



## Research Article

# Anti-Bacterial Activity of Honey Based on Floral Origin

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### Abstract

Modern research has documented the potent antimicrobial properties of honey, giving rise to the use of honey-based medical products for the topical treatment of wounds and burns. This study examined the influence of the floral origin related to the antibacterial efficacy of heat-treated honey. For each honey type of a specific floral origin, at least three geographically distinct samples were tested to determine the minimum bactericidal concentration against seven pathogenic bacterial strains. Results supported a significant degree of consistency supporting the role of the honey floral origin in affecting the antibacterial potency. Buckwheat honey performed the best, followed by avocado honey, and then manuka and blueberry honeys. These results support the role of the floral origin in determining the antimicrobial efficacy of honey and the potential therapeutic use of honey from buckwheat, avocado, and blueberry floral origins.

**Keywords:** Honey; Manuka; Floral; Antimicrobial

### Introduction

Honey has been used as a medicine for various ailments since ancient times by many cultures around the world [1]. Modern analysis is revealing the complex biochemical constitution of honey, including over 200 organic compounds including oligosaccharides, amino acids, enzymes, proteins, organic acids, pigments, phenolic compounds, vitamins, Maillard reaction products (MRPs), aromatic volatile oils, and numerous minerals [2,3]. Recent research documents the antimicrobial, antiviral, antioxidant, anti-inflammatory, anti-carcinogenic, and immunomodulating effects of honey [4,5], pointing to potential topical uses in the treatment of wounds, burns, and skin disorders such as eczema, as well as potential internal uses in a wide variety of gastrointestinal disorders, upper respiratory infections, metabolic and cardiovascular diseases, and even cancer [6,7]. Due to its broad-spectrum antimicrobial activity and effectiveness against antibiotic-resistant bacterial pathogens, as well as its ability to support and enhance the healing process, honey is being embraced by modern medicine with hospitals around the world using a growing number of honey-based medical products in the topical treatment of wounds and burns [8-12].

The major properties contributing to the antibacterial action of honey include low pH, high osmolarity, enzymatic production

of hydrogen peroxide by Glucose Oxidase (GOX), and various other non-enzymatic mechanisms that are still being discovered and investigated [13]. The potency of antibacterial action has been suggested to vary widely, generally attributed to several factors thought to independently influence each of the various antibacterial properties: geographic location, climate, and species of bee, floral source of pollen nectar, age, storage and processing conditions [14]. Medical grade honey produces high levels of non-enzymatic, broad-spectrum antibacterial activity, largely attributed to levels of methylglyoxyl (MGO) that are found to be markedly elevated in manuka honey from New Zealand or honey derived from similar *Leptospermum* species in Australia [14,15].

While floral origin and geographical location are generally considered to be crucial factors in determining antibacterial potency, only limited studies with inconclusive results have actually compared multiple samples of honey of known floral origin from different geographic locations [11,16-20]. While most studies have examined raw or minimally processed honey, it is important to investigate the antibacterial properties of honey following common industrial treatments. One of the most common treatments in the honey industry is thermal treatment which has been shown to affect the physicochemical, antioxidant and antimicrobial properties of honey [21,22]. The purpose of this study was to evaluate the antibacterial activity of heat-treated honey based on known floral origin, using samples from various

geographic locations for each of the different floral origins.

## Materials and Methods

### Honey Samples.

A minimum of three honey samples were collected from different geographical locations for each floral source. This resulted in 14 different floral sources from a total of 53 different geographical locations. Floral sources included manuka, goldenrod, blueberry, cranberry, buckwheat, clover, avocado, thyme, star thistle, mesquite, Japanese knotweed, alfalfa, basswood, and black locust (Table 1). Honey samples were obtained from North American beekeepers or purchased commercially online. Samples were predominantly monofloral, as confirmed and identified by personal communication with beekeepers based on the location of apiaries and availability of flora during the harvest season.

