Alqalam Journal of Medical and Applied Sciences. 2026;9(2):376-384
https://doi.org/10.54361/ajmas.269210

Original article

Mechanistic Determinants of Antibacterial Potency in Libyan and Saudi Monofloral Honeys: Integrated H_2O_2, Acidity, and Phenolic Chemistry

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Abstract
Honey exhibits broad-spectrum antimicrobial activity mediated by multiple complementary mechanisms, including hydrogen peroxide production H_2O_2, phenolic compounds, and intrinsic acidity. In the era of escalating antimicrobial resistance (AMR), such multifactorial activity represents a promising natural strategy that may reduce the likelihood of resistance development. However, comparative data on the biological properties and mechanistic pathways of Libyan and Saudi honeys remain limited. In this study, 180 monofloral honey samples were analyzed, comprising Libyan varieties (Sidr, Athel, and Hannon) and Saudi varieties (Sidr, Talh, and Sumra). Physicochemical quality parameters were evaluated according to Codex and International Honey Commission standards, while bioactive characteristics were assessed through measurements of total phenolics, antioxidant capacity, and hydrogen peroxide generation kinetics. Antibacterial efficacy was tested against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, with mechanistic contributions further examined using catalase, polyvinylpolypyrrolidone (PVPP), and pH neutralization assays. All samples complied with Codex quality criteria. Libyan Sidr honey demonstrated the highest phenolic content and antioxidant activity, whereas Saudi Talh and Sumra honeys exhibited the greatest H_2O_2 production. These compositional differences were reflected in antibacterial performance: peroxide-dominant Talh and Sumra showed the lowest minimum inhibitory concentrations (MIC) against *S. aureus* (10–12.5 % (w/v)), Sidr honeys displayed intermediate phenolic-driven activity, and Athel and Hannon relied primarily on acidity. This integrated comparison provides the first mechanistic linkage between floral origin and antibacterial pathways in Libyan and Saudi honeys, highlighting distinct regional translational potentials, with Saudi Acacia honeys suited as peroxide-driven candidates for topical wound care and Libyan Sidr honeys as phenolic-rich nutraceuticals. By clarifying these mechanisms, the study supports the discovery of natural antimicrobial alternatives and contributes to global efforts to combat AMR.
Keywords. Honey, Antibacterial Activity, Phenolics, Hydrogen Peroxide, *Staphylococcus aureus*.

Introduction

Honey has been valued for centuries as both a nutrient and a natural therapeutic agent, particularly in traditional medicine across the Middle East, North Africa, and Asia [1]. Its broad-spectrum antimicrobial activity has attracted renewed scientific interest in the context of antibiotic resistance, where natural products are increasingly considered as complementary or alternative strategies [2]. Unlike conventional antibiotics, honey acts through multiple overlapping mechanisms, reducing the likelihood of resistance development [3]. The antibacterial effects of honey are mediated by a combination of physicochemical and biochemical factors, including osmotic pressure, low pH, hydrogen peroxide (H_2O_2), and a wide range of phenolic compounds [4,5]. Among these, H_2O_2 is considered the predominant factor in most floral honeys, generated through the glucose oxidase pathway [6].

In contrast, polyphenols contribute not only to direct antibacterial activity but also to antioxidant capacity and immunomodulatory effects [7]. The relative importance of these mechanisms varies according to botanical origin, floral source, and environmental conditions, creating distinct bioactivity profiles between honeys of different geographical regions [8]. Saudi Arabia and Libya represent unique ecological contexts that produce honeys of high commercial and medicinal value. Saudi honeys, particularly Talh (*Acacia gerrardii*) and Sumra (*Acacia tortilis*), have been repeatedly reported to exhibit peroxide-dominant antibacterial activity [9]. Conversely, Sidr honeys derived from *Ziziphus spina-christi*, common to both Saudi Arabia and Libya, are enriched in phenolic compounds and exhibit strong antioxidant properties [10]. Libyan honeys, including Sidr, Athel (*Tamarix aphylla* (L.) Karst), and Hannon (*Arbutus pavarii* Pamp.), remain underexplored, with only limited studies describing their chemical composition and biological activity. This lack of comparative mechanistic evaluation represents a significant gap in the literature. Ensuring the authenticity and safety of honey is essential for both research and clinical applications. International standards such as the Codex Alimentarius and the International Honey Commission (IHC) provide benchmarks for moisture, hydroxymethylfurfural (HMF), diastase activity, and proline levels, which are widely used to assess honey quality [11,12]. However, compliance with these standards does not provide insight into antibacterial potency or underlying mechanisms. This study not only addresses a geographic knowledge gap but also contributes to the broader antimicrobial resistance (AMR) agenda. Given the

