Assessment of the anti-bacterial activity of different types of natural and commercial honeys on bacteria isolated from Wounds and Burns in Saudi Arabia

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Abstract: Present study aimed to investigate the antibacterial activity of different types of commercial honeys in Sudia Arabia on some bacterial strains isolated from wounds and burns in Jeddah, Sudia Arabia. Selected honeys were katad, Sidr, Nagd, Samra El-Madena, El-Nokhba, Bashayer, Wadi EL-Adwa, Wadi El-Debaa, Wadi-rk, El-Zemma, El-Begedi, Regal, El-Zytoun and Dahyana. Antibacterial activity was determined using Agar well diffusion method, Minimum Inhibition Concentration (MIC) was performed for all investigated honeys against the isolated bacterial strains, pH of each wound and burn was determined at the same time of taking swabs. Present study indicated that Katad, El-Begedi and El-Zemma honeys were the highest antibacterial activity against the isolated bacteria from wounds and burns, as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*., *staphylococcus epidermidis*, and *streptococcus pyogens.* MIC correlates with the antibacterial activity, katad, El-begedi and El-Zemma honeys were of lowest MIC. For all isolated bacteria lowest wounds and burns pH, more inhibition zone with mixed honey (50% Katad honey and 50% Dahyana honey).
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Key words: Sudia Arabia honeys; wounds and burns; bacteria; Agar well diffusion; MIC; pH

Introduction:

Honey is grant divine from God, used since ancient times in the treatment of all diseases, wounds, ulcers and burns. Honey is an extraordinarily healthy highly nutritious yellowish brown sweet viscid supersaturated fluid product of honey bees of the genera Apis and Meliponinae produced from the nectar of flowers (Eman *et al*., 2013).

Honey is a traditional topical treatment for infected wounds. It can be effective on antibiotic-resistant strains of bacteria. Honey is produced from many different floral sources and its antibacterial activity varies with origin and processing. Honey was used to treat infected wounds as long ago as 2000 years before bacteria were discovered to be the cause of infection. Honey was described as being "good for all rotten and hollow ulcers" (Gunther, 1959). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives (Molan, 1992).The current prevalence of antibiotic-resistant microbial species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey. Honey has been known to possess antimicrobial properties, as well as wound- healing activity (Bodeker *et al*., 1999; Moussa *et al*.,2011; Zainol *et al*.,2013).

Honey has been found to harbor an antioxidant activity (Atrooz, 2008). The antioxidant activity comes from the phenolics, peptides, organic acids and enzymes. Honey improves physical performance, resistance to fatigue and increases mental efficiency (FAO, 1996; Eman *et al*., 2013). In patients with wounds infected with antibiotic-resistant strains of bacteria, not responding to antibiotic therapy, good results have been achieved after application of honey (Wadi *et al*.,1987). The bacteria infecting the wounds were found to be resistant to ampicillin, oxytetracycline, gentamicin, chloramphenicol and cephadine. Wounds infected with MRSA have also been cleared of infection and healed by application of honey including a leg ulcer (Natarajan et al.,2001), cavity wounds (Dunford *et al*., 2000) and surgical wounds (Betts and Molan,2001**).** Thus Present study aimed to investigate the antibacterial activity of different types of commercial honeys in Sudia Arabia on some bacterial strains isolated from wounds and burns in Jeddah, Sudia Arabia.

Material and methods:

Honey samples:

fourteen samples of commercially available Honeys (katad, Sidr, Nagd, Samra El-Madena, El-Nokhba, Bashayer, Wadi EL-Adwa, Wadi El-Debaa, Wadi-rk, El-Zemma, El-Begedi, Regal, El-Zytoun and Dahyana) were obtained from middle city, Jeddah markets for Honey. Samples were stored at 4 ^{o}C in dark jars till used. For antibacterial tests, honey samples were used undiluted.

Isolation and Identification of Bacteria:

The study was carried out on 50 pus wound swabs and 50 burn wound swabs were collected from patients at special hospital located at Jeddah, Saudi Arabia. The swabs were held in contact with wounds for at least 5 seconds, the sterile swabs samples were places in sterile containers in -4^{O}C till cultured. The isolated bacterial strains were identified using API20E. The isolated bacterial strains were identified as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *staphylococcus epidermidis*, and *streptococcus pyogens*.

