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Antimicrobial and antinematodal activity of natural honey; an overview

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Abstract

Natural honey, a valued natural product is used for nutritional purpose as well as for the treatment of various clinical conditions ranging from wound healing to microbial infection. This review highlighted the importance of natural honey as antiviral, antibacterial, antiprotozoal, antifungal and antinematodal agent. These anti-infective properties are due to several components of honey including amino acids, proteins, enzymes, phenols, organic acids etc. Many in-vitro and in-vivo studies showed the antibacterial activity of honey against several veterinary and human pathogens like *Shigella*, *Salmonella*, *Escherichia coli*, *Helicobacter pylori.* The antibacterial activity of honey is characterized by high osmolarity, antioxidants compounds and low pH of honey. The sugar dependent high osmolarity is also found to be potent against fungal species *Candida albicans.* On the other hand, honey proteins have shown antiprotozoal agents against protozoal species *Entamoeba histolytica* and *Giardia lamblia.* Glycoproteins and glycopeptides of honey contribute to the nematicidal action of natural honey, which was revealed by death of *C. elegans* treated with honey proteins. Natural honey also possess antiviral activity.

Keywords: apitherapy, antibacterial, antifungal, antinematodal, antiviral

INTRODUCTION

Honey is natural mixture synthesized by honey bees from sweet deposits and nectars of plants [1]. Natural honey has been utilized for flavoring purpose as well as been used for the production of various food products and beverages. Therapeutic and pharmacological properties of honey have been useful for the treatment of several human ailments. The importance of honey has been emphasized in religious scriptures including Islam and Christianity [2]. The Chapter named ‘Al-Nahl’(means The Bee) of Holy Qur’an specifically demonstrates the importance of honey for the benefits of mankind. Prophet Muhammad (Peace be upon him) recommended the honey for wound healing and curative purposes. Holy Qur’an strongly directs its followers towards the consumption of honey in order to promote human health and nutritional needs. In verse number 68-69 of chapter 16, Holy Qur’an says;

"*And your Lord inspire to the bee, "Take for yourself among the mountains, houses and among the trees and [in] that which they construct. Then eat from all the fruits and flowers and follow the way your Lord lay down [for you]." There emerges from their bellies a drink, varying colors, in which there is a healing for the people. Indeed in that is a sign for a people who give thought".*

The vital role of honey and honey bee has been well demonstrated in several religious books of Christianity such as Bible, Exodus and Mathews. In the Holy Bible, the King Solomon quoted that: *"Eat honey my son, because it is good*" (proverbs 16:24:13) [2].

In ancient Hindu scripture honey has been esteemed as "the nectar of the Sun" [3]. In book of Vedas, the honeycomb has been described to be *"sweet, beautiful and golden like a Sun*". It is also manifested as a "blend of all the Nectar of many flowers" [4]. In India, honey has been used as an Ayurvedic medicine for thousands of years.

Since the time of ancient Egyptians, natural honey was utilized for the management and cure of several human disease. Long time ago, it was believed by ancient Greeks that honey is useful to increase the longevity and strength. Chinese and Russians has been used honey as a traditional medicine since hundreds of years ago. Likewise in twelfth century, Muslim scientists highlighted the medicinal effects of natural honey in management of several disease conditions like loss of muscle tissues, intestinal inflammation, abnormal bowels movement, irregular appetite and unbalance in body fluids [5].

In medieval ages, natural honey was used with different combinations in various countries of Europe such as Ireland, Germany and Finland. As a soothing medicine, natural honey had also been used for fastening the wounds healing of soldiers during the second Balkan war in 1913. Moreover, honey produced by sting less bees was used for the treatment of cough, cataract, flue and glaucoma. Manuka honey was found effective against infections of methicillin resistant Staphylococcus aureus (MRSA). The antibacterial and curative properties of honey were extensively reported in many scientific literature [5, 6].

