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Functional Properties of Brazilian Propolis: From Chemical Composition Until the Market

Andresa A. Berretta, Caroline Arruda,
Felipe Galeti Miguel, Nathalia Baptista,
Andresa Piacezzi Nascimento,
Franciane Marquele-Oliveira, Juliana Issa Hori,
Hernane da Silva Barud, Bianca Damaso,
César Ramos, Raul Ferreira and Jairo Kenupp Bastos

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Abstract

Propolis is a product obtained from resins and exudates of different plants from different regions in order to protect the comb, with peculiar organoleptic, chemical and biological properties. Considering this, this chapter presents the types of Brazilian propolis as the types available nowadays, their chemical compositions, as well as, some of their important biological properties enabling employing them as important health food, such as antimicrobial, antioxidant, and immunomodulation action. Various “*in vivo*” and clinical trial studies, conducted in different regions, on the safety and dosage of propolis, technologies used to obtain propolis extract, and several innovative presentations of this promising bee product are also presented in this chapter. Finally, this chapter aims to present the regulatory affairs, potential market for propolis around the world, and perspectives for a near future.

Keywords: Brazilian propolis, green, red, brown, chemical composition, antimicrobial, immunomodulatory, antioxidant, extraction process, innovation, regulatory affairs, potential market

1. Introduction and a brief history

The antique civilizations always used bee products as valuable therapeutic resources in their medicinal practices. The history of medicine of the Assyrian civilizations, Chinese, Tibetan, Inca, Egyptian, and also the Greco-Roman is very rich and possess records of centenary formulations, including propolis to treat or prevent diseases. Old Egyptians, Greeks, and Romans used propolis to treat wound, cutaneous lesions, ulcers, and chirurgical interventions [1].

In Egypt, propolis was used as one of the main ingredients used in the formulations to embalm cadavers. It was also used by Aristotle, Dioscorides, Pliny, and Galneo as an antiseptic and wound-healing. The Greeks, including Aristotle and Hippocrates, adopted it as an internal and external healing. Pliny Roman historian refers to propolis as a medicine to reduce swelling and relieve pain [2].

The term “propolis”, “pro” in favor of and “polis”, “city of bees”, which means in defense of the honey comb, was described in the sixteenth century in France [3], and in the seventeenth century, propolis was considered an official drug by London Pharmacopoeia [4]. In the subsequence centuries, propolis has attracted growing interest due to its medical properties, especially in Eastern Europe. In 1908, the first scientific article about propolis chemical properties and composition [5] was published, indexed on Chemical Abstracts. In 1968, the abstract of the first patent was published on Chemical Abstracts [6].

In South Africa, during the war in the end of the nineteenth century, propolis was largely used because of its healing properties [3] and in the Second World War it was used by several Soviet clinicians [2].

In the last decades, propolis has gained wide acceptance as traditional medicine in several parts of the world. This disseminated interest in propolis in several countries encouraged a large number of studies considering chemical and biological properties of propolis [7].

Nowadays, in the Brazilian market and in several other countries, it is possible to find propolis in different presentations, such as liquid or powder extract: in bottles, capsules, tablets, vaporizers, syrups, creams, and among others, aiming to act as an antimicrobial [8–10], antioxidant [11], immunoregulatory [12–14], anti-inflammatory [12, 15, 16], antiviral [17] agent, besides several other functions. A very large number of publications endorsing these biological benefits in “*in vitro*”, “*in vivo*,” and in some clinical trials, is be discussed further in this chapter.

Thus, this chapter presents recent studies about Brazilian types of propolis such as green, red, and brown, considering their chemical composition and some biological properties such as antimicrobial, antioxidant, and immunoregulatory, safety aspects, extraction process, and technology associated, regulatory aspects, potential market and challenges, as well as tendencies for a near future.

2. Chemical composition of Brazilian propolis

Propolis is formed by a complex set of components collected by *Apis mellifera* from different parts of plant resins (twigs, flowers, pollen, buttons, and exudates of trees) which are deposited in the hive with saliva and enzymes of the insect to seal the cracks and maintain the temperature (Figure 1A) [18].



Figure 1. Presentation of different types of Brazilian propolis. (A) *Apis mellifera* collecting Green propolis from *Baccharis dracunculifolia* species, (B) green propolis on the intelligent collector, (C) brown propolis and (D) red propolis. Figures (A) and (B) were gently donated by César Ramos, Natucentro Company. Figures (C) and (D) were gently donated by Felipe Galeti Miguel, Apis Flora Company. Both companies are associated with ABEMEL.

It consists of resin (50% of the mixture is composed by flavonoids and phenolic acids), wax (30%), essential oils (10%), pollen (5%), and other organic substances (5%). Among the present compounds, it can be consisted of hydrocarbons, alcohols, aliphatic and aromatic acids, esters and its derivatives, aldehydes, ketones, flavonoids, fatty acids, terpenoids, amino acids, sugars, lignans, vitamins, minerals, etc. [19].

The chemical composition of propolis differs significantly according to the geographic region where resins were collected due to the flora of each region, allowing the selection of different plants as source of resin [20].

When this product is derived from Europe or China, for example, the main plant metabolites found are flavonoids and phenolic acids, unlike the stemmed ones from southeastern Brazil, which, besides phenolic compounds, contain high amounts of terpenoids and prenylated derivatives of p-coumaric acid [21]. This difference in composition reveals the collection of resinous material, in temperate zones, from poplar, especially species of *Populus* and in

southeastern Brazil, especially from *Baccharis dracunculifolia* DC (Compositae), popularly known as “vassourinha do campo” [22].

Regarding Brazilian propolis, it was further divided into 12 classes according to Park et al. [23]: the first five ones originate from the south and have the colors yellow, light brown, dark brown, light brown, and greenish brown, respectively. Regarding propolis found in the Northeast, it was divided into six groups such as reddish brown, greenish brown, dark brown, yellow, dark yellow, and yellow. Finally, the last of these classes regards the kind of propolis that comes from the Southeast and is known to have a greenish brown or green color and so-called green propolis (**Figure 1B**) [23]. After 2007, the 13 types of propolis was added: this new kind comes from the mangroves of the Brazilian states of Sergipe, Alagoas, Paraiba, Pernambuco, and Bahia. Among the Brazilian propolis, the green, the brown (**Figure 1C**), and the red (**Figure 1D**) ones are the most studied and relevant to the Brazilian economy due to their biological activities and exports to other countries, such as Japan [24].

2.1. Green propolis

Green propolis is composed of large amounts of phenolic compounds such as artepillin C, baccharin, kaempferide, isosakuranetin, dihydrokaempferide, drupanin, *p*-coumaric acid, caffeic acid, aromadendrin, caffeoylquinic acid derivatives, and other compounds, such as the triterpene lupeol-3-(3'R-hydroxy)-hexadecanoate. The key source of these compounds is *B. dracunculifolia* [12, 25–30] (**Figure 2**).

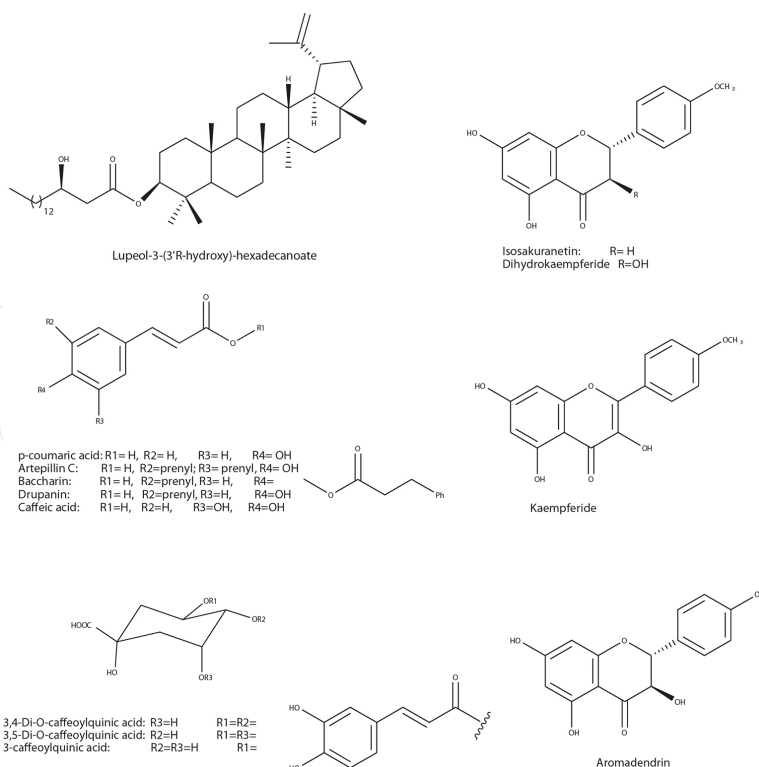


Figure 2. Chemical structures of compounds found in Brazilian green propolis.

Regarding the volatile compounds found in Brazilian green propolis, the major ones are sesquiterpenes, such as (E)-nerolidol, β -caryophyllene, spathulenol, and δ -cadinene. Furthermore, other compounds such as selina-3,7(11)diene, benzenepropanoic acid and longipinene were also identified. These compounds are responsible for the pleasant aroma in this bee product and are also responsible for many biological activities reported in the scientific literature. [31, 32]. (Figure 3).

Considering the large amount of artepillin C presents in green propolis (Figure 4), in addition to other biologically active compounds, the commercial value of Brazilian green propolis in the international market is high [16].

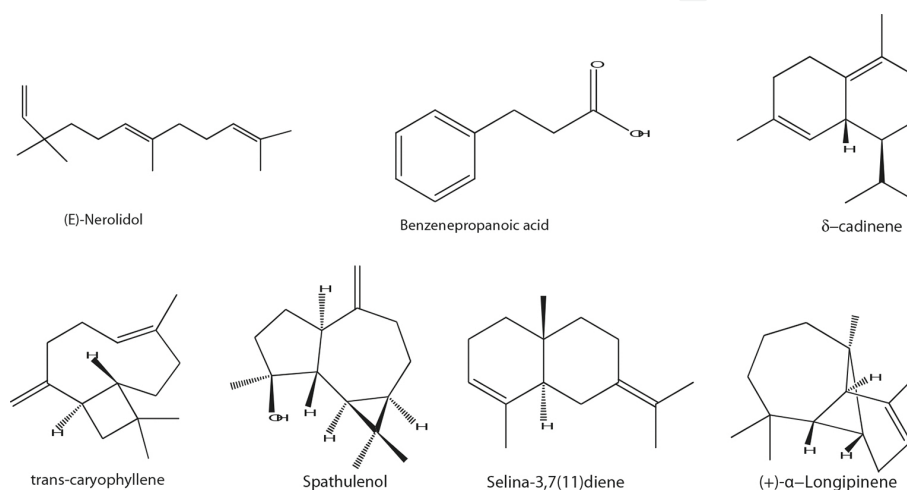


Figure 3. Volatile compounds from Brazilian green propolis.

