
Propolis: Biological and Pharmacological Activities. Therapeutic Uses of this Bee-product

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Abstract

This review aims to present an update of propolis properties, with emphasis on Brazilian samples. It also brings attention to points, such as standardization protocols and scientific evidence, that needed to be further investigated in order to broaden the potential therapeutic use of this bee product. Propolis became a traditional remedy in folk medicine. In recent decades a great interest has arisen in more knowledge about its composition and therapeutic activities. Its composition is very complex, varying with the geographic region, with more than 300 constituents having been identified to date. The biological activity of propolis is associated mainly with phenolic compounds such as flavonoids and derivatives of hydroxycinnamic acids. In recent years, Brazilian propolis became the subject of increasing interest, due to its unusual chemical composition when compared with samples from other countries. Several compounds isolated presented a variety of biological activities, such as microbicidal, antioxidant and antitumoral. Although there is a huge amount of information about the chemistry and biological activity of propolis, its application in human and veterinary therapies has hardly changed. Propolis is used both in human and veterinary clinics in Eastern Europe countries, Cuba and in Uruguay. Most of the therapeutic effect of this resin is associated with microbicidal properties and the ability to scavenge free radicals. A multidisciplinary study correlating chemical composition, basic plant sources in different geographic regions, as well as and biological and pharmacological activities, open the possibility of a classification of a limited number of "chemical types" of propolis which will be possible only with the combined effort of the national and international scientific communities.

Key-Words: propolis, bee product, natural product, biological activity, microbicidal activity, clinical use.

Background

Honeybees not only play an important role in the pollination of many crops, but also provide valuable products that are good for our health, such as honey, royal jelly, pollen and propolis. In some countries bee products are used successfully both in human and veterinary medicine and also as food complement. Many vegetables protect their leaves, flowers, fruits and buds by producing a resinous compound with potent antimicrobial, anti-putrefaction, waterproofing and heat-insulating properties. These resins are gathered from the gum of various plant sources by honeybees (*Apis mellifera* L.) that form pellets with their mandibles, probably mixing it with products of their salivary glands and with bee wax. So, propolis (bee glue) is an amalgamation of plant resins collected and transformed by bees, and is a strongly adhesive and resinous substance whose color varies from yellow-green to dark brown depending on its source and age. It is hard and brittle when cold, but becomes soft

and very sticky when warm. Bees use propolis as a glue to block holes and cracks in the beehive, to strengthen the border of their nests, to seal their honeycombs and to smoothe out internal walls. The resin also prevents growth of microorganisms and protects the hive entrance against intruders. It is also used as an embalming substance to cover bees' prey, preventing their decomposition, and the spread of diseases.

Propolis has been, used since ancient times, for example by Egyptians for embalming their dead. Also it was registered that Hippocrates (460-377 BC) prescribed its use to help heal external and internal sores and ulcers. Documentary evidence of its therapeutic use comes from the beginnings of the 20th century. It seems that the first use of propolis in the Modern Era was during the Boer War, when it was employed for disinfection of wounds and tissue regeneration (reviewed in Ghisalberti, 1979). Afterwards, it became a traditional remedy in folk medicine. Before the decade of 1980s, scientific reports about the composition and biological and therapeutic uses of propolis were not easily accessible, since most of them were published in Eastern European journals.

Chemical Composition

The chemical composition of propolis has been the subject of several reviews (Ghisalberti, 1979; Greenaway et al., 1991; Marcucci, 1995). Its composition is very complex and more than 300 constituents have been identified to date. Its composition varies with the season and the vegetation in the areas in which it is collected. Among these constituents we found wax, resins, balsams, essential oils, amino acids and sugars, with a prevalence of flavonoids and derivatives of cinnamic acid. Variable amounts of sugars are usually present, probably introduced accidentally during propolis manufacture and/or passage of bees over the resin. Propolis is rich in inorganic elements; some of them involved in fundamental enzymatic systems, which could be associated with its biological activities (Scheller et al., 1989). Volatile compounds mainly sesquiterpenoids are present in low concentrations, but their aroma and biological activity make them potential markers for propolis characterization (Bankova et al., 1994).

The biological activity of propolis is associated mainly with phenolic compounds such as flavonoids and derivatives of hydroxycinnamic acids. Flavonoids are a group of polyphenolic conjugated aromatic compounds, diverse in chemical structure and characteristics, potent antioxidants, free-radical scavengers and metal chelators (reviewed in Harborne & Williams, 2000). Quercetin and other flavonoids have been reported to act as anti-inflammatory agents, to prevent atherosclerotic plaque formation and platelet aggregation, to promote relaxation of cardiovascular smooth muscle and to display antiviral, carcinostatic, anti-ulcer and anesthetic activities. Flavonoids give color and high visibility for attracting pollinators, such as

insect and birds, essentials for vegetal reproduction, and are present in considerable quantities in food and beverages. Epidemiological studies revealed that a diet rich in flavonoids is positively correlated with increased longevity and decreased incidence of cardiovascular disease (reviewed in Cook & Samman, 1996). Besides these polyphenolics, propolis also contains other phenolic compounds, such as hydroxycinnamic acids such as caffeic acid (3,4-dihydroxi cinammic acid), that are found in almost every plant, and are also potent antioxidant agents (Clifford, 1999). The caffeic acid phenethylester (CAPE) is another active ingredient of propolis with a broad spectrum of biological activities, including antioxidation and tumor-cell cytotoxicity (Chen et al., 2001).

The materials available for the “manufacture” of propolis include substances actively secreted by plants as well as those exuded from wounds. For this reason propolis composition varies depending on the vegetation of a given geographic area. When sources of propolis appear to be absent, bees use “propolis substitutes” such as certain man-made products like paint, asphalt and mineral oils. In temperate zones, bud exudates from different poplar trees (*Populus* spp) are the main sources of propolis (reviewed in Bankova et al., 2000). Samples from these regions are characterized by similar chemical composition, the main constituents being flavonoid aglycones, aromatic acids and their esters. In tropical regions there are no poplars or birches, so obviously bees have to find new plant sources (Wollenweber & Buchmann, 1997; Martos et al., 2000). In Venezuela, Tomas-Barberan et al. (1993) isolated polyprenylated benzophenones that are the main components of the resin exuded by the flowers of *Clusia* species, the main source for propolis in the studied region. The investigations on tropical propolis concentrated almost exclusively on the resin produced by *Apis mellifera*, but in South America, there are also indigenous stingless bees from the subfamily Meliponinae, which collect resinous materials from plants and mix it with bee wax and soil, giving rise to geopropolis (Tomas-Barberan et al., 1993; Bankova et al., 1998; Velikova et al., 2000).

Compounds Isolated from Brazilian Propolis

Recently Brazilian propolis became the subject of increasing interest, were the main plant sources are *Araucaria* spp., *Baccharis* spp and *Eucalyptus* spp (reviewed in Bankova et al., 2000). Both the national and international markets of bee products are expanding in Brazil. The State of Paraná (South of Brazil) produces per year 8 thousand tons of honey, 37 tons of propolis and 900 tons of wax, valued in about US\$ 15 millions.

