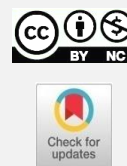




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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITIES FOR HADHRAMI HONEY ON GROWTH OF SOME PATHOGENIC BACTERIA

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ABSTRACT

Objectives: The majority of the Yemeni honey varieties are characterized by low moisture content, in addition to the various flora of Yemeni plant, which may not be found in many countries, which makes them of high medicinal importance and high monetary value.

Methods: This study was carried out the effect of three types of Hadhrami honey: Somur, Sidr (Baghya) and Meria against the growth of some pathogenic bacteria (*Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Enterobacter* sp., *Staphylococcus aureus*, and *Klebsiella* sp.).

Results: The results revealed that that Sidr honey gave the highest antibacterial activity against all bacteria tested, whereas the Somur and Meria honey were not recorded activity for growth *Klebsiella* sp. and Meria honey was not recorded activity for growth *E. coli*, respectively. When the antibiotics compared to types of honey antimicrobial activity, it was observed that the antimicrobial effect of Sidr honey was better than Imipenem antibiotic effect against *P. aeruginosa*.

Conclusion: Study concludes that inhibition of the studied strains was dependent on the type of honey source. It is concluded that Yemeni honey could potentially be used as therapeutic agents against bacterial infection particularly to the tested microorganisms.

Keywords: Antimicrobial activity of honey, sidr and meria, somur, Yemeni honey varieties.

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INTRODUCTION

Honey is the product of beekeeping that has great market potential. Honey contains more than 200 compounds comprising approximately 38% fructose, 31% glucose, 10% other sugar types, 18% water and 3% of other compounds. However, precisely the great mixture of compounds in this 3% is the product's greatest feature, with special reference to phenolic and carotenoids compounds¹. Honey is one of the most complete foods for humans, due to its therapeutic², antioxidant³, antimicrobial^{4,5}, antitumoral⁶, anti-inflammatory⁷, antiviral⁸, and antiulcer activities⁵. Most studies on the effects of honey are concentrated on the activities of bioactive compounds, especially phenolic compounds, in the human organism. The most relevant are those widely distributed in nature, including the phenolic acids and flavonoids⁹. Carotenoids were found in small concentrations in the dark honey (10 mg b-carotene Kg⁻¹) but they were not found in light

colored honey. This fact reveals the effect that carotenoids^{1,10} and phenolic compounds have in the honey color¹¹. The natural ingredients of honey show different activities against various microorganisms. Its activity is likely to be dependent on the grazing grounds and the weather conditions where the bees were raised, and on the natural structure of the blossom nectar¹². Honey has an increasing effect on the levels of anti-oxidants, iron and rare elements in blood¹³. The antibacterial activity of honey has never been reported nor any toxicity or side effects, low cost of maintenance, and local availability confer valuable advantages to using honey as an alternative antimicrobial therapy¹⁴. There are numerous reports of the antimicrobial activity of honey against a wide range of bacterial and fungal species^{8,12,14,15}. The antimicrobial activity could be attributed to osmotic effect of honey, the low pH of honey being between 3.2 and 4.5,¹⁶ hydrogen peroxide, defensin-1, as well as the presence of phytochemical factors¹⁷.

Thereby, the inhibitory activity caused by the osmotic effect of honey dilutions obviously depends on the species of bacteria. The major contributor to the antimicrobial activity of honey is hydrogen peroxide, and the different concentrations of this compound in different honey result in their varying antimicrobial effects¹⁸. Several types of bacteria, commonly involved in wound infections like *E. coli*, *S. aureus*, *P. mirabilis*, *Klebsiella* spp., *Streptococcus faecalis*, and *P. aeruginosa*, are susceptible to the antibacterial activity of honey regardless to their resistance to antibiotics¹⁹.

In vitro studies support the antimicrobial effect of honey against an extensive range of pathogens including β -haemolytic streptococci, methicillin-resistant *S. aureus* and *Pseudomonas* sp.²⁰. *In vivo* studies are less conclusive but honey has been used to treat burns²¹ and meningococcal lesions²². Subrahmanyam²³ compared between honey and silver sulphadiazine on the treatment of patients with burns and found less inflammation. This study aimed to investigate the antibacterial activities of three types of Yemeni Hadhrami honey against some pathogenic microorganisms (gram positive and gram negative bacteria) isolated from patients and compared between them with the effect of antibiotics.

