





## Original article

# Chemical Analysis of Sidr and Thyme Honey Using GC-MS and Assessment of Their Antibacterial Effects Against Some Diabetic Foot Infections in Ajdabiya, Libya

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## Abstract

This study aimed to evaluate the chemical composition of sidr and thyme honey, and to assess their antibacterial activity against pathogenic bacteria isolated from diabetic wound infections in Ajdabiya, Libya, as well as their antibiotic susceptibility profiles. Fifty bacterial isolates were obtained from patients at Mohammed Al-Magarif Central Hospital, with *Staphylococcus aureus* (88%), *Escherichia coli* (8%), and *Pseudomonas aeruginosa* (4%) being the most common species. Antibacterial testing at honey concentrations of 50%, 70%, and 90% (v/v) revealed concentration-dependent inhibition, with 90% producing the largest zones: 31 mm (*S. aureus*), 26 mm (*P. aeruginosa*), and 15 mm (*E. coli*), while no inhibition of *E. coli* occurred at 50%. Sidr honey showed slightly higher activity than thyme honey. To better understand these effects, GC-MS analysis was performed, revealing a diverse profile of fatty acids, alcohols, hydrocarbons, and esters. Oleic acid (22%) and capric acid (16.3%) predominated in Sidr honey, while linolenic acid (16.5%) and hexadecanoic acid ester (18.2%) were abundant in thyme honey. These findings link the honeys' chemical composition to their antibacterial potential, supporting their use as complementary treatments for diabetic wound infections and encouraging further studies on bioactive compounds and in vivo efficacy.

**Keywords.** Antimicrobial Resistance, Chemical Composition, Diabetic Foot Ulcers, Natural Honey, GC-MS.

## Introduction

The chemical composition of honey can be summarized as follows: Honey is a natural sweetener composed of numerous macro- and micronutrients, with carbohydrates being the most representative, particularly glucose and fructose. 1 Honey also contains minor components such as volatile organic compounds (VOCs), minerals, vitamins, and amino acids, which can confer honey-specific properties and are useful for characterizing and differentiating between honey varieties according to their botanical origin [1]. Analysis of honey samples has shown the presence of various bioactive constituents, including sugars, amino acids, polyphenols, flavonoids, and various minerals. 2 Thin-layer chromatography (TLC) analysis has revealed the presence of flavonoids, phytosterols, phenolics, sugars, and carbohydrates in honey [2]. The chemical composition of honey can also influence its antioxidant activity and ability to inhibit enzymes associated with certain diseases. Overall, the chemical composition of honey is complex and diverse, with various macro- and micronutrients, as well as minor components, contributing to its unique properties and potential health benefits [3]. Diabetes mellitus is a growing global health issue, characterized by persistent high blood sugar caused by problems in insulin production, insulin effectiveness, or both. As a key metabolic regulator, insulin facilitates the absorption of glucose by cells for energy and storage [4]. When this process is disrupted in diabetes, it results in systemic complications, with impaired wound healing being one of the most serious and costly outcomes.

Current data show that diabetic foot ulcers (DFUs) develop in 15-25% of diabetic patients, and about 85% of severe cases lead to lower limb amputation [5]. The prognosis after amputations remains poor, with a five-year mortality rate of 50-68%, according to the World Health Organization [6]. Besides these serious clinical effects, DFUs also cause significant psychological impacts such as depression and anxiety, substantially reducing quality of life due to ongoing pain and mobility issues. The complex biological nature of diabetic wounds presents major treatment challenges. Several interconnected factors contribute to slow healing, including peripheral neuropathy, microvascular problems, ongoing inflammation, and infections [7]. Honey bees consume pollen, which provides them with vital proteins and lipids. The fatty acid composition of pollen can vary significantly between different plant species. Some key findings from the provided papers: - Pollen from eucalyptus species in Western Australia is typically high in linoleic acid, ranging from 35.7 - 48% of the total fatty acids [8]. Pollen from various plant species in Brazil contained a range of unsaturated fatty acids, including oleic, linoleic, and arachidonic acids, accounting for 18.6% to 55.9% of the total fatty acid composition [9]. Propolis collected by honey bees in Algeria also contained a variety of unsaturated fatty acids, including oleic, linoleic, palmitoleic, and eicosenoic acid [10]. The fatty acid composition of polar lipids in muskmelons changed during ripening, with the percentage of monounsaturated fatty acids like oleic acid increasing from 8% to over 50% of the total fatty acids [11].