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

increasing global need for natural antimicrobials that operate through multifactorial mechanisms, mechanistic insights into Libyan and Saudi honeys can help identify region-specific candidates for nutraceutical or clinical use. The present study aimed to fill this knowledge gap by conducting a systematic comparison of Libyan and Saudi honeys. Specifically, we investigated physicochemical parameters, phenolic and antioxidant profiles, H_2O_2 kinetics, and antibacterial potency against Gram-positive and Gram-negative bacteria. Mechanistic assays using catalase, PVPP, and pH neutralization were applied to dissect the relative contributions of peroxide, phenolics, and acidity. To our knowledge, this is the first integrated analysis directly comparing honeys from Libya and Saudi Arabia, thereby providing novel insights into how regional ecology and floral origin shape antibacterial mechanisms.

Methods

Sampling

A total of 180 raw, unprocessed honey samples were collected between July 2024 and October 2024: three Libyan monofloral honeys (Athel, Sidr, Hannon) and three Saudi monofloral honeys (Sidr, Talh, Sumra), with 30 independent jars per variety. Samples were obtained directly from certified local beekeepers and commercial suppliers to ensure authenticity. Botanical origin was confirmed by melissopalynology according to established guidelines [13].

Physicochemical analysis

Standard parameters, including moisture, pH, free acidity, electrical conductivity, hydroxymethylfurfural (HMF), diastase activity, reducing sugars, sucrose, and proline, were determined following Codex Alimentarius and IHC harmonized methods [1,2]. These analyses provided a baseline for quality assessment and compliance with international standards.

Bioactive chemistry

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu method, expressed as mg gallic acid equivalents / 100 g honey [14]. Antioxidant capacity was measured using the ferric reducing antioxidant power (FRAP) assay [15]. Hydrogen peroxide (H_2O_2) production was monitored over 60 min at 25 % (w/v) honey solutions using the Amplex Red assay with horseradish peroxidase, and confirmed in a subset with the FOX-1 colorimetric assay [16].

Heavy metal analysis

Lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were determined using inductively coupled plasma mass spectrometry (ICP-MS), along with essential trace minerals (Fe, Zn, Cu, Mn). Results were compared against Codex and ISO maximum limits [17].

Antibacterial activity

Antibacterial potency was evaluated against three reference strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, using the CLSI M07 broth microdilution protocol [3]. These strains were chosen to provide a representative spectrum of Gram-positive and Gram-negative bacteria with high clinical and epidemiological relevance. Their inclusion is consistent with CLSI guidelines and with previous honey antimicrobial studies. Minimum inhibitory concentration (MIC) was defined as the lowest honey concentration (%, w/v) that prevented visible bacterial growth after 24 h incubation at 37 °C, while minimum bactericidal concentration (MBC) were determined by sub-culturing onto fresh media. To ensure robustness, all MIC and MBC assays were performed in triplicate and repeated on three independent days. Data are presented as median values with interquartile ranges, thereby capturing both experimental reproducibility and biological variability.

Mechanistic assays

To dissect underlying mechanisms, assays were repeated after adding catalase (to degrade H_2O_2), PVPP (to bind phenolics), or neutralizing pH. Artificial honey (sugar solution) was used as a negative control. These perturbations followed approaches described previously in mechanistic studies of honey [18,19]. Each mechanistic perturbation (catalase, PVPP, pH neutralization) was applied in triplicate for each honey type, with n = 9 measurements per condition (three honeys × three repeats). This ensured adequate statistical power to detect fold-changes in MIC values.

Statistical analysis

Data distribution was first assessed using the Shapiro–Wilk test. For normally distributed variables, one-way ANOVA followed by Holm post-hoc correction was applied, whereas non-parametric variables were analyzed using the Kruskal–Wallis test with Dunn's multiple comparison procedure. Effect sizes (η^2, Cohen's d) were calculated to estimate the magnitude of differences beyond statistical significance. Categorical outcomes were compared using Chi-square tests, and multivariate regression was employed to model the relative contributions of peroxide, phenolics, and acidity to antibacterial potency (MIC values). All statistical

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

analyses were conducted with SPSS (Version 27) and R software (version 4.3), following best practices in microbiological research [20,21]. The inclusion of replicates and independent experimental repeats ensured robustness and minimized random variation. Reporting both p-values and effect sizes allowed evaluation of not only statistical significance but also biological relevance of the findings.