Floral Origin	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Manuka						
<i>Leptospermum scoparium</i>	New Zealand	Australia	Australia	New Zealand	New Zealand	
Goldenrod						
<i>Solidago canadensis</i>	Upstate New York	Western New York	Ohio			
Blueberry						
<i>Vaccinium corymbosum</i>	Salem, Oregon	Michigan	New Jersey	Milwaukie, Oregon		
Cranberry						
<i>Vaccinium macrocarpon</i>	Wisconsin	Massachusetts	New Jersey			
Buckwheat			Squaw Lake, Minnesota			Elko New Market, Minnesota
<i>Fagopyrum esculentum</i>	Ohio	California		Michigan	Oregon	
Clover						
<i>Trifolium spp</i>	Ohio	Colorado	California	Mexico		
Avocado						
<i>Persea americana</i>	California	Mexico	Florida	Israel		
Thyme						
<i>Thymus vulgaris</i>	Spain	France	Greece			
Star Thistle						
<i>Centaurea solstitialis</i>	Michigan	Oregon	North Carolina			
Mesquite						
<i>Prosopis spp</i>	Arizona	Texas	Mexico			
Japanese Knotweed						
<i>Reynoutria japonica</i>	Pennsylvania	New York	Ohio	Massachusetts		
Alfalfa						
<i>Medicago sativa</i>	Wyoming	Michigan	Colorado			
Basswood	Squaw Lake, Minnesota	Elko New Market, Minnesota				
<i>Tilia americana</i>			Michigan			

Black Locust						
<i>Rabinia pseudoacacia</i>	New York	Tennessee	West Virginia	Pennsylvania	Illinois	

**Table 1:** Floral origins and geographical locations of honey samples. Table indicates the 14 different floral origins of the various honey samples used in this study and the geographical location from which they were obtained.

**Bacterial Strains.** A total of seven bacterial pathogens were tested, six human pathogens and one canine pathogen: *Staphylococcus aureus* (ATCC 14775), *methicillin-resistant Staphylococcus aureus* (ATCC BAA-44), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus mutans* (ATCC 35668), *Pseudomonas aeruginosa* (ATCC 35554), *Klebsiella pneumoniae* (ATCC 13883), and *Bordetella bronchiseptica* (ATCC 10580).

**MBC assay.** Honey samples were diluted with distilled water to 50% (v/v) and sterilized by autoclaving at 121 °C for 15 minutes. For bacterial growth inhibitory assays, 18-hour cultures grown at 37 °C in Tryptic Soy broth (TSB) ( $1 \times 10^6$  colony-forming units/ml (CFU/mL)) were diluted into TSB (1:1000 dilution) followed by the addition of the indicated concentrations of each sterilized honey sample or control (0%, 2.5%, 5%, 10%, 15%, 20%, and 25% (v/v)). The cultures were incubated at 37 °C with aeration (by continuous rotation at 30 rpm) for 24 hours. Minimum bactericidal concentrations (MBCs) were determined by serial dilution onto TSA plates and incubation for 24 hours at 37 °C. The MBC was identified by determining the lowest concentration of honey that reduced the viability of the bacteria by  $\geq 99.9\%$  compared to untreated and artificial honey samples.

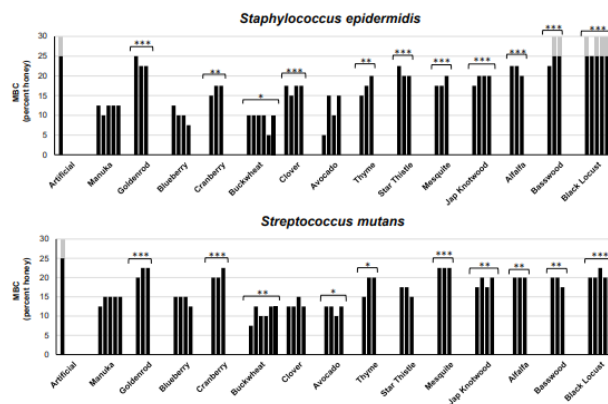
**Controls, Osmotic Effect, Hydrogen Peroxide, pH.** Manuka honey was utilized as a comparative control. Manuka honey is a monofloral honey, produced from the nectar of flowers of the Manuka tree, and has been studied significantly for its medicinal properties. As such, manuka honey is often considered the 'gold standard' for medicinal honeys and was therefore selected as the control for comparison to other floral honey sources. Five different samples of commercially available manuka honeys were obtained from New Zealand and Australia with varying degrees of marketed antimicrobial activity.

