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Evaluation of the Antibacterial Effect of Sidr Honey Produced by Tarhuna City Apiaries Against Escherichia coli

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تقييم التأثير المضاد للبكتيريا لعسل السدر الذي تنتجه نحل مدينة ترهونة ضد الإشريكية القولونية

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Abstract

This study evaluated the in vitro antibacterial effect of Sidr honey produced by apiaries in Tarhuna City against Escherichia coli. Four honey concentrations (100%, 75%, 50%, and 25%) were tested using 20 inoculated Petri dishes, with five dishes assigned to each concentration, while one additional untreated dish served as a negative control. Following honey application, the dishes were incubated for 24 hours, and antibacterial activity was assessed descriptively according to the presence or absence and relative size of visible inhibition zones. Undiluted honey at 100% produced the largest observable inhibition zone and demonstrated the strongest antibacterial effect. The 75% concentration also produced a visible but smaller inhibition zone. In contrast, no detectable inhibition was observed at concentrations of 50% and 25%, while the control dish showed bacterial growth without inhibition. The findings indicate that the antibacterial activity of Tarhuna Sidr honey against E. coli was concentration-dependent and became visibly detectable only at the two highest concentrations under the applied experimental conditions. Further standardized studies involving numerical inhibition-zone measurements, minimum inhibitory and bactericidal concentration assays, and chemical characterization are recommended.

Keywords: Sidr honey; Escherichia coli; antibacterial activity; inhibition zone; honey concentration; Tarhuna City; in vitro study.

الملخص

هدفت هذه الدراسة إلى تقييم التأثير المضاد للبكتيريا لعسل السدر المنتج من مناحل مدينة ترهونة ضد بكتيريا الإشريكية القولونية (*Escherichia coli*) في المختبر. اختُبرت أربعة تراكيز من عسل السدر، وهي 100% و75% و50% و25%، باستخدام عشرين طبق بتري مزروعًا بالبكتيريا، بواقع خمسة أطباق لكل تركيز، بالإضافة إلى طبق غير معالج استُخدم بوصفه مجموعة ضابطة سالبة. بعد إضافة العسل، حُضنت الأطباق مدة 24 ساعة، ثم قُيّمت الفعالية المضادة للبكتيريا وصفيًا وفقًا لظهور مناطق التثبيط أو غيابها وحجمها النسبي. أظهر العسل غير المخفف بتركيز 100% أكبر منطقة تثبيط وأقوى تأثير مضاد للبكتيريا، في حين أنتج تركيز 75% منطقة تثبيط واضحة ولكنها أصغر حجمًا. في المقابل، لم تُلاحظ مناطق تثبيط عند تركيزي 50% و25%، بينما أظهر طبق التحكم نموًا بكتيريًا دون تثبيط. تشير النتائج إلى أن تأثير عسل السدر المنتج في ترهونة ضد بكتيريا الإشريكية القولونية كان مرتبطًا بالتركيز، ولم يصبح ظاهرًا تحت ظروف التجربة إلا عند التركيزين الأعلى. وتوصي الدراسة بإجراء أبحاث أكثر توحيدًا تتضمن القياس الرقمي لأقطار مناطق التثبيط، وتحديد الحد الأدنى للتركيز المثبط والقاتل للبكتيريا، وتحليل المكونات الكيميائية الفعالة في العسل.

الكلمات المفتاحية: عسل السدر؛ الإشريكية القولونية؛ النشاط المضاد للبكتيريا؛ منطقة التثبيط؛ تركيز العسل؛ مدينة ترهونة؛ دراسة مختبرية.

Introduction

Antimicrobial resistance has become a major challenge to the effective treatment of bacterial infections, creating an urgent need to investigate naturally derived substances with potential antimicrobial activity. Among these substances, honey has attracted considerable scientific attention because of its long history of medicinal use and its complex biological composition. Honey is not merely a concentrated sugar solution; rather, it contains several interacting antibacterial factors, including high osmotic pressure, low water activity, acidic pH, hydrogen peroxide, phenolic compounds, flavonoids, antimicrobial peptides, enzymes, and other plant-derived constituents. The simultaneous action of these factors may inhibit bacterial growth through multiple mechanisms and may therefore offer a useful basis for developing complementary antimicrobial approaches (Mandal & Mandal, 2011; Bizerra et al., 2012; Brudzynski, 2021).