TYPES OF HONEY

On the basis of nectar, honey has been classified into floral and non-floral honey. Honey from floral source can be further divided into unifloral and multifloral honey, depending on the collection of nectar by from same flowers or from flowers of multiple species. Some non-floral honey such as honey dew honey is produced by bees that collect sugars from fruits or plant tissues, or from excretion of plant dwelling insects that found in the vein of evergreen plants. Honey dew honey is usually darker in color and bitter in taste [7].

Natural honey can also be classified into forest and apiary honeys. *Apis dorosta* and *Apis cerana* bees synthesize forest honeys and it is obtained by squeezing the honey combs. Due to presence of pollens, bee larvae, wax, and parts of honey bees; forest honeys are usually turbid and the suspended particles are removed by filtration process. Apiary honey is obtained from the hives of *Apis cerana* and *Apis mellifera* and extracted by the contemporary methods to remove impurities [5, 8].

Four species of honey bees are found in Pakistan i.e. *Apis cerana, Apis dorasta, Apis florea* and *Apis mellifera* [9]. *Apis cerana* and *Apis mellifera* are mostly found in the near the foot hills of North West Punjab, Baltistan, Khyber Pukhton khawa, Kashmir, FATA (Federally Administered Tribal Areas) as well as some areas of Sindh and Balochitan. *Apis dorosta* bees are mostly prefers to live in forest, foothills, semi desert area and plains. Whereas *Apis florea* habitat at 600 meter altitude and rarely found at 1500 meter height. *Apis florea* are usually absent in plains, subcoastal and foot hills area of north Himaliyas throughout the year [10].

In Pakistan, the annual production has been increased after the imports of honey bee *Apis mellifera*. Currently more than 400,000 colonies of honey bee are present in different areas of Pakistan that produced 21 kg of honey per bee colony [11, 12]. Colonies of *Apis mellifera* bees are mostly present in bee farms located in districts of Khyber Pukhtunkhwa and northern Punjab [10].

COMPOSITION OF HONEY

The natural honey is a viscous solution of sugars. Variations in the color, composition, aroma and flavor of honey is due to species of honey bees, flower source, geographical region and climate change. These variations also depends on processing, packaging, time of storage and weather conditions [13]. Sugars are the most abundant components of honey that comprise of monosaccharides (75%) such as glucose and fructose, disaccharides (10-15%) e.g. turanose, sucrose, trehalose, maltose, nigerose and kojibiose; and trisaccharides such as melezitose and maltotriose. In honey, fructose is considered as the most prolific monosaccharide, and the amount of glucose and fructose determine the origin of mono-floral honey. These monosaccharaides are responsible for hygroscopicity, energy values, granulation and viscosity of honey [12] (Table 1).

Natural honey also contains miniscule amount of organic acids (~0.57%) [14]. These organic acids are formed by enzymatic modification of sugars by enzymes originated from salivary glands of honey bee [15]. Like sugars, organic acids are also useful indicators for geographical and botanical origin of honey [15].

Table 1. Nutritional composition of honey. Adapted from [4].

	Blossom honey		Honeydew honey	
	Range	**Mean**	**Range**	**Mean**
Water	15 – 20	17.2	15 – 20	16.3
Total sugars		79.7		80.5
Monosaccharides				
Fructose	30 – 45	38.2	28 – 40	31.8
Glucose	24 – 40	31.3	19 – 32	26.1
Disaccharides				
Sucrose	0.1 – 4.8	0.7	0.1 – 4.7	0.5
Others	2.0 – 8.0	5.0	1.0 – 6.0	4.0
Trisaccharides				
Oligosaccharides		3.1		10.1
Erlose	0.5 – 6.0	0.8	0.1 – 6.0	0.1
Melezitose		< 0.1	0.3 – 22	4.0
Others	0.5 – 1.0	0.5	0.1 – 6.0	3.0
Minerals	0.1 – 0.5	0.2	0.6 – 2.0	0.9
Amino acids, proteins	0.2 – 0.4	0.3	0.4 – 0.7	0.6
Acids	0.2 – 0.8	0.5	0.8 – 1.5	1.1
Ph value	3.2 – 4.5	3.9	4.5 – 6.5	5.2

Variation in honeys is also due to organic acids that gives different flavors and colors to honey and are also responsible for providing chemical characteristics such as pH, electrical conductivity and acidity to honey [16]. Natural honey also contains small amount of more than 400 phenolic compounds, which are responsible for the antioxidant activity of honey [17].

