Chapter 7
The Chemical and Biological Properties of Propolis

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7.1 General Overview of Propolis

Bees produce several different products which have health benefits. There is no doubt that honey has a highly significant status in medical treatments, while other apian materials, such as wax, royal jelly and propolis, have fewer medical applications, despite the fact that propolis has been used by people since ancient times (Burdock 1998; Ghisalberti 1979). The term propolis comes from two Greek words, pro (which means for or in defence of) and polis (which means the city); thus propolis means in defence of the city or beehive (Ghisalberti 1979). Propolis is a sticky resinous substance, which is gathered from buds and the bark of trees. It is also known as "bee glue" as bees use it to cover surfaces, seal holes and close gaps in their hives, thus providing a sterile environment that protects them from microbes

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and spore-producing organisms, including fungi and molds (Wagh 2013). It is therefore considered to be a potent chemical weapon against bacteria, viruses, and other pathogenic microorganisms that may invade the bee colony. Also, bees use propolis as an embalming substance, to mummify invaders such as other insects, that have been killed and are too heavy to remove from the colony (Bankova et al. 2005a; Wagh 2013). Humans have certainly observed the behaviour of honeybees and the ways in which they use propolis in their hives, which might have inspired interest in the biological properties of bee glue. Therefore, it is not surprising that propolis has become a subject of interest in natural product studies in recent times. The literature on propolis is vast and even a lengthy review cannot cover everything, but can only give an indication of the areas of interest in propolis research. There have been many reviews of the chemical and biological properties of propolis over the years and the most recent ones are listed here: Bankova et al. (2016), Sforcin (2016), Bankova et al. (2014), Silva-Carvalho et al. (2015), Chandna et al. (2014), Toreti et al. (2013), Wagh (2013), Sforcin and Bankova (2011).

7.2 Propolis in History

The ancient Greeks, Romans, and Egyptians were the first to use propolis, with applications in wound healing and as a disinfection substance (Sforcin 2007). The long history of the use of propolis as a medicine is claimed to be as old as the use of other honeybee products, with the former being used from at least 300 BC (Ghisalberti 1979; Burdock 1998; Sforcin 2007).

According to Egyptian history, propolis was one of the main ingredients used in an embalming recipe for mummification, in which it serves as a preservative agent (Mejanelle et al. 1997; Kuropatnicki et al. 2013). Many other ancient civilizations, such as Chinese, Indian, and Arabian, all believed in the power of propolis to treat medical conditions like sores, ulcers, and some skin lesions, so it was used both internally and externally (Kuropatnicki et al. 2013). For a comprehensive review of the history of propolis use see Kuropatnicki et al. (2013). Despite such early use, propolis is often still considered a “folk medicine” and remains an unofficial drug in the field of pharmacy (Valenzuela-Barra et al. 2015; Kuropatnicki et al. 2013; Toreti et al. 2013). However, over the last two decades, its use has begun to gain scientific backing. It is considered to be a promising natural source for the discovery of new pharmaceutical products to treat several types of diseases. Thus, it has been subjected to intensive studies investigating its antioxidant, antimicrobial, anti-inflammatory, immune-stimulating, and anticancer properties (Banskota et al. 2001b). Nevertheless, propolis is still not considered an official conventional medicine in healthcare because of a lack of standardization of its composition due to the variability of its chemical components and thus its biological activity, which varies according to the different geographic locations of its collection (Silva-Carvalho et al. 2015). In addition, there is presently inadequate data regarding therapeutic efficacy from clinical trial studies involving propolis. As a result, there are a few propolis products which have undergone FDA approval (Fitzmaurice et al. 2011).

The bioactivity of propolis mainly depends on its chemical composition. Bankova stated that knowledge of the chemical composition of propolis leads to a prediction of

its biological activities (Bankova et al. 2005a). In general, the term biological activity describes the pharmacological activity of a substance in a living organism. However, when the therapeutic product is a complex mixture, the biological activity could be broadly based due to a multiplicity of active ingredients and thus the product could have many therapeutic indications (Jackson et al. 2007). This is the case with propolis, which contains many active components, leading to numerous pharmacological activities. Propolis has been demonstrated to be safe and non-toxic for human use. However, some cases of allergic reactions such as contact dermatitis have been reported by beekeepers. There is some variability in the toxic and safe dosages of propolis reported by different studies, probably due to a lack of standardized extraction methods (Burdock 1998). The effectiveness of propolis preparations is dependent on the method of preparation, including the solvents used during the extraction process (Silva-Carvalho et al. 2015). The following sections will give an overview of some of the biological activity studies that have been conducted on propolis samples from different parts of the world.

7.3 The Biological Properties of Propolis

7.3.1 The Antioxidant Properties of Propolis

Metabolic processes within the human body consist of multiple complex reactions which generate natural free radicals. The body also has natural enzymatic antioxidants, which include superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic antioxidants including lipid soluble, e.g. vitamin E, and water soluble, e.g. vitamin C and glutathione, compounds for defence against the harmful effects of the reactive oxygen species (ROS) (Valko et al. 2007). A free radical in the body is simply an atom or molecule containing one or more unpaired electrons in its outer orbital, such as the oxygen atom shown in Fig. 7.1. The unpaired electron

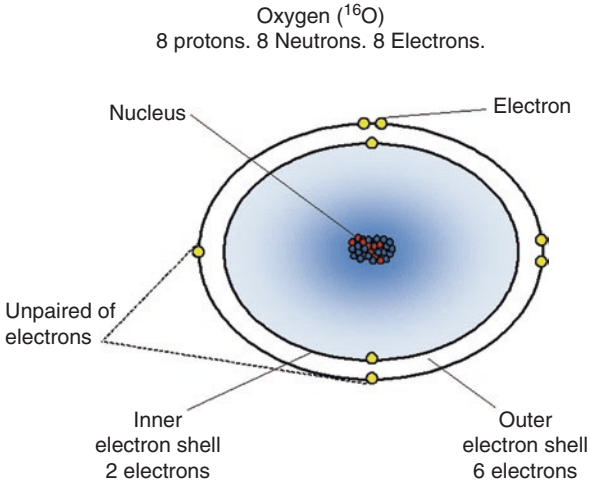

Fig. 7.1 Schematic showing the orbitals of the oxygen atom

allows the ROS to participate in many reactions with other free radicals. In low to moderate concentrations, ROS play vital roles in the biological processes of the human body, including stimulating pathways in response to changes in the extracellular environment (cellular signalling), mitogenic response, and immune response for defence against infections in the intracellular environment (Valko et al. 2007; Halliwell and Gutteridge 2015).

As can be seen in Fig. 7.2, oxygen molecules can accept energy in the form of electrons as an outcome of an inflammation process, leading to the production of oxygen-centred free radicals also known as ROS. The generation of ROS is

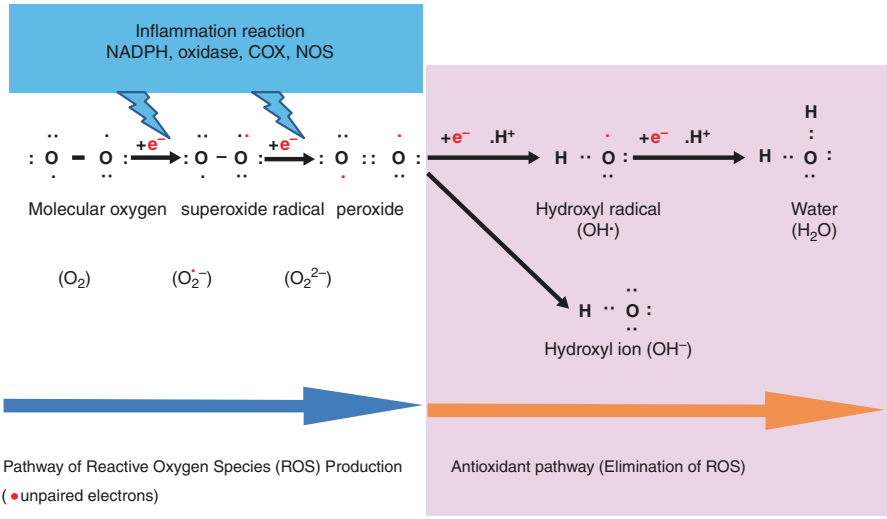

Fig. 7.2 Diagram illustrating the formation of Reactive Oxygen Species (ROS). When the oxygen molecule accepts an electron, it becomes a superoxide radical which, upon further electronation, produces peroxide. The latter can undergo further reactions with electrons and protons to produce potent hydroxyl radicals. Antioxidants act by donating protons (H+) to free radicals, leading to the formation of water, as shown in Fig. 7.3

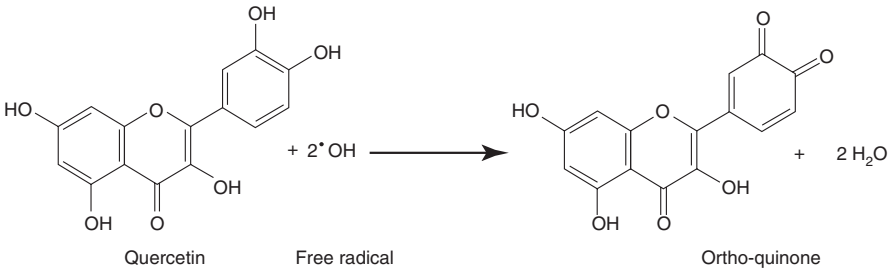

Fig. 7.3 Illustration of the antioxidant effect of quercetin, a flavonoid, on hydroxyl radicals. Quercetin reduces the free radical to water while it is oxidised to an *ortho*-quinone

regulated by the action of the enzyme RO synthase and over production results from both the mitochondrial electron transport chain and excessive production of NADH (Valko et al. 2007). Once formed, these ROS are highly reactive and produce a chain of deleterious reactions resulting in damage to cell structures (lipids, membranes, proteins and DNA) and modulation of many biological processes including inflammation and immune response. Oxidative stress occurs when there is an imbalance between the production of free radicals and physiologically active antioxidant metabolites in the body (Valko et al. 2007). The excessive production of ROS may be responsible for causing a large number of diseases, such as cancer (Kinnula and Crapo 2004), cardiovascular disease and inflammatory disorders such as rheumatoid arthritis. In addition, ROS can induce mutations or cause direct damage in DNA which leads to cell transformation and the possibility of developing a variety of malignant conditions (Valko et al. 2007). Also, active free radicals are the main factor involved in cellular aging and are responsible for the development of many CNS related medical conditions such as Parkinson's and Alzheimer's diseases. Antioxidant agents can serve as a defensive factor against free radicals in neuronal cells (Metodiewa and Kośka 1999; Martin and Grotewiel 2006; Valko et al. 2007).