Determination of antibacterial activity of tested Honey:

Agar well diffusion test (Stites *et al*.,1984**)** was used for determination of AMA. Muller Hinton agar medium was the medium of choice and the uniformly clear zone of inhibition was a confluent lawn of growth was measured in mm.

Agar well diffusion assay:

The plates were prepared using 20 ml of sterile Muller Hinton Agar. The surface of the plates was inoculated using a 100 µL of 0.5 McFarland standardized inoculum suspension of the isolated bacteria and allowed to dry. Wells, 8.0 mm in diameter, were cut from the culture media using a sterile metal cylinder, and then filled with the test honey. The plates were incubated at 37ºC for 24 hours for clear, circular inhibition zones around the wells. The diameter of zones of inhibition of the wells was measured by measuring them in millimeters (mm) in at least 2 directions perpendicular to each other (90°).. The mean of diameters of inhibition zone for each well and honey sample was calculated.

Minimum inhibitory concentration (MIC):

The MIC test was carried out according to **Patton** *et al*. (2006) and **Tan** *et al*. (2009) with slight modifications. isolated bacteria culture was prepared and adjusted to be equal to (1×10^8 cfu/ml) and further diluted by mixing 1 part of adjusted culture with 199 parts of Tryptic Soya Broth to meet 5×10^5 cfu/ml. Volumes of10 ml TSB was pipetted into five sterile screw-capped test tubes and labeled accordingly. Another empty tube served as the first tube of honey stock solution where it was used to prepare 50% (w/v) honey solution by weighing 5 g honey sample, made up to 10 ml with TSB, well mixed and filtered through 0.2 µm filters. A Two-fold serial dilution was prepared using all five pre-filled tubes together with four extra tubes containing honey dilutions of 5, 10, 15 and 20% (w/v). All tubes were vortex until uniformly mixed.

A volume of 190 µl of each honey dilution was aseptically transferred into 96 well flat-bottom microtitre plates (Nunc, Denmark) in eight replicates per dilution. The first two wells of every honey dilution served as dilution sterility controls (added with another10 µl of respective honey dilution) and six others were the test wells in which 10 µl bacteria culture was mixed. Row number 11 and 12 were reserved for batch sterility and growth controls. Volume of 200 µl TS broth was used as assay sterility control in all wells of row 11 while 10 µl bacteria culture in 190 µl TS broth served as the assay growth control in all wells of row 12. Plates were incubated in a shaker incubator (Stuart, UK) at 120 rpm, 37°C for 24 hours. The absorbance of the wells was read at 590 nm using microtitre plate reader (Bio-rad, US) after incubation. The percentages of inhibition of bacteria growth were calculated by using the following formula:

1 - (Absorbance of test well−Absorbance of corresponding control well) / (Absorbance of assay growth control−Absorbance of sterility control) x100 (Zainol *et al*., 2013**).**

Wound content pH:

From each wound or burn pH was measured using pH meter at the same time of taking swabs. Selected honeys were used original undiluted for examination the anti bacterial activity and factors affecting the efficacy as natural antibacterial.

Statistical:

Inhibition zones, Minimum Inhibitory Concentration (MIC) expressed as the mean of three replicates ± standard deviation.

Results:

Isolated bacteria from wounds and burns:

Gram positive bacteria were the most dominant (62.7%), followed by Gram negative bacteria (38.3%). *Staphylococcus aureus* was the most common organism, isolated (47.8%),

followed by *Pseudomonas aeruginosa* (23.0%), *Escherichia coli* (15.3%), *staphylococcus* (8.8%), were most isolated from wounds with percentage 70%,68% and 61% respectively while E.coli strain more predominant 83% in burns. Total isolation S.aureus was predominant (47.8%) while S.pyogenes was the least (6.1%) table 1.

