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Antibacterial activities of honey against *Escherichia coli* and *Staphylococcus aureus*: A potential treatment for bacterial infections and alternative to antibiotics

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Abstract
The global problem of antimicrobial resistance (AMR) has increased interest in natural products, such as honey in the treatment of diseases. This study evaluated the antibacterial activities of honey against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). This cross-sectional study was conducted from November 2020 to May 2021 using *E. coli* and *S. aureus* as indicator organisms. The antibacterial activities of honey were assessed using the agar well diffusion assay. The sensitivities of *E. coli* and *S. aureus* to honey were indicated by zones of inhibition which were measured using a ruler in millimetres. Data analysis was performed using Stata version 16.1. Honey produced a dose-dependent antibacterial activity against *E. coli* and *S. aureus*. Our study demonstrated that honey had stronger antibacterial activities against *E. coli* compared to *S. aureus*. This study highlights the need for further research on honey to investigate its potential use in treating bacterial infections.

Keywords: Antibacterial activities, antimicrobial resistance, *Escherichia coli*, honey, natural products, *Staphylococcus aureus*

Introduction
Honey, a natural product produced by honeybees, possesses both nutritional and medicinal properties [1-3]. Globally, honey has been used to cure infectious diseases due to its potent medicinal activity [4-5]. Given the escalating issue of antimicrobial resistance (AMR), there is an urgent need to promote drug discovery from natural products [6-10]. The inappropriate use of conventional antibiotics in humans, animals, and the environment have all contributed to the development and spread of AMR [11-17]. Antimicrobial resistance is a global public health problem that should be addressed using various strategies that include promoting drug discovery [7, 9, 18-24] and implementing antimicrobial stewardship programmes [25-35].
Some bacteria including *Escherichia coli* (*E. coli*) and *Staphylococcus aureus*(*S. aureus*) cause most infections that lead to increased morbidity and mortality [36-39]. *E. coli* has been known to be responsible for most cases of bacteraemia in developed countries surpassing other leading bacteraemia-causing pathogens such as *S. aureus* [40]. Due to the high prevalence of these microorganisms and other factors, antibiotics have been frequently overused and misused for their treatment [17, 41-44], resulting in the development of AMR among these microorganisms [45-49]. Consequently, infections caused by drug-resistant strains of *E. coli* and *S. aureus* are challenging to treat, thereby contributing to increased morbidity and mortality [38-39]. Moreover, drug-resistant infections increase medical costs and have adverse effects on the economy [50-51].
Honey, also called *Leptospermum scoparium* (*L. scoparium*) contains a variety of chemicals such as hydrogen peroxide and phenolics which are responsible for its antibacterial activity [52-55]. The higher the hydrogen peroxide content in honey, the better the antibacterial activity [52]. Other constituents of honey that are reported to be responsible for its antibacterial activity are phenols and flavonoids [56–58]. Honey also contains bee defensins-1 which is said to work together with hydrogen peroxide thereby potentiating the bactericidal effect of honey [59, 60]. These constituents have enabled honey to be effective in managing wound infections [61, 62]. Furthermore, honey has bactericidal and bacteriostatic activities against both Gram-positive and Gram-negative bacteria [63-70].

The antibacterial properties of honey vary depending on the floral source and geographic origin of the honey, as different plants produce different types and amounts of antibacterial compounds [71, 72]. For instance, Manuka honey from New Zealand is well-known for its potent antibacterial activity due to the high content of methylglyoxal [73]. Moreover, processing techniques such as pasteurisation and filtration can affect the antibacterial activity of honey by altering its chemical composition [74]. Evidence has shown that raw honey has greater antibacterial activity than processed honey, which may be due to the presence of enzymes and other beneficial compounds that are destroyed during processing [75]. Overall, understanding these factors is important for selecting the most effective honey for various applications in the healthcare and food industries.

The antibacterial activities of honey from different sources (i.e. Manuka honey, Tualang honey, and Sidr honey) were evaluated against both *E. coli* and *S. aureus* and demonstrated to be effective against both bacteria [1, 76]. Additionally, honey was used for topical treatment for infected wounds caused by S. *aureus*, and found that it was effective in reducing bacterial load and promoting wound healing [73, 77-81]. Furthermore, honey inhibits the growth of both *E. coli* and *S. aureus* in-vitro, and this effect was attributed to its high sugar content, low pH, and high hydrogen peroxide content [82]. Moreover, medically-graded honey that is enriched with antimicrobial peptides has antibacterial activity against resistant strains of bacteria [83]. Alongside this, honey has been proven to be effective against some drug-resistant pathogens [84]. These findings suggest that honey is a potential alternative treatment for bacterial infections caused by *E. coli* and *S. aureus*.

