Chapter 7

Propolis: Alternative Medicine for the Treatment of Oral Microbial Diseases

Vagner Rodrigues Santos

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54003

1. Introduction

Bees are arthropods of Hymenoptera order and are classified into two groups based on their type of life: solitary and social life. Propolis is produced by bees that live socially, from the harvesting of products derived from plants and used to seal and protect the hive against intruders and natural phenomena [1]. Propolis term derives from the Greek Pro, "opposite, the entry" and polis, "city or community" [2,3]. Propolis is a natural substance collected by *Apis mellifera* bees in several plant species. It has been used in folk medicine for centuries [2,4]. Characteristically, it is a lipophilic material, hard and brittle when cold, but soft, flexible and very sticky when warm. Hence the name "beeswax" [5]. It has characteristic odor and shows adhesive properties of oils and interact strongly with skin proteins [6]. The composition of propolis is complex [7,8]. Some factors, such as the botanical origin of propolis and its time of collection can influence the chemical composition of this resinous material [9]. The color of propolis varies from yellowish green to dark brown, depending on location - savannah, tropical forests, desert, coastal and mountainous regions - where it is produced. [10,11,12]. Propolis is used by bees to protect against the entry of microorganisms, fungi and bacteria in the hive, and as a sealing material for preventing the entry of light and moisture inside. It is also used to line the comb, to allow the deposition of eggs by the queen, and to embalm small dead animals (beetles and insects) that usually bees could not take into the hive, preventing its putrefaction.3,5,7].

Interest in the pharmacological action of natural products has grown and found significant popular acceptance. Among these products, propolis has been highlighted due to its applicability in the food industry and cosmetics, to be used as the active ingredient in several products, among which include toothpastes and skin lotions [13]. Also available in the form of a capsule (pure or combined), extract (hydroalcoholic or glycolic acid), mouthwash (combined with

melissa, sage, mallow and / or rosemary), lozenges, creams and powders (for use in or gargling internal use, once dissolved in water) [2].

Regarding the ethnobotanical aspect, propolis is one of the few "natural remedies" that continue to be used for a long period by different civilizations [14]. Propolis is widely used in popular medicine, especially in communities with inadequate public health conditions[15]. It was noticed that it can be more effective and less toxic than certain compounds. Significant decrease in H_2O_2-induced DNA damage in cultures treated with propolis demonstrated antioxidant activity of phenolic components found in propolis may contribute to reduce the DNA damage induced by H_2O_2 [16].

2. History

Propolis is a natural remedy that has been used extensively since antiquity. The Egyptians, who knew very well the anti-putrefactive properties of propolis, used it for embalming [17]. It was recognized for its medicinal properties by Greek and Roman physicians, such as Aristotle, Dioscorides, Pliny and Galen. The drug was used as an antiseptic and healing in the treatment of wounds and as a mouthwash, and its use in the Middle Ages perpetuated among Arab doctors [2]. Also, it was widely used in the form of ointment and cream in the treatment of wounds in battle field, because of their healing effect. This healing propolis property known as "Balm of Gilead," is also mentioned in the Holy Bible [18]. From the pharmacological point of view, propolis has been used as solid; in an ointment based on vaseline, lanolin, olive oil or butter, and in the form of alcoholic extract and hydroalcoholic solution. The proportion propolis/carrier may vary, in order to obtain bacteriostatic or bactericidal results [19]. In the 1980s and 1990s, a great number of publications occurred worldwide, highlighting Japan in number of published papers followed by Brazil and Bulgaria [6]. In Dentistry, there are studies investigating the pharmacological activity of propolissome situations, such as gingivitis, periodontitis, oral ulcers, pulp mummification in dogs' teeth and dental plaque and caries in rats [19]. Also, it has been used in dressings of pre and post-surgical treatment, oral candididosis, oral herpes virouses and oral hygiene. There was also the investigation of antiseptic and healing properties of propolis in subjects admitted to various hospitals and the results were extremely positive [20]. Thus, this natural product revealed great interest for the treatment of oral diseases [21]. Internationally, the first licensed commercial product containing propolis was registered in Romania in 1965. Worldwide, in the same period analyzed, it was found a total of 239 commercial licenses. In the 1980s, commercial licenses were predominant in the former USSR and satellite countries. Currently, 43% of commercial licenses are Japanese origin and 6.2% of them are products for dental treatment. In Japan, the scientific productivity reported for propolis increased 660% between the 1980 and 1990 [22]. The global interest in propolis research increased considerably in relation to its various biological properties [23-27]. Another incentive for conducting research on propolis is a high value on the international market, mainly in Japan, where a bottle of ethanol extract is sold at prices ten times higher than that prevailing in Brazil. Brazil is