Table 1: showing percentage of isolated bacteria from wounds and burns

Isolated bacteria	Percentage of isolation		Total %	gram
	wounds	burns		
Staphylococcus aureus	70%	30%	47.8	+ve
Pseudomonas aeruginosa	68%	32%	23.0	-ve
Escherichia coli	17%	83%	15.3	-ve
staphylococcus epidermidis	56%	44%	8.8	+ve
streptococcus pyogens	61%	39%	6.1	+ve

Antibacterial activity of honeys from different origins against isolated bacterial:

Present study displayed that all investigated honeys have antibacterial activity with different potency this was showed through zone of inhibition of different of honeys. Katad honey was the highest antibacterial activity with mean inhibition zone (20.2) while the lowest inhibition zone was Dahyana honey.

The most efficient honeys followed by Katad honey, were El-Begedi (17.6), El-Zemma (17.4) followed by Nagd (16.8), Regal (16.0), El-Nokhba (15.8), Samraa El-Madina (15.6), Bashayer and Wadi Rk (14.6), Wadi El-Adwa and Wadi El-Debaa (14.0), El-Zytoun (13.3) and Dahyana (12.4) table 2.

Table 2: Antibacterial activity of investigated honeys from different origins against isolated bacterial species from wounds and burns, determined by agar well diffusion.

Honey	inhibition zone mm ±SD					total
	S.aureus	*P. aeruginosa*	*E. coli*	*S. epidermidis*	*S. pyogens*	
katad	19.5±3.2	22.2±4.5	20.4±3.8	19.7±2.4	21.3±4.1	20.2±3.2
Nagd	16.0 ±1.0	17.3 ±1.2	16.3 ±1.5	17.3 ±2.1	18.3 ±1.2	16.8±2.3
Sidr	15.8 ±2.2	11.6 ±1.1	13.8 ±1.5	13.2 ±1.3	16.2 ±0.8	13.6±1.2
Samraa El-madina	16.7 ±0.6	14.3 ±0.5	15.3 ±0.5	17.0±0.0	16.0 ±1.0	15.6±2.5
El-Nokhba	17.3 ±0.5	15.3 ±0.5	14.3 ±0.5	17.6 ±0.5	16.6 ±1.2	15.8±2.1
Bashayer	14.8 ±0.3	14.2 ±0.3	14.0 ±1.0	16.2 ±0.8	15.0 ±1.0	14.6±1.2
Wadi EL-Adwa	12.7 ±1.2	13.7 ±0.6	15.7±0.6	15.7 ±1.2	15.0 ±0.0	14.0±2.3
Wadi El-Debaa	14.6 ±0.6	14.5 ±0.7	14.3 ±0.6	14.0 ±0.0	14.3 ± 0.6	14.0±3.2
Wadi-rk,	15.0 ± 0.6	13.3 ±0.5	16.2 ±0.8	15.2 ± 0.0	14.3 ±0.6	14.6±2.2
El-Zemma	18.7 ±0.6	16.8 ±1.1	18.7 ±0.6	19.3 ±0.6	16.0 ±1.0	17.4±3.1
El-Begedi	17.3 ±0.6	19.0 ±0.0	17.0 ±0.0	15.7 ±1.2	19.3 ±0.6	17.6±4.2
Regal	16.0 ± 0.5	17.2 ±0.6	15.3 ± 0.6	16.8 ±0.3	16.7 ±0.3	16.0±2.3
El-Zytoun	13.2 ±1.3	13.7 ±0.6	13.7 ± 0.6	13.3 ±0.6	15.7 ±2.5	13.3±3.1
Dahyana	10.3 ±0.6	11.2 ±0.3	12.3 ±1.2	14.0 ±2.0	15.3 ±0.5	12.4±2.1

The values are means of 3 replicates (well (8.0 mm) ± Standard deviation.

Inhibitory Concentration (MIC) of investigated honeys:

Present study revealed that the total MIC of honeys in Saudi Arabia ranged from 3.04% to 26.66%, the lowest total MIC was for Katad honey (3.04%) and the largest MIC was for Wadi-Rk honey (26.33%).concerning the isolated bacterial strains *S. pyogens* posses the lowest MIC (11.30%) followed by *P. aeruginosa* (12.44%), *S.aureus* (13.36%), *E. coli* (14.50%) and *S. epidermidis* (15.30%). The results indicated that S.pyogens isolated from wounds and burns was more susceptible for inhibition with (11.30 %) MIC by different types of honeys in Saudi Arabia in honey markets, followed by *P. aeruginosa*, (12.44%), *S.aureus* (13.36 %), *E. coli* (14.50 %) and *S. epidermidis* (15.30%) table 3.