MATERIALS AND METHODS

Bacterial strains

The bacteria strains that used in this study are most commonly involved in causing gastroenteritis, wound and burn exudates, urinary tract infection and ear secretions. Six bacteria strains (*P. aeruginosa*, *P. vulgaris*, *E. coli*, *S. aureus*, *Enterobacter* sp., and *Klebsiella* sp.) were isolated from different patients attending Al-Mukalla's Hospitals in Hadhramout-Yemen, and used throughout this study. The isolated bacteria were subcultured on Nutrient agar (Difco) and incubated aerobically at 37°C for 24 hours. Organisms were maintained in the laboratory on nutrient agar slopes at 4°C²⁴.

Honey samples

In this study, three Hadhrami honey samples were taken from Yemeni mountain nature were used: {Somur, Sidr (Baghya) and Meria}, and stored in the

dark at room temperature. The physical characterizations of honey samples such as pH which was measured using a pH meter (JeNWAY-3505), while the appearance was assessed in each sample by visual examination to determine the color.

Determination the minimal inhibitory concentration of the honey

The minimum inhibitory concentration (MIC) of honey was determined by using a different dilution for each type of honey that diluted with sterile distilled water. It was weighed 7gm/7mL from honey and the following concentration was prepared (1:4, 1:8, 1:10, and 1:20)²⁵.

Antibacterial activity

The well diffusion technique under aerobic condition was employed as previously described by Harris *et al.*,²⁶ About 20 ml of the sterilized medium was poured into each sterile Petri-dish (9 cm diameter) and allowed to solidify. Bacterial suspension for each strain tested was adjusted at 3×10^8 CFU/ml by McFarland scale which prepared by mixing 0.1 ml of 1.0% dehydrate barium chloride with 9.9 ml of 1.0% Sulfuric acid H₂SO₄ as described by McFarland.²⁷ 0.1 ml of the prepared bacterial suspension was spread evenly onto the agar surface using a cotton swab and kept in a refrigerator for 2 h. Wells (7 mm) were cut into the plates using sterile cork. Thereafter, all prepared plates were incubated at 37°C for 24 h²⁸. After that, the diameter of inhibition zone around the well was measured in mm.

Antibiotic susceptibility test

Antibiotic susceptibility tests were carried out by the Kirby- Bauer disk diffusion technique according to Clinical Laboratory Standard Institute guidelines²⁹. Mueller Hinton agar was used for growing the lawn of culture of the strains by spreading the culture onto the agar plate^{30,31}.

In this study, eight of different antibiotics disks (OXOID and HIMEDIA) were used against investigated bacteria. These antibiotics disks were: Aztreonam (AT 30 µg)-Imipenem (IPM 10 µg)-Gentamicin (GEN 10 µg)-Ceftazidime (CAZ 30 µg)-Piperacillin (PI 100 µg)-Amikacin (AK 30 µg)-Amoxicillin+Clavulanic acid (AMC 30 µg)-Cefuroxime (CXM 30 µg).

Table 1: Characteristics of types of honey tested.

Honey	The price of (1) kilogram		pH	Dark	Light
	In Yemeni Riyals	In USA Dollars			
Somur	5000	14	4.5	++	-
Sidr	20,000	55	3.8	-	++
Meria	2000	5	5.9	-	+++

Table 2: Inhibitory growth activity of Hadhrami honey against pathogenic bacteria.

Microorganisms	Mean diameter of inhibition zone (mm)±SD		
	Type of Honey		
	Somur	Sidr	Meria
<i>P. aeruginosa</i>	24±0.2	30±0.1	22±0.0
<i>E. coli</i>	15±0.10	23±0.3	-
<i>Enterobacter</i> sp.	11±0.1	21±0.2	10±0.11
<i>P. vulgaris</i>	19±0.0	20±0.0	20±0.0
<i>S. aureus</i>	13±0.18	16±0.28	11±0.2
<i>Klebsiella</i> sp.	-	17±0.01	-

Table 3: The minimal inhibitory concentration (MIC) of Somur, Sidr, and Meria honey against growth of microorganisms.

Honey Concentration	Microorganism	Mean diameter of inhibition zone (mm) results		
		Somur honey	Sidr honey	Meria honey
1: 4	<i>P. aeruginosa</i>	20	22	18
	<i>E. coli</i>	16	18	12
	<i>Enterobacter</i> sp.	10	13	10
	<i>P. vulgaris</i>	15	18	-
	<i>S. aureus</i>	10	12	13
	<i>Klebsiella</i> sp.	-	11	-
1: 8	<i>P. aeruginosa</i>	15	19	12
	<i>E. coli</i>	10	12	-
	<i>Enterobacter</i> sp.	-	10	-
	<i>P. vulgaris</i>	11	11	-
	<i>S. aureus</i>	-	10	10
	<i>Klebsiella</i> sp.	-	-	-
1:10	<i>P. aeruginosa</i>	10	15	10
	<i>E. coli</i>	-	10	-
	<i>Enterobacter</i> sp.	-	-	-
	<i>P. vulgaris</i>	10	10	-
	<i>S. aureus</i>	-	10	-
	<i>Klebsiella</i> sp.	-	-	-
1:20	<i>P. aeruginosa</i>	-	10	-
	<i>E. coli</i>	-	-	-
	<i>Enterobacter</i> sp.	-	-	-
	<i>P. vulgaris</i>	-	11	-
	<i>S. aureus</i>	-	-	-
	<i>Klebsiella</i> sp.	-	-	-

