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Original Article

Antibacterial Activity and Elemental Profiling of Sidr (*Ziziphus spina-christi*), Samar (*Acacia tortilis*), and Multifloral Jazan Honeys Against Methicillin-Resistant *Staphylococcus aureus*

Mohamed Eltaib Elmobark[1,*], Ahmad Salawi1, Abdullah Abdu Ali Hommdi[2], Nasser Khaled Mohammd Alhazmi[3], Mahdi Koriri[2], Alhussean Mohammed Sabei[2], Essa Mohammed Algathlan[2], Mohammed Abdullah Zakri[2], Turki Mohammed Kariri[2], Mohammed Ali Hamzi[2], Ahmed Mohammed Ibrahim Ogdi[2], Faisal Hassan Alami Hakami[2]

[1]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, SAUDI ARABIA.
[2]College of Nursing and Health Sciences, Jazan University Hospital, Jazan University, Ministry of Education, Jazan, SAUDI ARABIA.
[3]Medical Records Department, Jazan University Hospital, Jazan University, Ministry of Education, Jazan, SAUDI ARABIA.

ABSTRACT

Background: Antibiotic resistance is a major global health challenge, especially among multidrug-resistant pathogens such as Methicillin-Resistant *Staphylococcus aureus* (MRSA). Natural products, including honey, are increasingly investigated as alternative antimicrobial agents. This study evaluated the antibacterial activity of selected Saudi honeys and characterized their trace element composition. **Materials and Methods:** Three honey types-Sidr (*Ziziphus spina*-christi), Samar (*Acacia tortilis*), and Maraee (multifloral origin)-were collected from apisaries in the Jazan region, Saudi Arabia (*n*=3 each). Antibacterial activity against MRSA was assessed using agar well diffusion at 100%, 75%, and 50% (w/v). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by broth microdilution. Trace elements were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), followed by heatmap and principal component analysis. **Results:** All honey samples inhibited MRSA, producing inhibition zones of 19-34 mm. Sidr and Samar showed the strongest activity, with MIC values of 3.125% (v/v) and MBC values of 6.25% (v/v), while Maraee showed lower activity. ICP-MS revealed distinct elemental profiles among honey types, with all detected elements within international safety limits. **Conclusion:** Jazan honeys, particularly Sidr and Samar, demonstrate strong anti-MRSA activity and safe, distinct elemental profiles, supporting their potential as natural antimicrobial agents against resistant pathogens.

Keywords: Antibacterial activity, Honey, ICP-MS, Maraee honey, MRSA, Samar honey, Sidr honey, Trace elements.

Correspondence:
Dr. Mohamed Eltaib Elmobark
Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, SAUDI ARABIA.
Email: mohamedeltaib.me@gmail.com

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INTRODUCTION

Antibiotic resistance has emerged as a major global health challenge. The excessive and inappropriate use of antibiotics has contributed significantly to the development of resistance among pathogenic microorganisms. This problem is further exacerbated by the limited investment of pharmaceutical companies in the development of new antibiotics, largely due to high research and production costs combined with relatively low economic returns (Barlow and Nathwani, 2005; Gootz, 2010; Ventola, 2015). Consequently, antibiotic misuse has facilitated the emergence of new infections and the resurgence of previously controlled pathogens, leading to widespread outbreaks worldwide. Notable examples include *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Helicobacter pylori, Vibrio cholerae, Pseudomonas aeruginosa, Mycobacterium tuberculosis*, and *Salmonella* species, highlighting the urgent need for alternative therapeutic strategies (Abdallah, 2011; Abdallah and Hamed, 2019).

Since ancient times, honey has been recognized for its therapeutic properties, with historical evidence indicating that honey and other bee-derived products were widely used by ancient civilizations such as the Egyptians, Greeks, and Romans, particularly for wound treatment and gastrointestinal disorders (Eteraf-Oskouei and Najafi, 2013). Chemically, honey is a concentrated sugar solution consisting of approximately 82.4%

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carbohydrates (primarily fructose and glucose), 17.1% water, and about 0.5% minor bioactive components. These bioactive constituents include enzymes, vitamins, minerals, amino acids, phenolic compounds, flavonoids, carotenoids, ascorbic acid, α-tocopherol, and tannins (Rossi and Marrazzo, 2021). Although present in relatively small amounts, this bioactive fraction contributes significantly to honey's biological activities, including antioxidant, antimicrobial, antiparasitic, antidiabetic, and wound-healing properties, as well as protective effects on the gastrointestinal, cardiovascular, nervous, and respiratory systems (Cianciosi *et al.*, 2018).