Table 3: Minimum Inhibitory Concentration (MIC) of investigated honeys from different origins against isolated bacterial species from wounds and burns

Honey	Minimum Inhibitory Concentration (MIC) %					total
	S.aureus	*P. aeruginosa*	*E. coli*	*S. epidermidis*	*S. pyogens*	
katad	3.8	2.1	4.1	1.9	3.3	3.04
Nagd	7.6	5.6	8.2	5.9	3.5	6.16
Sidr	12.4	10.3	7.1	5.5	8.6	8.78
Samraa El-madina	9.8	12.7	21.5	18.3	11.3	14.72
El-Nokhba	13.5	15.3	17.6	15.6	8.2	14.12
Bashayer	17.8	14.3	22.6	27.1	12.5	18.86
Wadi EL-Adwa	14.4	17.1	28.7	31.4	21.9	22.70
Wadi El-Debaa	24.5	19.2	18.7	26.7	14.9	20.80
Wadi-rk,	23.8	24.3	31.2	34.5	19.4	26.66
El-Zemma	3.6	2.4	5.2	3.5	1.9	3.32
El-Begedi	1.7	4.2	3.2	4.5	2.6	3.10
Regal	4.1	3.2	4.4	2.3	3.9	3.58
El-Zytoun	18.9	22.1	17.6	23.8	28.4	22.16
Dahyana	31.2	21.4	12.3	13.5	17.4	19.16
total	13.36	12.44	14.50	15.30	11.30	--------

Factors affect the antibacterial activity of honeys:

pH of wound contents:

It was clear from the present study that inhibition zone of mixed honey (50% Katad honey and 50% Dahyana honey) was affected mostly by pH of investigated wound or burn. It was showed from table 3 that there was more large inhibition zone with low pH (3): (10.5), (7.9), (6.3),(13.6) and (15.4) for the isolated bacteria from wounds and burns *S.aureus, P.aeruginosa, E.coli, S.epidernids, and S.pyogens* respectively, And decreased gradually with increasing pH (9) in *S.aureus and P.aeruginosa* and not affected greatly in *E.coli, S.epidermis* and *S.pyogens* table 4.

Table 3: The effect of different pH on the antimicrobial efficacy of mixed honey on the inhibition growth of isolated bacterial strains from wounds and burns in Saudi Arabia [inhibition zone (mm)]

pH	Mean of inhibition zone (mm)				
	S.aureus	*P. aeruginosa*	*E. coli*	*S. epidermidis*	*S. pyogens*
3	10.5±2.2	7.9±0.8	6.3±2.3	13.6±2.4	15.4±3.2
4	7.2±1.7	6.5±0.6	6.2±2.1	13.3±2.1	12.7±3.1
5	6.4± 1.2	4.9±0.9	5.9±0.9	12.3±3.2	11.5±1.2
6	6.1±1.1	4.3±0.4	3.2±0.3	10.4±2.6	12.5±3.4
7	5.8±0.5	3.6±0.5	5.6±0.5	11.6±3.1	13.7±2.2
8	3.2±0.6	2.4±0.2	4.5±0.4	12.5±2.3	10.5±2.1
9	1.1±0.02	00.0±0.0	4.3±0.3	9.8±1.2	8.7±0.7

Discussion:

Honey has been used by humans to treat a variety of ailments, from gastric disturbances to ulcers, wounds and burns, through ingestion or topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained. Different honeys have different properties, which was known since ancient times. Much scientific research has been done, with emphasis of late on fighting infections in wounds (Grotte, 1998).

Honey was used in the medicine of many ancient communities **(**Molan, 2006**)**, including the ancient Egyptians. The ancient Chinese and Sumerians provided the first written prescriptions relating to the medical use of honey, found as clay tablets, dating back to 2000 B.C (Sulaiman *et al*.,2012).

