Chapter

Honey Composition, Therapeutic Potential and Authentication through Novel Technologies: An Overview

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Abstract

Honey is acknowledged as a natural functional food with additional health benefits. Due to its medicinal and therapeutic properties, honey is being used in both pharmaceutical and food industries to develop products as a remedy against various types of ailments. Honey contains polyphenols, flavonoids, and other key compounds that play an important role in human health. Honey possesses anticancer and antimicrobial properties as well as contains antioxidant and anti-inflammatory substance. Some studies also highlighted the antidiabetic properties of honey. It supports the respiratory system and contributes beneficial effects to the cardiovascular system. As a functional and nutraceutical food, honey plays a significant role. Due to the modernization and digitalization in this era, the role of novel technologies for characterization and authentication of honey cannot be ignored. Hence, the main purpose of this chapter is to review the latest studies related to honey's advantageous effects on human health and to highlight the novel technologies to detect the impurities in honey.

Keywords: honey, composition, therapeutic potential, novel technologies, authentication

1. Introduction

Honey is an ordinary product that can be found in nature and is the only concentrated sweetener. The composition of honey is thoroughly associated to its environmental conditions and botanical sources. Bees accumulate honeydew and transfer it into the mass, where enzymes act upon the sugars and transform these sugars into honey. In several countries, it has been used as a treatment of diseases for several centuries. It possesses the great potential or purpose of healing wound and burn injury. In addition to this, it has been recognized to be very effective in virtually all cases of infection [1]. Honey is also known as a natural sweetener with additional health benefits. It is frequently used as an important constituent of herbal medicines and considered as a nutraceutical agent. Since the last 1500 years, honey has been utilized in food and medicinal products [2].

In the past, honey has been utilized for medicinal and therapeutic purposes in a variety of cultures. Ancient Greeks have used it as cure for wound, fever, gout, and pain, and ancient Egyptians used honey for mummifying their deceased and also for the purpose of wound dressing as a topical balm [3]. The medicinal effect of honey is due to the manifestation of different antioxidant compounds, including phenolic acids and flavonoids. Various studies observed the antidiabetic, anticancer, and antimicrobial action of honey. The defensive impact of honey on cardiovascular, gastrointestinal, pulmonary, and nervous systems has also been demonstrated. Due to the excellent bio-functional properties of honey, many industries and local suppliers are adulterating honey by adding artificial sweeteners. In recent decades, the rate of adulteration of honey has increased in both developing and underdeveloped countries. Researchers and scientists have been using various new technologies to test honey adulteration like spectroscopic techniques, electronic tongue, microscopic analysis, immunoassays, and thixotropicity [4].

Honey contains protein contents, carbohydrates, pigments, moisture contents, enzymes, vitamins and minerals, phenols, and minor amounts of bioactive compounds such as carotenoid content, proline level, total flavonoid content, salicylic acid, naringin, and taxifolin. Normally, honey comprises about 80% of carbohydrates and 20% of water [5]. In all religious books, honey has been mentioned as an important food and medicine. It was not renowned as a significant therapeutic agent regardless of its extensive history or as beneficial medicine due to its wide range of activity and inadequate understanding of its properties. Now, honey is rapidly becoming a part of the pharmaceutical industry, and research is being done to check the medicinal properties of honey [6].

2. Sources

Manuka, Sidr, *Jelly bush*, *Pasture*, *Jungle*, and *Sumra* are common varieties of honey because of different botanical sources and environmental conditions [5]. Due to its wide range of antibacterial efficiency, *Manuka* honey has had key reputation. This beneficial Leptospermum sp. originates from Australia and New Zealand. In the pharmaceutical industry, this medical-grade honey has been effectively used to treat a wide range of diseases [7]. Almost 500 different species of honeybee have been found in Africa, America, Australia, and Southeast Asia. These varieties can be classified into *Melipona* and *Trigona*, the two main genera of honeybees. The *Melipona* genus is generally in majority than the common bee *Apis mellifera* [8].

