INTRODUCTION

Honey bees collect floral nectar from plants to produce natural sweet honey with a complex chemical composition. The major components of honey are simple sugars (~75% fructose and glucose), water (~20%), and sucrose (~3%–10%). Other constituents are complex sugars, minerals, vitamins, antioxidants, proteins, enzymes, phenolic compounds, and some unidentified substances (Alqarni, Owayss, & Mahmoud, 2016; Wright, Nicolson, & Shafir, 2018). In addition to its nutritional value, honey has also been used as...
traditional remedy in ancient and modern cultures for curing top-
ical burns, wounds, and numerous diseases (Abuharfeil, Al-Oran,
& Abo-Shehada, 1999; Al-Waili & Saloom, 1999; Eteraf-Oskouei &
Najafi, 2013; Molan, 2001; Samarghandian, Farkhondeh, & Samini,
2017). Supplementary hive products such as bee venom, royal
jelly, and propolis also have potential therapeutic properties and
are used in alternative medicine known as apitherapy (Basa, Belay,
Tilahun, & Teshale, 2016; Pasupuleti, Sammugam, Ramesh, & Gan,
2017). The chemical composition of honey varies with the source
plant of bee forage and geographical origin (Machado De-Melo et
al., 2018).

The antibacterial activity of honey was first recognized by Van
Ketel in 1892 (Dustmann, 1979), which was followed by numerous
studies concerning the antimicrobial properties of honey against a
broad-spectrum bacterial species (~60 species), including aerobes,
aerobes, and gram-positive (G+) and gram-negative (G−) bac-
teria (Bogdanov, 1997; Elbanna et al., 2014; Hannan et al., 2004;
Kwakman & Zaat, 2012; Lusby, Coombes, & Wilkinson, 2005;
Mandal & Mandal, 2011; Molan, 1992). The bactericidal and bac-
teriostatic potential of honey may be particularly profitable against
antibiotic-resistant bacteria (Patton, Barrett, Brennan, & Moran,
2006) and in synergizing with the antibiotic potential (Zakaria,
2015). Furthermore, honey also shows antimicrobial activity against
several other microorganisms, including viruses, fungi, and yeasts
(Maddocks & Jenkins, 2013; Saranraj & Sivasakthi, 2018). The devel-
opment of antibiotic resistance in microorganisms attracts the use
of alternative strategies such as using honey as antimicrobial agents
to reduce the global load of diseases and resistance (Ayukekbong,
Ntemgwa, & Atabe, 2017; S. Mandal, Pal, Chowdhury, & Debmandal,
2009; Patton et al., 2006).

In Saudi Arabia, honey consumption is gradually increasing, as
honey is a principle ingredient in foods and in folk medicines (Al-
Ghamdi & Adgaba, 2015; Alqarni, 2011; Alqarni et al., 2016). Many
locally produced and imported honeys are available in the Saudi

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**FIGURE 1** Location sites for honey collection in Saudi Arabia. Asterisks indicate the regions from where the honey samples were collected.
market. Sidr honey and Talh honey are two major honey types in Saudi Arabia and the Arabian Peninsula. These honeys are locally named with reference to their floral nectar source. Talh honey is produced from *Acacia gerrardii* Benth. trees and Sidr honey from *Ziziphus spina-christi* L. (Adgaba et al., 2017; Al-Ghamdi, 2007; Al-Khalifa & Al-Arify, 1999; Alqarni et al., 2016). *Ziziphus* and *Acacia* are the most common plants of economic importance in Saudi Arabia and are the major floral sources of high-valued expensive honeys (Alqarni, Hassan, & Owayss, 2015; Alqarni, 2015).

Our study aimed to evaluate the antimicrobial potential of the most preferred honeys in Saudi Arabia, Sidr and Talh honeys, against pathogenic bacterial and fungal strains. Their potential antimicrobial activity was also equated with that of antibiotics commonly used against the targeted microbial strains. This research pursuing the antimicrobial potential of honey types will be helpful in treating the pathogenic microorganisms threatening the public health and changing antibiotics into last-resort drugs.

### MATERIALS AND METHODS

#### 2.1 Honey Samples

Fresh samples (1 kg each) of the most preferred honeys for Saudi consumers, named Sidr (produced from *Z. spina-christi* L. trees: 11 samples) and Talh (produced from *A. gerrardii* Benth. trees: 20 samples), were collected from apiaries of selected regions in the Kingdom of Saudi Arabia (KSA) (Figure 1) for in vitro analysis of their antimicrobial activities against pathogenic bacterial and fungal strains. Each honey sample from the targeted region was divided for