Many scientific papers have been published on the antioxidant effects of propolis (Bittencourt et al. 2015; Olczyk et al. 2013; Piccinelli et al. 2013). The relationship between antioxidant activity and the chemical composition of propolis from different origins has been investigated by several authors (Isla et al. 2001; Kalogeropoulos et al. 2009; Mello and Hubinger 2012; Piccinelli et al. 2013). These studies confirmed that the significant antioxidant activity of propolis is related to the high content of polyphenolic compounds, such as flavonoids, in the sample. Additionally, it has been reported that the essential oil constituents of *Thymus vulgaris* (thyme) could act as antioxidant agents (Deans et al. 1992). Since one of the main components of propolis has been proven to be essential oils (Bankova et al. 2014; Marcucci 1995), it might be possible that these components contribute to its antioxidant effects. The study conducted by Kumazawa et al. suggested that propolis could act as an antioxidant agent due to the presence of anti-oxidative compounds such as kaempferol and phenethyl caffeate (Kumazawa et al. 2007). Their conclusion came following the investigation of antioxidant activities of various propolis samples from different geographical origins using the DPPH assay.

Another approach to verifying the antioxidant action for potential in human medicine is to analyse the potential alleviating effect of propolis in neurodegeneration by means of cell viability assays on neuronal cells (Imamura et al. 2006). It is well known that the main factor in CNS disorders is oxidative stress. Thus, antioxidant properties play a vital role in the management of CNS disorders induced by oxidative stress. Shimazawa et al. assayed and reported the neuroprotective effect of green Brazilian propolis both *in vitro* and *in vivo*. First, the *in vitro* assay was conducted by exposure of neuronal cell cultures to hydrogen peroxide (H_2O_2), followed by addition of propolis to the neuronal cells. On the other hand, the *in vivo* experiments studied the effect of propolis against lipid peroxidation in

the forebrain of mice and DPPH-induced free radical production (Shimazawa et al. 2005). Furthermore, a recent study found that Turkish propolis contains phenolic components which have the ability to minimize DNA damage by inhibiting the effects of H_2O_2 in cultured fibroblasts (Darendelioglu et al. 2016). An effective natural antioxidant agent such as propolis could provide a safe and novel treatment for oxidative stress-related diseases, especially in elderly people whose conditions tend to be complex in nature and include cases of neurodegeneration corresponding to aging. In addition, propolis could play a key role in the management and prevention of various disease conditions in which ROS have a causative effect, such as some inflammatory disorders, cancer, cardiovascular and immune diseases.

7.3.2 The Antimicrobial Activity of Propolis

Until now, the most widely investigated property of propolis is its antimicrobial activity, with hundreds of publications on this topic having appeared in the last 40 years (Bogdanov 2012). These findings explain why propolis plays such an important role in bee hives since it can be considered as a chemical weapon against pathogenic microorganisms (Fokt et al. 2010; Bankova 2005a). Different propolis types contain many chemical constituents responsible for their antimicrobial properties (Bankova 2005a) and it seems that the sum of the propolis antimicrobial components, rather than individual substances, is responsible for the observed antimicrobial effect (Kujumgiev et al. 1999; Bogdanov 2012). Propolis shows antibacterial (Silici and Kutluca 2005; Kujumgiev et al. 1999; Grange and Davey 1990), antifungal (Kartal et al. 2003; Kujumgiev et al. 1999; Ota et al. 2001), antiviral (Amoros et al. 1992a, b), antiprotozoal (Freitas et al. 2006; Dantas et al. 2006a, b), anti-tumour (Callejo et al. 2001; Komericki and Kränke 2009; Banskota et al. 2000; Su et al. 1994), anti-inflammatory (Khayyal et al. 1992; Dobrowolski et al. 1991; Fokt et al. 2010), local-anaesthetic (Marcucci 1995), antioxidant (Russo et al. 2002; Fokt et al. 2010; Kumazawa et al. 2007), immunostimulating (Dimov et al. 1992; Oršolić et al. 2004), cytostatic (Banskota et al. 1998) and hepatoprotective (Banskota et al. 2001a; Won Seo et al. 2003) activities.

There are many components which are responsible for the biological activity of propolis and these vary with propolis sample type and the solvents used in its extraction (Ugur and Arslan 2004). Flavonoids and esters of phenolic acids are generally regarded as bioactive compounds which are responsible for antimicrobial activity (Fokt et al. 2010). However, there are many other components with such activity; these are summarised in Tables 7.1 and 7.2 for different types of propolis and two of the main types, respectively.

Table 7.1 Biological effects of propolis components adapted from Bogdanov (2012)

Propolis active ingredient and propolis type	Biological activity
Polyphenols and flavonoids Mostly poplar and all propolis types	Antibacterial, antiviral, antifungal
Caffeic acid phenethyl ester (CAPE) and other caffeates Poplar and Bacharis	Antibacterial, antiviral, fungicidal
Caffeic acid (CA) Poplar and Baccharis	Antiviral
Terpenes Greece, Crete, Croatia, Brazil	Antibacterial, antifungal
Essential oils Brazil, Poland	Antibacterial
Furfuran lignans Canary islands	Antibacterial

Table 7.2 Biologically active ingredients in Poplar and Baccharis propolis adapted from Bogdanov (2012)

Biological activity	Propolis type, active ingredient
Antibacterial	*Poplar*: Different flavonones, flavones, phenolic acids and their esters
	Bacharis: Prenylated *p*-coumaric acids, labdane diterpenes
Antifungal	*Poplar*: Pinocembrin, galangin, benzoic acid, salicylic acid, vanillin
	Baccharis: Mono and sesquiterpenes, Artipellin C
Antiviral	*Poplar*: Polyphenols, phenyl carboxylic acids, and esters of substituted cinnamic acids, caffeic acid, quercetin, luteolin, fisetin, and quertecagetin
	Baccharis: Activity detected but no substances identified

7.3.2.1 Antibacterial Activity of Propolis

Antimicrobial activity is recognised as the most important property of propolis, particularly activity against bacteria. Several studies have been performed to evaluate this property against a large group of Gram-positive and Gram-negative bacteria; both aerobic and anaerobic types. The bacteria studied are summarized in Table 7.3. These bacteria were either from laboratory collections or isolated from clinical samples. The studies used propolis of different geographical origins and chemical composition, and employed different experimental approaches such as disc diffusion and disc dilution to investigate the antibacterial activity. In the disc diffusion method, antibacterial activity is determined by measuring the diameter of the bacterial growth inhibition zone in the agar layer surrounding a disc containing propolis extracts (Kujumgiev et al. 1999). The dilution method is used to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration

Table 7.3 Bacteria used in the determination of the antibacterial activity of propolis adapted from Fokt et al. (2010)

Type	Gram-positive	Gram-negative
Aerobic	*Bacillus* spp. • *B. cereus* • *B. subtilis*	*Aeromonas hydrophila*
		Brucella abortus
	Enterococcus spp. • *E. faecalis*	*Corynebacterium* sp. • *C. pseudotuberculosis*
	Micrococcus luteus	*Escherichia coli*
	Nocardia asteroids	*Helicobacter pylori*
	Rhodococcus equi	
	Staphylococcus spp. • *S. aureus* • *S. auricularis* • *S. capitis* • *S. epidermidis* • *S. haemolyticus* • *S. hominis* • *S. mutans* • *S. warnerii*	*Klebsiella pneumoniae*
		Salmonella sp. • *S. enteritidis*, • *S. typhi* • *S. typhimurium*
	Streptococcus spp. • *S. cricetus* • *S. faecalis* • *S pneumioniae* • *S. pyogenes* • *S. β-haemolyticus* • *S. mutans* • *S. sobrinus* • *S. viridians*	*Pseudomonas aeruginosa*
		Proteus spp. • *P. mirabilis* • *P. vulgaris*
		Shigella dysenteriae
Anaerobic	*Actinomyces naeslundii*	*Actinobacillus actinomycetemcomitans*
	Lactobacillus acidophilus	*Capnocytophaga gingivalis*
	Peptostreptococcus micros	*Porphyromonas* spp. • *P. anaerobius* • *P. gingivalis*
		Fusobacterium nucleatum
		Prevotella spp. • *P. intermedia* • *P. melaninogenica* • *P. oralis*
		Veillonella parvula

(MBC) which are, respectively, the lowest concentrations that inhibit visible bacterial growth and the lowest concentration that kills bacteria (Grange and Davey 1990; Stepanović et al. 2003). The vast majority of antibacterial activity studies were carried out using *in vitro* bioassays, as mentioned above. Although the composition of propolis differs considerably depending on its botanical origin, all examined types of propolis have revealed strong antibacterial activity (Kujumgiev et al. 1999; Bankova 2005b; Bankova et al. 2007). Also, the activity of propolis may depend on the type of bee collecting it since it was found that poplar propolis collected by *Apis mellifera*

caucasica had a higher antibacterial activity than that collected by *Apis mellifera anatolica* and *Apis mellifera carnica* (Silici and Kutluca 2005).

Tests for the antibacterial activity of propolis were carried out against a range of different pathogenic bacteria in several studies, as summarised in Table 7.3 (Banskota et al. 2001b; Burdock 1998; Ghisalberti 1979; Grange and Davey 1990). It has been reported that propolis is more active against Gram-positive pathogens, but many Gram-negative bacteria are also inhibited (see Table 7.3) (Fokt et al. 2010; Wagh 2013).

The data collected from a range of studies on the antibacterial properties of propolis support the fact that propolis is active mainly against Gram-positive bacteria and either displays much lower activity against Gram-negative ones or is not active at all (Marcucci 1995; Silici and Kutluca 2005; Kujumgiev et al. 1999; Drago et al. 2007; Grange and Davey 1990; Kartal et al. 2003; Dobrowolski et al. 1991; Fadaly and El-Badrawy 2001).

Such results can be seen in the study by Kujumgiev et al., who evaluated propolis samples from different geographic regions (tropical and temperate zones) against *Staphylococcus aureus* and *Escherichia coli*. All of the extracts exhibited significant antibacterial activity against *S. aureus* but none were active against *E. coli* (Kujumgiev et al. 1999).

However, it was reported that ethanolic extracts from propolis (EEP) completely inhibited the growth of *S. aureus*, *Enterococcus* spp. and *Bacillus cereus*, and moderately inhibited the Gram-negative organisms *Pseudomonas aeruginosa* and *E. coli*. (Grange and Davey 1990). The antibacterial activity of EEP from Brazilian propolis, collected during four seasons, was found to inhibit the growth of Gram-positive bacteria and higher concentrations of EEP were needed to inhibit Gram-negative bacterial growth, but the extracts had no effect on *Klebsiella pneumoniae*.

More recent research has revealed antibacterial activity of propolis against *Micrococcus luteus*, *Salmonella typhimurium* (Uzel et al. 2005) and *K. pneumonae* (Victorino et al. 2007); and although in earlier studies (Grange and Davey 1990) it was stated that *Listeria monocytogenes* is not sensitive to propolis, more recent studies revealed significant activity against this organism (Ozcan et al. 2004; Yang et al. 2006). It was also found that propolis showed strong antibacterial activity against 13 different bacterial plant pathogens (Basim et al. 2006).

The antibacterial effect of propolis is bactericidal (Grange and Davey 1990) and it is proposed to work by inhibiting bacterial mobility. In addition, it has been shown that the antibacterial activity of poplar propolis is based on inhibition of quorum sensing (QSI), with the flavonoid pinocembrin being an important QSI agent (Savka et al. 2015).

