



## Original article

## Antibacterial potential of some Saudi honeys from Asir region against selected pathogenic bacteria

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## ABSTRACT

Honey is a nutrient rich natural product and has been utilized as traditional and complementary medicine since ancient times. In this study, antibacterial activity of Sider (*Ziziphus spina-christi*), Dharm (*Lavandula dentata*), and Majra (*Hypoestes forskalii*) honey samples collected from Asir region of Saudi Arabia was *in vitro* evaluated at 80% and 50% w/v concentrations against five pathogenic bacteria i.e. *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Shigella flexneri*, and *Staphylococcus epidermidis*. Well diffusion assays to measure the average zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) values were employed in the experiments. All the tested honey samples showed antibacterial activity in a dose-dependent manner. Sider and Dharm exhibited a good antibacterial activity at high concentrations while, Majra honey of *Apis mellifera jemenitica* and of *Apis florea* showed comparatively low antibacterial activity. The average MIC values of Sider, Dharm from Rijal Alma, Dharm from Al-Souda, Majra (*A.m. jemenitica*), and Majra (*A. florea*) honey against all tested bacteria were 22%, 16%, 18%, 32%, and 28% (v/v) respectively. Dharm and Sider honeys showed better antibacterial activity than Majra honey. Saudi honey can be considered as a promising future antimicrobial agent and should be further investigated as an alternative candidate in the management of resistant bacterial pathogens.

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## 1. Introduction

Antimicrobial agents used to treat infectious diseases decrease the threats posed by microbes but, the development of resistant pathogens and their spread, diminishes the effectiveness of these agents gradually (Alqurashi et al., 2013). Bacterial resistance to antimicrobial agents is serious challenge to public health and all types of antibiotics including the major last-resort drugs (Levy and Marshall, 2004; Mandal et al., 2009). Excessive use of antibiotics throughout the world especially in underdeveloped and developing countries may spread resistance in the community and make the eradication of infectious diseases very difficult (Patel and Chauhan, 2017). Therefore, to search some novel

antimicrobial agents, scientists are keen to drug discovery from natural products that exhibit antibacterial properties. These circumstances demanded to reevaluate the therapeutic use of ancient remedies including honey (Bagde et al., 2013; Mandal et al., 2010).

Honey is a valuable functional food with a plenty of nutrients and it has been utilized as traditional and complementary medicine since ancient times. It has numerous beneficial biological activities, like antibacterial, antioxidant, anti-browning (Alvarez-Suarez et al., 2010; Chang et al., 2011), angiotensin converting enzyme (ACE) inhibitory (León-Ruiz et al., 2013), anti-inflammatory (Liu et al., 2013), antiparasitic (Zeina et al., 1997), and immunosuppressive (Michaluart et al., 1999). Recently, its therapeutic role in the treatment of burns, healing of infected and chronic wounds (Lay-Flurrie, 2008), skin ulcers, eye ailments, asthma, gastrointestinal disorders (Ferreira et al., 2009), and its medicinal effects like anticancer (Lopez-Lázaro, 2007), antimutagenic, antiproliferative, hepatoprotective and hypoglycaemic properties have been ascribed (Al-Waili et al., 2011). The main components of honey are fructose and glucose (~75%) with low quantities of sucrose and some polysaccharide sugars (Alqarni et al., 2014; Khan et al., 2016). However, minerals, proteins, phenolic compounds, and other minor components also greatly

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contribute to its biological activities (Moniruzzaman et al., 2013). Honey composition and biological activity differ according to its botanical origin and geographical location (Alzahrani et al., 2012).

Physical parameters (osmosis and acidity) and chemical properties of honey are mainly responsible for its antibacterial activity (Weston, 2000). The main constituent of honey that contributes for antibacterial activity is hydrogen peroxide. Glucose found in honey is oxidized by an enzyme glucose oxidase and results into the formation of hydrogen peroxide. The source of glucose and other sugars is floral nectar while the glucose oxidase is secreted by honey bee glands (León-Ruiz et al., 2013). This enzyme remains inactive till the honey is diluted, because the high sugar concentration stops the enzyme to work (Weston, 2000). Besides hydrogen peroxide some other molecules found in honey also contribute to its antibacterial activity and are called “non-peroxide” components. These substances are proteinaceous in nature like lysozyme (Snowdon and Cliver, 1996), flavonoids (flavones, flavonols, flavonones, and dihydroflavonols) and other phenolic compounds (cinnamic acids and their esters), methylglyoxal and bee peptides (Israili, 2014). Variation in antibacterial activity of a honey depends on its botanical origin (León-Ruiz et al., 2013), its type, and geographical location (Molan and Cooper, 2000).