| Honey type          | Botanical origin          | Sample code no. | Honey source | Apiary location |
|---------------------|---------------------------|-----------------|--------------|----------------|
| **Sidr honey (SH)** | *Ziziphus spina-christi* L.| SH1 AB           | AB           | Riyadh         |
|                     |                           | SH2 AB          | AB           | Northern Borders |
|                     |                           | SH3 AB          | AB           | Northern Borders |
|                     |                           | SH4 AB          | AB           | Riyadh         |
|                     |                           | SH5 AB          | AB           | Northern Borders |
|                     |                           | SH6 AB          | AB           | Riyadh         |
|                     |                           | SH7 AB          | AB           | Riyadh         |
|                     |                           | SH8 AB          | AB           | Riyadh         |
|                     |                           | SH9 AB          | AB           | Riyadh         |
|                     |                           | SH10 SMA        | Riyadh       |
|                     |                           | SH11 SMA        | Riyadh       |
| **Talh honey (TH)** | *Acacia gerrardii* Benth.  | TH1 AB          | AB           | Hail           |
|                     |                           | TH2 AB          | AB           | Riyadh         |
|                     |                           | TH3 AB          | AB           | Al-Qassim      |
|                     |                           | TH4 AB          | AB           | Hail           |
|                     |                           | TH5 AB          | AB           | Hail           |
|                     |                           | TH6 AB          | AB           | Al-Qassim      |
|                     |                           | TH7 AB          | AB           | Al-Qassim      |
|                     |                           | TH8 AB          | AB           | Hail           |
|                     |                           | TH9 AB          | AB           | Hail           |
|                     |                           | TH10 AB         | AB           | Riyadh         |
|                     |                           | TH11 AB         | AB           | Al-Qassim      |
|                     |                           | TH12 AB         | AB           | Riyadh         |
|                     |                           | TH13 AB         | AB           | Riyadh         |
|                     |                           | TH14 AB         | AB           | Al-Qassim      |
|                     |                           | TH15 RT         | Al-Baha      |
|                     |                           | TH16 RT         | Assir        |
|                     |                           | TH17 RT         | Makkah       |
|                     |                           | TH18 RT         | Makkah       |
|                     |                           | TH19 AB         | Al-Baha      |
|                     |                           | TH20 RT         | Hail          |

*Note: AB, apiaries of beekeepers; RT, retailer; SMA, self-monitored apiaries.*
three repeats. After running a triplicate measurement of antimicrobial activity, the mean value of these three repeats was calculated. The codes and regional data of these unifloral honeys are presented in Table 1. Two forms of honey samples, natural (nondiluted crude honey) and water-diluted honey (33% w/v) (Elbanna et al., 2014), were used for the examination of their potential antimicrobial action.

### 2.2 | Microbial Strains

The microbial pathogenic strains of two gram-positive bacteria (*Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* ATCC 8095), two gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella enteritidis* ATCC 13076) and one dermatophyte fungus (*Trichophyton mentagrophytes*), were obtained from the culture collection of the Department of Biology, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, KSA. Stock cultures of bacterial and fungal strains were maintained at 4°C on nutrient and potato dextrose agar slants, respectively.

### 2.3 | Assessment of Antibacterial Activity

Antimicrobial activities of each honey type (Sidr and Talh) were assessed using the well-diffusion bioassay technique (Elbanna et al., 2014). Sterilized Muller-Hinton or potato dextrose agar media (Oxoid) were poured into sterilized petri dishes, left to solidify at room temperature (25 ± 1°C), and swabbed with fresh bacterial or fungal strain cultures. Wells at the center of agar plates were made using a sterile cork borer (9 mm diameter) and filled with 300 µl of natural honey or water-diluted honey (33% w/v). To give honey enough time for diffusion, all plates were placed in a refrigerator (~5°C) for 2 hr and then incubated at 37°C for 24 hr (for bacteria) and at 28°C for 48–72 hr (for the fungus). The potential antimicrobial activities of honey treatments were expressed by measuring the diameter (mm) of a clear (inhibition) zone of each well, with distilled water taken as a control. In separate experiments, the antimicrobial activity of two broad-spectrum antibacterial (tetracycline and chloramphenicol) and two antifungal (flucoral and mycosat) antibiotics (Mast Diagnostic GmbH, Germany) were assessed against their respective microbial strains using the agar disk diffusion method and measuring the clear zone diameter (mm) of each disk (EFSA, 2012).

### 3 | RESULTS

#### 3.1 | Antimicrobial Activity of Honeys

In vitro antimicrobial activities of the most common unifloral honey types in Saudi Arabia (*Sidr* honey (SH) and *Talh* honey (TH)) were evaluated against pathogenic strains of gram-positive bacteria (*B. cereus*, *S. aureus*), gram-negative bacteria (*E. coli*, *S. enteritidis*), and dermatophyte fungi (*T. mentagrophytes*). Natural and water-diluted (33% w/v) forms of SH and TH were used for testing their potential antimicrobial activity. The data revealed that SH and TH honey types have significant differential antimicrobial potentialities against the