http://dx.doi.org/10.5772/54003

considered the third largest producer of propolis in the world, behind Russia and China only. Japan's interest for the Brazilian propolis is due to its therapeutic and organoleptic properties, and also the presence of minor amounts of heavy metals and other environmental pollutants [28,29]. In the last thirty years, various studies and scientific research were performed to clarify the medicinal properties attributed to propolis [30,31].

3. Classification / rating

There was an attempt to classify the Brazilian propolis into twelve types according to physical-chemical properties and geographical reports. However, to date, only three types of propolis had their botanical origin identified. The main types of botanical origin are South (three), Northeast (six) and Southeast (twelve), and they were reported as resins from *Populus sp*., *Hyptis divaricata* and *Baccharis dracunculifolia* (Figure 5), respectively. An attempt to classify propolis produced in Brazil according to botanical origin and chemical composition [32] has recognized 12 different types. It was suggested that *Hyptis divaricata* is the resin source of northeastern propolis, *Baccharis dracunculifolia* of southeastern propolis and poplar (*Populus nigra*) of southern propolis. This study by Park et al. [32] is indicative that just stating that a certain sample corresponds to 'Brazilian propolis' hardly means anything indicative of physical, chemical and biological characteristics, because a wide diversity of propolis types exist in a country as large as Brazil, housing a wide plant diversity and a complex honeybee genetic variation [3]. The different compounds present in Brazilian propolis were identified and quantified using high performance liquid chromatography (HPLC) technique. Established the process of separation by liquid chromatography, capable of identifying the major components of propolis samples (primary marker). Through the technique of HPLC and quantification of compounds identified by it, it was established a classification for Brazilian propolis based on the presence of markers (Table 1 and Table 2). The main feature of this classification relates to the speed in which this product bee can reach the market, from the field to the pharmaceutical and cosmetic industries, encouraging the use of these typing for the manufacture of their medicines and cosmetics, with established quality control, since all of these markers were separated in a concentration range types. That is, the classification is quantitative. Another important factor is that the classification will be possible to manufacture pharmaceuticals, cosmetics and oral hygiene products knowing the propolis type used and the quantities of bioactive components, features never reported before in publications and patents on propolis [33]. The Brazilian Cerrado is one of the richest areas in *Baccharis* sp. These plants are a group of woody perennial shrubs, which are dioecious with male and female inflorescences appearing on separate plants. Of the 30 different species of *Baccharis*, *Baccharis dracunculifolia* is the dominant source of propolis in southeastern Brazil (Sao Paulo State and Minas Gerais State), where most of propolis based products sold are produced [34]. Recently, it was founded a red type of propolis in hives located in mangrove areas in the Northeast. It was observed that bees collect exudate from the surface of red *Dalbergia ecastophyllum* (Linnaeus, Taubert) (Figure 6). Analysis and comparision of plant exudates and propolis samples demonstrate that the chromatographic profiles are exactly the same as the one found

for *D. ecastophyllum* [35]. The best way to find the plant origin of propolis would be by comparing the chemical composition of propolis with the alleged plant origin [36]. World Propolis constituents of are shown in Table 3.

4. Chemical composition

Table 1 and Table 2 show the chemical markers constituents of green and red Brazilian propolis, respectively, while Table 3 shows the chemical composition of various types of world propolis. The highest concentration of phenolic compounds was obtained using solvents with lower concentrations of ethanol and higher concentrations of crude propolis, but the highest concentration of flavonoids in the extract was obtained with higher concentrations of ethanol in the solvent [11]. Over 300 chemical compounds are described in various propolis origins [22]. Among the chemicals constituents, we can include waxes, resins, balsams, oils and ether, pollen and organic material. The proportion of these substances varies and depends on the place and period of collection [5,37]. The collected propolis in a bee hive, also known as crude propolis, in its basic composition, contains about 50% of plant resins, 30% of beeswax, 10% essential oils, 5% pollen, 5% debris of wood and earth [7,14,6]. Propolis also contains various organic acids, considerable amount of minerals (including, manganese, zinc, calcium, phosphorus, copper), vitamins B1, B2, B6, C and E, acids (nicotinic acid and pantothenic acid) and aminoacids [5,7,11,38]. These constitutive features may vary by region and period of the year [39, 40].

