Chapter 7

Natural bee products and their apitherapeutic applications

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Introduction

Modern pharmaceutical medicine is undergoing a major crisis because of the adverse effects of synthetic drugs on human health and increased resistance to micro-organisms. Increased rates of cancer, autoimmune diseases, chronic diseases, etc. have led to a search for new, reliable, non-synthetic, traditional and natural therapeutic products [1]. Science is now returning to natural products with new approaches in an endeavor to understand older medicinal applications.

The word "apitherapy" (bee therapy) derives from the Latin *Apis mellifera* L., meaning "honey-bearing bee." It represents a very ancient medical practice and is one of the areas in which bee products are used. Treatment encompasses a wide range of natural bee product therapies, in a range of modalities, and has come to be regarded as a complementary and traditional form of medicine in recent decades.

The honey bee has been of enormous importance to humanity for thousands of years. Civilizations throughout history have used honey for therapeutic purposes and for its nutritional properties, as well for its biological activity. Texts concerning apitherapy date back to ancient Egyptian and

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Sumerian inscriptions and writings. Honey was used in ancient Egypt in the treatment of joint inflammation. Ancient Egyptian vases, ornaments and jewelry show propolis being collected from trees, and even being used to mummify the dead. Ancient Sumerian tablet writings from 2100 to 2000 BC describe the use of honey in the form of medications and ointments [2]. Honey was of particular importance to the ancient Egyptians. Hippocrates wrote in 460–367 BC that, "Honey cleans wounds and ulcers of the lips, heals carbuncles and running wounds." The use of honey is also described in various religious texts, including the Veda and the Bible [2–5].

Recent years have seen great interest in functional food products, as well as bee products, due to growing interest in the physical and mental well-being of consumers. This chapter describes such products as honey, propolis, pollen, bee bread, royal jelly and bee venom.

Honey

Honey is produced by honey bees (*A. mellifera* L.) from the nectar and dews of different plants. However, its composition and biological active properties depend on many environmental factors. The dry weights of honey consist of 95% sugars, with the remaining 5% consisting of various peptides, proteins, enzymes, essential oils, organic acids, polyphenols, vitamins and minerals [4,6]. Numerous criteria for the purity of honey have been established by the international food codex agents (IHC, CEU, TSE, etc.). These include water content (moisture), acidity, pH, conductivity, color value, proline, diastase activity, sugar ratios (%C4/C13), and hydroxymethylfurfural (HMF) and insoluble particles [7]. However, although these criteria are critically important for the purity of the honey, they tell us nothing about its bioactivity potential. The bioactivity potential of honey depends on its antioxidant, antimicrobial (bacterial, fungal or viral), antiinflammatory, and antitumoral properties, which in turn derive from the strain of honeybee, geographical region, climate, and the nectar source involved [8] (Fig. 7.1).



FIG. 7.1 Honey sample.

Honey has been used throughout history, both as a foodstuff and as a therapeutic product due to its nutritional value and biological activity. The antimicrobial activity of honey was first scientifically reported in 1892 [8]. Honey was used to treat wounds suffered by Russian soldiers in World War I, and honey dilution was observed to increase antibacterial activity [9]. Hundreds of studies have since shown that honey possesses good and effective antimicrobial activity against many pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella zature*, *Salmonella typhimurium*, *Micrococcus luteus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Helicobacter pylori*, *Enterobacter cloacae*, *Moraxella catarrhalis*, etc.) and fungi (*Candida albicans*, *Aspergillus niger*, *Candida tropicalis*, etc.) [10–13]. Studies have shown that the antimicrobial activity of honey derives from four main factors, osmolality of sugar density, acidic medium (pH 3, 5–4, 5), H_2O_2 induced by glucose oxidase and many secondary substances, such as organic acids, polyphenols, and volatile compounds [7,14,15].

There are various different types of honey, including blossom, honeydew, monofloral, hetero floral, dark, light-colored, raw, organic, and adulterated honeys. Although all honey should be organic and pure, various factors, and particularly economic concerns, can reduce its naturalness. These include the use of antibiotics and synthetic agents, feeding bees with sugar syrups, and the use of agricultural pesticides, all of which can impair the organic or natural structure of honey. Honeys which are not treated under high heat (under 45 °C) are known as raw honey [16,17]. Honey is classified under two groups according to the flora involved, blossom and honeydew. Blossom honey is collected from flower nectars, while honeydew is collected from various different dews, or from insects or leaves, by *A. mellifera*. For example, pine honey is produced from the dew of *Marchalina hellenica*, and oak and fir are produced from secretions caused by sweating in the tree's leaves [18–21].