| Microbial strain | Zone of inhibition (ZOI) in mm ± SEM |
|------------------|-------------------------------------|
|                  | Sidr Honey (SH)                     | Talh Honey (TH)                      |
|                  | Natural | Water-diluted (33% w/v) | Natural | Water-diluted (33% w/v) |
| **G+ Bacteria**  |         |                         |         |                         |
| *Bacillus cereus*| 31.09 ± 0.84<sup>a</sup> | 36.45 ± 1.01<sup>a</sup> | 35.65 ± 0.53<sup>a</sup> | 41.65 ± 0.68<sup>a</sup> |
| *Staphylococcus aureus* | 29.45 ± 0.73<sup>a</sup> | 34.55 ± 1.08<sup>a</sup> | 32.00 ± 0.61<sup>a</sup> | 37.70 ± 0.70<sup>a</sup> |
| **G− Bacteria**  |         |                         |         |                         |
| *Escherichia coli* | 23.18 ± 0.83<sup>c</sup> | 27.09 ± 1.05<sup>c</sup> | 27.15 ± 0.67<sup>c</sup> | 31.20 ± 0.78<sup>c</sup> |
| *Salmonella enteritidis* | 19.36 ± 0.64<sup>d</sup> | 23.36 ± 0.79<sup>d</sup> | 23.35 ± 0.53<sup>e</sup> | 28.10 ± 0.67<sup>d</sup> |
| **Fungus**       |         |                         |         |                         |
| *Trichophyton mentagrophytes* | 25.91 ± 0.63<sup>b</sup> | 30.73 ± 0.98<sup>b</sup> | 25.75 ± 0.62<sup>d</sup> | 30.85 ± 0.78<sup>d</sup> |

Note: With the largest ZOIs, gram-positive bacteria are more sensitive to Sidr and Talh honey than the other microbes. Means with common letters are not significantly different (p ≤ .05) as analyzed by the ANOVA followed by the least significant difference (LSD) test. SEM: Standard error of mean.
tested microbial strains. The microbial strains were significantly inhibited as measured in terms of their zone of inhibition (ZOI), and a large ZOI reflects a high sensitivity of tested microbial strains. No microbial strains were resistant to any of the honey types.

The microbial strains presented differential sensitivity to the honey types. Gram-positive (G⁺) bacteria were more sensitive to both honey types (SH and TH), with significantly higher ZOI values than those of gram-positive (G⁻) bacteria and fungi (Table 2). *B. cereus* (G⁺) showed the greatest inhibition (largest ZOI) by SH (31.09 ± 0.84 mm and 36.45 ± 1.01 mm: natural and water-diluted, respectively) and TH (35.65 ± 0.53 mm and 41.65 ± 0.68 mm: natural and water-diluted, respectively). *S. aureus* (G⁺) presented the second most inhibition (ZOI) by SH (29.45 ± 0.73 and 34.55 ± 1.08 mm: natural and water-diluted, respectively) and TH (32.00 ± 0.61 and 37.70 ± 0.70 mm: natural and water-diluted, respectively). The least inhibition (smallest ZOI) was recorded for *S. enteritidis* (G⁻ bacteria) with SH (19.36 ± 0.64 and 23.36 ± 0.79 mm: natural and water-diluted, respectively) and TH (23.35 ± 0.53 and 28.10 ± 0.67 mm: natural and water-diluted, respectively). The descending sensitivity order was *B. cereus > S. aureus > T. mentagrophytes > E. coli > S. enteritidis* (Table 2). Extraordinarily, these measured ZOIs of microbial strains remained unchanged when plates were left for more than ten days, and no microbial growth occurred when new agar plates or broth media were inoculated with a loop sampling the clear zone, suggesting that both tested honey types (SH and TH) have a lethal bactericidal effect. The dermatophyte fungus (*T. mentagrophytes*) was equally sensitive to both honey types, natural honey (25.91 ± 0.63 and 25.75 ± 0.62 mm: SH and TH, respectively) and water-diluted honey (30.73 ± 0.98 and 30.85 ± 0.78 mm: SH and TH, respectively).

Antimicrobial activities of honeys were significantly amplified when natural honeys were diluted with water (33% w/v). A comparison of the antimicrobial activities of individual honey types, that is, natural SH versus water-diluted SH (Figure 2a) and natural TH versus water-diluted TH (Figure 2b) showed significantly higher inhibition in water-diluted honeys against all tested G⁺ and G⁻ bacterial strains, and fungal strains.
3.2 | Antimicrobial Activity of Antibiotics

The disk diffusion test for antibiotics evaluated the antimicrobial activity of two antibacterial (tetracycline and chloramphenicol) and two antifungal (flucoral and mycosat) antibiotics against their respective microbial strains. Our results indicated a significant difference among the antimicrobial activities of the tested antibiotics.

For antibacterial antibiotics, the largest ZOI was recorded for *S. aureus* (G⁺) against tetracycline (28.00 ± 0.67 mm) and for *B. cereus* (G⁺) against chloramphenicol (30.00 ± 0.71 mm). *S. enteritidis* exhibited the smallest ZOI (22 ± 0.79 mm) with tetracycline, whereas *S. aureus* showed the smallest ZOI (24 ± 0.70 mm) for chloramphenicol (Table 3). For antifungal antibiotics, mycosat was relatively more effective, having the largest ZOI (40.00 ± 0.75 mm), than flucoral (35.00 ± 0.79 mm) against the fungus *T. mentagrophytes* (Table 4). Of the antibacterial antibiotics, chloramphenicol was significantly more potent against *B. cereus* and *S. enteritidis*, and tetracycline was significantly more potent against *S. aureus*. However, the antimicrobial effects of these two antibiotics were significantly similar against *E. coli*. 