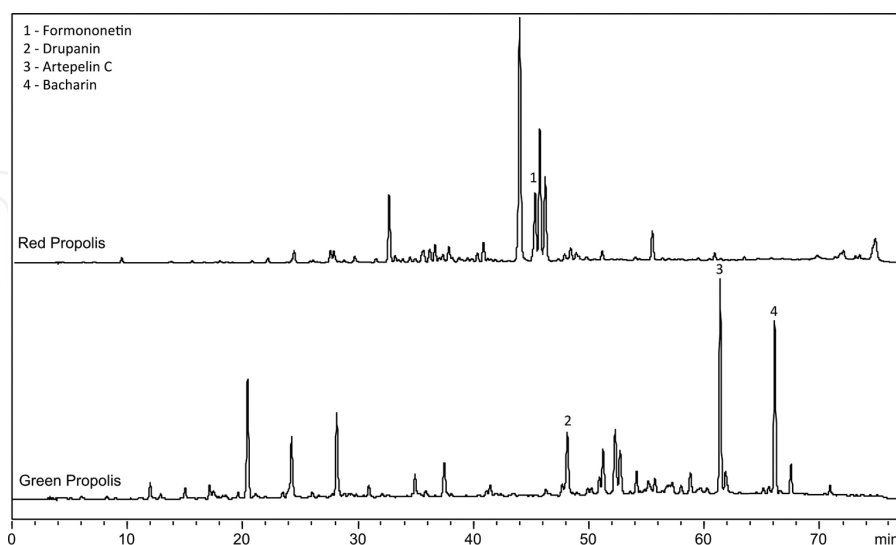


Figure 4. Chemical fingerprint for red and green propolis. Chromatographic profile was obtained using HPLC-DAD, Shimadzu Shim-pack CLC-ODS column (4.6 mm · 250 mm, particle diameter of 5 μ m, pore diameter of 100). Green propolis conditions followed according Berretta et al. [8] and red propolis, Cavendish et al. 2015.

2.2. Red propolis

Many of chemical compounds of red propolis, as well as green propolis, have been determined. Some of them are elemicin, isoelemicin, methyl isoeugenol, formononetin, biochanin A, isoliquiritigenin, liquiritigenin, medicarpin, homopterocarpan, quercetin, and vestitol. In the lipophilic extract, the majority of the compounds found are polyprenylated benzophenones—guttiferone E, xanthochymol, and oblongifolin A (**Figure 5**). Because the isoflavones—7 formononetin, biochanin A, pinocembrin, and medicarpin—are abundant in red propolis, they are used as chemical markers for identifying red propolis (**Figure 4**) [33, 34].

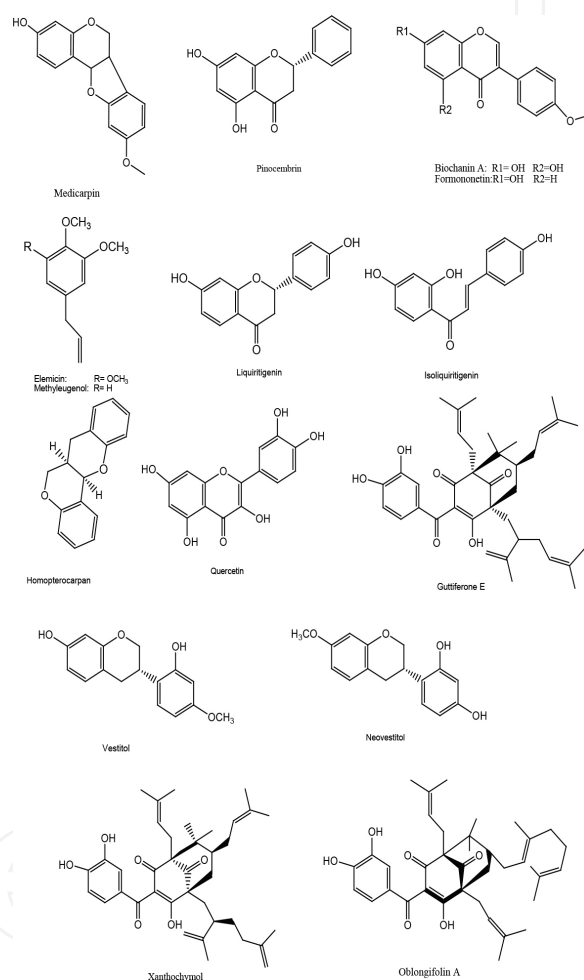


Figure 5. Chemical structures of some compounds found in Brazilian red propolis.

In addition to these compounds, De Mendonça et al. [33] identified caffeic acid, ferulic acid, umbellic acid, p-coumaric acid, genistein, kaempferol, catechin, dalbergioidin, epicatechin, daidzein, 2'-hydroxyformononetin, evernic acid, naringenin, calycosin, (7S)-dalbergiphenol, thevetiaflavone, cycloartenol, guttiferone C, and other compounds, using LC-Orbitrap-FTMS, a powerful tool to detect compounds because it does not require chromophores such as ultra-violet detector and it can detect very low amounts.

Red propolis has these chemical constituents, mainly due to the collection of resin from *Dalbergia ecastophyllum* by the bees. Its red color is due to the presence of cationic C₃₀ isoflavans, retusapurpurins A and B (**Figure 6**). This is characteristic of the propolis found in Brazilian Northeast, found especially in hives nearby mangroves in the states of Sergipe, Bahia, Alagoas, Paraiba, and Pernambuco. This bee product has attracted wide interest because of its numerous biological activities, such as cytotoxic against several cancer cell lines, antibacterial, antifungal, anti-cariogenic, antioxidant, antiproliferative, anti-inflammatory, and others [24, 33, 34]. In addition to its extracts, numerous biological activities of the isolated compounds have been described [24].

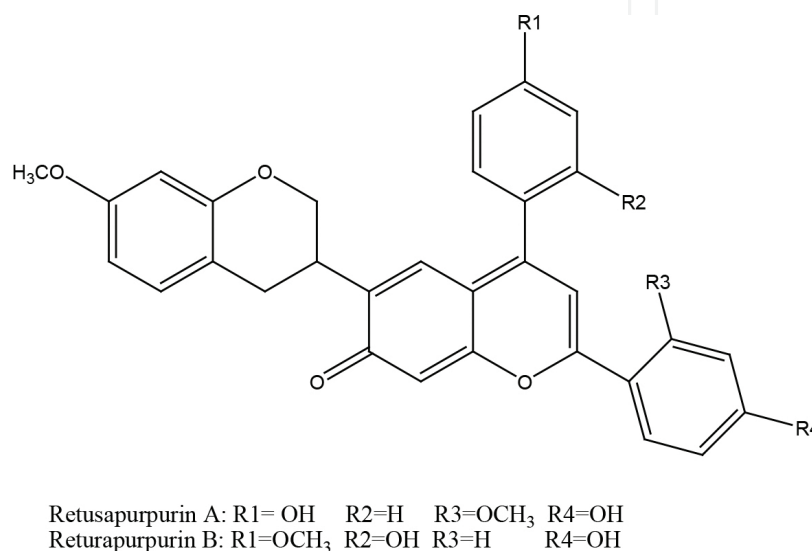


Figure 6. Chemical structures of isoflavans, retusapurpurins A and B.

Righi et al. [35], besides the compounds already described, found alkanes such as *n*-tricosane, *n*-pentacosane, *n*-heptacosane, *n*-nonacosane, *n*-hentriacontane, and *n*-tritriacontane in the apolar red propolis extract (hexane extract). They also identified other compounds as β -amirin, α -amirin, lupeol, methylguaiacol, trans-anethole, resorcinol, anisylacetone, *cis*-asarone, farnesol, and among others.

Nunes et al. [36] determined 34 volatile (**Table 1**) compounds in Brazilian red propolis and found that the major ones are trans-anethole, α -copaene, and methyl-*cis*-isoeugenol (**Figure 7**). They found that the chemical composition remains relatively constant during the year, considering that 17 out of the 34 compounds were detected every season of the year. But, the compounds δ -cardinol, β -gurjunene, isocaryophyllene, and δ -cadinene were found only in the sample collected in October and the alkanes, the 1,8 cineol, and α -selinene in the sample collected in July. This can be explained by the visitation of bees, in rainy seasons of shrubs and in dry seasons of woody plants: when the apiculture pasture changes, the chemical composition of propolis also changes. Similarly, as trans-anethole, the other red propolis compounds show biological activities such as analgesic, anesthetic, antigenotoxic, and antioxidant, making the volatile fraction pharmacologically interesting.

Class of the compound	Compound	Class of the compound	Compound
Monoterpene	<i>p</i> -Cymene	Sesquiterpene	α -Cubebene
	Limonene		α -Copaene
	1,8-Cineole		β -Gurjunene
	Linalool		β -Caryophyllene
Aromatic compound	Naphthalene		α -Bergamotene
Alcohol, aldehyde or ketone	4-Hydroxy-4-methyl-heptan-2-one		Farnesene
	6-Methyl-5-hepten-2-one		ϵ - β -Farnesene
Phenylpropanoid	Octanal		D-Germacrene
	Nonanal		α -Selinene
	<i>n</i> -Decanal		Isocaryophyllene
	Anisaldehyde		β -Bisabolene
	<i>n</i> -Dodecanal		δ -Cadinene
	<i>trans</i> -metil isoeugenol		Cadinene
	Estragole		δ -Cadinol
	<i>Trans</i> -anethole	Aliphatic hydrocarbon	Tetradecano
	Methyl- <i>cis</i> -isoeugenol		Pentadecano
	Elemicin		Hexadecano

Table 1. Compounds found in Brazilian red propolis.

