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Full Length Research Paper

Antibacterial activity of Saudi honey against Gram negative bacteria

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The antibacterial activity of local Sidr and Mountain Saudi honeys against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* were evaluated. Disc diffusion method, gel diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were used in this investigation. The findings indicated that both honey samples had growth inhibitory effect and all tested gram negative bacteria were sensitive to 40-80% concentrations. Increasing the honey concentration significantly (P≤0.05) increased the inhibition of growth of the tested bacteria. Sidr honey was more potent than Mountain honey in producing the inhibitory growth effect as an antibacterial agent. Sidr and Mountain honeys in different concentrations were more effective against *E.coli* than other bacteria. MIC of the two honey samples was 20 mg/mL while the MIC of *K. pneumoniae* was 40 mg/mL in the case of Mountain honey. The MBC of the two honey samples was 40 mg/mL and the MBC of *A. baumannii* that valued was 20 mg/mL. We are of the opinion that Sidr and Mountain Saudi honeys could potentially be used as therapeutic agents against bacterial infection particularly to the tested microorganisms.

Key words: Saudi honey, Sidr honey, mountain honey, Gram negative, antibacterial agents.

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health and all kinds of antibiotics, including the major last-resort drugs, as the frequencies of resistance are increased worldwide (Levy and Marshall, 2004; Mandal et al., 2009). The use of honey as a traditional remedy for microbial infections dates back to ancient times. The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature (Molan, 1992). Honey has previously been shown to have wound healing and antimicrobial properties, but this is dependent on the type of honey, geographical location and flower from which the final product is derived (Molan and Cooper, 2000). It is well established that honey inhibits a broad spectrum of bacterial species. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, Gram positives, and Gram negatives (Hannan et al., 2004). There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics (Patton et al., 2006). Sidr honey is made from bees who feed only on the nectar of the Sidr tree, which is native to the South Saudi Arabia and Yemen regions. The Sidr tree is considered sacred and has been used as a Natural medicine for centuries. Sidr honey is a "monofloral honey", a type of honey which has a high value in the marketplace because it has a distinctive flavor or other attribute due to its being

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predominantly from the nectar of one plant species. Sidr honey has wide medicinal applications and uses which include: liver diseases treatment, stomach ulcers, respiratory infections, diseases resulting from malnutrition, digestive problems, constipation, eye diseases, infected wounds and burns, surgical wounds (caesarian section), speedy recovery after childbirth, general health and vitality. Sidr honey has strong antioxidant and antibacterial properties (Alandejani et al., 2009) .Mountain honey has high antibacterial activity against gram positive and gram negative bacteria (Mekawey, 2010). A large number of honeys are available in the Saudi market and are either locally produced or imported from different countries. Some of them are traditionally used as remedy for several ailments. The antibacterial efficiency of local Saudi honeys has not been thoroughly evaluated (Eman and Mohamed, 2011).Therefore, the purpose of the present study was to evaluate and compare the *in vitro* inhibitory effect of Sidr and mountain Saudi honeys against the growth of four different gram negative bacteria.

MATERIALS AND METHODS

Sources of bacterial isolates and media

Isolates of *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* strains were obtained from the department of microbiology at the King Khalid Hospital (KKH) of Najran city, Saudi Arabia. The isolates were identified by an automated system (MicroScan Walkaway, Siemens) and the results were confirmed based on the standard microbiological techniques (Cheesbrough, 1988). Organisms were maintained in the laboratory on nutrient agar slopes at 4°C. The media used in this study were Mueller- Hilton broth, Mueller Hilton agar, and nutrient agar (Oxoid, England). The media were prepared according to the instructions of the manufacturer.

Source of honey and preparation of concentrations

Local Sidr and mountain honeys were purchased from market at Najran city, Saudi Arabia. Different concentrations of each honey constituting, 10, 20, 40, 60 and 80% (v/v) were made using sterile distilled water. This was done by dissolving the respective volumes: 1, 2, 4, 6, 8 mL of each honey into corresponding volumes of sterile distilled water to give a 10 mL preparation. Filter paper discs of 6 mm diameter were prepared according to the method of Cheesbrough (2000). The discs were impregnated with the different concentrations of each honey.

