International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (8), pp. 001-008, August, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Full Length Research Paper

Survey of the antibacterial activity of Saudi and some international honeys

Eman Halawani* and Mohamed Shohayeb

Taif University, Faculty of Science, Department of Biology and Biotechnology, Taif, Saudi Arabia.

Accepted 25 March, 2018

The antibacterial activities of 52 samples of 24 types of honey, either locally produced or imported were evaluated for their antibacterial activity. Manuka honey was included in the study for the sake of comparison. The antibacterial activity (estimated as phenol %) of 91.7% of the tested honeys ranged between 5.5 and 7.9%. There was no relationship between the potency of antibacterial activity and the color of honey. Locally produced Shaoka and Taify Sidr and the imported honeys, Yemeni Sidr, Black Seed, Clover and Orange Blossom, were more potent than Manuka honey. On the other hand, both Kashmiri and German acacia honeys were as potent as Manuka honey. Taking into consideration, the peroxide activity found in these honeys, which ranged between 4.8 and 15.6%, Taify, Shaoka, Black Seed, Yemeni Sidr, Orange blossom and Clover honeys had comparative antibacterial activities to Manuka honey. It was concluded that several honeys available in the Saudi market especially the locally produced Shaoka, and Taify Sidr, in addition to imported Yemeni Sidr, black seed, Clover and Orange blossom are as potent as Manuka honey. Therefore we recommend these honeys for use in the treatment of bacterial infections.

Key words: Saudi honeys, Shaoka honey, antibacterial activity of honey, Manuka honey.

INTRODUCTION

Honey has been used since ancient times in many cultures as an effective remedy (Alvrez-Suarez et al., 2010; Krell, 1996; Majno, 1975), cures bacterial infections (Chute et al., 2010; Dustmann, 1979; Namias, 2003; Natarajan et al., 2001; Wilkinson and Cavanagh, 2005) through its antimicrobial activity against a wide range of bacterial and fungal species (Molan, 1992a; Wilkinson and Cavanagh, 2005), widely used as a topical antibacterial agent for treatment of wounds, burns and skin ulcers (Fakoor and Pipelzadeh, 2007; McInerney, 1990; Subrahmanyam et al., 2001). Honey is a traditional remedy for dyspepsia, peptic ulcer (Kandil et al., 1987; Kumar et al., 2010; Tumin et al., 2005; Yoirish, 1977) and gastritis caused by enteropathogenic bacteria (Jeddar et al., 1985; Halawani, 2006).

The antimicrobial activity of honey could be attributed to several factors (Halawani, 2006; Kwakman et al., 2010; Molan, 1992a; Wahdan, 1998):

The first factor is the osmotic effect of honey. Honey is a saturated or super-saturated solution of a mixture of fructose and glucose sugars (84%), therefore, no fermentation occurs in honey. Inhibition by the osmotic (water-withdrawing) effect of dilute solutions of honey obviously depends on the species of bacteria (Molan, 1992a).

The second factor for the antimicrobial activity of honey is its acidity. The pH being between 3.2 and 4.5 is low enough to be inhibitory to many pathogens. However, if honey is diluted, especially by body fluids, the pH will not be low enough to effectively inhibit bacteria (Cooper et al., 2002; Molan, 1992b).

The third factor is the presence of hydrogen peroxide in honey. Hydrogen peroxide is produced enzymatically in honey by glucose oxidase enzyme secreted by bees into the nectar. Although, hydrogen peroxide has been used as an antiseptic (Turner, 1983), it is not now as popular because it causes inflammation and damage to tissues (Halliwell and Cross, 1994; Saissy et al., 1995; Watt et

*Corresponding author. E-mail: Halwani-e.m@hotmail.com.

al., 2004). In honey, the enzyme found is activated by dilution and the peroxide produced is too mild to cause tissue injury, and yet has antimicrobial activity (Bang et al., 2003; Bunting, 2001; Orrù et al., 2010).

The fourth factor is the presence of antibacterial phytochemical components (Molan and Russel, 1988; Mavric et al., 2008; Yao et al., 2004; Halawani, 2006).