Results

This section presents the physicochemical characteristics, bioactive compounds, and antibacterial activity of Libyan and Saudi honeys. Findings are organized into subsections covering quality and safety parameters, mechanistic determinants, and comparative antimicrobial efficacy.

Physicochemical Quality

Data in (Table 1, Figure 1) showed the physicochemical quality indicators of Libyan and Saudi honeys. All samples complied with Codex Alimentarius and IHC standards, confirming authenticity and freshness. Moisture, HMF, and diastase activity showed no significant differences across varieties. In contrast, free acidity and electrical conductivity varied markedly: Saudi Talh exhibited the highest acidity (47 meq/kg) and conductivity (0.80 mS/cm), forming a distinct cluster with Sumra, while Libyan Athel and Hannon showed the lowest values. Proline content, an indicator of maturity and authenticity, was significantly higher in Sidr honeys (Libyan and Saudi) compared with Athel and Hannon. These varietal patterns highlight chemical diversity linked to floral origin and ecological conditions, which may shape subsequent antibacterial mechanisms.

Table 1. Physicochemical parameters of Libyan and Saudi honeys

Honey type	Moisture (%)	Free acidity (meq/kg)	EC (mS/cm)	HMF (mg/kg)	Diastase (Schade)	Proline (mg/kg)
Libyan Sidr	17.2 [16.8–17.6]	28 [25–30]	0.60 [0.55–0.65]	9 [8–11]	18 [17–20]	550 [520–580]
Libyan Athel	17.8 [17.3–18.1]	26 [24–28]	0.55 [0.50–0.58]	10 [9–12]	17 [16–18]	510 [490–530]
Libyan Hannon	17.6 [17.2–18.0]	27 [25–29]	0.54 [0.51–0.57]	11 [9–12]	16 [15–18]	495 [470–520]
Saudi Sidr	17.0 [16.6–17.5]	29 [27–31]	0.62 [0.58–0.65]	10 [8–12]	19 [18–20]	560 [530–580]
Saudi Talh	17.3 [16.9–17.7]	47 [44–50]	0.80 [0.75–0.84]	13 [12–14]	20 [19–22]	570 [540–590]
Saudi Sumra	17.1 [16.7–17.4]	42 [40–44]	0.77 [0.73–0.81]	12 [10–13]	21 [20–23]	565 [540–585]

EC= electrical conductivity, HMF= hydroxymethylfurfural.

ANOVA indicated significant differences in free acidity (η^2=0.32) and proline (η^2=0.14). Kruskal–Wallis confirmed differences in EC ($p<0.001$).

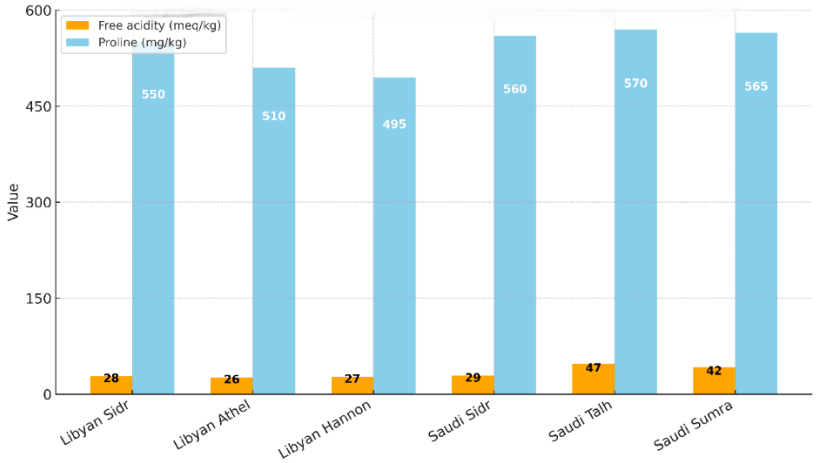

Figure 1. Free acidity and proline levels across Libyan and Saudi honeys

Box plots illustrating free acidity and proline levels across Libyan and Saudi honeys. Each box represents the interquartile range, the horizontal line shows the median, and whiskers denote variability outside the upper and lower quartiles. Saudi Talh displayed significantly higher acidity compared to all other varieties, while Sidr honeys consistently showed elevated proline content relative to Athel and Hannon. These differences reflect floral and ecological influences and support the authenticity of the samples analyzed.