Apis florae, Apis cerana, *Apis dorsata*, and *Apis mellifera* are famous species of honeybee found in Pakistan. *A. mellifera* is found in different parts of Pakistan, especially in KPK, and is one the most common in terms of quality of honey [9]. There are two primary floral sources of honey in Oman: *Ziziphus spina-Christi*, also known as *Sidr* locally, and *Acacia tortilis*, also known locally as *Sumera* [10].

3. Chemical composition of honey

Lim et al. [11] investigated the nutritional makeup of honey from various botanic sources. Results showed that the moisture content of honey ranged from 27 to 31 g/100 g of honey. The ash level of honey samples that were analyzed ranged from 0.15 to 0.90 g/100 g. Protein content was found to range from 0.2 to 0.8 g/100 g of honey. The carbohydrate level of honey samples was analyzed and found between 67.58 and 72.25 g/100 g. Another experiment conducted to examine the nutritional conformation of honey from various botanic sources. Results showed moisture around 18–19%, fructose 45–48%, glucose 29–31%, sucrose 2–4%, total sugar level (glucose, fructose, and sucrose) 77–82%, protein level (mg/kg) 0.76–0.80 mg, and ascorbic acid content 0.22–0.27 mg of honey [12].

Santos-Buelga and González-Paramás [13] hypothesized in 2017 that the chemical makeup of honey is influenced by its botanical source and place of origin. Due to abundance in sugar composition, honey is identified as a naturally sweet product. The composition is different depending on floral sources and processing along with certain natural factors.

3.1 Carbohydrates

The most profuse sugars are monosaccharides, which include fructose and glucose that comprise nearly 70% of the total sugar content in honey. The enzyme invertase present in honey is involved in the hydrolysis of sugars from nectar, resulting in the production of monosaccharide fructose and glucose [14]. Rest of sugars comprise 10–15% of the total sugar content of honey including disaccharides and trisaccharides. A daily dosage of 20 g of honey can provide 3% of one's daily energy needs [15]. In addition to fructose and glucose, honey is also known to contain oligosaccharides, maltose, isomaltose, maltulose, gentiobiose, kojibiose, laminaribiose, nigerose, and kojibiose. Furthermore, honey contains 4–5% fructo-oligosaccharides. Fructo-oligosaccharides are a good source of prebiotics that can support the digestive system, as they are indigestible. They also support the microbiota of intestine [16].

3.2 Proteins

The rough estimate of protein is about 0.5%, which includes amino acids and enzymes; however, the contribution of these proteins in meeting daily requirements is marginal. The main enzymes are amylase, invertase, and oxidase [17]. Proline, which makes up 80–90% of all the amino acids in honey, is the main amino acid found in it. In addition to this, other amino acids are also present (both essential and nonessential) alongside proline in honey [18].

3.3 Vitamins and minerals

Potassium (K) is the main mineral found in honey, with amounts ranging from 0.1 to 1.0%, followed by magnesium (Mg), sodium (Na), sulfur (S), calcium (Ca), and phosphorus (P). Other than these, trace minerals include copper, manganese, iron, and zinc. Some vitamins are also present including vitamin B6, pantothenic acid, B2 complex, thiamine (B1), vitamin C, nicotinic acid, and riboflavin [19, 20].

Bioactive compounds	Amount	Reference
Carotenoid content	0.6–6.2 mg/kg	[25]
Proline level	4.6 mg/kg	[26]
Total phenolic content	1.3–126 mg (GAE)/100 g	[25]
Total flavonoid content	1.9–4.2 mg (QE)/100 g	[26]
Salicylic acid	8.2–94.8 μg/100 g	[27]
Naringin	4–32 μg/100 g	[27]
Taxifolin	12–1920 μg/100 g	[27]

GAE: gallic acid equivalent QE: quercetin equivalent.

Table 1.
Bioactive compounds in honey.