Nº	Compounds	mg/g
1	Coumaric acid	3.56
2	Cinnamic acid	1.66
3	Quercetin	1.38
4	Kaempferol	1.77
5	Isorhamnetin	0,91
6	Sakuranetin	5.57
7	Pinobanskin-3-acetate	13.92
8	Chrysin	3.51
9	Galangin	9.75
10	Kaempferide	11.60
11	Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid)	82.96

BGP from *Baccharis dracunculifoila* (SBN97). HPLC test (Park et al.) [32].

Table 1. Chemical constituents markers of Brazilian green propolis sample

http://dx.doi.org/10.5772/54003

Number	Compounds	Contents (mg/g)
01	Rutin	0.7
02	Liquiritigenin	1.8
03	Daidzein	0.3
04	Pinobanksin	1.7
05	Quercetin	0.5
06	Luteolin	1.2
07	Dalbergin	0.4
08	Isoliquiritigenin	4.8
09	Formononetin	10.2
10	Pinocembrin	3.3
11	Pinobanksin-3-acetate	1.7
12	Biochanin A	0.5

from *Dalbergia ecastophyllum* (Alencar et al.) [41]

Table 2. Flavonoids and other chemical constituents of Brazilian red propolis

Compounds (percentage of content)			Authors
Fatty and aliphatic acids (24–26%)	Flavonoids (18–20%)	Microelements (0.5–2.0%)	Burdok et al. [7] Maciejewicz et al [43] Park et al. [32] Kumazawa et al.[44] Salatino et al. [3] Ozkul et al.[45] Eremia et al.[46] Machado et al.[47] Vandor-Unlu et al.[48] Wang et al.[49]
Butanedioic acid (Succinic acid)	Astaxanthin	Aluminum (Al)	
Propanoic acid (Propionic acid)	Apigenin	Copper (Cu)	
Decanoic acid (Capric acid)	Chrysin	Magnesium (Mg)	
Undecanoic acid	Tectochrysin	Zinc (Zn)	
Malic acid	Pinobanksin	Silicon (Si)	
D-Arabinoic acid	Squalene	Iron (Fe)	
Tartaric acid	Pinostrobin chalcone	Manganese (Mn)	
Gluconic acid	Pinocembrin	Tin (Sn)	
α-D-Glucopyranuronic	acid Genkwanin	Nickel (Ni)	
Octadecanoic acid (Stearic acid)	Galangin	Chrome (Cr)	
β-D-Glucopyranuronic acid	Acacetin		
9,12-Octadecadienoic acid	Kaemferide		
Tetradecanoic acid	Rhamnocitrin		
Pentanedioic acid	7,4′-dimethoxyflavone		
Glutamic acid	5-hydroxy-4′7-dimethoxyflavone		