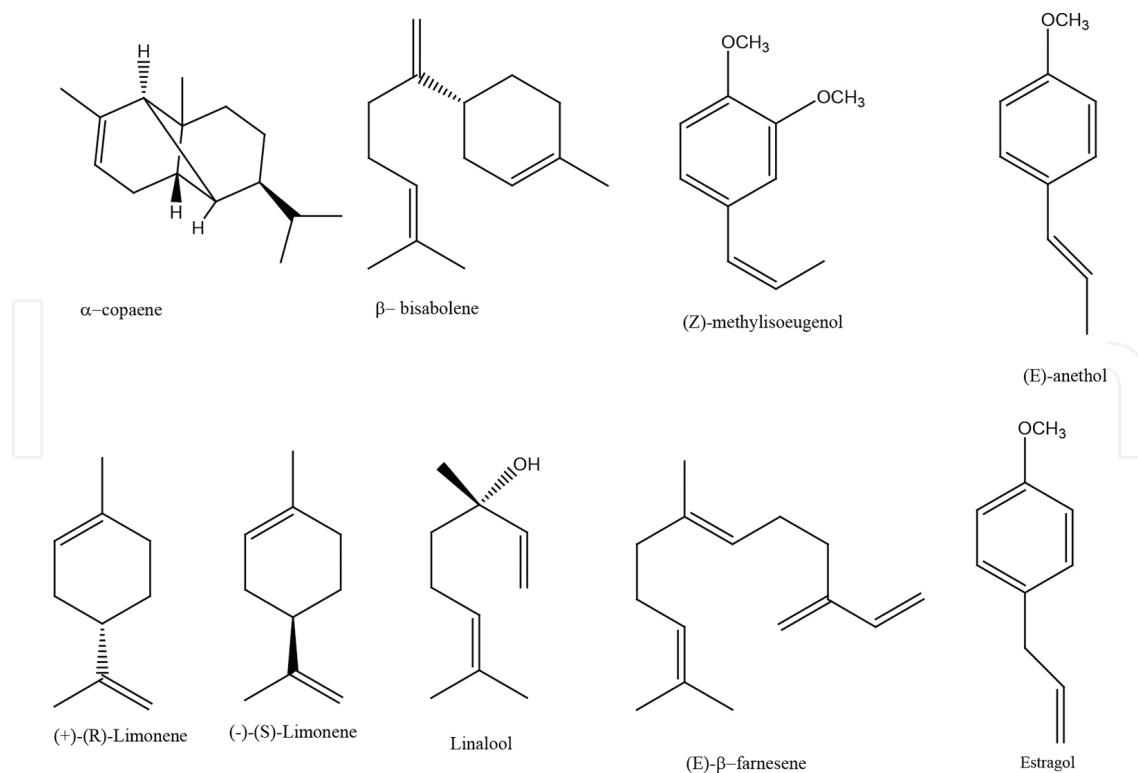


Figure 7. Some volatile compounds from Brazilian red propolis.

2.3. Brown propolis

The brown color is characteristic of propolis from different areas, but regarding Brazilian brown propolis, it is usually referred as the one that comes from the south of the country. Although many chemical compounds present in this product and their biological effects have already been identified, this type of propolis has not been well studied as red and green Brazilian propolis and many scientific reports on brown propolis are relatively old. Its botanical source seems to be mostly *Araucaria*, although some compounds found on it are also present in *B. dracunculifolia* [37].

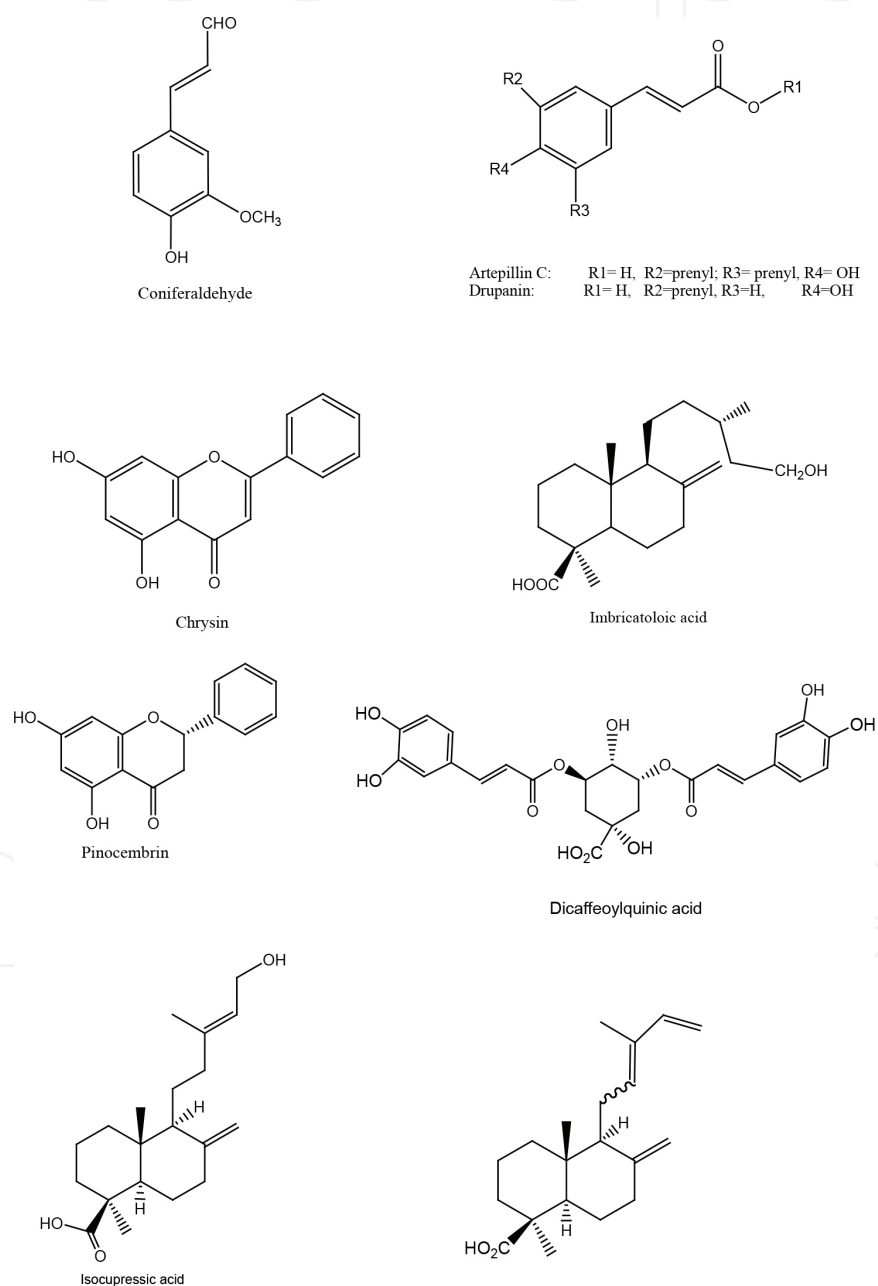


Figure 8. Chemical compounds of brown propolis.

The main compounds identified were: coniferaldehyde, 2,2-dimethyl-6-carboxyethenyl-2h-1-benzopyran, drupanin, pinocembrin, dicaffeoylquinic acid, and artepillin C, isocupressic acid, acetylisocupressic acid, imbricatoloic acid and a mixture of *cis* and *trans* isomers of communic acid [38–40] (**Figure 8**).

Among the numerous biological effects reported for brown propolis and its isolated compounds, it has been observed that both brown propolis and some of its isolated compounds have antimicrobial effect. In addition, it was possible to determine which compounds are responsible for such activity, highlighting the importance of chemically known product widely used by population [38, 41]. Moreover, brown propolis, as well as green propolis, has a significant preventive effect against oxidative stress in skin [42].

Brown propolis collected in Mato Grosso do Sul due to the significant amount of phenolic compounds in ethanol extract shows high antioxidant and antigenotoxic activities. Its volatile fraction is composed mainly of the sesquiterpenes spathulenol and (E)-nerolidol (**Figure 3**), which show an antimicrobial effect against *Cryptococcus neoformans*, *Enterococcus faecalis*, and *Staphylococcus aureus*. They were not mutagenic, considering that the antimicrobial activity is not because of DNA damage induction [43, 44]. The brown propolis collected from Mato Grosso also showed antimicrobial activity [45].

Therefore, considering that Brazil has a unique flora, among all types of Brazilian propolis three types of propolis are highly noticeable: green, red, and brown propolis due to their singular chemical composition, leading to their biological effects, culminating in the high value in the international market of Brazilian bee products.

3. Biological properties

3.1. Antimicrobial activity

Antimicrobial properties of Brazilian propolis are well-documented, including the antibacterial, antifungal, and antiviral activities. The biological activities of propolis are related to its chemical composition that varies with the collection period of the resin and the flora of the region visited by bees [46]. Therefore, in Brazil there are different types of propolis, since the different geographical regions of the country have a diversity of plant species. The most popular types of Brazilian propolis are green and red propolis.

Brazilian green propolis, whose most important plant source is *B. dracunculifolia*, has been extensively studied. Several studies have shown the activity of green propolis against several pathogenic bacteria, including Gram-positive bacteria (*S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *Kocuria rhizophila*) and Gram-negative bacteria (*Haemophilus influenzae*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, and *Prevotella denticola*) [9, 10, 46–48]. The last three bacteria cause periodontal diseases, which affect the periodontal tissues (tooth supporting tissues). Furthermore, green propolis is active against cariogenic bacteria, such as *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, *Streptococcus sanguinis*, and *Lactobacillus casei* [48, 49]. However, some Gram-negative bacteria are not

susceptible to green propolis, such as *Escherichia coli* and *Pseudomonas aeruginosa* [9, 10, 46]. *E. coli* can cause urinary tract infections and gastroenteritis, among others, while *P. aeruginosa* is associated with nosocomial infections, since it is an opportunistic bacterium.

Antifungal activity of green propolis has been reported against all three morphotypes of *Candida albicans* (yeast, pseudohyphae, and hyphae) [50]. At the cellular level, green propolis is able to induce apoptosis and secondary necrosis in yeasts, as showed in a study using *Saccharomyces cerevisiae* as a model organism [51]. Green propolis is also active against filamentous fungi (molds), such as *Trichophyton rubrum*, *Trichophyton tonsurans*, and *Trichophyton mentagrophytes* [52], which cause dermatophytosis. Ngatu et al. [53] reported the antimycotic effect of green propolis in patients with tinea pedis interdigitalis and tinea corporis caused by *T. rubrum*.

Green propolis also has the capacity to inhibit virus propagation. Shimizu et al. [54] reported that the ethanol extract of green propolis exhibited moderate efficacy in limiting herpetic skin lesions in mice infected with herpes simplex virus type 1 (HSV-1). Urushisaki et al. [17] showed the anti-influenza effect (H1N1 influenza virus) of the water extract of green propolis and its caffeoylquinic acids, which may have a cytoprotective action by affecting the internal cellular process. Takemura et al. [55] also reported the anti-influenza effect of the water and ethanol extracts of green propolis and their 3,4-dicaffeoylquinic acid, which enhance viral clearance by increasing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in the lungs of mice infected with H1N1 influenza virus.