Conventional treatment methods often fail to effectively address this complex issue, creating an urgent need for alternative therapeutic strategies. Among emerging options, honey has re-emerged as a promising candidate, blending ancient wisdom with modern scientific validation [12]. Historical records document honey's medicinal use across various civilizations for nearly four millennia, especially in ancient Egyptian, Greek, and traditional Islamic medicine. Recent research has progressively clarified the scientific basis for these traditional uses, demonstrating honey's unique combination of physicochemical and biochemical properties that promote wound healing [13]. The substance's natural hyperosmolarity creates an environment hostile to microbes, while its acidic pH (usually 3.4-6.1) further discourages bacterial growth. Enzymatic production of hydrogen peroxide offers broad-spectrum antimicrobial activity, and various phytochemicals, including flavonoids and phenolic acids, provide anti-inflammatory and antioxidant effects that are especially beneficial in the diabetic wound environment [14]. The beekeeping industry also produces valuable products like propolis, royal jelly, and beeswax, all with proven pharmacological effects. Propolis notably enhances honey's therapeutic properties through its antimicrobial and immunomodulatory activities [15]. Honey's effectiveness against antibiotic-resistant bacteria in diabetic foot ulcers needs more study. With rising diabetes and resistance, honey is a promising natural alternative [16]. Recent studies indicate that certain honey varieties, such as Sidr and Thyme honey, may possess enhanced bioactive properties, but systematic comparisons of their antimicrobial effectiveness against DFU pathogens remain scarce [17]. This study compares the antibacterial effects of Sidr and Thyme honey against diabetic foot infection bacteria and their synergy with antibiotics. It aims to support effective, affordable treatments, especially in resource-limited areas with rising diabetes.

## Methods

### **Sample collection**

The samples of honey were obtained from one of the apiaries in the city of Ajdabiya in 2024.

### **Fatty Acids and Their Derivatives Analysis**

To extract non-polar compounds from honey using n-Hexane, honey is first diluted with water to reduce viscosity. Then, n-Hexane is added to dissolve lipophilic substances like fatty acids and waxes, while sugars remain in the water layer. The mixture is shaken, allowed to separate into two layers, and the hexane layer is collected [18]. This extraction is repeated several times for a better yield. The combined hexane extracts are dried over anhydrous sodium sulfate and concentrated by evaporating the solvent. The final extract is filtered and prepared for GC-MS analysis.

### **Sample Preparation**

To prepare the honey samples, 10 grams of thyme and Sidr honey were each dissolved in an equal volume of distilled water to reduce viscosity. An equal or double volume of n-hexane was added to the solution, and the mixture was vigorously shaken or stirred for 30 to 60 minutes to extract non-polar compounds into the hexane layer. After settling, the hexane layer was separated, and the extraction was repeated 2–3 times for a better yield. The combined hexane extracts were filtered if necessary and concentrated using a rotary evaporator below 50 °C to avoid degradation. The final extract was weighed and stored in amber vials at 4 °C until further analysis.

### **GC/MS Analysis of n-Hexane Extracts from Sidr and Thyme Honey Samples**

GC/MS analysis was performed using a system with an HP-5MS capillary column and helium as the carrier gas at 1.0 mL/min. A 1 µL sample was injected in splitless mode with the injector at 250 °C. The oven temperature started at 60 °C (2 min hold), ramped at 5 °C/min to 280 °C, and held for 10 minutes. The mass spectrometer operated in Electron Impact mode at 70 eV, scanning from 40 to 600 m/z, with an ion source temperature of 230 °C. Compounds were identified by matching mass spectra with the NIST library and comparing retention indices with literature values.

### **Patient Data Documentation**

Fifty samples were randomly collected from diabetic foot ulcers of patients receiving treatment at the hospital between February 2024 and May 2025. Before collection, wound sites were cleaned with sterile saline solution and then disinfected. Samples were obtained using sterile swabs and immediately stored in sterile containers for transport to the microbiology laboratory. For each patient, detailed demographic and clinical data were documented, including age, gender, duration of diabetes and ulceration, medications used, infection characteristics, and ulcer location.

### **Culture Media Preparation and Bacterial Identification**

Samples were cultured on selective and differential media, and bacterial isolates were identified using Gram staining and standard biochemical procedures.