An artificial honey consisting of 40.5% fructose, 33.5% glucose, 7.5% maltose and 1.5% sucrose was tested against each bacterial strain at each of the various concentrations as a negative control and to test for potential osmotic effects (Nassar et al. 2012).

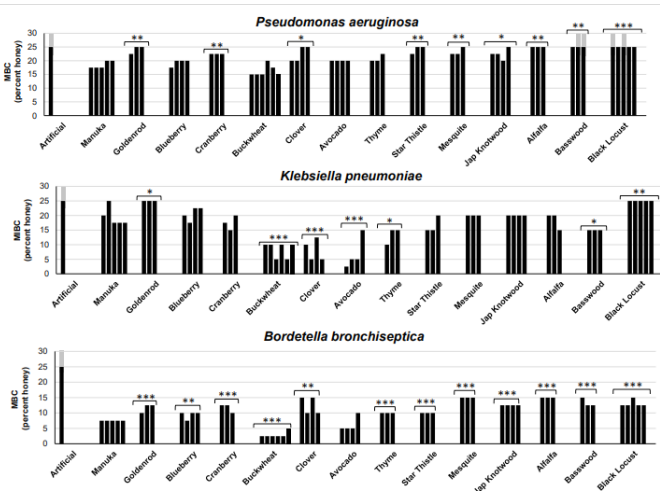
Hydrogen peroxide levels of honey solutions were tested before and after heat treatment using Peroxide 0-100ppm Test Strips (Indigo Instruments). Approximately half of the honey samples exhibited hydrogen peroxide activity initially, however after heat treatment there was no detectable hydrogen peroxide in any of the honey solutions (data not shown).

The pH of honey solutions were tested before and after heat treatment using Sigma pH test strips and samples diluted (1:10) in CO<sub>2</sub>-free water using a pH electrode. All honey samples had a final pH range of 6.8-7.5 (data not shown). The moisture content of the various honey samples was determined by drying the honey samples in a baking oven to completely remove any moisture. The moisture content of the various honey samples ranged from 16.5-18 percent.

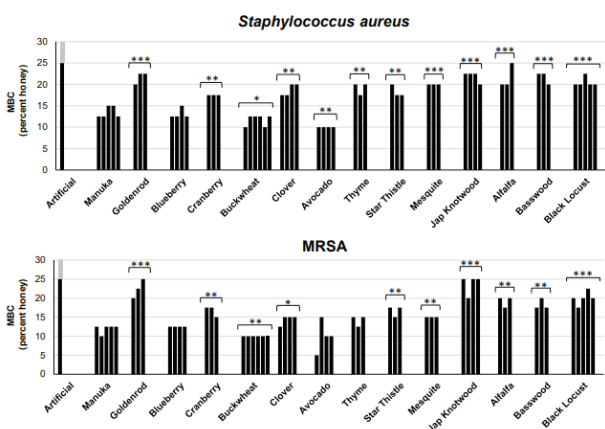
**Statistical analysis.** Statistical analysis was performed using one-way ANOVA. Statistically significant deviation of the various floral honey samples from manuka honey (stronger or weaker) was indicated with asterisks in Figures 1-3, with the p-value corresponding to the number of asterisks accompanying each honey type: \* p = 0.01-0.05; \*\* p = 0.001-0.01; \*\*\* p < 0.001.