Honey inhibits a broad spectrum of bacterial species. The antagonistic effect of honey to almost 60 bacterial species including aerobes, anaerobes, Gram positives, and Gram negatives has been reported (Hannan et al., 2004). In many studies antibacterial properties of honey have been reported against pathogenic bacteria including *Acinetobacter baumannii*, *Bacillus cereus*, *B. subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. typhimurum*, *Shigella flexneri*, *Shigella sonnei*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* (Al-Nahari et al., 2015; Al-Waili et al., 2013; Alqurashi et al., 2013; Deng et al., 2018; Hegazi et al., 2017; Kingsley, 2001; Nzeako and Hamdi, 2000; Rani et al., 2017; Wasihun and Kasa, 2016).

Beekeeping is an established practice and one of the most important economic activities for rural communities in the Kingdom of Saudi Arabia. Almost 5,000 beekeepers keep > 1,000,000 honey bee colonies with ~ 9,000 metric tons of honey production annually (Al-Ghamdi and Nuru, 2013). Taif, Baha, and Asir (mountainous regions) in the Southwest are bestowed with plenty of natural flora and are most suitable for beekeeping in the country (Alqarni et al., 2011). Many kinds of honey are specific to these areas and local people have been using honey as curative agent either alone or admixed with some medicinal plants for management of many diseases. Therefore, the aim of this study was to investigate antibacterial activities of different honey samples collected from Asir region, Saudi Arabia against various kind of bacterial pathogens.

## 2. Materials and methods

### 2.1. Honey samples

Five honey samples from various botanical origins i.e. two samples of Dharm (*Lavandula dentata*), two sample of Majra (*Hypoestes*

*forskaolii*) from different locations, and one sample of Sider (*Ziziphus spina-christi*) were used in the experiment and each sample was assigned a code either according to its location or collecting bee species (Table 1). The samples of raw honey except Majra (2) were collected from local beekeepers having native honey bees (*Apis mellifera jemenitica*) in traditional log hives placed at different locations (Fig. 1). All honey samples were stored in a refrigerator at 4 °C till the start of experiment. A loopful quantity of each honey sample was spread on nutrient agar medium to monitor the sterility of honey (Mulu et al., 2004).

### 2.2. Bacterial isolates and media

Bacterial strains used in this study were obtained from the Microbiological laboratory, Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia. They included Gram negative *Escherichia coli*, *Proteus mirabilis*, *Shigella flexneri*, and Gram Positive *Staphylococcus aureus*, *Staphylococcus epidermidis*. These bacteria were identified by standard bacteriological techniques following Harley (2004) and were maintained on nutrient agar slants at 4 °C. Nutrient agar and nutrient broth (HiMedia Laboratories Pvt. Ltd. India) were used in the experiment and prepared according to the instructions of the manufacturer.

### 2.3. Preparation of honey concentrations

Two different concentrations of each honey sample were prepared using sterile distilled water to test antibacterial activity. Ten gram of each honey was weighed in 50 mL beaker (Karter Scientific, USA) by using electric balance (Shimadzu Corporation, Japan) then 16 mL and 10 mL water was added to make 80% and 60% (w/v) concentrations respectively. Water and honey quantities required for different concentrations were calculated using the formula:  $C1 \times V1 = C2 \times V2$ .

### 2.4. Well diffusion assay for antibacterial activity

Antibacterial activity of various concentration of honey samples was determined by agar well diffusion assay. Bacterial isolates were inoculated in 10 mL nutrient broth and placed overnight in shaking incubator (Sheldon Manufacturing, Inc. USA) at 37 °C. Nutrient agar plates were made by following the instructions of manufacturer. Five wells (~6 mm diameter) were made in each nutrient agar plate by using distal end of sterile Pasteur pipette. Before making wells, each bacterial suspension (~10<sup>8</sup> colony forming unit (cfu)/mL) was spread on single agar plate with sterile cotton swap (Citotest Labware manufacturing Co. Ltd. China). One hundred micro liter of each honey sample was deposited into a separate well on the nutrient agar plate. These petri plates were incubated aerobically at 37 °C for 24 h in an incubator (Nüve Sanayi Malzemeleri, Turkey). The diameter of zone of inhibition around the outer surface of well was measured by following Barry and Thorsberry (1985).

**Table 1**

Honey type, botanical origin, collecting honey bee species, apiary location, sample code, and color designation of different honey samples used in the experiment.