The flavonoids galangin, pinocembrin and pinostrobin have been most associated with the antibacterial properties of propolis, as shown in Table 7.1 (Dimov et al. 1992), but it has also been reported that propolis samples containing only traces of flavonoids demonstrate antibacterial action (Tomás-Barberán et al. 1993). In addition, ferulic and caffeic acids, prenylated coumaric acid, benzophenone derivatives and diterpenic acids have also been reported as antibacterial compounds (Ghisalberti 1979; Burdock 1998; Castaldo and Capasso 2002; Kujumgiev et al. 1999; Popova et al. 2007; Mirzoeva et al. 1997).

In recent years, there has been considerable interest in using propolis in hospitals as an antibacterial agent due to the increase in antibiotic resistance (Bogdanov 2012). It has been shown that the components in propolis act synergistically against bacteria (Onlen et al. 2007; Orsi et al. 2006; Scazzocchio et al. 2006; Speciale et al. 2006; Stepanović et al. 2003). Several authors point out that the antimicrobial activity of propolis is related to its highly complex and variable constituents and their synergistic action (Bonvehí and Coll 1994; Burdock 1998; Freitas et al. 2006; Scazzocchio et al. 2006; Mirzoeva et al. 1997; Takaisi-Kikuni and Schilcher 1994).

Compounds which were active against *Mycobacterium marinum*, the closest genetic relative to *Mycobacterium tuberculosis*, were isolated from Saudi Arabian propolis. The strongest activity was found for the flavonoid psiadiarabin which showed an activity only 5 times less than that of the gentamycin control (Almutairi et al. 2014a). Twelve ethanolic extracts of propolis from different areas within Libya were tested against *M. marinum* in order to determine whether or not the observed activity was associated with specific components in the samples. The extracts showed moderate to strong activity against *M. marinum* (Siheri et al. 2016).

Commonly, the biological activity of a natural medicinal product decreases with increasing storage time, but Meresta (1997) stated that ethanolic solutions of propolis stored for 10–15 years had increased antibacterial activity (Meresta 1997).

7.3.2.2 Antiviral Activity of Propolis

Many recent reviews have reported on the various antiviral activities of propolis samples from different geographical origins against different strains of viruses, such as Adenovirus, HSV, Influenza A and B viruses, Newcastle disease virus, Polio virus, Vaccinia, Rotavirus, vesicular stomatitis virus (VSV), and Corona virus (Starzyk et al. 1977; Fokt et al. 2010; Bogdanov and Bankova 2012), as summarised in Table 7.4. Studies have reported that propolis has significant antiviral activity and interferes with the replication of some different viruses that cause human diseases, including *Herpes simplex*, *genitalis* and *zoster*, influenza and smallpox (De Castro 2001; Bogdanov 2012; Silva-Carvalho et al. 2015).

Studies over the past two decades have provided further important information on the antiviral properties of propolis. The effect of propolis on several DNA and RNA viruses, including herpes simplex type 1 (HSV-1), an acyclovir resistant mutant, herpes simplex type 2 (HSV-2), adenovirus type 2, VSV, and poliovirus type 2, was studied. The inhibition of poliovirus propagation was observed through a plaque reduction test and a multistep virus replication assay. At a concentration of 30 μg/ml, propolis reduced the titer of HSV by 1000, whereas VSV and adenovirus were less susceptible. The antiviral effect of propolis along with the major flavonoids found therein, such as galangin, kaempferol, chrysin, apigenin, luteolin and quercetin, against HSV was also studied. Flavonols were found to be more active than flavones, with the order of importance from least to most active being galangin, kaempferol and quercetin, demonstrating that activity increases with the number of hydroxyl groups in the molecule. The efficacy of binary flavone-flavonol combinations against HSV-1 was also investigated. It was concluded that synergism might occur between two or more compounds, leading to enhanced antiviral activity of propolis (Amoros et al. 1992a).

Table 7.4 Antiviral activity of the different propolis constituents from different geographical origins (1992–2016) adapted from Silva-Carvalho et al. (2015)

Propolis type/plant source/origin Type extract/isolated compounds	Species/cells/viruses	Antiviral effect
European type propolis Caffeic acid, p-coumaric acid, benzoic acid, galangin, pinocembrin, and chrysin	RC-37 cells, HSV-1 strain KOS	High anti-HSV-1 activity for both extracts when cells were treated prior to viral infection
European propolis PEE and PWE	RC-37 cells, HSV-2	High antiherpetic activity for both extracts when viruses were pre-treated prior to infection
Brazilian Brown propolis/HPE	HSV-2 strain propagated in Vero cells, female BALB/c mice	Effective against HSV-2 infection
Characteristic of Brazilian propolis Isopentyl ferulate isolatefrom and PEE	Influenza viruses	Suppression of influenza virus A/Hong Kong reproduction *in vitro*
Green Brazilian propolis/*B. dracunculifolia B. eriocladа*, *Myrceugenia euosma*/Brazil, PEE	Influenza A virus	Reduction of body weight loss of infected mice and virus yields in the bronchoalveolar lavage fluids of lungs
European propolis/Populus Nigeria/ France PEE	RC-37 cells, HSV-1 strain H29S, acyclovir resistant mutant HSV1-R strain H29R, HSV-2, adenovirus type 2, poliovirus type 2, and VSV	Reduction of titre of HSV, VSV and adenovirus, which was less susceptible; virucidal action on the enveloped viruses HSV and VSV
Geopropolis from the stingless bee *Scaptotrigona postica*/Brazil/ Hydromethanolic extract	African green monkey kidney cells (ATCC CCL-81); herpes simplex virus strain	Inhibition of HSV replication and entry into cells
Characteristic of Brazilian red and green propolis/synthesised/ Homoisoflavonoids, especially 3-benzyl-4-chromones	BGM (Buffalo GreenMonkey) cells, coxsackie viruses B3, B4, and A9 and echovirus 30	Good antiviral activity against the coxsackie viruse s B3, B4, and A9 andechovirus 30
European propolis/*P. trichocarpa* and *P. tremuloides*/Canada/PEE	HSV-1 and HSV-2 virus replicated in MDBK cells	Impairing the ability of the virus to adsorb or to penetrate the host cells
Green propolis/*Baccharis dracunculifolia* Characteristic of Brazilian green propolis. /Brazil/Water extracts, 3,4-dicaffeoylquinic acid (Isolated from Brazilian propolis)	Female BALB/c mice, Influenza A virus strain A/WSN/33 (H1N1)	Extension of the lifetime of mice. 3,4-dicaffeoylquinic acid which increases mRNA levels of tumor necrosis factor-related apoptosis-inducing and decreases H1N1 hemagglutinin mRNA

(continued)

Table 7.4 (continued)

Propolis type/plant source/origin Type extract/isolated compounds	Species/cells/viruses	Antiviral effect
Characteristic of Brazilian green pro polis Brazil/Melliferone, moronic acid, anwuweizonic acid and betulonic acid (isolated from Brazilian propolis)	H9 lymphocytes, HIV-1	Moronic acid inhibiting anti-HIV replication
Mediterranean propolis/*Populus* spp., *Eucalyptus* spp., and *Castanea sativa*/ Israel/PWE Characteristic of European propolis	Jurkat, uninfected human T-cell lines, and MT2 (HTLV-1 infected human T cells) cells	Inhibition of the activation of NF-κB-dependent promoter by Tax and prevention of NF-κB Tax binding to IκBα and its degradation
Nanometer propolis Flavone/ Nanometer propolis Flavone provided by Binzhou Animal Science and Veterinary.Medicine Academy of Shandong Province	Kidney cells (PK-15) Porcine parvovirus (PPV) Britain White guinea pigs	Inhibition of PPV infecting porcine kidney- (PK-) 15 cells. Restraining of PPV copy in lung, gonad, and blood, decrease of the impact of PPV on weight of guinea pigs and increase of hemagglutination inhibition of PPV in serum as well as improving the contents of IL-2, IL-6, and γ-IFN
European propolis/*Populus nigra* Brazil Green propolis/*Baccharis* USA and China Brazil/PEE	Peripheral blood mononuclear cells obtained from blood of healthy donors, microglial cells isolated from human fetal brain tissue, HIV-1AT, HIV-1SF162	Inhibition of HIV-1 variants expression
Propolis samples Turkey (Hatay region) Hatay, Turkey/PEE	HSV-1 and HSV-2 in Hep-G2 cell cultures	Quite effective against the replication of HSV-1 and HSV-2

A number of other studies have suggested an association between the antiviral activity of propolis and certain compounds which are found therein. Some flavonoids have an inhibitory effect on human immunodeficiency virus (HIV) infection and replication. It was found that luteolin was more active than quercetin, but less active than caffeic acid and some esters of substituted cinnamic acids found in propolis. Isopentyl ferulate significantly inhibited the infectious activity of influenza virus A. It has previously been observed that the antiviral activity is due to both the major constituents in propolis and the minor components such as 3-methylbut-2-enyl caffeate and 3-methylbutyl ferulate (Amoros et al. 1992a; Maksimova-Todorova et al. 1985; Vanden Berghe et al. 1986; Marcucci 1995). 3-Methylbut-2-enyl caffeate showed strong inhibition of HSV-1 growth (Amoros et al. 1992a, b).

In addition, it has been reported that some propolis constituents and their analogues (esters of substituted cinnamic acids) significantly inhibited infection by influenza virus A/Hong Kong (H3N2) (Serkedjieva et al. 1992). A study of the antiviral effect of caffeic acid, a constitutent of propolis, found that *Vaccinia* and adenovirus were more sensitive to caffeic acid than polio and parainfluenza viruses, but it exhibited only minor activity against influenza virus (Fokt et al. 2010).

The antiviral activity of aqueous and ethanol extracts of propolis and constituents, such as flavonoids caffeic acid, p-coumaric acid, benzoic acid, galangin, pinocembrin and chrysin, was tested against herpes simplex virus type 1 (HSV-1). Both propolis extracts demonstrated high levels of antiviral activity against HSV-1 in viral suspension tests; plaque formation was significantly reduced by >98%. Galangin and chrysin were proposed to greatly contribute to the observed activity (Schnitzler et al. 2010).

Besides its inhibitory effect on viral growth, propolis also shows virucidal action on enveloped viruses HSV and VSV (Marcucci 1995). The activity of Brazilian propolis against HSV-1 infection was studied after its oral administration to infected mice three times daily and on days 0–6 after treatment. The results revealed a significant effect on the development of herpetic skin lesions (Shimizu et al. 2011).

The antiviral activities of four propolis samples from Austria, Egypt, France and Germany were investigated against avian reovirus (ARV) and infectious bursal disease virus (IBDV). The results indicated that all propolis samples reduced the viral infectivity to a different degree and that the Egyptian propolis showed the highest antiviral activity against ARV and IBDV (Hegazi et al. 2000; El Hady and Hegazi 2002). The activity of 13 ethanol extracts of Brazilian green propolis against viruses was investigated. The extracts displayed antiviral activity against influenza virus *in vitro* and *in vivo*. The effect was attributed to the flavonoid and phenolic acid constituents (Shimizu et al. 2008).