TH displayed higher antimicrobial activity than SH against G⁺ and G⁻ bacteria but not against the fungal strain, where both honey types were significantly similar (Figure 3). The comparison of the antimicrobial activities between natural SH and natural TH (Figure 3a), and between water-diluted SH and water-diluted TH (Figure 3b) revealed that each form of TH was more effective than the respective form of SH against a single microbial strain. Figure 4 displayed the antimicrobial activity of the tested honeys with zone of microbial growth inhibition on the cultures of tested microbial strains.

**FIGURE 4** The zone of microbial growth inhibition on the cultures of bacteria and dermatophyte fungus obtained after adding natural and water-diluted honeys: (a) Talh honey and (b) Sidr Honey
TABLE 3 Antimicrobial activities of antibacterial antibiotics against mycobacterial strains

| Microbial strain | Antibacterial antibiotics | Diameter (mm) of inhibition zone ± SEM* |
|------------------|---------------------------|----------------------------------------|
|                  | Tetracycline (30 μg/ml)   | Chloramphenicol (30 μg/ml)             |
| G+ Bacteria      |                           |                                        |
| Bacillus cereus  | 25 ± 0.68b                | 30 ± 0.71a                            |
| Staphylococcus aureus | 28 ± 0.67a              | 24 ± 0.70c                           |
| G- Bacteria      |                           |                                        |
| Escherichia coli | 24 ± 0.62b                | 25 ± 0.78b.c                          |
| Salmonella enteritidis | 22 ± 0.79c           | 27 ± 0.67b                           |

*Well-diffusion assay. Means with the common letters within the same column are not significantly different from each other (p ≤ .05) as analyzed by the ANOVA followed by the least significant difference (LSD) test. SEM: Standard error of mean.

TABLE 4 Antimicrobial activities of antifungal antibiotics against the fungal strain

| Fungal strain | Antibiotics (Antifungal) | Diameter (mm) of inhibition zone ± SEM* |
|---------------|--------------------------|----------------------------------------|
|               | Trichophyton mentagrophytes |                                        |
|               | Flucoral (100 μg/ml)      | 35.00 ± 0.79b                         |
|               | Mycosat (100 μg/ml)       | 40.00 ± 0.75a                         |

*Disk diffusion assay. Means with the common letters within the same column are not significantly different from each other (p ≤ .05) as analyzed by the ANOVA followed by the least significant difference (LSD) test. SEM: Standard error of mean.

Of the antifungal antibiotics, mycosat showed significantly higher antimicrobial action against T. mentagrophytes than flucoral (Figure 5).

3.3 Comparison Among Antimicrobial Action of Honey and Antibiotics

It is apparent from the data analysis that the high antimicrobial activity (larger ZOIs) shown by bacterial strains particularly with water-diluted SH (Figure 6a) and water-diluted TH (Figure 6b) is significantly greater than that of the tested broad-spectrum antibacterial antibiotics (tetracycline and chloramphenicol). S. enteritidis (gram-negative bacteria) treated with water-diluted SH showed exception where ZOIs values were significantly lower than chloramphenicol but significantly at par with tetracycline (Figure 6a). Nevertheless, antifungal antibiotics exhibited significantly higher antimicrobial activity against the fungal strain than the tested water-diluted SH and TH honeys (Figure 7).

4 DISCUSSION

4.1 Antimicrobial activity of honeys

Saudi Sidr honey (SH) and Talh honey (TH) displayed substantial antimicrobial activities against tested pathogenic microbial strains. These primary findings strengthened the idea for using Saudi honeys as potential alternative broad-spectrum strategy to treat bacterial and fungal infections. Use of various types of honeys due to its antimicrobial effects has been published in numerous studies (Bradshaw, 2011; Israïl, 2014; McLoone, Warnock, & Fyfe, 2016). However, more extensive research is necessary for conclusive declaration as substituting broad-spectrum antibacterial drugs with these Saudi honeys. Some previous studies described the physiochemical properties of honeys and compared the antimicrobial action of various Saudi honey types with those of imported honeys in different experimental setups (Al-Nahari et al., 2015; Alqurashi, Masoud, & Alamin, 2013; Hegazi & Abd Allah, 2012). However, the present study compared the two most preferred local Saudi honeys for their antimicrobial potential against bacterial and fungal strains. It is concluded that TH possesses higher antimicrobial activity against bacterial strains than SH. These findings are confirmed by the noticeable higher acidity and total phenolic contents in TH than in SH (Alqarni et al., 2016). The phenolic contents in honey are directly connected with increased antibacterial activities (Alvarez-Suarez et al., 2010; Stagos et al., 2018). Another possible factor for the substantial antimicrobial activity of honey is probably the synergism between H2O2 and phenolic compounds that exert a pro-oxidant activity that may lead to the degradation plasmidic DNA (Poli et al., 2018). The difference in floral origin of tested honey types could be another potential dominant reason for their differential antimicrobial activities (Allen, Molan, & Reid, 1991; Elbanna et al., 2014; Willix, Molan, & Harfoot, 1992). In addition, the geographical location and seasonality could also influence the antimicrobial activity of different honey types (Al-Waili, Salom, Butler, & Al Ghamdi, 2011; Molan & Cooper, 2000). A previous study reported that SH had higher antibacterial activity than Somur and Meria honeys (A-Haik, Al-Haddad, Al-kaf, Hassan, & Edrees, 2018). In contrast, our study illustrated another honey (TH) exhibiting superior antibacterial activity against selected bacterial strains compared to SH.

