Honey: Types, Composition and Antimicrobial Mechanisms

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Abstract

Honey has been broadly recognized as a source of nourishment and medication by both old and new generations. It has been utilized by people to treat numerous illnesses through topical application for at least 2700 years, but recent researches have revealed the antiseptic and antimicrobial activities of honey. It has been

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seen as a powerful agent that can be used in various human pathologies. Different clinical examinations have affirmed that the use of honey to cutaneous injuries which are severely infected removes contamination from the injury and enhances tissue healing. Honey has been perceived as a successful antioxidant and antimicrobial agent for centuries. Utilized mainly for treating burns and surface wounds, it has thus been developed into clinical medicine, as medical grade honey. In spite of this, the underlying interest in the utilization of honey for antimicrobial treatment was significantly reduced, as antibiotics were discovered and used. Nonetheless, due to the alarming increment in the occurrence of organisms with antimicrobial resistance, specifically the expansion in multidrug resistance (MDR), the quantity of efficient antibiotics is decreasing at a larger rate than new medications are created. This serious situation has made numerous scientists to think back to the pre-antibiotic period for creating solutions, directing their consideration towards the mechanisms of action of antimicrobial activity of honey.

Keywords

Honey · Polyphenols · Constituents · Antimicrobial · Nectar · Apis mellifera

10.1 Introduction

Honey is having an extensive history of human utilization as an oldest sugar and nourishment source. Honey was referenced in the manuscripts of Egypt, China and India since 5500 BC. While the medical utilization of honey has been documented, at least since 2000 BC, it is recently that the utilization of honey in wound administration has become extensively available (Cooper et al. 2002). In both clinical medications and the safeguarding of nourishments, the utilization of natural items is turning into a perpetually well-known approach. Increase in their ubiquity is because of their powerful activities and normally extremely low lethality. As per the World Health Organization insights, in some developed countries, up to 80% of the population had utilized natural items in their primary health services (WHO 2014). Furthermore, 80% of individuals rely upon these sorts of treatment in Asian nations, for example, India and China. Products of natural origin can be utilized in the revelation of new antimicrobial medications as well as in treating many infectious diseases. Researchers have established that natural items are commonly more acknowledged by people; if these substitute methodologies are efficient, this may decrease the dependence on manufactured substances (Slover et al. 2009). In addition, the investigation of such natural products may prompt the detection of an active compound that might be utilized to evade some environmental risks or potentially, in mammalian cells have an ameliorative effect on certain diseases (Mahady et al. 2008). For these new active components, herbs, plant separates, honey and fundamental oils are mainly the well-known sources (Slover et al. 2009), and these items have been seen to be very efficient against a variety of inflammatory cases and bacterial contaminations (Molan 2009). Honey is one of the best examples of a naturally accessible product and is the main concentrated sugar found in nature. In many nations, it has been utilized for centuries as a cure for numerous diseases, long before the information existed on the reasons of contamination. Honey has been perceived to be powerful in practically all instances of disease and for the advancement of healing particularly in wounds and burns (Mandal and Mandal 2011). Throughout the past, honey has been utilized in various societies, with contrasting applications. The ancient Egyptians utilized honey for wound dressing as well as a topical ointment and for preserving their dead, while the old Greeks utilized it to treat fever, gout, pain and wound healing (Eteraf-Oskouei and Najafi 2013). The principal observations about the antimicrobial action of honey were made in the year 1892, and since then, honey was shown to have a wide range of activity, hindering both Gram-positive and Gram-negative microbes, including Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Listeria monocytogenes and their multidrug-resistant counterparts (Dustmann 1979; Laallam et al. 2015). The effectiveness of honey against these living beings is reliant on the kind of honey utilized, because of variations in geographical area, bee health, botanical origin and the processing of honey (Cokcetin et al. 2016; El Sohaimy et al. 2015; Sherlock et al. 2010). It is apparent that numerous diverse sorts of honey can be found throughout the globe as various regions will have diverse flora, which will have an effect on the preparation and activity of various kinds of honey. Moreover, it is feasible to recognize honey into two fundamental types: first, floral honey that is set up from the nectar of blossoms (blossom honey) and second, honeydew honey that is made from the plant secretions (living part) or the discharges of plant sucking insects (Sanz et al. 2005a; Bentabol et al. 2011).