Compounds (percentage of content)			Authors
2,3,4-trihydroxy butyric acid	5,7-dihydroxy-3,4′dihydroxyflavone		
Phosphoric acid	3,5-dihydroxy-7,4′-dimethoxyflavone		
Isoferulic acid			
	Sugars (15–18%)	Others (21–27%)	
	Sorbopyranose	Cyclohexanone	
	D-Erythrotetrofuranose	3-methyl,antitricyclo undec-3-en 10-one	
	D-Altrose	Cyclohexane	
	D-Glucose	Cyclopentene	
	Arabinopyranose	5-n-propyl-1,3 dihydroxybenzene	
	d-Arabinose	Butane	
	α-D-Galactopyranose	2(3H)-Furanone	
	Maltose	L-Proline	
	α-D-Glucopyranoside	2-Furanacetaldehyde	
	D-Fructose	2,5-is-3-phenyl-7-pyrazolopyrimidine	
Aromatic acids (5–10%)	Esters (2–6%)	Cliogoinol methyl derivative	
Benzoic acid	Caffeic acid phenethyl ester	Fluphenazine	
Caffeic acid	4,3-Acetyloxycaffeate	4,8-Propanoborepinoxadiborole	
Ferulic acid,	Cinnamic acid	1,3,8-Trihydroxy-6-methylanthraquinone	
Cinnamic acid	3,4 dimethoxy-trimethylsilyl ester	1-5-oxo-4,4-diphenyl-2-imidazolin-2-yl guanidine	
	3-Methoxy-4-cinnamate	3,1,2-Azaazoniaboratine/ Piperonal	
	Cinnamic acid	4 methoxy 3 TMS ester 3-Cyclohexene	
	2-propenoic acid methyl ester	1H-Indole	
Alcohol and terpens (2–3.3%) 1H-	Vitamins (2–4%)	Indole-3-one	
Glycerol	A, B1, B2, E, C, PP	2-Furanacetaldehyde	
Erythritol		Guanidine	
α-Cedrol		2(3H)Furanone	
Xylitol		1,3,8-trihydroxy-6-meyhylanthraquinone	
Germanicol			
Stigmast-22-en-3-ol			
Pentitol			

http://dx.doi.org/10.5772/54003

Compounds (percentage of content)	Authors
Ribitol	
Vanilethanediol	
Bicyclohept-3-en-2-ol	
Farneso	

Table 3. Propolis constituents according to Shawicka et al.[42].

However, the plant determines the chemical composition of propolis [4,39,40]. Today there are various substances known in propolis with distinct chemical structures from following classes: alcohols, aldehydes, aliphatic acids, aliphatic esters, amino acids, aromatic acids, aromatic esters, flavonoids, hydrocarbohydrates esters, ethers, fatty acids, ketones, terpenoids, steroids and sugars [21].The first studies to identify the active elements of propolis were performed in 1911 by researchers in Germany [50]: vanillin, cinnamic acid and alcohol. In the 1970s, [51] succeeded in isolating and identifying eleven elements, especially the most important type flavonoids, mainly flavones, flavonols and flavonones, terpenes, alpha-aceto butilenol and isovanillin. At the same time, [52] it was identified the unsaturated aromatic acids such as caffeic and ferulic acids. In the same decade, Kadakov et al.[53] reported the presence of thirteen amino acids in samples of propolis. The therapeutic effects are attributed to various phenolic compounds whichmake up the green propolis, which are widely distributed in plant kingdom. These flavonoids can be considered the main compounds [7,8], and also some phenolic acids and their esters, phenolic aldehydes, alcohols and ketones [54]. Flavonoids and caffeic acid phenethyl ester (CAPE) are phenolic compounds which have the ability to inhibit the growth and cell division and to increase membrane permeability interfering with microbial cell motility [13]. Despite being the most studied components of propolis, flavonoids are not solely responsible for the pharmacological properties. Several other components have been related to the medicinal properties of propolis [55]. Propolis from Europe and China contains many flavonoids and phenolic acids esters. Flavonoids are present only in small quantities in Brazilian propolis. The major components of propolis of Brazilian origin are terpenoids and ñ-coumarin prenylated acid derivatives [39]. In Southeastern Brazil there is plenty of the botanical species for production of green resin, which is the *Baccharis dracunculifolia,* also called "Rosemary's field", or "broom", which is a plant species typical of the Americas, due to the necessity of acid soil to grow. Rosemary easily develops in Brazil, both in planted areas and in abandoned spaces [34, 3,56]. The biodiversity needs to be investigated as a source of new bioactive substances, such as cinnamic acid derivatives, especially artepilin C, flavonoids and other pharmacological or functional properties [36].The renewed interest on the composition of Brazilian propolis is due to the fact that Brazil has a very diverse flora, tropical climate and Africanized *Apis mellifera* bees species that produce propolis during the period from April to September [5,32]. The typical constituents of Brazilian green propolis from *Baccharis dracunculifolia* are derived prenylated cafeochemic acid and cinnamic acid derivatives, such as artepilin C and baccharin. Brazilian green propolis is chemically different because it contains not only prenylateds of cinnamic acid, but also triterpenoid [57]. In dealing with the chemical composition and biological activity of green propolis, one can not point to a component of a