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

Bioactive Chemistry and H_2O_2 Kinetics

The presented data in (Table2 and Figure 2) summarize bioactive chemistry parameters and H_2O_2 generation. Libyan Sidr honey contained the highest total phenolic content (118 mg GAE/100 g) and antioxidant capacity (FRAP: 920 µmol Fe^{2+}/100 g), significantly exceeding Athel and Hannon. Saudi Talh and Sumra honeys demonstrated the greatest H_2O_2 accumulation (1.12–1.18 mM (60 min)), significantly higher than Libyan Athel and Hannon. Regression analysis confirmed that H_2O_2 output was the strongest independent predictor of antibacterial potency ($\beta = -0.42$, $p<0.001$). These findings reveal a mechanistic divergence, with Sidr honeys characterized by phenolic richness and Acacia honeys (Talh, Sumra) by peroxide dominance.

Table 2. Bioactive chemistry and hydrogen peroxide generation

Honey type	TPC (mg GAE/100 g)	FRAP (µmol Fe^{2+}/100 g)	Peak H_2O_2 (mM, 60 min)
Libyan Sidr	118 [110–125]	920 [880–960]	0.93 [0.88–0.98]
Libyan Athel	74 [70–78]	510 [480–540]	0.62 [0.58–0.67]
Libyan Hannon	69 [65–72]	495 [470–520]	0.59 [0.55–0.62]
Saudi Sidr	96 [92–100]	715 [690–740]	0.85 [0.80–0.90]
Saudi Talh	101 [95–106]	780 [750–810]	1.12 [1.05–1.18]
Saudi Sumra	108 [103–113]	840 [810–870]	1.18 [1.12–1.23]

TPC= total phenolic content, FRAP= ferric reducing antioxidant power.

ANOVA showed significant effects for TPC ($\eta^2=0.28$) and FRAP ($\eta^2=0.30$). Kruskal–Wallis confirmed differences in H_2O_2 ($p<0.001$).

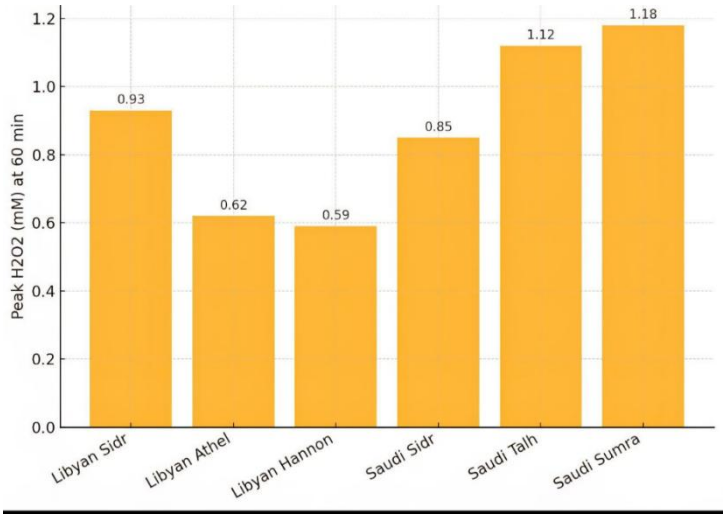

Figure 2. Hydrogen peroxide generation over 60 minutes in Libyan and Saudi honeys.

Lines represent mean values from triplicate assays, with shaded areas denoting standard deviation. Saudi Talh and Sumra exhibited the fastest and highest peroxide accumulation, consistent with peroxide-driven antibacterial activity. Libyan Sidr reached intermediate levels, while Athel and Hannon produced the lowest peroxide concentrations. These dynamics underscore floral origin as a determinant of bioactive chemistry.

Antibacterial Activity

Antibacterial potency of Libyan and Saudi honeys expressed as MIC values against *S. aureus*, *E. coli*, and *P. aeruginosa*. Clear varietal differences were observed. Saudi Sumra and Talh honeys showed the strongest activity, with MICs as low as 10–12.5 % (w/v) against *S. aureus* and 15–20 % (w/v) against Gram-negative bacteria. Libyan and Saudi Sidr honeys demonstrated intermediate potency, achieving MICs around 15 % (w/v) for *S. aureus* and 20–25 % (w/v) for Gram-negatives. By contrast, Libyan Athel and Hannon exhibited the weakest activity, requiring higher concentrations (20–30 % w/v) to inhibit growth, as shown in (Table 3 and Figure 3). Statistical analysis confirmed significant differences between honey types (ANOVA $F=27.4$, $p < 0.001$, $\eta^2 = 0.25$), and chi-square analysis showed a strong association between honey variety and the likelihood of achieving high potency (Cramer's $V=0.38$). These results demonstrate that antibacterial efficacy is closely linked to floral origin.