Although the composition of honey is affected by many environmental factors, climate, floral sources, and production techniques, the Codex Aliment Arius [22] standardized honey composition on the basis of floral origins. Moisture should be $<20\%$, free acidity should not exceed 50 milliequivalent acid/100 g, and electrical conductivity should not be >0.8 mS/cm in blossom honey [22]. Approximately 95% of honey dry matter is composed of carbohydrates. The main sugars in honey are fructose and glucose, and 20 or 25 different mono-, di-, tri, and oligosaccharides may also be present [7]. The fructose to glucose (F/G) ratio is usually higher than 1.20. The probability of honey crystallization decreases as the F/G ratio increases [23]. In general terms, dark honeys such as chestnut and oak honey have F/G ratios >1.5 and are not easily crystallized. Di-, tri- and oligosaccharides, such as melezitose, maltotriose and raffinose are usually found in dew honeys, and are used as honeydew markers [24].

The adulteration of honey is a serious problem. It is causing numerous economic concerns, and it is exceedingly difficult to discriminate between pure and adulterated honey, which in turn impacts on honey quality and safety. This adulteration can be performed in many different ways, including feeding sugar syrups, such as starch, sucrose and corn syrup to the bees. Adulterated honey loses its apitherapeutic activities, depending on the degree of adulteration. C13 isotope and C4 sugar analyses are today used to detect honey adulteration. However, while this technique is suitable for identifying honey made from sugar cane and corn syrup, it is not particularly suitable for beet sugars [23,25].

Honeys with high bioactivity are available for complementary medicine practitioners in developed countries. There are many different kinds of apitherapeutic potential honeys, such as Manuka, Tualang and chestnut, cider, and acacia honeys. Manuka honey has high antibacterial activity, which derives from its methylglyoxal (MGO) content, and is known as Unique Manuka Factor (UMF) [6,9,26].

One important component of honey is enzymes from honey bees. The presence and the activities of these enzymes provide valuable information about the natural status and freshness of the honey, since their activity decreases in old or heated honey. Diastase, invertase, glucose oxidase, and catalase are the main enzymes ın honey [27–29]. The Honey Codex (EU Directive 110/2001) sets a minimum value of 8 units on the Schade scale for diastase activity. However, in some types of honey, such as citrus honey, the minimum value is 3 units [29].

In general, dark honeys have high phenolic contents as well as high antioxidant capacities and other biological features [30–32]. The total phenolic content (TPC) of honey is a good marker for determining antioxidant capacities. In a study of Portuguese honeys, the phenolic contents of honey were measured in the three different colored honeys. TPC values were 22.61 ± 2.20 mg GAE/kg in light-color honeys, 406.23 ± 1.72 mg GAE/kg in amber-color honey and 727.77 ± 2.30 in dark-color honeys [32]. A similar study was performed in Turkey with monofloral honeys; TPCs of some dark-colored honeys such as chestnut, oak and heather were higher (>60 mg GAE/100 g) than those of some light-color honeys (<60 mg GAE/100 g) such as acacia, rhododendron, and lime honey [32].

Numerous scientific reports have indicated that honey is a good ointment agent for treating various kinds of wounds [33–36]. The healing effect of honey derives either from antimicrobial activities or else from the antiinflammatory potential of several flavonoids present in it. The antibacterial properties of honey are related to its acidity, high osmolality and hydrogen peroxide. Non-peroxide factors, such as lysozyme, phenolic acids and flavonoids, also contribute to its antibacterial properties [37].

Honey has been used as a foodstuff since ancient times. However, increasing obesity and insulin resistance due to excessive sugar intake have rather

limited the consumption of honeying in recent decades. The honey glycemic index (GI) may vary depending on botanical origin, since the sugar composition of honeys is dependent on floral sources [38–40]. The GI of German honey species has been reported to range from 75 to 82 [41], and between 44 and 69 in Turkish honeys [39]. The main factor determine GIand the insulin emic indexes the F/G ratio, which exhibits negative correlation with GI.

Propolis

Propolis, or bee glue, is the general name given to the resinous substance collected from plant buds and shoots by honey bees. The word means "defender of the city" in Greek, and the substance is responsible for the protection of bees and hives. Bees use propolis to seal holes and the honey combs and to fix the inner walls of the hive, as well as to prevent the bodies of intruders who have died within the hive decaying. It is used against a broad range of micro-organisms in the hive and in keeping intruders away. Propolis was also employed for mummification rituals in ancient times [42]. Today, propolis is used for many purposes, as an important apitherapeutic agent, and as a functional food additive [43]. The chemical composition of propolis varies depending on the geographic region, climate, environmental conditions and collecting season, and mode of production [43,44]. Propolis is normally a dark yellow or brownish resinous material whose chemical composition is dependent on many environmental factors. Raw propolis consists nearly entirely of resin (40–50%), wax (10–30%), essential compounds (2–6%), pollen (2–5%), polyphenols (2–20%) and numerous other organic molecules such as vitamins, and sugars [45–48]. These molecules are responsible for its biological active properties. Studies have shown that propolis has many biological active features. Although it's entire composition is responsible for propolis' quality and biological active properties, phenolic compounds are particularly responsible for the antioxidant, antimicrobial and antiinflammatory properties of propolis. Nearly 300 compounds have been identified in different raw propolis species, such as phenolic acids, flavonoids including flavones, flavanones, flavones and chalcone, stilbenes, steroids and lignans [42,49–51] (Fig. 7.2).

