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SHORT COMMUNICATION

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Screening for antibacterial activity of two jujube honey samples collected from Saudi Arabia

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ABSTRACT

Background: Honey produced by *Apis mellifera* is well-known of its nutritional and therapeutic effectiveness since ancient times. However, its properties vary according to the vegetation cover in each area, which requires scientific verification.

Materials and Methods: Two samples of natural jujube honey, from northcentral and southwestern Saudi Arabia, from Buraidah and Najran, respectively, was screened using disc diffusion test against seven referenced bacterial strains, namely, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, and *K. pneumoniae* ATCC 27736.

Results: Both samples showed significant antibacterial activity on Gram-positive bacteria and weak or no activity on Gram-negative bacteria although honey sample from Buraidah exhibited higher antibacterial efficacy overall.

Conclusion: According to the current study, the antibacterial characteristics of honey may be dependent on its source and origin. These honeys merit future follow-up in human trials.

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Introduction

The antibiotics resistance crisis is now a global health problem, and pathogens have developed a remarkable resistance due to the misuse and overuse of these drugs, the problem was compounded by the pharmaceutical companies' reluctance of developing more antibiotics due to low financial returns and high cost of scientific research, synthesis, and production process [1–3]. Regretfully, due to the misuse of antibiotics, new infections have arisen and also old pathogens have revived in the community and causing numerous outbreaks throughout the world, such as *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Helicobacter pylori*, *Vibrio cholera*, *Pseudomonas aeruginosa*, tuberculosis, and salmonellas, which requires finding new alternatives to the recent antibiotics urgently [4]. Natural products have been one of the main sources of medications since ancient times and until the recent history before entering high-throughput chemicals in drug production, the later has raised serious concern about its high risks on human health. Recently, the intensive investigations have approved numerous natural-product-based drugs as effective alternatives [5]. Honeybees (*Apis mellifera* L.) have been appeared on Earth in conjunction with the emergence of the flowering plants since more than 125 million years; honeybees produce various important products, some are initially synthesized inside this insect, such as royal jelly, beeswax, and venom; others are derived from plants and modified by this insect, such as honey and propolis [6]. Honey is one of the oldest natural products known of its nutritional and therapeutic properties since antiquity. It is used in traditional medicine for the treatment of cough,

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tonsils, skin burns, fatigue, inflammations, abdominal infections, wound healing, and many more [7,8]. In the literature, plentiful studies were conducted on the antimicrobial properties of natural honey [9]. However, the results are mostly heterogeneous, based on the fact that the antibacterial effectiveness of honey is diverged greatly according to the source from which it is initially collected, as honey is highly affected by the diversity of plant species in the region, as well as other indirect reasons such as conditions of collection and storage [10]. In the Middle East, Jujube honey are mostly coming from honeybees fed on the nectar of the flowers from *Ziziphus spina-christi* (L.), a wild tree, well-known since ancient Egyptian civilization, it was found prescribed on papyri scripts around 1900 BC as anti-swelling, anti-pain, and anti-fever [11]. The current investigation aimed to evaluate the antibacterial activity of two jujube honey samples (Claimed fed on jujube, *Ziziphus spina-christi*) collected from Buraidah in Northcentral Saudi Arabia and Najran region Southwestern Saudi Arabia. As local inhabitants in these areas believes that the jujube honey is of the high medicinal value, and accordingly sold at high price.

Materials and Methods

Honey samples

Two honey samples were used in this investigation, one was sold commercially as jujube honey (honey of *Ziziphus spina-christi*), from Buraidah town in northcentral Saudi Arabia. The second natural honey was collected from the local beekeeper in Najran region (southwestern Saudi Arabia) feeds from a native *Ziziphus spina-christi* tree.

Preparation of honey samples

Nearly, 100 ml of raw honey samples was fractionated using C18 Column (Hydrosphere C18) method.

It was loaded on a flash water preconditioned short C18 column (10 × 15 cm, LiChroprep_ RP-18, 40–60 µm, Merck, Germany) using tap water suction and eluted with 100% distilled H_2O (1.5 l), 40% (1.5 l), 60% (1 l), 80% (1 l), and 100% methanol, respectively. The 60% and 80% were separately concentrated under the reduced pressure to get the portions that were used in the current investigation.

