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Antimicrobial properties of natural honey: a review of literature

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Abstract: Health benefits of honey have been reported in a variety of conditions including microbial infections, wound healing, inflammation, glucose tolerance and analgesia. Honey is a supersaturated sugar solution mainly comprised of D-fructose, D-glucose, sucrose, maltose and higher sugars (~80% of solid mass). While other natural products i.e. alkaloids, flavonoids/isoflavones, glycosides, phenolics, peptides/proteins are present in minor quantities. A number of enzymes such as invertase, amylase and glucose oxidase have been found in honey. Antibacterial and antifungal activities of honey are well documented and characterized. These antimicrobial properties have been related to oligosaccharides, glycopeptides and peptides present in honey. Honey glucose oxidase provides a continuous and slow release of hydrogen peroxide at a level which is antibacterial but not tissue-damaging. Hydrogen peroxide produced by glucose oxidase plays important roles in inflammation, wound healing etc. The antimicrobial properties of honey have great potential for application in medicine as well as in food industry.

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INTRODUCTION

Natural honey is a supersaturated solution of sugars produced by bees of different *Apis* species[1]. In Indian subcontinent, at least four *Apis* species are found, i.e. *Apis dorsata*, *Apis cerana*, *Apis florae* and *Apis andreniformis*. Moreover, the *Apis Mellifera* bees imported from Europe are widely used in honey farms for large scale natural honey production. Natural honey has been used an effective medicine around the world since ancient time. It has had a valued set in traditional remedy for centuries. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut[1].

In the Holy Quran, Almighty Allah mentioned the special ability of honey to heal and cure disease. Allah said "And your Lord revealed to the bee: Make hives in the mountains and the trees in what they build. Then eat all the fruits and walk in the ways of your lord submissively. There comes forth form their bellies a beverage of many colours, in which there is healing for mankind. Verily in this, sign for those who give thought." (surah Al-Nahal; verses 68 and 69).

Honey is still used in folk medicine particularly where conventional and modern therapeutic agents fail. In recent time, the use of honey as therapeutic substance has been rediscovered by the medical profession and it is gaining acceptance as an antibacterial treatment of gastroenteritis ulcers, bed sores, and other surface infection[2].

Recently, scientific support has emerged with a proliferation in publications on the successful therapeutic use of honey in several general medical and surgical conditions. We have reported a number of interesting bioactivities including antinociceptive[3], immuno-modulatory[4] and nematicidal activities[5] in honey. Natural honey modulates physiological glycemic response compared to glucose[6]. In vitro studies indicated potentially beneficial effect(s) of honey on human platelets and blood coagulation proteins[7].

Honey has been of proven value in treating infected surgical wounds, burns and decubitus ulcers. Cavanagh et al. successfully applied honey in the postoperative management of patients who had undergone radical vulvectomy for vulval carcinoma[8]. Wound healing was accelerated and less bacterial colonization noted by local application of honey in patients who developed postoperative wound breakdown. Another study showed that skin grafting, surgical debridement and even amputation were avoided when local application of honey to wounds promoted healing whereas conventional treatment had failed[9]. These observations have been borne out by an animal model in which pure commercially available honey applied on mice healed wounds significantly faster than those of controls[10,11].

Honey is extremely viscous, and hygroscopic due to which it absorbs water from surrounding oedematous tissue, clean the wound and protect it from further infection[12]. Slough and necrotic tissue is gradually separated as a consequence, leaving healthy granulation tissue behind. A clinical study involving infants and children with gastroenteritis demonstrated that honey given with oral rehydration fluid shortens the duration of bacterial diarrhea[13]. It was concluded that honey can safely be used as a substitute for glucose in solution with electrolytes and is just as efficient as glucose in promoting sodium and water absorption from the gut.

Honey is gaining acceptance by the medical profession for use as an antibacterial agent for the treatment of ulcers and bed sores, and other surface infections resulting from burns and wounds[12,13]. In many cases it is being used with success on infections not responding to standard antibiotic and antiseptic therapy. Its effectiveness in rapidly clearing up infection and promoting healing is not surprising in light of the large number of research findings on its antibacterial activity[13].