The aqueous and ethanolic extracts of propolis were evaluated against HSV-1 and HSV-2. The anti-herpetic effect was analysed in cell culture and both propolis extracts exhibited high levels of antiviral activity against HSV-2. Infectivity was significantly reduced by 49% and direct concentration- and time-dependent antiherpetic activity was demonstrated (Nolkemper et al. 2010).

A hydromethanolic extract of geopropolis (HMG) was evaluated using viral DNA quantification experiments and electron microscopy. The study showed a reduction of viral DNA from herpes virus by about 98% under all conditions and concentrations of HMG tested (Coelho et al. 2015).

The antiviral activity of EEP of Turkish propolis on the replication of both HSV-1 and HSV-2 was investigated. HSV-1 and HSV-2 were suppressed in the presence of 25, 50, and 100 μg/mL of propolis extract when infection of a Hep-G2 cell line was examined. Synergistic effects of propolis with acyclovir were identified against these viruses. The results showed a significant decrease in the number of viral copies (Yildrim et al. 2016).

It was found that propolis suppresses the replication of human immunodeficiency virus type 1 (HIV-1), in addition to modulating immune responses. The antiviral activity of propolis samples against HIV-1 from several geographic regions was

investigated in CD4+ lymphocyte and microglial cell cultures. The results showed inhibited viral expression in a concentration-dependent manner (maximal suppression of 85 and 98% was observed at 66.6 μg/ml propolis in CD4+ lymphocyte and microglial cell cultures, respectively) (Gekker et al. 2005).

Propolis flavonoids act by preventing the virus from entering the host cell and by reducing intracellular replication activities. This process contributes to suppression of the growth and development of the virus. Other possible mechanisms of antiviral activity include inhibition of reverse transcriptase and stimulation of the immune system to fight back against the infection (Schnitzler et al. 2010; Boukraâ et al. 2013).

Propolis extracts were screened in a plaque reduction assay and exhibited anti-influenza activity. Mice were infected intranasally with the influenza virus, and the four extracts were orally administered at 10 mg/kg three times daily for seven successive days after infection; the EEP was found to possess anti-influenza activity and to ameliorate influenza symptoms in mice (Shimizu et al. 2008).

7.3.2.3 Antiprotozoal and Antihelminthic Activity of Propolis

Recently, attention has been focused on the antiparasitic activity of propolis since improvements on existing drugs against several tropical diseases caused by different protozoa are required. Numerous assessments have been performed using different *in vivo* and *in vitro* experiments to investigate the activity of raw propolis and active compounds isolated from propolis. Accordingly, significant effects against different parasitic species including *Cholomonas paramecium*, *Eimeria magna*, *Media perforans*, *Giardia lambia*, *Giardia duodenalis*, *Trichomonas vaginalis*, *Trypanosoma cruzi* and *Trypanosoma evansi* have been reported in the literature (Freitas et al. 2006; Falcão et al. 2013; Bogdanov 2012; Parreira et al. 2010). Several studies have been performed that show the activity of propolis and its components against a range of protozoan parasites which cause various human diseases, including *Trypanosoma brucei* which causes sleeping sickness and *Trypanosoma cruzi* which causes Chagas disease (Higashi and De Castro 1994; De Castro and Higashi 1995; Marcucci et al. 2001; Dantas et al. 2006a, b; Salomão et al. 2010; Falcão et al. 2013; Almutairi et al. 2014b; Siheri et al. 2014, 2016; Omar et al. 2016). Antiprotozoal effects of different propolis samples were reported against *Leishmania donovani*, which causes visceral leishmaniasis, and for other strains of leishmania (Duran et al. 2008; Pontin et al. 2008; Ozbilge et al. 2010; Monzote et al. 2011; Amarante et al. 2012; Da Silva et al. 2013; Siheri et al. 2016). Recent studies have reported antiprotozoal effects of propolis extracts against *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale*, *all of* which cause malaria (Olayemi 2014; Siheri et al. 2016). Propolis is also effective against *Entamoeba histolytica* and *Giardia lamblia*, which cause intestinal infections (dysentery and diarrhoea), as well as multicellular organisms such as intestinal worms, including helminths such as Schistosoma spp., cestodes such as tapeworms, nematodes such as roundworms, and trematodes such as flukes (Freitas et al. 2006; Issa 2007; Hegazi et al. 2007; Abdel-Fattah and Nada 2007; Noweer and Dawood 2008;

Alday-Provencio et al. 2015; Hassan et al. 2016). Some of the studies are described in more detail below.

Extracts of Portuguese propolis and its potential sources such as poplar buds were screened against different protozoa, including *Plasmodium falciparum*, *Leishmania infantum*, *Trypanosoma brucei* and *Trypanosoma cruzi* (Falcão et al. 2013). The toxicity of the extracts against MRC-5 fibroblast cells was also evaluated to assess toxic selectivity. The propolis extracts showed moderate activity against these parasites, with the highest inhibitory effect being observed against *Trypanosoma brucei* (Falcão et al. 2013).

Recently, extracts from 12 samples of propolis collected from different regions of Libya were tested for their activity against *Trypanosoma brucei*, *Leishmania donovani*, *Plasmodium falciparum* and *Crithidia fasciculate*, while the cytotoxicity of the extracts was also tested against mammalian cells. All of the extracts were active to some degree against all of the protozoa, exhibiting a range of EC_{50} values between 1.65 and 53.6 μg/ml (Siheri et al. 2016), while only exhibiting moderate to negligible cytotoxicity.

The activity of propolis against Chagas disease (caused by *Trypanosoma cruzi*) was assessed in comparison with crystal violet, a standard drug recommended to prevent the transmission of Chagas disease via blood (De Castro and Higashi 1995). The relationship between trypanocidal activity and the chemical composition of propolis has been widely investigated by several authors and these studies confirmed that Brazilian green propolis is highly active against *T. cruzi* transmission (Dantas et al. 2006b; De Castro and Higashi 1995; Higashi and De Castro 1994).

The activity of ethanol extracts from Brazilian (Et-Bra) and Bulgarian (Et-Blg) propolis against *T. cruzi* were tested and it was found that, although there were differences in the chemical composition between both extracts, they were both active against *T. cruzi*. The study also confirmed that in European samples biological activity was associated with the presence of flavonoids and aromatic acids and their esters. In Brazilian propolis, amyrins occur as components that might contribute to the anti-trypanosomal activity (Salomão et al. 2004; Higashi and De Castro 1994).

The activity of acetone and ethanol extracts of two Bulgarian propolis samples (Bur and Lov) against *T. cruzi* was evaluated. Both extracts showed similar chemical compositions with a high content of flavonoids and strong inhibitory activity against *T. cruzi* proliferative epimastigotes, which were more susceptible than trypomastigotes. While in the presence of blood, the activity of Et-Bur or Et-Lov against trypomastigotes was similar to that of the standard drug, crystal violet (Prytzyk et al. 2003). It was also found that two different samples from Bulgarian propolis showed significant activity against *T. cruzi in vitro* (Salomão et al. 2004, 2009; Dantas et al. 2006a, b).

Current therapy for *T. evansi* infections is not effective for the vast majority of animals with relapsing parasitemia and clinical signs. The susceptibility of *T. evansi* to a propolis extract *in vitro* and *in vivo* was evaluated. A dose-dependent trypanocidal activity of the propolis extract was observed *in vitro*. All trypomastigotes were killed within 1 h after incubation with 10 μg/ml of the extract. However, *in vivo* assessment of concentrations of 100, 200, 300 and 400 mg/kg administered orally

for 10 consecutive days presented no curative effect, and the rats died from the disease. However, rats treated with the two highest concentrations of propolis extract showed higher longevity than the other groups. Based on these data the study concluded that, despite the lack of curative efficacy observed *in vivo* at the concentrations tested, propolis extract can prolong life in rats infected with the protozoan (Gressler et al. 2012).

A comprehensive chemical profiling study was carried out on 22 African propolis samples collected from the sub-Saharan region. Results revealed that triterpenoids were the major chemical components in more than half of the propolis samples analysed in this study and some others were classified as temperate and Eastern Mediterranean types of propolis. Based on comparative chemical profiling, one propolis sample from southern Nigeria stood out from the others by containing prenylated isoflavonoids, which indicated that it was more like Brazilian red propolis (Zhang et al. 2014). This propolis was further investigated and ten phenolic compounds were isolated, including a new dihydrobenzofuran. All the isolated compounds were tested against *T. brucei* and displayed moderate to high activity. Some of the compounds tested showed similar activity against wild type *T. brucei* and two strains displaying pentamidine resistance. The Nigerian propolis from Rivers State showed some similarities to Brazilian red propolis and exhibited antitrypanosomal activity at a potentially useful level (Omar et al. 2016).

The chemical profile and antitypanosomal activity of Ghanian propolis against *T. brucei* was also investigated. Two compounds were isolated; a prenylated tetrahydroxy stilbene and a geranylated tetrahydroxy stilbene. These compounds exhibited moderate activity against *T. brucei*. In the same paper, isolation of a new phloroglucinone analogue from Cameroon propolis was reported. The compound was found to possess high potency, comparable to that of suramin (Almutairi et al. 2014b).

The EEP of Libyan propolis was tested for its activity against *T. brucei*. One of the samples was fractionated and yielded a number of active fractions. Three of the active fractions contained single compounds, found to be 13-epitorulosal, acetyl-13-epi-cupressic acid and 13-epi-cupressic acid, which had been identified previously in Mediterranean propolis. Two of the compounds had a MIC value of 1.56 μg/mL against *T. brucei* (Siheri et al. 2014).

The chemical composition and biological activity of a propolis sample collected from Saudi Arabia were investigated. A new diterpene, propsiadin, was isolated along with two flavonoids and a known diterpene, psiadin. The compounds had MICs in the range 30.9–78.1 μM against *T. brucei*. The propolis was thought to originate from *Psiadia arabica* and *Psiadia punctulata*, representing a new type of propolis (Almutairi et al. 2014a).

Leishmaniasis has been reported as an endemic disease in 88 countries in tropical and sub-tropical regions across the world, affecting more than 12 million people. There are no vaccines available for any form of the disease and the chemotherapy of this disease is still inadequate and expensive (Kayser et al. 2003; Croft et al. 2005). An intense search for potential natural products isolated from plants or propolis for the treatment of Leishmaniasis has been carried out during the last decades. The previous literature contains several reports on the activity of a variety of crude natu-

ral extracts against Leishmania, especially from plants collected in tropical zones (Croft et al. 2006).

Previous studies have reported that propolis samples from various origins possess activity as anti-leishmanial agents due to the presence of flavonoids and amyrins (Machado et al. 2007).

A study of propolis from Turkey investigated the effects of propolis against *Leishmania tropica* and it was observed with microscopic examination that propolis inhibited parasite growth at ≥32 μg/ml concentration. It was also found that the antileishmanial effects of propolis increased with increasing concentrations and incubation periods (Ozbilge et al. 2010).

The activity of *Baccharis dracunculifolia*, which is the most important plant source of Brazilian green propolis, against promastigote forms of *L. donovani* was investigated and IC_{50} values of 42 μg/ml were obtained. The extract also displayed high activity in a schistosomicidal assay (Parreira et al. 2010).