Following the classification of Bankova et al. (2000) for compounds isolated from tropical propolis, we will include in the present work those isolated from Brazilian samples that also had their biological activities investigated (*see next topic*). They were numbered and their formulae are displayed in Fig. 1 with names and references in Table 1.

Table 1: Bioactive compounds isolated from Brazilian propolis.

#	Compounds	References
1	3,5-diprenyl-4-hydroxycinnamic acid	Aga et al., 1994
2	3-prenyl-4-hydroxycinnamoloxyacetic acid	Aga et al., 1994
3	2,2,-dimethyl-6-carboxyethenyl-2H-1-benzopyran	Aga et al., 1994
4	2,2- dimethyl-6-carboxyethenyl-8-prenyl-2H-benzopyrane (<i>E</i> and <i>Z</i> isomers)	Boudourova-Krasteva et al., 1997
5	3-prenyl-4-hydroxycinnamic acid	Marcucci et al., 2001
6	<i>ent</i> -17-hydroxy-3,13 <i>Z</i> -clerodadien-15-oic acid	Matsuno et al., 1997 ^b
7	15-oxo-3,13 <i>Z</i> -kolavadien-17-oic acid and <i>E</i> -isomer (15-oxo-3 <i>Z</i> ,13 <i>E</i> -kolavadien-17-oic acid)	Matsuno et al., 1997 ^b
8	communic acid	Bankova et al., 1996 ^a
9	imbricatolonic acid	Bankova et al., 1996 ^a
10	isocupressic acid	Bankova et al., 1996 ^a
11	acetylisocupressic acid	Bankova et al., 1996 ^a
12	8(17),13 <i>E</i> -labdadien-15,19-dioic acid	Banskota et al., 1998
13	8(17),13 <i>E</i> -labdadien-15,19-dioic acid methyl ester	Banskota et al., 1998
14	3,4-dicaffeoylquinic acid	Basnet et al., 1996 ^a
15	3,4-dicaffeoylquinic methyl ester	Basnet et al., 1996 ^a
16	3,5-dicaffeoylquinic acid	Basnet et al., 1996 ^a
17	3,5-dicaffeoylquinic methyl ester	Basnet et al., 1996 ^a
18	3-caffeoylquinic (chlorogenic) acid	Tatefuji et al., 1996
19	4-caffeoylquinic acid	Tatefuji et al., 1996
20	5-caffeoylquinic acid	Tatefuji et al., 1996
21	4,5-dicaffeoylquinic acid	Tatefuji et al., 1996
22	3-acetoxymethyl-5-[(<i>E</i>)-2-formylethen-1-yl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran	Bankova et al., 1996 ^b Banskota et al., 1998
23	3-[4-hydroxy-3-(3-oxo-but-1-enyl)-phenyl]-acrylic acid	Basnet et al., 1997
24	benzofuran derivative A(1)	Banskota et al., 2000 ^a
25	benzofuran derivative B(2)	Banskota et al., 2000 ^a
26	coniferyl aldehyde	Banskota et al., 1998
27	betuletol	Banskota et al., 1998
28	kaempferide	Banskota et al., 1998
29	ermanin	Banskota et al., 1998

***p*-Coumaric Acid Derivatives**

In 1994, Aga et al. from a sample collected in the State of São Paulo (SP) and extracted with ethyl acetate, isolated three derivatives of *p*-coumaric acid: 3,5-diprenyl-4-hydroxycinnamic acid (**#1**); 3-prenyl-4-hydroxycinnamoloxyacetic acid (**#2**) and 2,2,-dimethyl-6-carboxyethenyl-2H-1-benzopyran (**#3**). From a methanolic extract from another SP sample the isomers *E* and *Z* of 2,2- dimethyl-6-carboxyethenyl-8-prenyl-2H-benzopyrane (**#4**) were isolated (Boudourova-Krasteva et al., 1997). Using a sample from the State of Paraná (PR) extracted with methanol, besides the isolation of **#1**, **#3** and **#4**, a new derivative 3-prenyl-4-hydroxycinnamic acid (**#5**) was also characterized (Marcucci et al., 2001). Also from a methanolic extract, but using a mixture of propolis samples from different regions in the South of Brazil, **#1**, named artepillin C. was isolated together with **#4**, which could be obtained by cyclization of the former compound (Matsuno et al., 1997^a, 1998). Based on the content of kaempferol derivatives, *p*-coumaric acid and prenylated coumaric acids, forty samples from the South and South East of Brazil were classified in four groups (Marcucci et al., 2000).

Diterpenic Acids

From the same methanolic extract employed to isolate **#1**, the following clerodane diterpenoids were isolated: *ent*-17-hydroxy-3,13*Z*-clerodadien-15-oic acid (**#6**), 15-oxo-3,13*Z*-kolavadien-17-oic acid (**#7**) and its *E*-isomer 15-oxo-3*Z*,13*E*-kolavadien-17-oic acid (Matsuno et al., 1997^b).

Labdane-type diterpenic acids (communic, imbricatoloic, isocupressic and acetoxisocupressic acids (**#8-#11**) typical from species of *Araucaria* were isolated from the methanolic extract of the PR sample (Bankova et al., 1996^a). Banskota et al. (1998), also using a methanolic extract, isolated besides compounds **#10** and **#11**, 8(17),13*E*-labdadien-15,19-dioic acid (**#12**) and its methyl ester (**#13**).

Caffeoylquinic Acids

From a water extract Basnet et al. (1996^a) characterized the compounds 3,4-dicaffeoylquinic acid (**#14**), its methyl ester (**#15**), 3,5-dicaffeoylquinic acid (**#16**) and its the methyl ester (**#17**). Also from a water extract of a sample from State of Amazonas State (North of Brazil), besides **#14** and **#16**, the following caffeoylquinic acids were also characterized: 3-caffeoylquinic (chlorogenic), 4-caffeoylquinic, 5-caffeoylquinic and 4,5-dicaffeoylquinic acids (**#18-21**) (Tatefuji et al., 1996).

Lignan

Using the same extract from a previous work (Bankova et al., 1996^a), Bankova et al. (1996^b) reported the isolation of the lignan 3-acetoxymethyl-5-[(*E*)-2-formylethen-1-yl]-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (**#22**). This lignan was also isolated from a methanolic extract from a different Brazilian sample by Banskota et al. (1998).

Flavonoids and Other Aromatic Compounds

The aromatic compound 3-[4-hydroxy-3-(3-oxo-but-1-enyl)-phenyl]-acrylic acid (**#23**), known commonly as propol, was isolated from a water extract of Brazilian propolis (Basnet et al., 1997). From a methanolic extract two benzofuran derivatives (**#24** and **#25**) and coniferyl aldehyde (**#26**) were isolated as well as the flavonoids betuletol (**#27**), kampferide (**#28**) and ermanin (**#29**) (Banskota et al., 1998, 2000^a). Bankova et al. (1996^a) also reported the isolation of compound **#26**. Flavonoids are present in small quantities in Brazilian samples (reviewed in Bankova et al., 2000).

Biological, Pharmacological and Therapeutic Activities of Propolis

Reports about the biological and pharmacological properties of propolis appeared in the early 1900's. Since this period, hundreds of papers dealing with this matter have been published (reviewed in Ghisalberti, 1979; Marcucci, 1995; Burdock,

1998). Although at an early stage, studies of propolis have been extensively carried out in Eastern Europe; nowadays many research groups have investigated the versatile properties of propolis extracts and components. A substantial part of these activities is connected with phenolic compounds, such as flavonoids and derivatives of caffeic acid.