The antibacterial effectiveness of honey is influenced by several characteristics, including its botanical source, geographical origin, moisture content, acidity, storage conditions, processing method, and concentration. These variables affect both the quantity and activity of its bioactive constituents. Consequently, honey samples obtained from different floral and geographical sources may exhibit substantially different physicochemical and antimicrobial properties. This variability makes it necessary to evaluate each locally produced honey under controlled experimental conditions rather than generalizing results obtained from honey produced in other regions (Al-Ghamdi & Ansari, 2021; Fahim et al., 2014; Hegazi et al., 2022).

Sidr honey is produced predominantly from the nectar of plants belonging to the genus *Ziziphus*, particularly *Ziziphus spina-christi*. It is widely valued in North Africa, the Middle East, and parts of Asia for its nutritional quality and traditional therapeutic applications. Previous studies have demonstrated that Sidr honey contains phenolic acids, flavonoids, organic acids, enzymes, volatile compounds, and other biologically active constituents that may contribute to its antibacterial, antioxidant, antibiofilm, and antivirulence properties. Nevertheless, the concentration and biological effectiveness of these constituents differ among

samples according to geographical and environmental conditions (Halawani & Shohayeb, 2011; Bazaid et al., 2023; El-Meihy et al., 2025).

Escherichia coli is a Gram-negative, facultatively anaerobic bacterium commonly present in the intestinal microbiota of humans and animals. Although many strains are harmless, pathogenic strains can cause gastrointestinal disease, urinary tract infections, wound infections, septicemia, and other serious conditions. The outer membrane of *E. coli* functions as a selective permeability barrier and may reduce the penetration of several antimicrobial compounds. However, experimental evidence indicates that honey may act against *E. coli* by altering membrane permeability, causing leakage of intracellular materials, inhibiting bacterial proteins, generating oxidative stress, and damaging bacterial DNA (Al-Sayaghi et al., 2022). These multiple mechanisms provide a biological explanation for the susceptibility of *E. coli* to sufficiently active honey preparations.

Several studies have reported that the antibacterial activity of honey against *E. coli* is influenced by concentration. Higher honey concentrations often produce larger inhibition zones because they contain greater amounts of active antibacterial components. However, this relationship is not always linear because moderate dilution may activate glucose oxidase and increase hydrogen peroxide production, in addition to improving honey diffusion through agar. Thus, differences in honey type, dilution procedure, bacterial strain, inoculum density, culture medium, incubation period, and assessment method may lead to variation among experimental findings (Owayss et al., 2020; Mudenda et al., 2023; Kassym et al., 2024).

Despite the traditional use and commercial importance of Sidr honey in Libya, limited evidence is available regarding the antibacterial activity of Sidr honey produced by apiaries in Tarhuna City. The biological properties of honey from other countries or Libyan regions cannot be directly applied to Tarhuna honey because local vegetation, climate, soil, harvesting practices, and storage conditions may influence its composition. Therefore, the present study evaluates the in vitro antibacterial activity of Sidr honey obtained from Tarhuna apiaries against *E. coli* at concentrations of 100%, 75%, 50%, and 25%. It specifically examines whether these concentrations produce visible inhibition after 24 hours and whether the observed antibacterial effect varies according to honey concentration.

Literature Review

Antibacterial Properties of Honey

The medicinal use of honey has been documented for centuries, particularly in wound care and the management of infections. Contemporary microbiological investigations have provided scientific support for some of these traditional applications by demonstrating activity against several Gram-positive and Gram-negative bacterial species. Mandal and Mandal (2011) explained that the antibacterial effect of honey results from the interaction of its physical and chemical properties, particularly osmotic pressure, acidity, hydrogen peroxide production, and phytochemical constituents. Similarly, Bizerra et al. (2012) emphasized that honey exerts antibacterial activity through multiple mechanisms rather than through a single active substance.

High sugar concentrations contribute to the antimicrobial activity of honey by reducing water availability and creating osmotic stress that limits bacterial growth. Its acidic environment may also interfere with bacterial enzyme activity and membrane function. When honey is diluted, glucose oxidase may become active and catalyse the production of hydrogen peroxide, which can damage bacterial proteins, membranes, and nucleic acids. In addition, phenolic acids, flavonoids, antimicrobial peptides, and volatile compounds may strengthen both peroxide-

dependent and non-peroxide antibacterial activity (Al-Ghamdi & Ansari, 2021; Brudzynski, 2021).