The diameter for the zone of inhibition (ZOI) indicates the sensitivity of microbial strains. All recorded diameters of the ZOIs in the present study were greater than 11 mm. This result aligns well with the declaration of Agbagwa and Frank-Peterside (2010) that “the diameter of inhibition zones less than 7 mm corresponds to resistant microorganisms and greater than 11 mm suggests that the microorganisms are sensitive to antimicrobial agent.” Thus, our findings are consistent in that all tested microbial strains were sensitive to tested honeys, and these honeys are proposed as prospective antimicrobial agents to benefit human health.

Both SH and TH showed broad-spectrum antimicrobial potential against G+ and G- bacteria and fungi, which is consistent with...
previous findings in which different honey types of diverse floral origins were reported with broad-spectrum activity against G+ and G- bacteria (Almasaudi et al., 2017; Al-Naama, 2009; Elbanna et al., 2014; Irish, Blair, & Carter, 2011; Lusby, Coombes, & Wilkinson, 2002; Radwan, El-Essawy, & Sarhan, 1984). The microbial strains presented differential sensitivity to the honey types: G+ bacteria were more sensitive than G- bacteria and fungi. Hegazi and Abd Allah (2012) reported Saudi honeys (20.30%) from twelve different floral sources (including Sidr) as effective antibacterial agents against G+(S. aureus, Streptococcus pyogenes, Corynebacterium pseudotuberculosis) and G-(Klebsiella pneumoniae, Pseudomonas aeruginosa, and E. coli) bacterial pathogens. These honeys were less effective against E. coli than the other bacteria and contradict our findings in which SH and TH were significantly effective against E. coli, similar to other tested microbial strains. In partial confirmation, Saudi Sidr honey was found to be more efficient than mountain honey against G- bacteria (E. coli, K. pneumoniae, P. aeruginosa and A. baumannii), with a high sensitivity of E. coli toward Sidr honey (Alqurashi et al., 2013). Saudi honeys named Shaoka (Fagonia cretica) and Taify Sidr (Z. spina-christi) were more potent than Manuka honey (Leptospermum scoparium) against single G- bacteria (S. enteritidis) in terms of ZOI equivalents in phenol percentages (7.3%, 8.4%, and 6.9%), respectively, and antimicrobial activity was independent of the honey color (Halawani & Shohayeb, 2011).

SH and TH presented lethal bactericidal and fungicidal effects because no further change in the inhibition zone was detected even after ten days. Al-Nahari et al. (2015) evaluated that the antimicrobial effect of Manuka honey (L. scoparium) against both antibiotic (imipenem)-resistant and antibiotic-sensitive bacteria (P. aeruginosa).
Manuka honey was bactericidal, but Seder and *N. sativa* honeys were only bacteriostatic. In contrast, SH was completely bactericidal against our tested bacterial strains.

Saudi honeys showed dose-dependent antibacterial activity: Sidr (*Z. spina-christi*) and Dharm (*Lavandula dentata*) were more potent at high concentrations (50%–80% w/v) against *E. coli*, *Proteus mirabilis*, *S. aureus*, *Shigella flexneri*, and *S. epidermidis* than Majra honey (*Hypoestes forskalii*) (Ghramh, Khan, & Alshehri, 2018). In contrast, only one concentration of water-diluted honey (33% w/v) was adopted from Elbanna et al. (2014) and substantially inhibited the tested microbial strains. Exploring the antimicrobial activity with a series of honey dilutions could be a potential future investigation to determine the dose dependency (if any).

SH and TH honeys also demonstrated equal fungicidal potential against a dermatophytic fungus (*T. mentagrophytes*) with high inhibition. This is in line with previous studies regarding the antifungal action of other honey types (Manuka, Medihoney, Nigerian, etc.) for some yeasts and fungi, such as *Aspergillus*, *Penicillium*, *Candida*, and common dermatophytes (*Anyanwu, 2012; Brady, Molan, & Harfoot, 1996; Carter, Blair, Irish, & Shokohi, 2006*). Conversely, fungi (*Aspergillus nidulans*) were less sensitive to honey samples, including Talh and Sidr, than bacteria (Al-Waili et al., 2013).