Plant bud resin is the main reported source of propolis. Poplar spp. (*Populus alba*, *Populus tremula* L. and *Populus nigra* L.) in particular have been reported as a primary source of propolis in temperate zones (Europe, North America, and non-tropical regions of Asia). Less important sources of European propolis are *Betula pendula* L., Acacia sp., *Aesculus hippocastanum* L., *Alnus glutinosa*, *Pinu*s sp. and *Salix alba* L. [52,53].

Since the chemical composition of propolis depends on the phytogeographic characteristics of the site of collection, and bees select different plants as sources of propolis in different habitats, propolis is known by regional names [54]. For example, Brazilian propolis is the most widely known and



FIG. 7.2 Raw propolis.

studied variety. There are 13 different kinds of Brazilian propolis, and green and red Brazilian propolis are particularly popular [42,55,56]. South Brazilian green propolis is collected from the native shrub *Baccharis dracunculifolia* L. [44] its green color derives from the chlorophyll collected by bees which is present in young tissues and non-expanded leaves of *B. dracunculifolia*. The green propolis is rich in flavonoids (e.g., ramnocitrin, eupalitin, and acacetin) and terpenoids (e.g., α- and β-amyrin, lupeol), 3,5-diprenyl-4-hydroxycinnamic acid (artepillin C) [56], 3-prenyl-4-dihydroxycinnamoiloxy cinnamic (baccharin), 3-prenyl-4-hydroxycinnamic acid (drupanin), aromadendrin-4′-*O*-methyl-ether and kaempferide [57,58]. Brazilian red propolis is collected from *Dalbergia* spp. (*Dalbergia ecastophyllum*) [59]. Biochanin A, formononetin, liquiritigenin, pinocembrin and daidzein are largely found in red propolis [44,59–61].

The global variety of biologically active propolis is wide. European propolis is mostly collected from poplar and contains aromatic acids (e.g., cinnamic acid, caffeic acid, ferulic acid), aromatic esters (i.e., cinnamic and caffeic acid phenyl esters (cape)), volatile compounds (e.g., geraniol, nerol, farnesol, β-eudesmol), aromatic compounds (e.g., vanillin), hydrocarbons (e.g., eicosane, tricosane, pentacosane), steroids (e.g., cholinasterol, fucosterol, stigmasterol), flavonoids (e.g., tectochrysin, pinobanksin, pinocembrin, chrysin, galangin, apigenin, kaempferol), fatty acids (e.g., palmitic acid, melissic acid, cerotic acids), micro- and macronutrients (i.e., Ca, K, Mg, Na, Zn, Fe, Mn, Al, Ba, and Cl), vitamins (i.e., B1, B2, B6, C, and E), and essential oils, which are mainly represented by monoterpenes and sesquiterpenes [62]. Propolis from Portugal [63,64], Spain [65], Turkey [51,65], Morocco [66], South Korea [67] and China [48] also contains numerous different bioactive compounds, and many different apitherapeutic functions have been reported. Almost every propolis, regardless of type, contains the following active substances: coumaric acid, ferulic acid, gallic acid, pinocembrin and galangin, caffeic acid, daidzein, quercetin, rutin, luteolin, and caffeic acid phenyl esters (CAPE) [62,68,69].

Raw propolis cannot be consumed as a feedstock, and must first be purified. Important pharmaceutical compounds such as different polyphenolic substances must be removed from the waxy material. The most suitable technique for the production of raw propolis is ethanol extraction, due to the fact that active substances are more easily soluble in ethanolic fraction [43,44]. Although 70% ethanolic solvent is the best extraction solvent for raw propolis, different polar and non-polar solvents are also useful, such as water, glycerol, polyglycerol, triacylglycerol, etc. [70]. Raw propolis extracts are first macerated, and then extracted using different extraction techniques, such as Soxhlet apparatus, shakers, magnetic stirrers, ultrasound extraction, and microwave extraction process [70]. In general, ethanolic propolis extracts are used in the form of tablets, drops, or sprays, and are also mixed with other natural products. The quality of raw propolis is measured by quantification of total phenolic, while total flavones/flavonols and total flavanones/dihydroflavonols are used to measure active principles [68].