Brudzynski (2021) presented honey as an ecological reservoir of antibacterial compounds originating from interactions among plants, nectar-associated microorganisms, and honeybees. This perspective suggests that honey composition is influenced by the biological environment in which it is produced. Therefore, differences in flora, microbial communities, climate, and beekeeping practices may generate substantial variation in antimicrobial activity, even among honeys classified under the same botanical name.

iological Characteristics of Sidr Honey

Sidr honey is principally associated with nectar collected from *Ziziphus* species and is among the most valued monofloral honeys in the Middle East and North Africa. Halawani and Shohayeb (2011) compared Sidr and Shaoka honeys with other local and imported honey samples available in Saudi markets. Their findings indicated that Sidr and Shaoka honeys demonstrated considerable antibacterial activity against pathogenic and food-spoilage bacteria. The study highlighted the importance of botanical origin in determining antimicrobial performance.

Fahim et al. (2014) examined the physicochemical characteristics and antimicrobial potential of honey samples obtained from different bee and botanical sources in Pakistan, including *Ziziphus jujube* honey. Their research associated differences in antimicrobial performance with differences in physicochemical composition. Likewise, Hegazi et al. (2022) characterized Sidr honeys obtained from different geographical origins and reported variability in their physicochemical properties, antioxidant capacity, phenolic composition, and antibacterial activity. These findings demonstrate that the term “Sidr honey” does not necessarily describe a chemically or biologically uniform product.

More recent research has extended the investigation of Sidr honey beyond planktonic bacterial growth. Bazaid et al. (2023) evaluated Saudi Sidr honey and reported antimicrobial, antibiofilm, anti-quorum-sensing, and antioxidant activities. These properties are important because biofilm formation and quorum sensing contribute to bacterial persistence, virulence, and reduced susceptibility to antimicrobial agents. El-Meihy et al. (2025) also examined the physicochemical characteristics, antioxidant capacity, and antibacterial activity of mixed Saudi Sidr honey, further demonstrating the connection between chemical composition and biological performance.

Evidence of Antibacterial Activity Against Pathogenic Bacteria

Studies conducted in different geographical settings generally support the antibacterial potential of Sidr and other natural honeys, although the degree of inhibition varies. Ekhtelat et al. (2016) investigated Iranian *Ziziphus* honey against foodborne pathogens and observed that its inhibitory effect depended on honey concentration and exposure duration. This finding indicates that antibacterial performance should be interpreted in relation to both treatment concentration and incubation conditions.

Owayss et al. (2020) compared Saudi Sidr honey derived from *Ziziphus spina-christi* with Talh honey derived from *Acacia gerrardii*. Both honey types inhibited the tested bacteria and fungus, but susceptibility differed among microorganisms. Gram-positive bacteria were generally more susceptible than Gram-negative bacteria, while *E. coli* demonstrated comparatively lower sensitivity. Interestingly, dilution enhanced the activity of some samples, which the authors associated with improved agar diffusion and increased hydrogen peroxide generation.

Al-Kafaween et al. (2023) compared Sidr and Tualang honeys with Manuka honey against *Staphylococcus aureus*. Although their study did not examine *E. coli*, it demonstrated that Sidr honey may influence bacterial growth and virulence-related characteristics. Similarly, Omran et al. (2023) investigated Egyptian Sidr honey and its interaction with conventional antimicrobial agents. Their findings supported the antimicrobial activity of Sidr honey and suggested that combining honey with selected antimicrobial agents may produce enhanced effects.

Farhana et al. (2023) evaluated raw and Sidr honey against antibiotic-resistant pathogenic bacteria. Their findings indicated that both honey types possessed activity against resistant bacterial strains, supporting continued investigation of honey as a potential source of antibacterial compounds. However, such in vitro findings should not be interpreted as evidence that honey can replace clinically approved antibiotics without additional safety, standardization, and clinical studies.

Antibacterial Activity Against *Escherichia coli*

The susceptibility of *E. coli* to honey has been demonstrated in several experimental investigations. Mudenda et al. (2023) used an agar-well diffusion assay to evaluate honey concentrations of 25%, 50%, 75%, and 100% against *E. coli* and *S. aureus*. They observed a concentration-dependent increase in inhibition, with the strongest antibacterial activity occurring at the highest honey concentration. Their results showed that honey produced greater inhibition of *E. coli* than of *S. aureus* in their experimental setting. However, ciprofloxacin generally produced stronger inhibition, demonstrating the importance of including a standard antibiotic as a positive control.

Kassym et al. (2024) focused on the antimicrobial contribution of honey phenolic compounds against *E. coli*. Their results demonstrated that phenolic constituents contribute to growth suppression and supported the interpretation that the antibacterial activity of honey cannot be explained by sugar concentration alone. The effectiveness of honey is therefore likely to result from the combined action of osmotic stress, acidity, hydrogen peroxide, phenolic compounds, and other biologically active substances.

