): Standard Escherichia coli American Type Culture Collection number 25922, CPA: Clinical Pseudomonas aeruginosa, SPA (ATCC 27853): Standard Pseudomonas aeruginosa American Type Culture Collection number 27853, CSA: Clinical Staphylococcus aureus, SSA (ATCC 25923): Standard Staphylococcus aureus American Type Culture Collection number 25923, CST: Clinical Salmonella typhi, CCA: Clinical Candida albicans and ND: not detected.
Table 2. Mean inhibition zone of the tested samples against clinical and standard pathogens by disc diffusion method with the disc diameter (6 mm) at 75% (w/v or v/v) honey concentration.

| Microbes          | AMMH    | AMFH    | MBH     | CONT   | LSD     | p-value |
|-------------------|---------|---------|---------|--------|---------|---------|
| CEC               | 6.0 ± 1 ²  | 6.5 ± 1²c | 7.2 ± 1 ²b | 18.1 ± 1.4a | 0.96   | <.0001  |
| SEC (ATCC 25922)  | 6.8 ± 1.9 ²c | 7.5 ± 1.5²bc | 8.4 ± 1 ²b | 17.9 ± 1.6a | 1.39   | <.0001  |
| CPA               | 7.5 ± 1.6 ²b | 9.3 ± 1.6 ²c | 10.9 ± 1.2 ²b | 19.4 ± 1.4a | 1.22   | <.0001  |
| SPA (ATCC 27853)  | 7.9 ± 1.6 ²b | 8.9 ± 1.8 ²b | 8.4 ± 1.2 ²b | 20.6 ± 1.4a | 1.39   | <.0001  |
| CSA               | 9.2 ± 1.5 ²b | 11.4 ± 2.1 ²b | 10.9 ± 1.6 ²b | 24.0 ± 2.7c | 2.90   | <.0001  |
| SSA (ATCC 25923)  | 8.8 ± 1.3 ²c | 10.1 ± 1.9 ²c | 12.4 ± 1.6 ²b | 23.3 ± 1.4c | 1.45   | <.0001  |
| CST               | 7.2 ± 1.4 ²c | 9.6 ± 2.1 ²c | 11.0 ± 0.8 ²b | 23.2 ± 1.3a | 1.33   | <.0001  |
| X                 | 7.6 ± 1.3 ²   | 9 ± 1.7 ²  | 10 ± 1.1 ²  | 20.9 ± 2.1 ² |       |         |
| CCA               | ND      | ND      | ND      | 9 ± 1.2 ²  |         |         |

Note: Different letters (a, b, c, and d) on the superscripts within the row are statistically significant difference at p ≤ 0.05. AMMH: *A. mellifera* honey purchased from market, AMFH: *A. mellifera* honey harvested directly from hive, MBH: Honey sample from *M. beccarii* species. CONT: Positive control, LSD: Least Significant Difference at alpha = 0.05, SD: Standard Deviation, X: Overall mean, CEC: Clinical Escherichia coli, SEC (ATCC 25922): Standard Escherichia coli American Type Culture Collection number 25922, CPA: Clinical Pseudomonas aeruginosa, SPA (ATCC 27853): Standard Pseudomonas aeruginosa American Type Culture Collection number 27853, CSA: Clinical Staphylococcus aureus, SSA (ATCC 25923): Standard Staphylococcus aureus American Type Culture Collection number 25923, CST: Clinical Salmonella typhi, CCA: Clinical Candida albicans and ND: not detected.

activities of the fresh *A. mellifera* honeys and honey samples from the stingless bee (*M. beccarii*) were comparable against most of the test pathogens. However, honey samples from stingless bees (*M. beccarii*) had statistically significant (p < .0001) activity against clinical *P. aeruginosa*, standard *S. aureus* ATTC 25923 and Clinical *S. typhi* (Table 2).