RESULTS AND DISCUSSION

The results of the physical characterizations and the prices of the three types of Hadhrami honey (Somur, Sidr and Meria) were recorded in Table 1. The results of antibacterial activity of different honey types against *P. aeruginosa*, *P. vulgaris*, *E. coli*, *S. aureus*, *Enterobacter* sp., and *Klebsiella* sp. were presented in Table 2. The Sidr honey was highly antimicrobial effective against all tested bacteria, which ranged between 10±0.11 mm to 30±0.1 mm, while the Somur and Meria honey showed no activity for the growth of *Klebsiella* sp. and Meria honey against *E. coli* (not inhibition zone). It was found that the Sidr honey has more influence antimicrobial activity, followed by Somur honey and then the Meria honey as the last one. These results are in agreement with reported by Othman²⁴ who recorded that the Yemeni Sidr honey has more effective than Egyptian honey against *Salmonella typhi*, *Neisseria meningitides*, *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *P. aeruginosa*, *H. influenza*, *Shigella flexneri*, and *P. vulgaris*. The experiment on El-Ariqi and El-Hamodi³² observed that the Sidr honey was the second one on the antimicrobial activities against *S. aureus*, *E. coli*, *Salmonella* sp., *Proteus* sp., and *P. aeruginosa*. On the other hand, Almasaudi et al.,³³ compared the effects of five types of honey (both imported and local Saudi honey) against *S. aureus*. It was found that the Manuka Honey showed the best results and had a bactericidal effect on both methicillin resistant and sensitive *S. aureus*. However, Sidr and Nigella sativa honey exerted a bacteriostatic effect. The present study showed varying degree of growth inhibition activity of three types of Yemeni honey against the tested microorganisms; these might be due to an advantage

for honey like osmotic effect, the effect of low pH, and these organisms are sensitive to hydrogen peroxide which are unsuitable for bacterial growth, represented as an inhibition factor in honey^{21,32,34}. This result was supported by a number of previous studies which have demonstrated that various honey, both commercially and locally produced, have antibacterial activity. A study by Nzeako and Hamdi³⁵ used six types of commercial honey and found that inhibition of *S. aureus*, *E. coli* and *P. aeruginosa*. Another study by Ceyhan and Ugar³⁶ investigated 84 types of honey against eight bacteria and two fungi. It was shown that the honey has a broad-spectrum activity against the used microorganisms. In addition, these authors found that the antibacterial activity of honey was greater than that which could be attributed to the sugar content of the honey. The antibacterial activity of honey has also been investigated for its potential use in reducing food-borne pathogens³⁷, preventing catheter exit/entry site infection³⁸, for the treatment of colitis³⁹, or even to protect the gastric mucousin *H. pylori* induced inflammation^{40,41}. The application of honey to wounds to animals in veterinary environments has also been noted⁴². Furthermore, the results of the current study revealed that most bacteria tested were sensitive at 1:4 concentration of all types of honey studied, except *Klebsiella* sp. was resistance to Somur and Meria honey. *P. aeruginosa* was more sensitive than others (Table 3). All bacteria were resistant to 1:20 concentration of all types of honey except *P. aeruginosa* and *P. vulgaris* were sensitive to Sidr honey with inhibition zone (10 mm and 11 mm), respectively. A similar result was previously reported by Shreef et al.,²⁵ who reported that most bacteria tested sensitive to 1:4 concentration more than other concentration (1:8, 1:10, 1:16, 1:20, 1:24) of both

natural and industrial honey. Also, Al-Nahari *et al.*,⁴³ studied antimicrobial activities of Saudi honey against *P. aeruginosa*. The results indicated that all types of honey tested exerted a full inhibition of bacterial growth at the highest concentration tested of 50% at 24 h of contact. Othman²⁴ showed that all the different concentrations of both honey samples (10 to 80%) showed growth inhibitory activity against *E. coli* more than other bacteria tested. It was recorded that all the tested bacteria were sensitive to Isis and Yemeni Sidr honey at 40 to 80% concentrations. The antibacterial activity of Yemeni Sidr honey was higher than those obtained by Isis honey. Variations seen in overall antibacterial activity were due to changes in the level

