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Original Research Article

Antimicrobial Activity of Different Honey Types Against *Escherichia coli*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Candida albicans*

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Abstract

***Objective**:* To assess the in vitro antimicrobial activity of eight types of local honeys from Green Mountain province, Libya on different microorganisms. ***Methods**:* 100% of different types (Undiluted) of honey were studied in vitro using, *Escherichia coli, Streptococcus pyogenes Pseudomonas aeruginosa and Candida albicans*, to determine the zone of inhibition against each type of microorganism. The antimicrobial activity of honey samples was compared with standard antibiotics like Ciprofloxacin, Amoxil/clavulunic acid, Tetracycline, Gentamycin, Ampicillin/cloxacillin, Erythromycin and Nitrofurantoin was determined by the disc diffusion method. ***Results**:* The diameter of zone inhibition of honey has various values tested ranged from 11-18.5 mm for *Pseudomonas aeruginosa*, 0-12 mm for both *Streptococcus pyogenes* and *Escherichia coli*, while with no effect for *Candida albicans*. ***Conclusions**:* The eight types of Libyan honey have a various antibacterial activity in-vitro.

Keywords: Antimicrobial Activity, Honey, *Escherichia Coli, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Candida albicans*.

INTRODUCTION

Disease causing bacteria have always been considered a major cause of morbidity and mortality in human. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents (Abdel-Massih *et al.*, 2010)**.** The emergence of resistant gram-positive bacteria presents a major challenge for the antimicrobial therapy of infectious diseases (Lusby, Coombes and Wilkinson, 2005),(Mundo, Padilla-Zakour and Worobo, 2004) .

Traditional importance and use of honey as therapeutics has been mentioned by the Egyptian and Sumerian physicians as early as 4000 years ago (Krell, 1996). Because honey inherits plants properties, its color, aroma, flavor, density and physical and chemical properties depend on the flowers used by bees, the weather conditions as well as processing influences its composition and properties (Alvarez-Suarez *et al.*, 2010).

Due to its unique taste, nutritional value and health-promoting properties, honey has a valued place in the human diet. Sugars, mainly fructose and glucose and minor amounts of oligosaccharides account for about 80% of its weight. As a consequence, it is an easily digestible and highly energetic food product. Consumption of 100 g of honey provides the body with about 320 kcal. However, the health-promoting properties of this product come mainly from the presence of other than sugar components: enzymes, peptides, free amino acids, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals and minerals (Terrab *et al.*, 2003).

Depending of botanical source, different types of honey are proposed for prophylaxis and treatment of different health problems (Hołderna-Kędzia and Kędzia, 2002). In particular, interesting and important issue is antimicrobial activity of honey. In fact, it is the only food product that without any technological processing, nor addition of preservatives, can be stored for a long period of time even several years, without any negative symptoms. Interestingly, the honey is not a sterile product, it contains some microorganisms, mostly bacteria, yeast and molds. However, the specific environment of this product, high osmotic pressure and high acidity prevents the development of microorganisms (Kwakman *et al.*, 2010). Moreover, it has been shown that some of bacteria that are present in the honey produce antimicrobial agents, bacteriocins, which can protect the product against development of other microorganisms and are be beneficial for consumers' health (Lee, Churey and Worobo, 2008).

Currently, many researchers have reported the antibacterial activity of honey and found that, natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic microorganisms (Lusby, Coombes and Wilkinson, 2005), (Mundo, Padilla-Zakour and Worobo, 2004).

Mechanisms of antimicrobial activity of honey are different from antibiotics, which destroy the bacteria's cell wall or inhibit intracellular metabolic pathways. The antibacterial activity is related to four properties of honey. First, honey draws moisture out of the environment and thus dehydrates bacteria. The sugar content of honey is also high enough to hinder the growth of microbes, but the sugar content alone is not the only reason for honey's antibacterial properties (Simon *et al.*, 2009). Second, the pH of honey is between 3.2 and 4.5, and this acidity is low enough to inhibit the growth of most microorganisms. Hydrogen peroxide produced by the glucose oxidase is the third and probably the most important antibacterial component, although some authors believe the non-peroxide activity to be more important. Lastly, several phytochemical factors for antibacterial activity have been identified in honey (Al-Waili, 2004).

