

## Evaluation of the Antibacterial Activity of Natural Honey Against Common Pathogenic Bacteria

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### تقييم الفعالية المضادة للبكتيريا للعسل الطبيعي ضد بعض الأنواع الشائعة من البكتيريا الممرضة

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#### Abstract

"This study aimed to evaluate the antibacterial activity of natural honey against selected common species of pathogenic bacteria, including both Gram-positive and Gram-negative strains. Eighteen bacterial isolates were obtained from clinical samples collected from Al-Qarawy Ben Nasser Hospital and identified using biochemical tests and the VITEK system. The antibacterial potential of natural honey was assessed using the agar well diffusion method at four different concentrations (25%, 50%, 75%, and 100%). Results demonstrated that honey exhibited a broad-spectrum antibacterial effect against "Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The inhibition zones ranged between 3–25 mm, with the strongest inhibition observed at the 100% concentration. Statistical comparison indicated no significant difference between Gram-positive and Gram-negative bacteria in their susceptibility to honey". The study concludes that natural honey possesses significant antibacterial properties that may serve as an alternative or complementary agent to conventional antibiotics, especially in combating antibiotic-resistant bacteria.

**Keywords:** Natural honey, antibacterial activity, pathogenic bacteria, Gram-positive, Gram-negative, antibiotic resistance.

#### الملخص

هدفت هذه الدراسة إلى تقييم الفعالية المضادة للبكتيريا للعسل الطبيعي ضد بعض الأنواع الشائعة من البكتيريا الممرضة، بما في ذلك السلالات الموجبة والسالبة لصبغة غرام. تم الحصول على ثمانية عشر عزلة بكتيرية من عينات سريرية من مستشفى القروي بن ناصر، وتم تعريفها باستخدام الاختبارات الكيميائية الحيوية ونظام (VITEK). جرى اختبار الفعالية المضادة للبكتيريا للعسل الطبيعي باستخدام طريقة الانتشار في الأوساط الصلبة (Agar Well Diffusion) بأربعة تراكيز مختلفة (25%، 50%، 75%، و100%). أظهرت النتائج أن للعسل الطبيعي تأثيراً واسع الطيف ضد Staphylococcus aureus و Streptococcus pneumoniae و Escherichia coli و Klebsiella pneumoniae و Pseudomonas aeruginosa. حيث تراوحت مناطق التثبيط بين 3 و25 ملم، وكان أعلى تأثير عند التركيز 100%. كما لم تُظهر النتائج فروقاً معنوية بين البكتيريا الموجبة والسالبة لصبغة غرام في درجة حساسيتها للعسل. خلصت الدراسة إلى أن العسل الطبيعي

يمتلك خصائص مضادة للبكتيريا تجعله عاملاً طبيعياً واعداً يمكن استخدامه كبديل أو مكمل للمضادات الحيوية، خصوصاً في مواجهة البكتيريا المقاومة له.

**الكلمات المفتاحية:** العسل الطبيعي، النشاط المضاد للبكتيريا، البكتيريا الممرضة، موجبة الغرام، سالبة الغرام، مقاومة المضادات الحيوية.

### Introduction

Antibiotic resistance has emerged as one of the most serious public health challenges of the 21st century. The widespread and often indiscriminate use of antibiotics has led to the development of resistant strains of pathogenic bacteria, rendering many conventional treatments ineffective. This alarming trend has created an urgent need for alternative antimicrobial agents derived from natural sources that can provide effective, safe, and affordable solutions for combating microbial infections. Among the natural substances being investigated, honey has gained increasing attention due to its broad-spectrum antibacterial activity and historical use in traditional medicine across cultures and centuries.

Honey is a natural product synthesized by *Apis mellifera* bees from the nectar of flowering plants. Its composition is complex and variable, depending on factors such as floral source, geographical region, and environmental conditions. Typically, honey consists of about 80% sugars (mainly fructose and glucose), 16–18% water, and small quantities of proteins, enzymes, amino acids, vitamins, minerals, and organic acids. These diverse components contribute to honey's unique physicochemical and biological properties, including its antimicrobial effects. The antibacterial potential of honey is attributed to multiple mechanisms, such as its high osmotic pressure, low pH, hydrogen peroxide production, and the presence of bioactive compounds like flavonoids, phenolic acids, and enzymes such as glucose oxidase and catalase.

