http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (1): 1-6, 1999 Research Article

Comparative Study Between Yemeni and Egyptian Types of Honey by Means of Antibacterial Activity

El-Fadaly, H., F.S. Abdilla* and E.E.Y. El-Badrawy**
Agriculture Microbiology Department, Faculty of Agriculture, Mansoura University, Egypt
*Plant Protection Department, Naser Faculty of Agriculture, Aden University, Yemen
**Home Economics Department, Faculty of Specific Education, Menyat El-Nasr, Egypt

Abstract

Six samples of Yemeni and Egyptian types of bee honey were chemically analyzed using different chemical standard methods. Yemeni honey types were Dhaba, Samer, and Merbeiy while Egyptian types were Citrus, Cotton and Clover. The antibacterial activity of these types of honey was examined using the original samples as well as the samples after heating at 50 and 100°C for 30 minutes against pathogenic bacteria. The tested bacteria were *Proteus* sp., *Shigella* sp., *E. coil*, *Listeria monocytogenes* and *Yersinia enterocolitica*.

Yemeni honey was higher in its mineral content and total sugars than Egyptian one. Total protein and total lipids were highest in Egyptian types of honey. Data also show that heating have no effect on the effectiveness of honey at its high concentration. The Yemeni types of honey were more active against examined bacteria than Egyptian ones and the values of the minimum inhibitory concentration were lower in case of Yemeni honey than Egyptian ones. Both Yemeni and Egyptian honey exhibited remarkable inhibitory effect against tested bacteria when using liquid cultures.

Introduction

The use of honey as a wound dressing is well established in ancient and traditional medicine. It's use as a tropical antibacterial agent is gaining acceptance for the treatment of surface infections such as ulcers and bed sores (Willix *et al*., 1992). Bee honey was used in treatment of external eye disease (Emarah *et al*., 1997). Honey from different plant sources vary in aroma, flavour, colour, density and in granulation characteristics. It was found that honey also vary in viscosity and in chemical content of sugars, water, enzymes, vitamins, minerals, acids, colloids and percentage of unknown compounds (Lindner *et al*., 1996). Many investigators examined the antimicrobial activity of honey using different concentrations (Obaseiki-Ebor *et al*., 1983; Jeddar *et al*., 1985; Oka *et al*., 1987; Allen *et al*., 1991). All human beings carry in their intestinal tracts *E. coil* and related Gram-negative bacilli capable of producing diseases in the urinary tract, in wounds and elsewhere (Allen *et al*., 1991). The purpose of this investigation is to compare between different types of honey by their efficacy on some pathogenic bacteria.

Materials and Methods

Honey samples: Three types of Yemeni honey namely, Dhaba, Samer, and Merbeiy were kindly taken from Naser Faculty of Agric., Aden Univ., Yemen. Three types of Egyptian honey, namely Citrus, Cotton and Clover were collected from Qalubyeia, Beni-Sweif and Dakahlyeia Governorates for the three types, respectively.

Bacteriological Procedures

Bacterial strains: Five pathogenic bacteria were provided by the Hungarian National Collection of Medical Bacteria, OKI, Budapest, Hungary. The selection of these strains was based on their economic importance as well as their negative roles for human beings in nature.

Cultivation media: For maintaining and counting the bacterial strains used in this investigation, the following specific cultivation media were used; Brilliant green agar medium was used for *Shigella* sp., at 37°C for 24 hr; MacConkey agar medium was used for *Proteus* sp., and *E. coli* at 37°C for 24 hr. Peptone sorbitol bile agar medium was used for *Yersinia enterocolitica* at 35°C for 48 hr (Klausner and Donnelly, 1991). *Listeria monocytogenes* was enumerated at 35°C for 48 hr using tryptose phosphate agar (Pini and Gilbert, 1988); Tryptone Soya Broth (TSB) was used for determination of the minimum inhibitory concentration (MICs). Tryptone Soya agar (TSA) was used for the agar diffusion method (Oxoid, 1982).