At the mechanistic level, Al-Sayaghi et al. (2022) demonstrated that honey can affect *E. coli* by interfering with bacterial membrane permeability, inhibiting cellular proteins, and inducing DNA damage. These effects confirm that honey acts at several cellular levels. Such multifactorial activity may explain why sufficiently concentrated honey can inhibit *E. coli* despite the protective function of its Gram-negative outer membrane.

Differences among studies remain evident. While Mudenda et al. (2023) reported detectable inhibition at concentrations as low as 25%, other investigations observed limited or absent activity at lower concentrations. These contrasting results may be attributed to differences in botanical origin, chemical composition, bacterial strains, honey dilution procedures, inoculum density, volume applied, agar diffusion, incubation conditions, and measurement methods. Therefore, findings obtained with one honey sample should not be generalized to honey produced in another location.

Evidence From Libya

The most directly relevant Libyan study in the reviewed reference list was conducted by Elfallah et al. (2025) in Ajdabiya. The researchers used gas chromatography–mass spectrometry to characterize Sidr and thyme honey and evaluated their antibacterial activity against bacterial isolates obtained from diabetic foot infections. The tested organisms included *S. aureus*, *P. aeruginosa*, and *E. coli*. Antibacterial activity increased with concentration, and the 90% concentration produced the greatest inhibition. For *E. coli*, the 90% concentration

produced a 15-mm inhibition zone, whereas the 50% concentration produced no detectable inhibition. Sidr honey exhibited slightly greater activity than thyme honey (Elfallah et al., 2025).

The Ajdabiya study provides important Libyan evidence that honey activity is related to concentration and chemical composition. Nevertheless, it examined honey obtained from a different geographical area, employed concentrations of 50%, 70%, and 90%, and tested clinical isolates associated with diabetic foot infections. It did not specifically evaluate Sidr honey obtained from Tarhuna apiaries at concentrations of 25%, 50%, 75%, and 100%. Its findings therefore support the rationale for the present study but do not replace the need to evaluate Tarhuna honey independently.

Synthesis of the Literature

The reviewed literature demonstrates that honey possesses antimicrobial activity arising from the combined effects of osmotic pressure, acidity, hydrogen peroxide, phenolic compounds, peptides, and other phytochemicals. Sidr honey has shown activity against several bacterial species, including antibiotic-resistant strains. However, the magnitude of this activity varies according to botanical and geographical origin, physicochemical composition, bacterial species, concentration, and experimental method.

A concentration-related pattern has been reported frequently, but the minimum concentration producing observable inhibition differs considerably among studies. In some experiments, diluted honey retained or even demonstrated enhanced activity, whereas in others, lower concentrations failed to inhibit bacterial growth. These inconsistencies demonstrate that antibacterial effectiveness cannot be predicted solely from the label “Sidr honey” or from findings obtained in another geographical region.

Research Gap

Within the literature reviewed for the present research, most studies of Sidr honey were conducted in Saudi Arabia, Iran, Pakistan, Egypt, or other non-Libyan settings. The available Libyan study focused on Sidr and thyme honey from Ajdabiya and used clinical isolates and concentrations that differed from those adopted in the present investigation. Consequently, limited evidence is available concerning the antibacterial activity of Sidr honey produced specifically by apiaries in Tarhuna City against *E. coli*.

A methodological gap is also evident. Previous studies used different bacterial strains, honey concentrations, dilution media, culture conditions, application volumes, and outcome measures, making direct comparison difficult. Some studies measured inhibition zones numerically, whereas others determined minimum inhibitory or bactericidal concentrations or assessed changes in bacterial cellular structures. There remains a need for locally focused evidence examining whether Tarhuna Sidr honey retains detectable antibacterial activity after progressive dilution.

The present study addresses part of this geographical and experimental gap by comparing four concentrations of Tarhuna Sidr honey—100%, 75%, 50%, and 25%—against *E. coli* under the same incubation conditions. It provides preliminary evidence about the concentration at which visible inhibition occurs. Nevertheless, because inhibition-zone diameters were evaluated descriptively rather than measured numerically, the study should be regarded as an initial investigation. Future research should include quantitative inhibition-zone measurements, equal numbers of control replicates, a standard antibiotic positive control, MIC and MBC assays, standardized bacterial strains, and chemical characterization of the tested honey.

Problem Statement

The increasing resistance of pathogenic bacteria to conventional antibiotics has created a growing need to investigate natural products with potential antibacterial properties. Sidr honey is traditionally recognized for its medicinal value; however, its antibacterial effectiveness may differ according to its botanical origin, geographical location, chemical composition, storage conditions, and concentration. Consequently, evidence obtained from Sidr honey produced in other geographical regions cannot be directly generalized to honey produced by apiaries in Tarhuna City.