Honey also contains vitamins especially vitamin C and vitamin B complex (i.e. pyridoxine, thiamine, riboflavin, niacin and pantothenic acids). The vitamins are originated from pollen grains of flowers and are well preserved in honey due to the acidic pH [19]. Thus the filtration of pollen grains from honey (for commercial purpose) results in the depletion of vitamin contents (depending upon the use) [20].

Proteins (including enzymes) and amino acids are found in miniscule quantities in honey. Their concentration in honey greatly rely on honey bee species that synthesize it e.g. honey produced by *Apis mellifera* comprises of approximately 0.2-1.6% proteins while honey produced by *Apis cerana* contains 0.1- 3.3% proteins [21]. Both plant and honey bee attributes as the source of proteins and amino acids in honey. Plant sources are pollens from flowers, tissue fluid and floral nectar; and bee sources are the secretions from the pharynx and salivary glands of honey bees [13]. Amino acids contributes about 1% of the total dissolved contents of honey and the most abundant amino acid is proline [22].

Honey also contains trace amount of minerals including calcium, iron, phosphorus, copper, sodium, manganese, potassium, zinc and magnesium [23]. The mineral content of honey depends on the soil and type of plant whose nectar is collected. These minerals serve as cofactor of enzymes. As compare to amino acids and vitamins, minerals are highly stable and cannot degraded by light, heat, pH and other factors [24].

Hence, natural honey contains several volatile and nonvolatile compounds that are transformed enzymatically into new products like furans, amino acids, alcohols and phenolic compounds by alteration in storage time and temperature. Similarly, millard reaction in honey transformed the reducing sugars into glucoseamine; this reaction is accelerated by heat and high temperature [25] (Figure 1).

MEDICINAL PROPERTIES OF HONEY

Due to impressive curative property, medicinal importance of honey has been widely studied and reported in scientific literature [26]. Many natural products found in honey are therapeutically important for the treatment of gastrointestinal diseases and wound healing by ancient Assyrians, Chinese, Egyptians, Greeks, and Romans [27].

Anti-microbial action of honey

The antimicrobial action of honey against various virulent and non-virulent microorganisms is useful for management of good health, especially in the clinical situation where the immune response is weak [28]. Natural honey has been considered as an antimicrobial agent having inhibitory activity against large number of pathogenic microbes such as bacteria, fungi, yeast, and nematodes [29], as well as some multi drug resistance microorganisms [28].

Antibacterial action of honey

Natural honey due to its antimicrobial action, eradicate the growth of several bacterial pathogens like *Escherichia coli, Shigella, Helicobacter pylori, Salmonella* etc. [30]. Oral administration of honey has been

found to be useful for the treatment of gastrointestinal infections like gastric ulceration, gastritis and duodenitis mostly caused by bacteria such as *Helicobacter pylori* and rotavirus [31]. At the early stage of bacterial infections, invading bacterial cells are attached on the plasma membranes of gastric mucosa. Natural honey is believe to inhibit the attachment of pathogenic bacteria to plasma membranes of gastric mucosal cells [33]. There are several explanations for this inhibitory activity of honey i.e. coating of honey on the bacterial cell surface that led to change in the electrostatic or hydrophobic property of bacterial cell surface molecules which eventually results in the inhibition of bacterial attachment to host cell surface [34]. The *H. pylori* is sensitive to honey (at 20% concentration) due to the presence of H_2O_2 [32]. Hence, natural honey can be considered as a therapeutic agent for the management of peptic ulcer [35]. Similarly natural honey has also been utilized for management of eye related disorders like blepharitis, conjunctivitis, keratitis, corneal injuries, chemical and thermal burns to eyes [36]. Thus, topical application of honey as an ointment can reduce the acute symptoms of ophthalmic disorders like redness, puffiness, swelling, pus discharge and elimination of bacterial infections [37].