No.	Local name	Honey sample code	Botanical origin	Collecting bee species	Apiary location	Designated color
1	Sider	SDR	<i>Ziziphus spina-christi</i>	<i>Apis mellifera jemenitica</i>	Rijal Alma	Light Amber
2	Dharm (1)	DHS	<i>Lavandula dentata</i>	<i>Apis mellifera jemenitica</i>	Al-Souda	Amber
3	Dharm (2)	DHR	<i>Lavandula dentata</i>	<i>Apis mellifera jemenitica</i>	Rijal Alma	Amber
4	Majra (1)	MJM	<i>Hypoestes forskaolii</i>	<i>Apis mellifera jemenitica</i>	Sarat Abidah	White
5	Majra (2)	MJF	<i>Hypoestes forskaolii</i>	<i>Apis florea</i>	Rijal Alma	Extra White

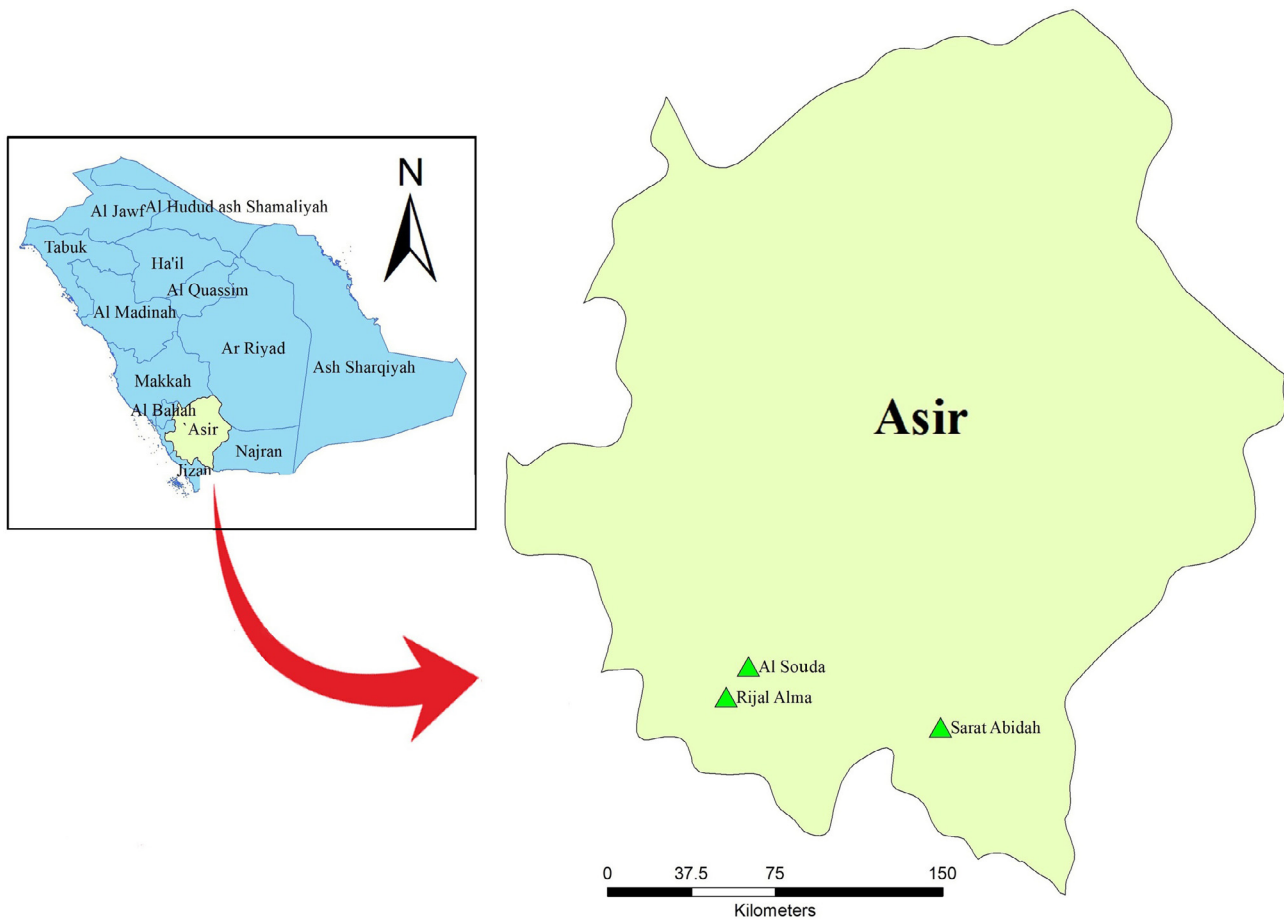


Fig. 1. Honey collection sites indicated by green triangles within the map of Asir region. Inset, location of Asir region within map of Saudi Arabia.