The activity of eighteen Cuban propolis extracts collected in different geographic areas were screened against *Leishmania amazonensis* and *Trichomonas vaginalis*. The study observed that all propolis extracts produced an inhibitory effect on intracellular amastigotes of *L. amazonensis*. Only five samples decreased the viability of *T. vaginalis* trophozoites at concentrations lower than 10 μg/ml (Monzote et al. 2011).

Brazilian green propolis was tested against *L. braziliensis* by experimental infection of mice. The results showed an IC_{50} value of 18.1 μg/ml against promastigote forms of *L. brasiliensis*. IC_{50} values were in the range 78–148 μg/ml against the M2904 strain of *L. brasiliensis* and the extract also had antiproliferative activity on *L. brazilensis* promastigotes at 100 μg/ml (Da Silva et al. 2013).

The EEP of Libyan propolis collected from North East Libya was found to be active against *L. donovani*, and four compounds, three diterpenes and a lignan, were isolated. These compounds exhibited moderate to strong activity against *L .donovani*, with IC_{50} values in the range 5.1–21.9 μg/ml (Siheri et al. 2014). These results were replicated in subsequent assays on *L. donovani* involving twelve extracts of Libyan propolis where IC_{50} values ranged from 2.67 to 16.2 μg/ml (Siheri et al. 2016).

The activity of methanolic extracts of ten Bolivian propolis samples was studied against *L. amazonensis* and *L. braziliensis*. The most active samples towards *Leishmania* species had IC_{50} values in the range 78–121 μg/ml against *L. amazonensis* and *L brasiliensis* (Nina et al. 2016).

It was reported that an ethanolic extract of European propolis showed activity against *Toxoplasma gonodi* (De Castro 2001).

The activity of Nigerian propolis was tested against *Plasmodium berghei* using mice experimentally infected with *P berghei*, with chloroquine as a positive control. The propolis significantly reduced the level of parasitemia in treated mice, and there was no significant difference from mice treated with chloroquine (Olayemi 2014).

Propolis extract inhibited the growth of the intestinal parasites *Giardia lamblia*, *Giardia intestinalis* and *Giardia duodenalis*. The extract decreased the growth of trophozoites and the level of inhibition varied according to the extract concentration and incubation times. Significant decreases in parasite growth were detected in

cultures exposed to 125, 250 and 500 µg/ml of propolis, respectively, for all incubation periods (24, 48, 72 and 96 h). Growth reduction of 50% was observed in cultures treated with 125 µg/ml of the extract, and concentrations of 250 and 500 µg/ml were able to inhibit growth by more than 60% (Freitas et al. 2006).

Mice were orally infected with axenically cultivated *Giardia lamblia* trophozoites. The trophozoite count in the intestines, measurements of interferon-gamma serum levels, and histopathological examination of duodenal and jejunal sections were carried out. The results showed that propolis as a prophylaxis resulted in a significant decrease in the intensity of infection. As a treatment, propolis caused a more significant decrease in trophozoite count than that obtained by metronidazole. However, mice treated with propolis alone showed a reversed $CD4^+$: $CD8^+$ T-lymphocyte ratio resulting in a strong immune enhancing effect, which resulted in an adverse increase in inflammatory response at the intestinal level. Combined therapy of metronidazole and propolis was more effective in reducing the parasite count than by each drug alone (Abdel-Fattah and Nada 2007).

Propolis was used as a foliar application or soil drench on fava bean plants. Propolis treatment increased total chlorophyll and carotenoid content and the magnitude of increase was more noticeable after applying a higher concentration (1000 mg/l). It was found that fava bean plants treated with propolis extract, either as a foliar application or soil drench, were able to overcome the inhibitory influence of nematode infection on chlorophyll formation (Noweer and Dawood 2008).

A study was carried out in BALB/c mice to investigate the synergistic effect of the EEP of Egyptian propolis and immunization with *Taenia saginata* crude antigen for the prevention of bovine cysticercosis. After 24 weeks of challenge the mice in G2 (given both EEP and immunisation) showed the highest level of protection (100%) with no cyst being detected as for mice in G1 (which received only immunisation). The latter showed just 33.3% protection. Additionally, the ELISA results in this study showed higher antibody titres in G2, with reduction in the alteration of liver and kidney functions, compared to mice in G1 (Kandil et al. 2015).

There are several papers on the antihelmintic effects of propolis extracts. Propolis inhibited the growth of the helminth parasite *Fasciola gigantica* (Hegazi et al. 2007). In tests against schistosomiasis in mice, a significant reduction in the number of schistosomules of 59.2% was obtained in the group treated with propolis compared to a reduction of 98.9% in the praziquantel treated group (Issa 2007). A study was carried out to evaluate the effect of Egyptian propolis against *Toxocara vitulorum*. Adult worms were incubated for 24 h in several concentrations of EEP (100, 50, 25, 12 and 6 µg/ml) and assessed by light and scanning electron microscopy following 24 h incubation. It was observed that the extract possessed anthelmintic efficacy and the mortality rate was concentration dependent: LC_{25} was 6.9 µg/ml, LC_{50} was 12.5 µg/ml, and LC_{90} was 53.4 µg/ml. The authors thus confirmed the nematodicidal effect of Egyptian propolis (Hassan et al. 2016).

7.3.2.4 Antifungal Properties of Propolis

The activity of an ethanolic extract of Italian propolis was tested against a range of zoophilic fungi and Candida species. The extracts were effective at 5% w/v in inhibiting fungal growth (Cafarchia et al. 1999).

The activity of Brazilian propolis against 80 strains of Candida yeast was studied: 20 strains of *Candida albicans*, 20 strains of *Candida tropicalis*, 20 strains of *Candida krusei*, and 15 strains of *Candida guilliermondii*. The propolis showed clear antifungal activity with the following order of sensitivity: *C. albicans* > *C. tropicalis* > *C. krusei* > *C. guilliermondii*. MICs were in the range 8–12 mg/ml. Patients with full dentures who used a hydroalcoholic propolis extract showed a decrease in the number of Candida in their saliva (Ota et al. 2001). In a further study patients treated with a commercial ethanol extract of propolis showed lesion regression similar to that observed in patients treated with nystatin (Santos et al. 2005).

An alcoholic extract of Brazilian propolis was tested against the fungal isolates *Candida parapsilosis*, C. *tropicalis*, *C. albicans* and other yeast species obtained from onychomycosis lesions. The concentration capable of inhibiting all of the yeasts contained 50 μg/ml flavonoids while 20 μg/ml flavonoids promoted yeast cell-death. Trichosporon sp. were the most sensitive species (Oliveira et al. 2006).

The antifungal activity of propolis ethanol extract (PE) and propolis microparticles (PM) obtained from a sample of Brazilian propolis was tested against vulvovaginal candidiasis (VVC). Yeast isolates obtained from vaginal exudates of patients with VVC were exposed to PE and PM, as well as to conventional drugs used in the treatment of VVC (Fluconazole, Voriconazole, Itraconazole, Ketoconazole, Miconazole and Amphotericin B). Some *Candida albicans* isolates showed resistance or dose-dependent susceptibility for the azole drugs and Amphotericin B. All yeasts were inhibited by PE and PM, with small variations, independent of the species of yeast. While the activity of the azole drugs was much higher than both PE and PM, the extracts inhibited resistant lines in the range 33–1100 to 174–5574 μg/ml, respectively (Dota et al. 2011).

The antifungal activity of propolis extracts from Argentinian propolis was tested against a range of fungi and yeasts. The most susceptible species were *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. All the dermatophytes and yeasts tested were strongly inhibited by different propolis extracts (MICs between 16 and 125 μg/ml). The main bioactive compounds in the extracts were found to be 2′,4′-dihydroxy-3-methoxychalcone and 2′,4′-dihydroxychalcone. Both were highly active against clinical isolates of *T. rubrum* and *T. mentagrophytes* (MICs and MFCs between 1.9 and 2.9 μg/ml) (Agüero et al. 2009).

7.4 Studies of the Protective Effect of Propolis on Bees

The role of propolis in protecting beehives from infection has become of increasing interest. Poplar propolis has been found to be active against various bee pathogens and pests, including *Varroa* mite (Simone-Finstrom and Spivak 2010). It has been observed that bee colonies exposed to *Ascophaera apis* (chalkbrood fungus) increased their foraging for poplar propolis and that increased propolis levels in the hive reduced the intensity of infection (Simone-Finstrom and Spivak 2012).

The sources of propolis in the vicinity of beehives in Minnesota were studied. Despite the availability of a wide range of poplar species, the bees foraged discriminately for resin from *Poplar deltoides* (Eastern Cottonwood) and *Poplar balsamifera* (Balsam Poplar). The compositions of the resins from these trees did not exhibit much seasonal variation in composition. The resins were active against *Paenibacillus larvae* in the range of 50–175 μg/ml (Wilson et al. 2013).

The differences between French bee colonies tolerant to *Varroa destructor* compared with colonies from the same apiary which were non-tolerant to the mites were also studied. The results indicated that non-tolerant colonies collected more poplar propolis than the tolerant ones but the percentage of four compounds, caffeic acid and three pentenyl caffeates, was higher in propolis from tolerant colonies (Popova et al. 2014).

The effect of a propolis envelope on beehive viability was studied by installing propolis traps in the 'treatment' hives. Brood areas were similar between treated and untreated groups but colonies with propolis traps had significantly more worker brood than controls. However, it was not possible to replicate this in a second experiment. Colony survivorship in the treatment group was significantly higher in the first year of the experiment but not replicated in the second year. There were no differences in Varroa or virus levels between treatment and control and only marginal differences in Nosema. The transcription of six key genes involved in the immune response were lower in the treatment group. The protein vitellogenin was higher in treatment bees, indicating improved nutritional status. The presence of the propolis envelope reduced the expression of immunogenic genes and thus reduced the costly investment of energy in the immune system of individual bees (Borba et al. 2015).

7.5 Wound Healing Properties of Propolis

Propolis, along with other honeybee products, has been widely used as an external treatment for wounds and burns. Propolis is believed to possess antimicrobial, antiseptic, anti-inflammatory, antioxidant and immunomodulatory effects (Castaldo and Capasso 2002). These properties probably enhance cell proliferation in the skin and activate remodelling of the skin tissue. The wound healing process is a natural reaction of the body involving a sequence of several biochemical factors and multiple cellular reactions which can be divided into four stages, namely, hemostasis (blood clotting), inflammation, proliferation of new skin tissue, and remodelling of mature tissue (Gurtner et al. 2008). Hemostasis is an initial natural reaction that occurs

immediately in the first few minutes of injury. During this stage, platelets in the blood accumulate at the site of injury where they release chemical signals. These signals cause fibrin, a blood protein, to form a mesh that works as a glue to bind the platelets, leading to the formation of thrombi (blood clots), which seal the injury and control bleeding. Immediately after formation of the blood clot the inflammation process starts and immune cells such as macrophages and inflammatory cells such as neutrophils secrete large amounts of pro-inflammatory cytokines and reactive oxygen species (ROS), respectively. The generated ROS arrive in the wound lesion and act as a defence system against pathogens. However, any excess amount of superoxides may damage the surrounding normal tissue, such as occurs in diabetes. The proliferative phase then occurs two or three days after the injury and involves the movement of epithelial cells until they meet each other from either migration side. This occurs via stimulation of fibroblasts in the dermis layer along with keratinocytes in the epidermis. New blood vessels start to form to provide nutrition and oxygen to repair the injured tissue. Then, proliferative fibroblasts begin to secrete matrix proteins and collagen to build up the extracellular matrix that acts as a connective tissue to form initial dermal granulation tissue. After that, the fibroblasts differentiate into myo-fibroblasts which cause contraction of the wound size due to increases in collagen synthesis relative to the proliferative process. Lastly, the remodelling and maturation phase occurs, which is characterised by scar formation as a result of replacement of type III collagen by type I collagen (Guo and DiPietro 2010; Gurtner et al. 2008). The wound healing process is illustrated in Fig. 7.4.