Despite the widespread local use and commercial importance of Sidr honey, there is insufficient laboratory evidence concerning the antibacterial activity of Sidr honey produced in Tarhuna against *Escherichia coli*. In particular, it remains unclear whether its inhibitory effect persists after dilution and whether different concentrations produce measurable differences in bacterial growth inhibition.

This knowledge gap limits the scientific assessment of Tarhuna Sidr honey and prevents reliable conclusions regarding the concentrations at which it demonstrates antibacterial activity. Therefore, the present study investigates the effect of four concentrations of Sidr honey—100%, 75%, 50%, and 25%—on the growth of *E. coli* after 24 hours of incubation. The research problem is centered on determining whether Tarhuna Sidr honey produces observable zones of inhibition and whether the magnitude of inhibition varies according to honey concentration.

Significance of the Study

This study is significant because it provides laboratory-based evidence regarding the antibacterial activity of Sidr honey produced by apiaries in Tarhuna City against *Escherichia coli*. Although Sidr honey is widely valued for its nutritional and traditional medicinal uses, the biological effectiveness of locally produced honey cannot be established solely from traditional use or findings reported for honey from other geographical regions. Evaluating Tarhuna Sidr honey under controlled laboratory conditions therefore contributes specific scientific information concerning its antibacterial properties.

The study also clarifies the relationship between honey concentration and bacterial growth inhibition by comparing four concentrations: 100%, 75%, 50%, and 25%. Determining which concentrations produce observable inhibition is essential because dilution can substantially influence the antibacterial performance of honey. The findings help identify the concentrations at which Tarhuna Sidr honey retains activity against *E. coli* and demonstrate the importance of concentration when interpreting its biological effectiveness.

From a microbiological perspective, the study contributes to the continuing investigation of natural substances that may contain biologically active antibacterial components. It also provides preliminary data that may support future studies involving the physicochemical characterization of Tarhuna Sidr honey, identification of its active compounds, determination of minimum inhibitory and bactericidal concentrations, and evaluation against additional bacterial strains.

Objectives of the Study

1. To evaluate the in vitro antibacterial activity of Sidr honey produced by apiaries in Tarhuna City against *Escherichia coli*.
2. To determine the effect of undiluted Sidr honey (100%) on the growth of *E. coli* after 24 hours of incubation.

3. To assess the antibacterial effect of Sidr honey diluted to a concentration of 75% against *E. coli*.
4. To investigate whether Sidr honey concentrations of 50% and 25% produce detectable inhibition of *E. coli* growth.
5. To compare the antibacterial effects of the four tested Sidr honey concentrations: 100%, 75%, 50%, and 25%.

Research Questions

1. Does Sidr honey produced by apiaries in Tarhuna City exhibit in vitro antibacterial activity against *Escherichia coli*?
2. Does undiluted Sidr honey at a concentration of 100% inhibit the growth of *E. coli* after 24 hours of incubation?
3. Does Sidr honey at a concentration of 75% produce a detectable inhibitory effect against *E. coli*?
4. Do Sidr honey concentrations of 50% and 25% inhibit the growth of *E. coli* under the specified experimental conditions?
5. Are there observable differences in antibacterial activity among the four tested concentrations of Sidr honey?

Previous Studies

Several studies conducted in Arab and international contexts have investigated the antibacterial activity of honey against pathogenic microorganisms. Particular attention has been given to Sidr honey because its botanical origin and bioactive composition may contribute to its antimicrobial properties. Findings from these studies indicate that the inhibitory activity of honey is influenced by its concentration, geographical source, physicochemical characteristics, bacterial species, and laboratory assessment method.

Owayss et al. (2020) conducted a study in Saudi Arabia to evaluate the in vitro antimicrobial activities of Sidr honey derived from *Ziziphus spina-christi* L. and Talh honey derived from *Acacia gerrardii* Benth. The honeys were tested against the Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, the Gram-negative bacteria *Escherichia coli* and *Salmonella enteritidis*, and the fungus *Trichophyton mentagrophytes*. Antimicrobial effectiveness was assessed by measuring the inhibition zones produced by natural honey and water-diluted honey at a concentration of 33% (w/v). Both honey types inhibited the tested microorganisms, although Talh honey generally demonstrated greater antibacterial activity. Gram-positive bacteria were more susceptible than Gram-negative bacteria, and *E. coli* displayed lower sensitivity than *B. cereus* and *S. aureus*. The study also found that dilution increased the antimicrobial activity of some honey samples, potentially because it improved diffusion through the agar and promoted hydrogen peroxide production.