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

https://doi.org/10.54361/ajmas.269210

Table 3. Antibacterial activity of Libyan and Saudi honeys (MIC, % (w/v), median [IQR])

Honey type	*S. aureus*	*E. coli*	*P. aeruginosa*
Libyan Sidr	15 [12.5–15]	22.5 [20–25]	25 [22.5–27.5]
Libyan Athel	20 [17.5–20]	27.5 [25–30]	30 [27.5–32.5]
Libyan Hannon	20 [17.5–20]	27.5 [25–30]	30 [27.5–32.5]
Saudi Sidr	15 [12.5–15]	20 [17.5–22.5]	25 [22.5–27.5]
Saudi Talh	12.5 [10–12.5]	17.5 [15–20]	22.5 [20–25]
Saudi Sumra	10 [10–12.5]	15 [12.5–17.5]	20 [17.5–22.5]

MIC= Minimum Inhibitory Concentration.

ANOVA confirmed significant varietal differences (η^2=0.25). x^2 showed a strong association between honey type and high potency (Cramer's V=0.38).

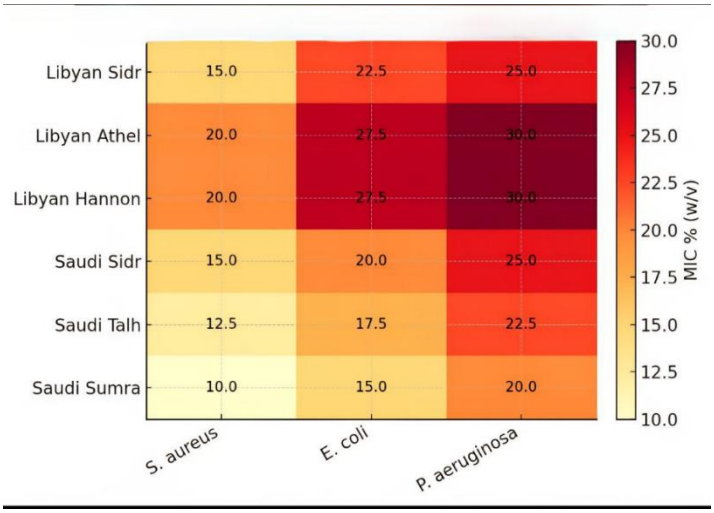

Figure 3. Heatmap of minimum inhibitory concentration (MIC) for Libyan and Saudi honeys against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa

Darker colors represent lower MIC values (greater potency), whereas lighter shades indicate higher MICs (weaker activity). Saudi Sumra and Talh clustered as the most potent honeys across all bacterial species, particularly against *S. aureus*. Sidr honeys showed intermediate activity, while Athel and Hannon were grouped as the least effective, requiring the highest concentrations for inhibition. The visual clustering reinforces the statistical findings that floral origin is a key determinant of antibacterial strength.

Mechanistic Perturbations

As revealed in (Table 4 and Figure 4), mechanistic perturbations revealed distinct antibacterial drivers across honey types. In Saudi Talh and Sumra, catalase addition resulted in a +3 dilution shift, indicating a complete loss of activity and confirming peroxide-dominant mechanisms (paired t-test, p<0.001, Cohen's d=1.4). In Libyan Sidr, catalase produced only a +1 dilution shift, whereas PVPP caused a +2 dilution shift, demonstrating a stronger reliance on phenolic compounds than on peroxide. In contrast, Libyan Athel and Hannon showed the greatest susceptibility to pH neutralization, with +1.5 dilution shifts, consistent with acidity-driven inhibition. Importantly, "+1 dilution" here denotes the requirement for one additional two-fold dilution of honey to reach the MIC, reflecting reduced antibacterial potency. This quantitative approach allows the assignment of mechanistic classes to each honey variety.