### 2.5. Determination of minimum inhibitory concentration (MIC)

MIC of honey samples was determined by following the method of Wasihun and Kasa (2016) with some modifications. Eight clean test tubes ( $13 \times 100$  mm) were placed in a stand. Nutrient broth was prepared according to manufacturer instructions and employed for preparation of serial dilution test tubes. Two mL of pure honey (100%) was added to a test tube which served as positive control while, another test tube received only 2 mL of nutrient broth but no bacterial suspension (negative control). For remaining six test tubes, a serial dilutions of honey sample were made that contained 2 mL final volume of nutrient broth to give the concentrations of 80%, 60%, 40%, 20%, 10%, and 5% (v/v). Each tube except negative control was inoculated with  $20 \mu\text{L}$  of bacterial suspension ( $\sim 10^8$  cfu/mL) and then incubated at  $37^\circ\text{C}$  for 24 h. The whole process was repeated for each honey sample in triplicate against all the bacteria. The MIC was observed by visual inspections for the presence and absence of growth (turbidity).

### 2.6. Statistical analysis

All results relating to antibacterial activity in terms of ZOI were expressed as the means of three replicates  $\pm$  standard deviation (SD). The analysis was made using Statistix 8.1 software. All pairwise comparison of means was performed using Tukey's Honest Significant Difference (HSD) test. Differences between means at  $p < 0.05$  were considered as statistically significant. The cluster analysis of five honey samples at 80% and 50% concentrations against five bacterial strains (Table 1) was performed in order to

know the similarity based on botanical origin of tested honey samples. Mean values of ZOI were used to compare the honey samples. The dendrogram was constructed using Ward's Linkage method with Euclidean distances. Discriminant analysis was performed using Past v.3.12 software.

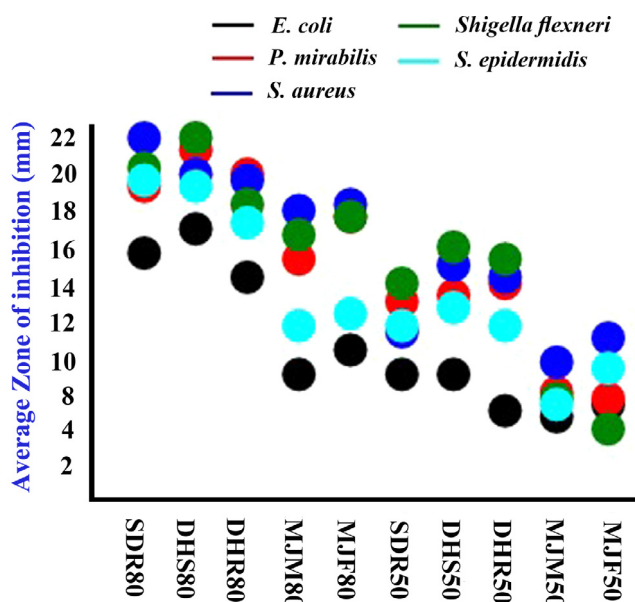
### 3. Results

The results of *in vitro* antibacterial activity (in terms of average ZOI) by different honey samples (Table 1) at two concentrations (80% and 50% w/v) against gram-positive (*S. aureus*, *S. epidermidis*) and gram-negative bacteria (*E. coli*, *P. mirabilis*, and *Shigella flexneri*) by well diffusion assay are summarized in Table 2 and their graphical representation was shown in Fig. 2. All the tested honeys inhibited the bacterial pathogens and significant differences were observed among most of the samples. The maximum ZOI ( $15.33 \pm 1.1$  mm) against *E. coli* was recorded from Dharm-1 honey (DHS) at the concentration of 80% (w/v). It was collected from Al-Souda region while, the minimum ZOI ( $5.00 \pm 1.0$  mm) was observed from Majra honey (MJM) of *A. m. jemenitica* at the concentration of 50% (w/v) collected from Sarat Abidah. The highest ZOI ( $19.67 \pm 0.6$  mm) against *P. mirabilis* was measured from DHS at the concentration of 80% (w/v) while, the minimum ZOI ( $6.00 \pm 1.0$  mm) was observed in Majra honey (MJF) of *A. florea* at the concentration of 50% (w/v) collected from Rijal Alma. MJM also showed minimum ZOI ( $6.33 \pm 0.6$ ) at the concentration of 50% (w/v) which was not statistically different from MJF. The highest ZOI ( $20.34 \pm 1.1$  mm) against *Shigella flexneri* was measured from DHS at the concentration of 80% (w/v) while, the minimum ZOI

**Table 2**

Measurement of average zone of inhibition (mm) produced by Saudi honey samples at different concentrations against five bacterial pathogens.