particular substance or class of substances that could be responsible for their distinct pharmacological activities. Isoliquiritigenin, liquiritigenin and naringenin, isoflavones, isoflavans and pterocarpans were detected in Cuban Red Propolis, Brazilian Red Propolis (BRP) and *Dalbergia ecastophylum* extract (DEE), whereas polyisoprenylated benzophenones guttiferone E/xanthochymol and oblongifolin A were detected only in BRP. Pigments responsible for the red color of DEE and red propolis were also identified as two C30 isoflavans, the new retusapurpurin A and retusapurpurin B [10]. Obviously, different samples at different combinations of substances are essential for the biological activity of propolis [58,14]. It is important to note that all investigations on the antibacterial activity of specific substances isolated from propolis showed that a single component does not have an activity greater than the total extract [59]. The chemical properties of propolis are of great relevance considering its pharmacological value as a natural mixture and as a powerful source of new antimicrobial agents, antifungal, antiviral and individual compounds [58, 60].

5. Therapeutic properties of propolis

Currently, it is known that Brazilian propolis shows several biological activities, such as antimicrobial, antiinflammatory, immunomodulatory, among others [12]. The composition of propolis is very complex. We can observe the following: antibacterial activity, conferred by the presence of flavonoids, aromatic acids and esters in its composition; bactericidal action resulting from the presence of cinnamic acid and coumarin; *in vitro* antiviral activity (herpes simplex, influenza), due to the action of flavonoids and aromatic acids derivatives, antiulcer (assistance in healing), immunostimulating, hypotensive and cytostatic actions [21]. The methods of extraction of propolis may influence its activity, from different solvents at different soluble extract components [6,61]. The composition of propolis can vary according to the geographic locations from where the bees obtained the ingredients. Two main immunopotent chemicals have been identified as caffeic acid phenethyl ester (CAPE) and artepillin C. CAPE and artepillin C have been shown to exert immunosuppressive function on T lymphocyte subsets but paradoxically they activation macrophage function. On the other hand, they also have potential antitumor properties by different postulated mechanisms such as suppressing cancer cells proliferation via its anti-inflammatory effects; decreasing the cancer stem cell populations; blocking specific oncogene signaling pathways; exerting antiangiogenic effects; and modulating the tumor microenvironment[62]. The good bioavailability by the oral route and good historical safety profile makes propolis an ideal adjuvant agent for future immunomodulatory or anticancer regimens. However, standardized quality controls and good design clinical trials are essential before either propolis or its active ingredients can be adopted routinely in our future therapeutic armamentarium [62].

5.1. Anti-inflammatory activity

As an anti-inflammatory agent, green propolis is known to inhibit the prostaglandin synthesis, activate the thymus gland, help the immune system by promoting the phagocytic activity, stimulating cellular immunity, and increasing healing effects on epithelial tissue. Additionally,

the propolis contains elements such as iron and zinc, which are important for the synthesis of collagen [63,35]. Recently it was reported that Artepillin C has an inhibitory effect on nitric oxide and prostaglandin E2 by modulating NF-êâ using the macrophage cell line RAW 264.7 [64]. The anti-inflammatory activity observed in green propolis seems to be due to the presence of prenylated flavonoids and cinnamic acid. These compounds have inhibitory activity against cyclooxygenase (COX) and lipooxygenase. It also appears that the caffeic acid phenethyl ester (CAPE) has anti-inflammatory activity by inhibiting the release of arachidonic acid from cellular membrane, removing the activities of COX-1 and COX-2 [65, 66]. Propolis also exhibits anti-inflammatory effects against models of acute and chronic inflammation (formaldehyde and adjuvant-induced arthritis, carrageenin and PGE 2, induced paw edema and granuloma pellete cotton). The exact mechanism of anti-inflammatory action of propolis is still unclear [2]. Treatment with 50 μM CAPE significantly reduced the levels of leptin ($p<0.05$), resistin ($p<0.05$) and tumor necrosis factor (TNF)-alpha ($p<0.05$) which are known to aid adipocytokines production in adipocytes. CAPE has inhibitory effects on 3T3-L1 mouse fibroblast differentiation to adipocytes. In 3T3-L1 cells, treatment of CAPE decreased the triglyceride deposition similar to resveratrol, which is known to have an inhibitory effect on 3T3-L1 differentiation to adipocytes. In conclusion, we found that CAPE suppresses the production and secretion of adipocytokines from mature adipocytes in 3T3-L1 cells [67]. The crude hexane and dichloromethane extracts of propolis displayed antiproliferative/cytotoxic activities with IC50 values against the five cancer cell lines ranging from 41.3 to 52.4 μg/ml and from 43.8 to 53.5 μg/ml, respectively. Two main bioactive components were isolated, one cardanol and one cardol, with broadly similar in vitro antiproliferation/cytotoxicity IC(50) values against the five cancer cell lines and the control Hs27 cell line, ranging from 10.8 to 29.3 μg/ml for the cardanol and < 3.13 to 5.97 μg/ml (6.82 - 13.0 μM) for the cardol. Moreover, both compounds induced cytotoxicity and cell death without DNA fragmentation in the cancer cells, but only an antiproliferation response in the the non-transformed human foreskin fibroblast cell line