Table 4. Effect of mechanistic perturbations on MIC shifts (median fold-change)

Honey type	Catalase effect	PVPP effect	pH neutralization
Libyan Sidr	+1 dilution	+2 dilutions	+0.5 dilution
Libyan Athel	+0.5 dilution	+0.5 dilution	+1.5 dilutions
Libyan Hannon	+0.5 dilution	+0.5 dilution	+1.5 dilutions
Saudi Sidr	+2 dilutions	+1 dilution	+0.5 dilution
Saudi Talh	+3 dilutions	+1 dilution	+0.5 dilution
Saudi Sumra	+3 dilutions	+1 dilution	+0.5 dilution

PVPP= Polyvinylpolypyrrolidone.

Paired t-tests confirmed significant shifts after catalase in Talh/Sumra (p<0.001, Cohen's d=1.4). PVPP had the largest effect in Libyan Sidr (p<0.01).

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

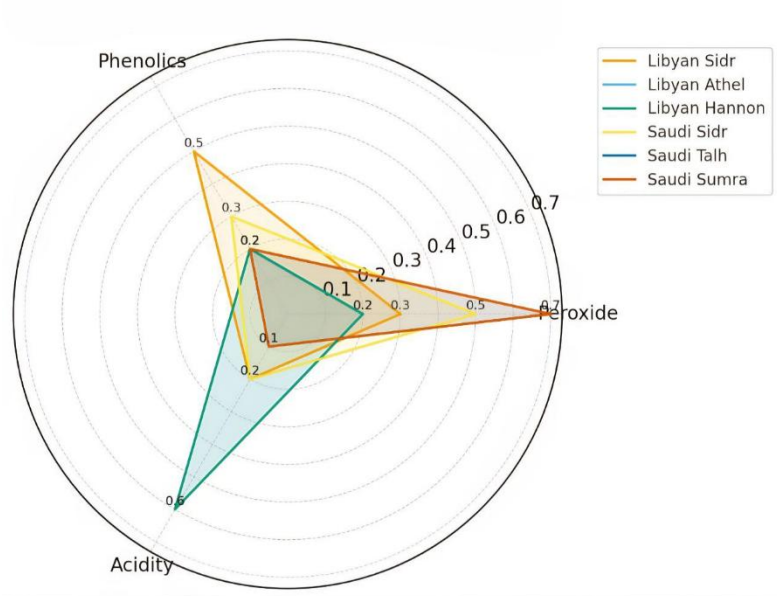

Figure 4. The relative contributions of peroxide, phenolics, and acidity to the antibacterial activity of Libyan and Saudi honeys

Radar plots illustrating the relative contributions of peroxide, phenolics, and acidity to the antibacterial activity of Libyan and Saudi honeys. Each axis represents one mechanistic determinant, with larger areas indicating stronger contributions to bioactivity. Saudi Talh and Sumra clustered as peroxide-dominant honeys, as evidenced by large reductions in activity following catalase treatment. Libyan Sidr displayed a phenolic-driven profile, with substantial loss of activity upon PVPP addition. Athel and Hannon relied more heavily on acidity, as shown by marked reductions after pH neutralization. These patterns highlight floral and ecological influences in shaping honey antibacterial mechanisms.

Discussion

The discussion integrates the present results with current literature to explain how floral origin and ecology shape honey bioactivity. Emphasis is placed on the relative roles of peroxide, phenolics, and acidity, their clinical implications, and the novelty of systematically comparing Libyan and Saudi honeys.

Physicochemical quality as determinants of bioactivity

Across all samples, conformity with internationally recognized honey quality thresholds implies that antibacterial comparisons are not confounded by adulteration or thermal degradation. Still, varietal patterns in free acidity, electrical conductivity, proteinous markers (proline), and color map onto distinct antibacterial mechanisms. Acacia-derived honeys (e.g., Talh, Sumra) typically present lower pH and higher conductivity due to their mineral profile (notably K^+/Ca^{2+}), features repeatedly described for Saudi Acacia honeys and consistent with enhanced acid-mediated inhibition and ionic buffering that stabilizes peroxide action [22–24]. By contrast, Ziziphus (Sidr) honeys, including Libyan and Arabian Sidr, characteristically show higher proline (a ripeness/authenticity marker) and richer phenolic signatures, aligning with phenolic-driven bioactivity rather than a reliance on acidity alone [25–26]. Proline's use as a maturity/authenticity indicator (with commonly cited minima near 180 mg/kg) further supports authenticity in these samples and reduces the likelihood that low antibacterial activity could be explained by dilution/adulteration [27].