Batch-to-batch variability is a common problem in the manufacture of propolis extracts. Since medicinal use of these extracts must rely on appropriate quality requisites, batch-to-batch reproducibility is essential to ensure consistent quality. Therefore, Berretta et al. [8] developed the propolis standardized extract (EPP-AF®), an ethanolic extract which contains green propolis and has batch-to-batch chemical reproducibility. Furthermore, it has several biological activities, including antibacterial and wound-healing activities [8]. **Figure 9** shows some results obtained by our research group, showing the antibacterial activity of EPP-AF® and extracts of brown, red and green propolis.

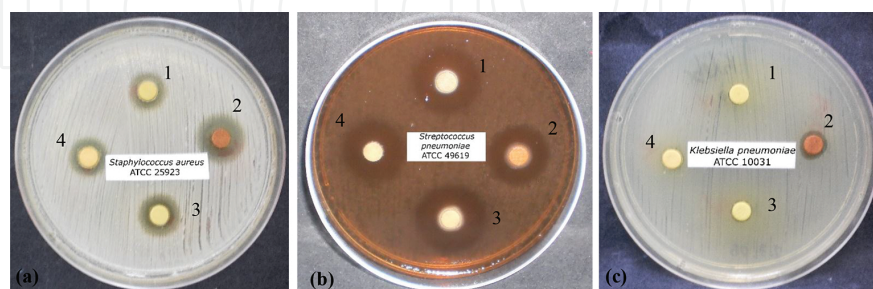


Figure 9. Zones of inhibition (disk diffusion method) provided by 1: Extract of brown propolis from the south of Brazil; 2: Extract of red propolis from the northeast of Brazil; 3: Extract of green propolis from the southeast of Brazil; 4: Propolis standardized extract (EPP-AF®); (a): *Staphylococcus aureus* ATCC 25923; (b): *Streptococcus pneumoniae* ATCC 49619; (c): *Klebsiella pneumoniae* ATCC 10031.

Our research group also has developed and evaluated different pharmaceutical forms of green propolis extracts, including propolis ethanolic extract (PEE), propolis water extract (PWE), propolis soluble dry extract (PSDE), and propolis matricial microparticles (PMM). With respect to antifungal activity (*S. cerevisiae* and *C. albicans*), PEE was the most potent followed by PWE, PMM, and PSDE [50]. The same results were obtained against *Lactobacillus* species (Gram-positive bacteria) (data not published yet).

Brazilian red propolis, in its turn, is produced from resinous exudates of *D. ecastophyllum*, found mainly in Northeastern Brazil (states of Alagoas, Bahia, Paraíba, Pernambuco, and Sergipe) [56]. Ethanolic extracts of red propolis showed activity against Gram-positive bacteria (*S. aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) [56–58]. These results are very interesting, since green propolis is not active against *E. coli* and *P. aeruginosa* [9, 10, 46]. Red propolis also has antifungal activity. Siqueira et al. [52] reported its activity against some dermatophytes (*T. rubrum*, *T. tonsurans*, and *T. mentagrophytes*).

Isoflavone formononetin is one of main chemical compounds in red propolis. Das Neves et al. [59] evaluated the activity of this compound against some bacteria (*S. aureus*, *S. epidermidis*, and *P. aeruginosa*) and yeasts (*C. albicans*, *Candida tropicalis*, and *C. neoformans*). The MIC value was 200 µg/ml for all bacteria and 25 µg/ml for the yeasts [59]. (6aS,11aS)-Medicarpin is the other chemical compound in red propolis, which exhibits a strong antibacterial activity, since MIC values of 16, 32 and 32 µg/ml were obtained against *S. aureus*, *B. subtilis*, and *P. aeruginosa*, respectively [57].

Kamuyama et al. [60] evaluated the use of green propolis to control microorganisms in minimally processed carrot. The study involved the comparison between: (i) carrot sanitation with 200 mg/l of total available chlorine, (ii) chlorinated solution “A” together with edible film with 0.4% propolis solution, and (iii) carrot sanitation with 0.4% propolis solution, prepared from 25% propolis alcoholic extract. Mesophilic and psychrotrophic aerobic bacteria, mold, and yeast were counted during the storage of samples of processed carrots at 10°C. The results demonstrated that the results for all treatments were similar to mesophilic and psychrotrophic bacteria. For mold and yeast count, the application of treatments (ii) and (iii), in the end of study, was similar to T0, suggesting that the use of propolis as a food preservative is viable and promising.

Borges et al. [61] evaluated the antibacterial and antifungal properties of different concentrations of a propolis hydroalcoholic extract in fresh pork sausage. This product is target of microbiological contamination, with consequent commitment of “shelf-life” and ability to cause diseases, factors that stimulate food companies to use synthetic preservatives as sodium nitrate, which possess high toxicity.

Interestingly, the results demonstrated that propolis extract (0.03 g/100 g of food) used showed greater antibacterial and antifungal results when compared to sodium nitrate.

3.2. Antioxidant activity

The propolis antioxidant property is one of the most studied biological activities worldwide. This biological property presents outstanding importance in the general benefits that propolis

may bring to human health as the free-radical scavenging capacity of propolis compounds may be closely related to the anti-inflammatory, antimicrobial, anticancer activities, as well as, prevention of atherosclerosis, skin damages, ageing, and among others.

Several antioxidant methods are available to study propolis, i.e., the DPPH assay, scavenging of hydroxyl radical by the deoxyribose assay, inhibition of lipid peroxidation, inhibition of chemiluminescence produced in the H₂O₂/luminol/horseradish peroxidase (HRP) system and inhibition of chemiluminescence produced in the xanthine/luminol/xanthine oxidase (XOD) system, and among others.

Propolis origin	Antioxidant activity/method	Reference
Brazil (marketed-standardized extract—PI 0405483-0)	0.016 µl/ml (IC ₅₀) inhibition of lipid peroxidation 0.22 µl/ml (IC ₅₀) inhibition of chemiluminescence produced in the H ₂ O ₂ /luminol/horseradish peroxidase (HRP) system 0.005 µl/ml (IC ₅₀) inhibition of chemiluminescence produced in the xanthine/luminol/xanthine oxidase (XOD) system 0.024 µl/ml (IC ₅₀) scavenging of hydroxyl radical by the deoxyribose assay	[11]
Campo grande Brazil (raw-material*propolis from stingless bees)	3 µg/ml (IC ₅₀) scavenging of DPPH No hemolysis—oxidative hemolysis inhibition assay 50–125 µg/ml—efficiency in inhibiting of AAPH-induced lipid peroxidation	[70]
Nan Province innorthern Thailand (raw-material)	Not indicated - scavenging of DPPH Observation: the authors inform the higher the ethanol amount in the ethanol aqueous solution, the higher the antioxidant activity	[71]
Alagoas, Brazil (brown propolis—raw-material)	8.01 µg/ml (IC ₅₀) scavenging of DPPH—ethanolic extract	[33]
Mediterranean propolis	Most prepared extracts inhibited lipid oxidation—oxidation of sunflower oil method	[72]
Brazil aqueus extract	0.62 µg/ml (IC ₅₀)—inhibition of lipid peroxidation employing brain homogenate	[27]

Table 2. Antioxidant activity of propolis from several regions in the world.

Some of these methods mimic the physiological conditions found in human body, this is the case of lipid peroxidation assay, in which membrane fractions (from mitochondria or brain) are used. In this method, the addition of iron salts triggers the decomposition of lipid peroxides into peroxy (LOO•) and alkoxy (LO•) radicals that can abstract hydrogen from polyunsaturated acyl chains and propagate lipid peroxidation. Any antioxidant capable of scavenging LOO• and LO• will decrease peroxidation. However, other methods can be considered more accessible, such as DPPH, which can easily demonstrate, in large scale, the antioxidant capacity of propolis samples from different sources or different batches. According to Marquele-

Oliveira et al. [62], this method could even be employed as an alternative for worldwide characterization and standardization of natural products. A good correlation of the DPPH method was observed against lipid peroxidation assay. This assay is based on the ability of DPPH, a stable free radical, to be quenched and thereby decolorize in the presence of antioxidants resulting in a reduction in absorbance values. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine. The extension of the reaction will depend on the hydrogen-donating ability of the antioxidants [63].

Propolis antioxidant properties have been fully investigated and both propolis raw material and propolis commercial extracts have been studied. **Table 2** shows examples of the antioxidant profile and the method employed for each sample, focusing on their collecting origin. Phenolic compounds have been reported as the main propolis compounds responsible for the antioxidant property. The antioxidant role of polyphenols results from the donation of hydrogen atoms from an aromatic hydroxyl group to the free radical, leading to stabilization of the radical [64]. During the evaluation of propolis fractions (from Brazil), Wang et al. [65] observed a strong inhibition of lipid peroxidation using rat liver homogenate at a concentration of 2 mg/ml, and this activity was related to the presence of flavonoids. However, it is known that other than phenolic compounds, flavonoids are involved in the antioxidant activity of propolis. So a series of phenolic compounds, including flavonoids, were assessed against the peroxidation of linoleic acid in a micellar solution. The results demonstrated that polyphenols in general present higher activity than BHT (butylated hydroxytoluene), a well-known antioxidant [66]. In a study using cell culture, artemillin C has been proposed as a strong candidate to be responsible for the antilipoperoxidative activity of Brazilian propolis [67].

Santos et al. [68] assessed the antioxidant activity of flavonoids and reported that the presence of structural groups, i.e., the B ring dihydroxyl, double bond in C2 and C3 in conjunction with the 4-oxo function, and the additional presence of hydroxyl groups in C3 and C5 (except for quercetin and 3'-O-methyl-quercetin), were the most potent inhibitors of lipid peroxidation using mitochondria. This antioxidant activity was also due to Fe chelation, which may explain the activity of flavonoids and polyphenols which do not have the above described structural groups [69].

After screening the antioxidant properties of propolis around the world, not only in the presented references, but also in the vast literature about this topic, one can observe a wide variation of responses. On the one hand, the antioxidant ability of each extract is related to the type and amount of phenolic compounds present in each extract, closely dependent on the propolis origin. But, on the other hand, no standardization regarding the solid soluble amount in each sample is presented, making comparisons among them not adequate. However, the presence of antioxidant activity in every propolis source studied is clearly observed and this activity has special importance to propolis biological properties.

Tian et al. [73] have shown that ethanolic propolis extract (EPE) protects endothelial cells from oxidized low-density lipoprotein (ox-LDL)-induced apoptosis and inhibits atherosclerotic lesion development. This research group has also demonstrated the effect of propolis extract on endoplasmic reticulum stress-C/EBP homologous protein pathway-mediated apoptosis. Apoptosis, especially in macrophages present in atherosclerotic lesions, is considered as a

prominent feature of advanced atherosclerotic plaques, suggesting that macrophage apoptosis is closely related to the atherosclerotic development and subsequent plaque rupture, which is the prominent event that results in the majority of clinical manifestations of acute coronary syndrome such as acute myocardial infarction and sudden coronary death [74]. Thus, protecting macrophages from apoptosis is believed as an effective approach to attenuate plaque instability and combat acute vascular events.

