

# Honey: a reservoir for microorganisms and an inhibitory agent for microbes

<sup>1</sup>Peter B. Olaitan, <sup>2</sup>Olufemi E. Adeleke, <sup>3</sup>Iyabo O.Ola.

<sup>1</sup>Department of Surgery, Ladoko Akintola University of Technology Teaching Hospital, Osogbo, Osun state, Nigeria.

<sup>2</sup>Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

<sup>3</sup>Department of Microbiology, University of Agriculture, Abeokuta, Ogun state, Nigeria.

## Abstract

**Background:** Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the medical profession. The use to which honey is put in medical care is increasing daily with many authors pointing out its importance and role in wound care. There have been reports that honey contains many microorganisms including bacteria and fungi.

**Objective:** The aim of this paper is to highlight the various uses, organisms commonly found in honey, how the organisms arrived in the honey and their effects on wounds and wound care. Would the presence of these organisms not constitute a limiting factor to the use of honey in wound management? This is what this review aims to answer

**Methods:** A literature search was done on honey using pubmed, google, local books and journals. Relevant journals were extracted and discussed with emphasis on the antimicrobial properties as well as microbial content of honey and the implications of these.

**Results:** The production of honey as well as the storing process account for the presence of microorganisms. Most of these organisms are said to be in inactive forms as they can hardly survive in honey because of its several properties including hygroscopicity, hyperosmolarity, acidity, peroxide content, antibiotic activities etc. However there is a need for caution in the use of honey in wound management.

**Conclusion:** We suggest that wounds to be treated with honey should be investigated i.e with a swab for the microorganisms present on the wound and their sensitivity to the honey before commencing honey treatment. This will help in carefully selecting wounds that might do well with honey treatment not withstanding other properties of honey that aid wound healing.

*African Health Sciences* 2007; 7(3): 159-165

## Introduction

Honey is the natural sweet substance produced by honey bees from nectar or blossoms or from the secretion of living parts of plants or excretions of plants, which honey bees collect, transform, and combine with specific substances of their own to ripen and mature<sup>1</sup>. It is also defined as the nectar and saccharine exudation of plants, gathered, modified and stored as honey in the honeycomb by honeybees, *Apis mellifera*<sup>2</sup>.

Honey in spite of its usefulness is known to contain certain microbes. It is in fact described as a reservoir for microbes. Some view the use of honey to treat infected wounds, with scepticism. For example, an editorial in the Archives of Internal Medicine in 1976 on medical folklore<sup>3</sup> ridiculed the use of honey, placing "honey from selected geographic areas" in the category of "worthless but harmless substances".

This reservoir for microbes' status however does not diminish the many important uses that honey is known for. Infact the antimicrobial status of honey is giving it a continued place in the management of wounds and injuries.

Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the medical profession, particularly where conventional modern therapeutic agents are failing<sup>4, 5</sup>. There are now many published reports<sup>4-9</sup> describing the effectiveness of honey in rapidly clearing infection from wounds, with no adverse effects to slow the healing process; there is also some evidence to suggest that honey may actively promote healing. In laboratory studies, it has been shown to have an antimicrobial action against a broad spectrum of bacteria and fungi. Remarkable among the bacteria is *Pseudomonas aeruginosa*, a notorious organism in the resistance to antimicrobial compounds.

Is there therefore a contradiction in the medical significance of honey-, which on one hand contains several microbes and on the other is active against many organisms?

### Correspondence

P.B. Olaitan

Department of Surgery,

LAUTECH Teaching Hospital

Osogbo, Osun state

Nigeria.

E-mail: emiolaitan@yahoo.com

Tel: 234 8033609292

## Composition

Honey primarily contains sugar and water. Sugar accounts for 95-99% of honey dry matter. Majority of these are simple sugars, fructose (38.2%) and glucose (31.3%), which represents 85-95% of total sugars. These are “simple” sugars, 6-carbon sugars that are readily absorbed by the body<sup>10</sup>. Other sugars include disaccharides such as maltose, sucrose, and isomaltose. Few oligosaccharides are also present.