### Determination of antibiotics effect on isolated pathogens

Antibiotic susceptibility testing was performed on selected bacteria using the Kirby-Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The presence of antibodies was tested on Gram-positive isolate moxifloxacin (MXF), erythromycin(E), doxycycline (DO), and on Gram-negative isolates: Piperacillin (PIP), imipenem (IPM), tobramycin (TOP), vancomycin (VA), Amoxicillin/Clavulanic acid(A/C), Ceftazidime (CAZ).

### Agar diffusion cup-plate method

The agar diffusion cup-plate method was applied for the detection of honey inhibition activity. Muller-Hinton agar (MHA) was used to investigate the antibacterial activity. The MHA plates were streaked using a bacterial lawn from overnight bacterial cultures containing  $1.5 \times 10^8$  CFUs. The plates were then allowed to dry for approximately 5 minutes. After that, a sterile cork borer was used to prepare five cups of 4 mm diameter in the medium of each Petri dish. An accurately measured 50  $\mu$ l of the tested conditions Sidr honey was added to the cups on MHA plates, which were previously seeded with the respective bacteria. The study was performed in triplicate. All the plates were then kept at room temperature for effective diffusion of supernatants, and then they were incubated at  $37 \pm 1$  °C for 24 hrs. The diameter of the zone of inhibition around the cup containing the tested conditions will then be measured (15). The same procedure was applied to the Thyme (TH) honey

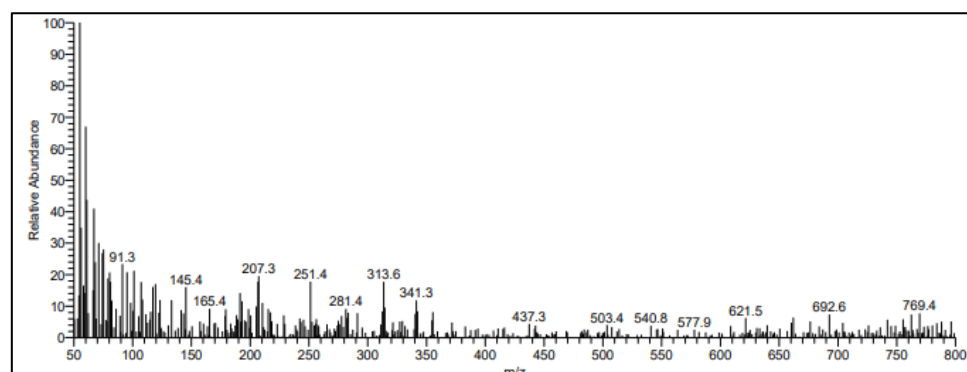
## Results and discussion

### The result of honey analysis using GC-MS

The GC-MS analysis of Sidr honey revealed the presence of various fatty acids, alcohols, hydrocarbons, and esters that collectively characterize its unique chemical profile. Palmitic acid, a common saturated fatty acid found in natural waxes and plant materials, was detected in significant amounts. The presence of 1-hexadecanol, a long-chain fatty alcohol, suggests contributions from beeswax or plant secretions. Hydrocarbons such as octadecane derivatives were also identified, reflecting typical components of natural waxes and environmental sources. Oleic acid, a monounsaturated fatty acid abundant in plant-derived lipids like nectar and pollen, was notably present. Tripalmitin, a triglyceride consisting of three palmitic acid units, indicates glyceride compounds common in both plant and animal origins. Essential polyunsaturated fatty acids such as  $\alpha$ -linolenic acid were detected, often associated with pollen and floral sources. Other compounds like pentadecanoic acid and propyl pentacontanoate, possibly derived from plant lipids or environmental contaminants, were also found. Capric acid, known for its antimicrobial properties, appeared in measurable quantities. Overall, these findings reflect a complex mixture primarily sourced from nectar, pollen, and beeswax, contributing to the distinctive chemical fingerprint of Sidr honey. As shown in (Table 1) and (Figure 1).