The fifth factor is the presence of defensin-1, which was recently found to contribute in the antibacterial activity of honey (Kwakman et al., 2010).

The sixth factor in the *in vivo* antibacterial activity of honey is the induction of increased lymphocyte and phagocytic activity. Recent studies showed that the proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentrations as low as 0.1% and phagocytes are activated by honey at concentrations as low as 0.1% (Abuharfeil et al., 1999). Honey at a concentration of 1% also stimulates monocytes in cell culture to release cytokines, tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activate the immune response to infection (Alvarez-Suarez et al., 2010; Tonks et al., 2001; Tonks et al., 2003).

A large number of honeys are available in the Saudi market and are either locally produced or imported from different countries. Some of them are traditionally used as remedy for several ailments. The antibacterial efficiency of honeys available in the Saudi markets, whether locally produced or imported, has not been thoroughly evaluated. On the contrary, Manuka honey, produced in New Zealand, has been extensively studied (Adams et al., 2008; Atrott and Henle, 2009) and is medically used worldwide (Molan, 2006; Robinson et al., 2009). In this study, 24 types of honeys available at the market were evaluated for their antibacterial activity.

MATERIALS AND METHODS

Bacteria

A clinical isolate of *Salmonella entritidis,* was obtained from the stock culture of the Department of Biology, Faculty of Science, Taif University, Saudi Arabia.

Honey samples

Fifty-two honey samples representing 24 sources of honeys (Table 1) were purchased from the local markets of Taif. Manuka honey (active Manuka honey 12+) was purchased from Superbee honey factory, New-Zealand. All honeys were kept at room temperature in dark glass containers.

Agar well diffusion assay of antibacterial activity of honey

Solutions of 2 to12% (w/v) phenol and 16% (w/v) honey samples were prepared in sterile distilled water. Sixty-four wells were cut using 6 mm cork borer into Muller-Hinton agar plates (240 × 240 × 18 mm) seeded with 10^4 CFU/ml of S. entritidis. Honey and phenol samples (50 µl) were applied in quadruplicate into wells using a quasi- Latin square template to ensure their random application. The plates were incubated for 18 h at 37°C and the mean diameter around each clear zone was calculated. A standard graph was plotted of the square of the mean diameter of inhibition zones of phenol concentrations and the obtained graph was used to calculate the equivalent antibacterial activity of phenol % for each type of honey (Allen et al., 1991).

Estimation of peroxide activity

To estimate the non-peroxide activity of honey, 32% samples were diluted with equal volumes of sterile distilled water containing 40 mg/20 ml catalase (Sigma, 4000 units mg/ml). Samples were applied to wells cut into large plates in quadruplicates as previously described (Allen et al., 1991).

Statistical analysis

Comparison between means was conducted using Analysis Variance (ANOVA), Minitab Software.

RESULTS

Evaluation of the antibacterial activity of honeys

Fifty-two samples of 24 types of honeys (Table 1) were evaluated for their antibacterial activity against *S. entritidis*. Honeys applied into 6 mm diameter wells produced inhibition zones ranging from 22.2 to 32.0 mm (Figure 1 and Table 2). The smallest inhibition zone was for Turkish Sidr while the largest was for Shaoka honey which is locally produced (Table 2) .The antibacterial activity of honeys was evaluated after the calculation of equivalent phenol %. As shown in Table 2, the antibacterial activities of honeys were equivalent to concentrations of phenol ranging between 4 to 8.4% w/v phenols. Thirteen types of honey were equivalent to 6 to 7% phenol, 5 types were equivalent to 7 to 8% while 4 types were equivalent to 5 to 6% phenol (Figure 2). Six honeys namely, Shaoka, Taify Sidr, Yemeni Sidr, Black Seed, Orange Blossom, and Clover had an equivalent of 7.2 to 8.4% phenol compared to 6.9% phenol in the case of Manuka (Table 2). Honey colours did not affect the activity of investigated honeys. Data in Table 2, show that orange blossom and Clover honeys which are lighter in colour had equivalent phenol % concentration of 7.9 , while a dark honey like Somra had an equivalent of phenol % of 6.2 (Table 2).