**Figure 1:** Antibacterial activity of honey samples from various floral origins against Gram (+) bacteria. The floral origin of each honey sample is indicated on the x-axis with each geographical source indicated by the individual bars. The graph represents the percent of honey required to achieve the MBC for each bacteria indicated. The maximum concentration of honey tested was 25% (v/v). A light grey bar above the black bar indicates those samples that did not achieve the MBC at 25% (v/v) honey (MBC was > 25% (v/v) honey). All experiments were done in duplicate. Statistical analysis was performed using one-way ANOVA. Statistically significant deviation of the various floral honey samples from manuka honey (stronger or weaker) was indicated with asterisks: \* p = 0.01-0.05; \*\* p = 0.001-0.01; \*\*\*p < 0.001.



**Figure 2:** Antibacterial activity of honey samples from various floral origins against Gram (-) bacteria. The floral origin of each honey sample is indicated on the x-axis with each geographical source indicated by the individual bars. The graph represents the percent of honey required to achieve the MBC for each bacteria indicated. The maximum concentration of honey tested was 25% (v/v). A light grey bar above the black bar indicates those samples that did not achieve the MBC at 25% (v/v) honey (MBC was > 25% (v/v) honey). All experiments were done in duplicate. Statistical analysis was performed using one-way ANOVA. Statistically significant deviation of the various floral honey samples from manuka honey (stronger or weaker) was indicated with asterisks: \* p = 0.01-0.05; \*\* p = 0.001-0.01; \*\*\*p < 0.001.



**Figure 3:** Antibacterial activity of honey samples from various floral origins against antibiotic-resistant *S. aureus*. The floral origin of each honey sample is indicated on the x-axis with each geographical source indicated by the individual bars. The graph represents the percent of honey required to achieve the MBC for each bacteria indicated. The maximum concentration of honey tested was 25% (v/v). A light grey bar above the black bar indicates those samples that did not achieve the MBC at 25% (v/v)

honey (MBC was > 25% (v/v) honey). All experiments were done in duplicate. Statistical analysis was performed using one-way ANOVA. Statistically significant deviation of the various floral honey samples from manuka honey (stronger or weaker) was indicated with asterisks: \* p = 0.01-0.05; \*\* p = 0.001-0.01; \*\*\*p < 0.001.

## Results

The goal of this study was to investigate the importance of the floral source in relationship to the antibacterial activity of honey. A minimum of three honey samples were collected from different geographical locations for each floral source. This resulted in 14 different floral sources (manuka, goldenrod, blueberry, cranberry, buckwheat, clover, avocado, thyme, star thistle, mesquite, Japanese knotweed, alfalfa, basswood, and black locust) from a total of 53 different geographical locations (Table 1). Samples were predominantly monofloral, as confirmed and identified by the beekeepers based on the location of apiaries and availability of flora during the harvest season.

The bactericidal activity of the various honey samples ranged in activity from 2.9 to >25% (v/v). While some variation was found amongst the different geographic samples of the same honey floral types, in most cases there was a high degree of consistency within each floral source against each bacterium (Figure 1 and 2). Traditionally, the antimicrobial activity of honey is thought to be due to various factors including low pH, high osmolarity, enzymatic production of hydrogen peroxide by glucose oxidase (GOX), and various other non-enzymatic mechanisms. The antimicrobial activity was not likely due to the osmotic effect of the honey since an artificial honey produced no observable antibacterial activity up to 25% (v/v) (Figure 1-3). In addition, all the honey samples had a consistent moisture content ranging from 16.5-18 percent, suggesting that the differences in antimicrobial activity was not due to variations in moisture content. Upon testing of the pH of the honey samples, all honey samples had a final pH range of 6.8-7.5 (data not shown). In addition, to eliminate variations in the enzymatic production of hydrogen peroxide, all honey samples were heated by autoclaving and found to have no detectable hydrogen peroxide activity after this process (data not shown). Therefore, this study focused on how the floral source influenced the non-enzymatic antibacterial properties of the honey samples.