Various pharmaceutical formulations of propolis are currently used for a range of different purposes, including as functional foods and cosmetics, such as candies, chocolates, candies, shampoos, skin creams, antiseptic solutions and toothpastes [71]. However, the application of ethanolic propolis extract is limited, because it is alcohol-soluble and has a strong flavor. Different encapsulation techniques are available for increasing the target or bioavailability of bioactive propolis particles. Encapsulating agents such as chitosan, maltodextrin, gum arabic, alginate, soy bean, pectin, etc. are employed for the conversion of propolis extract containing numerous hydrocolloids into dry powder using spray-drying methods [72,73].

Propolis possesses strong antibacterial activity and has a range of numerous pathogenic micro-organisms, including antifungals, and yeast [74–76]. This activity is reported to be due to flavonoids and aromatic acids and esters present in the resin [77], but the relationship between the structure and antibacterial activity of propolis constituents is unknown. Furthermore, propolis has been reported to exhibit synergism with a number of antibiotics against various bacterial species [78] and to be active against methicillin-resistant *S. aureus* [77]. The mechanism involved in this antimicrobial activity is reported to be complex and may be attributed to a synergism between phenolic and other compounds in the resin [75].

Propolis is a polyphenol source and an effective natural compound for the production of chemoprevention agents. In vitro and small-scale in vivo and clinical studies have shown that propolis extracts stops the proliferation of many different cancer cells and increases apoptosis [79]. Recent studies have generally attributed the numerous antitumoral effects of propolis to various different polyphenols, especially flavonoids, found in propolis extracts, including CAPE [80,81], rutin and quercetin [82], chrysin [83], artepillin C [5], Daidzein [67,84], Biochanin A [42,60], and luteolin [85], a flavonoid of interest in terms of identifying cancer-related signaling molecules.

As cancer cells develop multidrug resistance during chemotherapy, this opens a new avenue of research into cellular mechanisms of propolis components in combined treatments designed to overcome this problem. This antitumoral effect may be explained by different inhibition mechanisms, such as the ability to inhibit DNA synthesis in tumor cells, to induce apoptosis, and to activate macrophages to produce factors capable of regulating the functions of B, T and natural killer cells [75,79]. Chinese propolis has been reported to exhibit good cytotoxic activity on human breast cancer ER (+) and MDA-MB-231 (human breast cancer ER (−)) cells [86].

Propolis also possesses good antiinflammatory properties. Extracts exhibit wide antiinflammatory potentials, from diabetic wounds to liver diseases [87–89]. Recent studies have shown that many flavonoids found in propolis inhibit the formation of many interleukins and suppress various metabolic enzymes involved in inflammation, such as elastase, cyclooxygenase and lipoxygenase, hyaluronidase, tyrosinase, xanthine oxidase, and urease [89–94]. Antiinflammatory enzymes can reduce or halt inflammatory mediators (prostaglandins and leukotrienes) which are responsible for the maintenance of the inflammatory process. It also affects the fibrinolytic system, kinins, vasoactive amines (histamine and serotonin), and nitric oxide (NO) capable of leading to inflammation when physiologically altered [93].

Pollen and bee bread (Perga, ambrosia)

Honey bees gather pollens from flowers and pack these into granules. Honey bees use pollen as a source of proteins, lipids, sterols, vitamins, minerals and carbohydrates in the form of plain pollen or bee bread. A colony can collect 50–250 g of bee pollen in a day, and from 15 to 40 kg of pollen a year. Foraging bees carry the pollen to the hive in the form of pollen loads (lumps moistened with a little nectar or honey) in recesses on the shins of the hind legs known as "cages." The average weight of pollen carried by one bee is approximately 7.5 mg. Most pollen consists of a single grain, sometimes attached to two or more grains [95]. These range from 0.01 to 0.05 mm in diameter. Pollen grains differ considerably in shape and size. In the dry state, they are mostly spherical or spindle-shaped, while after swelling, they may be round, triangular, cylindrical, bell-shaped, or thorn-like in cross-section. Pollen analysis is an important marker indicating the authenticity of honey. The color of pollen loads is also variable and reflects the diversity of plant species from which the pollen is obtained. Pollen is usually various shades of yellow, gray-white, orange, reddish, greenish, or blue in color. Some differences in pollen color depend on whether it was collected from already open thecae or else cracked by the bee [95,96]. The pollens collected by bees from different flowers are combined with various enzymes produced by the bees themselves (such as amylase and catalase), thus being subjected to alteration and metabolization, finally emerging in the form of bee pollen granules [97] (Fig. 7.3).



FIG. 7.3 Pollen.