Peroxide antibacterial activity in honeys

The contribution of peroxide in the antibacterial activities of honeys was estimated after treatment of honeys with catalase enzyme (Table 3). Eight types of the

Table 1. Local and non-local honeys used in the study.

Serial no.	Type of honey	No. samples	Origin of honey		Floral source
1	Sidr	4	Local honeys	Taif	*Ziziphus spina-christi*
2	Somra	3		Taif	*Acacia tortilis*
3	Tobak	3		Taif	*Psiadia arabica*
4	Sharma	1		Taif	*Otostegia frticosa*
5	Dorm	1		Taif	*Lavandula dentata*
6	Doash	1		Taif	*Origanum majorana*
7	Morr	1		Taif	*Commiphora spp.*
8	Shaoka	4		Taif	*Fagonia cretica*
9	Black seed	3	Non-local honeys	Qasim	*Nigella Sativa*
10	Sidr	2		Yemen	*Ziziphus spina-christi*
11	Sidr	2		Kashmiri	*Ziziphus spina-christi*
12	Sidr	2		Turky	*Ziziphus spina-christi*
13	Orange blossom	3		Egypt	*Citrus spp.*
14	clover	1		Egypt	*Trifolium alexandrinum*
15	German acacia	3		Germany	*Acacia spp.*
16	black forest	3		Germany	-
17	German	3		Germany	-
18	Spanish	2		Spain	-
19	Australian	2		Australia	-
20	Swiss	1		Switzerland	-
21	Iranian	1		Iran	-
22	American	2		USA	-
23	Unidentified	2		-	-
24	Manuka	2		New Zealand	*Leptospermum scoparium*
	Total	52			

investigated honeys did not have a detectable peroxide activity (Table 3). Of these, 6 were locally produced and two (Manuka and American honeys) were imported (Table 3). The proportion of peroxide activity in Shaoka and Clover was 15.6 and 10.7, respectively (Table 3). Except in all other 14 honeys, the peroxide activity was less than 10% (w/v) of the total activity of honeys (Table 3).

Before the inactivation of peroxide, Shaoka was significantly ($p < 0.0007$ to 0.0001) more active than other studied honeys including Taify Sidr, Yemeni Sidr and Manuka honeys. Also, the activity of locally produced honeys like Taify Sidr, Black Seed and imported honeys like Yemeni Sidr, Orange blossom and Clover honeys were significantly ($p < 0.013$ - 0.0047) more active than Manuka honey. However, when the proportion of peroxide was deduced from the total phenol % antibacterial activity of each honey; Shaoka, Taify Sidr, Black seed, Yemeni Sidr, Orange blossom and Clover honeys had comparative activity to Manuka honey (Table 4).

DISCUSSION

In the present work, the antibacterial activity of 52 samples of honey representing 24 types of locally produced (8 types) and imported honeys (16 types) were evaluated for their antibacterial activities. One of the imported honeys, Manuka honey, which has a good reputation as a potent antibacterial (Adams et al., 2008; Atrott and Henle, 2009; Iurlina and Fritz, 2005), was included in the evaluation. Honey samples were screened for their antibacterial activity using agar diffusion technique. Shaoka honey which is locally produced gave the largest inhibition zone. Inhibition zones of different concentrations of phenol were used to draw a straight line graph which was used to quantitatively calculate the corresponding equivalent of phenol percent for each honey. Unlike other studies (Allen et al., 1991; Molan, 1992b; Wilkinson and Cavanagh, 2005), data obtained in this study revealed that the antibacterial activity of the majority of the investigated 24 types of honey did not show large

