



INHIBITING AND HEALING ACTIVITY OF HONEY AND MOLASSES (DATE SYRUP) IN CONTAMINATED WOUNDS AGAINST PATHOGENIC BACTERIA

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Abstract

The study includes two parts detection about the inhibiting activity in honey and molasses (date syrup) against pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) that caused wound contamination. First part was detect the inhibiting ability of natural honey and natural molasses by test the minimum inhibition concentration (MIC) on pathogenic bacteria in cultured plates and the second part by using rats to test their ability to heal the wound which contaminated with pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) used in the first part this contamination led to inflammation of cuts. The treatment step by natural honey and natural molasses was the last step for examination about the inhibiting activity of these two substances. The Results of honey inhibition activity test against (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) were detected with (8mm, 7mm and 6mm), respectively. But Molasses inhibition activity results against this bacteria *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were (3mm, 4mm and 5mm), respectively. To conform the results of inhibition activity for both honey and molasses, two antibiotics cefotaxime and meropenem depended already to inhibit the pathogenic bacteria and showed inhibition zone (4mm, 6mm and 3mm) for cefotaxime and (15mm, 13mm and 15mm) for meropenem against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* respectively. Rats divided into four groups, every one group include three individuals each individual infected with (*saphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). First group treated by natural honey, second group treated by natural molasses, third group treated by Cefotaxime and fourth by Meropenem. The period of healing wound with Meropenem which contaminated with the pathogenic bacteria which used in this study, was spent 10 days, but 14 days with Cefotaxime antibiotic treated the wound which contaminated with *Escherichia coli* and while 16 days with these were contaminated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Also, the healing period with honey which spent 12 days against *Escherichia coli* and *Pseudomonas aeruginosa* but 11 days against *Staphylococcus aureus*. About the Molasses treatment results, the treating extend till 14 days against *Escherichia coli* and *Pseudomonas aeruginosa* but 16 days for *Staphylococcus aureus*.

Key words: wounds, honey, molasses.

Introduction

The earlier U.S. Food and Drug Act defined honey as “the nectar and saccharine exudation of plants, gathered, modified and stored in the comb by honey bees (*Apis mellifera* and *A. dorsata*); is contains not more than 25% water, not more than 0.25% ash and not more than 8% sucrose. By far, the largest portion of the dry matter in honey consists of the sugars (Tomas *et al.*, 2001). This very concentrated solution of several sugars results in the characteristic physical properties of honey high viscosity, “stickiness,” high density, granulation tendencies, tendency to absorb moisture from the air and

immunity from some types of spoilage. Because of its unique character and its considerable difference from other sweeteners honey is an effective broad-spectrum antibacterial agent and have the ability of wound healing. Skin wounds, gastric ulcers and burns are treated by honey (Vonderohe, 1996).

The wound healing properties of honey include stimulation of tissue growth, enhanced epithelialization and minimized scar formation. These effects are ascribed to honey’s acidity, hydrogen peroxide content, osmotic effect, nutritional and antioxidant contents, stimulation of immunity and to unidentified compounds (Gheldof and *et*

Table 1: Antibacterial activity of honey.

Common name	Scientific name	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Natural Honey	<i>Apis mellifera</i>	7	8	6
German Honey	<i>Apis mellifera</i>	0	0	0
Local Honey	<i>Apis mellifera</i>	0	0	0
Turkish Honey	<i>Apis mellifera</i>	0	0	0
LSD value	-	2.19 *	2.66 *	2.08 *
* (P<0.05)				

al., 2002). Prostaglandins and nitric oxide play a major role in inflammation, microbial killing and the healing process (Diegelmann and Evans, 2004). Honey was found to lower prostaglandin levels and elevate nitric oxide end products. These properties might help to explain some biological and therapeutic properties of honey, particularly as an antibacterial agent or wound healer (Mundo and *et al.*, 2004).