In Zambia, honey is valued for its nutritional properties and has been anecdotally reported to possess medicinal properties, including antibacterial activity. However, there is limited published information on its activity against common bacteria like *E. coli* and *S. aureus*. A study reported that the application of honey on wounds contributes to quick healing [85]. Another study investigated the quality of honey harvested from various beehives in Zambia, focusing on parameters such as ash content, moisture, pH, total soluble solids, and soluble sugars, but did not specifically address antibacterial properties [86]. There is limited published information on honey activity against common bacteria like *E. coli* and *S. aureus*, and this call for further research to characterize Zambian honey and its potential antibacterial effects. Additionally, the chemicals responsible for its antibacterial activities should be elucidated. Therefore, this study evaluated the antibacterial activities of honey against *E. coli* and *S. aureus* in the Zambian context.

Materials and Methods

Study design, period, and site

A cross-sectional study was conducted from November 2020 to May 2021 in the Food and Drugs Laboratory Control at the University Teaching Hospital in Lusaka, Zambia. Pure honey (100% volume/volume) (v/v) was collected from Mulungushi University in the Kabwe district of Central Province, Zambia. This site was selected because it produces honey in small and large quantities which are widely sold to the Zambian population for nutritional purposes.

Preparation of honey samples

This study was carried out on unpasteurized, untreated natural honey without any preservatives and obtained straight from the blossoms of wildflowers in Kabwe district, Zambia. The extraction of raw honey was performed using water. Ten (10) grams (g) of honey was mixed with 25 mL of deionized water and centrifuged for 10 minutes at 3000 rpm at 25 °C. The supernatant was collected from the centrifuged tube into a 50 mL round-bottom flask by filtration and then dried at 50 °C using a rotary evaporator. To prepare the require honey concentrations of 25% (v/v), 50% (v/v), 75% (v/v), and 100% (v/v), we weighed the resulting product and then dissolved it in sterile deionized water before use, as described in an earlier study [87].

Sub-culturing and inoculation of bacteria

The bacterial strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were obtained and cultured on nutrient agar (Oxoid, Basingstoke, UK) in the Pathology and Microbiology Laboratory at the University Teaching Hospital in Lusaka, Zambia. The culturing of *E. coli* was done as reported in an earlier study that was done in Zambia [49]. Additionally, the culturing of *S. aureus* was done as reported in another previous study in Zambia [46]. The colonies of both *E. coli* and *S. aureus* were then counted.

After that, a sterile swab was used to pick the pure colonies *of E. coli* and *S. aureus* from the nutrient agar plates and then emulsified in 2 mL of normal saline. Further, to attain the required standard of 0.5 McFarland, we compared the turbidity of the inoculated normal saline to that of the standardised 0.5 Remel™ McFarland Turbidity (12076 Santa Fe Drive, Lenexa, KS 66215, USA).

We used a sterile swab to inoculate the bacterial suspensions onto the Mueller-Hinton agar plates (Oxoid, Basingstoke, UK).

Antibacterial agar well diffusion assay

The prepared honey concentrations (25% (v/v), 50% (v/v), 75% (v/v), and 100% v/v) were screened for antibacterial activity as reported by Khalil and others (2014) [88]. Briefly, wells measuring 6 mm in diameter and 3.2 mm in height were made in the Mueller-Hinton agar plates that contained inoculated bacteria. This was followed by adding 100 µL of a test dilution to each well. Thereafter, the plates were incubated at 37 °C for 24 hours as described in previous studies [88, 89]. A standard ciprofloxacin (5 µg) was used as a positive control while sterile deionized water was used as a negative control. The antibacterial activities of honey were evaluated by measuring the diameter of zones of inhibition (in millimetres) on the wells using a ruler, as was done in another study [90].

Data Analysis

The results of the zones of inhibitions were entered in Microsoft 2013 Excel spreadsheet (Microsoft Corp., Redmond, WA, USA)and analysed using Stata version 16.1(College Station, TX, USA). The presentation of data in the form of figures was done using Graph Pad Prism. Statistical significance was conducted at a 95% confidence level ($p<0.05$). One-way Analysis of Variance (ANOVA) was used to determine the association between the honey concentrations and the zones of inhibition.

Ethical approval

We obtained ethical clearance was from the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC), approval ID: 202031010119. The study was non-invasive and did not pose harm to humans. No identifiers were used and all study data was restricted to the investigators.

Results

The concentrations and zones of inhibition produced by honey and ciprofloxacin are shown in Table 1. An increase in the concentration of honey resulted into an increase in the antibacterial activity of honey. However, ciprofloxacin produced a higher antibacterial activity against both *E. coli* and *S. aureus* compared to honey (Table 1).