3.4 Polyphenols

Several polyphenols have been found in honey during the last few decades. According to a study, several varieties of honey contain 56–500 mg/kg of polyphenols. The major flavonoid in honey is chrysin, pinobanksin, and pinocembrin, while the minor flavonoids include kaempferol, galangin, quercetin, and isorhamnetin [21, 22]. An average amount of phenylacetic acid, leptosin, methyl syringate, and methoxyphenylacetic acid were found. Other constituents include various 1,2-dicarbonyl compounds [23].

3.5 Other components

Honey also contains polyphenols and organic acids like acetic acid, butyric acid, and citric acid [24]. The key flavonoids in honey are chrysin, pinobanksin, and pinocembrin, while the minor flavonoids include kaempferol, galangin, quercetin and isorhamnetin [21]. Over 600 different volatile compounds have been found in honey as shown in **Table 1**.

Gallic acid, pimaric acid, and pimaric acid isomers were discovered in the cerumen of stingless bees, which demonstrated the powerful antioxidant effect of honey [28]. According to Souza et al. [26], gallic acid is the most prevalent phenolic compound found in the Brazilian species *Apis mellifera*, with lesser levels of cinnamic, protocatechuic, quercetin, and p-coumaric acids being found.

4. Therapeutic properties of honey

4.1 Antioxidant effect

DPPH technique was used to check antioxidants in honey since it is a simple, exact, and precise technique [29]. The IC50 constraint, which addresses the concentration of the material necessary to hinder half of the free radicals, was used to resolve the antioxidant assessment in light of the scavenging activity against the free radical DPPH. As a result, honey's lower IC50 value indicates that it contains more antioxidants and has a greater ability to neutralize free radicals. For Sumer testing, honey samples had antioxidant levels between 7.8 and 48.6 mg/ml; for *Sidr* tests, between 33.8 and 72.3 mg/ml; and for multiflora tests, between 91.2 and 190.1 mg/ml. According to their more obscure variety and greater phenolic content, *Sumer* honey had the highest antioxidant levels of all the honey samples. A survey described the antioxidants (IC50) in *Acacia* from different countries; their quality ranged between 10.5 and 111.1 mg/ml. Comparative results were published in the study. Also described were the multiflora honey's antioxidant levels, which ranged from 4.4 mg/ml in Croatian honey to 358 mg/ml in Czech honey. Iranian honey had an IC50 of 5.9–89.7 mg/ml compared to 84.9–168.9 mg/ml for Portuguese honey [30].

4.2 Antimicrobial effect

Honey shows antibacterial action against several bacteria in different environments. Honey has exceptional antibacterial effect against MRSA and several varieties of Pseudomonas, which are frequently linked with burn and wound infections. Manuka honey shows efficiency against several pathogenic microorganisms, including *S. aureus*, *Salmonella*, *Enterobacter erogen*, and *Escherichia coli* (*E. coli*) [7]. Honey's antimicrobial properties are mostly related to its acidity, osmolality, glucose oxidase enzyme, and production of hydrogen peroxide. Gluconic acid is formed from glucose by this enzyme; hydrogen peroxidase is also produced as a by-product in this reaction. Hydrogen peroxide is responsible for antimicrobial activity against bacteria [31].

4.3 Anti-inflammatory effect

Quantitative analysis of total phenolics, flavonols, and flavonoids was conducted through HPLC for the determination of radical scavenging activity and the anti-inflammatory activity through in vitro studies. The phenolics were found to be 663.22 mg of the gallic acid per 100 g, while the flavanols and flavonoids were found to be 3.16 and 3.61, respectively [32]. An experiment demonstrated the effect of honey on reepithelialization through various cellular responses when loaded with nanofiber technology, which resulted in it showing antioxidant and anti-inflammatory properties. Further, the assay of markers including interleukins and cyclooxygenases confirmed its role in anti-inflammatory action [33].

4.4 Wound healing

A study was carried out to find the effect of a hydrogel prepared by using honey on the healing of wounds and antimicrobial activity. The gel was used in different concentrations and showed 75% antimicrobial activity and in vivo healing of burns in mice. The result was surprisingly amazing as this gel was 75% more effective than the commercial gel used for burns [34].