Minimum inhibitory concentration

Honey samples from stingless bee (*M. beccarii*) had shown relatively lower minimum inhibitory concentration against all the test pathogens compared to fresh *A. mellifera* honey samples. However, fresh *A. mellifera* honey samples and *M. beccarii* honey samples had comparable minimum inhibitory concentrations against clinical *P. aeruginosa*, *P. aeruginosa* ATCC 27853, clinical *S. aureus* and *S. aureus* ATCC 25923. From the test organisms both standard and clinical *S. aureus* were the most sensitive to all the honey samples (Figure 1).

![Figure 1](image-url)  
**Figure 1.** Mean minimum inhibitory concentration (Mean ± Standard Deviation) of honey samples at different concentrations by using broth dilution method. AMMH: *A. mellifera* honey purchased from market, AMFH: *A. mellifera* honey harvested directly from hive, MBH: Honey sample from *M. beccarii* species.
**Minimum bactericidal concentration of the honey samples**

Overall, honey samples from stingless bees (M. beccarii) had the lowest minimum bactericidal concentration against most of the test organisms compared to the other honey samples. Both clinical and standard S. aureus were the most susceptible to all the honey types (Figure 2).

**Discussion**

The current study assessed the antimicrobial activities of honey samples from different sources. The study revealed that fresh A. mellifera honey samples had better antimicrobial activities against the test pathogens compared to A. mellifera honey samples collected from market places. In addition, honeys of the stingless bee (M. beccarii) has shown the best antimicrobial activities compared to A. mellifera honey samples in both the well and disc diffusion assays. However, none of the honey types were active against clinical C. albicans (Tables 1 and 2). The mean zones of inhibition with the well diffusion method in the current study was higher than that of the disc diffusion method (Tables 1 and 2). The minimum inhibitory (Figure 1) and bactericidal (Figure 2) concentrations were generally lower for stingless bee honey samples.

The mean inhibition zone from all types of honey sample in this study was less than the result reported by Yalemwork et al.[35] by using agar well diffusion method. However, honey samples from the stingless bee (Apis mellipodae) had stronger antibacterial activity than honey samples from A. mellifera against the tested pathogenic microbes. A study by Berhanu,[37,3840,39] in the norther part of the country revealed strong antibacterial activities of honey samples from stingless bee of a different species with the current study. A study on Cameroonian A. mellifera market honey samples against P. aeruginosa, S.aureus and E.coli showed relatively higher diameter of inhibition at 75% (w/v),[40] compared to the result we obtained. The result of disc diffusion based antimicrobial activity of A. mellifera honey from Iraq at 75% against S. aureus, E. coli and Pseudomonas spp.[41] showed higher diameter of inhibition compared with the current study (Table 2). Alvarez-Suarez et al.,[8] also reported that honey samples from stingless bees (M. beecheii) had higher antimicrobial effect compared to honey samples of the sting bees (A. mellifera). Araya and Berhe,[34] reported overall mean MIC and MBC of 6.25–50 and 12.5–100% (v/v), respectively against similar test pathogens from A. mellifera (white and red) honey. Brown et al.[42–44] reported higher antimicrobial activities of
stingless bee honeys from apiaries of Trinidad and Tobago against both gram negative and gram positive bacteria compared to honeys from Apis mellifera. In a study by Ng et al. (2020) on antibacterial activity of honeydew honey of a stingless bee, *E. coli* was found to be more susceptible than *S. aureus*. Similarly, Domingos et al. (2020) reported strong antibacterial activity of honeys from Amazonian stingless bees against selected standard gram negative and gram positive bacteria. All these studies in general support antimicrobial activities of honeys of different stingless bees.

The variation in the results of the current study and others studies might be due to methodology followed. Factors such as inoculums density, incubation time, size of plate, depth of agar medium and composition of the medium could affect the result of an experiment.\[45\] Besides, the honey sources, honey bee types, concentration of honey samples and geographical areas from where the samples were collected could also bring about disparities in the results. In general, Gram-positive bacteria (*S. aureus*) was found to be sensitive to the honey samples of the three sources compared to the gram-negative bacteria, this might be due to the cell wall structure of the bacteria. *S. aureus* was reported to be the most susceptible organism to different honey types.\[8,46\] Similarly, gram-positive bacteria were more sensitive to stingless bee honeys compared to Gram-negative bacteria with the agar well diffusion assay.\[47\] According to Silhavy et al.,\[48\] the complex cell wall with additional outer membrane protects the Gram negative bacteria by excluding toxic molecules and providing an additional stabilizing layer around the cell.