Table 1: Concentrations of honey and ciprofloxacin and associated zones of inhibition

Honey concentration	*E. coli* zones of inhibition (mm)	*S. aureus* zones of inhibition (mm)
25%	15	13
50%	18	16
75%	20	18
100%	22	20
Ciprofloxacin 5µg	24	23

The antibacterial activities of honey against *S. aureus* are shown in Figure 1 while those against *E. coli* are shown in Figure 2.

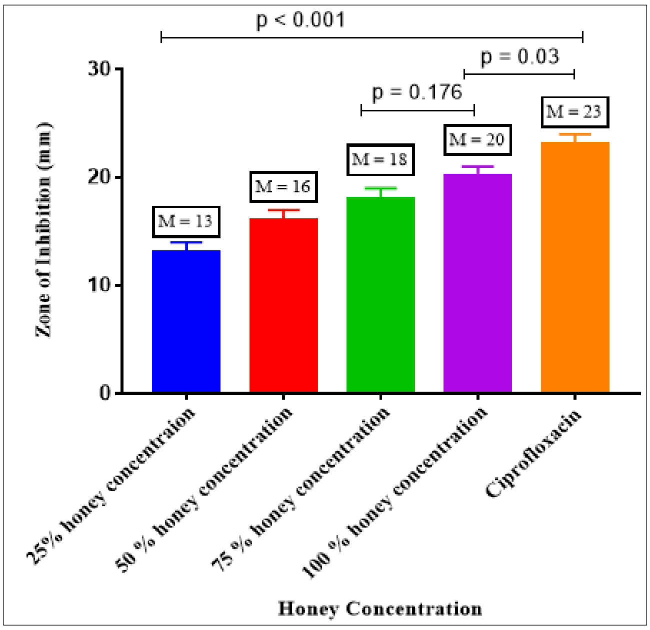

Fig 1: Antibacterial activities of honey against *Staphylococcus aureus* in comparison to ciprofloxacin

A One-way ANOVA was used to compare the mean zone of inhibitions produced by four different concentrations of honey against *S. aureus* with that produced by ciprofloxacin (Figure 1). There was a statistically significant difference in the mean zone of inhibition between honey concentrations and ciprofloxacin, $p<0.001$ (Figure 1). A Tukey's post hoc analysis found that at 25%, 50%, 75%, and 100% concentrations, honey produced smaller zones of inhibition compared to the standard medicine (ciprofloxacin). At the highest concentration of honey (100%), the mean zones of inhibition produced were significantly smaller in comparison to ciprofloxacin (mean = 20.0 mm vs. 23.0 mm; p = 0.03, respectively). This indicated that these concentrations produced lower antibacterial activities against *S. aureus*.

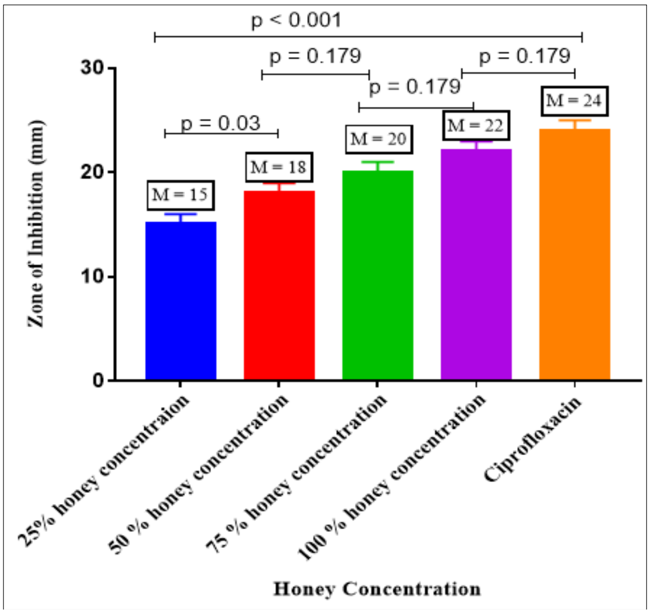

Fig 2: Antibacterial activities of honey against *E. coli* in comparison to ciprofloxacin

A One-way ANOVA was used to compare the mean zone of inhibition produced by four different concentrations of honey against *E. coli* with that produced by ciprofloxacin (Figure 2). There was a statistically significant difference in the mean zone of inhibition between honey concentrations and ciprofloxacin, $p<0.001$ (Figure 2). A Tukey's post hoc analysis found that at 25%, 50% and 75% concentrations, honey produced smaller zones of inhibition compared to the standard medicine (ciprofloxacin), indicating that these concentrations produced lower antibacterial activities. However, at 100% concentration, honey produced the mean zone of inhibition that was statistically similar to those produced by ciprofloxacin (mean = 22.0mm vs. 24.0mm; p = 0.179, respectively), indicating that at this concentration, honey has similar antibacterial activities as ciprofloxacin against *E. coli.*

Discussion

This study evaluated the antibacterial activities of Zambian honey against *E. coli* and *S. aureus* and found that it exhibited stronger antibacterial activities against *E. coli* compared to *S. aureus*. Additionally, the antibacterial activities of honey were found to be dose-dependent.