In this section we summarize the results about the biological and therapeutic activities of bee glue, with special emphasis on studies with Brazilian propolis. Propolis is usually separated from the wax by extraction into 70% ethanol, and is known as propolis balsam. Its biological effects are due, mainly, to more than 100 biologically active elements isolated from it; and possibly the components reinforce the effects of each other, a known phenomena in natural medicinal mixtures. The majority of the studies deal with extracts using ethanol, which throughout this review will be referred to as ethanolic extract of propolis (EEP). This is a generic name not associated with any standard procedure for its preparation. Furthermore, many works also deal with both the activities of compounds isolated from the resin and with commercial compounds, such as flavonoids and derivatives from caffeic acid. Especially in relation to EEP, I would like to stress that in several reports the method of the extract preparation and its concentration are not described, hampering comparison between results obtained by different researchers. It is important to point out that the microbicidal activity of a given extract depends on the solvent used in its preparation (Tosi et al., 1996).

Activity Against Bacteria and Fungi

The activity of propolis and its components against a broad range of bacteria has been a subject of intense research, with more than 90 papers published between 1950 and 1979 indicating that Gram+ bacteria are more susceptible than Gram- (Lavie, 1957; Cizmarik & Trupl, 1976^a; Scheller et al., 1977^a; Hegazi et al., 2000). However, this activity varies between samples collected from different geographic regions, and is dependent on the pH of the culture medium (Meresta & Meresta, 1980; Glinski & Meresta, 1993). Comparison between EEP obtained from different propolis samples and isolated compounds showed that the presence of flavonoids and derivatives of caffeic acid is associated with the bactericidal activity (Villanueva et al., 1964; Bosio et al., 2000). A synergistic effect of different propolis samples with commercial antibiotics was reported (Kivalkina & Gorshunova, 1973; Scheller et al., 1999); and also bacteria resistant to these drugs were susceptible to propolis (Shub et al., 1981).

The mechanism of antibacterial action of propolis has been the subject of only a few publications. Takaisi-Kikuni & Schilcher (1994) showed through electron microscopy and micro-calorimetric assays that EEP interferes with the division of *Streptococcus agalactiae* through the formation of pseudo-multicellular forms, cytoplasm

disorganization, inhibition of protein synthesis, leading to lysis of the bacteria. Mirzoeva et al. (1997) found that EEP and some of phenolic components affect the bioenergetical status of the membrane by inhibition of the membrane potential leading to increased permeability of the membrane to ions and to immobility of *Bacillus subtilis*.

Together with studies of bacteria, the fungicidal effect of European propolis samples was also investigated, indicating activity against different species including *Candida*, *Microsporum*, *Mycobacteria*, *Trichophyton*, *Fusarium* and other dermatophytes (Cizmarik & Trupl, 1976^b; Kujumgiev et al., 1999). A synergistic effect with conventional anti-mycotic drugs was also observed (Holderma & Kedzia, 1987; Scheller et al., 1998), and as in the case of bacteria, the fungicidal effect was associated with the presence of phenolic components (Metzner et al., 1977; Ravn et al., 1989).

Since several dental and periodontal disorders are associated with bacterial and fungal etiologies, different groups studied the *in vitro* effect of propolis against oral microorganisms (reviewed in Eley, 1999). Also, animal models were used to investigate the effect on dental pulp regeneration and on caries, and also as a dentifrice and mouth rinse inhibiting of plaque formation (Ilewicz et al., 1986; Ikeno et al., 1991; Ota et al., 1997).

More recently the microbicidal study of Brazilian propolis and of isolated compounds have received great attention. In four samples collected in SP and PR, the activity against *S. aureus* was concentrated in polar compounds fractions rich in phenols, and in the volatile fraction. This activity was similar to that of Bulgarian samples, in spite of the significant differences in the chemical composition between resins from both countries (Bankova et al., 1995). In two samples from indigenous bees of the genus *Melipona* collected in the States of Piauí and PR, the EEP and the volatile oils showed activity against *S. aureus* (Kujumgiev et al., 1999). These authors also reported the effect against *C. albicans* in six samples of Brazilian propolis, independent of differences in their chemical composition.

The activity against *S. aureus* and *C. albicans* of EEP in a sample collected in SP was similar to that of the secretions of local plants (*Baccharis dracunculifolia* and *Eucalyptus citriodora*) (Bankova et al., 1999), showing that knowledge about plant sources could be useful as a basis for the chemical standardization of propolis. Assays with SP samples collected in different periods showed no seasonal effect against bacteria; and as reported by other authors, higher activity was found against Gram+ bacteria in comparison with Gram- (Sforcin et al., 2000).

EEP from Brazilian propolis was also assayed against microorganisms isolated from human patients. Comparing 200 samples of bacteria the susceptibility order was: *S. aureus* > *S. typhimurium* > *E. coli*, whereas among 96 samples of fungus, the order was *C. albicans* > *C. guilliermondii* > *C. parapsilosis* (Fernandes Jr. et al., 1995).

In comparison between hydroalcoholic extracts prepared with different proportions of the solvents from a sample collected in the State of Minas Gerais, those showing higher activity against *S. aureus* presented an ethanol content between 60 and 70% (Park & Ikegaki, 1998). Interestingly, after fractionation of samples collected in different regions of Brazil, the most active fraction against *S. aureus* was that obtained in hexane (Fontana et al., 2000), suggesting to the authors that the bactericidal activity was due to a synergistic effect among compounds of different chemical groups, since hexane is not the ideal solvent for extraction of phenolic compounds.

The three derivatives of cinammic acid (Fig. 1) isolated by Aga et al. (1994) from an SP sample were active against *Bacillus cereus*, *Enterobacter aerogenes*, *Microsporum gypseum* and the cutaneous fungus *Arthroderma benhamiae*, with compound **#1** presenting a broad spectrum of activity against bacteria. From samples collected in PR, besides compounds **#1**-**#3**, a new derivative was characterized (**#5**) with all of them active against *S. aureus* e *Streptococcus faecalis*, yet presenting a low effect against *E. coli* (Marcucci et al., 2001). The four labdane-type diterpenic acids **#8**-**#11** isolated by Bankova et al. (1996^a) from a PR sample exhibited activity against *S. aureus*.

Recently, a series of studies about Brazilian propolis and microorganisms of odontological importance has been published. Formation of dental caries is associated with oral microorganisms and extracellular polysaccharides, synthesized from sucrose by glycosyltransferases from *Streptococcus* spp. EEP prepared from samples in different States in Brazil were active *in vitro* against *Streptococcus mutans* and *Streptococcus sanguis* and inhibited the activity of glycosyltransferases (Park et al., 1998; Koo et al., 2000). A sample from the State of Rio de Janeiro controlled the levels of microorganisms and the inflammatory process and led to healing of dental pulp in rats (Bretz et al., 1998). Propolis from the State of Rio Grande do Sul assayed in desalivated rats infected with *Streptococcus sobrinus* led to a statistically significant reduction in the number of caries in comparison with control group (Koo et al., 1999).