In another investigation, the intrinsic production of hydrogen peroxide was used to identify the mechanism involved in wound healing. The human keratinocyte cell lining was used, which showed that H_2O_2 through a specific aquaporin moves to the plasma membrane where it induces the entrance of extracellular calcium through a receptor and the Orai-1 channel due to calcium-ion-channel redox regulation and hydrogen peroxide production. The calcium route is involved in tissue regeneration during wound healing [35].

4.5 Antiulcer

A common disease affecting humans. A study was done to find out how honey affected artificially induced gastric ulcers. The mechanism was determined by using four groups of rat model through the examination of stomach macroscopically. The result showed that there was a reduction in mucosal NO, GSH, lipid peroxide, and superoxide dismutase (SOD). Honey significantly decreased the ulcer index, prevented lesion formation, preserved the stomach's glycoprotein content, and decreased plasma levels of IL-6 and TNF-alpha. Honey exerts its antiulcer effect due to certain enzymatic and nonenzymatic antioxidants and by reducing cytokine levels in the body [36].

Honey has also a protecting effect on oral ulcer. Hence, a study was conducted to determine an effective method for using honey. For that, three groups were created with one as control, while the other two were given honey, to one in adhesive form and to the other in gel form. Although there was complete healing in both of the groups, microscopically a significant difference was observed, with the gel having a higher mean value. So, it could be concluded that the therapeutic value of gel is more than that of the adhesive form in wound healing [37].

4.6 Antidiabetic

To ascertain the impact of honey consumption on individuals with type 1 diabetes, a randomized crossover trial was done. Twenty patients of 4–18 years of age were taken and were given a dietary intervention of honey in an amount of 0.5 ml per kg/day for 12 weeks. The research resulted in significant decline in the skinfold thickness, total cholesterol, fasting serum glucose, serum triglycerides, and LDL and elevation of C-peptide, thereby suggesting that a long-term use of honey may have a great impact on reducing type 1 diabetes [38]. Another randomized trial conducted on type 2 diabetes resulted in increased HbA_1c but decreased anthropometric measures [39].

4.7 Anticancer

Human cervical and breast cancer cell lines, as well as normal breast epithelial cell lines, were treated with honey for 72 h to study the anticancer potential of honey. This resulted in elevated lactate dehydrogenase, increased apoptosis in cancerous cells, decreased mitochondrial potential, and the activation of caspases 7 and 9, thus indicating mitochondrial-based apoptotic pathway in human cervical and breast cancer cell lines [40]. A clinical experiment demonstrated honey's effectiveness against head and neck cancer; following 6 weeks of radiation therapy, the honey group saw a lower proportion of oral mucositis than the control group [41].

4.8 Cardioprotective effect of honey

Heart is an important organ of the body. A study determined the effect of chronic intake of honey on cardiac arrhythmias in rat heart; honey was fed to the rats for 45 days; then, after giving anesthesia, their hearts were separated. The result of the ECG test showed that honey significantly declined the ventricular tachycardia and time of reverse ventricular fibrillation [42]. Honey also affects the HDL, LDL, VLDL, and total cholesterol, thus reducing the risk of heart disease. An animal trial showed a decrease in serum LDL and an increase in HDL, VLDL, and TG in comparison with