Several studies have demonstrated both bacteriostatic and bactericidal effects of honey, highlighting its potential as a natural therapeutic agent against antibiotic-resistant pathogens (Chhawchharia *et al.*, 2022; Green *et al.*, 2022; Hewett and Crabtree, 2022; Patton *et al.*, 2006). Among the various honey types, Sidr honey, derived from the nectar of the Sidr tree (*Ziziphus spina*-christi) native to southern Saudi Arabia and Yemen, is particularly valued for its medicinal properties and high commercial importance. As a monofloral honey, its nectar originates predominantly from a single plant species, contributing to its distinctive chemical composition and bioactivity. Although Saudi Arabia produces a wide variety of honeys traditionally used in folk medicine, systematic scientific investigations evaluating and comparing their antibacterial efficacy-particularly against multidrug-resistant pathogens such as MRSA-remain limited. Furthermore, data integrating both antimicrobial activity and elemental composition for Saudi honeys are scarce. Therefore, this study aimed to evaluate and compare the *In vitro* antibacterial activity and trace element profiles of Sidr, Samar, and Maraee honey against methicillin-resistant *Staphylococcus aureus* (MRSA).

METHODOLOGY

Collection of Honey Samples

Three honey types commonly produced in the Jazan region, Saudi Arabia, were collected directly from local apiaries. The botanical origin of each honey type was determined based on the dominant floral source during the flowering season and confirmation provided by experienced local beekeepers. The analyzed samples included Sidr honey (*Ziziphus spina-christi* L.; SDR), Samar honey (*Acacia tortilis*; SMR), and Maraee honey (mixed floral origin; MAR). Freshly harvested honey samples were collected during the production season and transferred into pre-cleaned, sterile, airtight glass containers to prevent contamination and physicochemical alterations. Basic physicochemical parameters, including pH and color, were measured to characterize the samples prior to elemental analysis (Kubera *et al.*, 2022). To maintain sample integrity and minimize degradation or compositional changes, all honey samples were stored in the dark at controlled room temperature (25°C) until further laboratory analysis. Prior to analytical measurements, each sample was thoroughly homogenized to ensure representative subsampling and analytical reproducibility.

Microscopic Analysis of Pollen (Melissopalynology)

The botanical origin of the honey samples was verified through melissopalynological analysis (Kubera *et al.*, 2022). A small amount of each honey sample was diluted with distilled water and centrifuged to concentrate pollen grains. The resulting sediment was mounted on microscope slides and examined under a light microscope at 400× magnification. Pollen identification was carried out based on morphological characteristics and comparison with published pollen reference atlases.

Physicochemical Characterization

Basic physicochemical properties of the honey samples, including color, density, acidity (pH), and electrical conductivity, were evaluated using calibrated digital instruments (Elmeihy, 2025). Honey color was assessed visually, while pH was measured using a calibrated digital pH meter. Electrical conductivity was determined using a conductivity meter to estimate mineral content. In addition, a qualitative observation of viscosity behavior in aqueous medium was performed by adding a small amount of honey to a glass of distilled water to observe its settling characteristics.

Preparation of Honey Solutions

Each honey sample (SDR, SMR, and MAR) was diluted in sterile distilled water to obtain different concentrations (100%, 75%, and 50% w/v). The solutions were prepared under aseptic conditions to prevent microbial contamination. Fresh dilutions were prepared immediately prior to each experiment to maintain consistency and ensure experimental reliability.