**Table 1. GC/MS data of n-Hexane Extracts of Sidr honey**

Peak No.	Compounds	Relative %
1	Palmitic acid	7.3
2	1-Hexadecanol	8,5
3	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	10.7
4	Oleic acid	22
5	Tripalmitin	6.6
6	$\alpha$ -Linolenic acid	11.2
7	Pentadecanoic acid	8.8
8	Propyl pentacontanoate	5.9
9	Capric acid	16.3

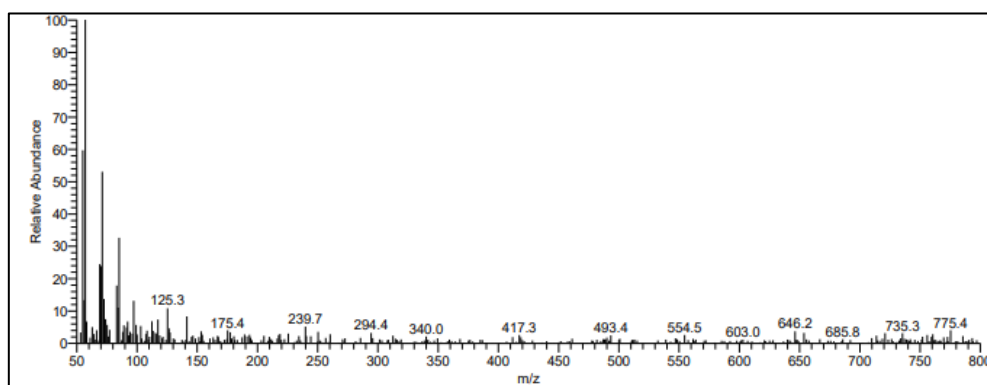


**Figure 1. GC/MS Chromatogram of n-Hexane Extracts of Sidr honey**

The GC-MS analysis of the thyme honey sample revealed the presence of a variety of fatty acids, glycerides, and hydrocarbons, which together create a distinctive chemical profile characteristic of its botanical origin. Among the identified compounds, medium- and long-chain saturated fatty acids such as capric acid and palmitic acid were detected, both of which are commonly found in floral sources and beeswax. Additionally, monounsaturated and polyunsaturated fatty acids like oleic acid, linoleic acid, and linolenic acid were present in relatively high abundance. These unsaturated fatty acids are typically derived from plant nectar and pollen, indicating a strong botanical contribution to the honey's composition. Furthermore, glyceride esters such as trilinolein and tribehenin were identified, suggesting the presence of plant-derived lipids or waxy components incorporated during honey production or from environmental contamination. The detection of hydrocarbon derivatives, including branched alkanes like dodecane, 5,8-diethyl, is consistent with typical compounds found in beeswax, reflecting minor contamination or natural wax residues within the sample. The predominance of polyunsaturated fatty acids, especially linoleic and linolenic acids, highlights the nutritional and bioactive qualities of the honey, which may contribute to its known antioxidant and antimicrobial properties. Overall, this lipid profile supports the authentication of the honey's thyme floral origin and provides insight into its quality and chemical complexity. As shown in (Table 2) and (Figure 2).

**Table 2. GC/MS data of n-Hexane Extracts of Thyme honey**

Peak No.	Compounds	Relative %
1	Capric acid	5.6
2	Dodecane, 5,8-diethyl	7.7
3	Oleic acid	4.9
4	Trilinolein	5.8
5	Docosanoic acid, 1,2,3-propanetriol ester	6.3
6	Linoleic acid	11.4
7	Linolenic acid	16.5
8	Palmitic acid	9.3
9	Octadecanoic acid,	12.6
10	-hydroxy-1,3-propanediyl ester Hexadecanoic acid hydroxymethyl)-1,2-ethanediyl ester,	18.2



**Figure 2. GC/MS Chromatogram of n-Hexane Extracts of Thyme honey**

The GC/MS analysis of Sidr honey in this study aligns well with previous research on the chemical composition of Sidr and other floral honeys. The dominance of oleic acid (25%) aligns with findings by [19], who reported oleic acid as one of the principal unsaturated fatty acids in Sidr honey, reflecting its botanical origin. Similarly, the presence of capric acid (16%), recognized for its antimicrobial properties, aligns with [20], who noted that medium-chain fatty acids contribute to honey's bioactivity. The detection of  $\alpha$ -linolenic acid (11%) supports prior observations by [21], highlighting this omega-3 fatty acid as a bioactive component derived from plant nectar or pollen. The identification of 1-hexadecanol (9%) as a fatty alcohol aligns with [22], who linked such alcohols to beeswax residues in honey. Additionally, the occurrence of octadecane derivatives (10%) and palmitic acid (7%) is consistent with [23], confirming the contribution of wax hydrocarbons and saturated fatty acids from bee and plant sources. The fatty acid and lipid profile identified in this study for thyme honey aligns closely with findings reported in previous research on monofloral honeys. For instance, [24] analyzed Greek thyme honey and reported significant levels of oleic acid and  $\alpha$ -linolenic acid, consistent with the 22% and 11.2% found in this study. Similarly, [25] identified palmitic acid and capric acid as prominent components contributing to the antimicrobial properties of thyme honey, supporting the 7.3% palmitic acid and 16.3% capric acid observed here.