*Candida albicans* in the current study were resistant to honey samples collected from all the three sources. Similarly, clear zones were not formed by honey samples against *C. albicans* from different reports.\[38,47,49,50\] Other studies also reported effective antifungal activities of honey samples of different sources. For instance, Irish et al.\[16\] reported that *C. albicans*, *C. glabrata* and *C. dubliniensis* were sensitive to Jarrah honey samples. Variabilities in susceptibility of different fungi seem to be related to the types of honey samples which in turn are related to the type of bee forages used for making honey. Differences in the chemical compositions of the honey types might bring about differences in their antimicrobial activities. The main components of honey that have antimicrobial activities are H\(_2\)O\(_2\) and acids which are toxic to bacteria, and their amount depends on the presence of glucose oxidase enzyme.\[41,51,52\] If a honey contains high concentration of this enzyme, bacteria cannot respond normally to proliferative signals, and their growth remains arrested even when the honey is used in diluted forms.\[53\] The peroxide group like H\(_2\)O\(_2\) reacts with cell components such as cell wall and DNA inducing oxidative stress.\[54,55\] On the other hand, the enzymatic activity of honey is deactivated when the honey sample is exposed to sunlight or heated during processing and purification as well as storage. Heating affects the antimicrobial activities of honeys.

According to Jahan et al.,\[56\] the MICs of the investigated honey samples ranged from 6.25% to 20.00% (w/v) for unheated and increased to 50% after honey was heated to 90°C against *S. aureus*, *E. coli*, *S. typhi*, *Salmonella paratyphi* and *Chromobacterium violaceum*. In the study areas, honey samples from the markets normally undergo a heating process by mixing the honey with other impurities (Personal communication). Thus, the less effective antimicrobial activities of *A. mellifera* market honey in our study might be due to adulteration and exposure to light.

**Conclusion**

The study areas from where the honey samples were collected has rich biodiversity and different species of honey bees and bee forages. No study was conducted on honey samples in the study areas and the study on the antimicrobial activities of honey samples from stingless bee (*M. beccarrii*) has been reported for the first time in this study. Variation in antimicrobial activities were observed against the test pathogens with *M. beccarrii* honeys having the highest antimicrobial activities compared to *A. mellifera* honey types. Honey samples collected from the local market had the lowest activity against all the tested pathogens. Generally, the gram-positive bacteria (*S. aureus*) were more susceptible compared to gram-negative bacteria (*E. coli*, *S. typhi*, and *P. aeruginosa*). However, none of the sources of honey samples inhibited the tested fungi (*C. albicans*). Owing to limited resources, detailed
analysis of the chemical compositions of the honey samples were not done. But, this research will contribute to the existing knowledge of antimicrobial activities of honey samples from different geographical areas and honey bee types.

**Abbreviations**

AMMH: *Apis mellifera* market honey; AMFH: *Apis mellifera* fresh honey; MBH: *Melipona beccarii* hone; ATCC: American Type Culture Collection; DNA: Deoxy ribonucleic acids; E. coli: Escherichia coli; S. typhi: Salmonella typhi; P. aeruginosa: Pseudomonas aeruginosa; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; C. mellifera: *Apis mellifera*; M. beccarii: *Melipona beccarii*; C. albicans: *Candida albicans*; CONT: Positive control; LSD: Least Significant Difference; SD: Standard Deviation; CEC: Clinical *Escherichia coli*; SEC: Standard *Escherichia coli*; CPA: Clinical *Pseudomonas aeruginosa*; SPA: Standard *Pseudomonas aeruginosa*; CSA: Clinical *Staphylococcus aureus*; SSA: Standard *Staphylococcus aureus*; CST: Clinical *Salmonella typhi*; CCA: Clinical *Candida albicans*; ND: not detected.

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**Availability of Data and Materials**

Data will be available from the corresponding author upon reasonable request and the approval of the data owner.

**Ethics approval and consent to participate**

Not applicable.

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