Microorganism and Inoculum Preparation

Methicillin-resistant *Staphylococcus aureus* (MRSA) used in this study was obtained from a clinical microbiology laboratory. The bacterial strain was subcultured in nutrient broth and incubated at 37°C for 24 hr to obtain fresh cultures. The bacterial suspension was then adjusted spectrophotometrically to match the turbidity of a 0.5 McFarland standard, corresponding to approximately 1-2 × 10^8 CFU/mL, and subsequently diluted to obtain the required inoculum concentration for antimicrobial testing. All experiments were performed in triplicate, and results were expressed as mean±Standard Deviation (SD).

Antibacterial Susceptibility Testing

The antibacterial activity of the honey samples (SDR, SMR, and MAR) was evaluated using the agar well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Chhawchharia *et al.*, 2022; Maeda, 2008). Mueller-Hinton agar plates were inoculated with standardized

bacterial suspensions using sterile cotton swabs to achieve uniform bacterial growth. Wells with a diameter of 6 mm were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 µL of honey solutions at the designated concentrations. The plates were incubated at 37°C for 24 hr, after which the Zone of Inhibition (ZOI) surrounding each well was measured in millimeters using a digital caliper.

Minimum Inhibitory Concentration (MIC) Determination

The Minimum Inhibitory Concentration (MIC) of the honey samples was determined using the broth microdilution method (Mama, 2019; Wadi, 2019). Two-fold serial dilutions of honey were prepared in sterile broth to obtain concentrations ranging from 100% to 0.78% (v/v). Each dilution was inoculated with 100 µL of the standardized MRSA suspension and incubated at 37°C for 24 hr. Following incubation, bacterial growth was evaluated visually. The MIC was defined as the lowest concentration of honey that completely inhibited visible bacterial growth.

ICP-MS Analysis

Trace element concentrations in honey samples were determined using inductively coupled plasma mass spectrometry (ICP-MS) following microwave-assisted acid digestion (Leme *et al.*, 2014). Prior to analysis, honey samples were thoroughly homogenized to ensure representative subsampling. Approximately 0.5 g of each sample was accurately weighed into acid-washed Teflon digestion vessels and mixed with 5 mL of ultrapure nitric acid (HNO_3, 65%, trace metal grade). The digestion was performed using a closed-vessel microwave digestion system under controlled temperature and pressure conditions to ensure complete mineralization of the organic matrix. After digestion and cooling, the solutions were quantitatively transferred and diluted to a final volume of 50 mL with ultrapure deionized water (18.2 MΩ·cm). Elemental analysis was carried out using an ICP-MS instrument operated under optimized plasma conditions, and calibration curves were established using multi-element standard solutions covering the expected concentration ranges. To correct for instrumental drift and matrix effects, appropriate internal standards were applied during analysis. Method accuracy was evaluated using Certified Reference Materials (CRMs) and spike-recovery tests, with recoveries ranging between 90-110%, confirming analytical reliability. Quality assurance procedures included reagent blanks, calibration verification standards, and triplicate sample measurements. Instrument detection limits were calculated as three times the standard deviation of procedural blanks, and elemental concentrations were reported as mean values expressed in Parts Per Billion (ppb).

Statistical Analysis

Descriptive statistics were used to summarize the concentrations of trace elements in the honey samples and were expressed as mean±Standard Deviation (SD). Differences among honey types were evaluated using one-way Analysis of Variance (ANOVA) to determine statistically significant variations in elemental concentrations. Multivariate analysis was performed to explore relationships between samples and elements. A heatmap was generated to visualize the distribution patterns of trace elements among the honey types. In addition, Principal Component Analysis (PCA) was applied to identify the main elements responsible for sample differentiation and to assess similarities related to botanical origin. A significance level of $p<0.05$ was considered statistically significant.

RESULTS

Properties of Honey

Microscopic examination of pollen grains confirmed the botanical origin of the honey samples. Sidr Honey (SDR) contained a high proportion of *Ziziphus spina-christi* pollen, while Samar Honey (SMR) showed predominantly *Acacia tortilis* pollen, confirming their monofloral origins. In contrast, Maraee Honey (MAR) exhibited a mixed pollen spectrum from multiple plant species, indicating its multifloral nature. Physicochemical characterization revealed differences in color and pH among the samples. SDR showed a golden-brown color with a pH of 5.88, Samar honey appeared reddish-brown tending to black with a pH of 3.86, while Maraee exhibited a dark red color with a pH of 4.10. In the water observation test, all honey samples settled at the bottom without immediate dissolution, indicating high viscosity typical of natural honey.