Although the composition of honey pollen is dependent on environmental conditions (e.g., flora, habitat, and season), in general terms, it consists of sugars, proteins, minerals and vitamins, fatty acids, and a small percentage of other components. Studies have shown that dried bee pollens contain proteins (5–55%), reducing sugars (13–55%), lipids (4–7%), crude fiber (0.3–20%), and minerals (0.2–1.0%), the remainder consisting of vitamins and polyphenols. Bee pollen is also a balanced foodstuff for bees and humans. Many essential elements, including Ca, Mg, Fe, Zn, and Cu, with a high K/Na ratio, vitamins including pro vitamin A (β-carotene), vitamin E (α-tocopherol), niacin, thiamine, biotin and folic acid, and many co-enzymes are found in honey bee pollens. Bee pollens also contain numerous saturated/unsaturated fatty acids, phospholipids (1.5%), phytosterols, i.e., β-sitosterol, β-sitosterol (1.1%) and terpenes. Different colored bee pollens may contain different qualities and quantities of carotenoids pigments (i.e., lycopene, and zeaxanthin) and polyphenols, phenolic acids, flavonoids, and anthocyanins [98–100].

Bee pollen has been used as a food and medicine from ancient times to the present day. It contains primary and secondary metabolites, and exhibits wide range of bioactive properties, such as antioxidant, antiinflammatory, anticarcinogenic, antibacterial, ant fungicidal, hepato protective, and antineurodegenerative activities [88,101–103].

One of the major secondary metabolites of bee pollen, similarly to honey and propolis, is polyphenols. The TPC of bee pollens is nectar source-dependent. Reported TPC values have been reported of between 10.8 and 17.64 mg GAE/g for walnut bee pollen [100], 28.87 mg GAE/g dry weight for chestnut pollen [101], and between 16 and 36 mg GAE/g for Turkish pollen [102]. Findings have shown that bee pollen contains approximately 0.30–3.0% polyphenols. When bee pollen total polyphenolic content was

compared with that of honey and propolis samples in ethanolic solvents in one study, the values were 10.76 mg GAE/100 g for honey, 54.27 GAE/g for bee pollen, 254.42 mg GAE/g for propolis [103]. The mechanism of the antioxidant activity is also related to the presence of polyphenols, which reduce many oxidant agents or scavenging and free oxygen radicals, such as superoxide, peroxide, and hydroxyl [98,104,105]. Flavonoids are low molecular weight subunits of polyphenols and have been identified as the most abundant and most common polyphenol in bee pollens. Quercetin, rutin, pinocembrin, caffeic acid, CAPE, apigenin, chrysin, galangin, kaempferol and isorhamnetin are the most abundant flavonoids in the pollen [95,106]. Pollens also exhibit antiinflammatory and cytotoxic properties [107].

Studies have shown that pollen extracts exhibit good hepatoprotective activity. Experimental animal studies have reported that bee pollen reduce liver enzymes (e.g., alanine, aspartate transaminase, acid phosphatase) in carbon tetrachloride-induced hepatic damage in rats [88,105,108]. Several studies have shown that bee pollen has greater antimutagenic activities against certain cancer cells. Different pollen extracts have also been reported to exhibit antiproliferative and cytotoxic effects on some cancer cells, such as K-562 cells, leukemia cells, or prostate cancer PC-3 cells [97]. In addition, pollen extracts have been shown to be capable of inducing apoptosis and stimulating secretion of tumor necrosis factors [109].

Bee pollen also exhibits antibacterial effects against many pathogenic micro-organisms, such as *S. aureus*, *Salmonella enterica*, *Candida glabrata*, *Listeria monocytogenes*, *E. coli*, *B. subtilis*, *Klebsiella* spp., *E. faecalis*, *P. aeruginosa* and *fungi Saccharomyces cerevisiae* and *Candida rugosa*, *A. niger* and *Rhizopus oryzae* [97]. All antimicrobial activities were concentration-dependent, and the antibacterial effect was probably associated with the properties of glucose oxidase, an enzyme produced by honeybees, and phenolic acids and flavonoids. The mechanism of action of flavonoids and phenols against bacterial and fungal cells involves the degradation of the cytoplasm membrane, leading to loss of potassium ions and initiation of cell autolysis [97,110].

Bee bread (Perga)

Bee bread, known asperga or ambrosia, is another highly valuable natural bee product. Honey bees store bee pollen in the colony in the form of bee bread, which is then consumed by adult bees and fed to the larvae. Bee bread is produced by lactic acid fermentation, probably performed by micro-organisms such as bacteria or yeasts or both, although the precise mechanisms are still unclear [96,111]. The pollen is stored in the hive, mixed with saliva, sealed with a drop of honey in the honeycomb cells, and matures after few weeks, resulting in a stable and more nutritious food for honeybees than fresh pollen [111]. Honey bee pollen for human consumption is harvested from hive traps



FIG. 7.4 Bee bread (perga) from a beekeeping frame.

and then stored [112]. It is very easy to use because it is very highly soluble in water, although the cellulosic membrane of bee pollen makes digestion difficult for humans. Honey bee bread has a high probiotic value and a higher yield than normal bee pollen, and is another new health-oriented product [96] (Fig. 7.4).