Water is the second most important component of honey. Its content is critical, since it affects the storage of honey. The final water content depends on numerous environmental factors during production such as weather and humidity inside the hives, but also on nectar conditions and treatment of honey during extraction and storage. Organic acids constitute 0.57% of honey and include gluconic acid which is a by product of enzymatic digestion of glucose. The organic acids are responsible for the acidity of honey and contribute largely to its characteristic taste.

Minerals are present in honey in very small quantities (0.17%) with potassium as the most abundant. Others are calcium, copper, iron, manganese, and phosphorus. Nitrogenous compounds among which the enzymes originate from salivary secretion of the worker honeybees are also present. They have important role in the formation of honey. The main enzymes in honey are invertase (saccharase), diastase (amylase) and glucose oxidase.

Vitamins C, B (thiamine) and B2 complex like riboflavin, nicotinic acid and B6 panthothenic acid are also found.

## Honey production and collection

Knowledge of the process of honey production is important to understand the various ways in which bacteria and other microorganisms may get into the honey.

Honey production begins with bees collecting nectar and pollen from flowers but only nectar is used to make honey. Nectar is mostly water with dissolved sugars and the amount of sugars varies greatly but is usually 25-70%. Nectar has been described as a “reward” given by the plant to attract bees. Nectar is sucked by honeybees by inserting its proboscis into the flowers nectary and passes it through the oesophagus to the thorax and finally to the abdomen. Pollen is transported back to the hive in the posture pollen baskets on the hind leg whereas the nectar is transported in the stomach. Back in the hive the nectar is placed into wax honeycomb cells and the excess water evaporates until the honey is approximately 83% sugar and 17% water. This takes few days. The cells are then covered with a layer of wax, which

is later removed when the bees need to eat the honey. When large amount of nectar are being collected, the bees speed up evaporation by using their wings to ventilate the hive. The sugar is also changed. Sugar in nectar is mostly sucrose, which has large molecules. The bees produce an enzyme (invertase), which breaks each sucrose molecule into glucose and fructose by evaporating the excess water, and converting the sucrose into smaller sugars. The bees therefore make the honey too concentrated for yeast and other microorganisms to grow<sup>11</sup>.

To extract honey, the cap is removed by the use of sharp knife, which has been warmed in hot water. The combs are then put into centrifuge and the honey is removed. Honey is sometimes removed by pressure that necessarily destroys the combs and that may reduce the amount of wax in honey<sup>12</sup>.

## Physical properties of honey

Honey has several important qualities in addition to composition and taste. Freshly extracted honey is a viscous liquid. Its viscosity depends on large variety of substances and therefore varies with its composition and particularly with its water content. Viscosity is an important technical parameter during honey processing because it reduces honey flow during honey processing, extraction, pumping, setting, filtration, mixing and bottling.

Hygroscopicity is another property of honey and describes the ability of honey to absorb and hold moisture from environment. During processing or storage however, the same Hygroscopicity can become problematic, causing difficulties in preservation and storage due to excess water content. Normal honey with water content of 18.8% or less will absorb moisture from air of a relative humidity of above 60%. The thermal conductivity of honey varies from 118 to 143x10 Cal/cm<sup>2</sup>/sec/°C<sup>13</sup>. One could therefore calculate the amount of heating, cooling and mixing necessary to treat a certain amount of honey i.e. before and after filtration or pasteurization.

The surface tension of honey varies with the origin of the honey and is probably due to colloidal substances. Together with high viscosity, it is responsible for the foaming characteristics of honey.

The colour in liquid honey varies from clear and colourless (like water) to dark amber or black. The various honey colours are basically all shade of yellow, amber; like different dilutions or concentrations of cameralized sugar, which has been used traditionally as a colour standard. Colour varies with botanical origin, age, and storage conditions, but transparency or clarity depends on the amount of suspended particles such as

pollen. Less common honey colours are bright yellow (sunflower), reddish undertones (chest nut), grayish (eucalyptus) and greenish (honeydew). Once crystallized, honey turns lighter in colour because the glucose crystals are white. Honey colour is frequently given in millimeters on a P fund scale<sup>14, 15</sup>. More recent but not widely practiced methods of colour description used spectral colour absorption of honey<sup>16</sup>. Honey crystallization results from the formation of monohydrate glucose crystals, which vary in number, shape, dimension, and quality with the honey composition and storage conditions. The lower the water and the higher the glucose content of honey, the faster the crystallization.