Antibacterial Activity of Honey Samples Against MRSA

Zone of Inhibition (ZOI)

The antibacterial activity of the honey samples against *Methicillin-resistant Staphylococcus aureus* (MRSA) was evaluated using the agar well diffusion assay. The mean Zone of Inhibition (ZOI) values produced by the three Saudi honey samples at different concentrations ranged from 19 to 34 mm, as illustrated in Figure 1. At the highest concentration (100% w/v), SDR exhibited the strongest antibacterial activity with a mean ZOI of 34 mm, followed closely by SMR with 33 mm, while Maraee exhibited a lower inhibition zone of 29 mm. A reduction in antibacterial activity was observed as the honey concentration decreased. At 75% (w/v), the inhibition zones were 31 mm (SDR), 30 mm (SMR), and 27 mm (MAR). Further reduction in activity was observed at 50% (w/v), where ZOI values decreased to 24 mm, 22 mm, and 19 mm for SDR, SMR, and MAR, respectively. Overall, SDR and SMR honey samples demonstrated stronger antibacterial activity against MRSA compared with Maraee honey, indicating differences in antimicrobial potency among the tested honey types.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) values of the honey samples against MRSA were determined using the broth microdilution method (Table 1). The MIC was defined as the lowest concentration of honey capable of completely inhibiting visible bacterial growth after incubation. As presented in Table 1, both SDR and SMR exhibited identical MIC values of 3.125% (v/v), indicating strong inhibitory activity against MRSA. In contrast, Maraee showed a higher MIC value of 6.25% (v/v), suggesting comparatively lower antibacterial potency. These results indicate that monofloral honey types (SDR and SMR) possess greater inhibitory activity against MRSA than the multifloral Maraee honey sample.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by evaluating bacterial growth following exposure to increasing honey dilutions (Table 1). The MBC represents the lowest concentration of honey capable of producing complete bacterial killing. As shown in Table 1, both SDR and SMR demonstrated an MBC value of 6.25% (v/v) against MRSA. In contrast, Maraee required a higher concentration of 12.5% (v/v) to achieve bactericidal activity. The results indicate that SDR and SMR honey samples possess greater bactericidal efficacy compared with Maraee honey. This enhanced antimicrobial effect may be associated with differences in botanical origin, phytochemical composition, and bioactive compounds present in the monofloral honey types. Significant differences in MIC and MBC values were observed among the honey samples ($p<0.05$).

Elemental Composition of Honey Samples

The concentrations of trace and heavy metals in the three honey samples-SDR, SMR, and Maraee -were determined using ICP-MS and expressed in ppb or µg/L. Significant variation in elemental composition was observed among the samples (Table 2). Aluminum (Al) concentrations ranged from 91.3 to 980 ppb, with the highest level in SDR and the lowest in Maraee. Cadmium (Cd) varied between 5.13 and 20.3 ppb, with the highest concentration in SMR. Copper (Cu) and manganese (Mn) showed marked variability, reaching 1300 ppb and 2450 ppb, respectively, in SMR, while the lowest levels were detected in Maraee. Lead (Pb) concentrations ranged from 0.0117 to 26.2 ppb, with the lowest value in SMR and the highest in Maraee. Selenium (Se) ranged from 316 to 490 ppb, whereas zinc (Zn) exhibited the highest overall concentrations, particularly in SDR (7340 ppb). In contrast, arsenic (As), beryllium (Be), chromium (Cr), and antimony (Sb) were not detected, indicating levels below the instrument detection limits. These findings highlight distinct elemental profiles among the analyzed honey samples (Table 2).