Measurement of inhibition zone: Holes were punched with a cork borer (6 mm) in plates of (TSA) freshly seeded with 1 ml of 24 hr. old bacterial suspension cultured in specific media mentioned above. The holes were then filled with the tested honey types in a concentration of 30, 40, and 50 percent. The plates were kept at 5°C for one hr to allow diffusion of the sample through the agar media. The incubation was carried out for 24 hr at 37°C. The diameter of clear zones surrounding the wells were measured and recorded expressing the antibacterial activity (Toda *et al*., 1989). Similarly, the control prepared free of tested material.

Determination of minimum inhibitory concentrations (MICs):

The MIC value for each representative bacterial strain was examined in a specific liquid medium amended with the test honey using a tube dilution technique as described by Simpson and Smith, (1992). After the incubation period, the test tube in which no growth can be recorded should contain the lowest inhibiting concentration of the tested extract. Three replicates were prepared for each bacterial strain.

Detection of the antibacterial nature of the honey: To verify the nature of the antibacterial effect of tested honey type whether is temporary or permanent, appropriate subculturing from MIC tubes were applied on plates of the same used media without addition of honey sample. After the incubation period, it was possible to determine the minimal bactericidal and/or bacteriostatic concentration (MBC), (Gardner and Provine, 1984).

Effect of honey type on bacterial growth in liquid cultures: Sterile specific culture media were amended with 20 percent of honey concentration were prepared for each bacterial strain. Medium was dispensed in 250 ml Erlenmeyer flasks of 50 ml aliquots per each flask in triplicates. Specific culture was used without additional honey sample as control. Each flask was inoculated with 1 mL of 24 hr old culture. The incubation was carried out at 150 rpm using shaking incubator (Lab-Line Incubator-Shaker, USA). During 24 hr incubation at 37°C, the growth was measured by plating appropriate dilution of each flask using a specific culture medium for counting the viable cells expressed in CFU mL^{-1} after 12, 18 and 24 hr. The bacterial growth of each strain was also traced in a honey free-medium as a control.

Chemical Analysis

Determination of chemical constituents in honey: Moisture, ash, total carbohydrates, reducing sugars, total lipids and total proteins were carried out according to AOAC (1990). Identification and determination of individual sugars was carried out by using TLC technique as described by Gauch *et al.* (1979). Specific rotation was estimated by using portable polarimeter.

Analysis of honey minerals: The mineral contents of honey were analyzed after White (1975).

Results and Discussion

Physical and Chemical Properties of Honey: Results in Table 1 show that there are remarkable differences in the chemical constitution between Yemeni and Egyptian honey types. Values of pH were higher in case of Yemeni honey than those of Egyptian honey. The highest moisture content of Yemeni honey is Merbeiy while Cotton honey is of Egyptian type being 17.13 and 17.8 percent, respectively. The water content of honey was influenced not only by humidity but also by the time of extraction from the comb in relation to ripening process by the bees (Chung *et al*., 1984). Egyptian honey was higher in total proteins, total lipids and ash than that of Yemeni ones. On the other hand, Yemeni honey showed to be highest in total and reducing sugars. The marked differences between both two types in the chemical composition could be attributed to the floral origin and environmental conditions. These variation were apparently due to many factors that were beyond the control of honey keeper, such as differences in soil and atmospheric conditions as well as in the type and physiology of each plant (Chung *et al.,* 1984). Data of minerals content (ppm) also showed that Ca, Na, Cu, and P were higher in Yemeni honey than that of Egyptian one (Table 2).

Antibacterial activity of honey types: Different types of honey investigated in this paper were first examined for the inhibition of bacterial growth and obtained results are shown in Table 3 and 4 for Yemeni and Egyptian types, respectively. Data in Table 3 showed that both *L. monocytogenes* and *Y. enterocolitica* were the most resistant strains while *Proteus* sp., *Shigella* sp. and *E. coil* were severely affected. Data also exhibited that Merbeiy honey was the most effective one while both Samer and Dhaba were almost the same. The same susceptibility of tested bacteiia towards examined honey was also found (Table 4). Data exhibited also that citrus honey was the most effective one among Egyptian types.

