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POTENTIAL ANTIBACTERIAL EFFECTS ON IRAQI HONEY ON BACTERIAL ISOLATES

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ABSTRACT
The *in vitro* antibacterial activity of two Iraqi honeys was tested against some bacterial isolates (*Staphylococcus aureus, Escherichia coli*, *Staphylococcus albus, Klebsiella sp.,* and *Peudomonasaeruginosa*). Both types of honey showed antibacterial activity against the tested organisms with a zone of inhibition (ZOI) ranging from 6.7 to 15.2 mm, while *P. aeruginosa*showed more resistance towards the two honey types witha lower ZOI ranging from 6.7 to 7.2 mm. The broth dilution assay gave aminimum inhibitory concentrations (MIC) value of 8 mg/mL, while the minimum bactericidal concentration (MBC) ranges between 16 mg/mL to 256 mg/mL. The study demonstrated that honey has antibacterial activity (bacteriostatic and bactericidal effect), similar to antibiotics, against test organisms and provides alternative therapy against certain bacteria.

KEY WORDS: Honey, antibacterial agent, Iraqi honey, antibiotic

INTRODUCTION

Honey has been cultivated by humans for many millennia, and according toCrane, (1983). Humans began hunting for honey at least 10,000 years ago. Honey was used to treat infected wounds as long as 2000 years before bacteria was discovered to be the cause of infections, serving to treat rotting and hollow ulcers (Gunther, 1934). Honey like other saturated sugar syrup has an osmolarity sufficient to inhibit microbial growth (Chirife *et al*., 1983). Honey is produced from many sources, and its antimicrobial activity varies greatly with origin and method of processing (Molan, 1992). The innate properties of honey make it naturally inhibitory to the growth of many micro-organisms (Mullai & Menon, 2007). The mechanisms of action have been investigated and range from inhibition of nucleic acid synthesis and inhibition of energy metabolism to inhibition of membrane function dependent on molecular structure of flavonoid molecules, however whether the proposed mechanisms are bactericidal or bacterostatic in nature remains unclear (Cushnie & Lamb 2005).Most types of honey generate hydrogen peroxide when diluted because of the activation of the enzyme glucose oxidase, which oxidizes glucose to gluconic acid and hydrogen peroxide (Schepartz and. Subers, 1964). It is suggested that hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects (Molan, 1992). Although the level of hydrogen peroxide in honey is very low, it is still effective as an antimicrobial agent. It has been reported that hydrogen peroxide is more effective when supplied by continuous generation through the glucose oxidase than when added in isolation (Pruitt & Reiter, 1985). Besides its antimicrobial properties, honey can clear infections in a number of other ways, including: boosting the immune system, having anti-inflammatory and antioxidant activities, and via stimulation of cell growth (Al-Jabri, 1992). Therefore, the purpose of the present study was to evaluate the *in vitro* antibacterial activity (bacteriostaticand bactericidal effect) of two Iraqi honeys (Eukalyptus and Rhamnous) against five different bacterial cultures: Staphylococcus aureus, Escherichia coli, Staphylococcus albus, Klebsiella sp., and Peudomonasaeruginosa.

MATERIALS AND METHODS

The two honey samples were taken from two different locations in Iraq. The first one Eukalyptuse honey (E), was taken from Karbala city (south of Baghdad), whereas the second type, Rhamnous honey (R), was taken from Whasout city (south east of Baghdad). They were used as found, comprising100% concentration each. The method described by AOAC (1984) was applied to estimate the honey moisture content, which involves weighing two grams of each honey type in an uncovered dish. The honey samples were then dried in an oven at 105ºC for 18 hours, and before cooling the samples they were weighed once more as per Hind, (2004).The rest of the honey was prepared by dissolving 10 gm of each type in 100 ml of distal water (w/v), to get 100mg/ml. Honey samples were prepared maintaining a minimum level of moisture (17%). A concentration of 0.3 ciprofloxacin, 2 mg/ml (ampoule) was used in addition to Tetracycline (Samarra drugs industry) as 250 mg was dissolved into 10 ml sterile distilled water to get asolution of 25 mg/ml. Five bacteria species were used in this study including *Staphylococcus aureus, Escherichia coli*, *Staphylococcus albus, Klebsiella sp.,* and *Peudomonasaeruginosa.* Different media have been used including Mueller Hinton and MacConky agars. Pure culture of microorganisms was grown on nutrient agar. Five colonies for each organism were used into Mueller Hinton and incubated at 37 ºC for 4 days, then diluted with sterile saline to a density visually equivalent to 10^6 cfu/ml according to the MacFarland standard.