Note: Values are expressed as mean±Standard Deviation (SD). SD values represent 3-9% of the mean based on replicate measurements. <LOD=below limit of detection; NA=not analyzed. All detected element concentrations were below the maximum permissible limits established by WHO, FAO, and

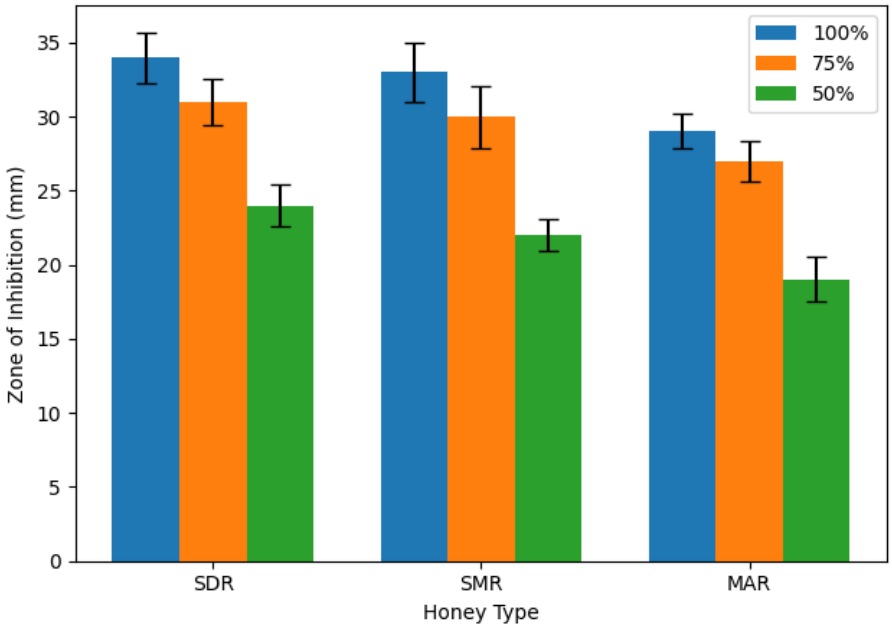


Figure 1: Antibacterial activity of Sidr Honey (*Ziziphus spina-christi* L.; SDR), Samar honey (*Acacia tortilis*; SMR), and Maraee honey (multifloral origin; MAR) against *Methicillin-resistant Staphylococcus aureus* (MRSA) at different concentrations. Bars represent the mean Zone of Inhibition (ZOI) obtained from replicate experiments, while error bars indicate the Standard Deviation (SD). The inhibition zones decreased with decreasing honey concentration, with SDR and SMR showing greater antibacterial activity than MAR.

Table 1: Antibacterial activity of Saudi honey samples against MRSA.

Pathogen	Bacterial concentration (CFU/mL)	Honey type	MIC (% v/v)	MBC (% v/v)
MRSA	2×10^4	SDR	3.125±0.81	6.25±1.10
MRSA	2×10^4	SMR	3.125±0.65	6.25±0.90
MRSA	2×10^4	MAR	6.25±0.34*	12.5±1.30*

Abbreviations: SDR=Sidr Honey (*Ziziphus spina*-christi L.); SMR=Samar honey (*Acacia tortilis*); Maraee honey (multifloral origin); MIC=Minimum inhibitory concentration; MBC=Minimum bactericidal concentration. Different superscript letters indicate statistically significant differences ($p<0.05$).

EU guidelines. Different superscript letters indicate statistically significant differences ($p<0.05$).

Heatmap

The heatmap (Figure 2) illustrates the distribution of trace element concentrations across the three honey types analyzed by ICP-MS. Color intensity represents the relative abundance of each element. Sidr Honey (SDR) is characterized by a notably high concentration of Zn, whereas Samar Honey (SMR) exhibits elevated levels of Cu and Mn. In contrast, Maraee Honey (MAR) shows relatively higher concentrations of Ba and Se. Overall, the heatmap reveals distinct elemental patterns among the honey samples, reflecting differences associated with botanical origin and environmental conditions.

Chemometric Analysis

Figure 3 presents the PCA loading plot illustrating the contribution of individual trace elements to the principal components derived from the ICP-MS dataset. Elements positioned farther from the origin exert a greater influence on sample differentiation. Zn and Al exhibit strong positive loadings on PC1, indicating their dominant contribution to the elemental profile of Sidr Honey (SDR). Cu and Mn also show positive loadings, reflecting their association with Samar Honey (SMR). In contrast, Ba, Pb, and Se are located on the negative side of PC1, suggesting a stronger relationship with Maraee Honey (MAR). Overall, the loading plot identifies the key elements driving the separation of honey samples according to their botanical and environmental origins.