Microorganisms

Seven referenced bacterial strains were used, representing both Gram-positive and Gram-negative microorganisms, inoculums were obtained from the Department of Pharmaceutics, Unaizah College of Pharmacy, Qassim University, Saudi Arabia. The Gram-positive bacteria include *S. aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, and *Bacillus cereus* ATCC 10876, while the Gram-negative bacteria were *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, and *K. pneumoniae* ATCC 27736.

Antibacterial activity testing

The antibacterial activity of honey samples was evaluated by disc diffusion method as previously mentioned [13]. Each bacterial strain was sub-cultured in nutrient broth and incubated overnight. Then, the fresh bacterial growth was considered as a working sample, which was adjusted using sterile normal saline (0.9%) to be equivalent to 0.5 McFarland to get a working bacterial suspension (about 1×10^8 CFU/ml). In aseptic conditions, 20 ml of autoclaved Mueller–Hinton agar was poured in a sterile Petri-dish (100 × 15 mm) and allowed to solidify and then 100 µl of the working suspension was pipetted and swapped over the Mueller–Hinton agar. Whatman filter paper No.1 was cut to 6-mm diameter discs and autoclaved using well tighten dry container. Dry sterile discs were saturated with the previously prepared honey samples in 60% and 80% methanol. Saturated discs were loaded over the inoculated Petri-dish. The pre-experimental test showed that 60% and 80% of the methanol (as a solvent) have no inhibitory effect on bacterial cells. Moreover, positive control disc containing Gentamicin (10 µg/disc) was also loaded. All plates were incubated for 24 hours at 37°C. The experiment was repeated two times and mean zone of inhibition was calculated using statistical package for the social sciences (SPSS) program.

Statistical analysis

Data was expressed as mean ± standard error of means, one way analysis of variance and graphing were performed using SPSS program, version 14.

Results

Table 1 and Figure 1 show the results of the antibacterial screening of two natural honey samples from Buraidah and Najran. In general, results revealed that both samples showed significant antibacterial activity on Gram-positive bacteria and weak or no activity on Gram-negative bacteria, compared with the referenced antibiotics, gentamicin. The honey sample from Buraidah exhibited higher

Table 1. Antibacterial activity of two honey samples*.

Honey sample	Gram-positive bacteria				Gram-negative bacteria		
	Sa	Se	Ef	Bc	Ec	Kp1	Kp2
A	15.5 ± 0.5	13.5 ± 0.5	17.5 ± 0.5	16.5 ± 0.5	7.0 ± 0.0	6.5 ± 0.0	7.0 ± 0.0
B	10.5 ± 0.5	10.5 ± 0.5	14.5 ± 2.5	10.0 ± 1.0	6.8 ± 0.2	7.2 ± 0.8	6.5 ± 0.0
C	7.5 ± 0.5	9.0 ± 0.0	7.0 ± 0.0	11.5 ± 1.5	6.0 ± 0.0	6.8 ± 0.2	6.8 ± 0.2
D	16.0 ± 1.0	15.5 ± 1.5	15.0 ± 1.0	14.0 ± 1.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
Gentamicin (10 µg/ml)	25.0 ± 0.0	23.0 ± 1.0	23.0 ± 0.0	25.0 ± 0.0	21.0 ± 1.0	12.5 ± 0.5	17.0 ± 0.0

*A and B = 60% and 80% methanol extract from honey (Buraidah), respectively. C and D = 80% and 60% methanol extract from honey (Najran), respectively. Sa = *S. aureus* ATCC 25923, Se = *S. epidermidis* ATCC 12228, Ef = *E. faecalis* ATCC 29212, Bc = *B. cereus* ATCC 10876, Ec = *E. coli* ATCC 35218, Kp1 = *K. pneumoniae* ATCC 700603, and Kp2 = *K. pneumoniae* ATCC 27736, 6.0 ± 0.0 = no activity (paper disc diameter).