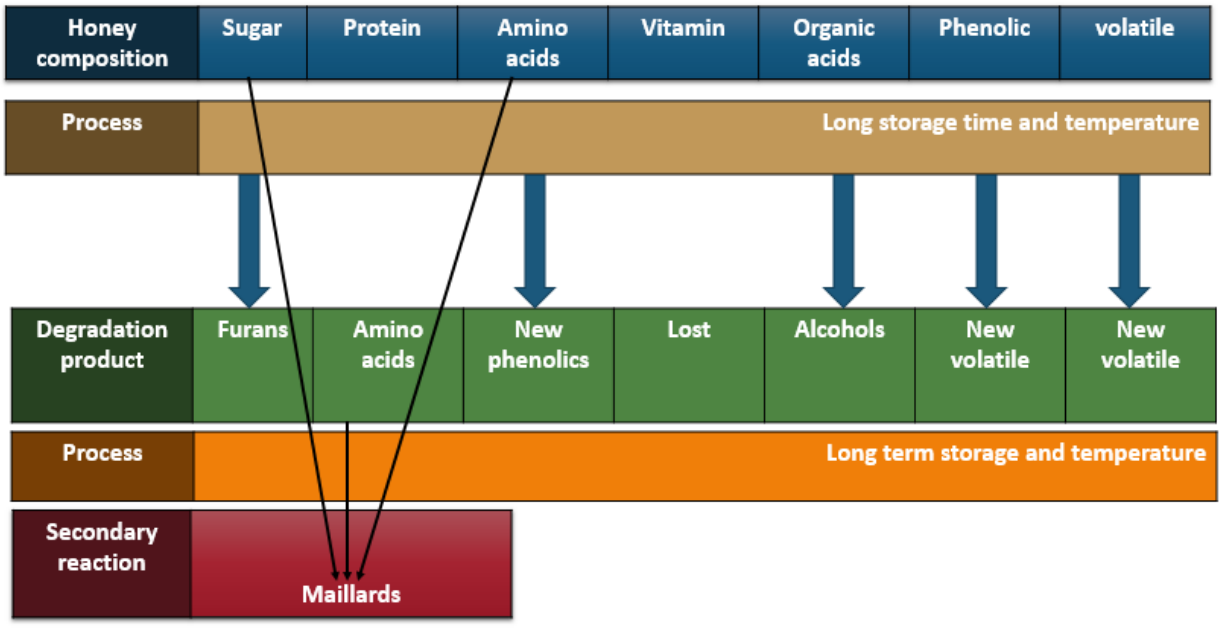

Figure 1. Showing compounds present in honey, processes that influence the stability of honey, degradation products and secondary reactions that may occur in honey. Adapted from da Silva, Gauche, Gonzaga, Oliveira, Costa, and Fett, (2016).

Natural honey has strong inhibitory effects against sixty different types of bacterial species including aerobic and non-aerobic as well as gram positive and gram negative bacteria [38]. Antibacterial activity of honey against *Strep. Pyogenes, Haemophilus influenzae, Pasteurella multicida, Corynebacterium diptheriae*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Vibrio cholera, Yersinia enterocolitica*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Pseudomonas aeruginosa* has been reported [39]. Some case studies reported the antibacterial activity of natural honey against methicillin-resistant *S. aureus* (MRSA) and gastric pathogens such as *H. pylori* [39]. On the basis of quantity used, honey exhibits both *bactericidal* and *bacteriostatic* effects. For instance, manuka honey and pesture honey at the doses of 8% and 5-11% respectively, shows bacteriostatic effects while at the concentration of 10% and 8-15% respectively, they are bactericidal [37].

Anti-fungal action of honey

Honey also possess considerable anti-fungal activity that varies with the quantity of honey used to treat infection; for example pure honey completely inhibits the growth of fungi, while diluted honey only suppresses process of toxin production from fungi [40]. Honey has significant antifungal effect against many fungal species including Aspergillus, Penicillin and some dermatophytes [41]. Candidiasis is the commonly occurring fungal infection, caused by *Candida albicans*. Natural honey is also found to be potent against infection of candida species and are used to treat candidiasis. Studies showed that the topical application of honey on fungal infected area can cure fungal infections like dandruff, dermatitis and seborrheic [42].