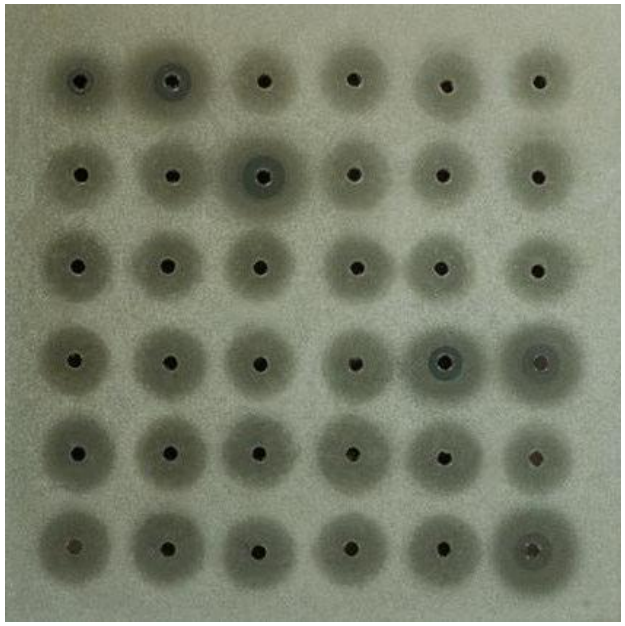

Figure 1. Representative part of a Muller-Hinton Agar plate seeded with *Salmonella entritidis* showing inhibition zones of different sizes around wells filled with 50 µl honey samples using a quasi-Latin square template (See methods).

Table 2. Inhibition zones and phenol % equivalent of 52 types of local and non-local types of honeys.

Serial no.	Type of honey	No. samples	Inhibition zone (Mean diameter ±SD)	Equivalent Phenol % (w/v)
1	Taify sidr	4	29.7 ± 0.34	7.3 ± 0.10
2	Somra	3	27.7 ± 0.80	6.2 ± 0.17
3	Tobak	3	26.5 ± 0.68	5.6 ± 0.14
4	Sharma	1	28.0 ± 0.80	6.4 ± 0.14
5	Dorm	1	27.7 ± 0.68	6.2 ± 0.18
6	Doash	1	26.7 ± 0.73	5.6 ± 0.15
7	Morr	1	26.0 ± 0.66	5.5 ± 0.15
8	Shaoka	4	32.0 ± 0.27	8.4 ± 0.13
9	Black seed	3	31.0 ± 0.57	7.9 ± 0.30
10	Yemeni sidr	2	29.5 ± 0.70	7.2 ± 0.07
11	Kashmiri sidr	2	29.2 ± 0.70	6.9 ± 0.17
12	Turkish sidr	2	22.2 ± 1.73	4.0 ± 0.16
13	Orange blossom	3	31.0 ± 0.17	7.9 ± 0.15
14	Clover	1	31.0 ± 0.70	7.9 ± 0.04
15	German acacia	3	29.0 ± 0.85	6.9 ± 0.18
16	German black forest	3	27.8 ± 0.51	6.3 ± 0.20
17	German	3	28.1 ± 0.91	6.5 ± 0.12
18	Spanish	2	27.6 ± 0.17	6.2 ± 0.04
19	Australian	2	27.5 ± 0.70	6.1 ± 0.15
20	Swiss	1	28.3 ± 0.68	6.6 ± 0.16
21	Iranian	1	28.7 ± 0.27	6.7 ± 0.06
22	American	2	26.6 ± 0.50	5.8 ± 0.11
23	Unidentified	2	28.2 ± 0.70	6.5 ± 0.16
24	Manuka	2	29.0 ± 0.56	6.9 ± 0.13