Water-diluted (33% w/v) honeys revealed an elevated antimicrobial activity as compared to nondiluted honeys. An enzymatic reaction of glucose oxidase is being active in water–honey medium. Hydrogen peroxide is produced when glucose oxidase oxidizes glucose to gluconic acid (Mandal & Mandal, 2011). Synthesis of hydrogen peroxide in water-diluted honeys could be the potential reason for elevated antimicrobial activity. This also explains why nectar (in plant or in bee stomach or in unripe honey) is not infected with microbes. The dilutions of honey between 30% and 50% (v/v) led to maximum levels of accumulated hydrogen peroxide (Bang, Bunting, & Molan, 2003), and the dilution range was similar to our tested honey dilution concentrations (33% w/v). However, the antimicrobial activity of honey is extremely complex and might be due to the involvement of multiple compounds and several nonperoxide components that are also reported to contribute to the unique antibacterial activity of honey, such as physico-chemical properties, osmotic pressure, acidic pH, and nonperoxide phytochemical components, including antioxidants and antimicrobial peptides (Ayaad, Shaker, & Almuhnaa, 2009; Brudzynski, 2006; Halawani & Shohayeb, 2011; Kwakman & Zaat, 2012; Mavric, Wittmann, Barth, & Henle, 2008; Molan, 1992; Molan & Russell, 1988; Simon et al., 2009). Elbanna et al. (2014) attributed the antimicrobial activity of three unifloral Egyptian honeys (~88%) to nonperoxide agents, whereas hydrogen peroxide contributed less (~12%) to the tested honeys. In contrast, some scientists reported a fourfold decline in the antimicrobial activity of honey upon dilution (Adeleke, Onakoya, & Ali, 2002; Olaitan, Adeleke, & Ola, 2007), possibly due the presence of catalase in water that neutralized the hydrogen peroxide (Szveda, 2017). Due to the presence of numerous compounds in honey, bacterial resistance is less likely to be developed in honey-treated bacteria (Carnwath, Graham, Reynolds, & Pollock, 2014; Machado De-Melo et al., 2018), favoring the use of honeys against microbial infections.

### 4.2 Antimicrobial activity of antibiotics

In the present study, broad-spectrum antibacterial (tetracycline and chloramphenicol) and antifungal (flucoral and mycosat) antibiotics were also effective against their respective microbes. Interestingly, the antibacterial activity of water-diluted SH and TH was greater than that of the tested antibacterial antibiotics. These findings should be considered as indicative rather than conclusive, as varied doses and two different testing methods were used for evaluation of antimicrobial activity. Karayil, Deshpande, and Koppikar (1998) and Elbanna et al. (2014) found that water-diluted honey inhibited the growth of certain pathogenic bacteria relatively more than some antibiotics. Although the tested antibiotics and bacterial strains were different from those in our study, the elevated effectiveness of water-diluted honey over tested antibiotics is in consistent with our findings. Agbagwa and Frank-Peterside (2010) found better antibacterial activity for SH than for imipenem (antibiotic) against a pathogenic G− bacterium (*P. aeruginosa*) and partially supported our results regarding superior antibacterial activity of SH compared with tested antibiotics. Nigerian honey samples (40% v/v) gave better antimicrobial activity against *P. aeruginosa*, *S. aureus*, *E. coli*, and *K. pneumoniae* than four antibiotics, namely amoxicillin, streptomycin, ceftriaxone, and erythromycin (Braide et al., 2012). Based on the published reports in literature (Israili, 2014; Liu et al., 2018), it is likely predictable that the use of honey in combination with antibiotics could synergize the antimicrobial activity. Müller et al. (2013) found a synergistic effect between Medihoney and rifampicin antibiotic on *S. aureus* but not between Manuka honey and rifampicin. Thus, further investigations with different honeys and common broad-spectrum antibiotics may unveil their...
synergism against microbes to establish their parallel use as an effective antimicrobial therapy.

4.3 | Honey as a promising therapeutic alternative to antimicrobial agents

Honey is traditionally used as therapeutic agent against skin infections and wounds caused by microbial pathogens (Israili, 2014; Liu et al., 2014; McLoone et al., 2016). Our results presented the potent antimicrobial prosperities of SH and TH against skin infection causing bacterial agents and dermatologically important filamentous fungi. These findings suggest the prospective use of Saudi honeys in the clinical treatments of different microbial infections. The antimicrobial activity of honey could be due to its various contents such as high sugar, total phenolic compounds and hydrogen peroxide levels. Furthermore, the bactericidal mechanisms of these content may include DNA degrading activity, interruption of cell division, alteration in the cell morphology and general loss of structural integrity of the microbial cell (Israili, 2014; Johnston, McBride, Dahiya, Owusu, & Nigam, 2018). The microbes may not develop resistance against honey in the same way as they develop for other commonly used antimicrobial agents. These features may make the honey a promising alternative to the commonly used antibiotics.

5 | CONCLUSION

Conclusively, Sidr and Talh honey samples have significant antimicrobial potential against gram-positive and gram-negative bacteria and dermatophytic fungi regardless of the sample origin. Talh honey was more potent against tested microbial strains than Sidr honey. Water dilution of honeys elevated the antimicrobial activity above that of natural nondiluted honeys. Microbial strains showed differential sensitivity, and G+ bacteria were more sensitive than G- bacteria and fungi. The in vitro antimicrobial activity of honeys was comparable with that of common broad-spectrum antibacterial antibiotics. Our findings are indicative of the potential antimicrobial quality of Saudi honeys considered in honey standards, and further investigations are necessary to standardize the Sidr and Talh honeys for their therapeutic applications as medical-grade honeys.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

AUTHOR CONTRIBUTIONS

Ayman A. Owayss and Abdulaziz S. Alqarni designed the field experiments and executed them with the contributions of Javaid Iqbal and Hael S.A. Raweh. Khaled Elbanna, Hussein H. Abulreesh and Sameer R. Organji planned and conceived the laboratory tests. Ayman A. Owayss. and Khaled Elbanna arranged the data and wrote the preliminary manuscript. Javaid Iqbal analyzed the results, constructed the graphs and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

ETHICAL STATEMENT

This study does not involve any human or animal testing. Informed Consent: Consent was obtained from all study participants for its submission in this journal.