Several studies have demonstrated that honey exhibits inhibitory effects against a wide range of bacteria, including both Gram-positive and Gram-negative species. Notably, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*—pathogens commonly associated with hospital-acquired infections—have shown varying degrees of sensitivity to honey. The antibacterial efficacy of honey depends largely on its concentration, botanical origin, and the type of bacteria tested. For example, undiluted or highly concentrated honey typically produces larger zones of inhibition in vitro compared to diluted samples, suggesting a dose-dependent effect. Furthermore, the antibacterial activity of honey can be influenced by its hydrogen peroxide content, as well as non-peroxide factors such as methylglyoxal (MGO) in Manuka honey.

The therapeutic potential of honey extends beyond its antimicrobial effects. It has been used in wound healing, burn treatment, and gastrointestinal disorders due to its anti-inflammatory, antioxidant, and tissue-regenerative properties. Recent research has focused on integrating honey into biomedical applications, including wound dressings, topical ointments, and as a natural preservative in pharmaceutical formulations. However, despite these promising findings, variations in experimental methodologies, types of honey tested, and bacterial strains used have led to inconsistencies in reported results. Therefore, further systematic studies are needed to evaluate the antibacterial potency of locally produced honey varieties and their potential applications in modern medicine.

This study aims to evaluate the antibacterial activity of natural honey against selected pathogenic bacteria, including both Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and Gram-negative species (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*). Using the agar well diffusion method, the research investigates the relationship between honey concentration and bacterial growth inhibition. The findings are expected to contribute to a better understanding of honey's antimicrobial efficacy and support its potential use as a natural alternative or complementary agent to conventional antibiotics, especially in the context of growing antibiotic resistance.

### Literature Review

Antibiotic resistance is now considered one of the most alarming global health concerns of the modern era. The indiscriminate and excessive use of antibiotics in both medical and agricultural settings has resulted in the evolution of multidrug-resistant bacterial strains, posing serious challenges to infection control and treatment outcomes (Amenu, 2013; Shenoy et al., 2012). This situation has prompted the scientific community to search for alternative natural antimicrobial agents that can either complement or substitute synthetic antibiotics. Natural substances of plant or animal origin, such as essential oils, propolis, and honey, have shown great promise due to their bioactive components and minimal side effects (Wasihun & Kasa, 2016).

Honey, in particular, has been recognized since ancient times as both a food and a therapeutic substance. It has been used in traditional medicine for the treatment of wounds, burns, and gastrointestinal disorders. Its ability to prevent bacterial infection and promote tissue regeneration has been well-documented in medical and

ethnobotanical literature (Molan, 1992; Irish et al., 2011). The biological activities of honey arise from its complex composition, which includes carbohydrates, amino acids, enzymes, vitamins, minerals, phenolic compounds, and organic acids. On average, honey contains about 80% sugars—primarily fructose (38%) and glucose (31%)—alongside approximately 16–18% water, trace proteins, and a diverse array of secondary metabolites (Carina et al., 2014).

The antimicrobial properties of honey are attributed to several synergistic mechanisms. Firstly, honey's **high osmotic pressure**, resulting from its high sugar concentration, inhibits bacterial growth by drawing water out of microbial cells through osmosis, leading to cellular dehydration. Secondly, honey's **low pH**—typically between 3.2 and 4.5—creates an acidic environment unsuitable for the growth of most pathogenic bacteria (Hegazi et al., 2017). In addition, honey contains **hydrogen peroxide ( $H_2O_2$ )**, which is generated enzymatically through the activity of glucose oxidase. Hydrogen peroxide acts as a potent antimicrobial agent by causing oxidative damage to bacterial cell walls and intracellular components (Osho & Bello, 2010; Dinkov, 2017).

Beyond hydrogen peroxide, honey's antibacterial effects are also enhanced by **non-peroxide factors** such as methylglyoxal (MGO), phenolic acids, flavonoids, and other phytochemicals derived from the nectar of flowering plants. These compounds possess strong antioxidant and antimicrobial properties, disrupting bacterial cell membranes and interfering with enzyme function (French et al., 2005; Taormina et al., 2001). The relative contribution of these components depends largely on the **botanical and geographical origin** of the honey. For example, Manuka honey from *Leptospermum scoparium* is renowned for its high MGO content and potent antimicrobial activity, while honeys derived from tropical plants may exhibit stronger peroxide-dependent effects (Mandal & Mandal, 2011).