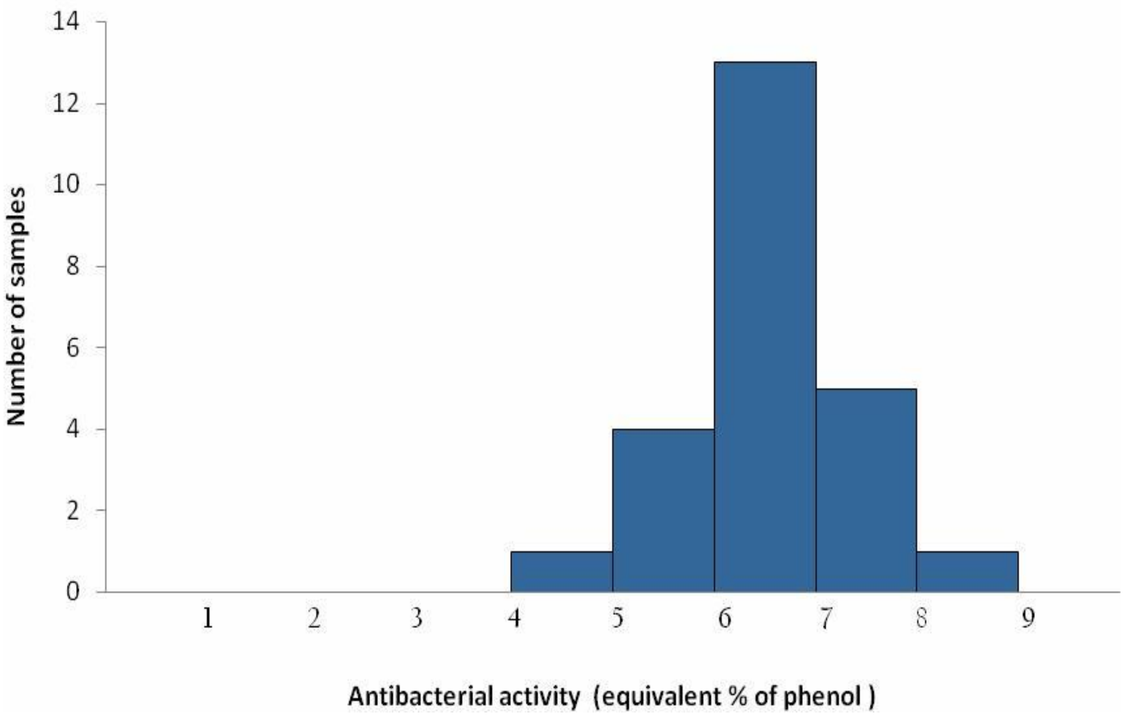

Figure 2. Distribution of antibacterial activity of honeys.

Table 3. Proportion of peroxide and non-peroxide activities calculated from equivalent phenol % of different types of investigated honeys.

Serial no.	Type of honey	No. samples	Proportion (%) of non-peroxide activity	Proportion (%) of peroxide activity
1	Taify sidr	4	92.6 ± 1.1	8.3 ± 0.14
2	Somra	3	100.0 ± 3.2	0.0 ± 0.12
3	Tobak	3	100.0 ± 2.3	0.0 ± 0.35
4	Sharma	1	100.0 ± 0.5	0.0 ± 0.12
5	Dorm	1	100.0 ± 0.6	0.0 ± 0.32
6	Doash	1	100.0 ± 2.8	0.0 ± 0.16
7	Morr	1	100.0 ± 3.3	0.0 ± 0.25
8	Shaoka	4	84.4 ± 7.1	15.6 ± 0.91
9	Black seed	3	90.9 ± 7.1	9.1 ± 0.43
10	Yemeni sidr	2	93.2 ± 2.3	6.8 ± 0.17
11	Kashmiri sidr	2	91.4 ± 2.2	8.6 ± 0.45
12	Turkish sidr	2	92.5 ± 2.3	7.5 ± 0.32
13	Orange blossom	3	90.3 ± 5.0	9.7 ± 0.35
14	Clover	1	89.3 ± 2.9	10.7 ± 0.38
15	German acacia	3	95.2 ± 2.3	4.8 ± 0.16
16	German black forest	3	93.5 ± 2.9	6.5 ± 0.05
17	German	3	98.8 ± 1.8	1.2 ± 0.07
18	Spanish	2	98.9 ± 3.3	1.1 ± 0.04
19	Australian	2	94.5 ± 2.7	5.5 ± 0.04
20	Swiss	1	98.9 ± 4.0	1.1 ± 0.04
21	Iranian	1	96.5 ± 3.2	3.5 ± 0.13
22	American	2	100.0 ± 1.5	0.0 ± 0.06
23	Unidentified	2	96.8 ± 2.4	3.2 ± 0.10
24	Manuka	2	100.0 ± 0.6	0.0 ± 0.07

Table 4. Antimicrobial activity of different honeys with and without peroxide activity, calculated as phenol percentage.