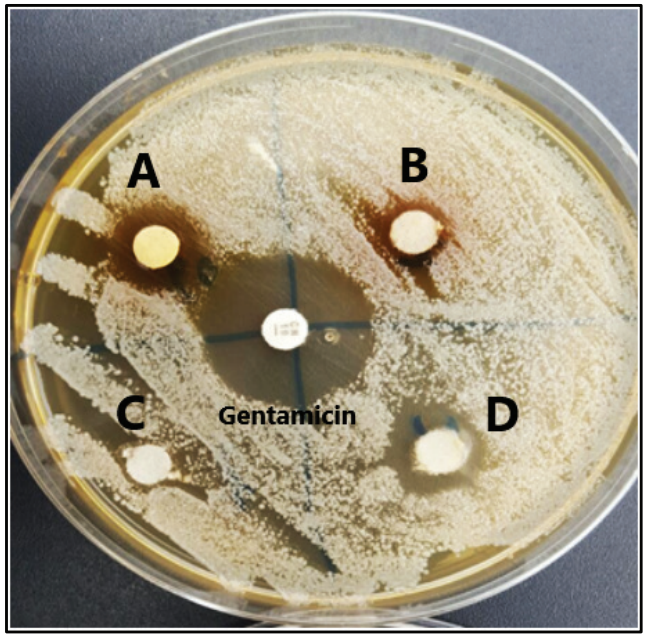

Figure 1. Representative photo showing bacterial susceptibility to honey samples and gentamicin using disc diffusion test.

antibacterial efficacy compared with that from Najran. Around 80% methanol extract of honey showed better results. In details, the most susceptible bacteria to Buraidah honey sample (using with methanol 80%) was *E. faecalis* (17.5 ± 0.5 mm), followed by *B. cereus* (16.5 ± 0.5 mm), *Staphylococcus aureus* (15.5 ± 0.5 mm), and *S. epidermidis* (13.5 ± 0.5 mm), respectively whereas, *E. coli* and *K. pneumoniae* strains showed weak antibacterial susceptibility ranging from 6.5 ± 0.0 to 7.0 ± 0.0 mm. As well, the most susceptible bacteria to Najran honey sample (with methanol 80%) was *S. aureus* (16.0 ± 1.0 mm), followed by *S. epidermidis* (15.5 ± 1.5 mm), *E. faecalis* (15.0 ± 1.0 mm), and *B. cereus* (14.0 ± 1.0 mm), respectively, while all the Gram-negative bacteria did not show any susceptibility (6.0 ± 0.0 mm).

Discussion

The current study provides the scientific evidence that natural honey samples from Buraidah and Najran have good antibacterial efficacy, which could have a potential inhibitory affect against diseases caused by Gram-positive bacteria. The Gram-negative bacteria were much resistant to honey extracts; it is known that the outer membrane surrounding the peptidoglycan layer of Gram-negative affect on the permeability of cell-membrane of the Gram-negative bacteria and may potentiate the other antibacterial molecules, as well [15]. Another study claimed that the antibacterial potential of honey is due to the enzymatic production of hydrogen peroxide, others cited that it could be attributed to its high osmolarity, i.e., low pH level and high sugar content [16]. However, the current study used 60% and 80% methanol, which indicated that this activity may be related to the bioactive contents of honey (Table 1). Overall, our findings are in agreements with some previous studies; it was published that some honey samples showed antibacterial activity against Gram-positives *S. aureus* and Methicillin resistant *S. aureus*, another sample revealed antibacterial activity against Gram-negative bacterium (*Acinetobacter baumannii*) [17]. Interestingly, many studies worldwide reported that, some honey samples showed wide-spectrum antibacterial activity against both Gram-negative and Gram-positive bacteria, clinical bacterial isolates from wounds, namely, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* exhibited high susceptibility to crude honey samples from Nigeria [18]. Eight natural monofloral and polyfloral honey samples collected from Algeria recorded remarkable antibacterial activity against urinary tract infections caused by *P. aeruginosa* and Enterobacteria [19].

Moreover, some samples of jujube honey collected from Saudi Arabia showed antifungal properties against *Candida albicans*, a major fungal pathogen of humans, and also have the ability to inhibit the biofilms formation [20]. Finally, variations in biological activities of honey samples is depending on many factors, such as the floral origin, geographical origin, humidity, temperature, climatic, and environment conditions [21], which make it difficult to be used as a drug unless the bioactive components are isolated, characterized, and produced in massive quantities using biotechnological approaches.

Conclusion

Honey has been considered as an important food and panacea for human beings presumably from early times. The findings of this study support the folkloric application of honey against some pathogenic diseases although they were much effective against the Gram-positive bacteria. The jujube honey sample from Buraidah showed higher activity, which is associated with the vegetation in this region (mostly *Ziziphus* spp.). Palynological assessment is important in the apitherapy research, in order to determine the origin of the collected nectar. Finally, more studies are recommended, such as isolation and characterization of the bioactive molecules of these honey samples. Studying the synergy action of natural honey and powerless antibiotics is also recommended, which could lead to revival of some weakened antibiotics due to differences in mode of actions.

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