The fermented pollen mixture is consumed as a food supplement. The recommended daily dosage for an adult human is 20–40 g, and there is no evidence of bee bread overdosing. Beneficial effects of diet supplementation with bee bread has been reported in animals and humans [96,113]. A diet containing protein-rich pollen and bee bread is has been shown to be suitable for children with lack of appetite. It is also especially beneficial for enhancing the immune system, for patients after surgery, and for elderly patients in whom malnutrition is a common cause of morbidity and mortality [96].

Bee bread specimens from Northeast Portugal have been shown to contain 32 flavonol glycoside derivatives, such as quercetin, kaempferol, myricetin, isorhamnetin and herbacetin derivatives, and to exhibit antitumoral activities against four cancer cells—MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) [113].

Further studies on bee bread are needed, since more is unknown on the subject than is actually known.

Royal jelly

Royal jelly (RJ) is secreted from the hypo-pharyngeal and mandibular glands of young worker bees (*A. mellifera*). One interesting property of RJ is that young female larvae fed on it develop into queen bees, while genetically identical female larvae fed on worker jelly develop into worker bees [114]. RJ is also a superfood consumed only by the queen. It is also used to feed the honeybee larvae after hatching and helps to nurture the brood. This special secretion is provided for the immature young larvae in the first 2–3 days of maturation, in addition to being used as a food specifically for the queen bee throughout her entire life cycle [115]. The question particularly requiring an answer is how queen bees can live for approximately 4 years, while the life span of the worker bees is only some 2 months. It has been suggested that the difference between queens and worker bees in some way involves the dietary complements present in the RJ. Although the mechanism is unclear, some form of epigenetic (i.e., nutrigenetic) changes may be involved [115]. Short chain-hydroxy fatty acids and RJ proteins are also thought to play an important role [116,117]. It is therefore believed that humans may be able to extend their own life spans by consuming RJ, similarly to queen bees. RJ is also believed to have an immunostimulatory effect, and has therefore been described as "a secret of life" or elixir (Fig. 7.5).

Commercial RJ is used in two different forms, fresh or lyophilized (Table 7.1). The white and viscous gel consists of water (50–60%), proteins (18%), carbohydrates (15%), lipids (3–6%), mineral salts (1.5%), and vitamins. The compositions of RJ is affected by many factors, such as type of production, seasonal changes, how the bees are fed, and bee breed [118].



FIG. 7.5 Royal jelly.

TABLE 7.1 Principal contents of fresh and lyophilized royal jelly [118].

	Fresh jelly	Lyophilized jelly
pH	3.5–5.5	–
Water %	60–70	<5
Proteins %	9–15	27–41
Lipids %	3–8	8–19
Sugars %	8–12	13–18
Minerals %	0.9–3.0	2–5
Other organic substances (vitamins, polyphenols, organic acids, hormones) %	0.5–1.0	0.9–1.5

The major RJ proteins (MRJPs) (royalactins) belong to a larger family consisting of nine identified proteins with molecular masses in the range of 49–87 kDa and with high homology among themselves [119]. All are glycoproteins, and water-soluble. Major RJ protein 1 (MRJP1) is the most abundant glycoprotein among the soluble proteins. It plays a vital role in honeybee nutrition and larvae development, and exhibits broad pharmacological activities in humans. In addition to its nutritional role, MRJP1 is a key factor responsible for cerebral functions in bees, such as learning, memory and social behaviors. The proteins also exhibit a wide range of pharmacological properties in bee colonies and human health [119,120]. Soluble MRJP1 is found in both monomeric and oligomeric forms, although it exists predominantly as an oligomeric structure under physiological conditions (Table 7.2).

The MRJP1 oligomer, also known as apalbumin, has significantly higher heat resistance and longer storage stability than the monomeric form [114]. Apisimin (M.W. 55, 40 kDa) is a serine-valine-rich peptide exclusively found in honeybees, and has a tendency to form oligomers. RJ peptides hydrolyzed with protease N and 14 antioxidative peptides have been isolated from RJP and shown to exhibit good antioxidative functions [121].