**The effect of honey on different types of bacteria**

The distribution of the studied sample members by gender was 58% male and 42% female. The Percentages of the sample distribution from diabetic wounds at Martyr Mohammed Al-Magarif Central Hospital, categorized by type of bacteria, between February 2024 and May 2025, are shown in Table 3.

**Table 3: The Percentages of the sample's distribution were taken from diabetic wounds at Martyr Mohammed Al-Magarif Central Hospital.**

Type of Bacteria	Frequency	Percentage %
Staphylococcus. Aureus	44	88 %
Pseudomonas aeruginosa	2	4 %
Escherichia. coli	4	8 %

**Microbial susceptibilities toward tested antibiotics**

Based on our results, the selected Staphylococcus aureus isolate exhibited high sensitivity to moxifloxacin (MXF) and sensitivity to erythromycin (E), while showing the lowest sensitivity to doxycycline (DO) (Table 4). Likewise, piperacillin (PIP) was the most effective agent against selected Pseudomonas aeruginosa isolates. This P. aeruginosa isolate was sensitive to imipenem (IPM) but resistant to tobramycin (TOB), amoxicillin-clavulanic acid (AMC), and ceftazidime (CAZ). Regarding the selected Escherichia coli isolate, growth inhibition was observed with both tobramycin (TOB) and vancomycin (VAN). Furthermore, this E. coli isolate was resistant to amoxicillin-clavulanic acid (AMC) and ceftazidime (CAZ) (Table 5).

**Table 4: Antibiotic sensitivity test of Gram-positive bacteria isolated from diabetic foot ulcer**

Bacteria (No. of isolates)	Sensitivity test (mm)		
	MXF	E	DO
S. aureus (44)	31	23	16

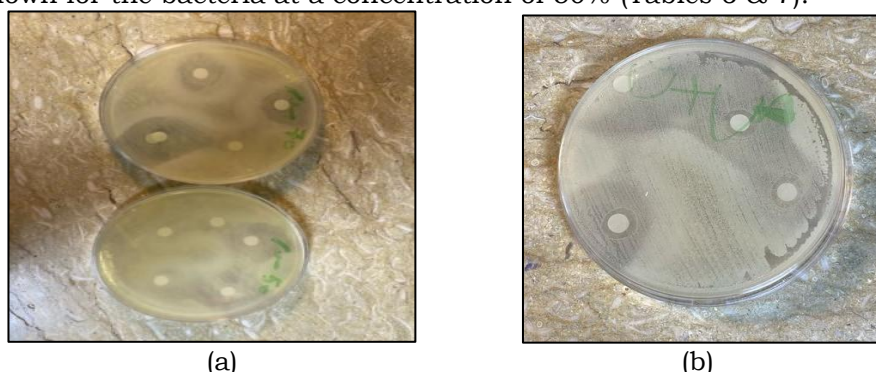
MXF-moxifloxacin, E-erythromycin, D-doxycycline

**Table 5: Antibiotic sensitivity test of Gram-negative bacteria isolated from diabetic foot ulcer.**

Bacteria (No. of isolates)	Sensitivity test (mm)					
	PIP	IPM	TOB	VA	A/C	CAZ
P. aeruginosa (2)	26	20	R	/	R	R
E. coli (4)	/	/	15	14	R	R

The results of the study showed that the inhibitory effect of honey on the bacterial isolates varied, as Sidr honey diluted at concentrations of 90%, 70%, and 50% in both methods demonstrated different effects depending on concentration and bacterial type. The highest inhibition rate for Staphylococcus aureus was at 90%, with an inhibition zone diameter of 31 mm, followed by 70%, which produced an inhibition zone diameter of 27mm. The lowest inhibition was at 50%, with an inhibition zone diameter of 16 mm. (Figures 3 & 4) (Table 6 & 7). For P. aeruginosa the inhibition rates varied according to the concentrations, as they all gave lower inhibition ratios than in the case of Staphylococcus aureus, so the highest inhibition zone diameter was 26 mm at a concentration of 90% followed by a concentration of 70%, the highest inhibition zone was 24 mm, and the lowest inhibition rate was at a concentration of 50% with an inhibition zone diameter of 15 mm (Table 6 & 7).