Antiviral action of honey

Natural honey is also considered as an effective antiviral agent. Al-Waili (2004) studied the antiviral effect of natural honey against herpes simplex virus and observed that application of honey on lesions of genital herpes is more effective as compared to the chemically synthesized drug acyclovir cream [40]. Honey has also been found to be effective against the lesions caused by rubella virus [40]. Among various types of honey, manuka honey is rich in methylglyoxal which possess potent antiviral activity against foot and mouth disease virus [43].

Nematicidal action of honey

Parasitic nematodes cause of a variety of infections in domestic animal, livestock and humans [44]. Nematode infections of animals and plants result in enormous economic burden. Common parasitic nematodes for humans are ascarids (Ascaris), pinworms (Enterobius), whipworms (*Trichuris trichiura*),

filarids, and hookworms [45]. These nematodes are common in developing countries causing high morbidity and mortality in both humans and domestic animals (Chan, 1997). Sajid and Azim (2009) found that natural honey has strong inhibitory activity on the growth and reproduction of model nematode *Caenorhabditis elegans.* They showed that at the concentration of 0.75-1.5%, natural honey has a potent paralyzing effect on all developmental stages of *C. elegans* [46]. Natural honey causes the defect in the egg laying and egg hatching process of *C. elegans*. This Anti *C. elegans* activity of honey was revealed by florescence and phase contrast microscopy, which showed cell death in the genital organs and intestinal lumen of *C. elegans* [29].

Florescence and phase contrast microscopic analysis revealed the anti-*C. elegans* activity of honey was due to anomaly in reproduction as manifested by defects in the process of egg-laying and -hatching by *C. elegans.* Florescence microscopic analysis unveil cell death in *C. elegans* genital organs and intestinal lumen [29]. Honey glycoproteins and glycopeptides were found as the main nematicidal molecules of honey rather than the sugars (sucrose, maltose, fructose and glucose) [29]. Anti-*C. elegans* honey proteins are likely to kill parasitic nematodes particularly those who belongs to the same phylogenetic clade as *C. elegans* (e.g. *C. onchophora* and *H. contortus*) [47].

Anti protozoal activity of honey

Anti protozoal activity of honey has also been reported. Protozoal parasitic infections are among the most common around the world. Oral consumption of honey has been recommended as a prophylactic treatment, for the control and prevention of parasitic diseases [48]. Waw and Alvieno (2012) identified anti-amoebic effectiveness of Tualang honey against the parasitic *Entamoeba histolytica,* which is the infectious agent of amoebic infection. The protozoan *Giardia lamblia* also known as *Giardia intestinalis* and *Giardia duodenalis* is the infectious agent of Giardiasis, the infection of human intestine manifested by severe diarrhea and malabsorbtion. The chromatographically separated honey proteins were found to be toxic against *Giardia lamblia* at the IC50 values ≥25μg/mL. Hence, honey (and honey proteins) due to their anti protozoal activity can be used as anti protozoal agent.

MECHANISM OF ANTIMICROBIAL ACTIVITY OF HONEY

Unlike mechanism of conventional antibiotics that kill bacteria by disrupting their cell wall or interfering with protein biosynthesis, natural honey mostly depends on certain factors for its antimicrobial activity such as osmolality, hydrogen peroxide, non-peroxide components and acidity [50]. High osmolality of honey is due to the presence of sugars, which allows honey to leave little or no water for survival of microbes and consequently lead to the death of microbes [51, 52, 53]. Generally, all chemical and physical properties of

honey are responsible for the rapid eradication of infections, stimulation of angiogenesis, wound healing, debriment of wound, epithelial tissue growth and suppression of inflammatory process [50, 54]. Similarly when natural honey applied on the wounds in the form of adhesive patches, honey draws all of the water from the wounds and leaves wounds dry. This condition lead to death of microbes [55].