Activity Against Virus

Propolis from different origins interferes with the reproduction of different viruses, such as those causing influenza and hepatitis B and also against herpes simplex virus (HSV) type I, avian and herpes zoster viruses (reviewed in Amoros et al., 1992). Further studies showed that propolis, flavonoids (quercetin and kaempferol) and the caffeolics (CAPE and isoprenyl caffeate) interfere with the replication of human immunodeficiency virus (HIV-1), inhibiting the enzyme HIV-1 integrase (Fesen et al., 1993; Harish et al., 1997; Xu et al., 2000). Compounds with the molecular moiety of caffeic acid were considered as new family of natural antiviral compounds (Serkedjieva et al., 1992).

Studying six samples of Brazilian propolis, besides the determination of bactericidal and fungicidal activity, Kujumgiev et al. (1999) reported that four of them were active against avian influenza virus, while two were inactive.

Activity Against Pathogenic Protozoa and Helminthes

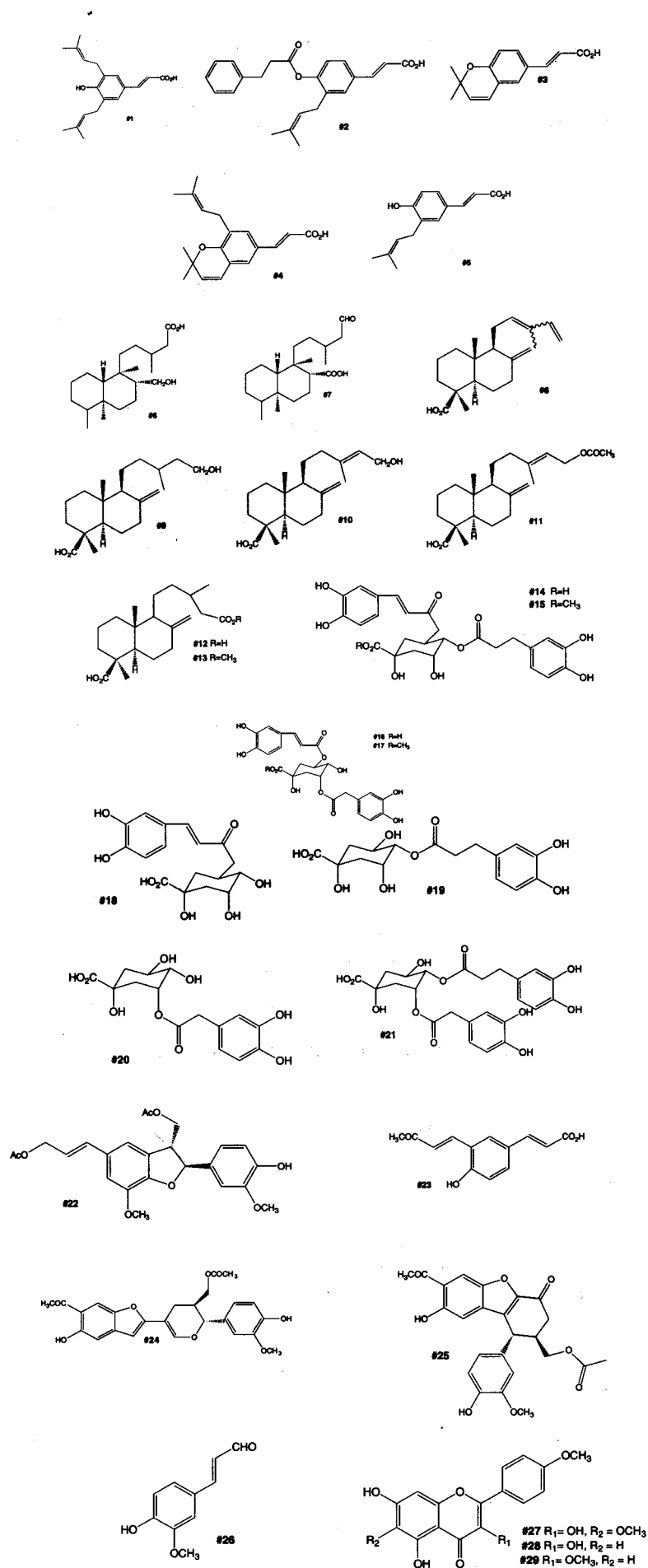
There are several reports about the effect of European propolis on protozoa that cause diseases in humans and animals such as trichomoniasis, toxoplasmosis, giardiasis, Chagas disease, leishmaniasis and malaria. EEP was active in mice infected with *Toxoplasma gondii* (Starzyk et al., 1977) and *in vitro* against different species of *Trichomonas* (Popeskovic, 1978; Starzyk, et al., 1977) and *Giardia lamblia* (Torres et al., 1990).

Against *Trypanosoma cruzi*, the etiological agent of Chagas disease, extracts prepared both in ethanol as in dimethylsulphoxide from a North American commercial sample were active *in vitro* against the three forms of the parasite and also against infection in macrophages and heart cells (Higashi & De Castro, 1994). In experimentally *T. cruzi*-infected mice, the oral administration of EEP up to 1.2 g propolis/kg per day or the extract offered *ad libitum* in the drinking water (up to 4 g/kg per day) or added to the food (up to 5 g/kg per day) did not reduce the parasitemia kinetics nor the survival rate of the animals (De Castro & Higashi, 1995). This negative result in *in vivo* experiments indicated the need of resin fractionation guided by biological activity and led to the purification of four phenolics compounds (#1, #3-5) (Fig. 1) isolated from Brazilian propolis that were assayed *in vitro* against *T. cruzi*, but showed levels of activity below that of crystal violet the standard drug (Marcucci et al., 2001).

Extracts from a Bulgarian sample were analyzed by the technique of high temperature high-resolution gas chromatography coupled with mass spectrometry (Pereira et al., 1999) and the EEP presented with a high content of flavonoids. Its activity against trypomastigotes of *T. cruzi* was similar to that of crystal violet. The acetone extract was even more active, and showed significant differences in the number and relative concentrations of propolis components in comparison with EEP. The methanolic extract presented a very low level of activity against trypomastigotes, which was associated with the high concentration of mono and disaccharides, probably resulting in a low concentration of potentially active compounds (De Castro et al., 2001).

In studies performed in Brazil, it was reported that EEP was effective against the proliferation and differentiation *in vitro* of *Leishmania donovani*, the agent of visceral leishmaniasis, and in experimental infections in hamsters caused a decrease in the parasite load in the liver and spleen and also recovery of the normal levels of leukocytes and polymorphonuclear cells (Sartori et al., 1994). The EEP from the Bulgarian sample, assayed against *T. cruzi* (De Castro et al., 2001), was also active against promastigotes of different species of *Leishmania* (Machado et al., 2000).

Figure 1.
Bioactive compounds
isolated from
Brazilian
propolis



The treatment of *Plasmodium berghei*-infected mice did not alter the course of infection (Woisky, 1995). On the other hand, it was reported that propolis had a prophylactic efficiency against malaria in endemic areas in Brazil (Gama, 1993) (see next topic).