The antibacterial potency of honey has been attributed to its strong osmotic effect, naturally low pH (Kwakman and Zaat, 2012), the ability to produced hydrogen peroxide which plays a key role in the antimicrobial activity of honey **(**Kačániová *et al*., 2011; Wahdan, 1998**)** and phytochemical factors. Numerous reports and clinical studies have demonstrated the antimicrobial activity of honey against a broad range of microorganisms, including multi-antibiotic resistant strains.

Regarding the isolated bacteria from wounds and burns, Present study revealed that Gram positive

bacteria were the most dominant (56.3%), followed by Gram negative bacteria (43.7%). *Staphylococcus aureus* was the most common organism, isolated (47.8%), followed by *Pseudomonas aeruginosa* (23.0%), *Escherichia coli* (15.3%), *staphylococcus* (8.8%), were most isolated from wounds with percentage 70%,68% and 61% respectively while E.coli strain more predominant 83% in burns. Total isolation S.aureus was predominant (47.8%) while S.pyogenes was the least (6.1%) the results nearly agree with that obtained by Alghalibi, ***et*** *al.*(2011) who reported that *Staphylococcus aureus* was the most common organism, isolated100 (47.8%), followed by *Pseudomonas aeruginosa* (23%), Candida albicans (5.3%), *Escherichia coli* (5.3%), Serratia plymuthica (3.8%), Proteus mirabilis (2.9%), Salmonella species (2.4%), Staphylococcus epidermidis (2.4%), Acinetobacter species (1.9%), Streptococcus faecalis (1.4%), Bacillus species (0.96%), Citrobacter freundii (0.96%), Klebsiella species (0.96), and Streptococcus pyogenes (0.96%) and (Ekrami and Enayat, 2007**)** who reported that The microorganisms causing infections isolated from burn patients in Iran were Pseudomonas aeruginosa (37.5%), Staphylococcus aureus (20.2%), and Acinetobacter baumanni (10.4%).

Agar well diffusion method is mainly a qualitative test for detecting the susceptibility of bacteria to antimicrobial activity of honey; however, the minimum inhibitory concentration (MIC) reflects the quantity needed for bacterial inhibition.

Regarding the antibacterial activity of investigated honeys, all used honey in the experiment have antibacterial activity against the isolated bacterial strains from wounds and burns with different potencies, present study revealed that, through zone of inhibition of different of honeys. Katad honey was the highest antibacterial activity with mean inhibition zone (20.2) while the lowest inhibition zone was Dahyana honey. The most efficient honeys followed by Katad honey, were El-Begedi (17.6), El-Zemma (17.4) followed by Nagd (16.8), Regal (16.0), El-Nokhba (15.8), Samraa El-Madina (15.6), Bashayer and Wadi - Rk (14.6), Wadi El-Adwa and Wadi El-Debaa (14.0), El-Zytoun (13.3) and Dahyana (12.4) the results nearly agree with that obtained by Hayam and Dalia (2011);Eman *et al*. (2013) for the anti bacterial activity of Egyptian honey**,** Zainol *et al*. (2013) for Malaysian honey and Moussa *et al*.,(2011) for Algarian honey.

The antibacterial action of honey may be due to H_2O_2 and non- peroxide antibacterial factors. The activity of H_2O_2 works when honey is diluted. Moreover, the hygroscopic feature of honey causes withdrawal of moisture from the surroundings by osmosis leading to microbial death (Eman *et al*., 2013). H_2O_2 is produced when glucose oxidase which is secreted from hypo pharyngeal gland of bee converts glucose in the nectar into gluconic acid and H_2O_2 (Effat,2012).other antibacterial factors of honey include low pH and presence of inhibine **(**Base,1982;Codon,1993**).**

The large variance in antibacterial potency of different honeys may be due to their floral source and geographical origin expressed as large discrepancy in results reported between authors and hospitals using honey in similar ways. Some have reported rapid clearance of infection in a range of different types of wound, with all wounds becoming sterile in 3-6 days (Cavanagh *et al*.,1970; Braniki *et al*.,1981), 7 days (Efem,1988 & 1993**)**, or 7-10 days (Armon,1980) Others have reported bacteria still present in wounds after 2 weeks (Harris,1994; Ndayisaba *et al*.,1993).

