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Antibacterial Effect of Honey: A Comparative Study Between Manuka And Sidr Prototypes

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ABSTRACT

Background: This study compares the antibacterial activity of Manuka honey (UMF 15+) against Escherichia coli, Acinetobacter spp., Klebsiella spp., Staphylococcus spp., Streptococcus spp., and Bacillus spp. All instruments were carefully rinsed before initiation.

Objective: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), as in microbiology and pharmacology, are standardized metrics used to evaluate how well an antimicrobial drug or agent works against specific pathogens were determined using the broth microdilution method with resazurin indicator.

Methods: Additionally, the agar well diffusion method was used to evaluate the inhibition zones produced by both honey samples.

Results: The results showed that Manuka honey was more antibacterial than Sidr honey against all tested bacterial isolates. The results demonstrated that Manuka honey showed a significantly lower value (12.5 µM (12.5 commercial on t50% - 50 commercial values)). The MIC values for honey are significantly lower (0%-12.5%).

Novelty: The specific new method, mechanism, theory, or finding that fills our research gap in existing literature, as the MBC values for Manuka honey remained lower across all bacterial isolates. In agar well diffusion assays, Manuka honey produced larger inhibition zones (18-24 mm) than the bacteria (15-19 mm), and both honey treatments further enhanced the effect against the bacteria. This antibacterial finding was certainly a unique contribution to our research discourse.

Conclusion: These results indicate the potential of honey, particularly Manuka honey, as a natural antibacterial agent due to its broad-spectrum anti-infective properties.

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1. Introduction

1.1. Characteristics of Manuka Honey

Manuka honey from New Zealand is often considered to be a medicinal product of special value due to its high level of antimicrobial activity. Therefore, the distinct authentication of its botanical origin is of great importance. Aside from the common pollen analysis, it is in this respect, particularly the analysis of the phenolic acids, flavonoids, and norisoprenoids, that is described as useful.[1] Manuka honey (MH) is a highly prized natural product from the nectar of *Leptospermum scoparium* flowers. Increased competition in the global market drives MH product innovations. Systematic review of this product, updates comparative and non-comparative studies to highlight nutritional, therapeutic, bioengineering, and cosmetic values of MH. MH is a good source of phenolics and unique compounds, including methylglyoxal, dihydroxyacetone, leptosperin glyoxal, methylsyringate, and leptosin. .[2] Based on evidence from in vitro, in vivo, and clinical studies, multifunctional bioactive compounds in MH possessed antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer activities. There are controversial topics related to MH, such as MH grading, safety/efficacy, implied benefits, and maximum contaminant levels. Artificial intelligence can optimize MH studies in chemical analysis, toxicity prediction, exploration of multifunctional mechanisms, and product innovation. .[3]

1.2. Characteristics of Sidr Honey

Sidr honey is a rare, premium monofloral honey harvested from the nectar of the ancient Sidr (jujube) tree. Renowned worldwide for its lavish buttery taste, rich floral aroma, and high antioxidant properties, it is frequently used in traditional remedies to boost immunity and soothe respiratory issues.[4]. Sidr honey primarily originates from the arid valleys of the Middle East, with the most famous and premium varieties sourced from Hadramout, Yemen, particularly from Wadi Do'an. It is also produced in Saudi Arabia, Pakistan, and other parts of the Arabian Peninsula. .[5] Its origin and characteristics are defined by the ancient Sidr tree (*Ziziphus spina-Christi*). This tree is also known as the Lote tree, Christ's thorn, or Jujube. .[6] It is an ancient desert tree often mentioned in religious texts, including the Holy Quran. .[7] The Sidr tree blooms for only 40 to 60 days a year because the harvest window is incredibly short and the trees grow in remote desert areas. As a result, authentic Sidr honey is one of the rarest and most expensive honeys in the world. .[8] The extraction process involves nomadic beekeepers employing ancestral, low-intervention techniques to harvest honey while the bees feed exclusively on the nectar of Sidr blossoms. The properties of Sidr honey include its monofloral purity, rich, buttery taste, and potent natural antibacterial and antioxidant activity.[9].