Honey has been chiefly utilized as a source of nourishment as well as therapeutic agent all the way through the history, across a wide and diverse variety of communities (White 1966). The antimicrobial properties of honey along with healing process and immune system activation are the major reasons for its universal recognition (de Abreu Franchini et al. 2007; Tonks et al. 2007). Furthermore, in spite of its extensive history as medication, honey was not perceived as a therapeutic agent by present day medication until recent past, perhaps because of limited understanding of its range and mechanism of antibacterial property (Blair et al. 2009). Numerous studies in the last two decades have looked into identification, mechanism of action and synergistic nature of its bioactive compounds (Blair et al. 2009; Tan et al. 2009; Brudzynski and Lannigan 2012; Majtan et al. 2014). In this manner, honey has recently become a part of conventional medicine for wound healing (Vandamme et al. 2013; Molan 2006; Bonn 2003). However, presently only a limited number of honey brands (Manuka, Medihoney) are accessible for the treatment of wound infections (Langemo et al. 2009; Simon et al. 2009). This identification is because of the high non-peroxide antibacterial activity of honey, identified specifically in Manuka honey (Allen et al. 1991). Manuka honey has been standardized according to phenol equivalence and labelled as Unique Manuka Factor (UMF) (Allen et al. 1991). A number of studies have found that honey also contains prebiotics, probiotics and zinc along with multiple antibacterial substances (Hernandez et al. 2005; Sanz et al. 2005b; Olofsson and Va'squez 2008; Robert and Ismail 2009; Vásquez et al. 2012). The existence of such valuable substances in honey has considerable clinical implications as far as treatment of diarrhoea is considered, since the existing treatment protocols for diarrhoea prohibits the usage of antibiotics and instead recommend the use of prebiotics, probiotics and zinc along with rehydration therapy (Guarino et al. 2008; Dinleyici et al. 2015; Dickinson and Surawicz 2014; Aachary and Prapulla 2011). The enhanced consumer utilization of complementary medicines has encouraged an expanding enthusiasm for nonconventional as well as traditional clinical medicines. One of the treatments that have gotten a lot of consideration is honey, and it has an extensive custom of utilization in different clinical frameworks (Majno 1975; Zumla and Lulat 1989), and over the previous decade, many researchers have centred their interest towards this natural product (Postmes et al. 1993; Greenwood 1993; Molan 1998; Moore et al. 2001). While honey has various therapeutic uses and has been utilized as a preservative agent for food, it is typically well identified for its benefits in wound treatment. Honey helps in maintaining moisture within wound environment that advances healing, and for the prevention of infection a protective barrier is provided by its high viscosity. Also, the mild acidic property of honey and release of lower levels of hydrogen peroxide help in wound healing and add to its antibacterial activity. It is this antibacterial property of honey which plays a vital role, in advancing healing of wound which is infected (Dunford et al. 2000; Lusby et al. 2002). There are two honeys in Australia, Manuka honey and Medihoney that are available as therapeutic honeys appropriate for treating ulcers, burns and infected wounds. Certainly, most of the available research right now in this field has been done utilizing either Manuka honey or Medihoney (Honey Scientific Report 1998). Both Manuka honey and Medihoney are obtained from *Leptospermum* spp., and it is not surprising that because of this reason, there is similarity in their activity. Earlier researches have revealed that both the honeys have a particular antibacterial property because of a non-hydrogen peroxide mechanism (Molan and Russell 1988; Weston 2000), that is, the Unique Manuka Factor (UMF). On the other hand, the distinction in Minimum Inhibitory Concentration (MIC) for antibacterial property among Unique Manuka Factor honeys and other honeys are often very little, usually <5% (Willix et al. 1992; Molan and Brett 1998), and the implication of this in medical domain is not clear. Yet, honey does have important prospective to help in healing of the wound, and this has been confirmed time and again (Molan and Brett 1998); Dunford et al. 2000; Natarajan et al. 2001). Manuka honey that