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REFERENCES

Abuharfeil, N., Al-Oran, R., & Abo-Shehada, M. (1999). The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytes, Food and Agricultural Immunology, 11(2), 169–177. https://doi.org/10.1080/0954010999843

Adeleke, O. E., Onakoya, T. M., & Alli, S. S. (2002). Exposure of bacterial isolates from different pathological sources to honey. African Journal of Medical and Pharmaceutical Sciences, 7, 79–83.

Adgaba, N., Al-Ghamdi, A., Tadesse, Y., Getachew, A., Awad, A. M., Ansari, M. J., ... Alqarni, A. S. (2017). Nectar secretion dynamics and honey production potentials of some major honey plants in Saudi Arabia. Saudi Journal of Biological Sciences, 24(1), 180–191. https://doi.org/10.1016/j.sjbs.2016.05.002

Agbagwa, O. E., & Frank-Peterside, N. (2010). Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria. African Journal of Microbiology Research, 4(16), 1801–1803.

Al-Ghamdi, A. A. (2007). Evaluation of various honeybee foraging activities for identification of potential bee plant in Riyadh, Saudi Arabia. Annals of Agricultural Science Faculty of Agriculture, Ain-Shams University, 52(2), 487.

Al-Ghamdi, A., & Adgaba, N. (2015). Beekeeping in the Kingdom of Saudi Arabia past and present practices. Bee World, 90(2), 26–29. https://doi.org/10.1080/0005772X.2013.11417527

Al-Haik, W., Al-Haddad, A. M., Al-kaf, A., Hassan, W., & Edrees, W. H. (2018). Antimicrobial activities for Hadhrami honey on growth of some pathogenic bacteria. Universal Journal of Pharmaceutical Research, 2(6), 7–12. https://doi.org/10.22270/ujpvr.v2i6.R2

Al-Khalifa, A. S., & Al-Arify, I. A. (1999). Physicochemical characteristics and pollen spectrum of some Saudi honeys. Food Chemistry, 67(1), 21–25. https://doi.org/10.1016/S0308-8146(99)00096-5

Allen, K. L., Molan, P. C., & Reid, G. M. (1991). A survey of the antibacterial activity of some New-Zealand honeys. Journal of Pharmacy and Pharmacology, 43(12), 817–822. https://doi.org/10.1111/j.2042-7158.1991.tb03186.x

Almasaudi, S. B., Al-Nahari, A. A. M., Abd El-Ghany, E. S. M., Barbour, E., Al Muhayawi, S. M., Al-Jaouni, S., ... Harakeh, S. (2017). Antimicrobial effect of different types of honey on Staphylococcus aureus. Saudi Journal of Biological Sciences, 24(6), 1255–1261. https://doi.org/10.1016/j.sjbs.2016.08.007

Al-Naama, R. T. (2009). Evaluation of in-vitro inhibitory effect of honey on some microbial isolate. Journal of Bacteriological Research, 1(6), 064–067.

