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Potential antimicrobial activity of different types of Libyan honeys

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Abstract:

Honey exhibits antimicrobial activity against a wide range of bacteria. The aim of the present work was to evaluate the antimicrobial effects of the Libyan honeys harmal (*Peganum harmala* L.) , red camphor (*Cinnamomum camphora*), white camphor (*Eucalyptus globule*), sarou (*Cupressus sempervirens*), athl (*Tamarix aphylla*) and kharoub (*Ceratonia silique*) on *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeuroginosa, Escherichia coli, Bacteriods* spp., *Sarcina* spp. and *Candida albicans.* Pathogens exhibited different sensitivities towards the honey samples. The results showed that *C. camphora* inhibited seven out of the nine tested microorganisms followed by *T. aphylla* honey, which inhibited six of them. The lowest effects were shown by *P. harmala* and *C. semperviren* honeys, where they only inhibited four different types of the tested microorganisms.

Introduction

Honey is a complex natural food produced from the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) feeding on plant nectar of blossoms, exudates of trees and plants or from honey bees feeding on honeydew produced by hymenoptran insects. Honey is a saturated solution of sugar of 31% glucose and 38% fructose and its colour and flavor vary considerably depending on it botanical and geographical origin (Gheldof *et al*., 2002) and of a moisture content of about 17.7% (Nagai *et al.*, 2006). In addition to minor component of phenolic acids, flavonoids, glucose oxidase, catalase, ascorbic acid, carotenoids, organic acids and α-tocpherol (Ferreres *et al*.,1993). Honey contains at least 181 components (White, 1975).

The use of honey for the treatment of diseases and wounds has been mentioned since ancient time (2100-2000 BC), where Aristotle (384-322 BC) described pale honey for sore eyes and wounds (Mandal and Mandal, 2011 and Vallianou *et al.* , 2014). Microorganisms such as *Staphylococcus aureus, Staphylococcus epidermis*, *Micrococcus luteus, Streptococcus uberis, Enterococcus faecalis, Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia* are frequently isolated from

human and animal skin wounds (Vuong and Otto, 2002; Nasser *et al.*, 2003; Halcon and Milkus, 2004; Altoparlak *et al.*, 2005 and Basualdo *et al*., 2007).

The healing effect of honey could be due to its physical and chemical properties (Rusell *et al.*, 1990 and Snow and Manley-Harris, 2004) and to its antioxidant and antimicrobial activity (Martos *et al.*, 2000; Escuredo *et al.*, 2012; Vandamme *et al.*, 2013; Isidorov *et al.*, 2015; Francine *et al*., 2016; Almasaudi *et al.*, 2017 and Leyva-Jimenez *et al*., 2019). Honey acts as an effective thermal insulator and a protective biofilm (Black and Costerton, 2010). Its antimicrobial activity is connected with its osmotic pressure which draws fluid from wounds, decreasing tissue edema (Molan, 2001). A possible reason for its activity depends on its ability to generate hydrogen peroxide by the bee derived enzyme glucose dehydrogenase (Saleh *et al.*, 2011). The strength of honey hydrogen peroxide is much lower than pharmacologic hydrogen peroxide, causing no damaging to the healing environment of a wound (Bang *et al*., 2003). Wound size is affected by pH value, Gethin *et al.* (2008) found that honey reduces the wound's pH and every 1 % reduction in pH is associated with a 1 % reduction in wound size. For wounds contaminated by methicillin-resistant *Staphylococcus aureus*, Manuka honey supported better wound healing than antibiotics (Gethin and Cowman, 2008). There are several studies indicating the effectiveness of honey in treating burns (Molan, 2001; Subrahmanyam, 1991 and Subrahmanyam *et al*., 2001).

Sukur *et al.* (2011) studied the effectiveness of Tualang honey in healing full-thickness burn wounds in rats. They found that topical application of honey on burn wounds contaminated with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* give better results of healing compared with other treatments.

The finding that the bacterium, *Helicobacter pylori* is a cause of stomach ulcers and the causative agent in many cases of dyspepsia has raised the possibility that the therapeutic action of honey for symptoms of dyspepsia may be due to Manuka honey's antibacterial properties. Somal *et al.* (1994) demonstrated that after an incubation period of 72 h.,5% Manuka honey completely prevent the growth of *H. pylori* (the causative organism of stomach ulcers). Similar invitro antimicrobial results of Manuka honey was reported against *Campylobacter* spp. (Lin *et al.*, 2009).