Burget<sup>17</sup> reported hyperosmotic effect of honey and thus hyperosmotic nature due to high concentration of solids and low moisture content prevent the growth of bacteria and yeast as it drains water out of the organisms, killing them by desiccation.

### Honey as food

Honey is most commonly consumed in its unpreserved state, i.e. liquid, crystallized or in the comb. In these forms, it is taken as medicine, eaten as food or incorporated as an ingredient in various food recipes<sup>17</sup>.

In confectionery production, honey is still included in many traditional products, which are consumed locally in considerable quantities and also exported. In gelatinous or gum product, honey can be used as flavouring agent.

In industrial sector, some honey milk products exist such as pasteurized and homogenized sweetened with honey for long time storage e.g. yoghurt with honey<sup>18</sup>.

In the industrial non-alcoholic beverage industry, the use of honey is relatively recent and expanded in 1990. Over 40 new honey drinks were introduced in Japan, of which one was introduced by coca cola bottling company of Tokyo. In many fruit juices, honey is added as flavouring and sweetener. In apple juice, it is also used to classify fruit juice<sup>19</sup>.

### Microbes in honey

Microorganisms that survive in honey are those that withstand the concentrated sugar, acidity and other antimicrobial characters of honey.

The primary sources of microbial contamination are likely to include pollen, the digestive tracts of honeybees, dirt, dust, air and flowers.

Microbes found in honeycomb are principally bacteria and yeast and come from the bees, the raw

materials (nectar) or from external sources. Larvae may be sterile initially but they are fed

nectar and pollen by workers and therefore subject to inoculation by the nectar, pollen and workers flora before pupation<sup>20</sup>.

Sackelt<sup>21</sup> has observed that *Bacillus*, *Micrococcus* and *Saccharomyces* species could be readily isolated from honeycombs and adult bees. A number of microbial species have been isolated from the faeces of bee larvae<sup>22</sup>.

*Bacillus* spp are most prevalent followed by Gram –variable pleomorphic bacteria. Mould, Actinomycetes, Gram-negative rods (probably of Enterobacteriaceae) and yeast have also been isolated while *Streptomyces* spp were recovered from one larva. This microbial load compares favourably with the intestinal microflora of adult honeybees, which is dominated by Gram variable pleomorphic bacteria of uncertain taxonomic status, *Bacillus* spp, *Enterobacteriaceae*, *Penicillium* spp., *Aspergillus* spp., and sometimes frequently *Torulopsis* spp<sup>23</sup>. Pollen may be the original source of microbes in the intestines of honeybees. It has been suggested that flowers and hives are more important sources of microbes than the soil<sup>24</sup>. Aerobic spore forming *Bacillus* were the most frequently encountered microbes on the external surface, crop and intestine of the honey bees<sup>25</sup>.

The intestine of bees has been found to contain 1% yeast, 27% Gram-positive bacteria including *Bacillus*, *Bacteridium*, *Streptococcus* and *Clostridium* spp; 70% Gram negative or Gram variable bacteria including *Achromobacter*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia coli*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Pseudomonas*<sup>26</sup>. The primary sources of sugar tolerant yeast are flowers and soil.

Secondary sources of microbial contamination in honey are human, equipments, containers, wind, dust e.t.c. Yeasts have been recovered from equipments in honey houses; contaminated equipments can introduce yeast into clean honey<sup>24</sup>. Possible routes of transmission into extracted honey would include air (in the house or while the honey was packed), food handlers (from skin infections, sneezing or faecal contamination).