These results proved that the heated samples either at 50°C or at 100°C showed more or less the same inhibition effect on the tested bacterial strains. Honey was evaluated by an *in vitro* study by many investigators. Obaseiki-Ebor *et al*. (1983) reported that different Gram-positive and Gram-negative pathogenic bacteria failed to grow in presence at 50% honey and most were inhibited by 40 percent.

Jeddar *et al*. (1985) found that most of pathogenic bacteria failed to grow in honey at concentration of 40 percent and above. According to the diffusibility of the tested honey sample through the solid medium, it can cause some disruption in the permeability of the outer membrane of Gram negative bacterial cell wall (Delves-Broughton *et al*., 1992). It was found that honeys from different floral sources varied in their antibacterial activity this is due to varying levels of hydrogen peroxides. This variation is also attributed to plants having different level of catalase enzyme (Allen *et al*., 1991).

Values of MICs and MBCs: Table 5 shows that the lowest concentration of honey type which capable to inhibit the growth of pathogenic bacteria. Data also showed that both Dhaba and Samer were identical in their MICs values while Merbeiy honey showed high. values of MIC with tested Incteria. The first three strains; *Proteus* sp., *Shigella* sp. and *E. coli* showed the same sensitivity towards Dhaba and Samer honey but more resistant with Merbeiy honey. The same trend was found with *L. monocytogenes* and *Y. enterocolitica* but they were more resistant.

In case of Egyptian honey types, Cotton honey showed to be more effective on all tested bacteria when both Citrus

Table 1: Same chemical analysis of examined honey types.

Types of honey		pH	Specific Rotation	Ash (%)	Moisture (%)	Total protein	Total lipid	Total sugar (%)	Reducing g sugar (%)	Glucose (%)	Fructose (%)	Sucrose (%)
Yemeni	Dhaba	3.2	-10.0	0.15	13.55	1.93	0.61	83.31	74.27	35.58	37.19	7.58
honey	Samer	3.9	-3.5	0.32	12.35	0.61	0.28	85.47	70.15	34.02	35.13	11:32
	Merbeiy	3.9	-10.6	0.24	17.13	0.36	0.49	80.41	72.08	28.28	40.80	5.25
Egyptian	Clover	2.7	ND	0.37	16.30	0.96	0.72	82.10	72.20	28.20	38.30	4.60
honey	Cotton	2.4	ND	0.58	17.80	1.85	0.89	77.30	68.50	31.40	33.50	6.40
	Citrus	1.8	ND	0.45	17.50	0.86	0.61	79.50	68.40	27.30	36.40	7.00

ND: Not detected.

Table 2: Mineral content (ppm) of tested honey types.

Type of honey		Ca	K	Na	Fe	Zn	Mn	Cu	P
Yemeni	Dhaba	112.91	621.0	239.2	66.98	10.04	5.26	0.95	227.2
honey	Samer	132.73	1608.0	160.3	5.13	38.47	3.84	4.49	685.3
	Merbeiy	82.86	1539.0	102.3	37.34	8.69	1.02	1.53	242.9
Egyptian	Clover	40.8	950.0	164.5	48.5	0.50	2.91	0.22	154.9
honey	Cotton	54.5	1150.0	141.4	60.4	0.71	1.62	0.49	211.0
	Citrus	80.0	1540.0	112.4	88.2	4.50	4.28	0.86	200.0

Table 3: The antibacterial activity of tested Yemeni honey types against some pathogenic bacteria.