Fresh RJ samples contain lipids (3–8%). The lipid composition is reported to be made up of 80–85% fatty acids, 5–6% waxes, 3–4% steroids and 0.4–0.8% phospholipids. The fatty acid fraction consists of 32% *trans*-10-hydroxy-2-decenoic acid (10-HDA), 22% 10-hydroxydecanoic (Fig. 7.4). The fatty acids are classified as short-chain (less than 6C), medium-chain (6–19C) and long-chain (more than 12C), of which medium-chain fatty acids (MCFAs) exist mostly in the free form [122]. The major fatty acids among the MCFAs

TABLE 7.2 The major royal jelly proteins [118].

Total royal Jelly proteins %	12.83%
MRJPs	11.09%
Apismin	0.13%
Royalactin	0.25%
Royalisin	0.83%
Jelleines	0.37%
Glucose oxidase	0.08%
Apolipophorin III-like	0.08%

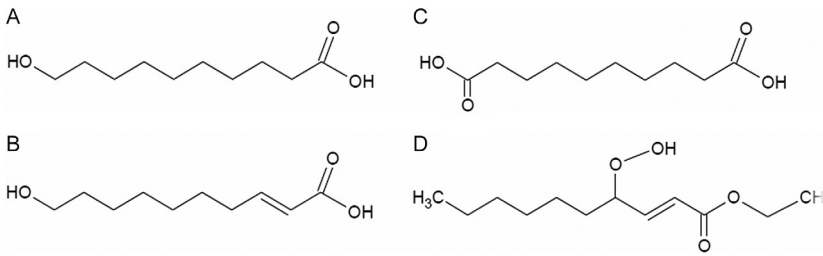

FIG. 7.6 Major Royal Jelly fatty acids: (A) 10-hydroxydecanoic acid (10-HSA); (B) *trans*-10-hydroxy-2-decenoic acid (10-H2DA); (C) decanedioic acid (sebacic acid); (D) 4-hydroperoxy-2-decenoic acid ethyl ester (C—H bonding).

are *trans*-10-hydroxy-2-decenoic acid (10-H2DA), 10-hydroxydecanoic acid (10-HDAA), and sebacic acid (SEA) (Fig. 7.1). *trans*-10-Hydroxy-2-decenoic acid (10-H2DA), an unsaturated hydroxyl fatty acid, is also present in predominant form [46].

It has been suggested that these the fatty acids, which are rarely encountered in other natural products, have wide biological active properties, ranging from neurodegenerative diseases to immunomodulatory effects [123]. In vitro studies have shown that 10-HDA inhibits many pathogenic micro-organisms, such as *E. coli*, *Salmonella*, *Proteus*, *B. subtilis* and *S. aureus* [118]. One of the fatty acids of 4-hydroperoxy-2-decenoic acid ethyl ester has been reported to protect against cell death via activation of the Nrf2-ARE and eIF2α-ATF4 pathways [120] (Fig. 7.6).

RJ consists of adenosine monophosphate (AMP) N1 oxide, adenosine, acetylcholine, polyphenols, and hormones, such as testosterone and progesterone,

while prolactin, and estradiol are other useful bioactive components reported to be present [123]. Randomized clinical studies have shown that RJ is effective in reducing premenstrual syndrome. Due to the specific hormones and fatty acids contained in RJ, it has been reported to be used in the context of various hormone replacement therapies in women. RJ has also been reported to exhibit estrogenic activities through interaction with estrogen receptors followed by endogenous gene expressions [124]. One randomized clinical trial study also indicated that RJ was effective in treating urinary problems and improving quality of life in postmenopausal women [125,126].

Animal studies have demonstrated that a RJ-rich diet reduces follicle-stimulating hormones (FSH) and increases estrogen concentrations in elderly mice [46,124,127].

RJ is also an important natural antiaging compound. The molecular mechanisms responsible for this antiaging activity are associated with six bioactive properties, antioxidant, antitumoral, neurotropic, antiinflammatory, phytoestrogen and antibacterial activities [123].

Further studies are now needed concerning RJ as a functional food, and which areas it might be used in and how, as well as investigating potential side-effects, etc.

Bee venom

Bee venom, apitoxin, is an important weapon that honey bees use for self-defense, and is produced in poison glands in the abdominal cavity. Approximately 0.3 mg bee venom is produced in the venom sac. Bees inject the poison with their stings, although male bees do not possess these. The substance is yellowish, bitter, pungent, and normally liquid. This valuable mixture contains at least 60 identifiable components. Bee venom has a very complex chemical structure consisting of various enzymes, proteins and peptides. Bee venom mainly consists of melittin, apamin, MCD peptide, histamine, hyaluronidase, and phospholipase-A2 [4]. It has also been reported to possess many biological active properties in humans including the immune system, central and peripheral nervous system, and cardiovascular system, antibacterial, fungicide, antiviral, antiinflammatory, antiarthritis, antitumoral, and antineurodegenerative effects, and potential for use in acupuncture and homeopathy [4,128,129]. Bee venom is currently the most the important agent in apitherapy.