1.3. Bacteria configuration

Bacteria are generally classified as either Gram-positive or Gram-negative, depending on differences in their peptidoglycan cell wall. Gram-positive bacteria are

composed of thick cell walls, approximately 20–80 nm, while Gram-negative bacteria possess thin cell walls, less than 10nm. .[10] Therefore, because Gram-positive bacteria have thicker cell walls than Gram-negative bacteria, antibiotics can have more difficulty accessing the peptidoglycan layer. But this is not possible for Gram-negative bacteria because they possess an outer cell membrane, which acts as a protective layer.[11]. Gram-negative bacteria are a major cause of morbidity and mortality in both humans and animals. Gram-negative bacteria are considered more prone to antibiotic resistance, leading to the emergence of many 'resistant-related' infections.[12].

Honey is a sweet, tasty natural product consumed for its high nutritional value and health benefits, including antioxidant, anti-inflammatory, and antimicrobial effects.[13]. Bees, through intuition, produce Honey from plant nectars, secretions, and excretions of plant-sucking insects.[14]. Regarding its nutrient properties, honey is an interesting source of natural macro- and micro-nutrients, consisting of a saturated solution of sugars, with fructose and glucose as the main contributors, along with a wide range of phenolic compounds.[15]. The composition of honey is slightly variable and depends on its floral source; seasonal and environmental factors also influence both its composition and biological properties.[16]. Several studies depict that the antioxidant potential of honey is associated not only with total phenolic concentration but also with color, with dark-colored honeys described as having higher total phenolic content and, consequently, greater antioxidant capacity.[17]. Manuka honey, derived from the blossoms of the Manuka tree in New Zealand, is known for its antibacterial properties and is used in various therapeutic applications, especially for wound healing.[18]. Its super antibacterial activity is mostly referenced to the methylglyoxal (MGO), with commercial Manuka honey products containing MGO concentrations ranging from 70 mg/kg to over 1100 mg/kg.[19].

While many components contribute to its efficacy, MGO is highlighted as the key active ingredient. Nevertheless, the relationship between MGO concentration and Manuka honey's antibacterial effectiveness is not fully understood at the moment. Research indicates that MGO modifies the structure of bacterial fimbriae and flagella, potentially reducing bacterial adherence and motility.[20]. This study aims to compare the antibacterial activity of Manuka honey (UMF 15+) and domestic Sidr honey against selected Gram-positive and Gram-negative bacteria.

2. Materials and Methods

2.1. Honey Preparation and Processing

Two types of honey were used in this study: Manuka honey (UMF 15+) with an MGO concentration of 514 mg/kg and domestic Iraqi Sidr honey. 20 ml of each honey prototype sample was placed in sterile screw-cap containers and stored at 25 °C in the laboratory. Initial filtration was performed through a sterile mesh to remove particulate matter; the samples were then refrigerated at 4°C. Both honey samples followed the same procedures as specified in the experimental design.

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2.2. Preparation of Honey Solutions (v/v)

Honey solutions were prepared on a volume/volume basis (v/v). The stock solution (100% v/v) consisted of undiluted honey. Serial twofold dilutions were prepared in sterile distilled water to obtain concentrations ranging from 50% to 0.19%.

2.3. Preparation of Bacterial Isolates

Three to five pure bacterial colonies were picked from the nutrient agar plate with an inoculating wire loop, suspended in 4–5ml of nutrient broth, and incubated at 37°C for 24 h. The bacterial suspension was diluted with sterile normal saline (0.85% NaCl) until it matched the turbidity of 0.5 McFarland Standards (1.5×10^8 CFU/ml)

2.4. The Minimum Inhibitory Concentration Preparation

The antibacterial activity of honey samples was evaluated using the microdilution method in sterile 96-well microtiter plates. Each well was filled with 100 µL of the freshly prepared honey sample dilutions, and 100 µL of bacterial suspension was added. Honey served as a negative control; I served as a bacterial growth control; the broth was used as a sterility control. Microtiter plates were incubated at 37°C for 20 hrs. After incubation, 20 µl of residue was added to all 2the 96 wells and incubated for two hours to observe any color changes. The MICs were determined by broth microdilution as the lowest concentrations at which no color change from blue to pink occurred in the resazurin assay, indicating complete inhibition of bacterial growth.[21]. The microtiter plate reader was used to measure the absorbance at 570 nm.[22].