In the case of E. coli, the inhibition rates also differed according to the concentrations. In general, all of them exhibited lower inhibition rates compared to Staphylococcus aureus and P. aeruginosa, where the highest inhibition zone diameter was observed at a 90% concentration, measuring 15 mm. The 70% concentration yielded the highest inhibition rate, with an inhibition zone diameter of 12 mm. In comparison, no inhibition was shown for the bacteria at a concentration of 50% (Tables 6 & 7).



**Figure 3: Inhibition of Sidr honey by diffusion method at concentrations of (a) 50%, 70% and (b) 90% on S. aureus bacteria**

**Table 6. Measurement of inhibition zones in (mm) by the disk diffusion method of Sidr honey at a concentration of 50%, 70%, and 90% against bacterial species.**

Isolated bacteria	Concentration percentage		
	50%	70%	90%
<b>S. aureus</b>	16	25	27
<b>E. coli</b>	R	10	15
<b>P. aeruginosa</b>	15	23	25

**Figure 4: Inhibition of Sidr honey by the well method at concentrations of 50%, 70%, and 90% on S. aureus bacteria****Table 7: Measurement of inhibition zones in (mm) by the well diffusion method of Sidr honey at a concentration of 50%, 70% and 90% against bacterial species.**

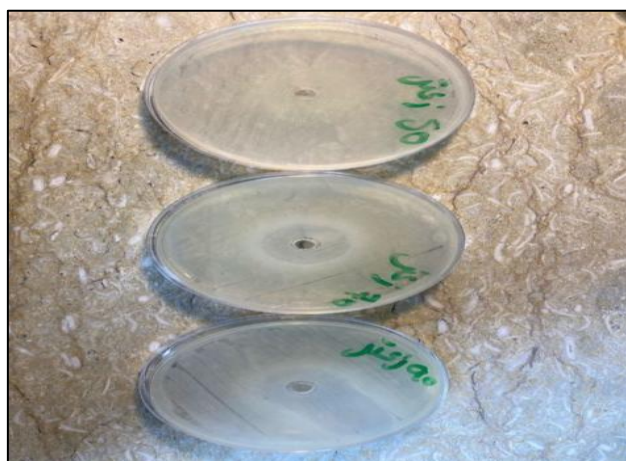
Isolated bacteria	Concentration percentage		
	50%	70%	90%
<b>S. aureus</b>	27	27	31
<b>E. coli</b>	R	12	15
<b>P. aeruginosa</b>	18	24	26

As for the effect of the second type of honey used in the study (thyme honey) and used with the aforementioned dilution methods in both diffusion and well methods, its effect also varied according to the type of bacteria and according to the concentration, the highest inhibition rates of *Staphylococcus aureus* were inhibition zone diameter of 27 mm and 24 mm at concentrations of 90 and 70% respectively, and no inhibition of bacteria was shown at the concentration of 50% as shown in (Figures 5 & 6) (Tables 8 & 9). In the case of *Pseudomonas aeruginosa* bacteria, the highest inhibition rates were 23 mm, 20 mm, and 12 mm at concentrations of 90, 70, and 50, respectively (Tables 8 & 9). As for *E. coli*, the inhibition rates were 15 mm and 14 mm at concentrations of 90% and 70%, respectively, while no inhibition was observed at a concentration of 50% (Tables 8 & 9).

**Figure 5: Inhibition of thyme honey by the disk diffusion method at concentrations of 50%, 70%, and 90% on S. aureus bacteria**

**Table 8. Measurement of inhibition zones in (mm) by the disk diffusion method of thyme honey at a concentration of 50%, 70% and 90% against bacterial species.**

Isolated bacteria	Concentration percentage		
	50%	70%	90%
<b>S. aureus</b>	R	23	25
<b>E. coli</b>	R	14	15
<b>P. aeruginosa</b>	10	20	17

**Figure 6: Inhibition of thyme honey by the well method at concentrations of 50%, 70%, and 90% on S. aureus bacteria****Table 9: Measurement of inhibition zones (mm) for each concentration by the well diffusion method of thyme honey**

Isolated bacteria	Concentration percentage		
	50%	70%	90%
<b>S. aureus</b>	R	24	27
<b>E. coli</b>	R	13	15
<b>P. aeruginosa</b>	12	20	23