All these physical and chemical factors give honey unique properties as a wound dressing: it has a rapid clearance of infections, rapid debridement of wounds, rapid suppression of inflammation, minimization of scarring, and stimulation of angiogenesis as well as tissue granulation and epithelium growth (Basualdo *et al.*, 2007), (Molan, 1992). As the majority of wound and burn infections are caused by *Pseudomonas aeruginosa* (*P.aeruginosa*), and *Streptococcus pyogenes* (*S. pyogens*), as well as the urinary tract infections caused by *Escherichia Coli* (*E.coli*) ; the action of honey against these organisms is of interest to both researchers and clinicians (Edwards-Jones, Greenwood and Group, 2003). Also, the incidence of fungal infections is increasing in both the community and hospital environments, with *Candida albicans* (*C albicans*) (Tortorano *et al.*, 2004). The aim of this study is to assess the antimicrobial effects of different local honey types on different clinical gram positive, gram negative bacterial and fungal isolates.

MATERIALS AND METHODS

Collection of honey

The eight types of honey were collected from local honey store Al-Nour in Shahat city in north-eastern, Libya: (Elkharob, Elrabee, Elhanoon, Elzaater, Elkafoor, Elseder, Dryas and Arar), Which then stored at refrigerator for bacteriological experiments.

Collection of bacterial Samples

Four clinical samples were isolated from different patients from Al-Thawra Hospital in El-Beida city, Libya (*E.coli*, *P.auerginosa*, *S.pyogenes and C albicans*), were aseptically swabbed with sterile swab. The samples were stored in an ice packed container as a mixed broth culture and taken to the laboratory for cultural assay.

Determination of antimicrobial activity of honey by disc diffusion method

The antimicrobial activity of the honeys was assayed using the disc diffusion method (DDM) as described by (Cheesbrough, 2006) on Muller Hilton agar. In this method, six (6) millimeter diameter discs cut out from N^{o}.1 Whatman filter paper were boiled for 30 minutes to remove any chemicals that may inhibit the growth of the microorganisms, and sterilized by autoclaving at 121°C for 15 minutes. The sterilized discs were soaked in different honey samples. The discs were thereafter, placed on plates has *Muller Hilton* agar and incubated at 37°C for 24 hours. The zones of inhibition were measured in millimeter as degree of susceptibility of the medical isolates to the honey samples and the means of the inhibition zones were calculated.

Sensitivity test of standard antibiotics

Sensitivity of the microbial isolates also was tested against antibiotic discs: Ciprofloxacin, Amoxil/clavulunic acid, Tetracycline, Gentamycin, Ampicillin/cloxacillin, Erythromycin and Nitrofurantoin.

STATISTICAL ANALYSIS

Data collected from the results were analyzed by using Oneway ANOVA, IBM SPSS version 24 statistics software. Simple means and standard deviation were computed as appropriate. A p value $\leq$ 0.05 was considered significant.

RESULT

The sensitivity of *S.pyogens*, *E.coli*, *P.aeruginosa* and *C albicans* against the undiluted (100%) honey samples studied was determined. Table 1 shows the diameter values of inhibition of isolated microorganisms. The results of antibiotics sensitivity test were illustrated in Table 2.

