Kuwait Research Journal of Agriculture and Life Science (KRJALS)

ISSN Print: 2790-6485 | ISSN Online : 2790-6477 Open Access

Antibacterial Effect of Al sider Honey and Mountain Honey Against E. coli and Staphylococcus aureus

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Article History
Received: 11.12.2025
Revise : 21.01.2026
Accepted: 24.01.2026
Published: 27.01.2026

DOI:10.61705/KRJALS.5.1.2026.12.21

Keywords: sider honey, mountain honey, E. coli, Staphylococcus aureus, antibacterial

Abstract:
Devotion has curdled to the custom of honey owed to its abundant assistances, and with the cumulative necessity to bargain safe, ordinary alternatives to contest certain categories of bacterial corruptions given the high cost of behavior, this study was directed to test honey's facility to kill bacteria in vitro. laboratory. The diffusion method was used to test the effectiveness of Sidr honey and mountain honey at three concentrations each on two forms of bacteria: E. coli and Staphylococcus aureus. The results exhibited that honey was highly effective as an antibacterial agent at all concentrations for mutually types of honey against the bacteria tested. This provides a good suggestion for using honey as an alternative strategy in combating certain modes of bacterial contamination.

Introduction

Honey is a syrupy, marvelous saturated solution of collected of glucose, carbohydrates, fructose, oligo- and polysaccharides, water, and the other substances, such as enzymes proteins, vitamins, phenolic compounds, minerals, and amino acids that are of strength and alimentary implication (Manickavasagam *et al*., 2024) . Honey is one of the most important natural sweeteners which detected in prehistoric times. However, since the production of sugarcane, the use of honey as a natural remedy has begun to decline. However, people continual to favor honey due to of its distinctive nutritional value, therapeutic qualities and pharmacological, including its antiallergic, anticancer, antidiabetic, and the antioxidant qualities (Zulpa *et al*., 2021). The production of valuable goods like honey, wax, pollen, propolis, royal jelly, and others depends heavily on beekeeping. This area of farming guarantees crop pollination and contributes to the notable rise in the amount and caliber of certain goods, particularly fruits and seeds (Greenop *et al*., 2020) and this is raises to a sugary material which includes amalgamation nectar gathered from flora,secretions from insects which feed on plants, or sweet releases from plants, along with the unique compounds which they generate themselves,then preservation inside the honeycomb, where the material matures, producing particular molecules and declining moisture levels. Honey serves as a important dietary item and is a significance of the interplay among bees and their role as pollinators in surrounding ecosystem, thereby bringing an essential service that aids maintain ecological balance (Leoni *et al*., 2021).

Honey's composition includes water, various

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sugars, enzymes, amino acids, flavonoids, organic acids, phenolic acids, minerals, vitamins, and volatile elements. The honeybee species involved, along with the variety and source of flowers, climatic and weather influences, and the honey's processing, packaging, and storage methods, all play a role in defining several attributes of honey, such as its color, taste, and scent (Abedi *et al*., 2021). becoming denser through water removal within the beehive. The chemical makeup of the honey is quite complicated and varies based on the plants from which it originates. Through history, it has assisted as both a nutritious and a therapeutic important source. Evidences suggests that human have been using honey for about 8000 years, as showed by artwork from the Stone Ages. Further the significant role of natural honey in customary healing practices, the recent decades have seen wide study of honey in laboratories and clinical settings by many researchers, leading to its leading to its acceptance in contemporary medical practices. It has been recognized that honey can inhibit growth of about 60 species of bacteria, along with certain species of fungi and viruses in contemporary medical practices. (Eteraf-Oskouei & Najafi, 2013).

In recent years, significant interest has rushed in the fields of medicine, nutritional, and the food sciences. This heightened attention stems from a rising understanding among consumers concerning the benefits of wholesome and functioning edibles, thereby starting scholarly explorations to the nutritional makeup of honey and its probable contributions to development wellness and warding off sicknesses. Consequentially, a vast group of investigations has raised, scrutinizing the widespread array of wellness-attractive qualities (Gupta *et al*., 2024). Moreover, the presence of energetic constituents in honey could assistance in modifying the body's custom of glucose and impede the improvement of resistance to insulin. Consequently, honey is progressively gaining acknowledgment as a wholesome sweetening alternative that can be included cautiously in everyday eating habits and the natural active ingredients in honey also play an important role in improving the function of the immune system, which enhances the body's ability to resist diseases by the immunomodulatory effects of honey are mediated through various mechanisms, including the activation of immune cells, stimulation of antibody production, and modulation of cytokine release (Takeuchi *et al*., 2011). Antibacterial resistance is a well-recognized, ever-emerging phenomenon which poses scary threats and challenges to humans' health worldwide. This is primarily because of the emergence of strains of bacteria that cannot be treated with presently available drugs. These bacteria are recognized as multidrug-resistant bacteria, and their appearance is attributed to reduced hygiene, globalization, and the unnecessary or incorrect use of antibiotics (Aldarhami, Bazaid, Qanash, Ahmad, Alshammari, *et al*., 2023). Honey has been used as an alternative method to combat antibiotic-resistant bacteria. It is clear that there are several types of honey that differ according to the environment, type of honey food, and geographical location, and each has its own antibacterial activity (Albaridi, 2019).