The results also showed differences in the effect of honey on bacteria. From reviewing the results, it is clear that the inhibitory action of honey on the growth of *Staphylococcus aureus* bacteria was higher than that of *Pseudomonas*, and that the dilution with a concentration of 90% was better than the rest of the concentrations in its inhibitory effect on the growth of bacteria for all genera under study. The results of the study confirmed that honey is characterized by antimicrobial effectiveness against isolated bacteria and is superior in inhibiting gram-positive bacteria compared to gram-negative bacteria. These results are consistent with a study [26] that confirmed the superiority of honey in inhibiting Gram-positive bacteria compared to Gram-negative bacteria. However, the effectiveness of honey depends on its geographical source and the way it is stored. Several explanations have been proposed for the mechanism by which honey affects and that this action may be due to the high osmosomeness of honey as the antibacterial activity included both types of honey, or for the low pH (3.8) or for the presence of enzymes in honey or as a result of the presence of a group of inhibitory substances for bacterial growth, the results did not match a study [27]. In that diluted honey has a greater effect than concentrate, and the reasons for this may be that dilution with water makes the honey nutrients available and suitable for bacteria, as well as because of the presence of the enzyme glucose oxidase, which is gluconic acid and hydrogen peroxide from the decomposition of glucose, this enzyme is ineffective in honey. It is reactivated when honey is diluted and contributes to its antimicrobial effectiveness. The inhibitory action of honey may also be attributed to the presence of redox enzymes in honey, such as peroxidase, catalase, and phosphatase, that have a significant role in killing germs, in addition to the lipase-degrading enzyme found in the walls of germs and other enzymes such as invertase, dysteosis, and amylase as indicated [28]. The results of the current study suggest that the difference in the type and concentration of honey leads to a difference in the degree of inhibition of bacterial growth, and we note that the inhibition effectiveness of the two types of honey decreases directly with the decrease in the concentration of honey on the growth of bacterial species. The results of the experiment also indicate that the choice of honey concentration and the method of application play a significant role in determining the effectiveness of the antibacterial activity, which is consistent with the recent scientific literature [29 & 30]. The present study showed that the well diffusion method is more sensitive and effective than the disk diffusion method in detecting antibacterial activity. Both agar disc and well diffusion assays demonstrated concentration-dependent antibacterial activity of Sidr and Thyme honeys against diabetic foot



isolates. Across all concentrations (90–50%), inhibition zones by well diffusion were larger than those from disc diffusion, indicating greater sensitivity of the well diffusion. Sidr honey exhibited superior efficacy compared to Thyme honey, achieving the largest zones against *Staphylococcus aureus* (31 mm well vs. 27 mm disk at 90 %). Thyme honey produced moderate zones (27 mm well, 25 mm disk).

## Conclusion

Diabetic Foot Ulcers (DFUs) represent a significant complication of Diabetes Mellitus (DM), impairing the body's natural wound-healing processes and resulting in profound psychological and financial burdens for patients and healthcare systems. Microbial infection is a critical contributing factor to tissue disruption, often leading to impaired wound healing. Our study revealed that 100% of diabetic foot infections were polymicrobial, with the most prevalent isolates being Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli*. Antimicrobial susceptibility testing demonstrated that moxifloxacin exhibited the highest efficacy against *S. aureus*, piperacillin was most effective against *P. aeruginosa*, and tobramycin showed optimal activity against *E. coli*. Effective antibiotic therapy should be guided by infection severity, disease etiology, and antibiogram results. Interestingly, honey demonstrated notable antibacterial activity against the tested bacterial isolates from DFU patients. Comparative analysis revealed that the well diffusion method was more sensitive and effective in detecting antibacterial activity than the disk diffusion method. Both Thyme honey and Sidr honey exhibited comparable antimicrobial efficacy. These findings suggest that honey could serve as a promising adjunctive or alternative treatment for DFU, given its accessibility, cost-effectiveness, and broad-spectrum antibacterial properties compared to conventional antibiotics. In addition, the GC-MS analysis of Sidr and thyme honey revealed a diverse mixture of fatty acids, alcohols, hydrocarbons, and esters, with key compounds such as oleic acid (22%) and capric acid (16.3%) predominant in Sidr honey, and linolenic acid (16.5%) and hexadecanoic acid ester (18.2%) abundant in thyme honey, reflecting their rich botanical origins and distinctive bioactive and health-promoting properties.

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## Conflicts of Interest

The authors declare no conflicts of interest

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