The major minerals of *Phoenix dactylifera* L. are selenium, copper, potassium and magnesium. It is contained a high percentage of carbohydrate, fat, minerals, protein, vitamins and a high percentage of dietary fiber. There are at least 15 minerals in dates. The percentage of each mineral in dates syrup varies from 0.1 - 916 mg/100 g date depending on the type of mineral. In many varieties potassium can be found at a concentration as high as 0.9% in the flesh. (Al-Shahib and Marshall, 2003).

Other minerals and salts that are found in various proportions include boron, calcium cobalt, copper, fluorine,

Table 2: Activity of antibiotics against (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*).

Antibiotic	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Cefotaxime (mm)	6.0	4.0	3.0
Meropenem (cm)	1.3	1.5	1.5
LSD value	2.061 *	1.736 *	1.328 *
* (P<0.05).			

Table 3: Antibacterial activity of molasses.

Common name	Scientific name	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Natural Molasses	<i>Phoenix dactylifera</i> L.	4	3	5
AL-tajy local molasses	<i>Phoenix dactylifera</i> L.	0	0	0
Iranian Molasses	<i>Phoenix dactylifera</i> L.	0	0	0
Karbala factory Molasses	<i>Phoenix dactylifera</i> L.	0	0	0
LSD value	-	1.945 *m	1.372 *	2.064 *
* (P<0.05).				

iron, magnesium, manganese, potassium, phosphorous, sodium zinc, aluminum, cadmium, chloride, lead and sulphur (El-Sohaimy and Hafez, 2010). Dates syrup contain elemental fluorine that is useful in protecting teeth against decay (Gad and *et al.*, 2010). Selenium, another element believed to help prevent cancer and important in immune

function, is also found in dates syrup. Date syrup had been assessed for the presence of antioxidant compounds such as anthocyanins, vitamins, carotenoids and phenolic compounds. Antibacterial properties were also exhibited by date palm extract, which could inhibit the growth of Gram positive and Gram negative bacteria (Hasnaoui and *et al.*, 2010; Tan and *et al.*, 2011).

Materials and Methods

Experiment of wounds healing on rats

Scalpel used to infect laboratory animals to make surface wounds at skin layer. The length of incisions were ranges between (1cm-1.5cm) and with a depth ranges from 1mm to 2mm on the side of the abdominal region in male and female rats that weight (200-250)g at room temperature and with age ranges from 5 to 6 months. After injury of the animals and happen of wounds the next step was infected these wounds by pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Then left for 24 hours that make hurts inflammation. (Jessica and Martha, 2010). After the inflammation the healing process be the last step to improve the honey and molasses ability for treating wounds and inhibiting the pathogenic bacteria.

The natural honey, molasses, Cefotaxime and Meropenem antibiotics that used for treatment the contaminated wounds. The incisions was infected by the following bacteria (*Staphylococcus aureus*, *Pseudomonas* and *E. coli*). The laboratory rats divided according to groups each groups contain three individuals as shown in (Table 4). (Hawk, *et al.*, 2005)

Minimum Inhibition concentration testing

The appropriate antimicrobial

Table 4: Comparison of the period time of inhibiting activity of the studied parameters (Meropenem, Cefotaxime, natural honey and molasses).

Type	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Meropenem	10	10	10
Cefotaxime	14	16	16
Natural honey	12	11	12
Natural molasses	14	16	14
LSD value	2.59 *	3.06 *	3.14 *
* (P<0.05).			

impregnated disks or the antibacterial substances (honey and molasses) placed on the surface of the agar. Then the dispenser put over the agar plate and firmly press the plunger once to dispense the disks or substance onto the surface of the plate. (Wayne, 2006). The dispenser off the plate left and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. (Jorgensen, 2007). The disk should not move if it has contacted the agar surface even if the disk is not in the proper location, because some of the antibacterial begins to diffuse immediately upon contact with the agar then incubated the plate for a full 24 hours before reading (Winn, 2006).

Results and Discussion

The results of Minimum Inhibition concentration against (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) were (7mm, 8mm and 6mm), respectively for natural honey table 1. But (6mm, 4mm and 3mm) for Cefotaxime antibiotic respectively and (13mm, 15mm and 15mm) respectively for Meropenem antibiotic table 2.

