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Survey of the Antibacterial Activity of Saudi and Imported Honeys

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Abstract: The antibacterial activity 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 colour 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 of 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 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 treatment of bacterial infections.

Key words: Saudi honeys · Shaoka honey · Antibacterial activity of honey · Manoka honey

INTRODUCTION

Honey has been used since ancient times in many cultures as an effective remedy [1, 2]. Honey cures bacterial infections [3] through its antimicrobial activity against a wide range of bacterial and fungal species [4]. Honey is widely used as a topical antibacterial agent for treatment of wounds, burns and skin ulcers [5-10]. Honey is a traditional remedy for dyspepsia, peptic ulcer [11,12] and gastritis caused by enteropathogenic bacteria [13].

The antimicrobial activity of honey could be attributed to several factors [4, 14]. 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 [4].

The second factor for the antimicrobial activity of honey is its acidity. The pH of honey 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 and the acidity of honey would not be an effective inhibitor of bacteria [15,16].

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. Hydrogen peroxide has been used as antiseptic [17], however, it is not now as popular because it causes inflammation and damage to tissues [18-20]. The enzyme found in honey is activated by dilution and, the peroxide produced is too mild to cause tissue injury and yet has antimicrobial activity [21,22].

The fourth factor in the antibacterial activity of honey is the presence of phytochemical factors [23-25]. The most direct evidence for the existence of non-peroxide antibacterial factors in honey is the persistence of antimicrobial activity in honeys treated with catalase to remove the hydrogen peroxide activity [22, 26].

The fifth factor in the 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% [27]. 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 [28, 29].

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A large number of honeys are available in the Saudi market. These honeys are either locally produced or imported from different countries. Some of these honeys 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 [30-33] and is medically used worldwide [31, 32]. In this study 24 types of honeys available at the market were evaluated for their antibacterial activity.

MATERIALS AND METHODS

Bacteria: A clinical isolates of *Salmonella entritidis,* was obtained from the stock culture of the Department of Biology, Faculty of Science, Taif University.

Honey Samples: Fifty-two honey samples representing 24 sources of honeys (Table 1) were purchased from the local markets of Taif were except for Manuka honey (Active Manuka honey 12+) which 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 2-12% (w/v) phenol and 16% (w/v) honey samples were prepared in sterile distilled water. Sixty-four wells were cut using 6mm cork borer into Muller-Hinton agar plates (240X240X18 mm) seeded with 10^4 CFU/ml of *Sl. 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 [26].

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 described above [26].

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 22.2-32.0 mm (Fig. 1, Table 2).

The smallest inhibition zone was for Turkish Sidr while the largest inhibition zone was for Shaoka honey which is locally produced (Table 2).

The antibacterial activity of honeys was evaluated after calculation of equivalent phenol % . As shown in Table 2, the antibacterial activity of honeys were equivalent to concentrations of phenol ranging between 4 -8.4% w/v phenol. Thirteen types of honey were equivalent to 6-7% phenol, 5 types were equivalent to 7-8% phenol and 3 types were equivalent to 5-6% phenol (Fig. 2).

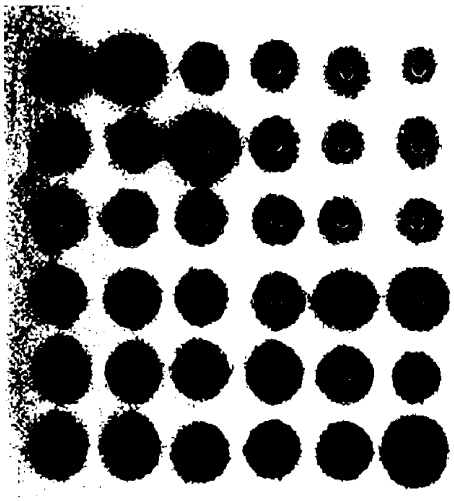

Fig. 1: Muller-Hinton Agar plate seeded with *Salmonella entritidis* showing different sizes of inhibition zones