Some recent studies have confirmed the therapeutic efficacy of propolis in different types of wounds, such as gastric ulcers, surgical wounds, infected wounds and burns (de Barros et al. 2007; Barroso et al. 2012; Martin et al. 2013). In addition, propolis accelerates the healing process in some medical conditions where there is a delay in wound healing, for example in patients of advanced age, or diabetic and immune-suppressed patients (McLennan et al. 2008).

According to several papers, propolis may aid wound healing by activation of all stages involved. Among these, there was a study which investigated the anti-inflammatory activity and wound healing potential of an alcohol extract of green Brazilian propolis. It was reported that the propolis sample could potentially control the processes involved in the early phases of wound healing, including hemostasis and inflammation (Moura et al. 2011). Several studies carried out *in vivo* have reported that propolis activity in the management of burns apparently occurs via regulation of the immune response during the inflammatory phase (Dimov et al. 1992; Mirzoeva and Calder 1996). Another study in rats showed that the skin penetration of a topical propolis ointment formulation on cutaneous wounds depends on the healing stage and mainly stimulates the proliferation phase of wound healing by stimulating the production of keratinocytes (Sehn et al. 2009). Propolis was also reported to be useful during the remodelling and maturation phase in which it stimulated the repair of granulation cells in burnt skin tissue (Olczyk et al. 2013).

A previous study of propolis in an induced diabetic rat model found that propolis showed significant effects in the acceleration of wound healing in diabetes by increasing the re-epithelialization process. The same study demonstrated a decrease in the inflammation phase, mainly by normalizing the physiological count of neutrophil and

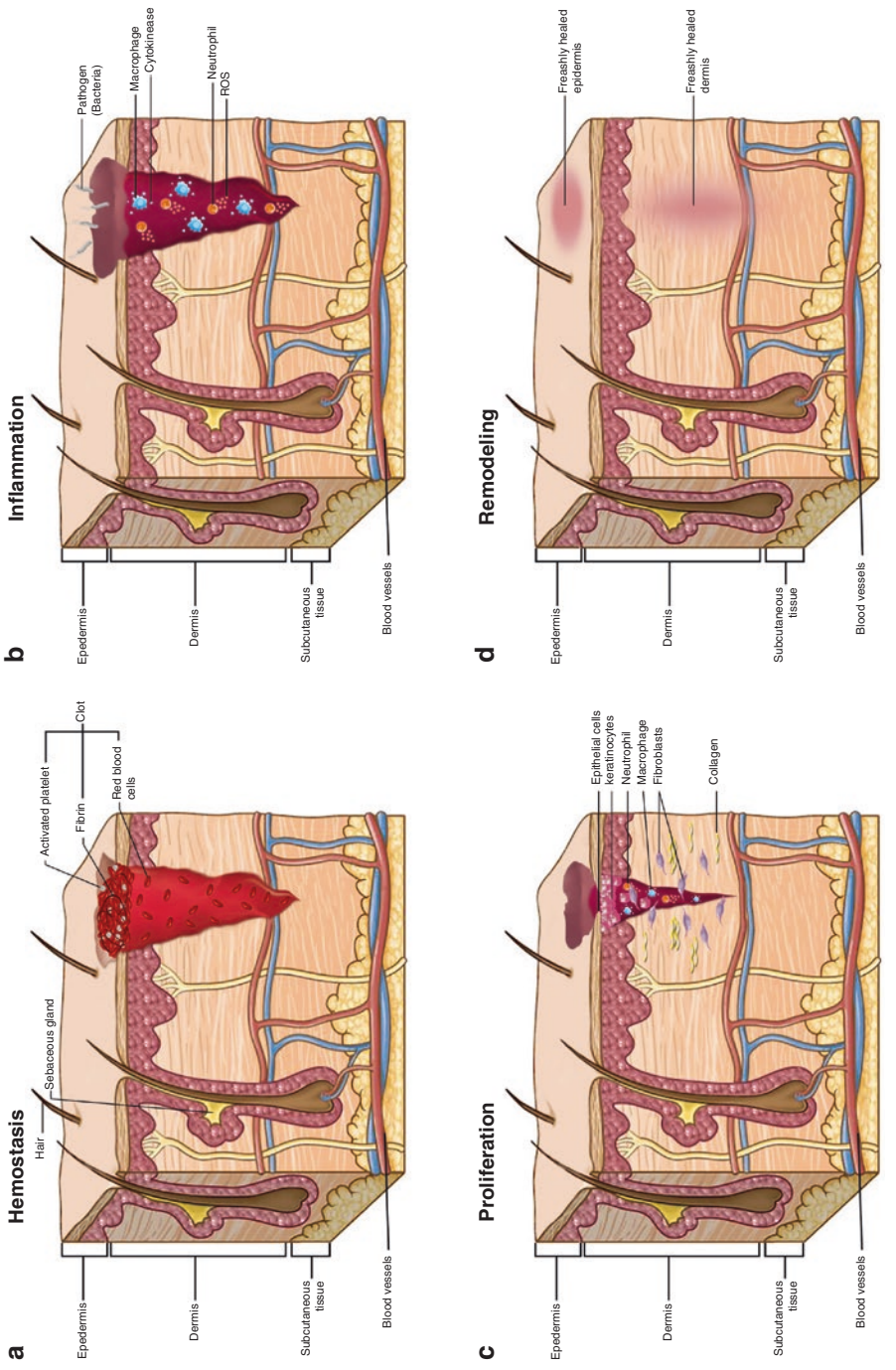

Fig. 7.4 Illustration of the process of wound healing

macrophage influx. As a result, persistent inflammation, which is frequently seen in diabetes, was prevented (McLennan et al. 2008). A recent study on wounds in a diabetic rodent model treated with a topical cream consisting of a mixture of natural products, namely: propolis, honey, royal jelly and olive oil, showed a significant beneficial effect on increasing the rate of wound healing due to antimicrobial, anti-inflammatory, and antioxidant activities (Rashidi et al. 2016).

Many scientific papers have investigated the chemical composition of propolis extracts, which could be responsible for wound healing. The study conducted by Olczyk et al. found that flavonoids have the ability to reduce lipid peroxidation and inhibit cellular necrosis (Olczyk et al. 2013). Moreover, flavonoids present in propolis reported to have antioxidant, anti-inflammatory, immunomodulatory and antimicrobial properties consequently aid in skin tissue repair and accelerate healing during wound treatment (Castaldo and Capasso 2002; Barroso et al. 2012). Another study pointed out that there is a close link between the presence of caffeic acid in propolis samples and the anti-inflammatory process *in vivo* (Rossi et al. 2002). This observation was supported by findings of a study by Song et al. which confirmed potent antioxidant and anti-inflammatory activities of caffeic acid, leading to wound healing in mice (Song et al. 2008).

In clinical trials on patients with similar minor burns, it was found that a propolis cream improved skin tissue healing and decreased wound inflammation more effectively compared to a topical silver sulfadiazine treatment (Gregory et al. 2002). Another clinical study on mouth wounds and dental sockets after tooth extraction found that the topical application of propolis stimulated epithelial cellular repair, but had no significant effect on wound healing in dental sockets (Filho and Carvahlo 1990). Thus, propolis is quite potent in accelerating wound healing because of its broad-spectrum activity encompassing all wound healing phases. Along with other bee products, propolis has multiple modes of action which give it a higher possibility of therapeutic success than drugs with a single mechanism of action.

7.6 Propolis in the Treatment of Diabetes and Cardiovascular Disease

Nitric oxide (NO) is believed to be an effector molecule that induces destruction of pancreatic β-cells resulting in type I diabetes. It has been reported that streptozotocin (SZO) induces destruction of pancreatic β-cells via a free radical mechanism which includes the production of NO. Rats were treated with water or methanol extracts of Brazilian green propolis. Untreated and propolis treated rats were treated with SZO. The rats treated with SZO alone developed elevated levels of glucose, suffered weight loss and had elevated triglyceride levels; propolis treatment inhibited these changes. It was believed that propolis acted via inhibition of nitric oxide synthase and free radical activity (Matsushige et al. 1996).

It was observed that an extract of Brazilian propolis was able to inhibit the postprandial rise in glucose levels in Sprague-Dawley rats. It was observed that the mech-

anism of action of the extract was via inhibition of maltase activity by the propolis extract, thus delaying the release of glucose from starch. The most active constituent in the propolis extract was 3,4,5-tri-caffeoylquinic acid (Matsui et al. 2004).

Type 2 diabetes has reached epidemic proportions in the developed and developing world. The anti-diabetic properties of propolis have been studied by several groups. Most commonly, rat or mouse models have been used. Type 2 diabetes was induced in rats by injection with alloxan and the rats were then treated by intragastric injection of water and ethanol extracts of Chinese propolis. Propolis treatment prevented elevation of glucose levels with time in treated compared to control diabetic animals, although levels remained well above those of normal controls. Also, propolis reduced the levels of oxidative stress and nitric oxide levels in treated compared with control diabetic animals (Fuliang et al. 2005).

SZO induced type I diabetes in rats was treated with a commercial ethanol extract of Brazilian green propolis by oral gavage. Treatment significantly increased body weight and reduced urinary albumin excretion in diabetic animals in comparison to untreated diabetic controls. In addition, treatment also increased levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CTA) and decreased malonyldialdehyde (MDA) in the renal tissue of treated diabetic animals. This suggested the potential of propolis for treating diabetic nephropathy (Abo-Salem et al. 2009).

Type I diabetes was induced in rats by injection of SZO. In the treated animals there were reductions in animal body weight and significant increases in serum glucose, triglycerides, total cholesterol and low density lipoprotein-cholesterol (LDL-C), and a decrease in serum high density lipoprotein-cholesterol (HDL-C) (51%) as compared to the control normal group. In addition, there was a significant elevation in malondialdehyde (MDA) and a marked reduction in glutathione (GSH), catalase (CAT), and pancreatic superoxide dismutase (SOD) in SZO-treated rats. Oral treatment of animals with a commercial ethanol extract of Brazilian green propolis showed reduced weight loss and alterations in glucose, lipids, lipoproteins, NO, GSH, CAT, pancreatic MDA, and SOD levels (El-Sayed et al. 2009).