2.5. Minimum inhibitory concentration reagent

The resazurin (Alamar Blue) solution was prepared by dissolving 0.015 g of resazurin in 100 mL of sterile distilled water. A vortex mixer was used until well dissolved, and the mixture was stored at 4°C for up to 1 week after preparation.[23].

2.6. Calculation of Inhibition Percentage

The percentage of bacterial growth inhibition was calculated using the formula:
Inhibition (%) = [(OD control − OD sample) / (OD control − OD blank)] × 100

2.7. Minimum bactericidal concentration (MBC)

The MBC was determined by the streak plate method after obtaining the MIC. From the MIC 96-well plates, 30 µL was taken and plated onto fresh nutrient agar; the plates were labeled and incubated at 37 °C for 24 h. The lowest concentration that did not produce any colonies was considered the MBC.[24].

2.8. Agar well diffusion assay

The agar well diffusion method was used to detect antibacterial activity of each

honey (Sidr and Manuka) against the six bacterial isolates. The bacterial suspension was spread onto Mueller-Hinton agar medium using a sterile cotton swab. Then it was left for 10 min. 6-mm-diameter wells were made in the previous agar layer. The agar discs were removed, 50µl of concentrated Manuka honey and domestic Sidr honey were added to each well by using a micropipette, and D.W was added to the middle well as a control. plates were incubated at 37 ºC for 18 hrs. and after that, the diameter of clear inhibition zones was measured in mm.[25].

3. Results and discussion

The antibacterial activity of Manuka honey (UMF 15+) and Sidr honey was broadly evaluated using MIC, MBC, resazurin-based microtiter assay, and well diffusion assay. The results from all methods were highly consistent and established a clear superiority of Manuka honey over Sidr honey. The resazurin assay exposed a concentration-dependent color change from blue to pink, reflecting bacterial metabolic activity.[25]. The wells treated with Manuka honey remained blue at lower concentrations than those treated with Sidr honey, indicating stronger inhibition of bacterial growth. Sidr honey exhibited an earlier color transition to pink, suggesting reduced antibacterial potency. Manuka honey showed significantly lower MIC values (12.5%- 1.56%) than Sidr honey (50%- 12.5%). Gram-positive bacteria, particularly Streptococcus spp. and Staphylococcus spp., exhibited greater susceptibility, as evidenced by lower MIC values and delayed metabolic activity in the resazurin assay. Gram-negative bacteria exhibited relatively greater resistance, particularly to Sidr honey, as shown in Table 1 and Figures 1 and 2 below:

Table 1: MIC, MBC of Manuka honeys UMF 15+ and Sidr honey tested against Bacterial isolates

Bacterial isolates	Manuka MIC (v/v%)	Manuka MBC (v/v%)	Sidr MIC (v/v%)	Sidr MBC (v/v%)
E.coli	6.25	12.5	25	50
Acinetobacter spp.	12.5	25	25	50
Klebsiella spp.	6.25	12.5	50	100
Staphylococcus spp.	3.125	6.25	12.5	25
Streptococcus spp.	1.56	3.125	12.5	25
Bacillus spp.	6.25	12.5	25	50