Materials and Methods

Study Design

This study employed an in vitro experimental laboratory design to evaluate the antibacterial effect of Sidr honey produced by apiaries in Tarhuna City against *Escherichia coli*. Four concentrations of Sidr honey—100%, 75%, 50%, and 25%—were tested under the same laboratory conditions. Five inoculated Petri dishes were assigned to each concentration, while one untreated inoculated dish served as the negative control. Following honey application, all dishes were incubated for 24 hours. Antibacterial activity was assessed through direct

observation of bacterial growth and the presence or absence of clear inhibition zones around the sites of honey application.

Study Area

The Sidr honey used in this study was obtained from local apiaries located in Tarhuna City, northwestern Libya. Tarhuna is characterized by environmental and botanical conditions that support beekeeping and the production of natural honey, including Sidr honey derived from *Ziziphus* trees. The experimental procedures were conducted in a microbiology laboratory under controlled conditions suitable for the cultivation of *Escherichia coli*, application of the different honey concentrations, incubation of the inoculated Petri dishes, and observation of bacterial growth inhibition after 24 hours.

Study Population

The study population consisted of laboratory cultures of *Escherichia coli* grown on solid culture media in Petri dishes. As this was an in vitro experimental study, the experimental units were the inoculated Petri dishes rather than human participants or animals. These dishes were used to evaluate the bacterial response to four concentrations of Sidr honey produced by apiaries in Tarhuna City. Bacterial growth in the treated dishes was compared descriptively with that observed in the untreated control dish after 24 hours of incubation.

Results

The antibacterial effect of Sidr honey produced by apiaries in Tarhuna City was evaluated against *Escherichia coli* at four concentrations: 100%, 75%, 50%, and 25%. A total of 21 inoculated Petri dishes were examined after 24 hours of incubation. The findings were evaluated descriptively according to bacterial growth and the presence or absence of a visible inhibition zone around the honey application site. No numerical measurements of the inhibition-zone diameters were available; therefore, no numerical values or statistical tests were introduced.

Table 1. Distribution of Petri Dishes According to Sidr Honey Concentration.

Experimental group	Sidr honey concentration	Number of Petri dishes	Treatment condition
Group I	100%	5	Treated with undiluted Sidr honey
Group II	75%	5	Treated with 75% Sidr honey
Group III	50%	5	Treated with 50% Sidr honey
Group IV	25%	5	Treated with 25% Sidr honey
Negative control	0%	1	Inoculated but not treated with honey
Total	—	**21**	—

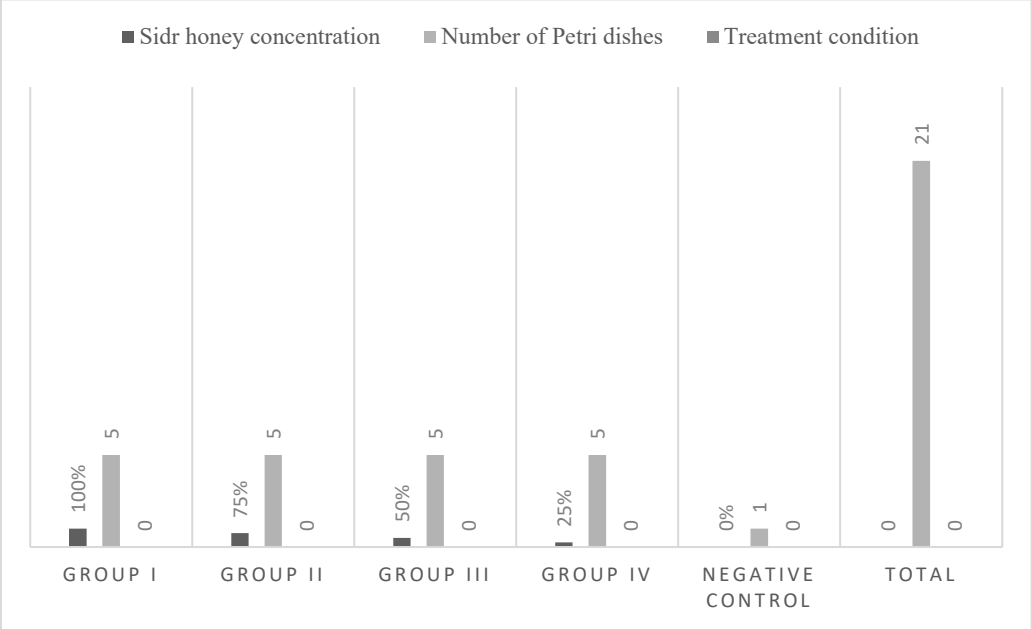

Figure 1. Distribution of Petri Dishes According to Sidr Honey Concentration

Table 1 shows that the 20 treated Petri dishes were distributed equally among four experimental groups, with five dishes assigned to each Sidr honey concentration. One additional dish was used as an untreated negative control. All groups were examined after the same incubation period of 24 hours.