Al-Nahari, A. A. M., Almasaudi, S. B., El-Ghany, E. S. M. A., Barbour, E., Al Jaouni, S. K., & Harakeh, S. (2015). Antimicrobial activities of Saudi honey against Pseudomonas aeruginosa. Saudi Journal of
eradicating staphylococcus aureus biofilms. Frontiers in Microbiology, 8, 2653–2653. https://doi.org/10.3389/fmicb.2017.02653
Liu, M., Lu, J., MÄ¼ller, P., Turnbull, L., Burke, C. M., Schlothauer, R. C., ... Harry, E. J. (2014). Antibiotic-specific differences in the response of Staphylococcus aureus to treatment with antimicrobials combined with manuka honey. Frontiers in Microbiology, 5, 779. https://doi.org/10.3389/fmicb.2014.00779
Lusby, P. E., Coombes, A., & Wilkinson, J. M. (2002). Honey: A potent agent for wound healing? Journal of Wound, Ostomy and Continence Nursing, 29(6), 295–300. https://doi.org/10.1097/00152192-200201000-00008
Lusby, P. E., Coombes, A. L., & Wilkinson, J. M. (2005). Bactericidal activity of different honeys against pathogenic bacteria. Archives of Medical Research, 36(5), 464–467. https://doi.org/10.1016/j.arcmed.2005.03.038
Machado De-Melo, A. A., Almeida-Muradian, L. B. d., Sancho, M. T., & Pascual-Maté, A., (2018). Composition and properties of Apis mellifera honey: A review. Journal of Apicultural Research, 57(1), 5–37. https://doi.org/10.1080/00218839.2017.1338444
Maddocks, S. E., & Jenkins, R. E. (2016). Honey: A realistic antimicrobial for disorders of the skin. Journal of Microbiology, Immunology and Infection, 49(2), 154–160. https://doi.org/10.2221/022121-1691(11)60016-6
Mandl, M. D., & Mandal, S. (2011). Honey: Its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine, 1(2), 154–160. https://doi.org/10.1016/S2221-1691(11)60016-6
Mandal, S., Pal, N. K., Chowdhury, I. H., & Debmandal, M. M. (2009). Antibacterial activity of ciprofloxacin and trimethoprim, alone and in combination, against Vibrio cholerae O1 biotype El Tor serotype Ogawa isolates. Polish Journal of Microbiology, 58(1), 57–60.
Mavric, E., Wittmann, S., Barth, G., & Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. Molecular Nutrition and Food Research, 52(4), 483–489. https://doi.org/10.1002/mnfr.200700282
McLoone, P., Warnock, M., & FYfe, L. (2016). Honey: A realistic antimicrobial for disorders of the skin. Journal of Microbiology, Immunology and Infection, 49(2), 161–167. https://doi.org/10.1016/j.jmii.2015.01.009
Molan, P. C. (1992). The antibacterial activity of honey: 1. The nature of the antibacterial activity. Bee World, 73(1), 5–28. https://doi.org/10.1080/0005772X.1992.11099109
Molan, P. C. (2001). Potential of honey in the treatment of wounds and burns. American Journal of Clinical Nutrition, 21(1), 13–19. https://doi.org/10.2165/00128071-200102000-00003
Molan, P. C., & Cooper, R. A. (2000). Honey and sugar as a dressing for wounds and ulcers. Tropical Doctor, 30(4), 249–250. https://doi.org/10.1177/004947550003000429
Molan, P. C., & Russell, K. M. (1988). Non-peroxide antibacterial activity in some New Zealand honeys. Journal of Apicultural Research, 27(1), 62–67. https://doi.org/10.1080/00218839.1988.11007083
Müller, P., Alber, D. G., Turnbull, L., Schlothauer, R. C., Carter, D. A., Whitchurch, C. B., & Harry, E. J. (2013). Synergism between medihoney and rifampicin against methicillin-resistant Staphylococcus aureus (MRSA). PLoS ONE, 8(2), e57679. https://doi.org/10.1371/journal.pone.0057679
Olaitan, P. B., Adeleke, O. E., & Ola, I. O. (2007). Honey: A reservoir for microorganisms and an inhibitory agent for microbes. African Health Sciences, 7(3), 159–165. https://doi.org/10.5555/ahs.2007.7.3.159
Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017). Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. Oxidative Medicine and Cellular Longevity, 2017, 1–21. https://doi.org/10.1155/2017/1259510
Patton, T., Barrett, J., Brennan, J., & Moran, N. (2006). Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. Journal of Microbiological Methods, 64(1), 84–95. https://doi.org/10.1002/j.mimet.2005.04.007
Poli, J. P., Guinoiseau, E., Luciani, A., Yang, Y., Battesti, M. J., Paolini, J., ... Lorenzi, V. (2018). Key role of hydrogen peroxide in antimicrobial activity of spring. Honeydew maquis and chestnut grove Corsican honeys on Pseudomonas aeruginosa DNA. Letters in Applied Microbiology, 66(5), 427–433. https://doi.org/10.1111/lam.12868
Radwan, S. S., El-Essawy, A. A., & Sarhan, M. M. (1984). Experimental evidence for the occurrence in honey of specific substances active against microorganisms. Zentralblatt Für Mikrobiologie, 139(4), 249–255. https://doi.org/10.1016/S0232-4393(84)80047-5
Rienzo et al., 2016Rienzo, J., Casanoves, F., Balzarini, M., Gonzalez, L., Tablada, M., & Robledo, C. (2016).InfoStat versión 2016 InfoStat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina. Retrieved from http://www.info Stat.com.ar/
Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: A review of recent clinical research. Pharmacognosy Research, 9(2), 121–127. https://doi.org/10.4103/0974-8490.204467
Saranraj, P., & Sivasakthi, S. (2018). Comprehensive review on honey: Biochemical and medicinal properties. Journal of Academia and Industrial Research, 6(10), 165–181.
Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U., & Molan, P. (2009). Medical honey for wound care–still the ‘latest resort’? Evidence-Based Complementary and Alternative Medicine, 6(2), 165–173. https://doi.org/10.1093/ecam/nem175
Stagos, D., Soulitsiotis, N., Tsadila, C., Papaeconomou, S., Arvanitis, C., Ntontos, A., ... Mossialos, D. (2018). Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. International Journal of Molecular Medicine, 42(2), 726–734. https://doi.org/10.3892/ijmm.2018.3656
Szweza, P. (2017). Antimicrobial activity of honey. In V. A. Arnaud de Toledo (Ed.), Honey analysis, Vol. 1 (pp. 215–232). InTech: Rijeka, Croatia.
Willix, D. J., Molan, P. C., & Harfoot, C. G. (1992). A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. The Journal of Applied Bacteriology, 73(5), 388–394. https://doi.org/10.1111/j.1365-2672.1992.tb04993.x
Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honey bees. Annual Review of Entomology, 63(1), 327–344. https://doi.org/10.1146/annurev-ento-020117-043423
Zakaria, A. S. (2015). Mechanism of antibacterial action of honey on pathogenic wound bacterial strains: A proteomic analysis. International Research Journal of Pharmacy, 6(1), 778–788. https://doi.org/10.7897/2230-8407.0611151

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