Several studies have empirically demonstrated honey's broad-spectrum antibacterial activity. Jawad (2011) found that undiluted honey inhibited the growth of both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas spp.*), producing inhibition zones up to 20 mm in diameter. Similarly, Osho and Bello (2010) compared different concentrations of honey (5–100%) and reported that antibacterial efficacy increased with concentration, indicating a dose-dependent relationship. Wasihun and Kasa (2016) further evaluated two types of honey—red and white—against multidrug-resistant bacteria and found that red honey exhibited stronger antibacterial effects, suggesting that color and composition are significant determinants of antimicrobial potential.

Moreover, studies such as those by Shenoy et al. (2012) and Hegazi et al. (2017) confirmed honey's effectiveness against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli*, and *Streptococcus* species—organisms that are commonly implicated in hospital-acquired infections. These findings are consistent with earlier work by Molan (1992), who reported that honey's antimicrobial activity varied depending on the bacterial strain and honey source. Importantly, the antibacterial effects of honey have been shown to persist even when diluted to moderate concentrations, although undiluted honey consistently exhibits the greatest inhibition (Taormina et al., 2001).

In addition to its antibacterial potential, honey's antimicrobial action is supported by its antioxidant, anti-inflammatory, and wound-healing properties. The combination of these biological activities enhances its therapeutic value in managing infected wounds and promoting recovery (Irish et al., 2011). Furthermore, because honey acts through multiple mechanisms—osmotic stress, acidity, enzymatic oxidation, and phytochemical toxicity—bacteria are less likely to develop resistance against it compared to single-target antibiotics (Hussain et al., 2015).

Despite the abundance of evidence supporting honey's antimicrobial potential, the variability in its composition remains a limitation to its standardization as a therapeutic agent. Factors such as floral origin, geographical location, bee species, and storage conditions can significantly influence the concentration of active compounds (Molan, 1992). Therefore, continuous investigation is essential to characterize the antibacterial potency of different honey varieties and determine optimal concentrations for therapeutic use.

In summary, the literature establishes that honey possesses significant antibacterial properties against a wide range of pathogenic bacteria. Its multifaceted mechanisms of action make it a promising natural alternative for managing infections, particularly those caused by antibiotic-resistant microorganisms. Building upon previous findings, the current study seeks to evaluate the antibacterial activity of natural honey collected from local sources against selected Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria at different concentrations. This research aims to contribute to the growing body of evidence supporting honey's clinical and microbiological significance as a natural antimicrobial agent.

## Materials and methods

### Isolation and identification of bacteria

Approximately eighteen bacterial isolates were collected from Al-Qarawy Ben Nasser Hospital and sent to the Reference Medical Laboratory in Misallatah for analysis. The bacteria were isolated from various clinical specimens, including stool, urine, sputum, nasal swabs, throat swabs, as well as burn and wound infections. The isolates were initially cultured on Blood Agar (Himedia, India) and incubated at 37 °C for 24 hours. Subsequently, well-isolated colonies were sub-cultured on MacConkey Agar, King A Medium, Pseudomonas Selective Agar, and Mannitol Salt Agar (Himedia, India). Finally, the purified colonies were transferred to Nutrient Agar (Himedia, India) for storage and further biochemical testing.

The bacterial isolates were preliminarily identified based on colony morphology, Gram staining, and a series of biochemical tests, including Catalase, Oxidase, Coagulase, Eosin Methylene Blue (EMB) Agar, Urease, Triple Sugar Iron (TSI), and IMViC tests (12, 13). Final identification of the bacterial species was confirmed using the VITEK automated system to ensure accurate and definitive classification. The identification results are summarized in Table 1.

### The collection and preparation of Honey

The honey samples were collected from markets Green mountain in sterile well-screwed container and kept in cool, dry and dark place in the laboratory. The honey was filtered with sterile mesh to remove any contamination and storage until use. The honey solutions were prepared as the following: 2.5ml of honey was mixed with 7.5 ml of sterile distilled water to achieve a 25% (v/v) solution; another 5 ml of honey was diluted with 5ml distilled water to achieve a 50% (v/v) solution, and 7.5ml of honey mixed with 2.5ml distilled water to achieve 75%(v/v) solution.