Additional studies investigated the potential use of topically and orally administered propolis extracts to prevent UV irradiation-induced oxidative stress in skin. Brazilian propolis extracts both green and brown successfully prevent UV-induced GSH (endogenous antioxidant) depletion *in vivo* and are both promising antioxidant systems against oxidative stress in skin [75].

Propolis also due to its antioxidant properties was tested against acute lung inflammation (ALI) caused by cigarette smoke (CS) *in vivo*. The researchers observed that propolis (P) treatment (200 mg/kg) normalized all biochemical parameters in the CS+P group compared with the CS group, including nitrite, myeloperoxidase level, antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase), reduced glutathione/oxidized glutathione ratio, and malondialdehyde. Additionally, TNF- α expression reduced in the CS+P group when compared with the CS group. They suggested, therefore, the potential antioxidant and anti-inflammatory role for propolis with regard to ALI caused by CS in mice [76].

Regarding the influence of the propolis antioxidant activity in food preservation, when combined with heat treatment in apple juice, propolis (0.1 mg/ml) reduced the thermal treatment time and temperature needed to inactivate 5 log₁₀ cycles of *E. coli*. No influence on organoleptic properties of the apple juice, which implies the possibility of obtaining a sensorially appealing, low-pasteurized apple juice with the functional properties provided by propolis was reached [77]. In another study, Costa et al. [78] studied the bifunctional biobased packing containing red propolis. In addition to the antimicrobial effect on coagulase-positive *Staphylococci* in cheese curds, the authors observed the reduced oxidation of butter during storage due to the antioxidant properties of propolis.

3.3. Immunoregulator

One of the biological effects of propolis is its immunomodulatory effect—by either enhancing or suppressing the immune system. This contradictory effect is probably due to its complex chemical variety, the presence in different geographic regions, and the different forms of extraction.

Little was known about the biological role of propolis until the 1990s, but recently numerous studies have been published, providing an important contribution to this research field.

Immunomodulatory as well as anti-inflammatory effects of propolis have been widely demonstrated both *in vitro* and *in vivo* [15, 79–82].

These effects are mainly related to its constituents, especially the phenolic compounds, including flavonoids as major components. Among the main types of flavonoids contained in

propolis are: pinocebrin, chrisin, and caffeic acid phenethyl ester (CAPE). In addition to flavonoids, propolis can also contain cinnamic acid derivatives such as caffeic acid and its esters, besides sesquiterpenes, quinones, and coumarins [83–85]. The typical constituents of Brazilian propolis, especially the Brazilian green propolis, are: caffeoylquinic acid and prenylated derivatives of cinnamic acid, such as artepillin C, p-coumaric acid, baccharin, and drupanin [23, 86, 87].

Despite the intensive search for the main constituent of propolis responsible for its immunomodulatory role, its effect seems to be associated with a combination of its different components [88].

Bachiega et al. [89] evaluated the propolis extract and its phenolic compounds, such as cinnamic and coumaric acids on cytokine production (IL-1b, IL-6, and IL-10) before or after macrophage challenge with LPS, to assess a possible immunomodulatory action. They observed a significant reduction in IL-6 and IL-10 in macrophages treated with the compounds only when the LPS was added before the stimulus, whereas the propolis extract was capable to inhibit the cytokine production both before and after the LPS addition. Thus, concluding that this efficiency could have occurred due to the synergistic effect of all compounds present in the extract [89]. On the other hand, the effect of polyphenolic compounds isolated from propolis and propolis extract was investigated on the growth and metastatic potential of a transplantable mammary carcinoma of CBA mouse. The results indicated that water-soluble extract of propolis (WSDP), caffeic acid (CA), quercetin (QU), and CAPE could be useful tools in the control of tumor growth in experimental tumor models [13].

The immunomodulatory activity of propolis extract was also investigated *in vivo* using the ovalbumin (OVA)-induced asthma model. Sy et al. [90] demonstrated that propolis extracts can suppress the serum levels of OVA-specific antibody IgE and IgG1 and attenuate the airway inflammation in treated mice, probably by the ability of propolis to modulate cytokine production. These findings suggest that propolis extracts may be a potential novel therapeutic agent for asthma [90].

Park et al. [91] evaluated another ethanolic extract of propolis (EEP) from Korea in an inflammatory animal model of hind paw edema induced by carrageenan. They observed a significant inhibition of the development of paw edema and increased vascular permeability coupled with an excellent analgesic effect in treated animals. They also showed a significant inhibitory effect on granuloma and exudate formation. The authors suggested that the anti-inflammatory effects of propolis observed might be due to its inhibitory effect on prostaglandin production [91].

In fact, Mirzoeva et al. [92] demonstrated the effect of another ethanolic extract of propolis in suppressing the prostaglandin and leukotriene generation by murine peritoneal macrophages *in vitro* and during zymosan-induced acute peritoneal inflammation *in vivo*. Furthermore, the authors described the caffeic acid phenethyl ester (CAPE) as being the most potent modulator of the arachidonic acid cascade among the propolis components examined [92].

Similarly, Borrelli et al. [93] investigated two ethanolic propolis extracts (EPE): with and without the caffeic acid phenethyl ester (CAPE) for their anti-inflammatory activity in rats

using carrageenan foot edema and carrageenan pleurisy models. They observed that only EPE with CAPE and CAPE alone significantly inhibited the carrageenan edema in the rat paw and the number of leukocytes in the pleural exudate in rats, suggesting that the anti-inflammatory activity of propolis is due to CAPE [93].

It is important to say that, despite Brazilian green propolis does not present CAPE in its composition, it presents a wide range of studies describing its beneficial properties such as antiulcerogenic, anti-inflammatory, antimutagenic, antifungal, angiogenesis, antioxidant, and immunomodulatory [14, 94–98]. Different from most European propolis extracts, which present flavonoids as the major component responsible for their effects, the biological activities of Brazilian green propolis are due to its high levels of phenolic acids such as artepillin C [99].

Studies with Brazilian green propolis have showed its role in inhibiting the development of pulmonary cancers [100], an antiviral activity *in vivo* [101], anticancer [102], an anti-inflammatory activity *in vivo* and *in vitro* [12, 16, 87], an antioxidant function in patients with type 2 diabetes mellitus [101, 102], antiherpetic activity [103], and among others [104, 105].

Despite several and growing studies involving the biological effects of Brazilian propolis, the detailed molecular and cellular basis of the action of propolis on immune cells is still unknown.

The administration of green propolis in animals subjected to chronic stress increased the generation of hydrogen peroxide, suggesting a modulation in the macrophage activation [106]. Machado et al. [12] verified an immunomodulatory effect of Brazilian green propolis extracts in acute and chronic inflammation models *in vivo* where the treated animals showed a decrease production of proinflammatory cytokines such as TNF- α and IL-6 and an increase in the IL-10 and TGF- β anti-inflammatory cytokines [12].

Most of the studies reported to date are associated with the immunomodulatory effect presented by propolis extracts with the modulation of the transcription factor NF κ B [107–110].

Recently, it has been demonstrated that Brazilian green propolis can also act in a new inflammatory pathway named inflammasome. The inflammasomes are a large molecular platform formed in the cell cytosol in response to stress signals, toxins, and microbial infections. Once activated, the inflammasome induces the molecule caspase-1, which in turn provokes the processing of inflammatory cytokines IL-1 β and IL-18. The Brazilian green propolis analyzed in this study (EPP-AF[®]) was capable of inhibiting the NLRP3 inflammasome and hence significantly reduces the IL-1 β secretion in mouse macrophages. Thus, indicating that Brazilian green propolis EPP-AF[®] extract has a significant role in regulating the inflammasomes [15].

In conclusion, the immunomodulation caused by propolis has been amply demonstrated in recent years, both in the stimulation and suppression of the immune system, making it potentially applicable as an alternative adjuvant therapy or even in the treatment of various diseases.

Table 3 presents a summary of the activities presented in Section 3.

Kind of propolis	Biological properties	References
Brown	Antigenotoxicity	[43, 44]
	Antimicrobial	[38, 41, 44, 45]
	Antioxidant	[42, 75]
Green	Antibacterial	[8–10, 46, 47, 49]
	Anticancer	[102]
	Antifungal	[51–53]
	Anti-inflammatory	[12, 15, 16, 87]
	Antimutagenic,	[96]
	Antioxidant	[27, 42, 75, 76, 103]
	Antiulcerogenic	[94]
	Antiviral	[17, 54, 55]
	Immunomodulatory	[12, 88]
	Inhibition of angiogenesis	[98]
Preservative	[60]	
Red	Antibacterial	[24, 56–59]
	Anticancer	[33]
	Anticariogenic	[24]
	Antifungal	[24, 52, 59]
	Anti-inflammatory	[24, 80]
	Antioxidant	[24, 33]
	Antiproliferative	[24]
	Immunomodulatory	[24]

Table 3. Biological activities presented in Section 3—summary.

4. Safety aspects

4.1. Nonclinical studies

Although propolis has been used for centuries around the world demonstrating to be safe, several scientific studies have been done in order to evaluate propolis safety by oral or topical route. Here we intend to present Brazilian propolis studies done in animals and, the studies found in humans, as clinical trials are not so numerous, despite the clinical trials did not focus on safety, we are presenting them in order to compare the dosages previously used and documented in humans.

Sforcin et al. [111] evaluated some biochemical parameters of animals treated with several different types of propolis aiming to study propolis safety and the differences in the propolis source could interfere in the results. The authors determined total proteins, glucose, urea,

creatinine, triglycerides, cholesterol, cholesterol-HDL, aminotransferases, and lactic dehydrogenase (LDH). The results demonstrated that all parameters were under standard values for the species studied and the propolis sources did not affect the results.

Reis et al. [95] evaluated the safety of propolis standardized extract (EPP-AF®), a Brazilian propolis composition that presents more than 50% of green propolis, by oral route in mice, in an acute model. DL50 was determined to be 3000 mg/kg after 24 h of treatment, and dosages under this value did not demonstrate intoxication signs in the animals. In the subchronic protocol (30 days) done in Wistar rats, there were no differences in the food and water intake, animals' weight and diuresis. Hematological and biochemical analysis did not show statistical differences between the treated (propolis 650 mg/kg) and placebo group. All parameters were in accordance with reference standards for the species studied. Microscopic analysis of all tissues did not show any differences with the placebo group, and it was not possible to detect any lesions, hemorrhages or cells infiltration, demonstrating the safety of oral administration of Brazilian propolis up to 650 mg/kg during 30 days of ingestion.