DISCUSSION

Honey has long been recognized for its diverse therapeutic properties, including antibacterial, antiviral, anti-inflammatory, antioxidant, and anti-obesity activities (Albaridi, 2019; El-Seedi, 2024; Nowar, 2023). In this study, Sidr, Samar and Maraee honeys demonstrated pronounced antibacterial activity against MRSA, with inhibition zones ranging from 19 to 34 mm. These findings are consistent with previous reports highlighting the antimicrobial potential of honey against pathogenic bacteria. For example, Halawani and Shohayeb reported inhibition zones of 22.2-32.0 mm against *Salmonella enteritidis*, which are comparable to the values observed in the present study (Halawani and Shohayeb, 2011). Notably, Sidr and Samar honey exhibited slightly greater antibacterial activity than Maraee honey, particularly at higher concentrations, suggesting that botanical origin and regional factors play a significant role in determining antimicrobial efficacy (El-Meihy *et al.*, 2025).

The MIC and MBC results further support the strong antibacterial potential of the tested honeys. Sidr and Samar honey demonstrated MIC values of 3.125% (v/v) and MBC values of 6.25% (v/v), whereas Maraee honey showed comparatively lower activity (MIC 6.25% and MBC 12.5% v/v). These findings align with previous studies reporting inhibition zones of 22-39 mm and MIC values ranging from 3% to 37% for honey against MRSA and other pathogens (Alqurashi, 2013; El-Meihy *et al.*, 2025; Mama, 2019; Wadi, 2019). Interestingly, the MIC values observed for Sidr and Samar honey were lower than those reported for Manuka honey (12.5%) and comparable to Ulmo honey (3.1-6.3%), suggesting that certain Saudi honeys may exhibit antibacterial activity comparable to internationally recognized medical-grade honeys (Rani, 2017; Sherlock, 2010). However, these observations should be interpreted cautiously, as the present study was conducted under *In vitro* conditions, and further validation is required.

In addition to antimicrobial activity, the trace element composition of the honey samples provides further insight into their physicochemical characteristics and potential origin. The ICP-MS analysis revealed distinct elemental profiles among the honey types, which may be partially associated with differences in antibacterial activity, alongside other bioactive components such as phenolic compounds and hydrogen peroxide. Variations in elemental composition are influenced by several factors, including botanical origin, soil composition, environmental conditions, and beekeeping practices (Bogdanov *et al.*, 2007; Golubkina *et al.*, 2016).

The presence of lead (Pb) in the analyzed samples may reflect anthropogenic contamination from sources such as vehicle emissions, industrial activities, or atmospheric deposition (Pacyna and Pacyna, 2001). The relatively higher Pb concentration observed in Maraee honey may indicate proximity to urban or roadside environments (Mahé *et al.*, 2021; Quiralte *et al.*, 2023). Similarly, the elevated Cadmium (Cd) concentration in Samar honey may be attributed to plant uptake from metal-rich soils or agricultural inputs (Bora *et al.*, 2023). Elements such as Barium (Ba), Manganese (Mn), and Copper (Cu) are closely associated with soil geochemistry, and their higher levels-particularly in Samar honey-suggest growth of nectar-producing plants in mineral-rich environments (Tutun *et al.*, 2019).

Table 2: Trace element concentrations (mean±SD) in Saudi honey samples compared with international safety limits.