In addition to osmolarity, acidic pH is another element for suppressing the growth of disease causing microorganisms. Natural honey has acidic pH around 3.2 to 4.5. The acidic pH of honey is due to the formation of gluconic acid by the oxidation of glucose through glucose oxidase enzymes [56]. The acidic pH of honey helps to suppress the growth of bacteria. Hydrogen peroxide is another potent antibacterial agent of honey that provides the free radicals, which are releases during the oxidation of glucose. Hence this catalytic effect of glucose oxidase is lethal for the growth of pathogenic and non-pathogenic bacteria, especially when honey is applied topically on wounds [58].

The oligosaccharide component of honey are very beneficial for human health. These oligosaccharides enhances the growth of lactobacilli and bifidobacterial, which are essential for maintaining the healthy microflora of human intestine. Lactobacilli and bifidobacterial provides protection against Salmonellosis and overgrowth of yeast in gut respectively. Lactobacilli also suppresses the growth of putrefactive bacteria and protect from colon cancer [60]. Natural honey also contain a wide range of other anti-microbial natural products in honey including syringic acid, flavonoids (pinocembrin) and volatiles (acetoin, acetic acid, acetone, valeric acid, ethyl salicylate, hyroxymethyl furfural and benzyl alcohol) [61, 62].

Bacteria in honey

Honey inhibits growth of microorganisms due to its high osmolarity, acidity and hydrogen peroxide. Nonetheless, honey encompasses microbes but less than other neutral-foods. Microbes in natural honey are those that can resist acidity, concentrated sugar, and other anti-microbial components. Conventional microbiology and PCR based studies reported several species of bacteria, yeasts and filamentous fungi associated with honeys of diverse botanical and geographical sources [63, 64] We characterized cultivable bacterial community of honeys produced in Pakistan. Bacteriological analysis as well as 16S rDNA sequencing and bioinformatics showed the presence of *Staphylococcus aureus, Micrococcus luteus, Streptococcus sp., Corynebacterium sp. Klebsiella pneumonia, Escherichia coli, Salmonella sp.* and *Proteus sp.* in honey samples tested [65].

Bacteria in honey bee

Bacterial flora of the gut plays an important role in honey bee physiology. Bacteriological examination using 16S rRNA sequences precisely identify and characterize the floral bacteria present in the gut of honey bee [66,67]. The organization of bacterial communities in digestive tract of *Apis melllifera* bee is relatively simple [68, 69, 70]. Sanger and next generation sequencing has been used to identify of distinct communities of bacteria present in alimentary canal such as Actinobacteria, Firmicutes, α- and γ-proteobacteria [71, 72, 73, 74, 75]. A recent study characterized the gut bacterial flora of honey bee in north-west Pakistan. According to this report, the gut bacteria are classified into phyla of Actinobacteria, Firmicutes and Proteobacteria. Majority of these bacterial isolates belong to the families of *Corynebacterium Enterococcus*, *Enterobacteriaceae, Ralstonia, Micrococcineae*, *Staphylococcus Bacillus*, *Sphingomonas* and *Ochrobactrum*. Moreover, several opportunistic pathogens like *Staphylococcus haemolyticus* group and *Sphingomonas paucimobilis* were also identified as local inhabitant of bee hive.

CONCLUSION

Natural honey has several therapeutically important components like sugars, amino acids, proteins, enzymes, antioxidants flavonoids, vitamins and phenolic compounds which are involved in prevention of human diseases and management of good health. Second, the presence of these components in honey can make it an effective drug for management of human ailments like wound healing, gastrointestinal disorders, immune system defects, ophthalmic disorders, oxidative stress and cancers. Along with theses disease natural honey also significantly showed its antinematodal and antimicrobial activities against many antibiotic resistant bacteria, virus and fungi. Therefore based on these facts, the use of honey as a medicine is highly recommended, and more studies are needed for better understanding of modes of action of honey components as well as to cover all medicinal aspects of honey.

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