No.	Bacterial Strain	Average zone of inhibition (ZOI) in "mm"									
		Concentration of honey sample (80% W/V)					Concentration of honey sample (50% W/V)				
		SDR	DHS	DHR	MJM	MJF	SDR	DHS	DHR	MJM	MJF
1	<i>Escherichia coli</i>	14.00 ± 1.0ab	15.33 ± 1.1a	12.67 ± 0.6b	7.33 ± 0.6c	8.67 ± 0.6c	7.33 ± 1.1c	7.33 ± 1.1c	5.33 ± 0.6d	5.00 ± 1.0e	5.67 ± 0.6d
2	<i>Proteus mirabilis</i>	17.67 ± 1.5bc	19.67 ± 0.6a	18.34 ± 1.5ab	13.67 ± 1.0d	16.00 ± 1.3c	11.33 ± 1.5e	11.67 ± 1.5e	12.33 ± 1.1de	6.33 ± 0.6f	6.00 ± 1.0f
3	<i>Shigella flexneri</i>	18.67 ± 1.3a	20.34 ± 1.1a	16.67 ± 1.3b	15.00 ± 1.0bc	16.00 ± 1.1b	12.34 ± 1.1d	14.34 ± 0.6c	13.67 ± 1.5 cd	6.00 ± 1.0e	4.33 ± 0.6e
4	<i>Staphylococcus aureus</i>	20.33 ± 2.1a	18.33 ± 1.5ab	18.00 ± 1.0ab	16.34 ± 1.1b	16.67 ± 2.0b	9.67 ± 1.5de	13.33 ± 1.1c	12.67 ± 2.6 cd	8.00 ± 1.0e	9.33 ± 1.5de
5	<i>Staphylococcus epidermidis</i>	18.00 ± 1.7a	17.67 ± 2.1a	15.67 ± 0.6b	10.00 ± 1.5d	10.67 ± 1.5d	10.00 ± 1.0d	11.00 ± 1.0d	10.00 ± 1.1d	5.67 ± 0.6f	7.67 ± 1.1e

NB: (i) ZOI are expressed as the average of three replicates ± SD. Means with same letters are not significantly different ( $p < 0.05$ ).(ii) SDR = Sider honey from Rijal Alma, DHS = Dharm honey from Al-Souda, DHR = Dharm honey from Rijal Alma, MJM = Majra honey collected by *Apis mellifera jemenitica*, and MJF = Majra honey collected by *Apis florea*.**Fig. 2.** Graphical representation of antibacterial activity of Saudi honey samples at 80% and 50% (w/v) concentrations against five bacterial pathogens, where SDR = Sider honey from Rijal Alma, DHS = Dharm honey from Al-Souda, DHR = Dharm honey from Rijal Alma, MJM = Majra honey collected by *Apis mellifera jemenitica*, and MJF = Majra honey collected by *Apis florea*.

(4.33 ± 0.6 mm) was observed from MJF at the concentration of 50% (w/v). MJM also showed minimum ZOI (6.00 ± 1.0) at the concentration of 50% (w/v) which was statistically similar to MJF. Sider honey (SDR) collected from Rijal Alma showed highest antimicrobial activity against *S. aureus* at 80% (w/v) concentration and its ZOI was measured 20.33 ± 2.1 mm, while, the minimum ZOI (8.00 ± 1.0 mm) was observed in MJM at 50% (w/v) concentration. SDR also showed highest antimicrobial activity against *S. epidermidis* at 80% (w/v) concentration and its ZOI was measured 18.00 ± 1.7 mm, while, the minimum ZOI (5.67 ± 0.6 mm) was observed in MJM at 50% (w/v) concentration. The MIC of SDR honey was observed at 40%, 20%, 10%, 20%, 20% (v/v); for DHS honey sample at 20%, 10%, 10%, 20%, 20% (v/v); for DHR honey sample was 40%, 10%, 20%, 20%, 20% (v/v); for MJM honey sample was 40%, 20%, 20%, 40%, 40% (v/v) and for MJF honey sample was 40%, 20%, 20%, 40%, 20% (v/v) against *E. coli*, *P. mirabilis*, *Shigella flexneri*, *S. aureus*, and *S. epidermidis* respectively. Similarly, the average MIC of each honey samples i.e. SDR, DHS, DHR, MJM, and MJF against all five tested bacteria was 22%, 16%, 22%, 32%, and 28% (v/v) respectively