is obtained from Leptospermum species inhibits the growth of Gram-positive microorganism, *Enterococcus faecalis,* while *E. coli* (Gram-negative microorganism) was seen to show more resistance towards honey treatment (Kumar et al. 2014). Several researches that were conducted on Chinese Buckwheat (Fagopyrum esculentum) and Manuka honey revealed a minimum inhibitory concentration of 60% (w/v) against *P. aeruginosa* and 5% (w/v) against *S. aureus* (Deng et al. 2018). Comparable outcome of linen vine honey revealed that *S. aureus* was more vulnerable than *P. aeruginosa* (Alvarez-suarez et al. 2010a). One more research assessing the efficiency of honey throughout a range of botanical origins recognized more vulnerability generally with respect to Gram-positive organisms, *Staphylococcus epidermidis* and *S. aureus*, and also reduced susceptibility or no impact towards the Gram-negative organisms, *P. aeruginosa* and *E. coli* (Matzen et al. 2018). One more investigation observing the antimicrobial action in case of Polish honey against *S. aureus* established a minimum inhibitory concentration of only 1.56% (w/v) (Grecka et al. 2018). However, many different investigations carried out on Gram-positive bacteria have revealed that they are more resistant to honey (Isla et al. 2011; Escuredo et al. 2012; Fyfe et al. 2017). Mohapatra et al. (2011) recognized that the Gram-positive microbes were less susceptible to honey than Gram-negative microbes, proposing the reason might be the high hydrogen peroxide concentration and osmolality of the samples. With respect to Rubus honey, the most susceptible organism was *Proteus mirabilis*, displaying an MIC of 7.8–31.3 mg/mL, while *S. aureus* showed an MIC range of up to 125 mg/mL (Escuredo et al. 2012). Besides, honeys of monofloral and multifloral origin were found to be more efficient against Gram-negative microbes compared to Gram-positive microbes, with *P. aeruginosa* establishing an MIC of 100 mg/mL, while as *S. aureus* showed an MIC of 250 mg/mL and *E. feacalis* extending from 200–250 mg/mL, also few honey samples do not show any effect on Gram-positive microbes (Isla et al. 2011). Besides, an investigation carried out on Egyptian honey recognized Sidr honey was the only efficient honey against *S. aureus*, having an MIC of 100% and just four honey samples out of six were efficient against *Streptococcus mutans*. All tested samples were found to be efficient against *K. pneumoniae and P. mirabilis*, having an MIC of 50% or less. Just one sample was not found to be efficient against *E. coli*, and three out of six samples were not efficient against *P. aeruginosa*; however, the MIC values of inhibitory samples were 50% or less (El-Borai et al. 2018). Also, it has been revealed that when given a treatment of a range of Scottish honey samples, *Acinetobacter calcoaceticus* was found to be the most affected, in comparison to *P. aeruginosa, E. coli*, and *S. aureus* (Fyfe et al. 2017). This diversity of results proposes that all honeys are not equivalent and their efficiency varies largely, delineating the importance of geographical area and botanical origin on the antimicrobial action displayed by a particular honey. Interestingly, through researches, it has been seen that no organism has acquired resistance against honey (Maddocks and Jenkins 2013). Furthermore, in methicillin-resistant Staphylococcus aureus (MRSA), sub-inhibitory dosages of honey have been found to re-establish oxacillin susceptibility (Jenkins and Cooper 2012). Initial investigations regarding honey have revealed some important aspects that result to its antimicrobial activity, these include low pH, polyphenolic compounds, hydrogen peroxide, high sugar content, and the detection of an “Inhibine” (Albaridi 2019; Dustmann 1979; Molan 1992). In addition, investigations exploring the reason for honey being a potent antimicrobial agent revealed that inhibine was a 1,2-dicarbonyl compound in the form of methylglyoxal which is an effective antimicrobial found primarily in Manuka honey (Mavric et al. 2008). Bee defensin-1, a bee-derived protein, a potential antimicrobial agent within honey was also identified through recent studies (Bucekova et al. 2019). This furthers the argument that honey contains a number of antimicrobial components, and their activity is not governed by a single antimicrobial agent. Furthermore, the effectiveness of honey as an antimicrobial agent is enhanced by synergistic operation of its multiple components.

