The Pharmaceutical and Chemical Journal, 2017, 4(3):37-42

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Antibacterial Potentiality of Water Extract of selected Honey Samples on Some Clinical Isolates

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Abstract The present study was carried to evaluate antibacterial activity of water extracts of honey against some selected bacteria (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *pseudomonas aeruginosa*) using microdilution method (MIC) at different concentrations (100-0.195mg/ml) and well diffusion method (200 mg/ml). The inhibitory potency of the honey from different sources varied on the test organisms and all the test organisms were susceptible to the honey extracts at different concentration. The water extracts of the sample 2 showed powerful antimicrobial activity against Gram-positive and Gram-negative bacteria and sample 1 showed acceptable degree in control of growth Gram-negative other than Gram-positive bacteria. The water extract from sample 2 showed powerful antimicrobial activity against *S. aureus* and *E. coli* with MIC value 6.25 mg/ml and inhibition zoon (38-29mm) respectively. These results were revealed the importance of tested extracts in control of some human pathogenic micro-organisms.

Keywords Antibacterial activity, microdilution method, well diffusion method, honey extracts, Gram-positive and Gram-negative bacteria

Introduction

Antibiotic resistance pathogens continue to increase worldwide but the rate of find of new antibiotics has steadily decreased over the last 20 years [1].

When injury occurs, tissues may be prone to bacterial infection leading to dark granulation of the tissues which can delay wound healing. Antibiotic resistance is a worldwide problem, and the presence of resistant strains of bacteria attributed to the extensive use of antibiotics [2]. Many forms of resistance spread with noticeable speed. World health leaders have described Antibiotic resistance pathogens as "nightmare bacteria" that pose a catastrophic threat to people in every country in the world. The failure of these antibiotics and the emergence the problem of resistance to antibiotics has resulted for man to search for more effective sources of natural products from plants and some insects [3]. There has been a recent impulse in interest for wound management products of natural origin including honey [4]. Honey is a natural sweet substance produced by honeybees from the nectar of blossoms or from the secretions of living parts of plants [5].

Honey is one of the oldest traditional medicines for the treatment of infected wounds, which has recently been rediscovered by the medical profession, particularly where conventional modern therapeutic agents fail [6]. The activity of honey for healing could be due to various physical and chemical properties [7].

The antimicrobial properties of honey are considered the most important characteristic of honey for healing of wounds [8]. The antibacterial activity of honey may be useful against antibiotic resistance bacteria e.g. *Staphylococcus aureus*, which is a major cause of wound sepsis in hospitals [9]. Its antibacterial potency has been referred to strong osmotic effect, naturally low pH [10].

Many studies have reported that Honey possesses powerful antimicrobial properties that can be utilized at low cost and at no risk [11], and also it has been shown to reduce aflatoxin B1 and B2 levels and inhibit the growth of *Aspergillus flavus* [12]. Also the honey possesses powerful properties that can be affected on the growth of microorganisms by bacteriostatic or bactericidal actions [13].

Renewed interest in honey for different therapeutic purposes including treatment of infected wounds has led to the search for new antibacterial and antifungal honeys [13]. The aim of this short study is to assess the in vitro the antibacterial and antifungal properties of two types of honey produced from different areas against four pathogenic microorganisms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*).

Materials and Methods

Chemicals

Mueller Hinton agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Agar (SDA), RPMI 1640 Media and Distilled water (D.W).

Table1: Culture media which used in this study

Chemicals	Manufacturer	Country
Mueller Hinton agar	Liofilchem	Italy
Mueller Hinton Broth	Liofilchem	Italy

Honey Samples

Two types of honey produced from different areas in Gaza strip. Honey samples were placed in glass containers and stored in the dark at room temperature until use.

Antimicrobial Susceptibility Test

Microorganisms

The microorganisms which have been used in this study are the bacteria (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *pseudomonas aeruginosa*) which were isolated from clinical samples delivered from El-Shifa Hospital.

Inoculum Preparation

The inocula were prepared by picking 3-5 morphologically identical colonies from overnight growth with a sterile inoculating wire loop. The actively growing culture was adjusted with sterile MHB to matches 0.5 McFarland standard **[18].**

Antimicrobial Test

Antibacterial Assay: The activity of extracts against microorganism was determined by the broth macrodilution method (96- well plates). Extracts were diluted a number of times through a sterile diluent (MHB) after were diluted the obtained concentration ranges were from (100 to 0.1953) mg/ml. Then added 10 µl of inocula of overnight growth microorganisms to each well except a positive control.

Extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37°C for 24 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5 triphenyl tetrazolium chloride (TTC) as indicator was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC [14].

Well Diffusion Method: Antibacterial activities of honey extracts were evaluated using well diffusion method on Mueller-Hinton agar (MHA). MHA agar plates were inoculated with bacterial strain under aseptic conditions and wells

were filled with 50 µl of the test samples and incubated at 37 °C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured [15].

Results

The results in Table 2 showed the antimicrobial activity of honey samples by microdilution method and well diffusion method against tested microorganisms (*S. aureus, E. coli, K. pneumoniae* and *p.aeruginosa*). From the evaluation it was found that the water extract from sample 2 showed powerful activity against Gram-positive Gram-negative bacteria. Sample 1 appears to be more inhibitor against Gram negative - than Gram-positive bacteria.

For *E. coli* the water extract of the two honey samples 1 & 2 showed powerful antimicrobial activity with MIC value (12.5 - 6.25 mg/ml) respectively and powerful inhibition zoon (28-29 mm) (Figure1&2).

Table2: Showed the antimicrobial activity of honey samples by microdilution method and well diffusion method against tested microorganisms.

A.A.A / Samples / M/Os	MIC (mg/ml)		Well diffusion method (mm)	
	Honey1	**Honey 2**	**Honey1**	**Honey 2**
S. aureus	100	6.25	21	38
E. coli	12.5	6.25	28	29
K. pneumoniae	50	12.5	35	37
p. aeruginosa	100	25	20	40

A.A Antimicrobial assay M/Os Micro-organisms

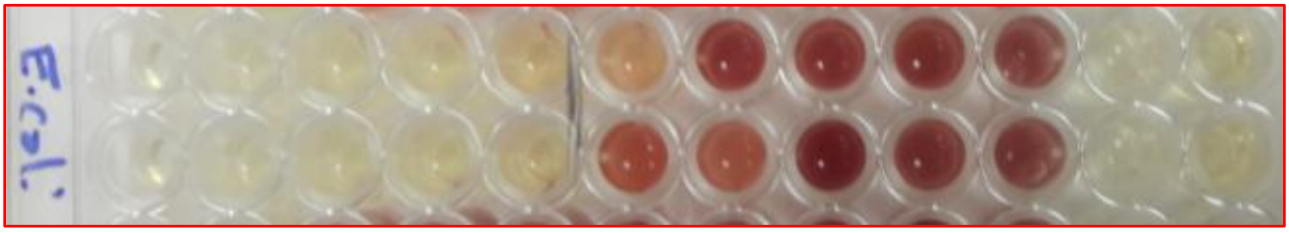

Figure 1: The effect of sample 1 against E. coli (MIC)

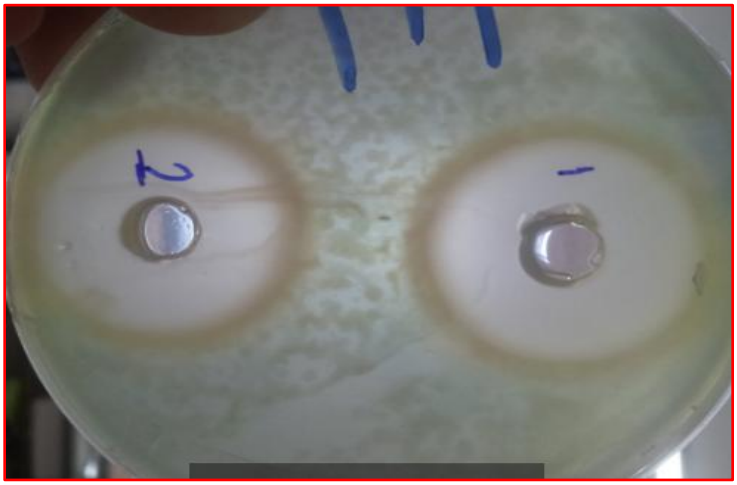

Figure 2: The effect of sample 2 against E. coli (well diffusion method)