(Table 3). Cluster analysis data based on antibacterial activity of all tested honey samples (80% and 50% w/v concentration) against five bacterial pathogens categorized honeys into three main groups. The first group delimited by Euclidian distance less than 5 comprised DHR, DHS, and SDR honey at 80% w/v. Second group (Euclidian distance > 6) formed by five honey samples divided in two subgroups, sub-group-I comprised DHR, DHS, and SDR at 50% concentration w/v while sub-group-II consisted of MJM and MJM honey samples (80% w/v). Third group (Euclidian distance < 3) formed by two honey samples MJM and MJF (50% w/v) (see Fig. 3).

#### 4. Discussion

This study showed the antibacterial potential of Saudi honeys collected from Asir region against five common bacterial pathogens. All of the honey samples displayed antibacterial activity, however the potential of each honey sample at different concentration varied against the tested bacterial strain. SDR at 80% w/v concentration showed significant antibacterial potential with maximum ZOI against *S. aureus*, *S. epidermidis*, and *Shigella flexneri* while DHS activity was better against *E. coli*, *P. mirabilis*, *S. epidermidis*, and *Shigella flexneri* at the concentration of 80% w/v. While with low concentrations (50% w/v) of both SDR and DHS exhibited the antibacterial activity but comparatively smaller ZOI were observed. These findings are in accordance with Alqurashi et al. (2013) who compared the Sider and mountain honeys from Saudi Arabia against gram-negative bacteria and found that tested honey samples had inhibitory effect at 40–80% concentrations against the bacteria used in the study. They observed significantly ( $p \leq 0.05$ ) increased inhibition activity against bacteria by increasing the honey concentration and found Sider honey more potent than Mountain honey. The antibacterial activity of all investigated honey samples against studied pathogens increased with increasing concentration in a dose-dependent manner and results were in line with Deng et al. (2018) who compared the antibacterial activity of buckwheat and manuka honey and found both honey samples inhibited the growth of bacterial pathogens at dose-dependent manner of all tested concentrations. DHR honey samples (80% w/v) showed statistically similar antibacterial activity like DHS (80% w/v) against *P. mirabilis* and *S. aureus* but its activity was observed comparative lower than DHS (80% w/v) against *E. coli*, *Shigella flexneri*, *S. epidermidis*. DHS and DHR had similar botanical origin i.e. *Lavandula dentate* but from different locations former from Al-Soda and latter from Rijal Alma. The difference in locations could be one of the reasons for variation in antibacterial activity as Molan and Cooper (2000) reported that variation in antibacterial activity of honeys could be many folds and depends on its geographical, season, and botanical source. Al-Waili et al.

**Table 3**  
Minimum inhibitory concentration (%v/v) of different Saudi honey samples from Asir region against bacterial pathogens.