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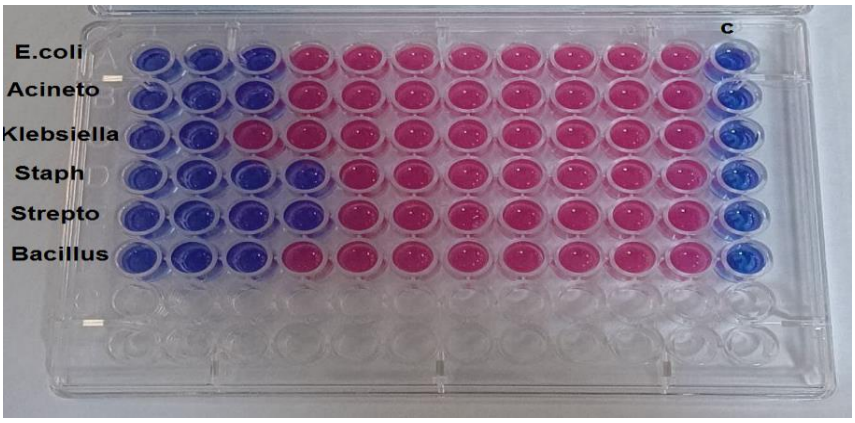

Figure 1: Resazurin microtiter assay showing color change indicating bacterial viability across different Sidr honey concentrations.

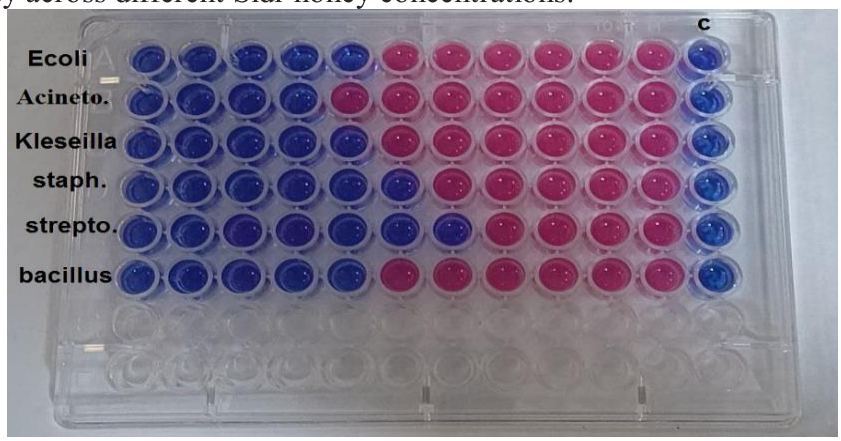

Figure 2: Resazurin microtiter assay showing color change indicating bacterial viability across different manuka honey concentrations.

Similarly, MBC values further confirmed the bactericidal nature of Manuka honey, with values nearly twofold higher than MIC, whereas Sidr honey required much higher concentrations to reach bactericidal effects. The well diffusion assay supported these findings, with Manuka honey producing larger zones of inhibition against all tested bacterial isolates than Sidr honey. The diameter of the inhibition zones was markedly larger in Gram-positive bacteria, indicating greater sensitivity. In contrast, smaller inhibition zones were detected with Sidr honey, mostly against Gram-negative bacteria, reflecting its moderately weaker antibacterial activity, as shown in Table 2 and Figures 3 and 4, as proved below:

Table 2: Zones of inhibition in mm in response to Manuka honey UMF +15 and Sidr honey

Isolates	Manuka honey	Sidr honey
E.coli	21	18
Acinetobacter	24	19

spp.		
Klebsiella spp.	18	15
Staphylococcus spp.	20	17
Streptococcus spp.	23	18
Bacillus spp.	21	17