10.2 Nomenclature and Classification of Honey

Honey is basically a sugar solution (saturated or supersaturated) which is prepared by honeybees and some other insects. Honeybees and the insects collect nectar from the flower and transform the nectar by adding up enzymes to it and then store it as a source of food, so that it can be used in dearth periods (Crane and Visscher 2009). Honey is predominantly prepared by honeybees (social insects) which have a perennial life cycle, although few other insects also contribute towards honey production. The bees are mostly categorized into various groups which include all honeybees (Apis spp.), Nectarina wasps in South America, as well as stingless bees (*Melipona and Trigona spp*.) and a number of honey ant species, particularly *Melophorus inflatus* that are found in Australia. In addition, other social wasps and bumblebees (*Bombus spp.*) are also present, but they generate a little amount of honey (Crane 1999). Honey is mainly classified into two categories (Fig. 10.1). These are nectar honey and honeydew honey.

10.2.1 Nectar Honey

The European Commission Council Directive (EU 1102001) characterizes nectar honey as a naturally occurring sweet compound that is prepared by *Apis mellifera*. Bees collect the plant nectar, convert it by combining with particular substances produced by them, deposit the nectar, dry out, store and leave in honeycombs, so that it can get ripened and mature (EU 1102001).

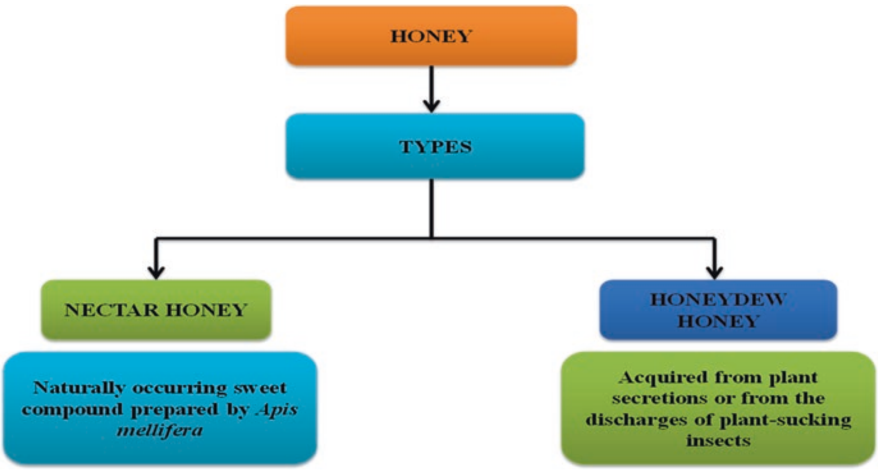

Fig. 10.1 Classification of honey

10.2.2 Honeydew Honey

The European Commission Council Directive (EU 1102001) characterizes honeydew honey as a source of nourishment that is acquired from plant secretions or from the discharges of plant sucking insects. These insects perforate the leaves or other plant parts and feed on the sap of the plants, discharge the excess in the form of droplets, which are later accumulated by the bees as honeydew (EU 1102001). The differentiation between nectar and honeydew honeys can be done with the help of pollen analysis, but through their physicochemical analysis, these can be better differentiated. The honeydew honeys have a high electrical conductivity, pH, ash, acidity and darker colour, as well as a high di- and trisaccharide concentration and a low monosaccharide concentration (Mateo and Reig 1998). Furthermore, honeydew honey contains algae and fungi cells, but their presence in not related to its origin (Bogdanov et al. 1997).

10.3 Honey Production

Apis mellifera (honeybee) is of enormous significance for people as it acts as a pollinator of both domestic and commercial crops and also provides honey which is of high nutritional value (Ratnieks and Carreck 2010; Potts et al. 2010). Honeybee loss because of the interfacing divers of diseases and pests, absence of genetic diversity, introduction of agrochemicals and apicultural mismanagement leads to extensive concern for the future potential of honeybees with respect to providing the services (Ratnieks and Carreck 2010; Potts et al. 2010). The composition and the quality of honey produced depend on numerous elements which includes bee health, geographical location of bee hive, flower composition, flowering phenology and yearly changes in local flora (Galimberti et al. 2014). Commercially, there are various kinds of honey that are available (creamed, chunk, comb, granulated or crystallized) with numerous diverse degrees of processing (heat processed, centrifuged, pressed, drained) (Anklam 1998). There are three castes within a honeybee hive: first is queen (alpha), second is worker (beta) and the third one is drone (gamma) bees (Havenhand 2010), and it is their aggregate effort which results in honey production. Honeybees prepare honey by utilizing nectar that is collected from flowering plants; nectar is a sugar containing liquid that is secreted by glands known as nectaries.

The worker bees travel up to 9 km in a single trip for collecting nectar (Havenhand 2010). The carbohydrate and sucrose that is present in nectar is hydrolysed to generate fructose and glucose (Kubota et al. 2004). The nectar is then ingested and regurgitated upon their arrival to the hive, by large number of worker bees inside the honey comb. The regurgitation and wing fanning process lead to evaporation which results in decreased water content, then the ripening of honey takes place with time (Fig. 10.2). Honeybees store honey as food for the winter season when there is non-availability of nectar or pollen. For human consumption,

Fig. 10.2 Process of production of honey

the excess honey can be extracted from the honey comb (Havenhand 2010). Kubota et al. (2004) depicted the way by which hypopharyngeal gland secretes glucosidase III in European bees. This glucosidase III is released into the nectar and is accountable for the release of hydrogen peroxide (Bucekova et al. 2014). In order to feed honeybee larvae, honeybees collect pollen grains as they visit flowering plants (Galimberti et al. 2014). Using nectar–saliva mixture, thick pellets of pollen are prepared from these pollen grains. Honeybees collect the exudates as an alternative to nectar, from sap-sucking insects. Honeydew is collected more often from insects that feed on sap of conifers and other anemophilous species (Oddo et al. 2004). For making propolis, the tree resin which is vigorously obtained from different plants is mixed with wax and later on stored in bee hive because it has antimicrobial properties (Wilson et al. 2013).

10.4 Honeybee: Life Cycle

Apis mellifera (honeybee), which is responsible for producing honey by collecting nectar from the flowers of different plants, is one of the eminent manifestations of God. In a bee hive, there are three kinds of bees: first, the only reproducing female that is the queen bee; second, the male bees known as drone bees; and third, the non-reproducing female bees called as worker bees. In order to produce eggs, the queen bee mates with the drones, fertilizes and resides the eggs in bee hive (comb) cells. Within 3–4 days, the eggs hatch to produce larvae. These larvae then grow to form pupae, and these pupae transform into new worker bees (female bees); the already existing worker bees then feed the new ones (Bishop 2005). A huge number of individual bees may be present in a characteristic bee colony which primarily comprise of the sterile worker bees (sterile female bees). Once the queen mates with the drones, the drones die, and the queen bee may have a life expectancy of almost 3–4 years (Bishop 2005; Rueppell et al. 2007). Honey is formed by the honeybees in their "honey stomach." The honeybees have two types of stomachs: normal stomach, which carries out the normal functions in honeybee, and the other one is particularly useful for preparing honey. Apis mellifera generally collects the nectar which is typically a sugar-rich transparent fluid comprising of mainly water (80%) and sugars (20%), from different flowers using their tongue (Zhu et al. 2016). In order to fill the "honey stomachs" with nectar, one worker bee usually visits approximately dozens of flowers. After that the processing of nectar is immediately done with the help of digestive enzymes which include catalase, amylase, acid phosphorylase and glucose oxidase, which converts sucrose into glucose and fructose (Zhu et al. 2016).