In rabbits infected with intestinal eimerias, addition of propolis to the drinking water reduced the number of oocysts on the feces (Hollands et al., 1984), and in guinea pigs infected with *Ascaris suum* and treated with propolis, a reduction in the number of larvae in relation to control animals occurred (Benkova et al., 1989).

Antitumoral Activity

There are several reports about the effect of propolis against tumor lineages (Hladon et al., 1980). Parallel studies with flavonoids showed that activity against HeLa cells presented the following susceptibility order: quercetin > rhamnetin >> galangin (Ban et al., 1983). The survival rate in mice bearing Ehrlich carcinoma and treated with EEP from a Polish sample was higher than in those treated with bleomycin (Scheller et al., 1989^a). Dietary administration of propolis in a female rat model of two-stage drug-induced carcinogenesis led to a significantly decreased incidence and multiplicity of mammary carcinomas (Kimoto et al., 1999). The majority of work in this area deals with propolis components, mainly flavonoids and caffeolols. Quercetin was shown to inhibit the proliferation of tumor cell lines and to potentiate the effect of conventional anti-tumor drugs (reviewed in Wang, 2000). Also dietary administration of flavonoids reduced the frequency of tumors induced by different carcinogens (Makita et al., 1996; Webster et al., 1996). CAPE that was also effective *in vitro* in different tumor lineages, led to inhibition of protein and nucleic acid synthesis and induction of apoptosis (Su et al., 1995; Nakayama et al., 1996). On the other hand, only a small effect was detected in normal cell counterparts (Grunberger et al., 1988).

In relation to components isolated from Brazilian propolis, compound **#1** (Fig. 1) was cytotoxic to tumoral cell lines, including leukemia cells (Matsuno et al., 1997^a). Apoptotic bodies and DNA fragmentation were observed in treated cell lines and although this compound inhibited the growth of mitogen-stimulated normal blood lymphocytes, it was not toxic towards normal lymphocytes (Kimoto et al., 2001). Treatment of mice with transplanted solid tumors led to suppression of tumor growth, apoptosis, necrosis, and activation of the immune system (Kimoto et al., 1998). Both propolis and **#1**, in models of drug-induced renal and pulmonary carcinogenesis, prevented the oxidative damage and development of pathology (Kimoto et al., 2000).

The clerodane diterpenoid **#6** (Fig. 1) inhibited the growth of a panel of tumor cells, while corresponding normal cells were less affected; and its topical application to mice with drug-induced skin tumors decreased the incidence of the tumors by inhibition of DNA synthesis (Mitamura et al., 1996). The benzopyranic

derivative (**#4**) and the two isomeric diterpenoids (**#7**) (Fig. 1) isolated by the same group were also active against human tumor lines (Matsuno et al., 1997^{a,b}), being the *Z* isomer of **#7** 7-fold more active than the *E* isomer (Hirota et al., 2000). This compound inhibited also a human breast cancer lineage; and this effect was associated with induction of apoptosis, a decrease in the level of the estrogen receptor, and inhibition of estrogen response element promoter activity, suggesting a potential role in breast cancer therapy (Luo et al., 2001).

From a methanolic extract of Brazilian propolis, a fractionation guided by cytotoxic activity led to identification of four compounds (**#26-29**) (Fig. 1) with high activity against murine carcinoma and human fibrosarcoma lineages (Banskota et al., 1998). From the same extract, the lignan **#22** and the benzofuranes **#24** and **#25** (Fig. 1) showed only a mild cytotoxic property against these two strains (Banskota et al., 2000^b). The methanolic extract was more cytotoxic than the aqueous one (Banskota et al., 2000^a).

Antioxidant Activity and Correlated Biological Properties

Free radicals are highly reactive species that are normally neutralized in the body by anti-oxidant enzymes and the nutrient-derived anti-oxidant molecules. Due to their reactivity, they can damage cellular components and are implicated in a variety of diseases. Several reports showed the *in vitro* anti-oxidative capacity of propolis using different types of assays (Scheller et al., 1990; Pascual et al., 1994; Krol et al., 1996; Moreno et al., 2000). This capacity is partially attributed to propolis radical scavenging properties of propolis, and to the high content of phenolics present in the resin (reviewed in RiceEvans, 2001). All the activities of propolis described below (see the following sub-items) were associated with its property of scavenging free-radicals.

Addition of Brazilian propolis to rat chow deficient in vitamin E led to increased levels of vitamin C in both the plasma and various organs of the animals and also to reduction of lipidic hydroperoxides in the intestine (Sun et al., 2000), indicating that propolis components behave as a hydrophilic antioxidant. From an aqueous extract of Brazilian propolis, fractionation guided by the antioxidative capacity led to isolation of the aromatic compound **#23** (Fig. 1) that was more potent than vitamins C and E, standard antioxidant agents (Basnet et al., 1997). Treatment of macrophages with **#23** led to reduction of the oxidation of low density lipoprotein and blockage of the activation of the transcriptional factor NF-kB, suggesting its involvement in neutralization of oxidative stress, preventing an apoptotic process (Claus et al., 2000).

Anti-inflammatory activity: Scavenging of free radicals, generated by neutrophils in inflammatory processes is the principal mechanism of anti-inflammatory

drugs. An aqueous extract of propolis inhibited the enzyme dihydrofolate reductase as several non-steroidal anti-inflammatory drugs; and the presence of caffeic acid was associated with the protective functions of propolis (Strehl et al., 1994).

In different *in vivo* murine models of inflammation, European propolis showed anti-inflammatory activity. These models included formaldehyde-induced arthritis, acute inflammation induced by zymosan and paw edema induced by prostaglandin E₂, carrageenan or radiation; and in some cases propolis presented an activity similar to conventional anti-inflammatory drugs (Scheller et al., 1989^b; Ledon et al., 1997). The topical treatment of rabbits after cornea cauterization with aqueous extract (Hepsen et al., 1999) or EEP (Ozturk et al., 2000) healed the lesion and reduced the inflammatory process, similarly to dexamethasone. Hepsen et al. (1999) suggested that this could be partially due to an inhibitory effect of propolis on cyclo-oxygenase and lipo-oxygenase activities. *In vitro* experiments complemented these findings, showing inhibition of the release of prostaglandins, leukotrienes and histamine by neutrophils and macrophages by propolis, with CAPE as the most potent modulator (Khayyal et al., 1993; Mirzoeva & Calder, 1996).

Among 14 Brazilian commercial extracts, four of them presented an anti-inflammatory effect similar to indomethacin in arachdonic acid-induced ear edema in mice (Menezes et al., 1999). Brazilian propolis showed a similar effect in rats in drug-induced paw edema and in a chronic inflammatory model (Park et al., 1996; Park & Kahng, 1999).

Hepatoprotective activity: Both in *in vitro* systems and in rodent models, different propolis formulations showed a hepatoprotective effect that was associated with the anti-oxidative properties of the resin (Rodriguez et al., 1997; Sugimoto et al., 1999). Rats with toxic liver damage treated with a pediatric formulation of propolis showed improvement in hepatic secretion of bile and cholic acids (Drogovoz et al., 1994).