Mani et al. [112] evaluated the safety of Brazilian propolis in distinct treatments: (i) rats treated with 1, 3, and 6 mg/kg/day during 30 days; (ii) rats treated with 1 mg/kg/day of propolis alcoholic or aqueous during 30 days, and (iii) rats treated with 1 mg/kg/day during 90 and 150 days, demonstrating that all levels of seric cholesterol, HDL-cholesterol, total lipids, triglycerides, aminotransferases (AST), and lactic dehydrogenase (LDH) of propolis treated group were similar to the control group. The authors suggested that Brazilian propolis in the dosages used during the period of treatment were safe (Table 4).

Type of study (oral route)	Dosage	Propolis source	Species	Dosage converted to human according FDA guideline (mg/day)*
Biochemical parameters, 150 days [112]	1, 3 and 6 mg/kg	Brazilian	Rats	67.74
Biochemical parameters, 60 days [113]	2000 mg/kg	Iranian	Rats	22,580
Acute safety study [114]	2000 mg/kg	Polish	Rats	22,580
DL50 determination [95]	3000 mg/kg	Brazilian	Mice	17,073
Biochemical parameters—30 days [95]	650 mg/kg	Brazilian	Rats	7338
Acute toxicity parameters**	2500 mg/kg	Brazilian	Wistar rats	28,225
Subchronic study (28 days)**	1000 mg/kg	Brazilian	Wistar rats	11,290
Subchronic study (28 days)**	100, 300 and 1000 mg/kg	Brazilian	Rabbits	22,580

* Conversion considering adult weight around 70 kg.

** Results of our group and not published yet.

Table 4. Safety non-clinical results for propolis administration for oral route.

According to Dobrowolski et al. [114], LD₅₀ for different sources of propolis varied from 2 to 7.3 g/kg in mice, suggesting a safe dose for humans of 1.4 and 70 mg/day (when using safety

factor of 1000). In conclusion, considering Brazilian propolis LD₅₀ as 17,073 mg/day for humans [95], the application of a safety factor of 10 suggested by FDA guidelines, we would have a safe dose of 1700 mg or 1.7 g/day of propolis for an adult.

4.2. Clinical studies

Khayyal et al. [115] evaluated propolis extract activity in asthmatic patients with oral administration of 260 mg of propolis/day, for 2 months. The results demonstrated reduced night attacks (2.5 attacks/week for 1/week) and improved ventilatory functions, as a consequence of a decrease of TNF-, ICAM-1, IL-6, and IL-8, and an increase of 3· of IL-10, besides a decrease of prostaglandins E2, F2, and leukotriene D4.

Cohen et al. [116] evaluated 430 children aged 1–5 year old. Treated group (*n*=215) received a mixture of echinacea (50 mg/ml), propolis (50 mg/ml), and vitamin C (10 mg/ml), during 12 weeks, and compared to the placebo group. Children aged 1–3 year old received 5.0 ml, 2·/day, orally while children aged 4–5 year old received 7.5 ml. They were benefits in the incidence and severity of respiratory tract infections, with a decrease of 55% in the number of sick children, 50% in the incidence of respiratory diseases, and 60% decrease in the number of days with fever.

Type of study (oral route)	Dosage	Dosage converted to human adult (mg/day)	Reference
Double-blind study with children—prevention to respiratory infections	50 mg/ml—10 ml/day (children 1–3 year old)	2482.27	[116]
Double-blind study with children—prevention to respiratory infections	50 mg/ml—15 ml/day (children 4–5 year old)	2876.71	[116]
Pilot clinical trial with asthmatic volunteers	2–3 tablets/day with 88.4 mg propolis each	265.20	[116]
Pilot clinical trial with healthy volunteers—prophylactic study	500 mg propolis (2 capsules/day)	500.0	[117]
Pilot clinical trial—recurrent stomatitis	500 mg propolis/day	500.0	[118]
Propolis for wound healing	500 mg propolis/day	500.0	[119]
Clinical trial with asthmatic patients	1 sachet with 260 mg propolis/day	260.0	[115]
Pilot clinical trial with Brazilian propolis for treatment of <i>Helicobacter pylori</i>	20 drops, 3× /day	~350.0	[120]

Table 5. Clinical trials done with propolis administrated for oral route.

Brätter et al. [117] evaluated the oral administration of 500 mg of propolis for 13 days in healthy volunteers focusing on the evaluation of the immune response (TNF- α , IL-6, and IL-8). There was an increased ability in the cytokines secretion, however, without plasmatic levels. Then, prophylactic administration of propolis depends on the immune system reactivity and time, with no adverse effects.

Samet et al. [118] tested the oral administration of 500 mg of propolis in a randomized, placebo-controlled double-blind study, in which it was possible to demonstrate the benefits of propolis treatment in the repeated stomatitis, especially important in cases of resistance to treatment.

Finally, Zedan et al. [119] evaluated the administration of 500 mg of propolis/day in 45 patients aiming to offer an alternative treatment to cutaneous healings. The study compared propolis with echinacea and placebo, and propolis demonstrated to be more efficient than the other groups, especially in usual and superficial healings (**Table 5**).

Jasprica et al. [121] studied the antioxidant effects of propolis (propolis soluble in water and maltodextrin, 0.65 g of propolis, presenting 2.5% of flavonoids, equivalent to 16.25 mg expressed as galangin, Specchiasol, Italy) when administered in healthy volunteers ($n=47$, women and men), 3 doses/day (total daily dose of 48.75 mg of flavonoids) for 15 and 30 days, with the following parameters under investigation: superoxide dismutase, glutathione peroxidase, and catalase, malondialdehyde, total cholesterol, low- and high-density lipoprotein cholesterol, triglycerides, glucose, uric acid, ferritin and transferrin, and all routine red blood cell parameters. Interestingly, only men with 30 days of treatment presented differences in malondialdehyde (decrease), superoxide dismutase activity (increase), and a few changes in some parameters of red blood cell were detected.

Considering all the previously presented studies, it is possible to suggest that propolis dosages ranging from 260.0 mg to 2.87 g, which have already been used in humans can be considered safe. The biological results observed also varied much. Considering that propolis around the world is largely used as a supplement or functional food, it is reasonable to assume that dosages within this range will probably be safe, since none of the articles suggested any damage or complications for the volunteers. Regarding the antioxidant evaluation proposed by Jasprica et al. [121] in humans and the literature available until now, it is likely that the propolis dosage used was very high for this purpose. Some data previously published suggested that propolis can have a "pro" or "anti" action and, because several *in vitro* and *in vivo* studies demonstrated antioxidant actions at certain doses, it is possible that the best result may be achieved using dosages around 500 mg/day successfully, but further investigation is needed.

5. Extraction technologies and innovation products

5.1. Extraction technology

Propolis is a very complex material depending on the vegetation present in the area visited by bees. Besides the propolis source, the extraction process associated with solvents used will definitely provide a completely different extract [20]. It is well established that chemical compounds possess several particularities such as solubility, volatility, partition coefficient oil/water, pKa, and therefore, different solvents will probably extract different compounds [122]. Depending on the temperature and equipment necessary, volatile compounds can be lost and then, it is common to use cold procedures such as maceration or percolation, and when it is necessary to use higher temperatures, it is important to be careful in order to preserve every compound of interest.

Park and Ikegaki [122] studied propolis extracts obtained from water with 96% ethanol solution as solvents. The results demonstrated that propolis extract obtained with 80% ethanol grade showed higher absorption at 290 nm. Using ethanol solution at 60%, higher quantities of isosakuranetin, quercetin, and kaempferol were extracted, while pinocembrin and sakuranetin were better extracted with ethanol solution at 70% and kaempferide, acacetin, and isorhamnetin were most extracted with ethanol solution 80%. More expressive antimicrobial activities were found in propolis extracts from 60 to 80% of ethanol solution extraction and higher antioxidant results came from propolis extract obtained with ethanol 70–80%.

The most common extraction process for propolis to oral administration is the alcoholic (70%) extraction using maceration, percolation and/or turboextraction. The ratio of propolis raw material:extract used is completely variable and usually, in Brazil, 1:3–4, i.e., 1 part of propolis raw material may offer 3 or 4 parts of extract, considering the production of a liquid extract with at least 11%w/v of propolis dry matter. Of course, this ratio may vary and it is completely dependent on the quality of propolis raw material used.

Jorge et al. [46] studied green propolis extracts obtained from four different locations in São Paulo and Minas Gerais States, using the same extraction process, i.e., hydroalcoholic solution 70% with maceration for 30 days. Although all samples evaluated were Brazilian green propolis using the same extract, different results were found for drupanin, baccharin, and artemillin C during the same month, in spite of seasonal differences. Therefore, it is possible to conclude that, using the same floral source, solvent and process extraction, different regions, and seasonal variations also offer a different chemical composition. Interestingly, these differences did not affect the safe of propolis ingestion [111].

Besides maceration, percolation or turboextraction, Trusheva et al. [123] compared ultrasound extraction and microwave-assisted extraction with the maceration process. The careful analysis of the results obtained with each process demonstrated that statistical differences were found for total phenolics and propolis total extractable matter for ultrasound process (30 min) and microwave ($2 \cdot 10$ s) when compared to maceration extraction. For ultrasound, higher total phenolics were found (52.3%) in comparison to maceration (43.2%) while the reduced total extractable matter (53.3% versus 55%). In turn, microwave offered reduced values for total phenolics (40.4–0.6% versus 43.2%) and expressively higher amounts of the total extractable matter (75% versus 55%). Flavonoids analysis did not show important differences among the procedures evaluated. It is important to consider the time of extraction in each process, since maceration takes around 72 h, and ultrasound was effective with 30 minutes and microwave $2 \cdot 10$ s. Another important thing is to define the objective of the extraction: for analytical purposes, it is more practical and cheaper to use ultrasound or microwave, however, for industrial scale, these latter may not be easily implemented.