Microorganisms found in honey have been identified<sup>22</sup>. They include bacteria, yeasts and moulds (table1). Most bacteria and other microbes cannot grow or reproduce in honey i.e. they are dormant and this is due to antibacterial activity of honey. Various bacteria have been inoculated into aseptically collected honey held at 20°C. The result showed loss of bacterial viability within 8-24 days<sup>27</sup>. It is only the spore forming microorganisms that can survive in honey at low temperature. The spore count remained the same 4 months after. *Bacillus cereus*,

*Clostridium perfringens* and *Clostridium botulinum* spores were inoculated into honey and stored at 25°C. The *Clostridium botulinum* population did not change over a year at 4°C. At 65°C however, no spore were found after 5 days of storage. It has been observed that if honey is diluted with water, it supports the growth of non-pathogenic bacterial strains and killing of dangerous strains<sup>22</sup>. Solution of less than 50% honey in water sustained bacterial life for longer periods but never exceeding 40 days. It has therefore been concluded that the probability of honey acting as a carrier of typhoid fever, dysentery and various diarrhoea infections is very slight<sup>21</sup>.

**Table 1. Microorganisms found in Honey**

Bacteria	Yeasts	Moulds
<i>Alcaligenes</i>	<i>Ascosphaera</i>	<i>Aspergillus</i>
<i>Achromobacter</i>	<i>Debaromyces</i>	<i>Aliphia</i>
<i>Bacillus</i>	<i>Hansenula</i>	<i>Betisia alvei</i>
<i>Bacteridium</i>	<i>Lipomyces</i>	<i>Cephalosporium</i>
<i>Brevibacterium</i>	<i>Nematospora</i>	<i>Chaetomium</i>
<i>Citrobacter</i>	<i>Oosporidium</i>	<i>Coniothecium</i>
<i>Clostridium</i>	<i>Pichia</i>	<i>Hormiscium</i>
<i>Enterobacter</i>	<i>Saccharomyces</i>	<i>Peronosporaceae</i>
<i>Escherichia coli</i>	<i>Schizosaccharomyces</i>	<i>Peyronelia</i>
<i>Erwinia</i>	<i>Trichosporium</i>	<i>Triposporium</i>
<i>Flavobacterium</i>	<i>Torula</i>	<i>Uredianaceae</i>
<i>Klebsiella</i>	<i>Torulopsis</i>	<i>Ustilaginaceae</i>
<i>Micrococcus</i>	<i>Zygasaccharomyces</i>	
<i>Neisseria</i>		
<i>Pseudomonas</i>		
<i>Xanthomonas</i>		

### Antimicrobial properties of honey

The antimicrobial properties of honey have been known to humans for centuries<sup>28</sup>. Honey was used to treat infected wounds as long ago as 2000 years before bacteria were discovered to be the cause of infection. In c.50 AD, Dioscorides described honey as being “good for all rotten and hollow ulcers”. Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives<sup>29</sup>. An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium*, as well as all the common dermatophytes<sup>30</sup>. The current prevalence of antibiotic-resistant microbial species has led to a re-evaluation of the therapeutic use of ancient remedies, including honey<sup>31</sup>. Aristotle (384-322 BC), when discussing different honeys, referred to pale honey as being “good as a salve for sore eyes and wounds”<sup>32</sup>.

The numerous reports of the antimicrobial

activities of honey have been comprehensively reviewed<sup>6</sup>. Honey has been found in some instances by some workers to possess antibacterial activities where antibiotics were ineffective<sup>4,5</sup>. Pure honey has been shown to be bactericidal to many pathogenic microorganisms including *Salmonella spp*, *Shigella spp*; other enterophthogens like *Escherichia coli*, *Vibrio cholerae* and other Gram negative and Gram positive organisms<sup>6,7</sup>. High antimicrobial activity is as a result of osmotic effect, acidity, hydrogen peroxide and phytochemical factors<sup>8</sup>.