The suspension was then seeded evenly onto the surface of the Mueller Hinton agar plates in triplicates with a sterile swab. Using a sterile 6 mm diameter cock borer, 5 wells were cut in the agar to which concentrations of honey were added, as well as the drugs (ciprofloxacin and tetracycline) which were considered as the controls. All plates were incubated at 37ºC for 48 h and they were examined at 24 h and 48 h for zone of inhibition. Volumes of the two types honey were added to sterile tubes containing 10 ml of Mueller Hinton broth to get several concentrations:1, 2, 4, 8, 16, 32, 64, 128, 256, 512 mg/ml. 50rations:1, 2, 4, 8, 16, 32, 64, 128, 256, 512 mg/ml. tube. The tubes containing the bacterial cultures and the candida culture were incubated for 24 and 48 h respectively at 37ºC.Two controls were used: The first one represent a row of positive control tubes containing the growth medium and each of the microorganisms, whereas the second represent a negative control, which consist of a row of tubes containing two types of honey with no organisms. We also determined the minimum bactericidal concentration (MBC).

RESULTS & DISCUSSION

It is evident from table (1) that the inhibition effect of honey 100% concentration (E) was more potent than honey (R). The unequal activity between the two types of honey may be due to their differences in the amount of flavanoids and phenolic acids (D'Arcy, 2005). On the other hand the two types of honey were more potent than tetracycline concerning inhibition zone diameters. The inhibitory activity against test microorganisms is of interest because these organisms cause infection.

Comparing the two types of honey with the two antibiotics with regards to inhibition, obtained that Ciprofloxacin is the best followed by honey (E), honey (R) and then Tetracycline.

TABLE 1: Antibacterial activities of two types of honeys at different concentrations compared with ciprofloxacin and tetracycline

Organisms	Zone inhibition diameters /mm			
	Honey E	Honey R	Tetracycline	Ciprofloxacin
Staphylococcus aureus	11.6	10.7	10.3	25.3
Escherichia coli	13.4	13.3	14.5	29.7
Staphylococcus albus	15.2	13.5	0.0	24.5
Klebsiella sp.	10.2	9.9	5.8	28.4
Peudomonas aeruginosa	7.2	6.7	7.2	36.3

TABLE 2: Antibacterial activity of honey using the broth dilution method

	MIC (mg/ml)		MBC (mg/ml)	
Organisms	Honey E	Honey R	Honey E	Honey R
Staphylococcus aureus	16	32	32	64
Escherichia coli	64	64	128	128
Staphylococcus albus	8	16	16	32
Klebsiella sp.	64	128	64	128
Peudomonas aeruginosa	128	128	256	256

MIC= Minimum inhibitory concentration
MBC= Minimum bactericidal concentration

The antibiotic effect of honey may 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, which represent the "inhibition" factor in honey (Postmes *et al*., 1993). Results revealed that the *S. albus*was the most susceptible microorganisms according to MIC, while *Peudomonasaeruginosa*was the most resistant pathogen (Table 2). This pathogen was found to be particularly resistant to honey in a previous study (Wahdan, 1998). The superiority of honey may be attributed to its ability to stimulate the activity of B-lymphocytes and T-phagocytes (Abuharfeil *et al*., 1999). Furthermore, Tonks *et al*., (2001) report that honey has stimulated monocytes in cell culture to release cytokines, which stimulate the immune response to infection. In addition, the low PH of honey and its glucose content may assist the macrophages to be more active to destroy the bacteria. Results suggest that the two honey types used, contain biocomponents whose antibacterial activities are highly comparable with those of the two regular antibiotics (tetracycline and ciprofloxacin).

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