Element (ppb)	Maximum recommended level (ppb)	Sidr Honey (SDR)	Samar honey (SMR)	Maraee honey (MAR)
		Mean ± SD		
Ag	-	NA	NA	NA
Al	10000	980 ± 49[a]	830 ± 42[b]	91.3 ± 4.6 [c]
As	100	<LOD	<LOD	<LOD
Ba	-	<LOD	102 ± 6 [b]	402 ± 24 [a]
Be	-	<LOD	<LOD	<LOD
Cd	50	5.13 ± 0.31 [b]	20.3 ± 1.22 [a]	17.5 ± 1.05 [a]
Cr	50	<LOD	<LOD	<LOD
Cu	10000	489 ± 29 [a]	1300 ± 78 [b]	23 ± 1.4 [c]
Mn	-	765 ± 46 [a]	2450 ± 147 [b]	32.3 ± 1.9 [c]
Mo	-	<LOD	1.28 ± 0.07	<LOD
Ni	200	<LOD	31.1 ± 1.9	<LOD
Pb	100	9.8 ± 0.59 [a]	0.0117 ± 0.001 [b]	26.2 ± 1.6 [c]
Sb	-	<LOD	<LOD	<LOD
Se	1000	411 ± 25 [c]	316 ± 19 [b]	490 ± 29 [a]
Zn	50000	7340 ± 367 [a]	1560 ± 94 [b]	733 ± 44 [c]

Abbreviations: SDR = Sidr honey (*Ziziphus spina-christi* L.); SMR = Samar honey (*Acacia tortilis*); Maraee = Maraee honey (multifloral origin); LOD = Limit of detection.

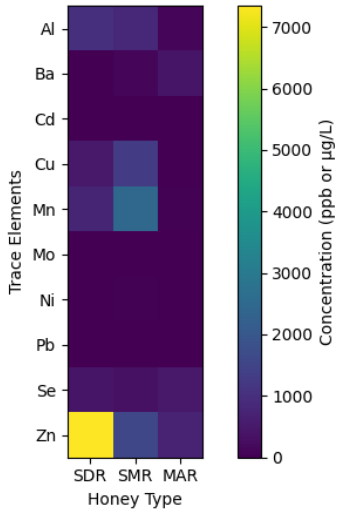


Figure 2: Heatmap showing the distribution of trace element concentrations in Sidr Honey (*Ziziphus spina*-christi L.; SDR), Samar honey (*Acacia tortilis*; SMR), and Maraee honey (multifloral origin; MAR) determined by ICP-MS. Color intensity corresponds to element concentrations (ppb or µg/L). SDR is characterized by high Zn levels, SMR by elevated Cu and Mn, and Maraee by relatively higher Ba and Se concentrations, indicating distinct elemental profiles associated with botanical and geographical origin.

Botanical characteristics and processing conditions may also contribute to differences in elemental composition (Reimann *et al.*, 2001). The high Zinc (Zn) concentration detected in Sidr honey may partly result from contact with metallic equipment during honey extraction or storage (Stecka *et al.*, 2014). In addition, plant species differ in their capacity to accumulate trace elements from soil. For instance, Sidr honey showed higher Zn and Al concentrations, whereas Samar honey exhibited elevated Mn and Cu levels, while Maraee honey contained higher Ba and Se concentrations, likely reflecting regional geological characteristics. Despite these variations, all measured element concentrations were below WHO, FAO, and EU safety limits, indicating that the analyzed honey samples are safe for human consumption (Naccari *et al.*, 2025). Moreover, these elemental signatures may serve as useful markers for honey authentication and geographical traceability.