Disc diffusion method

The disc diffusion technique was employed as previously described by Bauer et al. (1966). Discs impregnated with the different concentrations of each honey were employed in the study. 0.5 McFarland standard was prepared by the method of Koneman et al. (1992) and 5 mL was put into a sterile test tube. An inoculum of each isolate was prepared from subculture of bacterial suspension. Briefly, it was prepared as follows: 4–5 colonies of the isolates were emulsified in sterile normal saline and the turbidity adjusted to 1.5×10^8 CFU/mL (corresponding to 0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the Mueller Hinton agar plates. The plates were allowed to dry for 3 to 5 min. Thereafter, all discs were placed on the plates and pressed gently to ensure complete contact with agar. A distance of at least 15 mm was maintained from the edges of the plates to prevent overlapping of inhibition zones. A ciprofloxacin disc (5 µg) was used as the positive control. 15 min following placement of the discs, the plates were incubated for 24 h at 37°C. They were then examined and the diameter of the zone of inhibition was measured in mm. The experiment was repeated in triplicates for each isolate.

Gel diffusion method

Mueller Hinton agar plates were prepared according to the manufacturer's instructions. Using a sterile 6 mm borer, wells were cut in the agar. The medium surface was cultured by swabs from bacterial suspension (1.5×10^8 CFU/mL) in triplicates. Different honey concentrations (10, 20, 40, 60 and 80%) were added to the wells. The plates were incubated at 37°C for 24 h under aerobic condition and then examined for inhibition zone (Barry and Thornsberry, 1985).

Minimal Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC is defined as the lowest concentration of honey that is able to inhibit the growth of bacteria. Mueller Hinton broth was employed for the determination of MIC in serial dilution tests tube preparation. Serial dilutions of the two honey samples were made in test tubes that contained 1 mL of Mueller Hinton broth medium to give a final concentration of 80, 40, 20, 10, 5, 2.5, 1.25 and 0.62 mg/mL. 20 µl of the test organisms (1.5×10^8 CFU/mL) was dispensed into the tubes. Negative control tube just contained 1 mL of honey but no organisms. Positive control tubes contained only 1 mL broth medium and each of the organisms but no honey. The tubes were incubated at 37°C for 24 h. After incubation, turbidity of each tube was visually inspected. Clear test tube indicated break point (Mackie and McCartney, 1996). From the tubes showing no visible sign of growth/turbidity in MIC determination, test microorganisms were inoculated onto sterile nutrient agar plates by streak plate method. The plates were then incubated at 37°C for 24 h. The least concentration that did not show growth of test organisms was considered as the MBC.

Statistical analysis

Data analysis results were expressed as means ± standard deviation and differences between means were analyzed statistically using an analysis of variance (ANOVA) according to Fisher's PLSD test. Differences were considered significant when $P \leq 0.05$.

RESULTS

The inhibition zone diameter (IZD) of different Sidr and mountain honey concentration (80-10%) were determined for *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* by disc diffusion test (Figure 1). The highest