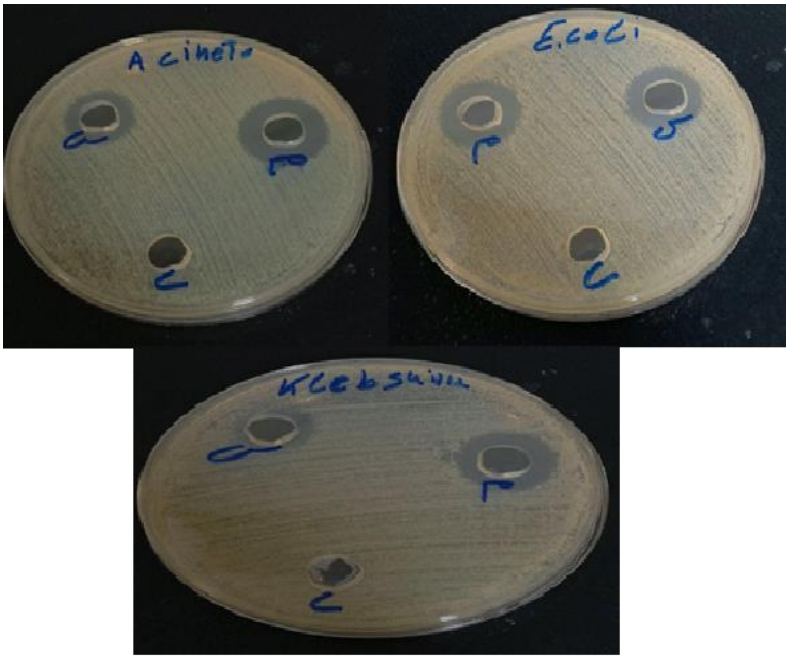

Figure 3 - Comparison of Zones of inhibited growth of gram-negative bacterial isolates due to the presence of Manuka honey and Sidr honey

Figure 4 - Comparison of Zones of inhibited growth of gram-positive bacterial isolates due to the presence of Manuka honey and Sidr honey

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The strong amalgamation amongst the well diffusion assay, MIC/MBC, and resazurin results confirms that the antibacterial effect of honey is both concentration-dependent and measurable across different methodological approaches. The larger inhibition zones observed with Manuka honey are consistent with its lower MIC values and sustained blue coloration in the resazurin assay, indicating effective inhibition of bacterial growth and metabolism.

In terms of bacterial susceptibility, Gram-positive bacteria were commonly more sensitive to both types of honey, especially Manuka honey. This can be attributed to the absence of an outer membrane, which facilitates penetration by antimicrobial honey. In contrast, Gram-negative bacteria exhibited greater resistance due to the presence of a lipopolysaccharide-rich outer membrane, which serves as a barrier to antimicrobial agents.[26].

The superior antibacterial activity of Manuka honey can be explained by its unique chemical composition, particularly the presence of methylglyoxal (MGO), which contributes to its non-peroxide antimicrobial activity. Additionally, factors such as low pH, high osmolarity, and the presence of phenolic compounds enhance its overall efficacy. In contrast, the antibacterial activity of Sidr honey largely depends on hydrogen peroxide production, which is less stable and may be affected by environmental conditions.[27].

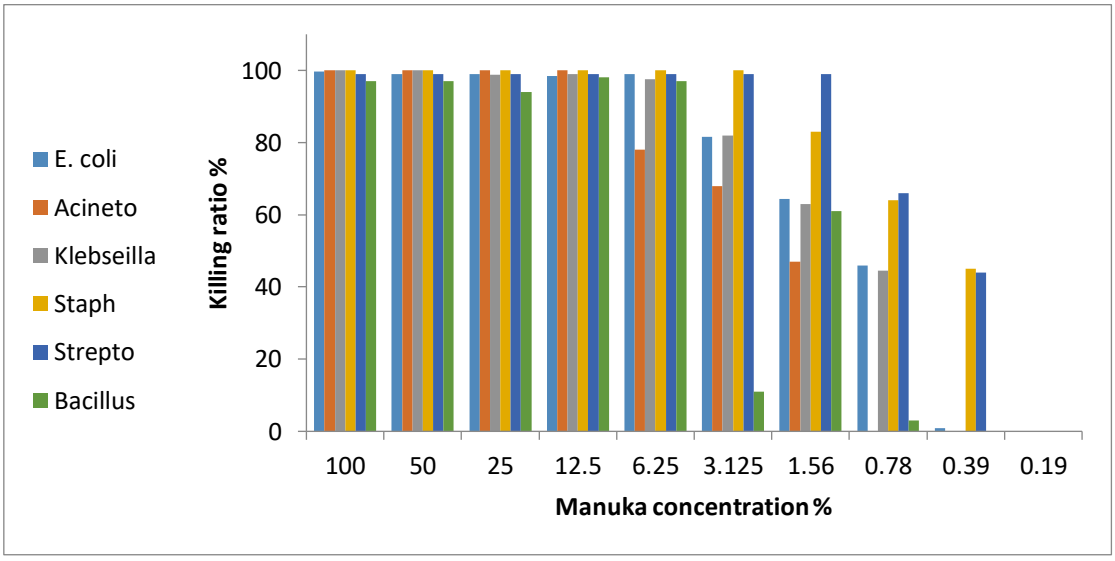

Figure 5: Effect of Manuka Honey Concentration on the Killing Rate of Selected Bacterial Species