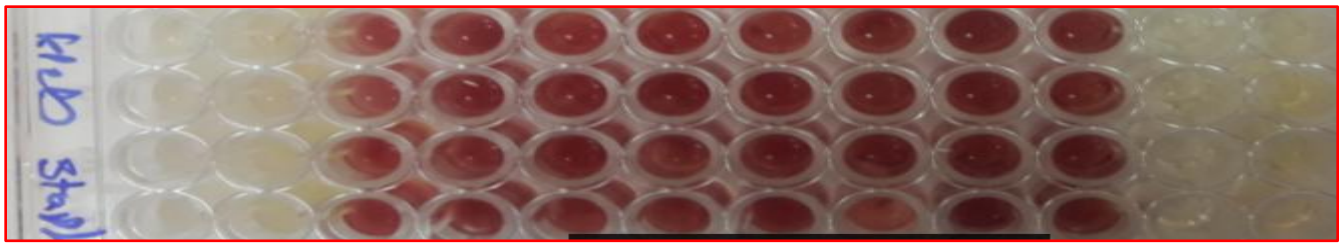

Figure 3: The effect of sample 1 against S. aureus and K. pneumoniae (MIC)

Also the water extract of the two honey samples (1&2) showed antibacterial activity against *K. pneumoniae* with MIC value (50-12.5 mg/ml) (Figure 3), and powerful inhibition zoon (35-37 mm). For *p. aeruginosa* the two extract showed antimicrobial activity with MIC value (100-25 mg/ml) and inhibition zoon (20-40mm). Sample 2 showed powerful activity against *S. aureus* with MIC 6.25 mg/ml (Figure 3), and inhibition zoon 38mm.

Figure 4: The effect of sample 2 against p. aeruginosa (Well diffusion method)

Discussion

Although antibiotics are one of the main methods used in resistance bacterial infections, the use of antibiotics has become threatened due to appearance of resistance strains [14]. Obviously the wide use of antibiotics in the treatment of bacterial infections has led to spread of resistant strains so we need to find a source of bioactive substances that could possess broad spectrum of activity when it is combined with antibiotic to be able resist the pathogenic microorganism. The antibacterial activity of honey has been known since the 19th century which was first reported in 1892 [8]. The intrinsic properties of honey as antimicrobial agent have been attributed to multiple components including high sugar concentration, low pH, osmolarity, and enzymatic generation of hydrogen peroxide via glucose oxidase [16, 17]. The high sugar concentration and it's moisture content causes osmotic stress to microbial cells and low pH is undesirable for the growth of many micro-organisms [17]. Moreover, the honey components, such as aromatic acids or phenolic compounds, may also contribute to the overall antimicrobial activity [16]. *Candida albicans* and other pathogens can cause life-threatening infections, especially in immune-compromised patients. Treatment with currently available antifungal agents may lead to sharp side-effects and emergence of resistant strains [18]. Antibiotic resistance in multi-drug resistance bacteria is becoming a major problem in the treatment of many infections [19, 14]. Many in vitro, studies have found honeys possess antibacterial and antioxidant activity which form part of the functional physico-chemical properties of honey considered necessary for wound healing [20, 4]. This current study was aimed to detect the effectiveness of water extracts of two honey samples toward a number of bacteria especially (which have multi-resistance characteristic against antibiotics) for producing new antimicrobial agent of great benefit to mankind.

The result of this study revealed that the tested honey samples provided more powerful antimicrobial activity against selected bacteria (*S. aureus, E. coli, K. pneumoniae* and *p.aeruginosa*). All extracts showed a decrease in MIC to test samples which mean that the selected honey samples contain bioactive antimicrobial agents that might inhibit bacteria by different mechanisms. The excellent antibacterial activity of honey against clinical bacterial isolates indicates the usefulness of honey in clinical practice against bacterial infection and these results are in agreement with the previous studies [21, 22 & 18]

Generally, most natural extracts appear to be more inhibitor against Gram-positive than Gram-negative bacteria [23], which could be due to the difference in the structure of the bacterial cell wall [24].This agreement contradicts our results because the sample 1 showed powerful antimicrobial activity against Gram-negative than Gram-negative bacteria and extract from sample 2 showed powerful antimicrobial activity against all tested microorganisms (Gram-positive and Gram-negative bacteria).

Conclusion

We can be concluded from *in vitro* studies that honey has intrinsic properties contribute to the applicability of honey for medical purposes. Also honey samples contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. In vitro, our results revealed that honey samples proved their effectiveness with acceptable degree in control of growth some pathogenic microorganism however in vivo experiments are needed to confirm these results.

Acknowledgments

The authors are grateful to Department of biology and Biotechnology, Islamic University for providing excellent research facilities and El-Shifa Hospital for providing clinical bacteria strains.

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