Various properties disturb the antibacterial possible of honey including its low ratio of water content, high viscosity, acidity and H_2O_2 content. Additionally, various mixtures are associated with honey and offer its antibacterial activity, including phytochemicals, peroxidase glycopeptides peptides, and proteins that are notable features of honey and are related with its antimicrobial effects (Figure 1) (S. Almasaudi, 2021).

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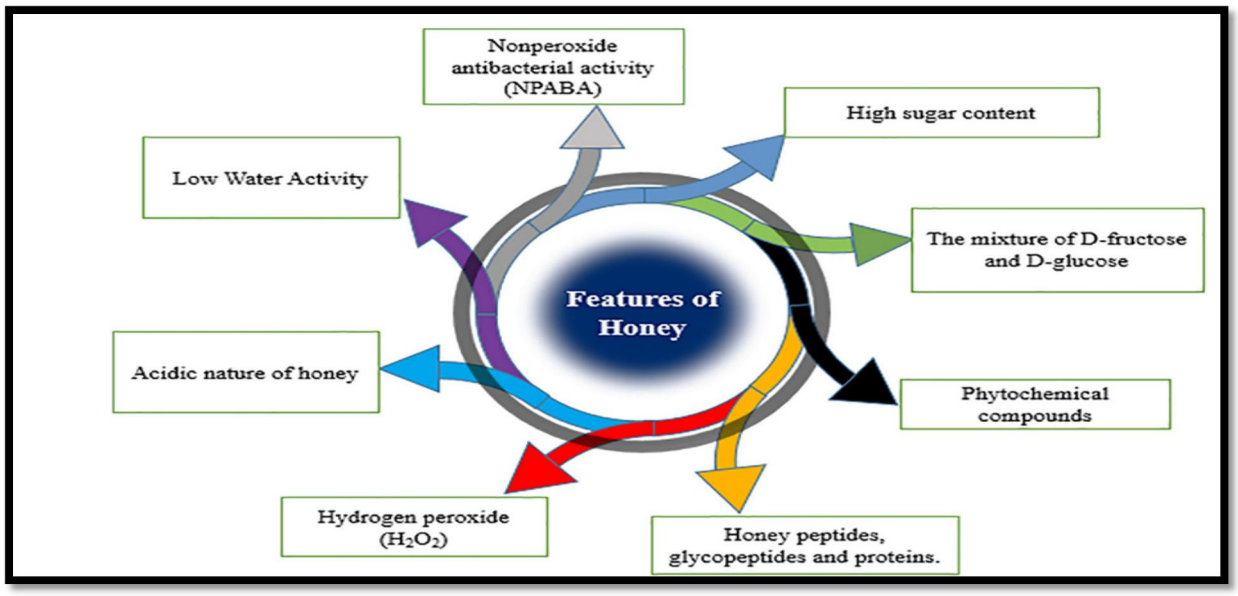

Figure 1: Schematic diagram showing the proprieties that contribute to the antimicrobial activity of honey

Materials and Methods

A pathogenic bacterial isolate from Al-Sadr Teaching Hospital in Najaf were used *E. coli* and *Staphylococcus aureus*, the bacterial isolates were diagnosed using the VITEK system. The isolates of bacteria were reactivated using nutrient broth media by incubating them in the medium at a temperature of 37°C. for 24 hours , The two types of honey that used in the experiment were: natural Al Sidr honey and natural mountain honey (both types of honey were obtained from the markets of Mashhad in the Islamic Republic of Iran) as shown in (figure 2and 3). Concentrated and diluted honey were used at a 50% (v/v) ratio for both types by mixing 5 ml of honey with 5 ml of distilled water. Honey solutions were prepared immediately before inoculation to ensure that there was no decomposition and loss of hydrogen peroxide in the solutions used in the test.