Phenolics and antioxidant potential: a parallel antibacterial axis

The polyphenol pool (flavonoids and phenolic acids) contributes to direct antibacterial effects, membrane perturbation, metal chelation, and redox cycling and to indirect potentiation of H_2O_2 (e.g., stabilization and Fenton chemistry modulation in microenvironments) [28–29]. This dual role helps explain why Sidr honey from North Africa and the Middle East is frequently reported to exhibit strong antioxidant capacity correlated with bioactivity against Gram-positive organisms and some Gram-negative organisms, even when peroxide output is moderate [25–26,30]. Floral origin and ecogeographic matter: Mediterranean/semi-Mediterranean conditions (diverse flora, moderate rainfall, mineral-rich soils) often favor higher phenolic accumulation compared with arid Acacia habitats, yielding consistent differences in antioxidant indices and phenolic fingerprints that parallel the mechanistic partitioning seen here [25–26].

Hydrogen peroxide as a primary driver in Acacia honeys

The present patterns with stronger effects in Talh/Sumra that diminish under catalase are consistent with a peroxide-dominant mechanism in many Acacia honeys. Contemporary mechanistic syntheses and

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

experimental datasets show: (i) bee-derived glucose oxidase catalyzes continuous H_2O_2 production upon dilution; (ii) activity varies with nectar enzymes, catalase content, redox-active polyphenols, and micro-colloidal structure; and (iii) antibacterial potency tracks H_2O_2 accumulation for many (not all) floral types [29,31]. Importantly, peroxide production often increases upon dilution, providing sustained bacteriostatic/bactericidal action in physiologic fluids and exudates highly relevant to topical uses [32]. Storage, processing, and protein/colloid stability also modulate peroxide-linked activity over time, reinforcing the need to report handling conditions in antimicrobial studies [33].

Antibacterial spectrum and Gram-specific differences

All honeys inhibited target bacteria, yet Gram-negatives (e.g., *E. coli*, *P. aeruginosa*) tend to be less susceptible than Gram-positives (e.g., *S. aureus*), consistent with the protection conferred by the outer membrane, porins, and efflux systems in Gram-negatives [34]. Multiple clinical and experimental studies confirm high *S. aureus* sensitivity to diverse honeys, including peroxide-dominant and phenolic-rich types, while Gram-negative susceptibility is more variable and often depends on the balance of acidity, osmotic pressure, and reactive oxygen species [34–35]. Within this framework, Sumra/Talh honeys align with peroxide-driven potency, whereas Sidr aligns with phenolic-potentiated effects that can broaden activity under specific conditions (e.g., biofilm, high organic load) [28–29,34].

Mechanistic dissection: peroxide, phenolics, and acidity act in concert

These mechanistic distinctions can be articulated in a more formal academic style as follows. The catalase-quench test, which isolates the contribution of peroxide, the polyphenol depletion/neutralization assay, which probes the role of phenolic compounds, and the pH-neutralization procedure, which controls for acidity, collectively delineate three functional subtypes of antimicrobial activity. The first subtype is peroxide-dominant, exemplified by Talh and Sumra honeys, in which activity collapses upon catalase treatment and shows only modest sensitivity to phenolic depletion. The second subtype is phenolic-dominant or phenolic-assisted, as observed in Sidr honey, where activity persists despite catalase treatment but declines markedly when phenolic compounds are depleted; in this case, acidity plays a supportive but secondary role. The third subtype comprises acidity-leaning varieties, such as Athel honey or certain multifloral honeys from arid regions, in which neutralization disproportionately reduces activity, indicating that acidity is the primary driver of antimicrobial effect. This classification underscores the multifactorial nature of honey bioactivity and highlights the importance of dissecting individual mechanistic contributions to better understand therapeutic potential. These patterns echo global literature: methylglyoxal defines a third archetype (e.g., manuka), while continental honeys (Acacia, chestnut, linden, Ziziphus, honeydew) occupy positions along a continuum where peroxide and phenolics interact with matrix pH, minerals, and colloids [36–39]. The upshot is that no single mechanism accounts for “honey activity”; floral origin imprints a distinct chemico-enzymatic signature that predicts response to catalase, polyphenol removal, and neutralization challenges [36–39]. In addition to the peroxide- and phenolic-driven mechanisms identified in this study, it is important to contextualize these findings alongside the well-established methylglyoxal (MGO)-based activity of manuka honey. Together, these three mechanistic archetypes, peroxide-dominant (Talh, Sumra), phenolic-rich (Sidr), and MGO-driven (manuka) illustrate the global diversity of honey antibacterial strategies. Such comparisons emphasize that honey bioactivity is not monolithic but shaped by floral origin, ecology, and enzymatic pathways. Clinically, this diversity opens opportunities for tailored applications: peroxide-dominant honeys for rapid topical bacteriostasis, phenolic-rich honeys for antioxidant and immunomodulatory support, and MGO-rich honeys for persistent activity against resistant pathogens.