Once the worker bee arrives at the bee hive, the nectar is spitted into the mouth of different bees, thus starting the process of regurgitation. This process vitally helps in the production of honey that is the final product from raw nectar, with the help of the impact imparted by the digestive enzymes secreted by bees. The process of regurgitation proceeds for around 20 min, and then the final product (raw honey) is spitted into the honey comb cells. This final product is quite vulnerable for the attack by different microbes, as it has a high moisture content of about 80%. Then the honeybees flutter the wings to decrease excessive moisture, which creates a strong draft resulting in evaporation, thus reducing the moisture content to <20%. When the honey is dried, the honeybees seal the cells of the comb with wax in order to store it for future consumption. It is by the activity of honeybees that the wax is also produced from honey (Nicolson and Human 2008). There are usually two techniques that can be utilized for the extraction of honey from the hive. The first method is a conventional technique in which bees are calmed down or moved away from the bee hive by applying smoke in the hive. The moment bees are calmed down or moved away from the bee hive, honey is extracted by squeezing the hive. In the second method, the combs are placed in a metallic bowl soon after the bees are moved away, and then the burning coal is placed on the combs which results in melting down of honey and bee wax. These are then drained out from a hole, where honey is collected. However, the above-mentioned conventional techniques are not so effective and are being taken over by the modern techniques (Ediriweera and Premarathna 2012). Mechanical extractor which works on the concept of centrifugal force is a contemporary honey extraction method. It comprises a container that has a frame basket, this basket spins and tosses out the honey from the comb without damaging it and, hence, can be reutilized again by the honeybees.

10.5 Honey and Its Composition

Honey is a complex mixture consisting of water, carbohydrates and other minor compounds (Garcia et al. 1986; Cortes et al. 2011). The composition of honey is affected by the flower type as unifloral and polyfloral, as well as climatic and regional parameters (Cortes et al. 2011). Honey majorly comprises carbohydrates (82.4%), water (17.1%), amino acids (0.5%), minerals, vitamins and various minor compounds (Table 10.1) (Garcia et al. 1986; Montenegro and Fredes 2008; Cortes et al. 2011). In association with the geographical and botanical origin, there are various heavy metals that have been extracted from honey (Cortes et al. 2011). Alvarez-Suarez et al. (2010a, b) revealed that honey approximately contains 181 substances and, therefore, is a supersaturated solution. Honey was known as a significant carbohydrate sweetener before producing industrial sugar (Bogdanov et al. 2008; Alvarez-suarez et al. 2010b). Honey is being used as a nutrient as well as a medicine in many human societies (Bogdanov et al. 2008). The particular colour, flavour, texture and aroma of honey depends on a variety of factors; these include the flower type, honeydew and plants, their foraging habits, the physiological behaviour of the bee, post-collection processing and climatic conditions. Honey is prepared throughout the world, and it estimates approximately to 1.2 million tons per year, but it still constitutes only about less than 1% of the total sugar production worldwide (Bogdanov et al. 2008). Honey consumption varies worldwide; in European Union, it is 0.3–1.8 kg per capita, and in Argentina and China, it is 0.1–0.2 kg per capita annually. Researchers have so far been successful in isolating about 600 compounds from honey. Typically, the compounds that are present in honey can perhaps be obtained straight away from the plant source, by converting them with the help of metabolic activities of the bee, or from handling, heating, storage and microbial and environmental contamination (Manyi-Loh et al. 2001). The isolated chemicals belong to different chemical families, which include aldehyde, alcohol, hydrocarbon, ketone, norisoprenoids, furan and pyran, acid, cyclic compounds, benzene and its derivatives, ester, sulphur and terpenes. Due to the temperature at which honey has been stored and other storage conditions, the volatile compounds of honey may vary. In addition, the geographical location and the floral composition may greatly influence the composition of honey. During the storage process of honey, the volatile substances are produced by nonenzymatic activity, and the heat labile compounds may get destroyed, which results in change in the organic components of

Table 10.1 Different components of honey and their concentration

Component	Concentration
Water	17.1%
Carbohydrates	82.4%
Fructose	38.5%
Glucose	31%
Amino acids	0.5%
Protein	0.25%
Gluconic acid	0.23–0.98%

honey (Manyi-Loh et al. 2001). Honey is a nutritive compound as it is composed of numerous contents. Despite health benefits of honey, it can also get contaminated with pesticides, heavy metals and antibiotics that are present in the environment (Bogdanov 2006). There are some plants which contain poisonous compounds such as pyrazolidine and diterpenoids; bees can also use these for collecting nectar and may result in honey contamination (Edgar et al. 2002; Bogdanov et al. 2008). A brief description of some important components of honey is given below:

10.5.1 Carbohydrate

Honey being a highly saturated sugar solution generally comprises around 17.1% water. The major sugar is fructose which constitutes about 38.5%, followed by another sugar glucose which is about 31%, and disaccharides, trisaccharides and oligosaccharides in small quantities are also present (Crane 1976). Honey is the most suitable sweetener that is being utilized by the consumers, as an alternate to other sweeteners because of its unique flavour and higher nutritional value (Cortes et al. 2011). Besides 25 different forms of oligosaccharides, the most common sugars that are found in honey are monosaccharide, glucose and fructose (Siddiqui 1970). The major oligosaccharides include turnose, panose, sucrose, maltose, palatinose, 6-kestose, 1-ketose and trehalose (Bogdanov et al. 2008). The fructose and glucose present in honey soon after digestion provide instant energy to the body. However, as per the human standards, honey should be considered as a food supplement and not as a complete food. A dosage of 20 g of honey can provide only about 3% of daily energy requirement.

10.5.2 Protein, Enzymes and Amino Acids

Honey comprises about 0.25% protein content which majorly consists of amino acids and enzymes. Invertase, glucose oxidase and diastase are the key enzymes that are present in honey. As per the recommended daily requirement for human consumption, the consumption of honey as a protein source is not adequate (Bogdanov et al. 2008). Although the amino acid content in honey is often small, the extensive range of almost 18 amino acids (essential as well as nonessential) that are found in honey is exclusive and varies by floral origin. The main amino acid found in honey is proline, and lysine is the second most common amino acid. Tyrosine, glutamic acid, phenylalanine and aspartic acids are the various other amino acids that are found in honey. The glucose oxidase reaction yields glutamic acid as a product. While as the proline and other amino acids are contributed by the nectar, pollens or by the bees themselves (Crane 1976). Numerous enzymes which include glucose oxidase, acid phosphatase, invertase, diastase (amylase) and catalase are also found in honey (Crane 1976). Glutamic acid and hydrogen peroxide are prepared from glucose by glucose oxidase reaction, and it also results in the production of glucolactone that occurs in equilibrium with gluconic acid. The hydrogen peroxide found in honey thus acts as a contributing factor towards the antimicrobial activity of honey. The sucrose is converted to glucose and fructose with the help of invertase enzyme. The bees add invertase to the nectar in the form of fructo-invertase or gluco-invertase (Ensminger et al. 1983). After the extraction of honey, it contains a little quantity of invertase enzyme, and this enzyme may continue its activity there. However, high temperature results in inactivation of invertase enzyme.

10.5.3 Vitamins, Minerals and Other Compounds

Besides carbohydrates, proteins and enzymes, honey additionally contains different quantities of trace elements and minerals which include calcium, sodium, magnesium, zinc, potassium, phosphorous, copper, manganese, iron, selenium and chromium (Bogdanov et al. 2008). There are certain essential vitamins that are found in trace amounts; these include niacin, riboflavin, thiamine, phyllochinon, ascorbic acid, pyridoxine and pantothenic acid.

10.5.4 Polyphenols

Polyphenols are the finest vital groups that are found in plants, incorporating almost 8000 varied recognized structures (Bravo 1998; Estevinho et al. 2008). Polyphenols are affirmed to exhibit anti-inflammatory, antiatherogenic, analgesic activities, immune modulating, anti-carcinogenic and anti-thrombotic activities (Vinson et al. 1998). The possible indicators of the botanical source of honey are phenolic acids and flavonoids which are basically the phenolic compounds of honey (Yao et al. 2003). There are numerous diverse mechanisms which include singlet oxygen quenching, metal ion chelation, hydrogen donation, free radical scavenging and substrate carrying out for radicals such as hydroxyl and superoxide, to which the antioxidant activity of phenolic compounds is related to (Kucuk et al. 2007; Pandey and Rizvi 2009). The phenolic compounds that are isolated from honey can be categorized as flavonoles (quercetin, fisetin, kaempferol, myricetin, galangin), flavones (luteolin genkwanin, wogonin, apigenin, acacetin, tricetin), flavanones (hesperidin pinobanksin, pinocembrin naringenin, naringin), phenolic acid (vanillic acid, p-hydroxybenzoic acid, caffeic acid, gallic acid, syringic acid, p-coumaric acid, cinnamic acid, chlorogenic acid, ferulic acid, rosmarinic acid and derivative forms), tannins (ellagic acid) and coumarins (coumarin), as recognized by Abubakar et al. (2012).

10.5.5 Flavouring Agents

The aroma profile is the most significant attribute for the assessment of genuineness and organoleptic nature of any food product. As far as the consumer point of view is concerned, the flavour of honey is the most essential criteria. It is the volatile and semi-volatile organic components on which the aroma of the honey mostly depends (Jerkovic et al. 2006). The colour, taste and flavour of the honey may perhaps differ depending upon the botanical origin. Sugar is one of the most essential flavouring agents. The aroma of honey is dependent on factors like the type and quantity of amino acids and has also found to be connected with various types of phenolic compounds that are separated from various kinds of honey (Bogdanov et al. 2007). There are about 56–500 mg/kg polyphenols present in honey, and these depend usually on the type of honey (Al-mamary et al. 2002). Chrysin, luteolin, quercetin, galangin and apigenin are some of the important phenols that are found in honey (Tomas-Barberan et al. 2001). In general, the dark colour honey will have a prominent flavour compared to mild coloured honey which will have a mild flavour (Castro-Varquez et al. 2003; Kaskoniene et al. 2008).