### Antibacterial activity of honey

The antibacterial activity of honey was tested by agar well diffusion method (5,11) the test was performed as the following: 4-5 colonies of the tested bacterial isolate were picked up from an overnight culture plate. Then the colonies emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of the McFarland No.0.5 turbidity standard. After that a sterile swab dipped into the bacterial suspension, and the surface of a Mueller-Hinton agar plate (Himedia-India) was inoculated by bacterial isolate.

Finally, three wells were cut in the agar by a sterile cork borer of 6mm $\phi$ , the agar were removed with sterile needle. The three wells of each plate then filled with honey solutions, two wells were filled with 150  $\mu$ l of 25% (v/v), 50% (v/v) and 75%(v/v) solutions of honey, the fourth well was filled with 150 $\mu$ l of honey without any dilution(100%). The plates were incubated at 37°C for 24 hours.

### Results and discussion

The results of the agar well diffusion assay demonstrated that natural honey inhibited the growth of all bacterial isolates tested in this study, including both Gram-positive and Gram-negative species. This confirms that honey possesses a broad-spectrum antibacterial activity against a variety of pathogenic microorganisms.

The inhibition zones observed for Gram-negative bacteria ranged between **3–23 mm** (Table 2), while those for Gram-positive bacteria ranged between **7–25 mm** (Table 3). These results indicate that honey effectively suppressed bacterial growth across all concentrations tested. However, the degree of inhibition varied according to the concentration of honey. The **100% (undiluted) concentration** produced the largest inhibition zones, whereas the inhibitory effect decreased progressively with lower concentrations (75%, 50%, and 25%).

For Gram-negative bacteria, the inhibition zones ranged from **17–23 mm** at 100% honey concentration, **12–16 mm** at 75%, **5–11 mm** at 50%, and **3–6 mm** at 25%. Similarly, for Gram-positive bacteria, the inhibition zones ranged from **22–25 mm** at 100%, **19–21 mm** at 75%, **12–14 mm** at 50%, and **7–9 mm** at 25%. Statistical analysis revealed no significant difference in susceptibility between Gram-positive and Gram-negative bacteria (Table 4). These findings suggest that honey exerts comparable antibacterial effects on both bacterial groups.

The present results are consistent with those of **Jawad (2011)**, who reported that honey inhibited both *Staphylococcus aureus* (Gram-positive) and *Pseudomonas spp.* (Gram-negative). Similarly, **Mandal (2011)** observed inhibition zones of approximately 13–14 mm for *E. coli*, 15–16 mm for *P. aeruginosa*, and 20–21 mm for *S. aureus* using undiluted honey. In another study, **Hussain et al. (2015)** found that honey concentrations of 40–50% or higher produced the greatest antibacterial effects. Likewise, **Osho and Bello (2010)** compared different honey concentrations (5%, 25%, 50%, and 100%) against *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. aureus*, and *Bacillus subtilis* and reported the highest inhibition zones at 25% and 100%.

Further supporting evidence is provided by **Taormina et al. (2001)**, who found that floral honey exhibited inhibitory effects against *E. coli*, *Shigella sonnei*, *Salmonella typhimurium*, *Listeria monocytogenes*, *S. aureus*, and *Bacillus cereus*, with the degree of inhibition depending on both honey concentration and bacterial species.

**Wasihun and Kasa (2016)** also reported that red honey showed stronger antibacterial activity than white honey,

suggesting that chemical composition and pigment-related compounds may enhance antimicrobial potency. Similarly, **Hegazi et al. (2017)** demonstrated that honey inhibited *S. aureus*, *S. mutans*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, and concluded that both pathogen type and honey source influence antimicrobial activity.

**Shenoy et al. (2012)** further revealed that honey could inhibit *P. aeruginosa* growth even when diluted. They found that lower concentrations (20–50%) achieved bactericidal effects within 24 hours, while higher concentrations (75–100%) killed bacteria within 12 hours, indicating that higher concentrations accelerate antimicrobial action. **Molan (1992)** explained such variability by noting that honey samples differ in their antibacterial potency due to variations in floral source, environmental conditions, and bacterial susceptibility.