			Tested bacterial strains				
			Proteus sp.	*Shigella sp.*	*E. coli*	*L. monacytagenes*	*Y. enterocolitica*
Concentration (%) of tested Yemeni honey type							
Dhaba	30°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ +	+ +	+ +	+	+
		H_2	+	+	+	+	+
	40°C	0	+ + + +	+ + +	+ + + +	+ +	+ +
		H_1	+ + +	+ + +	+ + +	+ +	+ +
		H_2	+ + +	+ + +	+ + +	+	+
	50°C	0	+ + + +	+ + + +	+ + + +	+ + +	+ + +
		H_1	+ + + +	+ + + +	+ + + +	+ + +	+ + +
		H_2	+ + + +	+ + + +	+ + +	+ +	+ +
Samer	30°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ +	+ +	+ +	+	+
		H_2	+	+	+	+	+
	40°C	0	+ + + +	+ + + +	+ + + +	+ +	+ +
		H_1	+ + +	+ + +	+ + +	+ +	+ +
		H_2	+ + +	+ + +	+ + +	+	+
	50°C	0	+ + + +	+ + + +	+ + +	+ + +	+ + +
		H_1	+ + + +	+ + + +	+ + +	+ + +	+ + +
		H_2	+ + + +	+ + + +	+ + +	+ +	+ +
Merbery	30°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ +	+ +	+ +	+	+
		H_2	+	+	+	+	+
	40°C	0	+ + +	+ + +	+ + +	+ + +	+ + +
		H_1	+ + +	+ + +	+ + +	+ + +	+ + +
		H_2	+ + +	+ + +	+ + +	+ + +	+ + +
	50°C	0	+ + + +	+ + + +	+ + + +	+ + +	+ + +
		H_1	+ + + +	+ + + +	+ + + +	+ + +	+ + +
		H_2	+ + + +	+ + + +	+ + + +	+ + +	+ + +

and Clover honey gave the same inhibition power being typical values of MICs. Table 5 also indicated that the nature of antagonistic action of tested honey types against examined pathogens. As can be seen, Yemeni honey showed static action on *Proteus* sp., *Shigella* sp and *E. coli* while bactericidal effect was noticed in case of both *L. monocytogenes* and *Y. enterocolitica.* For the Egyptian honey types, bacteriostatic effect was observed with only *Proteus* sp. and *Shigella* sp., while the cidal action was found in case of the three other tested strains.

Thp observed growth (sign +) of bacterial strains were detected after reseeding the MIC tubes on the agar plates containing honey free specific culture medium. The explanation of this phenomenon is that the concentration used inhibit the bacterial growth but after that the cell can persist and resume again the growth once the agent is removed. No growth (sign -) reveals that the honey kill the cells therefore they have an irreversible and permanent effect. The measuring of MICs and MBCs values rather than inhibition zone measurement suggest the real effect of such honey sample *in vivo* against the microbial cells (Cederlund and Mardh, 1993).

The presence of Na^+ and other minerals in honey may be the main cause of the inhibition effect. Giunta *et al*. (1984) reported that the intracellular content of Na^+ in Gram-negative bacteria is maintained chiefly by a tightly coupled Na^+/H^+ exchange system which can be blocked and consequently alters the cellular pH which intern prevents proliferation of cells. The bactericidal effect of honey was also confirmed by White (1975) who owed it to the presence of substances called "inhibin" that result from the accumulation of hydrogen peroxide which has produced by natural glucose-oxides° system in honey. Chue *et al*. (1994) reported

Table 4: The antibacterial activity of tested Egyptian honey types against some pathogenic bacteria