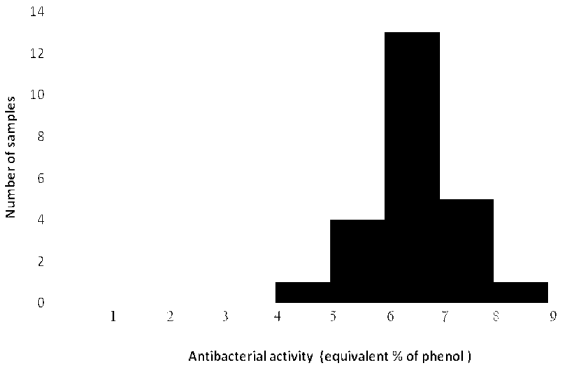

Fig. 2: Distribution of antibacterial activity of honeys

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	Zizphus 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 dentat
6	Doash	1		TAif	Origanum marjorana
7	Morr	1		TAif	Commphora spps
8	Shaoka	4		Taif	Fagonia critica
9	Black seed	3		Qasim	Nigella Stiva
10	Sidr	2	Non-local honeys	Yemen	Zizphus spina-christi
11	Sidr	2		Kashmiri	Zizphus spina-christi
12	Sidr	2		Turky	Zizphus spina-christi
13	Orange Blossom	3		Egypt	Citrus spps
14	Clover	1		Egypt	Trifolium alexandrinum
15	Accacia	3		Germany	Acacia spps.
16	Black Forest	3		Germany	-
17	honey	3		Germany	-
18	Spanish	2		Spain	-
19	Australian	2		Australia	-
20	Swiss	1		Switzerland	-
21	Iranian	1		I ran	-
22	American	2		USA	-
23	Unidentified	2		-	-
24	Manuka	2		New Zealand	Leptospermum scoparium
	Total	52			

Table 2: Inhibition zones and phenol % equivalent of 51 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 Accacia	3	29.0 ± 0.85	6.9 ± 0.18
16	German Black Forest	3	27.8 ± 0.51	6.3 ± 0.20
17	German honey	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

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 Accacia	3	95.2 ± 2.3	4.8 ± 0.16
16	German Black Forest	3	93.5 ± 2.9	6.5 ± 0.05
17	German honey	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 percent

		Activity (phenol % w/v)	
Serial No.	Type of honey	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 Accacia	6.9 ± 0.18	6.6 ± 0.22
16	German Black Forest	6.3 ± 0.20	5.9 ± 0.16
17	German honey	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

Six honeys namely, Shaoka, Taify Sidr, Yemeni Sidr, Black seed, Orange blossom and Clover had an equivalent of 7.2-8.4 % phenol compared to 6.9 % phenol in 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 of honeys was estimated after treatment of honeys with catalase enzyme (Table 3). Eight types of the investigated honeys did not have a detectable peroxide activity (Table 3). Of these 6 were locally produced and two types (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). In all other 14 honeys except, 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 ($p < 0.0007$-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 [30, 33, 34], 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 [15, 26], data obtained in this study revealed that the antibacterial activity of the majority of the investigated 24 types of honey, did not show large variations. The equivalent phenol % 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 the colour and antibacterial activity of honey, as was previously suggested [4, 25]. 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 % respectively).

Inhibition zones produced by Manuka honey were equivalent to 6.9% phenol. Other investigated imported honeys like Orange blossom, Clover and a locally produced honeys like Shaoka, Taify Sidr and Black seed, showed higher antibacterial activity which was equivalent to 7.3-8.4 % 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 [14]. However, on wounds catalase produced by tissues destroys peroxide and hence, the antimicrobial activity of honeys is diminished [23]. Therefore, only Manuka honey lacking peroxide activity is selected for medicinal use [25].

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. A part 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 ($p < 0.013$-0.0001) more active than Manuka honey, when the proportions of peroxide activity in honeys were deduced from the total phenol % 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 [26]. 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 is screened, there is a probability that some of them might lack peroxide activity.

The identification of antimicrobial phytochemicals in honeys has gained the interest of several research workers [14, 30, 33], It would be interesting to identify the antibacterial phytochemicals of Shaoka or other local or non-local potent honeys, included in this study.

It can be concluded that several locally produced and imported honeys available in 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.

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