Our study revealed that honey had antibacterial activities against *S. aureus*. A previous study in Zambia demonstrated that honey healed wounds quicker but did not indicate the organisms that were responsible for the wound infections [85]. Therefore, the activity of honey against *S. aureus* and other wound-infecting pathogens could be the reason why honey was found to heal wounds quicker when applied to the infected area. Similarly, another study reported similar antibacterial effects at lower concentrations of 10% and 20% v/v, with activities on both antibiotic-sensitive and methicillin-resistant *S. aureus* (MRSA) [91]. In Kazakhstan, a study found that honey had good activity against MRSA and *Enterococcus faecalis* [89]. Concurrently, another investigation in Iran found that honey possessed antibacterial activity against sensitive and MRSA with minimum inhibitory concentrations ranging from 18-100% v/v [87]. These results align with a Scottish study which found honey to be effective against MRSA [77]. In Australia, Manuka honey was found to have antibacterial activities against *S. aureus* due to the presence of phenols and methylglyoxal [92]. A study in Ethiopia demonstrated that honey had antibacterial activity against tetracycline-resistant *S. aureus* and MRSA [93, 94]. The antibacterial activity of honey could be due to its composition of various chemicals [1, 52, 58, 95-97]. As such, honey may be a potential therapy for infections caused by resistant strains of bacteria such as MRSA.

Our study further found that honey exhibited high antibacterial activity against *E. coli*, which aligns with previous findings demonstrating honey's effectiveness against *E. coli* [59, 98-100]. The antibacterial activity of honey could be due to the presence of hydrogen peroxide which is a disinfectant and a strong oxidizing agent [39, 55]. Hydrogen peroxide is produced by the glucose oxidase enzyme, which is found in an inactive form in honey but is activated after the honey is diluted with water [58]. The antibacterial activities seen in honey can also be attributed to the presence of high levels of phenolic compounds which have long been reported to have activity against microorganisms including *E. coli* [97, 101]. In Scotland, honey was reported to have antibacterial activity against *E. coli* due to its phytochemical composition such as the novel fatty diacid glycoside derivatives [77]. A study in Egypt revealed that honey was effective against antibiotic-resistant *E. coli* [100], similar to reports from Ethiopia [94]. This makes honey a valuable potential alternative or source of antibiotics after further investigations.

For both *E. coli* and *S. aureus*, the antibacterial activity was dose-dependent, with activity more on *E. coli* than *S. aureus*. This means that as the concentration was increased, honey produced higher antibacterial activity against the two pathogens. Our findings are similar to those reported in a previous study [58]. Our study findings also corroborate those from Pakistan in which honey produced a dose-dependent activity against both *E. coli* and *S. aureus* but greater activity against *E. coli* [99]. Conversely, a study in Kenya reported different findings in which honey produced high antibacterial activity against *S. aureus* compared *to E. coli* [102]. This could due to the different sources of honey that our study used compared to what was used in Kenya. While our study reported important findings, there is a need to explore the different honey sources in Zambia to investigate whether the source of the honey could influence the antibacterial activities.

Our research aligns with previous studies conducted in various regions, which have also reported honey's antibacterial activity against *E. coli* and *S. aureus*, including antibiotic-resistant strains such as MRSA. Furthermore, our findings emphasize the importance of conducting additional investigations to identify the chemical components responsible for honey's antibacterial properties and to better understand the underlying mechanisms of action.

Ultimately, this study highlights the potential of honey as a viable alternative or complementary therapy for bacterial infections caused by *E. coli* and *S. aureus*. Further research is warranted to explore the full therapeutic potential of honey and to promote its use in clinical settings following the establishment of safety and efficacy guidelines.

Conclusion

This study found that the honey has antibacterial activities against *E. coli* and *S. aureus*, with more pronounced effects on *E. coli* than *S. aureus*. The antibacterial activity was found to be dose-dependent for both bacterial strains, indicating that higher concentrations of honey produced stronger inhibitory effects. While the specific components responsible for honey's antibacterial activity in Zambia have not been fully elucidated, our findings contribute to the growing body of evidence supporting the therapeutic potential of honey for treating bacterial infections and possibly drug-resistant infections.

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