The antibacterial efficacy of honey can be attributed to several factors, including its **high osmotic pressure**, **low pH**, and the presence of numerous bioactive compounds such as **hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**, **lysozyme**, **organic acids**, **phenols**, **fatty acids**, **flavonoids**, and **vitamins** (Molan, 1992; Carina et al., 2014). In this study, the greatest inhibition zones were observed at 100% honey concentration, confirming that dilution reduces antibacterial potency. This can be explained by the osmotic nature of honey: it is a saturated sugar solution with approximately 15–20% water, resulting in a **low water activity (a<sub>w</sub> ≈ 0.5–0.6)** that prevents microbial growth, whereas most bacteria require a water activity above 0.9 for survival (Amenu, 2013).

Finally, it should be noted that the **chemical composition of honey** can vary significantly depending on factors such as the floral source of nectar, the season and time of collection, the species and health of the bee colony, and geographic conditions (Hegazi et al., 2017). These variations influence the levels of bioactive compounds and therefore affect the overall antimicrobial potential.

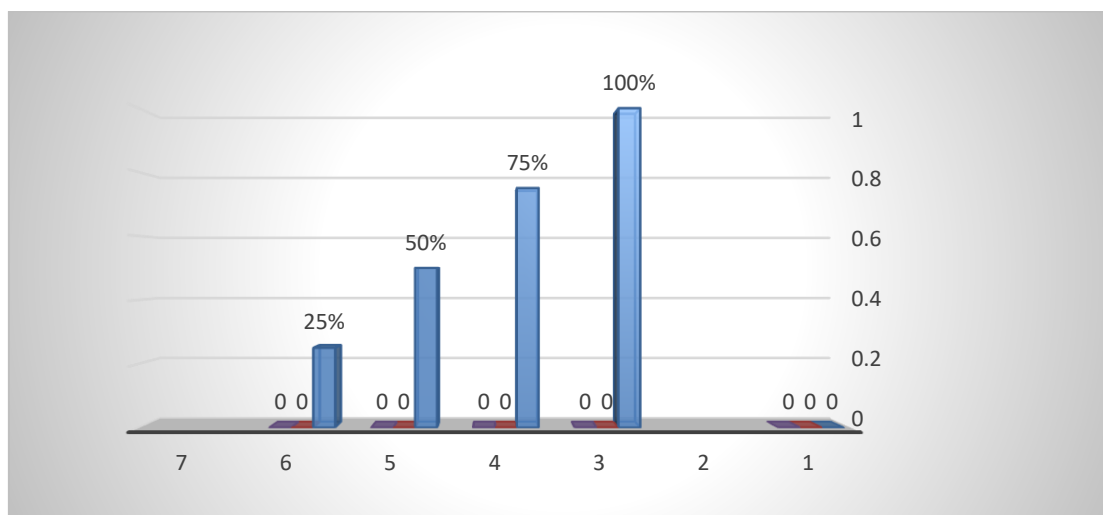
In conclusion, the findings of this study confirm that natural honey exhibits strong antibacterial activity against both Gram-positive and Gram-negative bacteria, particularly at higher concentrations. These results support the potential application of honey as a natural antimicrobial agent and highlight its promising role as an alternative or complementary treatment to conventional antibiotics

**Table 1-** Explain and bacterial isolation that included in the study and identification

| N.S | The isolates             | Identification |
|-----|--------------------------|----------------|
| 1   | E.coli                   | 96%            |
| 2   | E.coli                   | 98%            |
| 3   | E.coli                   | 96%            |
| 4   | E.coli                   | 99%            |
| 5   | E.coli                   | 95%            |
| 6   | Klebsiella pneumoniae    | 97%            |
| 7   | Klebsiella pneumoniae    | 99%            |
| 8   | Klebsiella pneumoniae    | 97%            |
| 10  | Pseudomonas aeruginosa   | 98%            |
| 11  | Pseudomonas aeruginosa   | 95%            |
| 12  | Pseudomonas aeruginosa   | 99%            |
| 13  | Staphylococcus aureus    | 98%            |
| 14  | Staphylococcus aureus    | 99%            |
| 15  | Staphylococcus aureus    | 96%            |
| 16  | Streptococcus pneumoniae | 97%            |
| 17  | Streptococcus pneumoniae | 98%            |
| 18  | Streptococcus pneumoniae | 98%            |