The clearing of infection seen when honey is applied to a wound may reflect more than just antibacterial properties. Recent research shows 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%<sup>35</sup>. Honey (at a concentration of 1%) also stimulates monocytes in cell culture to release cytokines, tumour necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which activate the immune response to infection<sup>33,34</sup>. A wide range of MIC values (the minimum concentration of honey necessary for complete inhibition of bacterial growth) have been reported in studies comparing different honeys tested against single species of bacteria: from 25% to 0.25% (v/v)<sup>35</sup>; >50% to 1.5% (v/v)<sup>36</sup> 20% to 0.6% (v/v)<sup>37</sup>, 50 to 1.5% (v/v)<sup>38</sup>.

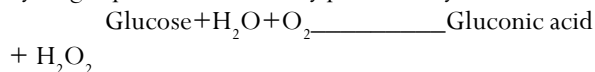
The osmotic effect of honey has been described<sup>17</sup>. Honey is a supersaturated solution of sugars, 84% being a mixture of fructose and glucose. The strong interaction of these sugar molecules will leave very few of the water molecules available for microorganisms. The free water is measured as the water activity ( $a_w$ ). Mean values for honey have been reported, from 0.562 to 0.62.

Although some yeasts can live in honeys that have high water content, causing spoilage of the honey, the water activity ( $a_w$ ) of ripened honey is too low to support the growth of any species and fermentation can occur if the water content is below 17.1%. Many species of bacteria are completely inhibited if water activity is in the range of 0.94 to 0.99. These values correspond to solutions of a typical honey ( $a_w$  of 0.6 undiluted) of concentrations from 12% down to 2%(v/v). On the other hand, some species have their maximum rate of growth when the ( $a_w$ ) is 0.99, so inhibition by the osmotic (water drawing) effect of dilute solutions of honey obviously depends on the species of bacteria.

Honey is characteristically acidic with a pH of between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens<sup>39</sup>. The minimum pH values for growth of some common pathogenic

species are: *Escherichia coli* (4.3), *Salmonella spp* (4.0), *Pseudomonas aeruginosa* (4.4), *Streptococcus pyogenes* (4.5)<sup>40</sup>. Thus in undiluted honey the acidity is a significant antibacterial factor.

Hydrogen peroxide is produced enzymically in honey. The glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar. The hydrogen peroxide and acidity produced by the reaction:



serve to preserve the honey. On dilution of honey, the activity increases by a factor of 2500 to 50,000, thus giving "slow-release" antiseptics at a level, which is antibacterial but not tissue damage<sup>27</sup>. Other workers<sup>41</sup> have however shown a reduction in antibacterial activity of honey on dilution to four times.

Phytochemical factors have been described as non-peroxide antibacterial factors, which are believed to be many complex phenols and organic acids often, referred to as flavonoids. These complex chemicals do not break down under heat or light or affected by honey's dilution. The stability of the enzyme varies in different honey. There have been reports of honeys with stability well in excess of this variation showing that there must be an additional antibacterial factor involved (i.e. do not break down under heat or light or affected by dilution). The most direct evidence for the existence of non-peroxide antibacterial factors in honey is seen in the reports of activity persisting in honeys treated with catalase to remove the hydrogen peroxide activity.

Several chemicals with antibacterial activity have been identified in honey by various researchers<sup>39</sup>.

Antibacterial activity of honey varies between different types of honey<sup>8,9</sup>. It has been observed that there are different types of honey and a method has been used to determine the "inhibine number" of honey as a measure of their antibacterial activity. The "inhibine number" is the degree of dilution to which a honey will retain its antibacterial activity<sup>41</sup> representing sequential dilutions of honey in steps of 5 percent from 25% to 5%. Major variation seen in overall antibacterial activity are due to variation in the level of hydrogen peroxide that arises in honey and in some cases to the level of non peroxide factors<sup>42</sup>. Hydrogen peroxide can be destroyed by components of honey, it can be degraded by reaction with ascorbic acid and metal ions and the action of enzyme catalase which comes from the pollen and nectar of certain plants, more from the nectar.

Although it appears that the honey from certain plants has better antibacterial activity than from

others, there is not enough evidence for such definite conclusion to be justified because the data are from small numbers of samples. Thus it is important that when honey is to be used as an antimicrobial agent, it is selected from honeys that have been assayed in the laboratory for antimicrobial activity. It is also important that honeys for use as an antimicrobial agent be stored at low temperature and not exposed to light, so that none of the glucose oxidase activity is lost although all honey will stop the growth of bacteria because of its high sugar content.