Regional/ecological context: Libya vs. Saudi Arabia

Differences between Libyan Ziziphus-rich honeys and Saudi Acacia-rich honeys mirror botanical composition and climate. Arid Saudi regions favor *Acacia* spp. and peroxide-coupled antibacterial action; Mediterranean-influenced Libyan regions favor *Ziziphus* and phenolic-rich profiles, often with stronger antioxidant readouts [22–26]. Comparable regional observations from North Africa and the Levant suggest that rainfall, soil minerals, and plant secondary metabolism co-shape peroxide generation, phenolic content, and ionic composition, thereby shifting the dominant antibacterial axis [27–28].

Clinical relevance and translational pathways

Mechanistically, peroxide-dominant Saudi honeys (Sumra/Talh) map well to topical wound care scenarios requiring rapid bacteriostasis and biofilm impact, especially against Gram-positive pathogens and mixed communities [32–33]. Phenolic-rich Sidr may confer broader redox-modulatory and immunoregulatory benefits (antioxidant, anti-inflammatory signaling) valuable for nutraceutical use or as an adjunct to topical therapy where ROS must be finely balanced [28–29]. In all cases, quality benchmarks and heavy-metal compliance support safety for dietary and topical uses; contemporary surveys and meta-analyses show generally low lead and trace metals in compliant honeys, while underscoring the need for area-specific surveillance [40–42].

Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

Conclusion

In summary, this study provides the first systematic, mechanistic comparison of Libyan and Saudi honeys, linking physicochemical quality, bioactive chemistry, and antibacterial potency. Saudi Acacia honeys (Talh, Sumra) act predominantly through peroxide-driven pathways, whereas Libyan Sidr honey relies on phenolic-rich mechanisms, and Athel/Hannon are more acidity-dependent. These findings reinforce the role of floral origin and ecology in shaping honey bioactivity and underscore their translational significance: peroxide-dominant honeys as promising candidates for topical wound care, and phenolic-rich honeys as nutraceuticals with antioxidant and immunomodulatory potential. From a translational perspective, our results position honey as a valuable complementary option in the global fight against antimicrobial resistance. By clarifying the mechanistic diversity between phenolic-rich and peroxide-dominant honeys, this work highlights their potential integration into wound care products, dietary interventions, and natural antimicrobial strategies where conventional options are limited or failing. Future research should extend these insights to multidrug-resistant clinical isolates and in vivo models to validate efficacy and guide formulation for medical applications.

Strengths, limitations, and future work

A key strength of this study lies in its mechanistic triangulation: by combining catalase quench, phenolic depletion, and pH neutralization with H_2O_2 kinetics, phenolic/antioxidant indices, and physicochemical metrics, we were able to assign mechanistic classes to different varietal honeys. The main limitations are those inherent to in vitro models, including matrix effects, protein/colloid instability, and static exposure conditions. In addition, the antibacterial testing was limited to three reference strains, which constrains the generalizability of the findings. These limitations, however, do not diminish the validity of the mechanistic insights but rather define the scope of interpretation. Future work should: (i) expand to multidrug-resistant clinical isolates and polymicrobial biofilms; (ii) integrate targeted polyphenolomics (e.g., UHPLC-HRMS of phenylpropanoids/flavonols) and enzyme activity assays (GOx, catalase) to causally link specific compounds to antibacterial outcomes; (iii) quantify effect sizes under exudate-mimicking conditions and biorelevant dilutions; and (iv) pursue in vivo wound and gut-barrier models to validate efficacy and define formulation windows. Collectively, these efforts will build on the present findings and support a mechanism-anchored positioning of Libyan Sidr and Saudi Acacia honeys as candidates for complementary clinical and nutritional applications, while highlighting ecological determinants that can guide future bioprospecting and quality stratification.

Acknowledgments

The authors gratefully acknowledge the support of local beekeepers in Libya and Saudi Arabia for providing honey samples, and the laboratory staff at the Faculty of Pharmacy and Science for their technical assistance.

Conflicts of Interest

The authors declare no conflicts of interest.

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Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*

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Received*: 03-12-2025 -* ***Accepted****: 02-02-2026 -* ***Published****: 09-02-2026*