**Table2-** the inhibition zone(mm)of G-ve bacteria with different honey concentration

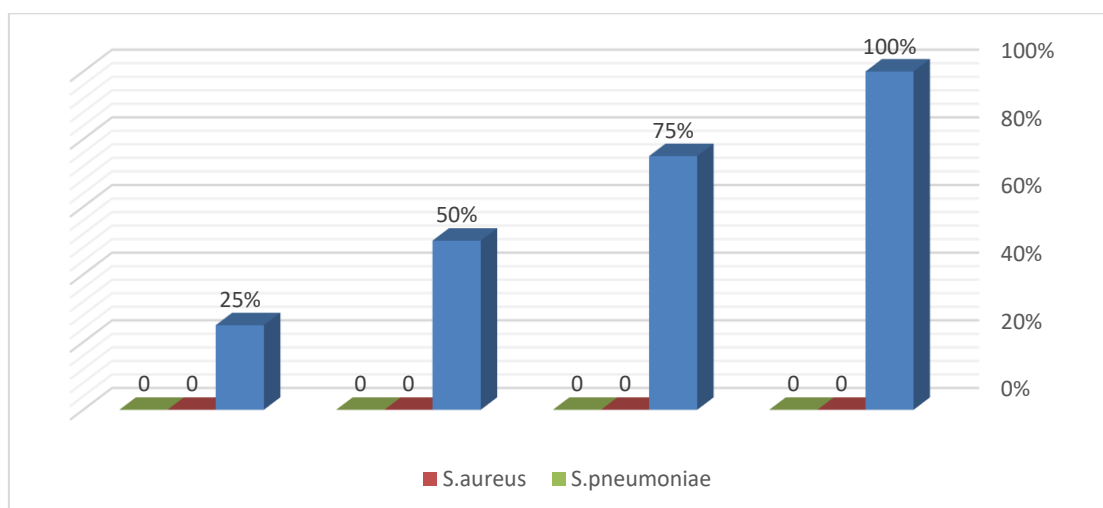
| G-ve bacteria | percentage | 100% | 75%  | 50%  | 25% |
|---------------|------------|------|------|------|-----|
| E.coli        |            | 23mm | 14mm | 11mm | 6mm |
| k.pneumoniae  |            | 17mm | 12mm | 5mm  | 3mm |
| p.aeruginosa  |            | 20mm | 16mm | 10mm | 5mm |



**Figure 1-** the results of the Gram –ve with different concentrations

**Table3-** the inhibition zone(mm)of G+ve bacteria with different honey concentration

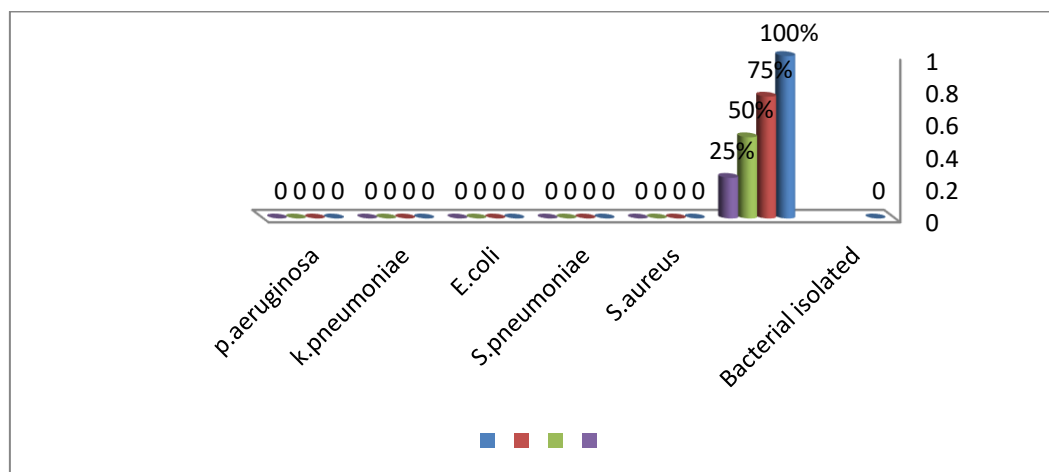
| G+ve bacteria | 100% | 75%  | 50%  | 25% |
|---------------|------|------|------|-----|
| S.aureus      | 22mm | 21mm | 14mm | 7mm |
| S.pneumoniae  | 25mm | 19mm | 12mm | 9mm |