Honey's antibacterial properties on different microorganisms

The empirical application of honey on open wounds, burns or use of honey in syrups does show that it stops the growth of many microorganisms. Many of these microorganisms have been isolated and identified.

Mundo *et al*<sup>10</sup> discovered that the antimicrobial activity of honey was more with *Pseudomonas* and *Acinetobacter spp*, both with resistance to some antibiotics like gentamicin, Ceftriazone, Amikacin and Tobramicin than other bacteria tested. This was attributed to inhibitory effect of ascorbic acid in honey on aerobic microorganisms. *Staphylococcus aureus* and *Streptococcus spp* were also found to be sensitive to honey.

Undiluted honey has been found to stop the growth of *Candida spp* while *Clostridium oedemantiens*, *Streptococcus pyogenes* remained resistant<sup>28</sup>. Some species of *Aspergillus* did not produce aflatoxin in various dilutions of honey while honey has been found to stop the growth of *Salmonella*, *Escherichia coli*, *Aspergillus niger* and *Penicillium chrysogenum*<sup>29</sup>.

Wounds infected with *Pseudomonas*, not responding to other treatment, have been rapidly cleared of infection using honey, allowing successful skin grafting<sup>46</sup>. Obaseki *et al*<sup>44</sup> found that *Candida albicans* strains are sensitive to honey while Obi *et al*<sup>45</sup> reported the inhibitory effect of pure honey against local isolates of bacteria agents of diarrhoea. At concentration of 50% and above, honey excellently inhibited the growth of *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Plesiomonas shigelloides*, *Aeromonas hydrophila*, *Salmonella typhi*, *Shigella boydi* and *Clostridium jejuni*<sup>44</sup>.

## Conclusion

While honey easily gets contaminated during the process of its production by bees and microorganisms also get introduced into honey by activities of man including equipment, containers, wind and dust, the status of the microorganisms found in honey is dormant. It is the spore forming microorganisms that survive in honey by remaining dormant i.e suspended without growth<sup>26</sup>.

Non-spore forming bacteria ie vegetative forms are not normally present in honey because they cannot survive. Ten species of non-spore forming intestinal bacteria inoculated into pure honey survived only a few hours<sup>21</sup>. It is possible therefore to assert that the microorganisms found in honey undergo gradual extinction in honey due to its inhibitory properties as highlighted earlier in this discourse. It is also recognized that spores are dormant forms of certain microorganisms. The fact that spores cannot transit into vegetative forms and still remain alive in honey persistently is supportive of the inhibitory role of honey on microorganisms. The failure to take into account the large variance in antibacterial potency of different honeys may contribute, in part, to the large discrepancy in results reported between 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<sup>46</sup>, 7 days<sup>47</sup>, 7-10 days<sup>48</sup>. Others have reported bacteria still present in wounds after 2 weeks<sup>49</sup>, 3 weeks<sup>50</sup> and 5 weeks<sup>51</sup>.

There is however the need for caution. Is it possible for the spores in honey to transform to active microorganisms and therefore become pathogenic after honey has been applied to the wounds especially with dilution of the initial high osmolarity and other properties that inhibit microorganisms?

We suggest that swabs from wounds should be cultured, microorganisms isolated and their sensitivity to honey assessed before commencing treatment with honey. This is important not only because of the varying activities of honey but also because of varying microorganisms they may contain. Any honey should not just be used on just any wound without this preparation. This will ensure judicious use of honey for organisms against which it is likely to be active and diminish the possibility of infecting the wounds rather than destroying the microbes. More works may need to be done to give an answer to the conflict.