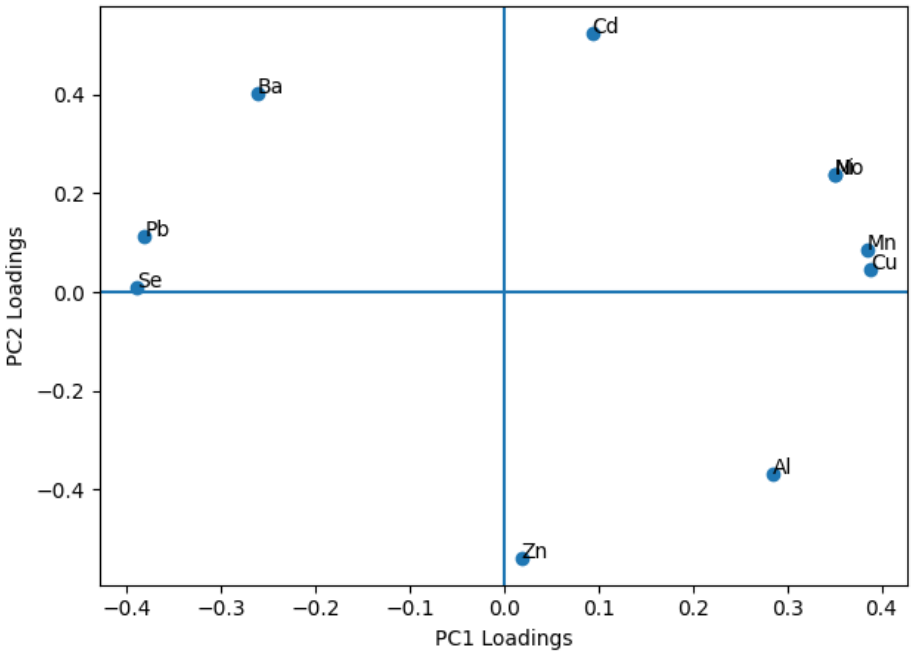


Figure 3: PCA loading plot showing the contribution of trace elements to the principal components derived from ICP-MS analysis of honey samples. Elements located farther from the origin have a greater influence on sample separation. Zn and Al exhibit strong positive loadings on PC1, while Cu and Mn also contribute to the variability associated with Samar honey. In contrast, Ba, Pb, and Se are positioned on the negative side of PC1, indicating their association with Maraee honey and reflecting differences related to geological and environmental conditions.

CONCLUSION

This study has some limitations that should be acknowledged. The antibacterial activity was evaluated against a single bacterial strain (MRSA), which limits the generalizability of the findings to other microorganisms, particularly Gram-negative bacteria. Additionally, the experiments were conducted under *In vitro* conditions, which may not fully reflect *in vivo* environments. Furthermore, the study did not investigate specific bioactive compounds responsible for antibacterial activity, such as phenolic compounds or hydrogen peroxide, which could provide deeper insight into the underlying mechanisms.

This study demonstrated that Saudi honeys from the Jazan region, particularly Sidr (*Ziziphus spina-christi*) and Samar (*Acacia tortilis*), exhibit pronounced antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), as evidenced by substantial inhibition zones and low MIC and MBC values. Elemental profiling revealed distinct trace element patterns among the honey types, reflecting variations in botanical origin and environmental conditions, while all detected elements remained within international safety limits. These findings highlight the potential of Saudi honey as a safe and effective natural antimicrobial agent. Further research is warranted to elucidate the underlying bioactive mechanisms and to validate these effects through *in vivo* and clinical studies.

ABBREVIATIONS

ANOVA: Analysis of Variance; **CFU:** Colony-Forming Units; **CLSI:** Clinical and Laboratory Standards Institute; **CRM:** Certified Reference Material; **EU:** European Union; **FAO:** Food and Agriculture Organization; **HNO_3:** Nitric Acid; **ICP-MS:** Inductively Coupled Plasma Mass Spectrometry; **LOD:** Limit of Detection; **MAR:** Maraee Honey (multifloral origin); **MBC:** Minimum Bactericidal Concentration; **MIC:** Minimum Inhibitory Concentration; **MRSA:** Methicillin-resistant *Staphylococcus aureus*; **NA:** Not Analyzed; **PC1:** Principal Component 1; **PCA:** Principal Component Analysis; **ppb:** Parts Per Billion; **SD:** Standard Deviation; **SDR:** Sidr Honey (*Ziziphus spina-christi* L.); **SMR:** Samar Honey (*Acacia tortilis*); **WHO:** World Health Organization; **ZOI:** Zone of Inhibition; **µg/L:** Microgram Per Liter; **µL:** Microliter; **Al:** Aluminum; **As:** Arsenic; **Ba:** Barium; **Be:** Beryllium; **Cd:** Cadmium; **Cr:** Chromium; **Cu:** Copper; **Mn:** Manganese; **Mo:** Molybdenum; **Ni:** Nickel; **Pb:** Lead; **Sb:** Antimony; **Se:** Selenium; **Zn:** Zinc.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHORS CONTRIBUTION

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be responsible for all aspects of the work.

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