The aim of the present work was to evaluate the antimicrobial effects of the Libyan honeys Harmal (*Peganum harmala*), red camphor (*Cinnamomum camphora*), white camphor (*Eucalyptus globule*), sarou (*Cupressus sempervirens*), athl (*Tamarix aphylla*) and Kharoub (*Ceratoniasilique*) on *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Enterococcus faecalis, Pseudomonas aeuroginosa, Escherichia coli, Bacteriods* spp., *Sarcina* spp. and *Candida albicans.*

Materials and Methods

The present investigation was carried out at the Beekeeping Research Section, Plant Protection Research Institute, Giza, Egypt.

1.Honey samples:

Six types of Libyan honeys of mono and multi-floral source were collected from selected beekeepers during the harvesting periods and from local markets in Western Libya. The honeys of mono-floral source were harmal (*P. harmala*, red camphor (*C.camphora*), white camphor (*E. globule*), sarou (*C. sempervirens*), athl (*T. aphylla*) and kharoub (*C. silique*). Honey samples were kept in dark at room temperature prior to analysis. The samples were investigated microscopically to determine their containing of pollen grain types (Table, 1).

2.Bacterial strains:

Bacterial strains and *C. albicans* were kindly donated by the Microbial Genetic Department, Genetic Engineering and

Biotechnology Division, National Research Center, Giza, Egypt.

3. Assay of antimicrobial activity:

Antimicrobial activity of honey samples was determined by the disc diffusion method (Collins *et al.*, 1995). A concentration of 20% of each kind of honey in distilled water was prepared in clean sterile test tube and kept in refrigerator at 4^{o}C to be used for microbiological test.

4.Preparation of the microbial culture:

The tested organisms were inoculated in the appropriate liquid media and incubated at 37 ^{o}C for 24 h. The microbial culture was used for the preparation of seed layer by inoculating the agar medium with 2% (v/v) of the microbial culture, thoroughly mixed and immediately used as the seed layer of plates.

5.Preparation of plates:

The appropriate agar medium was distributed at the rate of 7 ml portion in petri dishes. After solidification 5 ml of the seeded agar was distributed over the surface of the base layer and left for 15 min to solidify. The previously prepared filter paper discs (each disc was moistened with exactly 0.05 ml of the diluted honey) placed side down on the seeded agar and gently pressed with a tip of sterile forceps. Discs were placed symmetrically around the center of the dish. Plates were incubated at 37 ^{o}C for 24 hours. for *P. aeruginosa* and *M. leutus*, plates were incubated at 30 ^{o}C. Antimicrobial activity was determined measuring the diameter of inhibition zones around the discs to the nearest mm (Table, 2).

Three replicates were prepared for each honey sample. As a positive control method, the antibiotic tetracycline (30 µg) was used, while sucrose sugar solution (20%) was used as a negative control method.

6. Statistical analysis:

Results are expressed as mean $\pm$ standard deviation. ANOVA were applied at a confidence level of 95%.

Results and discussion

The results of inhibition effects of different honey samples in comparison to control are shown in Table (2). It was observed that all honey samples inhibited the growth of *C. albicans* with different degrees, where P<0.001. *Bacteroids* spp. was the most resistant bacteria, where it was only inhibited by *C.camphora* honey with an inhibition zone of only 5.0$\pm$0.67 mm. Except *C. silique* honey all honey samples affect the growth of *E. faecalis* with different degrees. *B. subtilis* was moderately inhibited by *T. aphylla* and *C. silique* honeys with inhibition zones of 11.33$\pm$0.57 and 11.66$\pm$0.57 mm, respectively. *C. camphora* inhibited seven out of the nine tested microorganisms followed by *T. aphylla* honey, which inhibited six of them.The lowest effects were shown by *P.harmala* and *C. semperviren* honeys, where they only inhibited four different types of the tested microorganisms. *Escherichia coli, P. aeruginosa* and *Bacteriods* spp. were found to be resistant to the antibiotic tetramycine (+ve control), while 20% sucrose sugar solution (-ve control) had no inhibitory effect on all bacterial strains.

The antimicrobial activity of honey is mainly contributed to the high osmolarity and acidity. In addition, hydrogen peroxide, volatiles, organic acids, flavonoids, phenolic compounds, wax, pollen, propolis are important factors that provide antimicrobial properties to honey. Shin and Ustunol (2005) stated that the sugar composition of honeys from different floral source are responsible for the inhibition of various intestinal bacteria. According to Moumbe *et al.* (2013) the minor components of honey including proteins, minerals, phytochemicals and antioxidants are responsible for the antimicrobial activity of honey in the treatment of infections, burns, wounds and ulcers.