Serial no.	Type of honey	Activity (phenol % w/v)	
		Total activity	Activity without peroxide
1	Taify sidr	7.3 ± 0.10	6.8 ± 0.13
2	Somra	6.2 ± 0.17	6.2 ± 0.26
3	Tobak	5.6 ± 0.14	5.6 ± 0.16
4	Sharma	6.4 ± 0.14	6.4 ± 0.14
5	Dorm	6.2 ± 0.18	6.2 ± 0.15
6	Doash	5.6 ± 0.15	5.6 ± 0.15
7	Morr	5.5 ± 0.15	5.5 ± 0.18
8	Shaoka	8.4 ± 0.13	7.1 ± 0.45
9	Black seed	7.9 ± 0.30	7.2 ± 0.47
10	Yemeni sidr	7.2 ± 0.07	6.7 ± 0.16
11	Kashmiri sidr	6.9 ± 0.17	6.3 ± 0.33
12	Turkish sidr	4.0 ± 0.16	3.6 ± 0.45
13	Orange blossom	7.9 ± 0.15	7.1 ± 0.35
14	Clover	7.9 ± 0.04	7.0 ± 0.25
15	German acacia	6.9 ± 0.18	6.6 ± 0.22
16	German black forest	6.3 ± 0.20	5.9 ± 0.16
17	German	6.5 ± 0.12	6.4 ± 0.23
18	Spanish	6.2 ± 0.04	6.1 ± 0.12
19	Australian	6.1 ± 0.15	5.8 ± 0.11
20	Swiss	6.6 ± 0.16	6.5 ± 0.06
21	Iranian	6.7 ± 0.06	6.5 ± 0.11
22	American	5.8 ± 0.11	5.8 ± 0.15
23	Unidentified	6.5 ± 0.16	6.3 ± 0.21
24	Manuka	6.9 ± 0.13	6.9 ± 0.17

variations. The equivalent phenol percent concentrations for the majority (91.7%) of types of honey ranged between 5.5 and 7.9%.

It was also noticed in this investigation that there was no relationship between color and antibacterial activity of honey, as was previously suggested (Molan and Russel, 1988; Molan, 1992a). Some honeys of light coloration like orange blossom and clover, were more active as antibacterial (7.9% phenol), than darker studied honeys like Turkish Sidr and Somra (4.0 and 6.2 phenol percent, respectively).

Inhibition zones produced by Manuka honey were equivalent to 6.9% phenol. Other investigated imported honeys like orange blossom, clover, and locally produced honeys like Shaoka, Taify Sidr and Black Seed, showed higher antibacterial activity which was equivalent to 7.3 to 8.4% phenol.

One of the factors for which honeys exhibit antibacterial activity is the presence of peroxide. On dilution of some types of honey, glucose oxidase generates hydrogen peroxide at levels lethal to bacteria (Brudzynski, 2006; Kwkman et al., 2010; Wahdan, 1998). However, on wounds catalase produced by tissues destroys peroxide and hence the antimicrobial activity of honeys is diminished (Bang et al., 2003). Therefore, only Manuka honey lacking peroxide activity is selected for medicinal use (Molan and Russel, 1988). The screened honeys were tested for the contribution of peroxide in their antibacterial activity. While some local honeys like, Somra, Dorm, Tobak and Doash, had no peroxide activity, Taify Sidr and Shaoka had 8.3 and 15.6% peroxide activity respectively. Apart from Manuka and American honeys, other imported honeys had different percentages of peroxide activities which ranged between 4.8 and 9.1%. Although, before the inactivation of peroxide, the activity of locally produced honeys like, Shaoka Sidr, Taify Sidr, Black Seed and imported honeys like Yemeni Sidr, Orange blossom and Clover honeys were significantly ($p < 0.013$ to 0.0001) more active than Manuka honey, when the proportions of peroxide activity in honeys were deduced from the total phenol percent antibacterial activity of each honey, Shaoka, Taify Sidr, Black seed, Yemeni Sidr, Orange blossom and Clover honeys had comparative activities to Manuka honey. In a previous study, although some samples of Manuka honey did not have peroxide activity, 62% of Manuka honey samples screened in New Zealand had peroxide activities (Allen et al., 1991).