Concerning the MIC of selected honeys as antibacterial against isolated bacterial strains from wounds and burns, present investigation displayed that, the total MIC of honeys in Saudi Arabia ranged from 3.04% to 26.66%, the lowest total MIC was for Katad honey (3.04%) and the largest MIC was for Wadi-Rk honey (26.33%).concerning the isolated bacterial strains *S. pyogens* posses the lowest MIC (11.30%) followed by *P. aeruginosa* (12.44%), *S.aureus* (13.36%), *E. coli* (14.50%) and *S. epidermidis* (15.30%). The results indicated that S.pyogens isolated from wounds and burns was more susceptible for inhibition with (11.30 %) MIC by different types of honeys in Saudi Arabia in honey markets, followed by *P. aeruginosa*, (12.44%), *S.aureus* (13.36 %), *E. coli* (14.50 %) and *S. epidermidis* (15.30%). The MICs of various types of honeys for various pathogenic bacterial strains have been determined by many authors **(**Cooper, 2001; Mandal and Mandal, 2011). The results of the present study nearly coincide with Zainol *et al*.(2013) who reported that there is correlations between MIC and Equivalent Phenol Concentration EPC value of Malaysian honey were proven to be dependent on bacteria species and honey origin.

The MIC (minimum inhibitory concentration) of the honeys was found to range from 1.8% to 10.8% (v/v), indicating that the honeys had sufficient antibacterial potency to stop bacterial growth if diluted at least nine times, and up to 56 times in the presence of *Staphylococcus aureus* [(Willix *et al.,* 1992), the most common wound pathogen In another study with 58 clinical isolates of *Staphylococcus aureus* (Cooper *et al*.,1999) the MIC ranged from 2% to 4% (v/v). In a study of 20 isolates of *Pseudomonas* from infected wounds (Cooper and Molan,1999) the MIC of these two honeys was found to range from 5.5% to 9.0%.

Concerning factors affecting antibacterial activity of honey, Molan and Cooper (2000) reported that the difference in antimicrobial potency among the different honeys can be more than 100-fold, depending

on its geographical, seasonal and botanical source. present study showed that inhibition zone of mixed honey (50% Katad honey and 50% Dahyana honey) was affected mostly by pH of investigated wound or burn. It was showed from table 3 that there was more large inhibition zone with low pH (3): (10.5), (7.9), (6.3),(13.6) and (15.4) for the isolated bacteria from wounds and burns *S.aureus, P.aeruginosa, E.coli, S.epidernids, and S.pyogens* respectively, And decreased gradually with increasing pH (9) in *S.aureus and P.aeruginosa* and not affected greatly in *E.coli, S.epidermis* and *S.pyogens*., it was clear that the pH of wound mostly affect the inhibition zone of growth of different isolated bacteria from wounds and burns. This is may be due to that, one of the most important mechanisms of antibacterial activity of honeys is the low pH **(**Base,1982; Codon,1993**).** The pH of intact skin is acidic with values between 4 and 6, while plasma pH is approximately 7.4. Wounds often demonstrate a higher pH than 7.4, and as they heal this decreases. Pathogenic bacteria often require higher pH levels to proliferate and some evidence exists that colonized / infected wounds maintain a high pH (Lusby *et al*.,2005; Al-Waili *et al*.,2005; Manisha and Shyamapada 2011**)** the pH of wound and pH of used honey interfere with each other affecting the antibacterial activity of honey.

From the present study it was concluded that all investigated honeys in Saudia Arabia, katad, Sidr, Nagd, Samra El-Madena, El-Nokhba, Bashayer, Wadi EL-Adwa, Wadi El-Debaa, Wadi-rk, El-Zemma, El-Begedi, Regal, El-Zytoun and Dahyana possesses antibacterial activity on the isolated strains of bacteria from wounds and burns in Jeddah with different potencies due to differences in flowral and geographical origin. Katad honey was the highest antibacterial activity followed by El-Begedi and El-Zemma honeys, the MIC seems to correlates with the antibacterial activity the total MIC of honeys in Saudi Arabia ranged from 3.04% to 26.66%, the lowest total MIC was for Katad honey. The antibacterial activity greatly affected by pH of wound and burns, the results indicated that the wound and burns of low pH record more large inhibition zones.