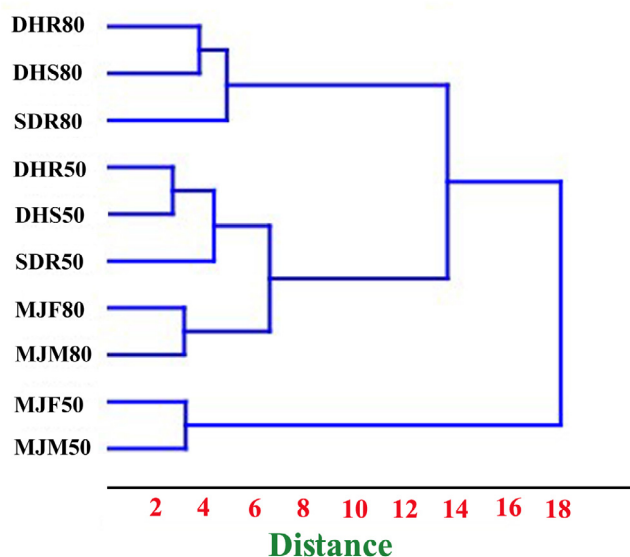
Bacterial Strain	Honey dilutions (v/v %)								Honey Sample	MIC value % (v/v)
	100 (+Control)	80	60	40	20	10	5	0 (Control)		
<i>E. coli</i>	-	-	-	+	++	+++	+++	+++	SDR	40
<i>P. mirabilis</i>	-	-	-	-	+	++	+++	+++	SDR	20
<i>Shigella flexneri</i>	-	-	-	-	-	+	++	+++	SDR	10
<i>S. aureus</i>	-	-	-	-	+	++	+++	+++	SDR	20
<i>S. epidermidis</i>	-	-	-	-	+	++	+++	+++	SDR	20
<b>Mean MIC value for SDR honey</b>										<b>22</b>
<i>E. coli</i>	-	-	-	-	+	++	+++	+++	DHS	20
<i>P. mirabilis</i>	-	-	-	-	-	+	++	+++	DHS	10
<i>Shigella flexneri</i>	-	-	-	-	-	+	++	+++	DHS	10
<i>S. aureus</i>	-	-	-	-	+	++	+++	+++	DHS	20
<i>S. epidermidis</i>	-	-	-	-	+	++	+++	+++	DHS	20
<b>Mean MIC value for DHS honey</b>										<b>16</b>
<i>E. coli</i>	-	-	-	-	+	+++	+++	+++	DHR	20
<i>P. mirabilis</i>	-	-	-	-	-	++	+++	+++	DHR	10
<i>Shigella flexneri</i>	-	-	-	-	+	++	+++	+++	DHR	20
<i>S. aureus</i>	-	-	-	-	+	++	+++	+++	DHR	20
<i>S. epidermidis</i>	-	-	-	-	+	++	+++	+++	DHR	20
<b>Mean MIC value for DHR honey</b>										<b>18</b>
<i>E. coli</i>	-	-	-	+	++	+++	+++	+++	MJM	40
<i>P. mirabilis</i>	-	-	-	-	+	++	+++	+++	MJM	20
<i>Shigella flexneri</i>	-	-	-	-	+	+++	+++	+++	MJM	20
<i>S. aureus</i>	-	-	-	+	++	+++	+++	+++	MJM	40
<i>S. epidermidis</i>	-	-	-	+	+	++	+++	+++	MJM	40
<b>Mean MIC value for MJM honey</b>										<b>32</b>
<i>E. coli</i>	-	-	-	+	++	+++	+++	+++	MJF	40
<i>P. mirabilis</i>	-	-	-	-	+	++	+++	+++	MJF	20
<i>Shigella flexneri</i>	-	-	-	-	+	++	+++	+++	MJF	20
<i>S. aureus</i>	-	-	-	+	++	+++	+++	+++	MJF	40
<i>S. epidermidis</i>	-	-	-	-	+	++	+++	+++	MJF	20
<b>Mean MIC value for MJF honey</b>										<b>28</b>

NB: (-, no growth; +, minimum growth; ++, moderate growth; +++, heavy growth, grey color indicates the MIC).

(MIC = minimum inhibitory concentration, SDR = Sider honey from Rijal Alma, DHS = Dharm honey from Al-Souda, DHR = Dharm honey from Rijal Alma, MJM = Majra honey collected by *Apis mellifera jemenitica*, and MJF = Majra honey collected by *Apis florea*).

(2011) reviewed different antibacterial studies of honey and reported that variations in antibacterial properties of honey depend on its geographical origin. MJM and MJF honey samples showed statistically less antibacterial activity as compared to SDR, DHS, DHR honey samples. Both MJM and MJF honey samples (80% w/v) showed similar ZOI against *E. coli*, *S. aureus*, and *S. epidermidis*, while MJF showed comparatively better activity than MJM against *P. mirabilis* and *Shigella flexneri*. Similarly, MJM and MJF honey samples (50% w/v) showed statistically similar ZOI against *P. mirabilis* and *Shigella flexneri* and MJF was better than MJM against *E. coli*, *S. aureus*, and *S. epidermidis*. Both Majra honey samples were light in color (MJM = white; MJF = extra white) which could be the possible reason for their low antibacterial activity when compared to darker honey samples i.e. SDR, DHS, and DHR. The darker honey mostly had higher phenolic content and its antioxidant power (Escuredo et al., 2013; Piljac-Žegarac et al., 2009; Sant'Ana et al., 2014). Similar results were also reported by Alvarez-Suarez et al. (2010) that Cuban unifloral honeys with higher phenolic contents demonstrated higher antibacterial activity. Wasihun and Kasa (2016) while evaluating the antibacterial activity of Ethiopian honey against multidrug resistant bacteria found that red honey showed better antibacterial activity than