Our results are in agreement with other published studies, showing that some kinds of honey have an inhibitory effect against the fungus *C. albicans* and the bacteria *S. aureus, B. subtilis, K. pneumoniae, E. faecalis, P. aeuroginosa, S. coli, Bacteriods* spp. and

Sarcina spp. (Basualdo *et al.*, 2007; Mercan *et al*., 2007; Al-Haj *et al.,* 2009; Sherlock *et al,*. 2010; Francine *et al.*, 2016; Almasaudi *et al.*, 2017 and Leyva-Jimenez *et al.,* 2019).

The results of this study are similar to the results obtained by Mohapatra *et al.* (2011), who reported that honey was effective against gram-positive bacteria *S. aureus, B. subtilis, E. faecalis* and gram-negative bacteria *E. coli* and *P. aeruginosa.*

The inhibitory effect of honey against *S. aureus*, *E. coli* and *K. pneumonia* is of great importance due to the fact that *Streptococcus* species and coliforms are recognized pathogens. In this work the growth of *Pseudomonas aeruginosa* was inhibited by 3 honey samples (*C.camphora*, *E. globule* and *C. sempervirens*). This type of bacteria is always found in wounds, especially those related to burns causing a variety of systemic infections, particularly in victims with severe burns (Yau *et al.*, 2001). Irish *et al.* (2011) noted that temperature, the time of storage, and the nature of flower's nectar may explain the different antimicrobial activities of different honeys. Our data are in agreement with the findings obtained by McCarthy (1995), who reported that, honey from different floralsources varies greatly in their antibacterial activity. Rybak and Szczęsna (1996) found that the minimum concentrations of honey which inhibit the growth of *B.subtilis* were 5-10%. Molan *et al.* (1988) reported significant differences between different kinds of floral honey in their activities on *S. aureus* at dilutions of 1/4, 1/8 and 1/16 original strength. Radwan *et al.* (1984) reported that honey from *Acacia mellifera* inhibits the growth of *E.coli*. Molan and Russell (1988) found that pollen present in honey could be the source of the antibacterial aromatic acids, which causes the component to act individually or synergically to prevent bacterial resistance (Cooper *et al.*, 2010). In addition to pollen, propolis is also found in honey. The antimicrobial and anti-inflammatory activity of European propolis is associated with the presence of flavonoids, flavones, and phenolic acids and their derivates (Bankova, 2005).

Table (1):Types and floral sources of Libyan honeys.

No. of samples	Local name of honey	Floral source
Sample 1	Harmal	*Peganum harmala*
Sample 2	Red camphor	*Cinnamomum camphora*
Sample 3	White camphor	*Eucalyptus globulu*
Sample 4	Sarou	*Cupressus sempervirens*
Samples 5	Athl	*Tamarix aphylla*
Sample 6	Kharoub	*Ceratonia silique*

Table (2): The diameter and standard deviation (in mm) of inhibition zones of different bacterial strains by honey samples compared to control.

Bacterial strains	*Peganum harmala*	*Cinnamomum Camphora*	*Eucalyptus globulu*	*Cupressus Sempervirens*	*Tamarix aphylla*	*Ceratonia Silique*	Tetracycline	Sucrose solution
Escherichia coli	10.33±0.57^{b}	11.00±0.00^{b}	11.66±0.57^{b}	0.00	0.00	0.00	0.00	0.0
Enterococcus faecalis	21.0± 0.0^{c}	23.66±1.52^{c}	5.33±57a	24.0±0.00^{c}	12.0±1.00^{b}	0.00	23.66±1.52^{c}	0.0
Staphylococcus aureus	0.00	0.00	0.00	21.33±1.15^{c}	6.0±0.5^{a}	5.66±1.15^{a}	22.0±0.1.5^{c}	0.0
Pseudomonas	0.0	10.33±0.57^{b}	10.66±0.57^{b}	6.66±0.57^{a}	0.00	0.00	0.00	0.0
Bacillus subtilis	0.00	0.00	0.00	0.00	11.33±0.57^{b}	11.66±0.57^{b}	21.33±1.15^{c}	0.0
***Bacteroids* spp.**	0.00	5.0±0.67^{a}	0.00	0.00	0.00	0.00	0.00	0.0
***Sarcina* spp.**	22.0 ±0.67^{c}	10.0± 1.12^{b}	0.00	0.00	11.0±0.00^{b}	11.0±0.00^{b}	23.66±1.52^{c}	0.0
Klebsiella pneumoniae	0.00	6.0±.00^{a}	11.0.± 0.00^{b}	0.00	6.0±0.50^{a}	5.0.±0.00^{c}	6.0±0.00^{a}	0.0
Candida albicans	20.33±0.57^{c}	20.66±0.57^{c}	10.66±1.15^{b}	11.0±0.00^{b}	20.66±1.15^{c}	21.33±0.57^{a}	22.0±67^{c}	0.0

Different letters indicate significant difference ($P < 0.001$).

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