## References

1. Codex Alimentarius Commission. Codex Standards for sugar (honey). Supplement 2 to codex Alimentarius Volume 111. Food and Agriculture Organisation of the United Nations and World Health Organisation, Rome 1989.
2. Bassey IH. In: Bee keeping, digest of selected literature on bee keeping, honey and bee wax processing, Libriservice limited, Lagos, Nigeria 1986; 22.
3. Soffer A. Editorial: Chihuahuas and laetrile, chelation therapy, and honey from Boulder, Colo. *Arch Intern Med* 1976; 136(8): 865-6.
4. Stokes ES Rid way GI Wren GM. *Clinical Microbiology* (7<sup>th</sup> ed) 1993; Arnold, London. 20-30.
5. Subramanyam M. Tropical application of honey in treatment of burns. *Br. J Surg* 1991; 78: 497-498.
6. Radwan S El- Essawy A Sarhan MM. Experimental evidence for the occurrence in honey of specific substances active against Microorganisms. *Zentral Microbiol* 1984; 139:249-255.
7. Ibrahim AS. Antibacterial action of honey. *Bull Islam Med* 1985; 1: 363-365.
8. Jeddar A Kharsany A Ramsaroop UG Bhamjee A Hafejee IE Moosa A. The Antibacterial action of honey. *South Afri. Med. J.* 1985; 67: 257-258.
9. Molan PC Smith IM Reid GM. A comparison of the antibacterial activities of some New Zealand honeys. *Journal of Agricultural Research* 1988; 27: 252-256.
10. Moundoi MA Padila-Zakour OI Worobo RW. Antimicrobial activity of honey against food pathogens and food spoilage microorganisms. Department of Food Sciences and Technology, Cornell University, NYSAES, W North St, Geneva, NY 14456, 2001; VOL.1: 61-71.
11. Pamphlet of Honey Bee. *American Magazine*. April 21, 2002:4pp
12. Zwaenopoeel C. *Honey: Facts and Folklore*. Alberta Beekeepers Association, Edononton, Canada. 1984; 24.
13. White JW. Physical Characteristics of honey. In: *Honey, a comprehensive survey*. Crane(ed), Heineman London; UK 1975; 157-206.
14. White JW. Honey. In: *The Hive and the honeybee*. Dadant and Sons Inc., Hamilton Illinois, USA, 1957; 491-530.
15. Crane B. *A book of honey*. Oxford University Press, Oxford UK 1980; 198
16. Rodriguez LC. Determination Espectro-fotometrica del color de las mieles. *Vida apic*. 1985; 16: 24-29.
17. Burgget DM. Osmotic pressure. In: Morse R and Hooper T(eds). *The Illustrated Encyclopedia of Bookkeeping*. EP. Dutton, New York, 1985.
18. Ebisu T. Maeda N Matsubara H and Kato K. Honey powder. Composition as additives for foods and Pharmaceuticals. *Japanese Patent Number* 1988; 63(25): 157-943.
19. Spanish Dairy Corporation. Preparation of yoghurt with honey. In: *International Symposium on Apitherapy, Madrid*. 1975; 55-56.
20. Lee CY AND Kime RW. The use of honey for clarifying apple juice. *J Apic Res*. 1984; 23(1): 45-49.
21. Sackelt WG. Honey olorado State Univ. as a carrier of intestinal diseases. *Bull. CExp. Stad* 1919; 252: 1-18. 15.
22. White P.B. The normal flora of the bee. *Agricultural research service*. US Department of Agriculture Washinton AC 1996; 301-309.
23. Gilliam M and Prest DB. *Microbiology of Lanial honey bee (Apis mellifera)*. *Journal of Invertebrate Pathology*. 1987; 49:70-75.
24. Root AL. *The ABC and XYZ of bee culture*. Edward Arnold publication Ltd. London, 1993; 5-28.
25. El-leithy MA El-sibael KB. Role of microorganisms isolated from bees, its ripening and fermentation of honey. *Egyptian Journal of Microbiology* 1992; 75:679-681.
26. Tysset C Durand C De Rantline de la Roy. Contribution to