Figure 5 illustrates the relationship between Manuka honey concentrations and their antibacterial activity against a range of bacterial species, including E. coli, Acinetobacter, Klebsiella, Staphylococcus, Streptococcus, and Bacillus. The antibacterial effect is expressed as the percentage killing rate. At higher concentrations (100% to 12.5%),

Manuka honey consistently demonstrates a strong bactericidal effect, with killing rates approaching or reaching 100% against most tested organisms. This indicates that high concentrations of Manuka honey exhibit potent antimicrobial properties, likely due to its high osmolarity, low pH, hydrogen peroxide content, and bioactive compounds such as methylglyoxal. As the concentration decreases (6.25% to 1.56%), a gradual reduction in antibacterial activity is observed. While some bacterial species remain relatively sensitive, others show partial resistance, reflected by lower killing percentages. This suggests variability in susceptibility among bacterial species. At the lowest concentrations (0.78% to 0.19%), the antibacterial effect is significantly diminished or completely absent for most organisms. This indicates that below a certain threshold, Manuka honey is no longer effective as an antimicrobial agent.

Overall, the results demonstrate a clear concentration-dependent antibacterial effect of Manuka honey, with higher concentrations being significantly more effective in inhibiting or killing bacterial growth. Differences among bacterial species may be attributed to structural and physiological variations, such as differences in cell wall composition and resistance mechanisms.

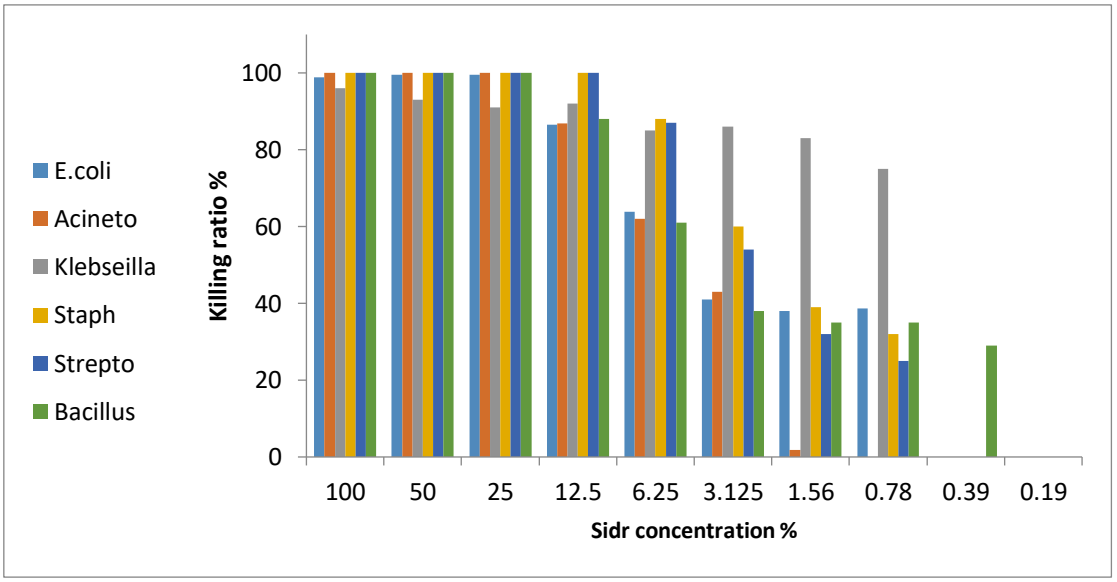

Figure 6: Effect of Sidr Honey Concentration on the Killing Rate of Selected Bacterial Species