			Tested bacterial strains				
			Proteus sp.	*Shigella sp.*	*E. coli*	*L. monorytogenes*	*Y. enterocolitica*
Concentration (%) of tested Egyptian honey type							
Citrus	30°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ +	+ +	+ +	+	+
		H_2	+	+	+ + +	+	+
	40°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ + +	+ + +	+ + +	+ +	+ +
		H_2	+ + +	+ +	+ +	+	+
	50°C	0	+ + + +	+ + + +	+ + +	+ +	+ + +
		H_1	+ + + +	+ + + +	+ + +	+ +	+ + +
		H_2	+ + + +	+ + + +	+ + +	+ +	+ +
Cotton	30°C	0	+ +	+ +	+ +	+ +	+ +
		H_1	+	+	+	+	+
		H_2	+	+	+	+	+
	40°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ + +	+ + +	+ + +	+ +	+ +
		H_2	+ +	+ +	+ +	+	+
	50°C	0	+ + + +	+ + + +	+ + +	+ +	+ + +
		H_1	+ + + +	+ + + +	+ + +	+ +	+ + +
		H_2	+ + + +	+ + + +	+ + +	+ +	+ + +
Clover	30°C	0	+ +	+ +	+ +	+ +	+ +
		H_1	+	+	+	+	+
		H_2	+	+	+	+	+
	40°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ + +	+ + +	+ + +	+ +	+ +
		H_2	+ + +	+ + +	+ + +	+ +	+ +
	50°C	0	+ + +	+ + +	+ + +	+ +	+ +
		H_1	+ + +	+ + +	+ + +	+ +	+ +
		H_2	+ + +	+ + +	+ + +	+ +	+ +

0: Original sample, H_1: Sample heated at 50°C for 30 minute. H_2: Sample heated at 100°C for 30 minute, + + + +: 0>18 mm, + + +: 0>14 mm, + +: 0>12 mm, +: 0>8 mm

Table 5: Values of minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of different tested honey types

			Tested bacterial strains				
			Proteus sp.	*Shigella sp.*	*E. col*	*L. monocytmenes*	*Y. enterocolitica*
Yemeni honey	Dhaba	MIC%	25	25	25	30	30
		MBC**	+	+	+	-	-
	Samer	MIC%	25	25	25	30	30
		MBC	+	+	+	-	-
	Merbeiy	MIC%	30	30	30	35	35
		MBC	+	+	+	-	+
Egyptian honey	Citrus	MIC%	35	35	35	35	35
		MBC	+	-	+	+	+
	Cotton	MIC%	30	30	30	30	30
		MBC	+	-	+	+	+
	Clover	MIC%	35	35	35	35	35
		MBC	-	-	+	+	+

Table 6: Values of minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of different tested honey types

	Yemeni honey						Egyptian honey					
	Dhaba		Samer		Merbeiy		Citrus		Cotton		Clover	
Tested bacterial strains	MIC*(%)	MBC**	MIC (%)	MBC	MIC (%)	MBC	MIC (%)	MBC	MIC (%)	MBC	MIC (%)	MBC
Proteus sp.	25	+	25	+	30	+	35	-	30	-	35	-
Shigella Sp.	25	+	25	+	30	+	35	-	30	-	35	-
E. coli	25	+	25	+	30	+	35	+	30	+	35	+
L. monocytmenes	30	-	30	-	30	-	35	+	30	+	35	+
Y. enterocolitica	30	-	30	-	30	-	35	+	30	+	35	+

*As percentage; **, +: observable growth; -: No growth

Table 7: Inhibition of bacterial growth as affected by honey type (20%) added to liquid culture medium

		Viable cell count, CFU x 10^5/ml									
		Proteus sp.		*Shigella sp.*		*E. coil*		*L. monocytogenes*		*Y. enterocolitira*	
Incubation Time (hr.)		YH	EH	YH	EH	YH	EH	YH	EH	YH	EH
12	Control	11.7		8.8		18.6		14.5		22.3	
	Treated	7.2	9.6	6.3	7.8	14.2	15.3	10.6	11.2	19.6	19.4
	Inhibition fold	0.62	0.82	0.72	0.89	0.76	0.82	0.73	0.77	0.83	0.87
18	Control	13.6		10.2		19.3		16.1		23.9	
	Treated	7.5	8.0	6.7	8.1	14.6	15.5	10.9	11.9	19.3	20.2
	Inhibition fold	0.55	0.59	0.66	0.79	0.76	0.81	0.68	0.74	0.81	0.85
24	Control	14.9		12.4		22.2		18.2		25.7	
	Treated	11.2	12.4	9.5	11.3	20.3	21.2	13.6	15.2	22.5	23.2
	Inhibition fold	0.75	0.83	037	0.91	0.91	0.95	0.75	0.84	0.88	0.90