Therefore, there is a possibility that peroxide activity also varies from one local honey sample to another. If a larger number of samples of each locally produced honey are screened, there is a probability that some of them might lack peroxide activity.

Since the identification of antimicrobial phytochemicals in honeys has gained the interest of several research workers (Atrott and Henle, 2009; Mavric et al., 2008; Snow, 2008; Wahdan, 1998), It would be interesting to identify the antibacterial phytochemicals of Shaoka, other local or non-local potent honeys, included in this study. It can be concluded that several locally produced and imported honeys available in the Saudi market like, Shaoka, Taify Sidr, Yemeni Sidr, Black seed, Clover and Orange blossom are potent antibacterial honeys and therefore, could be recommended for use in treatment of bacterial infections.

ACKNOWLEDGMENT

This research was financially supported by Taif University, Grant 2\429\135. The authors are grateful to Dr. H. Sabbagh for his technical assistance.

REFERENCES

Abuharfeil N, Al -Oran R, Abo-Shehada M (1999). The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytes. Food Agric. Immunol., 11: 169-177.

Adams CJ, Boult CH, Deadman BJ, Farr JM, Grainger MN, Manley-Harris M, Snow MJ (2008). Isolation by HPLC and characterization of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. Carbohydr. Res., 343: 651-659.

Allen KL, Molan PC, Reid GM (1991). A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmacol., 43: 817-822.

Atrott J, Henle T (2009). Methylglyoxal in manuka Honey: Correlation with Antibacterial Properties. Czech J. Food Sci., 27: 163-165.

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M (2010). Contribution of honey in nutrition and human health: a review. Mediterranean J. Nutr. Metab., 3: 15-23.

Bang LM, Buntting C, Molan P (2003). The effect of dilution onthe rate of hydrogen peroxide production in honey and its implications for wound healing. J. Altern. Complement. Med., 9: 267-273.

Brudzynski K (2006). Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. Can. J. Microbiol., 52: 1228-1237.

Bunting CM (2001). The production of hydrogen peroxide by honey and its relevance to wound healing. MSc thesis. University of Waikato.

Chute RK, Deogade NG, Kawale M (2010). Antimicrobial activity of Indian honey against clinical isolates. Asiatic J. Biotech. Res., 1: 35-38.

Cooper RA, Molan PC, Harding KG (2002). The sensitivity to honey of Gram -positive cocci of clinical significance isolated from wounds. J. Appl. Microbiol., 93: 857-863.

Dustmann JH (1979). Antibacterial Effect of Honey. Apiacta, 14: 7-11.

Fakoor M, Pipelzadeh MH (2007). A study on the healing effect of honey on infected open fracture wounds. Pak. J. Med. Sci., 23: 327-329.

Halawani EM (2006). A study on *Salmonella typhimurium* causing food poisoning in Al-Taif city and antibacterial effect of *Nigella sativa*, Honey and Camels urine. Ph.D Thesis. Taif University, Saudi Arabia.

Halliwell B, Cross CE (1994). Oxygen-derived species: their relation to human disease and environmental stress. Environ. Health Perspect., 102(10): 5-12.

Iurlina MO, Fritz R (2005). Characterization of microorganisms in Argentinean honeys from different sources. Intl. J. Food Microbiol., 15: 297-304.

Jeddar A, Kharsany A, Ramsaroop UG, Bhamjee A, Haffejee IE, Moosa A (1985). The antibacterial action of honey; an *in vitro* study. S. Afr. Med. J., 67: 257-258.

Kandil A, El-Banby M, Abdel-Wahed GK, Abdel-Gawwad M, Fayez M (1987). Curative properties of true floral and false non-floral honeys on induced gastric ulcers. J. Drug Res., 17: 103-106.

Krell R (1996). Value-added products from beekeeping. FAO Agric. Sev. Bull., No. 124. Retrieved from: htt/www.fao.org.

Kumar KP, Bhowmik D, Biswajit C, Chandira MR (2010). Medicinal uses and health benefits of honey: An overview. J. Chem. Pharm. Res., 2(1): 385-395.