10.5.6 Water Content

The determination of water content of honey is a significant quality parameter to avoid the spoilage of honey which can occur because of fermentation process. The moisture content affects the shelf life and the quality of honey; therefore, it is not like several other parameters which are alternatively accepted (Bogdanov et al. 2004). The International Honey Commission (IHC) has set a highest concentration of 20 g of water per 100 g of honey, for any honey sample to be acknowledged for honey trade. The other parameters of honey like glucose crystallization and viscosity are directly affected by its moisture content (Bogdanov et al. 2004). The moisture content of honey can be assessed with the help of many techniques which include Karl Fischer titration, gravimetric technique or refractive index (Sanchez et al. 2010).

10.5.7 Organic Acids

Honey contains mostly 30 organic acids (Mato et al. 2003), although the main organic acid that is found in honey is gluconic acid. It is found in the range of 0.23–0.98%, which is produced with the activity of the enzyme glucose oxidase (White 1975).

10.6 Honey: Antimicrobial Activity

Honey obtained from several plant source shows intense antimicrobial action (Szweda 2017). Manuka honey exhibits efficient action against *Salmonella aureus, Escherichia coli, Enterobacter aerogenes* and *S. typhimurium* (Lusby et al. 2005; Visavadia et al. 2006). Hovenia monofloral honey has been reported capable of showing antibacterial action Gram-negative and -positive bacteria that are found in various foodstuffs (Park et al. 2020). Researches on buckwheat honey also revealed that it showed strong antibacterial activity (Dżugan et al. 2020). Antibacterial property is an essential trait of honey for its selection in medical purpose and also serves an important criterion to assess honey in terms of quality (Godocikova et al. 2020). The osmotic pressure of honey is usually high because of its high sugar concentration, resulting in low water activity (Aw), of range 0.562–0.62 (Bogdanov et al. 1997), which makes the osmolarity to play a fundamental function in determining the antimicrobial action in case of undiluted honeys. When the water activity is between 0.94 and 0.99, it completely inhibits the growth of numerous bacterial species (Molan 1992). Acidity is another factor that plays a major role in determining the antimicrobial property of honey. Though it was considered to have a major role in antibacterial activity, current investigations have confirmed the acidity as a minor role player in determining the antibacterial activity of honey (Molan 1992).

10.6.1 Antimicrobial Activity: Mechanisms

Although a number of factors are responsible for the antimicrobial action of honey, the enzymatic glucose-oxidation reaction and few physical properties of honey contribute majorly towards its antimicrobial activity. Other factors comprise low pH/acidic environment, low protein content, low water content/high osmotic pressure, high carbon to nitrogen ratio, viscosity, and low redox potential that limits dissolved oxygen and other phytochemicals/chemical agents (Snowden and Cliver 1996). Dr. Peter Molan, the most well-known honey researcher, has carried out investigation on honey-related antimicrobial components and came out with major findings that are available on the website of University of Waikato, Hamilton New Zealand. The major findings include:

- The water activity (Aw) of honey is low, resulting in small amount of water availability that limits the bacterial and yeast growth. If the Aw of honey is in the range of 0.94–0.99, it will encourage the growth of many bacterial species. The Aw of ripened honey is in the range of 0.56–0.62, which prevents yeast growth. The bacterial species that grow rapidly at an Aw of 0.99 will not be affected with diluted honeys which have a higher value of Aw.
- The acidic property of honey inhibits the growth of several pathogens. For certain pathogens usually causing wound infections, the minimum pH value ranges from 4 to 4.5. This antibacterial efficiency of honey resulting from its acidity gets reduced whenever the honey is diluted particularly with body fluids which raise its pH.
- Honeybees secrete glucose oxidase, an enzyme that helps in the preparation of honey from nectar. It transforms glucose into gluconic acid and hydrogen peroxide in the presence of water and oxygen. During the ripening of honey, sterilization and preservation are carried out by gluconic acid and hydrogen peroxide. The pure honey has an insignificant content of hydrogen peroxide and active glucose oxidase. The hydrogen peroxide is quickly decomposed into oxygen and water by ascorbic acids and transition ions, while in case of low pH, the enzyme

is inactivated. On the other hand, there occurs a 2500- to 50,000-fold increase in enzyme activity on dilution of honey and formation of a slow release antiseptic which do not damage the tissue.