Propolis water extract was also obtained by some authors [12, 17, 98, 124] using completely different procedures, demonstrating some interesting biological activities that had been previously studied for propolis alcoholic extract, such as anti-inflammatory [12], inhibition of inflammatory angiogenesis [98], and antiviral [17]. Although the demonstration of these interesting results, chemical characterization was poorly explored in the manuscripts, except by Urushisaki et al. [17] that presented the caffeoylquinic derivatives as the most important

compounds of this extraction process; however, the manuscript does not present the extraction process used. De Moura et al. [98] performed the extraction from propolis raw material properly crushed in water maceration (500 ml) with temperature around 70°C (30–60 min), two fractions were obtained from the same propolis raw material, followed by filtration. The filtrate was then lyophilized. A similar procedure was used by others too. Nafady et al. [124] in turn, used an innovative process with β -cyclodextrin as encapsulate agent. To obtain this extract, 10 g of crushed propolis was dispersed in 1 l of water containing 10 g of β -cyclodextrin previously dissolved. The inconvenient of this procedure is the elevated costs involved in the acquisition of β -cyclodextrin besides the limitation of the propolis concentration obtained in the final product. Machado et al. [12] proposed the extraction using hydroalcoholic solvent (70%) as usual, however, the solvent was evaporated and after a hydrolysis step the propolis soft extract was then resuspended in water, in this last case, the obtained extract demonstrated similar chemical results of the alcoholic extract in the moment of preparation.

5.2. Nanoparticles and innovation products

Nowadays, finding natural additives has increased the efforts both to obtain bioactive compounds from natural raw materials and develop stable and functional derivative products. The former mentioned properties attributed to propolis are valuable and find applications in several industries, such as pharmaceuticals, agrochemical, and food. The growing interest in propolis has also promoted technological development for the suitable application of propolis.

Propolis in the powder form, for example, exhibits several advantages as increased concentration of propolis dry matter, higher chemical stability of the compounds, and longer preservation of the biological properties. Additionally, the powder form also permits the production of presentations with higher compliance in therapeutics, i.e., sachets, tablets, and capsules. The drying process may also involve the encapsulation of the product resulting in micro/nanoencapsulation systems, which can minimize sensory flavor and odor and control the release of the active compounds.

Propolis dry extract was obtained by Da Silva et al. [125] by employing arabic gum and octenyl succinic anhydride (OSA) starch as carriers by spraydrier. The process allowed obtaining propolis in the powder form with preserved antioxidant activity, stability, and low hygroscopicity. Microencapsulated propolis extract obtained by complex coacervation was reached and presented inhibitory activity against *S. aureus* [126]. Bruschi et al. [127] obtained gelatin microparticles containing propolis extractive solution by spray-drying technique. The microencapsulation by spray-drying technique maintained the activity of propolis against *S. aureus*. In another study, the effect of spray drying parameters on the chemical and biological properties of alcoholic extract of green propolis was investigated [128]. Several parameters of the process demonstrated to influence the polyphenol and flavonoid content, as well as the antioxidant activity, but under an optimized condition, the dried propolis extract showed significant antioxidant activity, with 50% lipid peroxidation inhibition at concentrations ranging from 2.5 to 5.0 mg/ml.

More recently, Marquifável et al. [129] aimed to develop a propolis dry extract with high propolis (~40%w/w of propolis dry matter) and artemillin C contents by employing a combi-

nation of silicon dioxide with arabic or modified starch and silicon dioxide by spray-drier. They have successfully obtained a standardized propolis extract with high amount of propolis, flavonoid content, expected amounts of artemisinin, and with maintained antibacterial activity, and obtained microparticles with both excipients used. Recently, results of the same group obtained dry extracts of propolis with 70–80% of dry matter; however, the microparticles were not obtained (data not published yet), and then, the odor, color, and taste are not similarly reduced as it is possible to observe when microparticles are obtained (**Figure 10**). Although microparticles were not obtained with 70–80% of propolis dry matter, this extract is the most concentrated one found in the market until now and can be used in several products with very good results, for example soft or hard capsules or tablets.

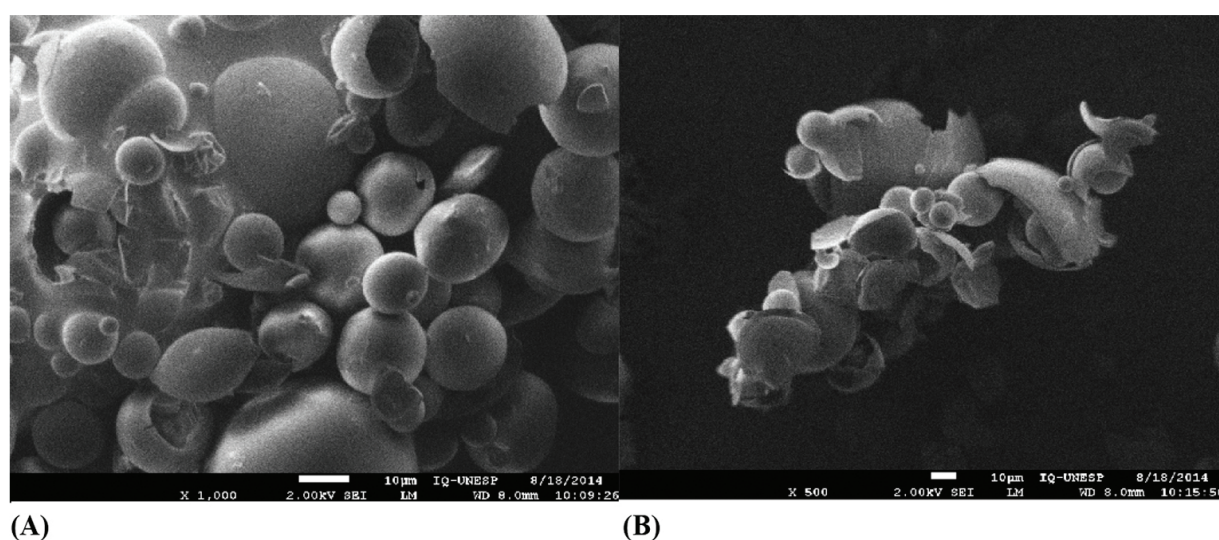


Figure 10. Propolis standardized water extracts of green propolis; C: propolis standardized extract (EPP-AF®).

In general, the propolis powder extracts obtained by spray-drying technique investigated in the literature demonstrated the formation of particles at the micrometer scale, from 1 to 10–20 μm . On the other hand, as nanotechnology can offer new opportunities for propolis application, in another line of research, nanosized particles have been developed. Patil et al. [130] have obtained and characterized silver nanoparticles containing propolis [130]. Propolis nanoparticles have also been obtained employing lipid carriers. Our research group has focused on developing solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) encapsulating propolis. **Figure 11** shows atomic force microscopy (AFM) images of propolis-loaded NLC. Additional studies were also conducted covering NLC surface with chitosan. The chitosan-covered particles presented positive residual surface charge [$\approx +40$ mV], while the uncoated ones presented negative charges [≈ -30 mV]. Particles were anisometric in shape and approximately 150–200 nm in size. The images demonstrate the particle surface and confirm the nanometric size of the particle. Additionally, no roughness was observed on the particle surfaces.

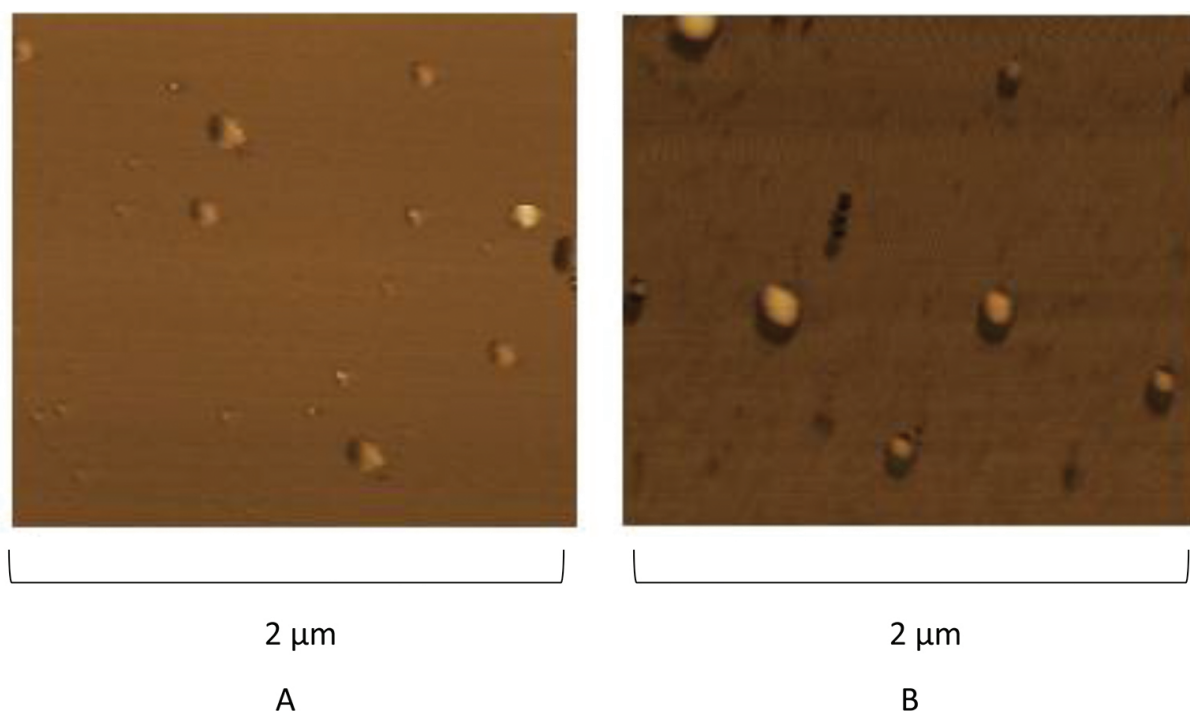


Figure 11. Atomic force microscopy (AFM) images of propolis-NLC (A) and chitosan coated propolis-NLC.

Several important applications can be carried out with innovative propolis extracts. Different presentations of propolis were previously showed as in a liquid presentation without alcohol, usually using propylene glycol or polyethylene glycol, propolis powders in different systems or concentrations, micro or nanoparticles, and others, as soft or hard capsules, with immediate or sustained release systems. Considering the applications, it is possible to formulate capsules, tablets, pills, or others with a specific amount of propolis dry matter, or with a focus on some groups of compounds (total flavonoid or polyphenols) and finally, on a biomarker or a group of these substances such as artemillin C, drupanin, or baccharin, all presentations completely applicable to functional or supplement food, or medicines. Besides oral administration, it is possible to use all of these propolis presentations in topical products, as previously published by Berretta et al. [8] who presented a propolis thermoreversible gel to treat cutaneous lesions or burns, Barud et al. [131] with a propolis biomembrane for the same application, or Berretta et al. [50] that demonstrated the benefits of the application of a propolis mucoadhesive gel in vulvovaginal candidiasis. Several other products can be found in the market and in the literature, such as mouthwashes, toothpastes dental creams, among others.

6. Regulatory affairs

Functional food and natural health products have become an important part of people's daily diet, contributing to the general health of the population and boosting the global food industry. Therefore, its importance is reflected in the interest in regulation of health claims and standards from industry stakeholders and policymakers.