It was obvious that, the Elkharoob and Elsedr (18.5mm) similarly gave the highest inhibition zone followed by Elrabee and Arar (17.5mm), Alhanoon and Elkafoor (16mm), Elzaater and Dryas (11mm) with *P.aeruginosa*. Elkharoob honey (12 mm) gave the highest antibacterial activity against *S.pyogens*, followed by Elrabee (10mm),then Arar and Elkafoor (8mm), after that Elzaater (6mm) and later no inhibition zones were observed with Dryas, Elhanoon and Elsedr. It was clear that, *E.coli* was inhibited by only two types of honey; Arar (12mm) and Elrabee (9mm). On the other hand, the other types of honey have no effect against *E.coli*. In this study, *C albicans* was not inhibited by any type of honey.

Table-1: Activity of various honeys against *E.coli, S. pyogens, P. aeruginosa* and *C albicans*

		Zone of inhibition (in mm)		
	E.coli	**S.pyogens**	**P.aeruginosa**	***C albicans***
Honey type				
Elrabee	9	10	17.5	0
Elhanoon	0	0	16	0
Elzaater	0	6	11	0
Elkafoor	0	8	16	0
Elsedr	0	0	18.5	0
Elkharoob	0	12	18.5	0
Dryas	0	0	11	0
Arar	12	8	17.5	0

The results of this study were also compared with the inhibition zone diameters of commonly used antibiotics (Table 2). All the tested bacteria were affected by Gentamicin with zones of inhibition measuring 13 mm, 19 mm and 10 mm for *E.coli*, *S.pyogens* and *P.aeruginosa*, respectively. *E.coli* and *S. pyogens* were affected by Amoxil /clavulunic acid, Ampicillin/cloxacillin and Nitrofurantoin with zones of inhibition measuring 12 mm, 25 mm; 15 mm, 17 mm and 14 mm, 17 mm, respectively. Ciprofloxacin revealed a large zone of inhibition measuring 30 mm similarly for *S.pyogens* and *P.aeruginosa*. Analysis of tetracycline showed no effect on all tested microorganisms. The result of *C albicans* showed no zones of inhibition.

Table-2: The results of antibiotics sensitivity test were illustrated

		Zone of inhibition (in mm)		
	E.coli	S. pyogens	P.aeruginosa	*C albicans*
Antibiotic types				
Ciprofloxacin	0	30	30	0
Gentamicin	13	19	10	0
Amoxil/clavulunic acid	12	25	0	0
Tetracycline	0	0	0	0
Ampicillin/cloxacillin	15	17	0	0
Erythromycin	17	0	0	0
Nitrofurantoin	14	17	0	0

DISCUSSION

Many studies have evaluated the antimicrobial properties of honey against a range of Gram-positive as well as Gram-negative bacteria (Lee, Churey and Worobo, 2008), (Mandal *et al*., 2010).

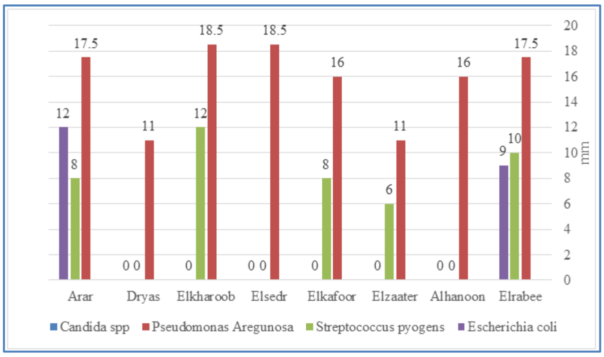

Fig-1: Susceptibility of *E.coli, S.Pyogens, P.aeruginosa and C albicans* to various honeys samples

This study attempted to assess the value of honey from different botanical sources as an antimicrobial therapeutic agent. Elkharoob, Elrabee, Elhanoon, Elzaater, Elkafoor, Elseder, Dryas and Arar are common types of honeys consumed in Libya, especially in Green Mountain. These different types of honey showed different antimicrobial activities against clinically isolated microorganisms (Figure 1). The variation in the antimicrobial potential of honey used in the present study as compared to the others, might be due to differences in growth rate of pathogens, inoculum size and the test method itself, as well as source of the microorganisms (Mandal *et al.*, 2010).