Against the Gram (+) bacteria, *Staphylococcus epidermidis* and *Streptococcus mutans*, manuka MBCs averaged 12.0% and 14.5%, respectively (Figure 1). Buckwheat MBCs were significantly lower than manuka in both cases, averaging 9.2% and 10.8%, respectively. Avocado MBC was also significantly lower than manuka in the case of *S. mutans* (11.9%). Avocado and blueberry had similar activity as manuka against *S. epidermidis*, as did blueberry, clover, and star thistle against *S. mutans*. The activity of the honey samples of the same floral source from different geographical locations was very consistent (Figure 1). For example, when testing against *S. epidermidis*, buckwheat



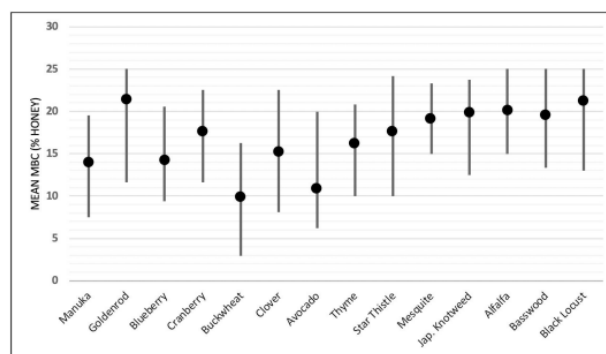
MBCs ranged from 5-10% (v/v) for the 5 samples obtained, clover ranged from 15-17.5% (v/v) across 4 samples, and black locust ranged from 25->25% (v/v) for 5 samples (Figure 1). Across all the honey samples tested, the same floral source from different geographical locations performed very similarly in regards to the MBC values. This strongly supports that the honey floral origin was a consistent and contributing source for a non-enzymatic antibacterial component.

The Gram (-) pathogen *Pseudomonas aeruginosa* was the most resistant to the antimicrobial activity of the honey samples (Figure 2). MBCs averaged 4.3-5% higher with *P. aeruginosa* compared to *Klebsiella pneumoniae*. Against *P. aeruginosa*, manuka MBCs averaged 18.5%, with blueberry, buckwheat, avocado, and thyme not being significantly different (Figure 2). Against *K. pneumoniae*, the MBCs of buckwheat, clover, and avocado were significantly lower than manuka (8.3%, 8.1%, 6.9%, and 19.5%, respectively), while blueberry and cranberry had similar activity as manuka (Figure 2). *B. bronchiseptica* was particularly sensitive to all honeys tested, especially buckwheat with an average MBC of 2.9% (Figure 2). Manuka MBC averaged 7.5%, which was lower than most other honeys, although avocado had a similar level of activity. Again, for these Gram (-) bacteria, the MBCs of each geographically distinct floral source were very similar, supporting a major role of the floral source in determining the antibacterial activity of a honey.

Bacterial antibiotic resistance has become a major medical concern. Since the honey samples tested were shown to have antibacterial activity against *S. aureus* (Figure 1), antibacterial testing was performed against a broad-spectrum antibiotic resistant strain of *S. aureus* (MRSA). Against *S. aureus* and MRSA, manuka MBCs averaged 13.5% and 12%, respectively (Figure 3). Buckwheat MBCs were significantly lower against both strains (11.7% and 10%, respectively) and blueberry was similar to manuka against both strains (Figure 3). On average, MBCs for the various honey samples were 1.7% lower against the methicillin-resistant strain than the non-resistant strain. This suggests that the mechanism of antibiotic resistance for the MRSA strain did not provide resistance against the active constituent(s) present in the honey samples. This may support the potential use of these antimicrobial honeys, especially buckwheat, against *S. aureus* and MRSA infections since the antibiotic resistance does not provide improved resistance against these honey varieties.