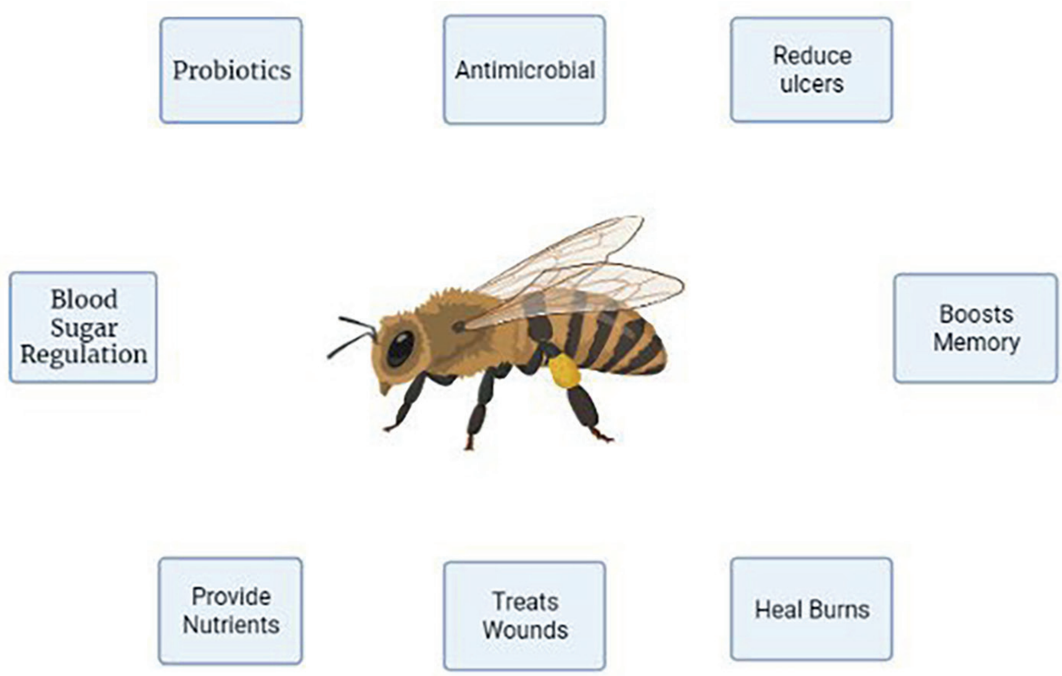

Figure 1.
Nutritional properties of honey.

the control, concluding that the honey can improve lipid profile and decrease the cardiac disease risk (**Figure 1**) [43].

5. Honey as a functional food

Honey can play a useful part in the food industry as functional and nutraceutical substance. Honey-based edible coatings are being utilized in the food industry for fruit preservation because of the antimicrobial and dehydrating properties of honey [44]. Honey can be used as treatment for coughs and sore throat when mixed with lime juice. Research showed the beneficial impact of honey in the food industry. Honey can impart desired characteristics like texture, flavor, and color, especially in pastes, butters, and spreads. Honey is being used in the baking industry as a natural sweetener because of its unique flavor and supportive chemical properties. It would improve the appearance, nutrition, and taste when use in food production [45].

Honey is one of the most important functional foods, which contains protein, minerals, and vitamins. It maintains the face and skin while strengthening memory and the immune system. The sweetness level of honey is higher than sucrose (table sugar) because honey captures its sweetness from glucose and fructose. Honey imparts 33 more calories as compared to sucrose. Due to the presence of oligosaccharides, honey can individually support the gut microbiota by accelerating the growth of probiotic *bifidobacteria* and *lactobacilli*, consequently improving host metabolic relationship [46].

Ramya and Anitha [47] prepared muffins with the addition of honey along with regular sugar. Results showed that there is increase in volume of muffins containing honey as an ingredient. The organoleptic properties of muffins exhibited substantial variation in different parameters like color, flavor, texture, and their overall acceptability. Results showed momentous variation of muffins relieved with honey as linked to control. Spray drying of sweeteners is also an emerging technology in the food sector.

Honey is also produced as spray-dried honey. Using honey as natural sweetener, numerous products are being developed in food industries. Deneesha Madunimani et al. [48] developed a cinnamon-based ready-to-eat drink using honey as sweetener.

6. Novel techniques to detect impurities in honey

For several decades, natural honey of bee has been the topic of research purpose. However, with its biological, prophylactic activity and diversity, and nutritional properties, it still surprises scientists. As a result, techniques for assessing its quality are continuously improved and changed in an effort to dispense with costly and dangerous reagents, speed up analysis, improve accuracy, and lighten the workload. Many scientists are looking for methods that will make it quicker and easier to find contaminants in honey [49].