The hypolipidemic effect of propolis in a mouse obesity model induced by a high fat-diet was observed in C57BL/6N mice fed a high-fat diet and given Brazilian propolis extract intragastrically. Propolis inhibited weight gain and the formation of visceral adipose tissue. In addition, propolis reduced the levels of free fatty acids and glucose in serum, and triglycerides in liver and serum. Realtime PCR results suggested that the anti-obesity effects of the propolis extract could be attributed to reduced expression of fatty acid synthesis genes in the liver (Koya-Miyata et al. 2009).

An extract of Brazilian red propolis was found to promote the differentiation of pre-adipocytes into adipocytes *in vitro* via its activity on peroxisome proliferator-activated receptor γ (PPAR γ). Several genes associated with adipocyte formation were upregulated, thus providing a potential mechanism for the observed effects of propolis in promoting lipid metabolism (Iio et al. 2010).

Diabetes was induced in rats by a single dose of SZO (35 mg/kg). Rats with a high level of glycaemia were treated with an extract of Brazilian green propolis. Treatment over seven days had no effect in reducing blood glucose or free fatty acid

levels, or in reducing weight loss (Sartori et al. 2009). This contrasts with other findings. However, in other studies longer term administration of SZO was carried out, thus inducing more severe type I diabetes. In another study, type I diabetes was induced in rats treated with a single dose of SZO (60 mg/kg) and propolis was found to reduce blood glucose and increase insulin levels in treated diabetic rats. Animals treated with propolis had a reduced level of thioabarbituric acid reactive substances in their serum. There was no effect of propolis treatment on calcitonin or parathyroid hormone levels (Al-Hariri et al. 2011).

The effect of propolis on SZO induced type I diabetes in rats was studied. Chinese and Brazilian propolis extracts (10 mg/ml) were administered orally. Propolis was found to inhibit weight loss in diabetic rats and to reduce blood glucose levels. There was some reduction in glycated haemoglobin levels and oxidative stress was reduced in propolis treated rats in comparison to untreated controls (Zhu et al. 2011).

Type 2 diabetes was induced in a rat model by a combination of a high fat diet and injection of low dose SZO. Animals were treated with Chinese propolis encapsulated in cyclodextrin by spray drying and the propolis was administered via gavage. The propolis was able to decrease blood glucose levels, improve lipid profiles, and improve insulin sensitivity in the treated animals in comparison to untreated diabetic controls (Li et al. 2011).

Type I diabetes was induced in mice by treatment with alloxan. The mice were treated by intra-peritoneal administration of extracts from Croatian propolis. The lifespans of the propolis treated mice were significantly increased in comparison to untreated controls and they also gained weight in comparison with controls. The propolis treatment reduced the degree of cellular vacuolisation in the livers of treated mice due to protection against reactive oxygen species and improved fatty acid metabolism. The treated mice had less infiltration of lymphocytes and eosinophils into their kidney tissues (Oršolić et al. 2012).

The ob/ob mice are genetically obese and serve as a model of type 2 diabetes. An extract of Brazilian green propolis was administered via intra-peritoneal injection. Treatment had no effect on body weight or food intake but blood glucose and plasma cholesterol levels were lowered in comparison to untreated mice, and glucose tolerance and insulin sensitivity were improved. The propolis-treated mice showed lower weight gain in mesenteric adipose tissue. It was proposed that Brazilian propolis improved diabetes in *ob*/*ob* mice through modulation of immune cells in mesenteric adipose tissues (Kitamura et al. 2013).

The effect of oral administration of propolis on Otsuka Long-Evans Tokushima Fatty rats, which have the symptoms of type 2 diabetes, was studied. Glucose and insulin levels and systolic blood pressure were all lowered in the treated rats compared to controls after eight weeks of treatment. Interstitial fluid pH was higher in ascites, liver, and skeletal muscle after propolis treatment, suggesting that the effects of propolis might be mediated via suppression of metabolic acidosis (Aoi et al. 2013).

BALB/c mice were treated with S961 peptide, an antagonist of the insulin receptor, in order to induce type 2 diabetes. The mice were treated orally with an ethanolic extract of Indonesian propolis. Propolis treatment was found to decrease

blood glucose levels and levels of interferon-γ via a reduction in the level of activated T-cells, thus potentially reducing T-cell mediated damage (Rifa'i and Widodo 2014).

The effect of Brazilian green propolis, and four flavonoids isolated from it, on systolic blood pressure in spontaneously hypertensive rats was observed. The four flavonoids all lowered systolic blood pressure after 28 days with isosakuranetin being the most potent. In addition, the four flavonoids and various fractions of Brazilian green propolis were found to relax aorta from spontaneously hypertensive rats in a concentration dependent manner (Maruyama et al. 2009).

The effect of Brazilian propolis on the development of atherosclerosis in rabbits fed high levels of cholesterol was also studied. The cholesterol-enriched diet promoted increases in serum total cholesterol (TC), triglycerides, low density lipoprotein cholesterol (LDLC), concentrations of thiobarbituric acid-reactive substances (TBARS), and a decrease in high density lipoprotein-cholesterol (HDLC) and glutathione (GSH) levels compared to the control group. Administration of propolis reduced levels of TC, LDLC, triglycerides and TBARS, while increasing HDLC and GSH. Propolis lowered the levels of endothelial damage and thickened foam cells in aorta and reversed the damage to the kidneys induced by the high cholesterol diet as observed by histopathology (Nader et al. 2010).

7.7 Anti-cancer Effects of Propolis

The anti-cancer effects of propolis have been widely studied in *in vitro* and animal models. There is an overlap between anti-cancer effects and the immunomodulatory effects of propolis, which largely seems to exert its anti-cancer activity via immunomodulation. The anticancer properties of propolis have been recently reviewed (Watanabe et al. 2011).

A methanol extract of Netherlands propolis had antiproliferative activity towards murine colon 26-L5 carcinoma with an EC_{50} value of 3.5 mg/ml. Fractionation of the extract resulted in the isolation of four flavonoids, seven cinnamic acid derivatives and glycerol derivatives. The isolated compounds were tested for their antiproliferative activity. The three most active had EC_{50} values of 0.288, 1.76 and 0.114 μM towards colon 26-L5 carcinoma (Banskota et al. 2002).

Polyphenolic compounds isolated from propolis and a water soluble extract of Croatian propolis were investigated for their effects on the growth and metastatic potential of mammary carcinoma in mice. Metastases in the lung were generated by intravenous injection of tumour cells. Oral dosing of the compounds and extract (50 mg/kg) significantly decreased the number of tumour nodules in the lung (Oršolić et al. 2004).

The effect of propolis in combination with paclitaxel against induced experimental breast cancer was investigated in female Sprague Dawley rats. Administration of paclitaxel and propolis effectively suppressed breast cancer, which was revealed by a decrease in the extent of lipid peroxidation with an increase in the activities of

superoxide dismutase, catalase and glutathione peroxidase, and the levels of glutathione, Vitamin C and Vitamin E when compared to animals treated with either paclitaxel or propolis alone. Thus, it was proposed that co-administration could reduce the side-effects of paclitaxel (Padmavathi et al. 2006).

The effects of ethanolic extracts of two samples of Brazilian propolis and the bud resin of *B. dracunculifolia* on the proliferation of human prostate cancer cells was studied. The Brazilian propolis extracts showed significant inhibitory effects on the proliferation of human prostate cancer cells. Inhibition was achieved through regulation of protein expression of cyclins D1 and B1, and cyclin dependent kinase, as well as expression of the p21 protein (Paredes-Guzman et al. 2007).

An ethanolic extract of green Brazilian propolis (EEBP) significantly reduced the number of newly formed vessels in an *in vivo* angiogenesis assay. The ethanolic extract of propolis (EP) also showed antiangiogenic effects in an *in vitro* tube formation assay. The major constituent of EP, artepillin C, was found to significantly reduce the number of newly formed vessels in an *in vivo* angiogenesis assay. Thus, artepillin C, at least in part, is responsible for the antiangiogenic activity of EEBP *in vivo* and might prove effective in counteracting tumour angiogenesis (Ahn et al. 2007).

The prenylated flavanoid propolin G, isolated from Taiwanese propolis, was able to efficiently induce apoptosis in brain cancer cell lines, glioma and glioblastoma, with IC_{50} values of 5 and 7.5 μg/ml, respectively, against the two cell lines. The results suggested that apoptosis might have occurred through a mitochondrial- and caspase-dependent pathway. Propolin G and Taiwanese and Brazilian propolis extracts were also found to protect against oxidative stress in rat primary cortical neurons (Huang et al. 2007).

The activity of extracts of three Mexican propolis samples against human colonic, human lung, and cervical cancer cells was examined. The extracts exhibited strong anti-proliferative activities and analysis of DNA isolated from treated cells showed the presence of intra-nucleosomal DNA cleavage (Hernandez et al. 2007).

An extract of propolis was found to inhibit the proliferation of U937 cells in a dose-dependent manner by inducing apoptosis and blocking cell cycle progression in the G2/M phase. Western blot analysis showed that propolis increased the expression of p21 and p27 proteins, and decreased the levels of cyclin B1, cyclin A, Cdk2 and Cdc2, causing cell cycle arrest. The results suggested that propolis-induced apoptosis was related to the selective activation of caspase-3 and induction of Bcl-2/Bax regulation (Motomura et al. 2008).

A methanol extract of red Brazilian propolis was found to kill 100% of nutrient starved human pancreatic cancer cells at a concentration of 10 μg/mL. Forty three compounds were isolated from the extract with the most potent compound producing 100% kill at a concentration of 12.5 μM. The mechanism of cell death was believed to be via necrosis. The targeting of nutrient deprived cells presents a method of targeting cells which are resistant to nutrient starvation (Li et al. 2008).

A methanolic extract of propolis from Myanmar inhibited the growth of human pancreatic cancer cells preferentially under nutrient-deprived conditions (NDM), with a PC_{50} value of 9.3 μg/mL. Two new cycloartane-type triterpenes, 13 cycloartanes, and four known prenylated flavanones were isolated from the extract. One of

the newly identified triterpene acids exhibited the most potent preferential cytotoxicity (PC_{50} 4.3 μM) in a concentration- and time-dependent manner and induced apoptosis-like morphological changes of PANC-1 cells within 24 h of treatment (Li et al. 2010).

The anticancer effect of ethanol extracts of Chinese and Brazilian propolis on four human colon carcinoma cell-lines was studied. The extracts of both Chinese and Brazilian propolis caused a marked dose-dependent growth inhibition of the cells, with IC_{50} values in the range 4–41 μg/ml (Ishihara et al. 2009).

The effect of propolis on the production of IL-2, IF-γ, IL-4 and IL-10 cytokines by T-helper cells in melanoma-bearing mice submitted to immobilization stress was studied. Spleen cells were assessed for cytokine expression. Stress induced a higher rate of tumour growth, while propolis-treated mice, stressed or not, showed melanoma development similar to the control. Cytokine production was inhibited in melanoma-bearing mice and propolis administration to melanoma-bearing mice submitted to stress promoted IL-2 and IFN-γ production, indicating the activation of antitumor cell-mediated immunity. Propolis also stimulated IL-10 expression and production (Missima et al. 2010).