A water extract of Brazilian propolis showed strong hepatoprotective activity against CCl₄-toxicity in rats and D-galactosamine/LPS-induced liver injury in mice (Basnet et al., 1996^a). This extract also protected pancreatic β -cells against streptozotocin in rats, with an activity similar to nicotinamide (Matsushige et al., 1996^a). *In vitro* studies with rat hepatocytes treated with CCl₄ guided the fractionation and isolation of four dicaffeoyl quinic acid derivatives (**#14-#17**) (Fig. 1), that were more potent than the standard drug glycyrrhizin (Basnet et al., 1996^b). In studies with human leukocytes, the hepatoprotective effect of these derivatives was associated with inhibition of interleukin 1 (IL-1) generation and nitric oxide synthase activity (Matsushige et al., 1996^b).

Labdane-type diterpenic acids (**#10-13**) isolated from a methanolic extract showed a protective effect in mouse hepatocytes against death induced by

D-galactosamine/tumor necrosis factor- α (TNF- α), that was correlated to a pronounced effect against free radicals (Banskota et al., 2000^a).

Cardioprotective activity: Cancer therapy with doxorubicin (Dox) is limited by development of cardiomyopathy due to generation of free radicals through self-reduction of the drug. In this context, rats with Dox-induced cardiomyopathy treated with propolis presented reduction of creatine phosphokinase, aspartate aminotransferase, blood and tissue glutathione levels, and thiobarbituric-acid-reactive substances, effects similar to those caused by rutin, a known cardioprotective flavonoid (Chopra et al., 1995).

Radioprotective activity: Administration before or after γ -irradiation of EEP, prepared from a Polish propolis sample, protected experimental mice, returning leukocyte counts and spleen plaque-forming activity to normal levels. All the animals survived while non-treated mice died within 2 weeks (Scheller et al., 1989^b). Treatment of rats with an aqueous extract before and after exposure to γ -irradiation markedly reduced the anti-inflammatory response to carrageenan, the malondialdehyde concentration in plasma, normalized the acid phosphatase level and stimulated the release of superoxide dismutase (El-Ghazaly & Khayyal, 1995).

Tissue Regeneration Activity

The regenerative property of European propolis applied to tissues, such as cartilage, bone, conjunctive and epithelium, has been reported in animal models (Scheller et al., 1977^b; Stojko et al., 1977). This property was associated with scavenging of free radicals, an anti-inflammatory effect and a general stimulation of tissue metabolism (Kaminski et al., 1977). Another ointment composed of propolis, urea and trypsin applied to burned areas in experimental animals by Troshev et al. (1990) led to tissue regeneration with good rate and quality. Standardization of preparation of a propolis extract and also of skin-healing formulations of propolis as ointments, emulsions and gels have been investigated (Arvouet-Grand et al., 1994; Vennat et al., 1998). The wound-healing properties of this extract were assayed in rabbits (Arvouet-Grand et al., 1993^a).

Gabrys et al. (1986) considered that, besides phenolic compounds, the high level of aminoacids in Polish propolis is also involved in regenerative processes, such as arginine-stimulating protein synthesis and proline, the main component of collagen and elastin in the conjunctive tissue.

An ointment containing Brazilian propolis, confrei and honey implanted in rats inside a polyethylene tube led to regeneration of conjunctive tissue (Magro Filho et al., 1987). Using both extract and ointment prepared from Brazilian samples in the model of surgical lesions in mice, a rate of cicatrization higher than in untreated animals was observed (Damian et al., 2000).

Immunomodulatory Activity and Allergic Reaction

In *in vitro* and *in vivo* models an immunostimulatory activity of propolis has been described. Administration of EEP from a Polish sample by intraperitoneal route (ip)

led to a dose-dependent increase in antibody production after immunization of mice with sheep red blood cells (Scheller et al., 1988).

Using an aqueous extract, propolis administered by oral route enhanced the survival rate of mice infected with different species of bacteria and with *C. albicans*, even in studies with *Klebsiella pneumoniae* when the animals were treated with the immunosuppressor cyclophosphamide (Dimov et al., 1992). Since the extract showed no effect on the growth of *K. pneumoniae*, the authors suggest that the *in vivo* protection was due to stimulation of defense mechanisms. The stimulation of macrophage phagocytosis and of IL-1 and decrease of TNF levels by this extract reinforced their hypothesis of a non-specific activation of macrophages (Dimov et al., 1991). *In vitro* it was further observed inhibition of the classical pathway and of the alternative complement pathways with the effect dependent on the source of complement, the time and temperature of the assay, while in mice, interference on the alternative pathway was strongly dependent on the route of administration (Ivanovska et al., 1995^a). Administration of propolis (ip) plus cinnamic acid and lysine (CN:Ly) also protected the animals from inoculation with *K. pneumoniae* and the activity of CN:Ly was associated with increased proliferation of thymic and splenic lymphocytes and release of IL-1 and IL-2 (Ivanovska et al., 1995^b).

A Brazilian propolis extract induced increased cytotoxicity of natural killer (NK) cells against murine lymphoma and stimulated antibody production (Sforcin, 1998). The *in vivo* treatment of mice with EEP caused an increase of IFN- γ levels, associated with a more efficient response to infections (Orsi et al., 2000).

The caffeoylquinic acid derivatives isolated from Brazilian propolis (**#18-21**) (Fig. 1) presented stimulatory activity peritoneal macrophages, with increase on their spreading and mobility (Tatefuji et al., 1996). Compound **#1** (Fig. 1) besides microbicidal (Aga et al., 1994) and antitumoral activities (Matsuno et al., 1997^a), *in vivo* led to activation of the immune system, associated with the enhancement of T helper cells (Kimoto et al., 1998).

Like pollen, propolis may be dangerous to people with hypersensitivity to it. The resin can act as a contact allergen, both in occupational (beekeepers, beeswax modeling, string instrument makers) and in non-occupational uses (pharmaceutical and cosmetic products) (reviewed in Hausen et al., 1987^a, Hausen, 2001). Several works dealt with the isolation and characterization of propolis allergens that are in most of the cases associated with derivatives of caffeic acid, such as LB-1 consisting of 3 isomeric pentenyl caffeates: 3-methyl-2-butenyl caffeate, 2-methyl-2-butenyl and 3-methyl-3-butenyl caffeate (Hausen et al., 1987^b; Hausen & Wollenweber, 1988). The catecholic moiety of these esters was found to increase the sensitizing capacity in comparison with esters of ferulic acid, which are also present in propolis. Hansson et al. (1995) analyzed the reaction of 3-methyl-2-butenyl caffeate with proteins, such as glutathione and cysteine, and observed the formation of thiol adducts. These authors

suggest that this caffeate is a pro-hapten that forms a complete antigen after oxidation to caffeate quinone and addition to nucleophilic proteins. The mechanism of the hypersensitivity response is associated with the formation of haptens, that triggers an anaphylatic process, mediated by IgE. The effector cells of IgE stimulation are mastocytes, which by release of histamine and other chemical mediators, recruit eosinophils. For cell-mediated responses, T cells, upon stimulation by allergens, liberate pro-inflammatory cytokines. The transductional mechanisms coupled with the hypersensitivity response to propolis are not completely elucidated but probably involve increase in intracellular calcium levels associated with the reactional cascade of phospholipase C and/or D (N. Paulino, personal communication).