Composition of honey

Natural honey primarily contains sugar and water. Sugar accounts for 95-99% of honey dry matter[14]. Majority of these simple sugars are D-fructose (38.2%) and D-glucose (31.3%), which represents 85-95% of total sugars. These are simple 6-carbon sugars that are readily absorbed by the body. 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[14,15]. 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 bye product of enzymatic digestion of glucose. The organic acids are responsible for the acidity of honey and contribute largely to its characteristic taste [15-17]. Minerals are present in honey in very small quantities (0.17%) with potassium as the most abundant. Others minerals are calcium, copper, iron, manganese, and phosphorus[18]. The blossom honey has lower mineral content than honeydew honey. These minerals have important roles in the formation of honey. Vitamins C, B (thiamine) and B2 complex like riboflavin, nicotinic acid and B6 panthothenic acid are also found in honey[19].

Sugars are the principal constituents of honey, which aside from determining its nutritious and energetic value, also influences some of its important physical characteristics such as crystallization, hygroscopicity and viscosity. Ash value indicates the botanical origin; Temperature effect is recognized by the production of 5-hydroxymethyl furfural (HMF). The HMF is inversely proportional to the quality of honey, which depends on pH and moisture value of honey, heat process after harvesting and storage time temperature. Since HMF is formed during acid hydrolysis of sucrose, the presence of high levels of this compound suggests the possibility that the honey has been adulterated with invert syrup[20].

Proteins and enzymes in honey

Honey contains a number of proteins and eighteen free amino acids[21,22]. The presence of proteins in natural honey has been known for many years in addition to carbohydrates, vitamins and minerals[23]. Honey contains approximately 0.5% proteins[21]. Nineteen bands of honey proteins have been detected by silver staining in SDS-PAGE[24]. Different proteins of diverse molecular weight are found in natural honey depending upon the species of the harvesting honey bees[21]. Most of the enzymes are added by honey bees during the process of natural honey ripening[14]. Relative quantity of natural honey proteins is measured as a quality indicator. The proteins in natural honey originate from the nectar, pollen and honeybee.

Determination of the quantity of plant protein and honey bee proteins in natural honey is significant; as this proportion can be an index for quality control of natural honey[23]. The presence of an array of proteins in honey has been considered to be a useful indicator of the geographical and floral origins[22]. Recently, mass spectrometry of honey proteins has utilized for determining geographical origin of honey[25]. Because different regions have distinct and characteristic floral communities, analyses of honey proteins might be advantageous compared to the other compounds for differentiating the floral and geographical origins[22,25,26]. The honey proteins that come from the honeybees have much higher molecular weight than that of proteins of plant origin[27]. The honey bees contribute a protein into natural honey, called defensin-1 which is present in almost all honeybees' immune systems[28]. Researchers have related honey's anti-bacterial properties to honey peptides, glycopeptides and proteins. This protein might one day be used to cure infections and to develop new drugs that could combat antibiotic-resistant bacteria.

Considerable work has been done for characterization of honey proteins[22,29,30]. A number of reports mention the use of electrophoretic and immunological techniques to separate honey proteins into several bands[31]. Comparatively less considerations has been given to purification and characterization of the natural honey proteins. Few reports describe purification of honey proteins using chromatographic techniques including gel filtration chromatography and ion-exchange chromatography[22]. Characterization of the

fractionated of natural honey proteins has provided useful information regarding structure and function(s) of natural honey proteins/enzymes.

The enzyme content of honey is one of the characteristics that make it beneficial to human health. The main enzymes in honey are invertase (saccharase), diastase (amylase) and glucose oxidase[1]. These enzymes are derived from the hypopharyngeal glands of worker honey bees. The Invertase converts sucrose to glucose and fructose; Glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide; and Amylase hydrolyzes starch. The enzymes in honey which originate from plants are Catalase, Acid phophotase, Amylase etc.[32]. The Glucose oxidase present in honey originates from hypopharyngeal gland of honey bees[33].

The D-glucose present in honey is converted by Glucose oxidase into glutamic acid and hydrogen peroxide. Glucose oxidase is almost inactive in full strength honey, however, becomes active on dilution with wound exudates[34]. This provides a slow release of hydrogen peroxide at a level, which is antibacterial but not tissue-damaging. Glucose oxidase present in honey is not involved only in the inhibition of pathogenic microbes but also participate in wound and burn healing. Hydrogen peroxide of honey produced by Glucose oxidase play important roles in inflammation, stimulation of tissue growth, eptheialization, analgesia and wound debriding action.