YH = Yeineni honey (Dhaba); EH = Egyptian honey (Cotton)

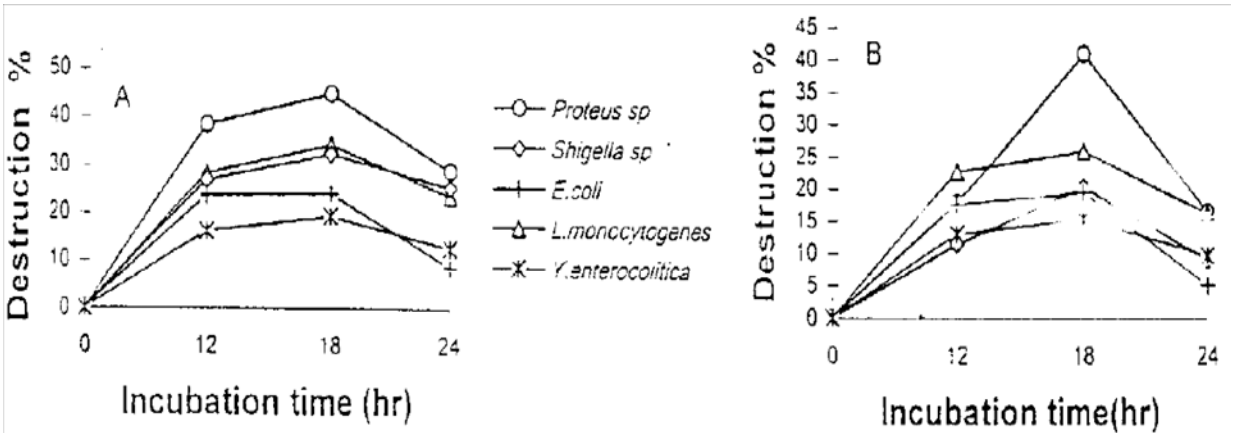

Fig. 1: Destruction percent of bacterial growth as affected by 20 percent of tested honey types A, Yemeni honey, B, Egyptian honey

that honey had a so potent bactericidal effect, that it did not allow microbes to survive after few hours. They owed this to its high sugar concentration (hygroscope effect).

Pattern of bacterial growth in presence of honey: In a liquid culture, the pattern of each bacterial strain was recorded in Table 6 using Dhaba and Cotton honey as a sample of Yemeni and Egyptian type, respectively. It is obvf)us from tabulated results that both two types achieved remarkable inhibition fold in the bacterial growth. Data also showed that Yemeni honey exhibited more inhibition fold than Egyptian one with each tested bacterial strain during the three interval times determined (Table 7). This may be ascribed to the high values of total and reducing sugars as well as the high content of minerals in Yemeni honey types. On the other hand, the destruction percent was also calculated and obtained values was plotted in Fig. 1. Graphed results exhibited that *Proteus* sp., was the most severely affected strain after 18 hr. incubation at 37°C that the destruction percents were 44.9 and 41.2 per cent by Yemeni (Dhaba) and Egyptian honey (Cotton), respectively. On the other hand, *E. coil* showed to be the most resistant bacteria since the destruction percents were 8.6 and 4.5 per cent after 24 hr by Dhaba and Cotton honey, respectively.

The results of Oka *et al.* (1987) proved the presence of seven chemotherapeutic agents and antibiotics such as

tetracyclines in honey by chromatography. They attributed them to be a cause of antimicrobial activity of bee honey. Sanchez *et al.* (1973) found that honey itself has been proven to stimulate the human neutrophilic phagocytosis of microorganisms.

References

AOAC., 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., pp: 200-210.

Allen, K.L., P.C. Molan and G.M. Reid, 1991. A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmacol., 43: 817-822.