In this chapter, we examine propolis product regulations and policies in many important producing and consuming countries around the world. The goal of this study is to incentive legislators to update the regulation on propolis products in order to improve information available to consumers so they can make better choices and also be provided with healthier and more innovative options.

The regulatory climate worldwide appears to be tending toward propolis classification into the health food products category, although this category also has different names, registration requirements, and allowed claims throughout the world.

Nevertheless, there are still some countries that categorize propolis as a conventional food together with the other bee products, such as honey, royal jelly, and bee pollen. That is the case of Brazil, where the product is regulated by the Ministry of Agriculture with very stringent regulation that limits the product's presentations, information to consumer and does not allow health claims. In 2005, the Brazilian "National Health Surveillance Agency" (ANVISA) published a technical note allowing the registration of propolis as a topical medicine with the claims of anti-inflammatory, antiseptic, and wound healing [132]. The publication of another regulation [133] reinforced the same rules but, due to the very strict rules for medicines, although Brazil is one of the biggest propolis markets, there are no propolis medicines registered to this date.

In the United States, propolis is encompassed together with a wide range of substances by the definition of a dietary supplement in the Dietary Supplement Health Education Act of 1994 (DSHEA) [134]. The use of function claims is also regulated by the above-mentioned regulation that established some special regulatory requirements and procedures for claims of general well-being. These claims are not preapproved by FDA, but the manufacturer must have substantiation that the claim is truthful and not misleading and must submit a notification with the text of the claim to FDA no later than 30 days after marketing the dietary supplement with the claim.

In the European Union (EU), propolis belongs to the food supplement group, regulated by the Directive 2002/46/EC [135], which defines the category as concentrated sources of nutrients or other substances with a nutritional or physiological effect. Since 2006, EU has been engaged in assessing generic health claims to surpass local regulation of member states and after this harmonization product's labels can only bear health claims authorized by the European Food Safety Authority (EFSA) [136], which evaluates scientific data on claims provided by the applicant. Up to this date, there is still no authorized health claim for propolis.

In Australia, all food supplements fall within the category of "complementary medicines" under the *Therapeutic Goods Act 1989* and the supporting *Therapeutic Goods Regulations 1990* [137], in which the substances are evaluated according to their level of risk. It includes vitamin, mineral, herbal, aromatherapy, and homeopathic products. A positive list of low-risk substances that may be used has been established and propolis is one of them. It can be used as an active, excipient, or component in all listed medicine formulations. Propolis products can make indications for health maintenance and health enhancement or certain indications for non-serious, self-limiting conditions. It is the manufacturer responsibility to hold evidence to support

any indications as well as any other claims made for the medicine (according to Requirements of section 26A of the Act).

Food supplements in Canada are regarded as “Natural Health Products” under the Natural Health Products Regulations (SOR/2003-196) [138–141] and may contain a wide range of substances, such as vitamins and minerals, herbal remedies, homeopathic medicines, traditional medicines, and probiotics. All products must be safe to use as over-the-counter products and not need a prescription to be sold. Propolis is positive listed to be used orally in multiple pharmaceutical dosage forms as a source of antioxidants for the maintenance of good health and to help relieve sore throat and/or other mouth and throat infections. It can also be used topically to assist in minor wound healing.

Japan is one of the first countries to move toward regulating functional foods. There are lists containing a broad range of substances that are not restricted to medicinal use and can therefore be used in food supplements. Propolis in this scenario can be used as an authorized excipient under the Food Sanitation Act 2010, as regular health food without any claims or as an active of a “Food for Specified Health Uses” (FOSHU) [142] with health claims.

The Republic of Korea defines functional food significantly differently from other countries, restricting functional food to nutraceuticals. They are regulated under the Health Functional Food Act of 2004 [143] and there is a positive list in the Health Functional Food Code with 37 categories. Propolis preparations in all forms are allowed and may include two health claims: antioxidant activity and antimicrobial activity in oral cavity.

The People’s Republic of China is another example of an Asian country that uses a product-specific system of registration. The State Food and Drug Administration in China (SFDA) [144] regulates these food supplements as “health foods” and maintains positive and negative lists of substances that may be used in health foods. Propolis is in the positive list. There are 27 categories of health function claims approved by the SFDA, but the regulatory process for achieving approval of these health claims is very strict and expensive, requiring the applicant to conduct nonclinical or even clinical studies through an approved agency in addition to the regular scientific literature review.

With this brief regulatory framework on propolis products, we have presented different policies and regulations around the world and we hope that policymakers can improve the regulatory scenario in the near future in order to accelerate and foster innovation in the sector.

7. Propolis nowadays market and potential

The green propolis has gained preference in the world market since the 1980s, unveiling new horizons for the product. The growing interest of the market for green propolis in the context of international food trade follows the increasing trend in search of healthier habits, which have gained ample space in people’s daily lives all over the world.

In the Japanese market, green propolis has a high commercial value: according to SEBRAE, the price of 1 kg of this product in 2010 was around \$ 87 and the same amount of honey was

priced at about 3 dollars. In Tokyo's market, this product is even more valued: a bottle of green propolis, in 2010, was sold for around \$ 150 [145, 146]. In 2008, it was estimated that in Japan, 700 million dollars a year started to be moved by green propolis [147]. Japan's interest in green propolis is justified not only by consumption: one of the most important examples of its use is as an adjuvant in the treatment of cancer; but also by Japanese research related to the chemical composition and biological activities of this type of propolis, especially studies with artemipillin C [16, 147].

The high demand for green propolis, especially from Asian countries, such as Japan, is essential for sustaining the economy that revolves around this product, which is fairly lucrative. Green propolis is produced mainly in the southeastern region, highlighting the state of Minas Gerais, where there are over 8000 beekeepers, which produces more than 35 tons of propolis per year [148]. These data show the importance of production and exports of green propolis, which is one of the pillars of Brazilian apiculture economy.

Red propolis found in the Brazilian state of Alagoas has been internationally certified by the Brazilian National Institute of Industrial Property (INPI) as the only producer of this kind of propolis in the world and most of its compounds were not found in other types of Brazilian propolis, which makes it a singular bee product [24]. Due to that, its commercial value is internationally high. It has been reported that a kilogram of this product can cost around R \$ 500. Its importance to the Brazilian economy and to red propolis producer states is immeasurable. Many propolis producers are being qualified and thereby, they are improving their product quality and the production process. Like green propolis, red propolis is also highly exported to Japan due to its chemical composition and biological effects [149].

Brazil is currently the world's third largest producer of propolis, second only to Russia and China [150]. Although it represents 10–15% of world production, Brazil fulfill about 80% of Japanese demand. Minas Gerais State (Brazil) Beekeepers Federation data show that the propolis produced in the Midwest region of the state is considered the best in the world by the Japanese market, where the kilogram of product has jumped from \$ 5 to \$ 200 in recent years [150].

The propolis production in Brazil is estimated at around 140 tons, and the major part is destined for international market, both in raw form and as finished products. It is estimated that 100 tons are green propolis and 40 tons other types of propolis. About 80% of the green propolis produced in Brazil comes from the Midwest region of Minas Gerais State, close to the source of the São Francisco river at Serra da Canastra, region where are the highest number of producers. Despite the great Brazilian beekeeping potential, the current production is not enough to fulfill a growing global demand. The Brazilian honey bees are Africanized, presenting defensive and disease-resistant features, with no need to use chemical treatments as in other countries, which ensures Brazilian bee products excellent quality and free from contamination.

Many research fronts have been opened in the pursuit of development and adaptation of professional management techniques in the production of green propolis. In addition to the improvement actions and training of producers in beekeeping management practices, a group

of green propolis producers in the Midwest region of Minas Gerais in the Source of the São Francisco River created an independent association supported by governmental agencies, universities, researchers, and local private institution.

This association aims to establish the technical and scientific cooperation between the scientific community and the beekeepers, aimed at regional development, improving the quality and increasing the amount of green propolis produced in the region. Among the main projects carried out, stand out the training of beekeepers in the professionalization of beekeeping, conservation and cultivation of *B. dracunculifolia* fields, periodical replacement of old to younger queen bees, and others projects. It is believed that the interaction of technical and practical knowledge of beekeepers in conjunction with the application of scientific knowledge by researchers and universities will contribute significantly to a comprehensive training in the professionalization of Brazilian beekeepers to fulfill the goal of maintaining the quality and increase the amount of green propolis produced in the region.

Trade promotion strategies are being constantly designed and implemented by the Brazilian Association of Honey and Propolis Exporters (ABEMEL) and the Brazilian Trade and Investment Promotion Agency (APEX-Brasil) to disseminate Brazilian bee products around the world. The result is the increasing demand from Asia, Europe, and North America countries.

In recent years, Brazil has been prominent on the international scene by winning important prizes at the World Beekeeping Awards of Apimondia, the main world beekeeping event that brings together representatives of over 130 countries and is held every 2 years. In the last editions, Brazil won gold and silver medals in the category honey and gold in the category propolis.

8. Future perspectives

Considering all information presented here, it is easy to imagine the important potential of propolis in the health of the population and in the Brazilian and international market, especially because of the important biological activities and safety demonstrated with scientific reports as "*in vitro*", "*in vivo*," and in some clinical trials. It is possible to generate several innovative products in different fields considering food, cosmetics, and medicines, and this choice obviously will be related to propolis dosages, formulations, and indications.

It is important to consider the investment in more clinical trials aiming to explore the benefits observed with traditional use and in animal studies, in order to refine the dosages and formulations, with regard to the development of medicines. Besides clinical studies, another important area is the improvement in the investments in productivity in field, since the Brazilian propolis available nowadays is not enough to supply all the countries that may be interested in working with this fabulous natural material produced by bees with the support of Brazilian Biodiversity. And finally, the effort of beepers and entities, such as ABEMEL, is crucial to stimulate and support this work.

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Author details

Andresa A. Berretta^{1,2,3*}, Caroline Arruda², Felipe Galeti Miguel¹, Nathalia Baptista¹, Andresa Piacezzi Nascimento¹, Franciane Marquele-Oliveira¹, Juliana Issa Hori¹, Hernane da Silva Barud⁴, Bianca Damaso^{3,5}, César Ramos^{3,5}, Raul Ferreira¹ and Jairo Kenupp Bastos²

*Address all correspondence to: andresa.berretta@apisflora.com.br

1 Apis Flora Indl. Coml. Ltda., Ribeirão Preto, Brazil

2 Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

3 Brazilian Association of Honey Exporters, ABEMEL, Rio Claro, Brazil

4 Uniara, University of Araraquara, Araraquara, Brazil

5 Natucentro Própolis, Bambuí, Brazil

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