- the study of intestinal microbial infection of healthy honeybees: Inventory of bacterial population by negative organisms. Department of Agriculture, SEA-AR, Eastern region research centres, Philadelphia, 1991; 21-55.
27. Al Somai N, Coley KE, Molan PC, Hancock BM. Susceptibility of *Helicobacter pylori* to the antibacterial activity of Manuka Honey. *Russian Medical Journal*; 1994, 87:9-12.
  28. Efem SE. Recent advances in the management of Fournier's gangrene: Preliminary observations. *Surgery*, 1993; 113:200-204.
  29. Molan PC. The antibacterial activity of honey. 1. The nature of the antibacterial activity. *BeeWorld* 1992; 73(1): 5-28.
  30. Brady NF, Molan PC, Harfoot CG. The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Pharm Sci* 1997; 2: 1-3.
  31. Select Committee on Science and Technology. Report no. 7: Resistance to antibiotics and other antimicrobial agents. London: House of Lords, 1998
  32. Aristotle. *Historia Animalium (350 BC)*. Oxford: Oxford University, 1910.
  33. Abuharfeil N, Al-Oran R, Abo-Shehada M. The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytes. *Food Agric Immunol* 1999; 11: 169-77.
  34. Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP. Stimulation of tnf-alpha release in monocytes by honey. *Cytokine* 2001; 14(4): 240-2.
  35. Abbas T. Royal treat. *Living in the Gulf* 1997; 50-1.
  36. Dustmann JH. Antibacterial effect of honey. *Apiacta* 1979; 14(1): 7-11.
  37. d'Agostino Barbaro A, La Rosa C, Zanelli C. Attivit antibatterica di mieli Siciliani [Antibacterial activity of Sicilian honeys]. *Quad Nutr* 1961; 21(1/2): 30-44.
  38. Buchner R. Vergleichende Untersuchungen antibakteriellen Wirkung von Blüten- und Honigtauhonigen [Comparative study of the antibacterial activities of honey]. *S?deutscher Imker* 1966; 18: 240-1.
  39. Allen KL, Molan PC, Reid GM. A survey of antibacterial activity of some New Zealand honey. *J Pharm* 1991; 43:817-822.
  40. O'Grady FW, Lambert HP, Finch RG, Greenwood D. *Antibiotic and Chemotherapy* (7<sup>th</sup> ed), Churchill Living Stone, New York, 1997.
  41. Adeleke OE, Onakoya TM and Alli SS. Exposure of bacterial isolates from different pathological sources to honey. *African J Medical and Pharmaceutical Sciences*. 2002; 7: 79-83.
  42. Willix DJ, Molan PC, Harfoot CJ. A comparison of the sensitivity of wound infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. *J Appl. Bacteriol*, 1992; 73:388-394.
  43. Wood B, Rademaker M, Molan P. Manuka honey, a low cost leg ulcer dressing. *N Z Med J* 1997; 110(1040): 107.
  44. Obaseiki-Ebor EE, Afonja TC. In Vitro evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of antimycotic agents. *J. Pharm. Pharmacol.* 36:283-284.
  45. Obi CL, Ugoji EO, Edwin SA, Lawal SF, Anyiwo CE. The antibacterial effect of honey on diarrhea causing bacterial agents isolated in Lagos, Nigeria. *Afr. J Med. Sci.* 1994; 23:257-260.
  46. Cavanagh D, Beazley J, Ostapowicz F. Radical operation for carcinoma of the vulva. A new approach to wound healing. *J Obstet Gynaecol Br Commonw* 1970; 77(11): 1037-40.
  47. Efem SE. Clinical observations on the wound healing properties of honey. *Br J Surg* 1988; 75(7): 679-81
  48. Armon PJ. The use of honey in the treatment of infected wounds. *Trop Doct* 1980; 10(2): 91
  49. Harris S. Honey for the treatment of superficial wounds: a case report and review. *Primary Infection* 1994; 2(4): 18-23.
  50. Subrahmanyam M. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. *Burns* 1998; 24(2): 157-61.
  51. Wadi M, Al-Amin H, Farouq A, Kashef H, Khaled SA. Sudanese bee honey in the treatment of suppurating wounds. *Arab Medico* 1987; 3: 16-8.