References:

1. **Alghalibi, S. M. S.; Humaid, A.A.; Alshaibani, E. A. S. and Alhamzy, E. H. L (2011):**:Microorganisms Associated With Burn Wound Infection in Sana'a, Yemen Egypt. Acad. J. biolog. Sci., 3(1): 19-25.
2. **Al-Waili NS, Akmal M, Al-Waili FS, Saloom KY, Ali A. (2005):** The antimicrobial potential of honey from United Arab Emirates on some microbial isolates. Med Sci Monitor; 11: 433-438.
3. **Armon PJ. (1980):**The use of honey in the treatment of infected wounds. *Trop Doct*; **10**(2): 91.
4. **Atrooz, O.M., M.A. Al-Sabayleh and S.Y. Al-Abbadi, (2008):** Studies on physical and chemical analysis of, various honey samples and their antioxidant activity. Journal of Biological Science, 8: 1338-1342.
5. **Base, B.,(1982).** Honey or sugar in treatment of infected wounds.Lancet, 1:8278.
6. **Betts JA, Molan PC. (2001)**: A pilot trial of honey as a wound dressing has shown the importance of the way honey is applied to wounds. 11th Conference of the European Wound Management Association,; Dublin, Ireland.
7. **Bodeker, G.C., T.J. Ryan and C.K. Ong, (1999**): Traditional approaches to wound healing. Clin Dermatol., 17: 93-8.
8. **Braniki FJ. Surgery in Western Kenya. *Ann R Coll Surg Engl* (1981)**; 63: 348-52.
9. **Cavanagh D, Beazley J, Ostapowicz F. (1970):** Radical operation for carcinoma of the vulva. A new approach to wound healing. *J Obstet Gynaecol Br Commonw*; **77**(11): 1037-40.
10. **Condon,R.E.,(1993).**Curious interaction of bugs and bees. Surgery, 113:234-235.
11. **Cooper R. (2001):** How does honey heal wounds? In: Munn P, Jones R, editors. Honey and Healing. UK: International Bee Research Association.
12. **Cooper RA, Molan PC, Harding KG. (1999):** Antibacterial activity of honey against strains of Staphylococcus aureus from infected wounds. J R Soc Med; 92(6): 283-5.
13. **Cooper RA, Molan PC. (1999):** The use of honey as an antiseptic in managing Pseudomonas infection. *J Wound Care*; **8**(4): 161-4.
14. **Dunford C, Cooper R, White RJ, Molan P. (2000):** The use of honey in wound management. *Nurs Standard*; **15**(11): 63-8.
15. **Efem SE. (1988)**: Clinical observations on the wound healing properties of honey. *Br J Surg*; **75**(7): 679-81.
16. **Efem SE. (1993)**:Recent advances in the management of Fournier's gangrene: preliminary observations. *Surgery*; **113**(2): 200-4.
17. **Effat, M.M.,2012** AT. Talbina prophetic Medicinal Treasure (in Arabic). Dar- alfarouq,Cairo, Egypt pp:98-108.
18. **Ekrami Alireza & Enayat Kalantar (2007)**: Bacterial infections in burn patients at a burn hospital in Iran Indian J Med Res 126, December, pp 541-544.
19. **Eman A. Khairy, Riham H. Hedia, Sohad M. Dorgham and M. Effat (2013):** Comparative Studies on Antimicrobial Activities (AMA) of Different Types of Honey Using Bacteria from

Animal Origin International Journal of Microbiological Research 4 (1): 50-55.

20. **FAO (Food and Agriculture Organization, 1996, Rome.**

Grotte, L.B. (1998). "Honey as a Dressing for Wounds, Burns, and Ulcers: A Brief Review of Clinical Reports and Experimental Studies". *Primary Intention* 6 (4).