Other *in Vitro* and *in Vivo* Activities

EEP showed a dose-dependent anti-mutagenic activity in studies with *Salmonella typhimurium* exposed to different drugs (Jeng et al., 2000), and it was suggested that propolis could block cytochrome P-450 activity and/or interact with mutagens leading to inactive complexes.

A low toxicity of EEP was detected in experimental animals; for example, the LD₅₀ was higher than 7 g/kg for mice (Arvouet-Grand et al., 1993^b) and higher than 15 g/kg for rats (Kedzia et al., 1990). EEP showed no immunogenic or allergic properties for rabbits that received oral doses between 75 and 7500 mg propolis/kg every other day for two weeks (Scheller et al., 1977^c). When the extract (100-500 mg/kg) was administered to rats and mice, it caused a reduction in blood pressure, a sedative action and maintenance of serum glucose levels (Kedzia et al., 1990).

The addition of pollen and propolis to the diet of rats with nutritional ferropenic anemia produced a positive effect on weight gain, improved the digestive utilization of iron and the regeneration efficiency of hemoglobin (Haro et al., 2000).

In murine models, protection against stomach ulcers have been reported (Kedzia et al., 1990). In experimental dogs, the local application of propolis as an oil mixture cured external otitis (Heinze et al., 1996). Diterpenic acids isolated from Brazilian propolis (#10-13) (Fig. 1) also showed activity against *Helicobacter pylori* (Banskota et al., 2001).

A complete anesthesia of the rabbit cornea was described using EEP and this effect lasted for 1 h, being more effective than procaine (Prokopovich, 1957). A Cuban EEP showed an analgesic effect in mice using both the model of acetic-acid-induced pain and the hot plate assay (Ledon et al., 1997). Park & Kahng (1999) reported also analgesic effect of a propolis extract assessed by tail-flick test in rats, an effect comparable to those of prednisolone and acetyl salicylic acid. EEP from a sample collected in the South of Brazil showed an anti-hyperalgesic effect in mice treated with acetic acid, kaolin or zymosan, measuring the number of abdominal

constrictions. This extract also significantly inhibited capsaicin-induced pain and reversed the hyperalgesia induced by bradykinin (De Campos et al., 1998).

Three *p*-coumaric acid derivatives isolated from a sample from PR (#1, #3 and #5) (Fig. 1) presented *in vitro* a relaxant effect on smooth muscle using preparations of guinea pig trachea (Marcucci et al., 2001).

Therapeutic use of propolis

Most of the reports about clinical use of propolis come from countries in Eastern Europe, as well as Cuba and Uruguay. I tried to develop this subject in terms of separate topics, but they are interrelated; and it must be kept in mind that the therapeutic effect of this resin is mostly based in its microbicidal properties. In recent years a renewed interest in the therapeutic activities of propolis has arisen due to the role of antioxidants in preventive medicine.

Parasitic infections

In the 1980s several reports described the clinical use of propolis in the treatment of viral infections. For 35 years the Stefan S. Nicolau Institute of Virology (Roumenia) used bee-product preparations for the treatment of mainly influenza and herpes (Esanu, 1984). Local application of "Nivcrisol-D", a propolis product from this Institute, showed an excellent effect on recurrent cutaneous and zoster herpes, with decrease of duration of the local eruption and reduction of pain (Giurcaneau et al., 1988). Treatment of common cold infections caused a 2.4-fold shorter mean recovery time in relation to the placebo group (Szmeja et al., 1989).

The treatment of infection by *Trichophyton* on the hairy zone of the head with an ointment containing propolis led to results considered excellent in 96 out of 110 patients (Bolshakova, 1975).

In Cuba, a preparation named "Propolisina" was effective against giardiasis in children (n=48) and adults (n=90), with cure rates between 52% and 60%, while with the standard drug tinidazole this value was 40% (Miyares et al., 1988).

Although treatment with propolis of experimental animals infected with *Plasmodium berghei* did not alter the course of infection (Woisky, 1995), there is a report by a beekeeper about the use of propolis in an endemic area of human malaria (*Plasmodium falciparum*) in the North of Brazil (Gama, 1993). He mentioned that propolis was employed as an insect repellent, that its daily use prevented himself from contracting malaria, and that in the absence of specific drugs, administration of EEP to infected people led to recovery from the crisis and also precluded recurrence of the disease.

In the veterinary clinics, intra-mammary infusion of a propolis preparation named "Provet" (Apipharma, Hungary) was effective in the treatment of bovine mastitis

due to different pathogens, but not against Gram+ bacteria (Kegl et al., 1993). Treatment with a dimethylsulfoxide extract from Brazilian propolis resulted in clinical and microbiological cure in 84.8% of cases of bovine mastitis that involved the alga *Prototheca zopffi* (Langoni et al., 1995).

Gynecology

The results of *in vitro* studies (Starzyk, et al., 1977; Popeskovic, 1978) were confirmed by successful treatments of vaginal inflammations with *Trichomonas* etiology. The use of intra-vaginal tablets containing propolis in the treatment of trichomoniasis showed a fast cure with a great reduction of the inflammatory process (Suchy et al., 1974). Patients with gynecological infections when treated with propolis solution (n=45) showed a higher percent of cure, with the rate of recovery in cases of vaginitis at 80% and in cases of cervical erosion, 39%. Using sulphadevaginol (n=45), the percentages were, respectively, 59% and 20% (Zawadzki et al., 1973). In another report the treatment of bacterial vaginitis with EEP led to a higher percent of cure (92%) than with sulphadevaginol (64%), and for mycotic vaginitis it was 80%, similar to nystatin (Krasnodebski et al., 1986). The same extract when applied to patients (n=27) submitted to an electro-coagulation procedure due to cervicitis led to total recovery after 8 weeks, while for those that did not use the extract (n=50), the recovery was only 13%. The authors associated the results obtained with propolis with tissue regeneration, anti-inflammatory response, and microbicidal effect (Krasnodebski et al., 1986).

In Cuba, patients with acute cervicitis and positive vaginal smears for gynecological infections were treated with vaginal dressings containing 5% of propolis. This treatment led to 100% of negativation of the smears, achieving 90% of the patients a total epithelization of the cervix within 10 days of treatment (Santana Perez et al., 1995). The association of propolis with conventional drugs in cases of diffuse inflammation and uterine cervix ulcerations (n=137) led Roman et al. (1989) to report good to excellent results in 57% of the cases and satisfactory results in 20%.

In Uruguay different formulations of propolis applied to patients with herpes simplex, buccal or vaginal, reduced the recovery period, parallel infection and in several cases, no recidive was reported (Fierro, 1995).

In a controlled multi-center study, the treatment of men and women with recurrent genital HSV type 2 infections with an ointment of Canadian propolis was more effective in healing herpetic lesions, and in reducing local symptoms than with an acyclovir and a placebo ointments (Vynograd et al., 2000).

Odontology

Propolis use in dental practice in Eastern Europe and Cuba is associated with antiseptic activity, together with anti-inflammatory and anesthetic effects. EEP

mixed with toothpaste and with a mouthwash preparation improved their antiseptic properties and also the treatment of gingivitis (Panzeri et al., 1998). Murray et al. (1997) analyzed the effect of a propolis-containing mouth rinse on inhibition of plaque formation and found that the results were only marginally better than in the untreated group.