Figure 6 demonstrates the antibacterial activity of Sidr honey at different concentrations against the same panel of bacterial species, including E. coli, Acinetobacter, Klebsiella, Staphylococcus, Streptococcus, and Bacillus, expressed as the percentage killing rate. Overall, Sidr honey exhibits a concentration-dependent antibacterial effect similar to that observed with Manuka honey; however, its efficacy is comparatively lower at equivalent concentrations. At higher concentrations (100% to 12.5%), Sidr honey shows moderate to high antibacterial activity, but the killing rates are generally lower than those recorded for Manuka honey, indicating reduced bactericidal potency. As the concentration decreases (from 6.25% to 1.56%), the decline in antibacterial activity is more pronounced

than in Manuka honey. Several bacterial species exhibit partial resistance at these intermediate concentrations, with noticeably reduced killing percentages. At the lowest concentrations (0.78% to 0.19%), Sidr honey shows minimal to no antibacterial activity against most of the organisms tested. This suggests that Sidr honey requires higher concentrations to achieve bactericidal activity comparable to Manuka honey. The relatively lower antimicrobial efficacy of Sidr honey may be attributed to differences in its chemical composition, particularly to lower levels of methylglyoxal and other bioactive compounds that contribute to antibacterial activity in Manuka honey. These findings highlight that, although both types of honey possess antimicrobial properties, Manuka honey is more potent under the same experimental conditions.

A comparison between Manuka and Sidr honey reveals that both exhibit concentration-dependent antibacterial activity against the tested bacterial species (E. coli, Acinetobacter, Klebsiella, Staphylococcus, Streptococcus, and Bacillus). However, Manuka honey consistently demonstrates superior antibacterial efficacy across all tested concentrations. At higher concentrations (100% to 12.5%), both types of honey show strong bactericidal effects; nevertheless, Manuka honey achieves higher killing rates, often approaching complete inhibition of bacterial growth. In contrast, Sidr honey, while effective, shows comparatively lower killing percentages under the same conditions. As the concentration decreases (from 6.25% to 1.56%), the difference between the two becomes more pronounced. Manuka honey maintains relatively high antibacterial activity against several bacterial species, whereas Sidr honey exhibits a more rapid decline in efficacy, indicating reduced potency at intermediate concentrations. At the lowest concentrations (0.78% to 0.19%), Manuka honey retains partial antibacterial activity against certain organisms, whereas Sidr honey shows minimal or no detectable effect. This further supports the higher antimicrobial strength of Manuka honey. The enhanced antibacterial activity of Manuka honey is likely attributed to its unique chemical composition, particularly its high content of methylglyoxal and other non-peroxide antimicrobial factors. In contrast, the antibacterial activity of Sidr honey relies more on general factors such as osmotic pressure, hydrogen peroxide production, and acidity, which may be less potent under diluted conditions. Overall, these findings indicate that Manuka honey is more effective than Sidr honey as an antibacterial agent under identical experimental conditions, making it a stronger candidate for therapeutic and antimicrobial applications.

4. Conclusion

This study demonstrated that Manuka honey (UMF 15+) exhibits significantly higher antibacterial activity than Sidr honey against six isolates of both Gram-positive and Gram-negative bacteria, as indicated by the MIC and MBC values. Gram-positive bacteria were more susceptible to both types of honey, particularly Manuka honey, whereas Gram-negative bacteria showed relatively greater resistance due to their cell wall structure. Despite this, Manuka honey maintained effective antibacterial activity against all tested isolates. The findings highlight the potential of Manuka honey as a natural and effective antimicrobial agent that could be used as an alternative or complementary approach to

antibiotics, particularly in the face of growing antimicrobial resistance. Further scope for research could include regional precipitation data for the experimental honey values, as manuka honey comes from New Zealand, which is far from the arid region of Iraq, where the manuka honey was tested. This particular anomaly can spur further research and discussion in the near future on how the environment affects the antibacterial activity of honey.

List of Abbrevations: **MH:** Manuka honey**; MGO:** methylglyoxal; **MBV:** Minimum bactericidal concentration.

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