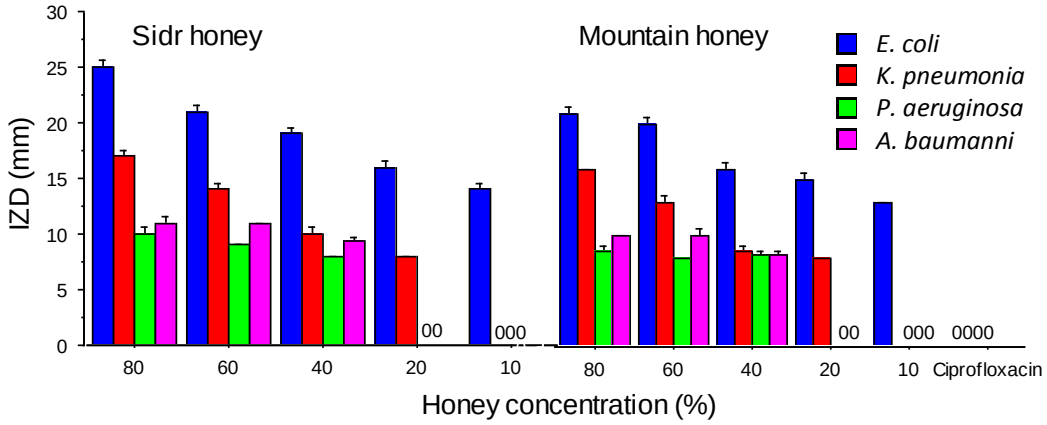

Figure 1. Inhibitory growth activity of Sidr honey, mountain honey and ciprofloxacin against *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* using disc diffusion test.

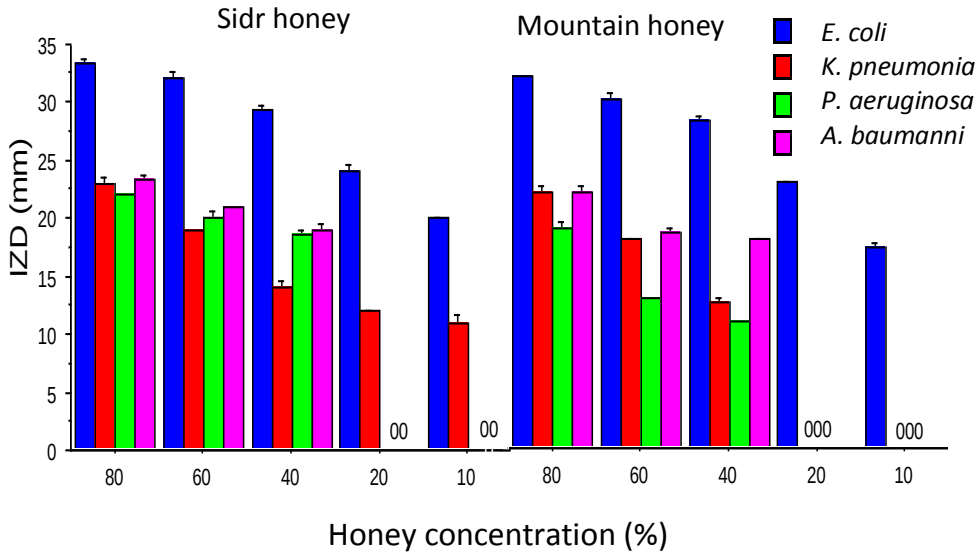

Figure 2. Growth inhibitory activity of Sidr and Mountain honeys against *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* using gel diffusion test.

inhibition zone (25.0 ± 0.58 mm) was recorded from Sidr honey against *E. coli* at the concentration of 80% while mountain honey showed slightly lower inhibition zone (21.0 ± 0.58 mm) against *E. coli* at the same concentration. The Sidr and mountain honeys had more inhibitory growth effect on *E. coli* at 10% concentration with IZD 14.0 ± 0.58 mm and 13.0 ± 0.00 mm respectively. The IZD of Sidr honey against *K. pneumoniae* ranged from 17.0 ± 0.58 to 8.0 ± 0.00 mm at concentration of 80 to 20% while in mountain honey, it ranged from 16.0 ± 0.00 to 8.0 ± 0.00 mm. The lowest IZD was detected for mountain honey against *P. aeruginosa* (8.67 ± 0.33 to 8.33 ± 0.33 mm) at concentration ranging from 40-80%. *K. pneumoniae, P. aeruginosa* and *A. baumannii* were resistant to Sidr and mountain honeys at 10% concentration. All the test bacteria were resistant to ciprofloxacin with IZD (0.00± 0.00 mm). The results of the *invitro* susceptibility of the test organisms to Sidr and Mountain honey samples by gel diffusion test are presented in Figure 2. In all the cases of microorganisms tested, 80% concentration of the two honey samples produced a greater IZD; from 34.33 ± 0.89 to 22.0 ± 0.00 for Sidr honey and(32.0 ± 0.00 to 19.0 ± 0.58 for Mountain honey. Sidr and mountain honey at concentration of 10% had more growth inhibition against *E. coli* with IZD of 20.0 ± 0.00 and 17.33 ± 0.33 mm respectively. As shown in Figures 1 and 2, all the tested bacteria were susceptible to Sidr and

Table 1. MIC and MBC of Sidr and mountain honeys *against E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii*.