Adulterants in honey are typically discovered using physicochemical techniques. Chemical analyses using diastase, fructose, sucrose, glucose, and HMF can be used to detect the adulteration of honey by cane sugar syrup, invert sugar syrup, and crystallized cane sugar. Physicochemical characteristics of the honey, including color, moisture, fructose, free acidity, electrical conductivity, glucose, sucrose, and HMF, can be used to classify it geographically [50]. Because detecting honey adulteration is difficult, new adulterant detection technologies are continually being developed.

6.1 Spectroscopic techniques

Many adulterants in food can be detected using infrared (IR) spectroscopy, which is considered superior than other approaches. There is little to no sample preparation required, and just a small number of samples are needed for analysis. Additionally, the technique is regarded as simple-to-perform, nondestructive, quick, and inexpensive. Because of this, the technique may be portable and enable on-site analysis of adulterants in honey. Another alternative for on-site use is Raman spectroscopic analysis, which is nondestructive and requires little sample preparation. The apparatus can be made portable and is comparable to IR spectroscopy in terms of cost, simplicity, and speed. An advantage over IR is that there is no fluorescence interference on the samples [51]. In addition to this, fluorescence spectroscopy may also be an important candidate for the authentication of the honey based on the flourophores that provide the excitation and emission bands at specific wavelength combination.

6.2 Electronic tongue

Food for mankind is judged by our senses, which assist us in determining the product's acceptability and quality. Emerging technology known as "biomimetics" will further research by simulating human senses to create things like an artificial tongue. Although an electronic tongue has been used in numerous food assessments, only a few research have used it to analyze honey. For instance, electronic tongue was utilized to research the botanical and geographic sources of honey, as well as the physiochemical characteristics of both pure honey and honey adulterants [52, 53].

6.3 Microscopic analysis

Microscopic analysis is used to find adulterants as well as identify the botanical and geographic origins of honey. It is more accurate to combine microscopic analysis for adulterants with additional procedures such as PCR, HPLC, and physicochemical analysis. Particularly in developing countries where alternative technologies are prohibitively expensive, microscopic methods may be helpful [54].

6.4 Immunoassays

An analytical method known as an immunoassay relies on the idea of immunology and is based on the interaction of an antigen and an antibody. The body produces an antibody, a glycoprotein, in response to exposure to an antigen, which is a foreign body. In a favorable environment, these antigens trigger the production of antibodies. Immunoassay is a technique for detecting foreign entities (antigens) in a sample matrix, which can be proteins or smaller molecules. A new method of checking contaminants has been developed based on enzymes and honey proteins [54].

6.5 Thixotropicity

Thixotropy, viscoelastic and flow behavior, creep, shear stress, crystal formation, and nitrogen concentration can all be used to identify carbohydrate adulterants like sucrose syrups, fructose, and glucose. The ability to identify adulterants is influenced by the honey's solubility, temperature, and length of storage. The sensitivity of adulterant detection utilizing viscoelastic behavior is unknown, despite the fact that it is effective in identifying the presence and absence of carbohydrate adulterants in honey. More improved detection technologies are necessary for quantification. More research is required to determine the thixotropicity of honey with other adulterants before choosing a method that would be considered viable for a honey adulterant kit. After establishing a carbohydrate adulterant detection tool, glucose, sucrose syrup, and fructose adulterants could be recognized [55].

7. Conclusion

Honey can be considered a natural antioxidant medicine, and it is one such promising nutraceutical antioxidant. The phenolic compounds, which include polyphenols and flavonoids, are involved in preventing or lowering the risk of a number of human disorders, including ulcers, tumors, chronic inflammation, diabetes, cancer, and cardiovascular diseases. Studies show the beneficial effect of honey in wound healing. In the food industry, honey is used as a functional and nutraceutical ingredient in the preparation of novel food products. Some novel technologies like spectroscopy, electronic tongue, microscopy, immunoassays, and thixotropicity may be considered helpful to detect adulterants in honey. This chapter provides significant indications about the use of honey in both food and medical sectors.

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