21. **Gunther RT. (1934):** *The Greek Herbal of Dioscorides*. New York: Hafner, (reprinted 1959).

22. **Harris S. (1994);** Honey for the treatment of superficial wounds: a case report and review. *Primary Intention* **2**(4): 18-23.

23. **Hayam M. Hamouda and Dalia S. Marzouk (2011):** Antibacterial Activity of Egyptian Honey from Different Sources International Journal of Microbiological Research 2 (2): 149-155.

24. **Kačániová, M., Vukovic, N., Bobková, A., Fikselová, M., Rovná, K., Haščík, P., Čuboň, J., Hleba, L., Bobko, M. (2011):** Antimicrobial and antiradical activity of Slovakian honeydew honey samples. In *The Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, 2011, no. 3, p. 354-36.

25. **Kwakman PH, Te Velde AA, de Boer L, Speijer D, Vandenbroucke- Grauls CM, Zaat SA.(2010):** How honey kills bacteria. FASEB J,24: 2576-2583.

26. **Lusby PE, Coombes AL, Wilkinson JM. (2005):** Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res; 36: 464-467.

27. **Mandal Manisha Deb, Shyamapada Mandal (2011):** Honey: its medicinal property and antibacterial activity Asian Pac J Trop Biomed 2011; 1(2): 154-160.

28. **Manisha Deb Mandal, Shyamapada Mandal (2011):** Honey: its medicinal property and antibacterial activity Asian Pac J Trop Biomed 2011; 1(2): 154-160.

29. **Molan PC, Cooper RA.(2000):** Honey and sugar as a dressing for wounds and ulcers. Trop Doct; 30: 249-250.

30. **Molan PC. (1992):** The antibacterial activity of honey. 1.The nature of the antibacterial activity. *Bee World*; **73**(1): 5-28.

31. **Moussa Ahmed, Aissat Saad, Djebli, Noureddine, Boulkaboul Aboud, Abdelmalek Meslem and Khiati Baghdad (2011):** The Influence of Starch of Ginger on the Antibacterial Activity of Honey of Different Types from Algeria against Escherichia coli and Staphylococcus aureus International Journal of Microbiological Research 2 (3): 258-262.

32. **Natarajan S, Williamson D, Grey JA, Harding KG, Cooper RA. (2001**): Healing of an MRAS-colonised, hydroxyurea-induced leg ulcer with honey. J Dermat Treat; 12: 33-6.

33. **Ndayisaba G, Bazira L, Habonimana E, Muteganya D. (1993):** Clinical and bacteriological outcome of wounds treated with honey. *J Orthop Surg*; **7**(2): 202-4.

34. **Patton T, Barrett J, Brennan J, Moran N (2006):** Use of spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. J Microbiologocal Methods, 64:84–95.

35. **Stites,D.P., Stobo,H.H., Fudenberg and J.V., Wells, (1984).** Basic and clinical immunology, 5th edition. Large Medical Publications.

36. **Sulaiman Alnaimat, Milton Wainwright and Khalid Al'Abri (2012**): Antibacterial potential of honey from different origins: Acoparison with Manuka,Journal of Microbiology, Biotechnology and Food Sciences*:* 1 (5) 1328-1338.

37. **Tan HZ, Abdul Rahman R, Gan SH, Halim AS, Hassan SA, (2009)**: The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complement Altern Med, 9:34.

38. **Wadi M, Al-Amin H, Farouq A, Kashef H, Khaled SA**. (**1987**): Sudanese bee honey in the treatment of supprating wounds. *Arab Medico*; **3**: 16-8.

39. **Wahdan, H. (1998);** Causes of the antimicrobial activity of honey. In *Infection*, vol. 26, no. 1, p.26-31.

40. **Willix DJ, Molan PC, Harfoot CG. (1992):** A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *J Appl Bacteriol*; **73**(5): 388-94.

41. **Zainol Mohd Izwan, Kamaruddin Mohd Yusoff and Mohd Yasim Mohd Yusof (2013):** Antibacterial activity of selected Malaysian honey. BMC Complementary and Alternative Medicine, 13:129.