Bacteria	MIC(mg/mL)		MBC (mg/mL)	
	Sidr honey	Mountain honey	Sidr honey	Mountain honey
E. coli	20	20	40	40
K.pneumoniae	20	40	40	40
P.aeruginosa	20	20	40	40
A.baumannii	20	20	20	20

*MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Mountain honey at concentration of 40 to 80%. The IZD produced by Sidr honey was significantly broader than those of mountain honey. Furthermore, increasing the honey concentration significantly ($p<0.05$) broaden the IZD. The MIC of Sidr honey against the tested microorganisms was 20 mg/mL, while in the case of Mountain honey, the MIC was 40 mg/mL for *K. pneumonia* and 20 mg/ mL for the other bacteria .The MBC value for *E. coli, K. pneumoniae,* and *P. aeruginosa* was 40 mg/mL but the MBC value for *A. baumannii* was 20 mg/mL (Table 1).

DISCUSSION

In our study, two Saudi honey samples were tested for their antimicrobial activity on *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii*. The present study showed varying degree of *in vitro* growth inhibition activity of Sidr and Mountain honeys against the tested organisms. These might be due to the osmotic effect, the effect of pH, and the sensitivity of these organisms to hydrogen peroxide which are unsuitable for bacterial growth, represented as an inhibition factor in honey (Postmes et al., 1993; Minisha and Shyamapada, 2011). All the different concentrations of both honey samples (10 to 80%) showed growth inhibitory activity against *E. coli*. This contrasts with the result reported by other workers (Hegazi, 2011; Hegazi and Fyrouz, 2012) who reported that the different types of Saudi honey were less inhibitory against *E. coli* than other bacteria. All the tested bacteria were sensitive to Sidr and Mountain honeys at 40 to 80% concentrations. The antibacterial activity of Sidr honey was higher than those obtained by Mountain 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 nonperoxide factors. The content of nonperoxide factors was obviously related to the floral source and sometimes accounted for the major part of the antibacterial activity in honey (Molan and Russell, 1988). Molan and Cooper (2000) reported that the difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source. The IZD in case of gel diffusion test were greater than those of disc diffusion test. This result was in agreement with those previously reported by Mohammed et al. (2008). The different concentrations of the two honey samples had good growth inhibitory effect on the tested microorganisms. Similar result was previously reported by Mohapatra et al. (2011) for *E. coli* and *P. aeruginosa*, (Agbaje et al. 2006) for *E. coli*, *K. pneumoniae* and (Hern et al. 2009) for *A. baumannii*. By visual inspection, the MIC assay showed that a lower MIC was observed with Sidr honey (20 mg/mL) for the tested microorganisms while those of mountain honey ranged from 20 to 40 mg/mL. The present findings are supported by Kwakman et al. (2008). The MBC value of both honey samples was in the range of 20 to 40 mg/mL.The lowest MBC value (20 mg /mL) was against *A. baumannii*. The present findings are consistent with the results reported by (Hern et al., 2009). Comparing the mean ± standard deviation of the inhibition diameters of the tested bacteria at different honey concentrations, we observed that there was statistically significant difference in the values ($P\leq0.05$) between microorganisms at all the honey concentrations. Our results further show that there was an increase of inhibition zone for the tested microorganisms with increase in the concentration of honey. This was obvious by statistical analysis which revealed that there was significant difference in the values ($P\leq0.05$) between the different honey concentrations.

Conclusion

The present study reveals that local Sidr and mountain Saudi honeys were effective in inhibiting the *in vitro* growth of *E. coli, K. pneumoniae, P. aeruginosa* and *A. baumannii* .Sidr honey was more potent than mountain honey in inhibiting these bacterial growths *in vitro*. Both honey samples in the different concentrations were more effective against *E. coli* than the other bacteria.

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