LSD results of honey of the pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) were (2.19, 2.66 and 2.08) respectively, In compare with the two types of antibiotics (Cefotaxime and Meropenem) that used to treat contaminated wounds by pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*), results of LSD were (2.061, 1.0736 and 1.328).

The results of Minimum Inhibition concentration against (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) were (4mm, 3mm and 5mm) respectively for natural Molasses, table 3. But (6mm, 4mm and 3mm) for Cefotaxime antibiotic respectively and (13mm, 15mm and 15 mm) respectively for Meropenem antibiotic plates 3 (10-12)

LSD results of natural Molasses of the pathogenic bacteria. In compare with the two types of antibiotics

Cefotaxime and Meropenem that used to treat contaminated wounds by pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*), in (Table 3). There is significance differences $P < 0.05$ among the studied parameters.

LSD of *Escherichia coli* is 2.59 for the four substances (honey, molasses, cefotaxime and meropenem) and 3.06 the result related to *Staphylococcus aureus* also its 3.14 of *Pseudomonas aeruginosa*. So it gives indicate there is significance difference. $P < 0.05$.

Conclusion

From the current results the following can be concluded:

1. Raw honey has inhibiting activity against pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*).
2. Natural molasses (date syrup) has inhibiting ability against pathogenic bacteria. (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*).
3. Processed honey and molasses have no inhibiting activity against pathogenic bacteria. (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*).
4. Raw honey have inhibiting activity better than Cefotaxime antibiotic and molasses against pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*).

References

- Al-Shahib, W. and R.J. Marshall (2003). The fruit of the date palm: its possible use as the best food for the future?. *Food Sci.*, **54(4)**: 247-59.
- Diegelmann, R. and M.C. Evans (2004). Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci.*, **9**: 283-289.
- El-Sohaimy, S.A. and E.E. Hafez (2010). Biochemical and Nutritional Characterizations of Date Palm Fruits (*Phoenix dactylifera*). *Journal of Applied Sciences Research.*, **8**: 1060-1067.
- Gad, A.S., A.M. Kholif and A.F. Sayed (2010). Evaluation of the Nutritional Value of Functional Yogurt Resulting from Combination of Date Palm Syrup and Skim Milk: *American Journal of Food Technology.*, **5(4)**: 250-259.
- Gheldof, N., X.H. Wang and N.J. Engeseth (2002). Identification and quantification of antioxidant components of honeys

- from various floral sources, *J. Agric. Food Chem.*, **50**: 5870-5877. of dates: a review. *Food Sci. Nutrition.*, **48(10)**: 878.
- Hasnaoui, A., M.A. Elhoumaizi, A.A. Sehraou and A. Hakkou (2010).* Chemical composition and microbial quality of main varieties of dates grown in figuig oasis of Morocco. *Int. J. Agric. Biol.*, **12**: 311-314.
- Hawk, C.T., S.L. Leary and T.H. Morris (2005). *Formulary for Laboratory Animals*; 3rd, Ed.
- Jorgensen, J. and D. Turnidge (2007). Susceptibility test methods: dilution and disk diffusion methods, 1152-1172.
- Mundo, M.A., I.O. Padilla-Zakour and R.W. Worobo (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int. J. Food Microbiol.*, **97(1)**: 1-8.
- Tan, Y.N., M.K. Ayob, M.A. Osman and K.R. Matthews (2011). Antibacterial activity of different degree of hydrolysis of palm kernel expeller peptides against spore-forming and non spore forming bacteria.
- Tomás-barberán, F.A., I. Martos, F. Ferreres, B.S. Radovic and E. Anklam (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture.*, **81(5)**: 485-496.
- Vonderohe, W. (1996). Characterisation of honeydew honey guided with specific saccharides. *Apidologie.*, **27(4)**: 270-272.
- Wayne, P. (2006). Performance standards for antimicrobial disk susceptibility tests: *Approved standard.*, **9(1)**: 1-26.
- Winn, R. *et al.*, (2006). *Diagnostic text of microbiology*. 6th ed, 945-1021.