the white color honey sample. Our results showed that antibacterial activity of each honey and bacterial pathogen was different. Similar results were also found by Hegazi et al. (2017) who evaluated the potential antibacterial activity of 10 Saudi Arabian honey and concluded that the potential activity was differing according to bacterial pathogen and honey type. They found that the tested honey samples inhibited the growth of bacterial strains of medical importance and that honey could be used as complementary antimicrobial agent against selected pathogenic bacteria. *P. mirabilis* and *Shigella flexneri* were more susceptible at low concentrations (10–20% v/v) of honeys while, *S. aureus* and *S. epidermidis* were susceptible at the honeys concentration ranging from 20% to 40% v/v. MIC values for *E. coli* were observed high for all the tested honey samples that depicted less susceptibility of this bacterium. Similar reports were also presented by Hegazi and Allah (2012) while investigated the antimicrobial activity of 12 Saudi Arabian honeys. The reasons for less susceptibility of *E. coli* to tested honeys could be the low permeability of its cell wall, resistance, and mutation Wasihun and Kasa (2016). MIC values in this study indicated that all tested honey samples have potential antibacterial activities and results were similar to other studies (Ahmed et al., 2014; Getaneh et al., 2013; Wasihun and Kasa,



**Fig. 3.** Dendrogram (Ward's Method-Euclidean distances) of the honey samples at 80% and 50% (w/v) concentrations, based on antibacterial activity against five bacterial pathogens (*Escherichia coli*, *Proteus mirabilis*, *Shigella flexneri*, *Staphylococcus aureus*, *S. epidermidis*), where SDR = Sider honey from Rijal Alma, DHS = Dharm honey from Al-Sooda, DHR = Dharm honey from Rijal Alma, MJM = Majra honey collected by *Apis mellifera jemenitica*, and MJF = Majra honey collected by *Apis florea*.

2016). Differences in growth rate, low permeability of bacterial cell wall, nutritional requirements, temperature, inoculum size, difference in honey samples, and applied test method could attribute the variations in bacterial susceptibility (Molan and Betts, 2000). In the hierarchical cluster analysis of honey samples based on the antibacterial potential, the first group was at high concentration and showed identical antibacterial potential. Honey from same botanical origins were clustered together while the SDR with different botanical origin was at different branch. Cluster analysis also differentiated the honey samples based on botanical origins. This botanical differentiation of honey was also observed in second group of cluster where SDR honey was again on different branch. Majra honey samples at 80% w/v concentrations shared the second group of 50% w/v concentration of DHR DHS, SDR honey samples while Majra honey at 50% w/v concentration joined the third group. It clearly indicated that Majra honey samples had comparatively low in antibacterial activity than Dharm and Sider. Mazol et al. (2016) also made a cluster analysis of honey samples based on phenolic compounds, antioxidant potential, and antibacterial activity of honey samples from Poland against five bacterial strains and their results were almost comparable with our results.

## 5. Conclusions

Antibacterial potential of honey samples collected from Asir region of Saudi Arabia was investigated at different concentrations in this study. Sider (*Ziziphus spina-christi*) and Dharm (*Lavandula dentata*) exhibited a good antibacterial activity at both 80% and 50% w/v concentrations against tested bacteria (*P. mirabilis*, *Shigella flexneri*, *S. aureus*, and *S. epidermidis*) except *E. coli* as the average ZOI against this bacterium was comparatively small. Majra (*Hypoestes forskalii*) honey samples collected from *A. m. jemenitica* and *A. florea* showed antibacterial activity against all the tested bacteria at high concentration (80% w/v) but their antibacterial potential was lower than Sider and Dharm honeys. Majra honey antibacterial effects were minimum against *E. coli* and *S. epidermidis*. Majra honey which was collected from *A. florea* performed

slightly better than the Majra honey collected by native bee *A. m. jemenitica*. Similar proportionately results of all honey samples at 50% w/v concentration were also observed. Dharm and Sider honey samples were darker while Majra samples were white in color. It indicated the low phenolic contents in Majra honey resulting its low antibacterial activity. Botanical origin, geographical characteristics, honey bee species, and plant phytochemicals in honey may contribute in its antibacterial potential and could be source of variations in antibacterial activity among different honey samples. It is obvious that Saudi honey attributes a considerable antibacterial activity and in future more studies can be designed to isolate and synthesize the antibacterial agent as medicine from honey.

## Ethical statement

This study does not have any involvement with animals that need approval of the ethics committee.

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