In more recent time, isolated hydrogen peroxide lost favor because of inflammation and tissue damage[35]. The hydrogen peroxide concentration produced in honey activated by dilution is typically around 1 mM/L about 1000 time less than the 3% solution commonly used as an antiseptic[36]. At this concentration hydrogen peroxide act as a novel intracellular and intercellular messenger capable of promoting growth responses and stimulating expression of early growth gene important in wound healing. Hydrogen peroxide is produced by enzymatic reaction in honey. The hydrogen peroxide and acidity produced by the reaction serve to preserve the honey.

$$\text{Glucose} + H_2O + O_2 \rightarrow \text{Gluconic acid} + H_2O_2$$

Antimicrobial properties of natural honey

The antimicrobial properties of honey have been known to humans for centuries[9,10,12,37]. Honey was used to treat infected wounds as long ago as 2000 years before bacteria were discovered to be the cause of infection[1]. Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives[13,36,38-40]. The antifungal action has also been observed for some yeasts and species of *Aspergillium* and *Penicillium*, as well as all the common dermatophytes[41]. Honey has been found to possess antibacterial activities where antibiotics were ineffective[42]. Pure honey has been shown to be bactericidal to many pathogenic microorganisms including *Salmonella* Species, *Shigella* Species, *Escherichia coli*, *Vibrio cholerae* and other Gram negative and Gram positive organisms[41,43].

The antimicrobial activity of honey has been reported to be due to osmotic effect, acidity, hydrogen peroxide and phytochemical factors[29,36,44,45]. Antibiotic-resistant pathogenic microorganisms posture a very stern risk to public health. Resistance not only is problematic in hospitals; resistant bacteria are now documented among numerous groups in the public[46,47]. Frequencies of bacterial antibiotics resistance are growing wide-reaching while very few new antibiotics are being advanced[48,49]. Therefore marginal antibacterial, antifungul and antiviral tactics are needed[50]. Honey has broad-spectrum action against pathogenic bacteria and fungi[51,52]. Researchers showed effectiveness of natural honey in handling of chronic wound infections not responding to antibiotic therapy[51,53].

Considerable amount of the antimicrobial compounds have been found in natural honey[54,55]. Deficient information of the antimicrobial factors in honey and the impact of these factors to the bactericidal activity hinder overall applicability of natural honey. Researchers have attempted to resolute the mechanism of action of antimicrobial activity of honey and evaluated the contribution of honey components to the bactericidal activity against pathogenic bacteria including *S. aureaus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio cholera, Yersinia pestis and E. coli*[28]. The implications of these findings have discreet application in medicine for the likely usage of honey as antimicrobial and food preservative.

The clearing of infection seen when honey is applied to a wound may reflect more than just antibacterial properties[56]. Application of natural honey for the inhibition of microorganisms might be a substitute way in some suitable cases for topical application for certain partially systematic infections. Recent research 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%[57]. 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 responses[58]. 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 different species of bacteria. These MIC values are 0.25-25% (v/v); 1.5-50% (v/v)[59-61]; and 0.6-20% (v/v)[62].

The mixture of D-fructose and D-glucose in honey forms strong interaction with water[63]. These sugar molecules will leave very few of the water molecules available for microorganisms[64]. The free water is measured as the water activity (aw). Values of 'aw' for honey have been reported between 0.479-0.557 with 0.521 as the mean value[65]. Although some yeast can live in honeys that have high water content, causing spoilage of the honey, the water activity (aw) 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%[13,66]. Many species of bacteria are completely inhibited if water activity is in the range of 0.94-0.99[67]. These values correspond to solutions of a typical honey (aw of 0.6 undiluted) with concentrations from 2-12% (v/v). On the other hand, some species growth at the 'aw'=0.99[68]. Therefore microbial inhibition by the osmotic (water drawing) effect of dilute solutions of honey obviously depends on the species of bacteria[9].