Table 2. Observed Effect of Sidr Honey Concentrations on Escherichia coli

Sidr honey concentration	Number of dishes	Bacterial growth after 24 hours	Inhibition zone	Relative antibacterial effect
100%	5	Growth inhibited around the application site	Present; largest observed zone	Strongest
75%	5	Growth inhibited around the application site	Present; smaller than at 100%	Moderate
50%	5	Bacterial growth remained observable	Not detected	No detectable inhibition
25%	5	Bacterial growth remained observable	Not detected	No detectable inhibition
Negative control	1	Normal bacterial growth	Absent	No inhibition

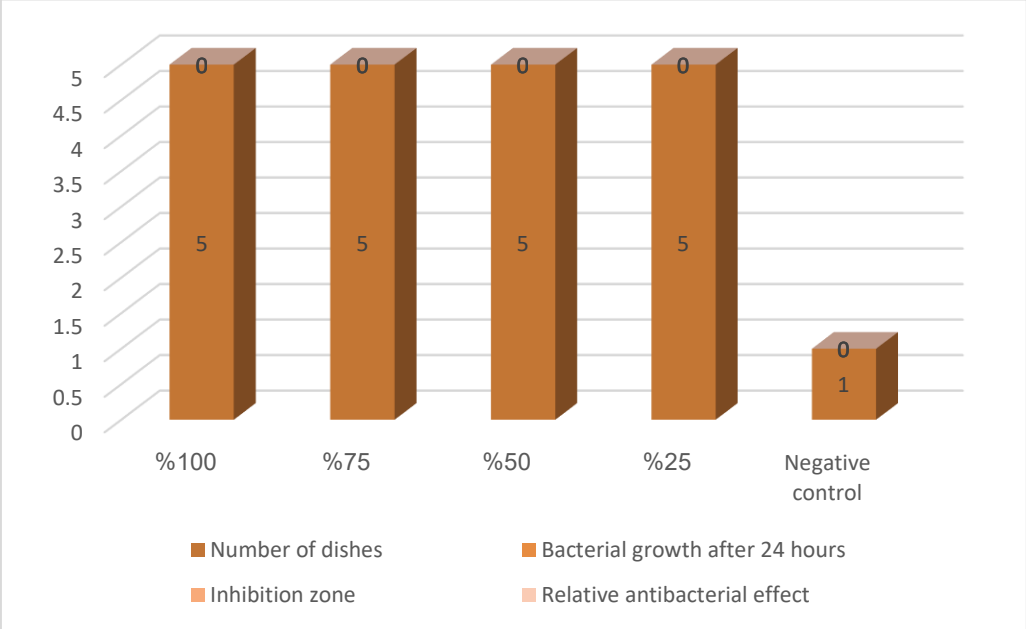

Figure 2. Observed Effect of Sidr Honey Concentrations on Escherichia coli**.**

As presented in Table 2, undiluted Sidr honey at 100% produced the strongest observable antibacterial effect against *E. coli*. This concentration formed the largest clear zone around the site of honey application. Sidr honey at 75% also produced a visible inhibition zone, but its relative diameter was smaller than that observed with the 100% concentration.
In contrast, concentrations of 50% and 25% did not produce detectable inhibition zones after 24 hours. Bacterial growth remained observable in these two groups. The untreated control dish also demonstrated bacterial growth without the formation of an inhibition zone, confirming that inhibition in the 100% and 75% groups was associated with Sidr honey treatment.