**Figure 2-** the results of the Gram +ve bacteria with different concentrations

**Table4-**comparison between Gram +ve bacteria and Gram –ve bacteria to determine activity of honey on microorganism

| Bacterial group         | Bacterial isolated | Inhibition zone(mm) with different honey concentration |      |      |     |
|-------------------------|--------------------|--------------------------------------------------------|------|------|-----|
|                         |                    | 100%                                                   | 75%  | 50%  | 25% |
| Gram-positive bacteria  | S.aureus           | 22mm                                                   | 21mm | 14mm | 7mm |
|                         | S.pneumoniae       | 25mm                                                   | 19mm | 12mm | 9mm |
| Gram-neagative bacteria | E.coli             | 23mm                                                   | 14mm | 11mm | 6mm |
|                         | k.pneumoniae       | 17mm                                                   | 12mm | 5mm  | 3mm |
|                         | p.aeruginosa       | 20mm                                                   | 16mm | 10mm | 5mm |



**Figure 3-** the results of the Gram +ve bacteria and Gram –ve with different concentrations

### Conclusion

The findings of this study clearly demonstrate that natural honey possesses strong antibacterial properties against both Gram-positive and Gram-negative bacteria. Using the agar well diffusion method, honey showed significant inhibitory effects on the growth of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The results revealed that the antibacterial activity of honey increased with concentration, reaching its maximum at 100% undiluted honey, while the inhibition zones decreased progressively at lower concentrations.

The study also indicated that there was no significant difference in susceptibility between Gram-positive and Gram-negative bacteria, suggesting that honey exhibits a broad-spectrum antimicrobial effect. These findings are consistent with previously published research and confirm that honey can serve as a potential natural alternative or complementary agent to conventional antibiotics.

The antibacterial mechanisms of honey are attributed to multiple factors, including its high osmotic pressure, low pH, hydrogen peroxide production, and the presence of bioactive compounds such as flavonoids, phenolic acids, and enzymes. The variability in antibacterial potency across different studies is likely due to differences in honey type, floral source, geographical location, and physicochemical properties.

In conclusion, natural honey represents an effective and safe natural antimicrobial substance that can be utilized in the management of bacterial infections, particularly in cases involving antibiotic-resistant strains. Further studies should focus on the standardization of honey samples, detailed chemical characterization, and in vivo evaluations to enhance its clinical application as a therapeutic antimicrobial agent.

### Recommendations

Based on the findings of this study, the following recommendations are proposed to enhance the scientific understanding and practical application of natural honey as an antibacterial agent:

- Further Chemical Characterization:**  
Future studies should analyze the detailed physicochemical composition of honey samples, including pH, sugar profile, hydrogen peroxide content, and phenolic compounds, to better correlate chemical properties with antibacterial efficacy.
- Comparative Studies Between Honey Types:**  
It is recommended to compare different types of honey (e.g., floral, mountain, and Manuka honey) collected from various geographic and botanical sources to determine which types exhibit the strongest antibacterial activity.
- In Vivo Experimental Studies:**  
Additional in vivo studies should be conducted using animal models or clinical trials to confirm the antibacterial effects of honey under physiological conditions, ensuring safety and therapeutic effectiveness.
- Combination Therapy:**  
Investigate the potential synergistic effects of honey when combined with conventional antibiotics or herbal extracts, which may enhance antimicrobial efficacy and reduce antibiotic resistance.
- Standardization and Quality Control:**

Establishing standardized protocols for testing, processing, and labeling natural honey is essential to ensure consistency in its antibacterial properties and clinical reliability.

6. **Public Health and Clinical Application:**

Encourage the use of natural honey in topical formulations for wound healing, burn treatment, and skin infections as a cost-effective and safe alternative, especially in regions with limited access to antibiotics.

7. **Educational and Awareness Programs:**

Increase public and healthcare professional awareness of the medicinal value of honey and its role as a natural antimicrobial agent, promoting its safe and rational use.

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## Compliance with ethical standards

### Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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