Figure 2: Al Sidr honey

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Figure 3: Mountain honey

Agar diffusion method was applied, researchers declare that this is the most appropriate method for testing the antibacterial activity of natural products such as essential oils and honey, it offers several significant advantages, such as simplicity, low cost, the ability to test huge numbers of microorganisms, and ease of interpreting the results. (Bouacha *et al.*, 2023). 0.1 ml of activated bacteria equal to the turbidity of a McFarland 1.5 x 10^8 cell/ ml tube were spread onto the plates, which were then left to dry at room temperature for approximately 15 minutes. Five holes were then made using a sterile 6 mm cork drill. The holes were filled with the four prepared concentrations, and the fifth hole was filled with distilled water as a negative control (Ejaz *et al*., 2023) The plates were then incubated at 37°C for 24 hours. Three replicates of the test were performed. The diameter of the inhibition zone was then measured with a ruler. The results were interpreted based on common criteria used in the initial screening of natural substances and extracts. A substance or extract that produced an inhibition zone diameter of 12 mm was considered ineffective, while a diameter of 6-12 mm was considered moderately effective, and a diameter exceeding 16 mm was considered effective (Nader & Azhar, 2010).

Results and Discussion

Agar diffusion well method using two types of natural honey: Al Sidr honey and Mountain honey were performed on *E. coli*, r reprehensive of Gram-negative bacteria, and *Staphylococcus aureus*, reprehensive of Gram-positive bacteria., and the honey showed good effectiveness in inhibiting the growth of the bacterial isolates used in the test. Al Sidr honey has shown an excellent effect on *E. coli*, the diameter of inhibition zone of concentrated Al sider honey was 45 mm while for diluted honey it was 36 mm. Concentrated Mountain honey on *E. coli* was 42 mm, while for diluted honey it was 36 mm. as shown in (figure 4)

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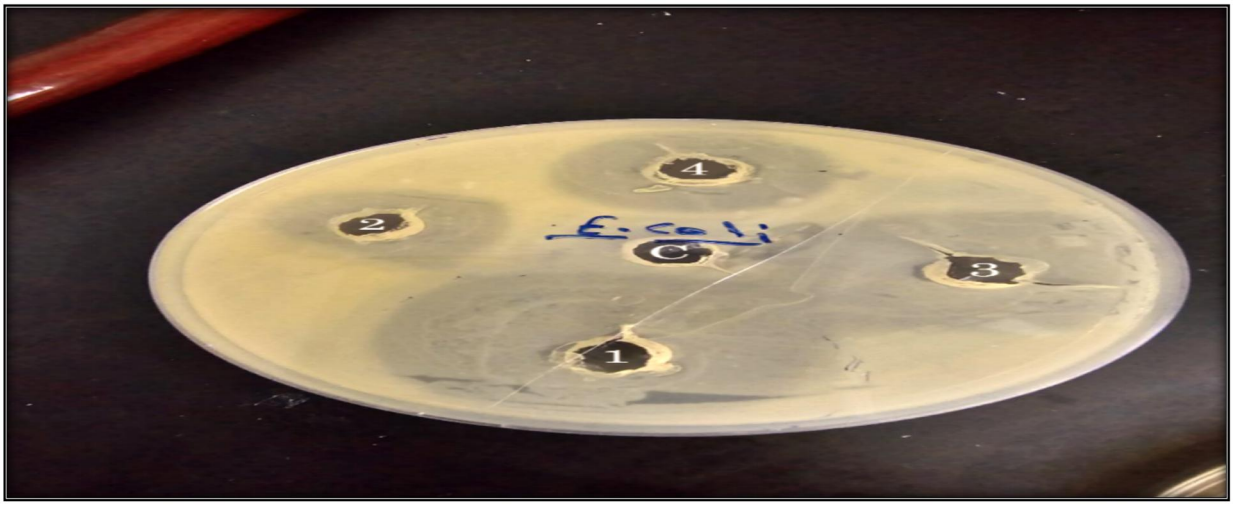

Figure 4: The inhibitory effect of Al Sidr honey and Mountain honey on *E. coli* ,1= concentrated Al Sider, 2= diluted Sider, 3= concentrated Mountain, 4= diluted Mountain c= Control

Staphylococcus aureus also affected by sider honey with 50 mm diameter of inhibition zone for concentrated sider honey and 37mm for the diluted, Mountain honey was effected on *Staphylococcus aureus*, as shown in (figure 5).



Figure 5: The inhibitory effect of Al Sidr honey and Mountain honey on *Staphylococcus aureus* 1=concentrated Sider, 2=diluted Al Sider, 3=concentrated Mountain, 4=diluted Mountain C=control

Concentrated and diluted Mountain honey on *Staphylococcus aureus* was 25 mm and 42 mm, respectively as shown in (table 1). The diameter of the inhibition zone indicates that honey has an excellent antibacterial effect. According to the SPSS test, there are no significant differences at the probability level of 0.05.