To obtain a more overall understanding on how the different floral honey samples performed as broad antibacterial agents, the mean and ranges of MBCs of each floral honey against all the tested bacteria was determined and graphed in Figure 4. These results also supported the importance of the floral source in determining the antibacterial properties of the honey (Figure 4). As expected, based on previous studies, manuka honey performed quite well, with an average MBC of 13.9% across all bacteria tested, with individual average MBCs ranging from 7.5-19.5% depending on the bacteria (Figure 4). The floral origins of buckwheat and

avocado outperformed manuka overall with buckwheat having an average MBC of 9.9% across all bacteria tested, and individual average MBCs ranging from 2.9-16.3% depending on the bacteria, and avocado with an average MBC of 10.9% across all bacteria tested and individual MBCs ranging from 6.3-20%. Blueberry also performed well with an overall average MBC of 14.2%. Certain floral honey sources were consistently less effective in regards to their antibacterial properties including goldenrod, mesquite, Japanese knotweed, alfalfa, basswood, and black locust (with average MBCs of 21.5%, 19.2%, 19.9%, 20.2%, 19.6% and 21.4%, respectively) (Figure 4).



**Figure 4:** Mean and ranges of MBCs of each floral honey sample. The mean and ranges of MBCs for each floral honey sample was calculated based on all the bacteria tested in Figures 1 and 2. The floral origin of each honey sample is indicated on the x-axis with the mean MBC (percent honey (v/v)) indicated on the y-axis.

## Discussion

In addition to other factors, it's often suggested that the antibacterial potency of honey depends on the floral origin. Previous studies that purport the influence of floral origin often fail to analyze multiple samples of each honey type from various geographic locations as a way to show statistically significant correlation between floral origin and potency of antibacterial activity [16-19,23]. This study strongly supports that the floral origin was a major factor in the bactericidal activity of heat-treated honey samples.

One finding of major significance was the performance of buckwheat and avocado honeys, both of which outperformed manuka honey against each bacterium tested, with the exception of avocado against *P. aeruginosa*. These results are supported by the literature, where buckwheat honey has been shown to exhibit antibacterial activity comparable to or more potent than manuka [23-29]. Buckwheat has been shown to have higher concentrations of proteins, phenols, and minerals [27], and darker honeys are generally considered to be highly medicinal and have been found to have higher concentrations of pollen, total phenolics, minerals, and 5-hydroxymethylfurfural (HMF) [4]. Both buckwheat and avocado are dark honeys and ideal candidates for further research

into their therapeutic use.

Currently, the literature is lacking in studies that examine the antibacterial effects of honey against canine pathogens. In our study, MBCs against *B. bronchiseptica*, the canine pathogen responsible for kennel cough, were very low. The average overall MBC was 10.8% compared to 16.1-22.1% for the human pathogens, with buckwheat honey producing bactericidal effects at 2.9%. This suggests a strong therapeutic potential for a buckwheat honey-based kennel cough treatment that warrants further study.

Several studies have investigated the non-enzymatic antibacterial activity of honeys, but few have done so with heat-treated honey [19,22]. In most studies, the GOX-dependent hydrogen peroxide activity is removed by the addition of catalase [30]. Heat application is typically avoided in order to keep the honey as unaltered as possible, leaving intact other potentially heat-sensitive components that contribute to overall antibacterial action, such as defensin-1, polyphenols, or MGO [31]. Sterilization of honey is important for medical application since therapeutic products must be sterilized for safety purposes and one of the most common treatments in the honey industry is thermal treatment [21]. Our results support the antibacterial activity of heat-treated honey at levels comparable to previous studies using non-heat-treated honey [16,27,32].

The presence of active constituent(s) present in honey contributed by the floral source is currently under investigation. Defensin-1 is an antimicrobial peptide that is found in some honeys in various quantities. It has been shown to be active against gram-positive bacteria, making it one of a multitude of factors contributing to bactericidal potency [33]. One study showed defensin-1 to be resistant to conventional heating up to 55 °C for 24 hours [34]. The phenolic content of honey varies by sample and is thought to potentially contribute to the overall antimicrobial activity. Antibacterial phenolic acids that have been identified in honey include caffeic,  $\beta$ -coumaric, ferulic, syringic, and methylsyringic, as well as flavonoids such as quercetin, isohamnetin, luteolin, galangin, kaempferol, naringenin, hesperetin, pinocembrin, and chrysin [35]. Although it is generally recognized that the concentrations of individual phenolic compounds are too low to be antimicrobial themselves [18,36], it has been suggested that the combination of phenolics may indirectly contribute through synergistic effects to overall antibacterial activity [33].