Honey is characteristically acidic with a pH in the range of 3.2 - 4.5, which is low enough to be inhibitory to many animal pathogens[70]. The minimum pH values for growth of some common pathogenic species are *Escherichia coli* (pH 4.3), *Salmonella* Species (pH 4.0), *Pseudomonas aeruginosa* (pH 4.4), *Streptococcus pyogenes* (pH 4.5)[71]. Thus in undiluted honey the acidity is a significant antibacterial factor. The antimicrobial properties of honey offer the potential to treat both the fungal and bacterial infections. Several mechanisms have been suggested to explain the antimicrobial activity of honey[13,72,73]. It has also been claimed that honey contains lysozyme, a well-known antibacterial enzyme[74].

Researchers believe that honey Glucose oxidase is the source of hydrogen peroxide, which is a potent antibacterial agent[75-77]. Some floral sources provide additional antibacterial components by way of plant-derived chemicals in the nectar, such as flavonoides and aromatic acids[78,79].

On dilution of honey, the activity of Glucose oxidase increases by a factor of 2500-50,000, thus giving "slow-release" antiseptics at a level, which is antibacterial but not damaging for tissues[37]. Some researchers have however shown a reduction in antibacterial activity of honey on dilution. Phytochemical factors have been described as non-peroxide antibacterial factors[10], which are believed to be complex phenols and organic acids often, referred to as flavonoids. These natural products do not decompose under heat, light or affected by dilution[80]. The most direct evidence for the existence of non-peroxide antibacterial factors in honey was 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[81].

Antibacterial activity of honey varies among different types of honey[10,82,83]. Due to different types of honey, 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 representing sequential dilutions of honey in steps of 5 percent from 5-25%[84]. Major variations 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[85]. Hydrogen peroxide can be transformed by components of honey. It can be degraded by reaction with ascorbic acid and metal ions and the action of Catalase originated from the pollen and nectar of certain plants.

Apparently, honeys from certain plants have better antibacterial activity. However, not enough evidences for this notion is available due to the data are from small numbers of samples[86]. Thus, it has been suggested that honey to be used as antimicrobial agent, should be 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[76,87].

Most of the reports on the antibacterial activity of honey do not allow a distinction to be made between bacteriocidal and bactriostatic property. Although no growth may have been seen over the period of observation, sometimes up to four days, in

the absence of other evidence this only can be taken to be a bacteriostatic action, even if termed a bactericidal action by some researcher[13]. A bactericidal action only can be concluded to have been observed in those studies where subculturing in a honey-free medium after initial exposure to honey shows no subsequent growth[88,89].

Honey trade

Honey export is an important trade in the world market. The leading honey-producing countries are the USA, Canada, Australia, Argentina, Mexico and China. The contribution of Pakistan in honey export is insignificant. The province Khyber Pakhtoonkhwa has good global position and climate conditions for production of natural honey and exporting it to Western countries and Middle East. In order to have a respectable place in the world market of honey, the honey produced in Pakistan must fulfill international quality standards. Different kinds of honey differ for their color, flavor and density[90]. Only slight deviation in the color, flavor and aroma from the usual quality associated with the brand can cause the product to be rejected by the consumer. In order to have uniform standard of honey, an International Honey Commission (IHC) was found in 1990. Its main objective was to revise the methods and standards for honey. International honey standards are specified in a European Honey Directive and in Codex Alimentarius Standard for honey[91,92]. According to the definition of Codex Alimentarius Commission Standards (2001), any food ingredient (other than honey) should not be added into honey, nor should any particular constituent be removed from it. Honey shall not have any objectionable matter, flavor, aroma from foreign matter during its processing and storage with no fermentation or effervescence. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired. Certain quality parameters are used to determine the honey quality. Countries strictly following these quality standards earn an appreciable amount of foreign exchange through honey export. The most important is the water-sugar relationship due to its effect on silt against fermentation and granulation[93].

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CORRIGENDUM

This is to correct the date of receiving and the acceptance of manuscript entitled: "Comparison of denaturing and non-denaturing gel electrophoresis methods for RNA analysis" authors: *K. M. Lodhi, M. A. Lodhi, S. Burgado, P. Petty, R. Bazzelle and R.L. Grier IV*, page 159-161, published in PJBMB 43(3), September, 2010. The manuscript was received on August 8, 2010 and accepted for publication in PJBMB on August 30, 2010.