Kwakman PH, Te Velde AA, De Boer L, Speijer DV, Enbroucke-Grauls CM, Zaat SA (2010). How honey kills bacteria. FASEB J., 24: 2576-2582.

Majno G (1975). The Healing Hand. Man and Wound in the Ancient World. Harvard University Press Cambridge, Massachusetts.

Mavric E, Wittmann S, Barth G, Henle T (2008). Identification and quantification of methylglyoxal as the dominant Antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zaeland. Mol. Nutr. Food Res., 52(4): 483-489.

McInerney RJ (1990). Honey- a Remedy Rediscovered. J. Royal Soc. Med., 83: 127-130.

Molan PC (1992a). The Antibacterial Activity of Honey. 1. The Nature of the Antibacterial Activity. Bee World, 73: 5-28.

Molan PC (1992b). The Antibacterial Activity of Honey. 2. Variation in the Potency of the Antibacterial Activity. Bee World, 73: 59-76.

Molan PC (2006). The evidence supporting the use of honey as a wound dressing. Int. J. Lower Extremity Wounds, 5: 40-54.

Molan PC, Russel KM (1988). Non-peroxide antibacterial activity in some New Zealand honeys. J. Apic. Res., 27: 62-67.

Namias N (2003). Honey in the Management of Infections. Surg. Infect., 3: 219-226.

Natarajan S, Williamson D, Gery J, Harding KG, Cooper RA (2001). Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. J. Dermatol. Treatment, 12: 33-36.

Orrù G, Del Nero S, Tuveri E, Ciusa M, Pilia M, Erriu M (2010). Evaluation of antimicrobial-antibiofilm activity of a hydrogen peroxide decontaminating aystem used in dental unit water lines. Open Dent. J., 4: 140-146.

Robinson V, Dodd S, Thoma S (2009). Standardized antibacterial honey (Medihoney™) with standard therapy in wound care: randomized clinical trial. J. Adv. Nurs., 65: 565-575.

Saissy JM, Guignard B, Pats B, Guiavarch M, Rouvier B (1995). Pulmonary edema after hydrogen peroxide irrigation of a war wound. Intens. Care Med., 21: 287-288.

Snow M (2008). Isolation by HPLC and characterization of the bioactive fraction of New Zealand manuka (*Leptospermumscoparium*) honey. Carbohydr. Res., 343: 651-659.

Subrahmanyam M, Hemmady A, Pawar SG (2001). Antibacterial activity of honey on bacteria isolated from wounds. Ann. Burns Fire Disast. 14: 198-201.

Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP (2001). Stimulation of tnf-alpha release in monocytes by honey. Cytokine, 14: 240-242.

Tonks AJ, Cooper RA, Jones KP, Blair SJ, Parton GH, Tonks A (2003). Honey stimulates inflammatory cytokine production from monocytes. Cytokine, 17: 21-23.

Tumin N, Halim NA, Shahjahan M, Noor INJ, Sattar MA, Khan AH, Mohsin SS (2005). Antibacterial activity of local Malaysian honey, Malays. J. Pharmaceut. Sci., 3: 1-10.

Turner FJ (1983). Hydrogen Peroxide and Other Oxidant Disinfectants (3rd ed). Philadelphia: Lea and Febiger, pp. 240-250.

Wahdan H (1998). Causes of the antimicrobial activity of honey. Infection, 26: 30-35.

Watt BE, Proudfoot AT, Vale JA (2004). Hydrogen peroxide poisoning.

Toxicol. Rev., 23: 51-57.
Wilkinson JM, Cavanagh HM (2005). Antibacterial Activity of 13 Honeys Against *Escherichia coli* and *Pseudomonas aeruginosa*. J. Med. Food, 2: 100-103.
Yao L, Jiang Y, Singanusong R, Datta N, Raymont K (2004). Phenolic acids and abscisic acid in Australian Eucalyptus honeys and their potential for floral authentication. Food Chem., 86: 169-177.
Yoirish N (1977). Curative properties of honey and bee venom. San Francisco New Glide Publication, p. 198.