Table 3. Qualitative Ranking of the Tested Sidr Honey Concentrations

Rank	Sidr honey concentration	Observed inhibition	Interpretation
1	100%	Largest visible inhibition zone	Highest antibacterial activity
2	75%	Visible inhibition zone smaller than at 100%	Detectable antibacterial activity
3	50%	No visible inhibition zone	No detectable activity under the experimental conditions
4	25%	No visible inhibition zone	No detectable activity under the experimental conditions

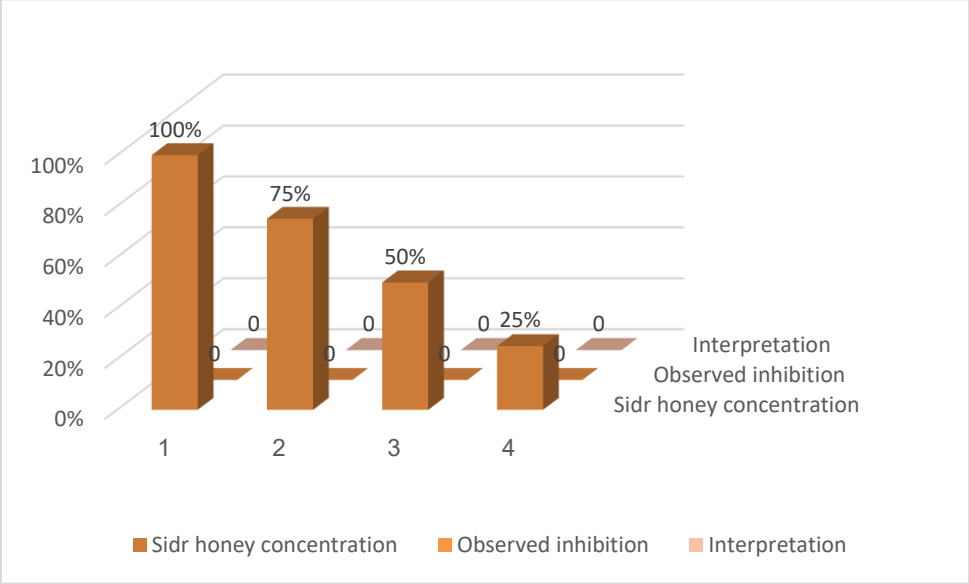

Figure 3. Qualitative Ranking of the Tested Sidr Honey Concentrations

Table 3 demonstrates a concentration-related pattern in the observed antibacterial activity. The 100% concentration ranked first, followed by the 75% concentration. No detectable inhibition was observed at 50% or 25%. These observations indicate that the antibacterial activity of the tested Tarhuna Sidr honey became visibly detectable only at the two highest concentrations under the conditions of this experiment.

Discussion

The antibacterial effect of Sidr honey produced by apiaries in Tarhuna City was evaluated against *Escherichia coli* at concentrations of 100%, 75%, 50%, and 25%. A total of 21 Petri dishes were used: five dishes for each concentration and one untreated dish as a negative control. All dishes were examined after 24 hours of incubation.

The results showed that the antibacterial effect of Sidr honey varied according to concentration. The 100% concentration produced the strongest antibacterial activity and formed the largest visible inhibition zone around the site of honey application. This clear area indicated that bacterial growth had been inhibited near the applied undiluted honey.

The 75% concentration also produced a visible inhibition zone against *E. coli*. However, the zone was smaller than that observed with the 100% concentration, indicating that its antibacterial effect was comparatively weaker.

In contrast, the 50% and 25% concentrations did not produce visible inhibition zones after 24 hours. Bacterial growth remained observable around the sites where these concentrations had been applied. The untreated control dish also showed normal bacterial growth without any inhibition zone.

The findings demonstrated that Sidr honey produced in Tarhuna exhibited a concentration-dependent antibacterial effect against *E. coli*. Undiluted honey produced the strongest inhibition, followed by the 75% concentration, whereas 50% and 25% produced no detectable inhibition after 24 hours. This may be related to the higher levels of antimicrobial factors in concentrated honey, including acidity, osmotic pressure, hydrogen peroxide, and phenolic compounds.

These findings agree with previous studies reporting stronger inhibition of *E. coli* at higher honey concentrations. However, the absence of visible zones at 50% and 25% indicates only that inhibition was not detectable using the applied method. Overall, the results provide preliminary evidence of the antibacterial potential of Tarhuna Sidr honey, although further quantitative studies are required to determine its MIC, MBC, and active chemical components.

Recommendations

1. Future experiments should measure inhibition-zone diameters accurately in millimetres using a ruler, calliper, or digital measuring instrument.
2. The experiment should be repeated independently to confirm the reproducibility of the observed concentration-related effects.
3. Equal numbers of negative control replicates should be included to correspond with the five replicates used in each treatment group.
4. A recognized antibiotic should be included as a positive control to compare the antibacterial activity of Sidr honey with a standard antimicrobial agent.
5. Additional concentrations between 50% and 75% should be evaluated to identify more precisely the concentration at which detectable inhibition begins.

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Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.