Cederlund, H. and P.A. Mardh, 1993. Antibacterial activities of non-antibiotic drugs. J. Antimicrob. Chemother., 32: 355-365.

Chue, T.C., A.B. Wu and C.Y. Chen, 1994. Sugars content of honey from wild and kept honey bees. J. Food Drug Anal., 2: 97-103.

Chung, W.C., M.W. Kim, K.J. Song and E.H. Choi, 1984. Chemical composition in relation to quality evaluation of Korean honey. Korean J. Food. Sci. Technol., 16: 17-22.

Delves-Broughton, J., G.C. Williams and S. Wilkinson, 1992. The use of the bacteriocin, nisin, as a preservative in pasteurized liquid whole egg. Lett. Applied Microbiol., 15: 133-136.

Emarah, M., L. Missoten and M.A. Khalaf, 1997. The use of bee honey in the treatment of external eye diseases. Proceedings of the International Symposium on Apitheropy, March 8-9, 1997, National Research Center, Cairo, Egypt.

Gardner, P. and H.T. Provine, 1984. Manual of Acute Bacterial Infections. 2nd Edn., Little Brown and Co., Boston, Toronto, pp: 328-331.

Gauch, R., U. Leuenberger and E. Baumgartner, 1979. Quantitative determination of mono-, di- and trisaccharides by thin-layer chromatography. J. Chromatogr. A, 174: 195-200.

Giunta, S., C. Pieri and G. Groppa, 1984. Amiloride, a diuretic with *in vitro* antimicrobial activity. Pharmacol. Res. Commun., 16: 821-829.

Jeddar, A., A. Kharsany, U.G. Ramsaroop, A. Bhamjee, I.E. Haffejee and A. Moosa, 1985. The antibacterial action of honey. An *in vitro* study. S. Afr. Med. J., 67: 257-258.

Klausner, R.B. and C.W. Donnelly, 1991. Environmental sources of *Listeria* and *Yersinia* in Vermont dairy plants. J. Food Prot., 54: 607-611.

Lindner, P., E. Bermann and B. Gamarnik, 1996. Characterization of citrus honey by deuterium NMR. J. Agric. Food Chem., 44: 139-140.

Obaseiki-Ebor, E.E., T.C.A. Afonya and A.O. Onyekweli, 1983. Preliminary report on the antimicrobial activity of honey distillate. J. Pharmacy Pharmacol., 35: 748-749.

Oka, H., Y. Ihai, N. Kawamura, K. Uno and M. Yamada, 1987. Improvement of chemical analysis of antibiotics: XII. Simultaneous analysis of seven tetracyclines in honey. J. Chromatogr. A, 400: 253-261.

Oxoid, 1982. The Oxoid Manual of Culture Media, Ingredients and Other Laboratory Services. 5th Edn., Oxoid Ltd., London.

Pini, P.N. and R.J. Gilbert, 1988. A comparison of two procedures for the isolation of *Listeria monocytogenes* from raw chickens and soft cheese. Int. J. Food Microbiol., 7: 331-337.

Sanchez, A., J.L. Reeser, H.S. Lau, P.Y. Yahiku and R.E. Willard *et al*., 1973. Role of sugars in human neutrophilic phagocytosis. Am. J. Clin. Nutr., 26: 1180-1184.

Simpson, W.J. and A.R.W. Smith, 1992. Factors affecting antibacterial activity of hop compounds and their derivatives. J. Applied Bacteriol., 72: 327-334.

Toda, M., S. Okubo, R. Hiyoshi and T. Shimamura, 1989. The bactericidal activity of tea and coffee. Lett. Applied Microbiol., 8: 123-125.

White, J.W., 1975. Composition of Honey. In: Honey: A Comprehensive Survey, Crane, E. (Ed.). Heinemann, London, pp: 157-206.

Willix, D.J., P.C. Molan and C.G. Harfoot, 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J. Applied Bacteriol., 73: 388-394.