Another active compound is MGO which has been found in several honey types, although generally found in honey derived from *Leptospermum* floral origins. Even amongst manuka honey samples, which are of *Leptospermum* floral origin, the concentrations of MGO can vary considerably and it has been shown that MGO is not solely responsible for manuka honey's antibacterial action [24,33,37]. MGO in honey is the product of nonenzymatic conversion of dihydroxyacetone (DHA), and its antibacterial activity has been shown to actually increase over time and when stored at higher temperatures. Other potential nonenzymatic factors contributing to the diverse antibacterial

action of honey have been proposed including transition metals [31,33,37].

In our study, the recorded MBCs against the human pathogens tested ranged from 6.25% - >25% (v/v), which is comparable to other studies that examined total antibacterial activity of non-heated honey [16,27,32]. This study investigated fifty-three honey samples from fourteen different floral sources and various geographical locations for their antibacterial activity. Bactericidal potency was strongly correlated with the floral origin of the honey, with several honey samples performing statistically better than manuka honey ( $p > 0.01-0.001$ ). Buckwheat honey displayed the most potent bactericidal activity, with an overall mean MBC of 9.9% (v/v), followed by avocado with an overall mean MBC of 10.9% (v/v), manuka with an overall mean MBC of 13.9% (v/v), and blueberry with an overall mean MBC of 14.2% (v/v).

The bacteria included in this study are important human pathogens, and their increasing antibiotic resistance is growing problem worldwide [38]. The ability of some honeys to kill antibiotic resistant bacteria is well documented, as is the concentration-dependent nature of honey's bactericidal action [11,12,26]. This study demonstrated MRSA to be susceptible to every honey type tested, at varying concentrations with average MBCs 1.7% lower than those against the non-resistant *S. aureus*. A previous study suggested that unlike conventional antibiotic drugs, resistance to honey cannot be induced [15]. Honey has also been shown to disrupt or inhibit biofilm formation [28,39], neutralize bacterial endotoxins [5], degrade bacterial DNA [25], damage bacterial cell membranes and even enhance the efficacy of bacteriophage action [40]. The therapeutic potential of honey is vast, due to its complex makeup which gives rise to various overlapping antibacterial mechanisms [23]. Further research into the development of therapeutic medicinal products utilizing specific floral-sourced, heat-treated honey is warranted.

## Limitations

Further research is needed to fully confirm the role of the floral origin related to the antimicrobial properties of honey. Although well documented regarding the geographical and floral source of the honey used in this study, these are wild bee hives for which the bee keepers know the predominant floral source in the area, but certainly other floral contaminants are possible. In addition, chemical constituent characterization would add to the understanding and value of the research. Due to the number of samples, each honey sample was only tested once, although across a variety of doses done concomitantly, to provide confidence in the approximate MBC value.

## Conclusions

Honey has been utilized as medicine by people around the world since ancient times. In addition to other factors, it's often suggested that the antibacterial potency of honey depends on the floral origin. By obtaining honey samples from a wide variety of

geographic sources of known floral origin and characterizing their anti-microbial activity, this study strongly supports that the floral origin was a major factor in determining the bactericidal activity of honey samples. Of highest activity were honey samples from buckwheat, avocado, and blueberry floral origins, supporting the potential therapeutic value of these floral honeys.

## Declarations

**Author's contributions:** EZ conducted the research experiments. EZ and JL planned and evaluated the results. EZ wrote the manuscript. JL edited the manuscript. JL was the principle investigator

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**Data availability:** No additional information is supplied as a supplementary file. Additional questions or information may be obtained by contact the Corresponding author, Jeffrey Langland.

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