Tan *et al.*, 2009 stated that, honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing. Also, it might be the fact that the type of honey produced by honeybees is dependent on the natural vegetative flowers blooming in different seasons and in different places, and thus the flowers from which bees gathered nectar to produce the honey may contribute to the difference in the antimicrobial activities of honey (Mulu, Tessema and Derbie, 2004). Several earlier authors reported that, the antimicrobial activities have been attributed to its high acidic nature (pH being 3.2-4.5), high osmotic effect, hydrogen peroxide concentration and its phytochemical nature; beside this, it has been reported that methylglyoxal, which is present in high concentration in honey, is directly responsible for its characteristic antibacterial property (Mundo, Padilla-Zakour and Worobo, 2004), (Adams *et al.*, 2008), (Atrott and Henle, 2009).

The antimicrobial effect of honey samples against *E. coli* and *S. pyogens* was found different that in turn indicated difference in the sensitivity of these bacteria to the antimicrobial activity of honey (Mandal and Mandal, 2011). The current study showed that, honey has less antimicrobial activity against *E.coli* as compared with the other test microorganisms. A study conducted in 2003 Al-Jabri *et al.*, had revealed that the Black Forest honey had highest activity with *P.aerugiriosa* and least with *E.coli.*

In this study, there is a significant difference in susceptibility to honey was observed for *E.coli* compared to the isolated microorganisms, whereas no significant difference in susceptibility to all honey samples was observed for *P.aeruginosa*. The antibacterial effect against *E.coli* was also shown in vitro and in vivo by other authors, results obtained for in vivo experiments indicated that consumption of honey produced lesser growth of *E.coli* (Shamala, Jyothi and Saibaba, 2002).

A study conducted in 2016 by Wasihun and Kasa, showed that *S.pyogenes* was inhabited after treating with honeys. Another study by Efem and Iwara, 1992, showed a moderate activity (6-11 mm considered as moderately sensitive) of honey on *S.pyogenes*, which is similar to the result in present study with Elkharoob, Elrabee, Elkafoor, Arar and Elzaater honeys.

Notably, the ability of all local honeys to inhibit *P.aeruginosa* growth was observed in this study, these results demonstrated that there is a similarity with previous conducted reports, a study performed by Wilkinson and Cavanagh, 2005, indicated that *P.aeruginosa* was inhabited after treating with several floral species of honeys. It was also reported that, *P.aeruginosa* and *E.coli* species are inhibited by honey (Hegazi *et al.*, 2001). According to a report by Cooper, Halas and Molan, 2002, found a potential effect of tested honey against *P.aeruginosa* . Further study performed by Shenoy *et al.*, 2012; Wasihun and Kasa, 2016, stated that Indian and Ethiopian origin honeys respectively have antimicrobial activity against *P.aeruginosa* isolates.

Inadequate researchers have found honey to have an inhibitory effect against *C albicans* in vitro (Theunissen, Grobler and Gedalia, 2001), (Efem SE *et al*., 1992), (Adeleye IA *et al*., 2003). One of these studies performed *in vitro* demonstrated that, honeys have activity at the high concentration and concentration dependent (Koc *et al.*, 2009) (Efem and Iwara, 1992). Recently, a study in 2018, by Morroni *et al.*, indicate that three different types of honey demonstrate potent inhibitory activity against all clinical isolates tested, that included representatives of Gram-positive, Gram-negative bacteria, and *C. albicans*. Whereas, the result in this study showed no inhibition effect of honey on *C albicans*.

CONCLUSION

All collected honeys showed varied antibacterial activities, and only *C albicans* was resistant to tested honeys. Alternative natural products that have been tested could be a source for antibacterial agents that can serve as an alternative to the expensive synthetic antibacterial agents used in treatment of various bacterial infections if adequately explored. In light of the enormous pressure during the last years, for recovery of new medicinal products, further work is required to be done in order to evaluate the honey antibacterial capacity against various pathogens.

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