Extracts from two Portuguese propolis samples were tested for their anticancer properties on human renal cell carcinoma cells, with IC_{50} values of 56.5 and 56.1 μg/ml. The extracts were much less toxic against normal human renal cells (Valente et al. 2011).

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is an important endogenous anti-cancer cytokine that induces apoptosis selectively in tumour cells. However, some cancer cells are resistant to TRAIL-mediated apoptosis. Phenolic and polyphenolic compounds sensitize TRAIL-resistant cancer cells and augment the apoptotic activity of TRAIL. The cytotoxic and apoptotic effects of an ethanolic extract of Brazilian green propolis (EEP) was examined against LNCaP prostate cancer cells. The extract sensitised TRAIL-resistant prostate cancer cells to TRAIL-mediated apoptosis, and when prostate cancer cells were co-treated with 100 ng/ml TRAIL and 50 μg/ml EEP, the percentage of apoptotic cells was increased to 65.8%—several fold greater than the level induced by TRAIL alone (Szliszka et al. 2011a, b).

An ethanolic extract of stingless bee propolis from Thailand was tested for antiproliferative activity against five cancer cell lines and two normal cell lines. The cell viability (%) and IC_{50} values were calculated. The hexane extract provided the highest *in vitro* activity against the five cancer cell lines and the lowest cytotoxicity against the two normal cell lines. Fractionation of the extract produced a fraction with high antiproliferative activity with IC_{50} values in the range 4.09–14.7 μg/ml against cancer cells but with limited activity against normal cells (Umthong et al. 2011).

PC-3 prostate cancer cells were incubated with dimethyl sulfoxide and water extracts of Turkish propolis and the treatments were found to significantly reduce cell viability to 24.5% and 17.7%, respectively. Statistically significant discriminatory peaks in the proteomic profiles were observed between control PC-3 cells and those treated with the dimethyl sulfoxide extract of propolis. Surface enhanced laser desorption ionization time of flight mass spectrometry was used to examine changes in the proteome as a result of the propolis treatments and it was found that

the treatment promoted changes in the proteome, suggesting that this might be a mechanism for the cytotoxic action (Barlak et al. 2011).

Extracts of *Apis mellifera* propolis collected in Thailand were assayed for cytotoxic activity against five human cancer cell lines and a control cell line. Crude hexane and dichloromethane extracts of propolis displayed anti-proliferative/cytotoxic activities with IC_{50} values across the five cancer cell lines ranging from 41.3 to 52.4 μg/ml and from 43.8 to 53.5 μg/ml, respectively. Two main bioactive components, a cardanol and a cardol, were isolated and IC_{50} values across the five cancer cell lines ranging from 10.8 to 29.3 μg/ml were obtained for the cardanol and <3.13 to 5.97 μg/ml for the cardol. Both compounds induced cytotoxicity and cell death without DNA fragmentation in the cancer cells, but only an anti-proliferation response in the control Hs27 cells (Teerasripreecha et al. 2012).

An ethanolic extract of Brazilian red propolis was found to significantly reduce the viability of MCF-7 breast cancer cells through the induction of mitochondrial dysfunction, caspase-3 activity, and DNA fragmentation, but did not affect these factors in a control cell line. In addition the extract was found to promote apoptosis via endoplasmic reticulum stress (Kamiya et al. 2012).

7.8 Immunomodulatory Effects of Propolis

The immunological effects of propolis have been recently reviewed (Sforcin 2007).

A water soluble extract of propolis (WSP) was used to modulate the alternative complement activation pathway (AP) in mice. The extract was administered via the oral, intravenous, and intraperitoneal routes. The most significant effect was obtained from intraperitoneal administration which inhibited the AP and also caused a moderate fall in zymosan-induced paw odema (Ivanovska et al. 1995). In a related study, the WSP was found to inhibit the complement pathway (CP) to a greater extent than AP in human serum, whereas in mice the reverse was the case (Ivanovska et al. 1995).

Paracoccidoides brasiliensis affects individuals living in endemic regions, such as certain areas in Brazil, through inhalation of airborne conidia or mycelial fragments. Peritoneal macrophages isolated from BALB/c mice were stimulated with Brazilian or Bulgarian propolis and subsequently challenged with *P. brasiliensis*. There was an increase in the fungicidal activity of macrophages treated with either propolis extract (Murad et al. 2002).

Macrophages were pre-stimulated with extracts of Brazilian or Bulgarian propolis and subsequently challenged with *Salmonella typhimurium* at different macrophage/bacteria ratios.

To assess the bactericidal activity, the number of colony-forming units of *S. typhimurium* after 60 min was counted. Propolis from Brazil and Bulgaria enhanced the bactericidal activity of macrophages, depending on its concentration, with Brazilian propolis being more effective than that from Bulgaria (Orsi et al. 2005).

The effect of intraperitoneal or dietary administration of propolis on innate immune response of gilthead seabream was evaluated. Fish were intraperitoneally injected with 5 mg water (WEP), ethanol (EEP) or both WEP and EEP extracts of propolis. Humoral (alternative complement activity and peroxidase content) and cellular (leucocyte peroxidase, phagocytosis, cytotoxicity and respiratory burst activity) immune responses were evaluated in both cases. The results suggested that propolis had limited immunostimulatory effects although intraperitoneal administration was more effective than dietary intake (Cuesta et al. 2005).

The adjuvant capacity of an ethanol extract of green propolis associated with inactivated Suid herpesvirus type 1 (SuHV-1) vaccine preparations was tested. Mice inoculated with the SuHV-1 vaccine plus aluminium hydroxide plus 5 mg propolis extract produced higher levels of antibodies when compared to animals that received the vaccine plus aluminium hydroxide without propolis. The SuHV-1 vaccine with propolis extract alone did not induce a significant increase in antibodies, however, in this case the cellular immune response increased based on an increase in the expression of mRNA for IFN-γ. Using propolis as an adjuvant increased the percentage of animals surviving challenge with a lethal dose of SuHV-1 (Fischer et al. 2007).

The interaction between stress and immunity has been widely investigated and has been found to involve the neuroendocrine system and several organs. The effect of propolis on activated macrophages in BALB/c mice submitted to immobilization stress was investigated and histopathological analysis of the thymus, bone marrow, spleen and adrenal glands was also carried out. Stressed mice showed higher hydrogen peroxide generation by peritoneal macrophages, and propolis treatment potentiated hydrogen peroxide generation and inhibited nitric oxide (NO) production by these cells. Histopathological analysis showed no alterations in the thymus, bone marrow and adrenal glands, but increased germinal centers in the spleen. Propolis treatment counteracted the alterations found in the spleen of stressed mice (Missima and Sforcin 2008).

The effect of Turkish propolis samples on peripheral blood mononuclear cells (PBMC) was studied. All propolis samples decreased mitogen-induced neopterin release and tryptophan degradation in both stimulated and non-stimulated PBMC. In addition, TNF-α and IFN-γ release in stimulated PBMC was inhibited by propolis extracts (Girgin et al. 2009).

The effect of oral propolis administration in mice on IL-2, IFN-γ, IL-4 and IL-10 production by T-helper 1 (Th-1) and T-helper 2 (Th-2) cells was studied. Propolis administration to mice did not affect IL-2, IL-4 and IL-10 expression and production, but IFN-γ production was inhibited in the splenocyte cultures, when both unstimulated or stimulated by conclavin A. These findings support the proposed anti-inflammatory effects of propolis (Orsatti et al. 2010a, b).

The antimicrobial activity of Brazilian stingless bee (*Melipona fasciculata*) geopropolis against oral pathogens and its effects on *Streptoccus mutans* biofilms was studied. Activity was observed against *S. mutans* and *Candida albicans* but not against *Lactobacillus acidophilus*. The propolis extract increased the levels of anti-inflammatory cytokines IL-4 and IL-10 in the blood of mice which were treated in their oral cavity with a gel containing propolis (Liberio et al. 2011).

The effect of propolis on Toll-like receptor (TLR-2 and TLR-4) expression and on the production of pro-inflammatory cytokines (IL-1β and IL-6) was evaluated. Male BALB/c mice were treated with propolis (200 mg/kg) by oral gavage for three consecutive days. TLR-2 and TLR-4 expression was increased in the peritoneal macrophages of the propolis-treated mice. TLR-2 and TLR-4 expression and IL-1β and IL-6 production were also upregulated in the spleen cells of propolis-treated mice. This suggests that propolis activates the initial steps of the immune response by upregulating TLR expression and by the production of pro-inflammatory cytokines in mice (Orsatti et al. 2010a, b).

The effect of propolis on chronically stressed mice was assessed by evaluating TLR-2 and TLR-4 expression by spleen cells, and corticosterone levels. Propolis administration to the stressed mice prevented inhibition of TLR-2 and TLR-4 expression. No effect was seen on the corticosterone levels among the groups. It was concluded that propolis exerted an immunomodulatory action in chronically stressed mice by upregulating TLR-2 and TLR-4 mRNA expression, thus supporting the immune system (Orsatti and Sforcin 2012).

An extract of Brazilian green propolis, given orally at a dose of 5 mg/kg, was shown to inhibit the production of pro-inflammatory cytokines and promote the production of anti-inflammatory cytokines in mice challenged either with a cotton pellet granuloma implanted in subcutaneous tissue or by intranasal administration of lipopolysaccharide (Machado et al. 2012).

Cinnamic acid (CA) is a major component of propolis. The effects of CA were evaluated on human monocytes by assessing the expression of Toll-like receptors HLA-DR and CD80. Cytokine production (TNF-α and IL-10) and the fungicidal activity of monocytes were also evaluated. CA was found to downregulate TLR-2, HLA-DR, and CD80 and upregulate TLR-4 expression by human monocytes. High concentrations of CA inhibited both TNF-α and IL-10 production, and induced a higher fungicidal activity against *Candida albicans*. TNF-α and IL-10 production was decreased by blocking TLR-4, while the fungicidal activity of monocytes was not affected by blocking TLRs (Conti et al. 2013).

The antileishmanial and immunomodulatory effects of Brazilian propolis were evaluated in an experimental *Leishmania braziliensis* infection. In the immunomodulatory assay, macrophages were pre-treated with propolis and then infected with *L. braziliensis in vivo*. Supernatants from liver cells and peritoneal exudates of BALB/c mice were pretreated with propolis and infected with *Leishmania* promastigotes, and TNF-α and IL-12 were measured. Macrophages incubated with propolis showed a significant increase in interiorization and greater killing of parasites. Increased TNF-α production was seen in mice pretreated with propolis, whereas IL-12 was downregulated during the infection. Brazilian propolis showed direct action on the parasite and displayed immunomodulatory effects on macrophages (Da Silva et al. 2013).

The expression of TLR-2, TLR-4, human leukocyte antigen-DR and cluster of differentiation (CD) 80 by human monocytes was assessed following incubation with Brazilian propolis. In addition, production of TNF-α and IL-10 was measured. Propolis was found to upregulate TLR-4 and CD80 expression, and inhibited TNF-α and IL-10 production at 100 μg/ml, but stimulated their production at lower concentrations. The propolis also increased the fungicidal activity of monocytes. Propolis did not affect cell viability (Bufalo et al. 2014).

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