However, the generation of peroxide is not responsible for the entire observed antibacterial action. A number of other constituents found in honey with antibacterial property are found in small amounts and do not contribute significantly towards its antibacterial activity. These include benzyl alcohol, 3,4,5-trimethoxybenzoic acid, terpenes, 1,4-dihydroxybenzene, pinocembrin, 3,5-dimethoxy-4-hydroxybenzoic acid, 2-hydroxybenzoic acid, methyl-3,5-dimethoxy-4-hydroxybenzoate, and 2-hydroxy-3-phenylpropionic acid. Different researches provided the evidence for the presence of non-peroxide antimicrobial factors. One such research is treating the honey with heat resulting in inactivation of glucose oxidase, and another is treating the honey with catalase resulting in the elimination of peroxide activity. All kinds of honey does not have the same antimicrobial potential because of the differences in concentration of non-peroxide factors as well as peroxide production owing to difference in floral source and honey processing. Certain factors like metal ions, catalase and ascorbic acid can destroy hydrogen peroxide while as glucose oxidase enzyme may get wiped out by light and heat. The antibacterial potential can be assessed by determining the Inhibine Number, and it is the degree of dilution up to which the antibacterial activity of honey can be retained.

The majority of the researchers nowadays represented the antimicrobial activity of honey in terms of minimum inhibitory concentration (MIC). It is defined as the minimum concentration of honey that is essential for absolute inhibition of microbial growth (Saranraj and Sivasakthivelan 2012). A variety of studies carried out on a large number of honey samples showed a broad range of antimicrobial activity and several with low level of activity (Allen et al. 1991). While there is still lot more work to be done to clearly recognize the antimicrobial action of all honeys, there are few researches which revealed high levels of antimicrobial activity in honeydew honey from coniferous forests of Central Europe and Manuka honey obtained from *Leptospermum* species of New Zealand, as it had the maximum levels of non-peroxide activity among 26 different samples of honey with diverse floral origin. It strongly inhibited the growth of *Escherichia coli and Staphylococcus aureus* (Willix et al. 1992). An in vitro study conducted for comparing the antibacterial potential of pasture honey and Manuka honey on coagulase positive strains (*Staphylococcus aureus*) collected from contaminated wounds reported slight difference in their sensitivity towards both the honeys. However, Manuka honey which has non-peroxidal antibacterial action and pasture honey which has a high peroxide generation were both efficient at low concentrations of 2–3% v/v and 3–4% v/v, respectively (Cooper et al. 1999).

Today most of the researches have revealed the antimicrobial activity of honey in many microbial strains including clinical isolates, with the help of in vitro antimicrobial assays. There are only few studies that have revealed the antimicrobial activity of honey in vivo, with relation to wound infections. Cooper et al. (2001) reported, in a 38-year-old female who was suffering from a *S. aureus* infected recalcitrant surgical wound, when treated with Manuka honey impregnated dressings and oral coamoxiclav resulted in wound healing and bacterial clearance after 7 days of commencement of the treatment. For 3 years, the wound had failed to respond to any conventional treatment before the commencement of honey/antibiotic therapy. More researches have reported many controversial findings. Gethin and Cowman (2008) studied the treatment of Manuka honey or hydrogel in 108 patients suffering from venous leg ulcers. They found out that the Manuka honey efficiently eradicated methicillin-resistant *Staphylococcus aureus* (MRSA) from 70% of MRSA-infected wounds, and the hydrogel eliminated MRSA only from 16% of wounds. Jull et al. (2008) during a clinical trial of 368 patients treated with Manuka honey impregnated dressings or usual care revealed no major difference in incidence of venous leg ulcer infections. One more study reported that when patients undergoing peritoneal dialysis were treated with Medihoney antibacterial gel or the topical antibiotic mupirocin, no major difference was reported with respect to development of peritoneal dialysis-related infections (Johnson et al. 2014).

10.7 Conclusion

Today, researchers give more consideration to medications with natural origin and consider that the products with natural origin may prove to be efficient therapeutics compared to synthetic ones. Honey is the most significant natural product, being utilized for diverse medicinal purposes since long back. Although, honey has a significant role in conventional medication, scientists also believe that honey can also be used as an efficient medicine in treating various kinds of diseases. Researchers have revealed that honey has a significant antimicrobial activity. The antimicrobial and wound healing effectiveness of honey is specific to season, flower and region. All honeys do not necessarily show the same antimicrobial potential as it can vary due to the difference in pH, quantity of active principles, sugar content, different vulnerability of a variety of bacterial strains and storage conditions, though some honey samples do not show any significant antimicrobial activity. The use of honey does not result in developing antibiotic resistance in microorganisms, unlike some other traditional local chemotherapeutics, and hence can be used continuously. In case of highly drug-resistant bacterial infections, honey has the capability to immensely reduce the requirement of last resort drugs, since currently the antimicrobial resistance in case of honey is not seen. The utilization of honey in future will be prolonged to a large extent. This is because of the increase in multidrug-resistant organisms (MDR) which causes infections that cannot be treated even with multiple classes of antibiotics, predominantly because honey has been found to have the capability of reversing certain antibiotic resistance mechanisms. Hence, the renewal of this substitute antimicrobial agent represents potential therapeutic avenue in controlling the rising frequency of antibiotic-resistant bacterial infections.

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