Honey bee venom can be collected in two different ways, surgical and electro-shock methods. In surgical collection, bee venom is obtained from the venom gland by squeezing each individual bee until a droplet emerges from the sting. This method is very difficult and time-consuming, and is little used today. The electro-shock method employs a cooling system with a standard electro-shock collecting apparatus in order to preserve more of the volatile compounds. Various different electrical collecting techniques are

available. This technique makes it possible to obtain a much higher quantity of bee poison at one time [130]. Electrically-equipped glass trays are placed in front of the beehives. Following the application of a low-ampere electroshock, the bees deposit their poisons on the tray. The bee venom then comes into contact with the air, and quickly dries and crystallizes. The amorphous bee venom, also known as raw venom, is a dirty yellow in color, and is either crystallized or directly stored in deep freezes.

Studies have also demonstrated that bee venom is an allergen agent also responsible for allergic disorders, such as asthma, allergic rhino conjunctivitis, and atopic eczema, through the generation of allergen-specific CD4+ T cells [131]. In some cases, bee sting leads to an increase in specific immunoglobulins (IgE), interleukin-4 (IL-4) and IL-13, specific cytokines, by overstimulating the immune system, frequently resulting in death [131,132].

Honeybee venom consists of numerous enzymes, peptides, and proteins, a variety of smaller molecules (amino acids, catecholamine's, sugars, and minerals), and lipids, although the main components are proteins and peptides.

The main peptides and proteins are melittin, apamin, MCD peptide, secapine, pamine, minimine, adolapine, procamine A, B, protease inhibitor, tertiapine, cardiopep, and melittin F.

The main enzymes identified to date in bee venom are phospholipase A2, phospholipase B, hyaluronidase, phosphatase, α-glucosidase, lysophospholipase, and acid phosphomonoesterase. Histamine, dopamine, noradrenalin and aminobutyric acid are some of the identified biogenic amines in bee venoms. Glucose and fructose are the main sugars and P, Ca and Mg the main minerals [4,129]. Some pheromones of iso-pentyl acetate; *n*-butyl acetate; isopentanol; *n*-hexyl acetate; *n*-octyl acetate; 2-nonanol; *n*-decyl acetate; benzyl acetate; benzyl alcohol; (2)-11-eicosen-1-ol are also present in bee venom [133]. Bee venom has also been used as a supportive therapy in recent years, especially for rheumatoid arthritis, multiple sclerosis, cancer, and shingles [134–136].

The main pharmacological component of bee venom, constituting approximately 50% of its dry matter, is melittin. This water-soluble protein consists of 26 amino acids with a molecular weight of 2840 Da. Many studies have reported that melittin is a very active peptide exhibiting antiviral, antibacterial, antifungal, antitumoral and antiparasitic activities [133]. The proposed activity ion of melittin has been attributed to its non-selective cytolytic peptide structure, which physically and chemically disrupts all prokaryotic and eukaryotic cell membranes [133] (Fig. 7.7).

Melittin has also been shown to exhibit a variety of anticancer effects, either in preclinical cell culture or animal model systems [133], including lung cancer cells (A549 and NCI-H460) [136], ovarian cancer cells [137], angiogenesis of human hepatocellular carcinoma BEL-7402 [138], prostate cancer cells [134], and liver cancer cells [139].

Bee venom and melittin are of significant potential in the treatment of autoimmune diseases (such as rheumatoid arthritis, lupus erythematosus,



FIG. 7.7 Bee venom powder.

and multiple sclerosis), and some neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease and ALS [131].

Google Scholar currently contains >18,200 studies investigating the relation between honey bee melittin and cancer. The results suggest that melittin is an attractive candidate for cancer chemotherapy in the near future.

Conclusion

In this chapter, I have endeavored to describe the structures and functions of honey, propolis, pollen, bee bread, and bee venom and bee milk from bee products containing a significant proportion of natural products and with high apitherapeutic value. The use of these products is as old as human history, and has recently attracted considerable interest in the fields of natural nutrition, supplementary foods, and ethno-therapy in traditional and complementary medicine. Since the composition of bee products varies depending on geographical, floral and production patterns, their biological active properties are also variable. All organically produced bee products are therefore potentially therapeutic for humans, and further scientific research is now needed to reveal their exact functions.

Abbreviations

10-HDA 10-hydroxydecanoic acid
CAPE caffeic acid phenethyl ester
GAE gallic acid equivalent
HMF hydroxymethylfurfural
MGO methylglyoxal
MRJPs major RJ proteins

RJ royal jelly
TPC total phenolic content
UMF Unique Manuka Factor

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