EEP from a Polish sample was used in cases of dentin hypersensitivity, dental caries for direct capping of pulp, as well as in endodontics (Ilewicz et al., 1982). Toothpaste made from an alcoholic solution of propolis and zincoxyde exerts effects on the capping of the dental pulp similar to zinc eugenate, being the treatment monitored by X-ray analysis (Ionita et al., 1990). Kosehko and Kosovich (1990) suggested the addition of EEP to the filler for root-canal filling, since clinical and X-ray examinations have demonstrated its high efficacy in acute and chronic forms of periodontitis. This propolis-filler showed also an anesthetizing effect, did not stain the tooth crown and promoted the regeneration of the bone structures. In Cuba, a propolis formulation named "Propolan" also was effective for the treatment of chronic gingivitis (Martinez-Silveira et al., 1988). A mouth rinse containing 5% Brazilian propolis aided the repair of intra-buccal surgical wounds and exerted pain-killing and anti-inflammatory effects in patients submitted to sulcoplasty (Magro-Filho & De Carvalho, 1994).

Dermatology

Propolis has a wide use for dermatological problems, applied as an ointment or as an alcoholic solution. It has the capacity of wound healing and promotion of tissue regeneration in problems such as eczema, dermatitis and second degree burns (Khachaturov & Gudkov, 1969). It is also employed in cosmetology as face creams, ointments, lotions and solutions (reviewed in Hausen et al., 1987^a). An ointment containing 3% of propolis led to excellent results on the treatment of dermatitis (n=109) with bacterial etiology (Chlorazak et al., 1971). In the treatment of 460 patients with panaritium, abscesses or infectious wounds propolis was added to the routine procedure, enhancing both anti-inflammatory and microbicidal activities (Tsarev et al., 1985).

In 22 cases of hip joint with aseptic necrosis of thighbone, intra-articular injections of EEP from a Polish sample gave good results (Przybylski & Scheller, 1985), suggesting to the authors that the use of propolis together with the conventional treatment in advanced stages of necrosis and also in early-stage cases of the illness that no consent to surgery was given by the patients.

In Cuba the treatment of children with infected surgical wounds or with mucositis or dermatitis showed the effect of propolis on tissue regeneration and against pathogenic microorganisms (González, 1999). In Uruguay, patients with infected wounds that received local treatment with propolis together with a systemic antibiotic presented better progress than those treated only with the antibiotic (Fierro, 1995).

In Brazil it was also reported that propolis caused a bactericidal effect and accelerated tissue regeneration in patients with decubitus scabs (Azevedo et al., 1986). Also different propolis formulations were employed in dressings after plastic surgery and in the treatment of infected lesions, leading to healing of the scars and wounds (Mujalli, 1999).

Otorhinolaryngology and Ophthalmology

Of special interest is the activity of propolis against bacterial and yeast pathogens isolated from human infections, such as respiratory tract ones (Focht et al., 1993). There are several reports about its beneficial use in otorhinolaryngologic diseases (Aliev, 1968; Palos et al., 1989), such as acute and chronic inflammations of the ear, pharyngitis, rhynopharyngolaryngitis and rhinitis (Kachnii, 1978; Crisan et al., 1995). The introduction of an emulsion containing propolis into the maxillary sinuses of patients with chronic suppurative sinusitis, led to sterilization of the sinuses in 92.4% of cases, higher than when the treatment was performed with penicillin or streptomycin (82.6%) (Sytnik & Kovalik, 1984).

In Cuba, an eyewash containing propolis (LRA-20) was applied to calves (n=20) suffering from infectious keratoconjunctivitis leading, after 2 days, to the cure of all the animals, while treatment with cloramphenicol (n=20), after 72 h the cure rate was only 75% after 3 days of treatment (Martinez et al., 1992).

Geriatry

Patients (n=75, 67-98 years old) with chronic bronchitis and emphysema received EEP (33 mg/kg/day), prepared from a Polish sample, while 58 other cases were treated with placebo (Scheller et al., 1984). Applying psychological tests, the authors considered that propolis caused a significant improvement of intellectual function and physical conditions, without adverse lateral effects. In aging subjects with impairment of immunological functions, oral administration of EEP (250 mg/2x day) restored several of these functions, increasing the enzymatic activity of granulocytes, the number of T-lymphocytes and immunoglobulin production (Scheller & Fanckiewicz, 1984). Stimulation of the immune response was also reported after treatment of patients with prostate inflammation with EEP plus polyvaccine and levamisole (n=50) (Scheller et al., 1985), with the results significantly better than in another group (n=50) treated with antibiotics and antiflogistics. Also in patients with fibrotic alveolitis, propolis led to normalization of the number of T lymphocytes and activation of granulocytes (Scheller et al., 1989^c).

Other Clinical Applications

EEP was effective in the treatment of acute and chronic colitis, acute gastric and duodenal ulcers (Korochkin & Poslavskii, 1986). Recently it was reported

in Brazil that the use of propolis and royal jelly led to a beneficial effect in the treatment of 85% of clients (n=34, 29-68 years) suffering from hypertension (Facchinhi, 2000^a). This apitherapeut using these two bee products plus pollen produced a reduction of glucose levels in 73% of the cases (n=26, 22-59 years) (Facchini, 2000^b).

Concluding remarks

Although there is a huge amount of information about the chemistry and biological activity of propolis, its application in human and veterinary therapies has hardly changed. Experimental studies showed several different properties, specifically anti-microbial, anti-inflammatory, anti-tumoral, anti-ulcer, immunostimulatory and anesthetic. The reports about clinical uses of propolis showed that its efficacy resides mainly in cases of microbial contamination, showing excellent therapeutic indexes on the treatment of giardiasis, viral infections, vaginitis and odontologic and otorhinolaryngologic disorders. Due to its ability to scavenge free radicals, propolis also displays a protective role in different organs and injured tissues, acting as an anti-inflammatory agent. More recently, a potential effect of propolis on the immune response has emerged, with experimental studies showing its unspecific stimulation of release of cytokines and nitric oxide by macrophages, and of NK cell cytotoxicity.

On the other hand, the broad spectrum of properties attributed to propolis can arouse skepticism or even incredulity among researchers in the medical area, as if it could be a remedy for all diseases (Toth, 1985). Systematic investigations of the mechanism of propolis action are urgently needed. Up to now, studies in this line are scarce, but they converge to two points, that are probably interrelated, microbicidal activity and neutralization of free radicals.

The biological activities of propolis are mainly associated with phenolic compounds; and the mechanism of action may depend on the synergism between different components. The main problem is the striking variability of its chemical composition depending basically on its plant sources. Propolis composition, unlike products derived from medicinal plants, is extraordinary variable, creating a substantial problem for the medical use of propolis and stating a need for its standardization. This variability hampers a chemical standardization of propolis based on its "active principles". However, recently a request for a patent was submitted to the Brazilian agency Instituto Nacional de Propriedade Industrial (INPI) regarding propolis categorization based on the chemical composition determined by gas and liquid chromatography techniques (M. C. Marcucci, personal communication). Investigations correlating chemical composition, the basic plant sources in different geographic regions, and biological and pharmacological activities open the possibility of a classification of a limited number of "chemical types" of propolis. This multidisciplinary approach is only possible with the combined effort of the national and international scientific communities.

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