of hydrogen peroxide achieved and in some cases to the level of non-peroxide factors²⁴. The content of non-peroxide factors was obviously related to the Yemeni floral source and sometimes accounted for the major part of the antibacterial activity in honey⁴⁴. Molan and Cooper⁴⁵ reported that the difference in antimicrobial potency among the different honey can be more than 100-fold, depending on its geographical, seasonal and botanical source. This result was in agreement with those previously reported by Mohammed *et al.*,⁴⁶. The different concentrations of the two honey samples had good growth inhibitory effect on the tested microorganisms.

Table 4: The antibiotic susceptibility test for tested bacteria.

Antibiotics	Mean diameter of inhibition zone (mm)					
	Bacteria tested					
	<i>Klebsiella</i> sp.	<i>P. vulgaris</i>	<i>Enterobacter</i> sp.	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Piperacillin	11	15	13	18	10	19
Amikacin	15	16	13	15	11	16
Imipenem	17	18	20	17	19	22
Cefuroxime	11	10	0	15	0	0
Aztreonam	14	12	20	20	19	18
Amoxicillin + Clavulanic acid	13	10	20	18	11	0
Gentamicin	10	17	20	17	19	16
Ceftazidime	0	0	12	14	0	0

A similar result was previously reported by Mohapatra *et al.*⁴⁷ for *E. coli* and *P. aeruginosa*⁴⁸, for *E. coli*, *K. pneumoniae* and for *Haemophilus influenza*⁴⁹. The less inhibition effect of the two tested honey against *K. pneumoniae* and *S. aureus* was in agreement with Patricia *et al.*,⁵⁰ who reported that the overall poor activity of the honey against *S. aureus* was unexpected as previous reports which recorded that Maunka honey has an excellent activity against this organism. For example, Cooper and Molan²⁰ who also used an agar dilution method and demonstrated that the minimum inhibitory concentration for Maunka honey against 58 strains of *Staphylococcus* sp. was 2-3% (v/v) and for pasture honey 3-4% (v/v). In this study, the antibiotic susceptibility was tested and the highest percentage of the sensitivity to Imipenem for most bacteria were recorded. The highest inhibition zone of Imipenem was (22 mm) for *P. aeruginosa* (Table 4), while the highest inhibition zone of Sidr honey was (30 mm) that indicated the antimicrobial effect of honey was higher than antibiotic effect. A similar result was previously reported by Al-Nahari *et al.*,⁴³ for Manuka honey UMF +10 was the most effect on antimicrobial resistance and had an effect on modulation of Imipenem resistant *P. aeruginosa*. The resistance of pathogenic microorganisms to antibiotics is a serious global health concern⁵¹. On another hand, Al-Naama⁵² showed that honey, like antibiotics, has certain organisms sensitive to it, and provides alternative therapy against certain bacteria and is also shown to have an antimicrobial action against a broad spectrum of bacteria (both gram-positive and -negative bacteria). Honey contains compounds with antioxidant and antibacterial capacities, such as phenolic compounds and

carotenoids⁵³. Honey bees add an enzyme, called glucose oxidase, to the collected nectar during the honey-making process, which converts the glucose in the honey into hydrogen peroxide (H₂O₂) and gluconic acid. H₂O₂ is toxic to many microbes⁵¹.

Mohapatra *et al.*,⁴⁷ showed that the honey has an antibacterial effect against both gram-positive bacteria (*S. aureus*, *B. subtilis*, *B. cereus*, *Enterococcus faecalis*, and *Micrococcus luteus*) as well as anti-gram negative bacteria (*E. coli*, *P. aeruginosa*, and *S. typhi*). This effect was either bacteriostatic or bactericidal depending on the type of honey tested. There are countless varieties of honey being produced worldwide, and some may have superior antimicrobial activities that are yet to be discovered.

CONCLUSION

The results indicated that three types of honey affected the test organisms differently. Also it was evident that the antibacterial effect of different types of honey is type and concentration dependent. Sidr honey was more potent than Somur and Meria honey in inhibiting the bacterial growths *in vitro*. Consequently, using honey for the treatment of infections may be worth perusing.

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AUTHOR'S CONTRIBUTION

Al-Haik WM: designed the study. **Al-Haddad AM:** acquired the data. **Al-kaf AG:** analyzed the data and interpreted the results. **Edrees WH:** drafted the article. All authors revised the article and approved the final version.

DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

None to declare.

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