Table (1): Average diameters of inhibition halos resulting from the effect of Sidr honey and mountain honey on *E. coli* and *Staphylococcus aureus*

S.aureus	*E.coli*	Concentration	Honey type
50 mm	45 mm	100%	Concentrated sider
37 mm	30 mm	75%.	Diluted sider
25 mm	42mm	100%.	Concentrated mountain
42 mm	36mm	75%.	Diluted mountain

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The findings revealed that honey had a pronounced antibacterial effect against both Gram-positive and Gram-negative bacteria, these results were consistent with the findings of a number of researchers Desoky & Hamouda, 2023; Koohsari & Yazdani, 2025; Sundari *et al*., 2025; Wadi, 2022).

The effectiveness of honey is attributed to its content of many compounds such as sugars, amino acids, phenols, flavonoids, minerals, vitamins, and antioxidants (Alshammari, *et al*., 2023; Eteraf-Oskouei & Najafi, 2013; Tafere, 2021). The hydrogen peroxide in honey also plays a major role in its antibacterial activity, as it breaks down bacterial DNA and affects the bacteria's sensitivity to oxidative stress, its growth stages, and its survival strategy (for spore-forming species) (Brudzynski *et al*., 2011). Honey has been used since ancient times to treat and prevent infections; therefore, the active ingredients in honey must be in high concentrations because its use in combating infections must be fast-acting(Kwakman *et al*., 2011). In a previous study on the effect of pure honey on bacterial growth, researchers found that all bacteria exposed to high concentrations of honey (50% and above) were unable to grow. The mechanism was explained as follows (Jeddar *et al*., 1985):

1- Cell shrinkage and disturbance due to the osmotic effect of honey
2- Low acidity
3- Presence of other unspecified antibacterial substances in honey

In this study, Al Sidr honey appeared to be more effective at higher concentrations against *Escherichia coli* and *Staphylococcus aureus*. This can be explained by the fact that the antibacterial activity of honey involves several biological factors that affect it in different ways on bacterial growth, particularly non-peroxide compounds such as phenolic compounds and antioxidants, is known to disrupt bacterial cell membranes and interfere with their vital functions on a dose-dependent basis. Studies have shown that these phenolic compounds can possess antibacterial activity even in the absence of hydrogen peroxide, and contribute significantly to inhibiting the growth of *E. coli* at high concentrations of honey(S. Almasaudi, 2021; Al-Sayaghi *et al*., 2022) . In contrast, mountain honey was noticed to be more effective at lower concentrations, while its efficiency decreased or it showed smaller inhibition zones at higher concentrations, particularly against *S. aureus.* This is explained by the fact that many traditional honey varieties rely heavily on production Hydrogen peroxide is produced by the enzyme glucose oxidase extant in honey. Upon dilution, this enzyme is activated to produce H_2O_2, a potent antibacterial agent (Brudzynski, 2023). This substance readily diffuses throughout the agar medium, showing a greater inhibitory effect on bacterial growth. The concentration of honey increases to high levels The high viscosity and low free water content reduce the diffusion of active agents (including H_2O_2) in the medium, thus decreasing their apparent effectiveness in laboratory tests such as agar diffusion method (Mandal & Mandal, 2011).

Furthermore, bacterial structural and physiological differences play an important role, as Crack-negative *E. coli* possesses an additional outer membrane rich in glycolipids, which forms a barrier to the entry of chemicals. Therefore, it is highly susceptible to high osmotic pressure and membrane-attacking compounds at high concentrations (Almasaudi *et al*., 2017).

Gram-positive bacteria lack an outer membrane but have a thick cell wall of peptidoglycan. This makes them less sensitive to pure osmotic pressure but more susceptible to oxidizing agents and enzymes that can diffuse better in dilute solutions (Kwakman *et al*., 2010).

In short, the composition differs between Sidr honey and mountain honey, and this can be explained by the different molecular structure of each type of Al Sidr honey and Mountain honey:

1- Al Sidr honey exerts its effects gradually at high concentrations across a wide range of biological compounds.
2- Mountain honey relies more heavily on peroxide mechanisms that are controlled upon dilution, resulting in higher apparent efficiency at lower concentrations in in vitro diffusion studies.

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In general, the mechanisms of antibacterial activity in different types of honey are very complex and vary depending on the type of bacteria (Kwakman *et al*., 2012).

Conclusion

Honey is a natural sweetener and a complex substance rich in various nutrients that have a positive effect on human health. Al sidr honey and mountain honey showed good ability to inhibit the growth of Escherichia coli (a Gram-negative bacterium) and Staphylococcus aureus (a Gram-positive bacterium) at both high and low concentrations, with no statistically significant differences between the two types. This opens the door for deeper studies that promote the use of honey as a natural alternative to chemical treatments.

Acknowledgement

The researchers express their thanks and gratitude to the laboratory staff and the administration of Al-Sadr Teaching Hospital in Najaf for their assistance in obtaining the bacterial isolates used in the study.

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