(Hs27, ATCC No. CRL 1634) used as a comparative control.However, these compounds did not account for the net antiproliferation/cytotoxic activity of the crude extracts suggesting the existence of other potent compounds or synergistic interactions in the propolis extracts. This is the first report that *A. mellifera* propolis contains at least two potentially new compounds (a cardanol and a cardol) with potential anti-cancer bioactivity. Both could be alternative antiproliferative agents for future development as anti-cancer drugs [68].

5.2. Antimicrobial activity

5.2.1. Antibacterial and antifungal activity

Previous studies have shown that green propolis extracts inhibit the *in vitro* growth of *Streptococcus mutans* [5,69,8,59]. This microorganism is etiologically related to the formation of dental caries in humans and animals. Propolis showed efficient antimicrobial activity against *Pseudomonas* sp and *Staphylococcus aureus* [70]. Propolis antimicrobial effect is directly proportional to its concentration [54]. Propolis ethanolic extracts exhibited significant antimicrobial activity against many pathogens from the oral cavity, including *Porphyromonas gingivalis*,

Prevotella intermedia, *Tannerella forsythia*, *Fusobacterium nucleatum* [(69,24,71], which is the main microbiota involved in periodontal disease related to plaque. Gram-positive bacteria are more sensitive than Gram-negative bacteria to propolis extracts [72]. So far, no data is available to answer this observation. Gram-negative bacteria have a cell wall chemically more complex and a higher fat content, which may explain the higher resistance [73,74]. Antibacterial activity of green propolis derives mainly of flavonoids, aromatic acids, esters present in resins, galangin, pinostrobin, and pinocembrin which have been known as the more effective agents against bacteria. Ferulic acid and caffeic acid also contribute to the bactericidal action of propolis [5]. A simple analogy can not be made to the mode of action of classic antibiotics. There are no reports considering the resistance to bacterial constituents of propolis, and these properties may influence the success of antibiotic therapy in the oral cavity [63]. The solvent used for propolis extraction (ethanol, chloroform, methanol, propylene glycol, for example) can influence its antimicrobial activity. In fact, oily preparations have high antimicrobial activity, while solutions of glycerin showed little inhibition of Gram-positive and ethanolic solutions and propylene glycol showed good activity against yeasts [74]. Several studies have reported synergistic activity of propolis associated with various antibiotics, including activity against strains resistant to benzylpenicillin, tetracycline and erythromycin. These studies concluded that propolis has significant synergistic action, which may constitute an alternative therapy for microbial resistance, but dependent on its composition [75,9,76]. Propolis has also shown fungistatic and fungicidal activity *in vitro* against yeasts identified as cause of onychomycosis [35]. Although propolis is not widely used in conventional health care, is recommended for use as home remedies in the treatment of oral candidosis, denture stomatitis and skin lesions by numerous books and articles in the popular press [77,78]. Although some studies have focused on showing the antifungal activity of propolis extract, few have shown their effects on morphology and structure of *Candida albicans* [79,80]. Combinations of some drugs, antimycotic with propolis (10%) increase their activity against the yeast *Candida albicans*. The greatest synergistic effect against various strains were obtained when propolis is combined with other antifungal agents [5]. Siqueira et al.[81] demonstrated the antifungal activity of aqueous and alcoholic extracts of the green propolis and the alcoholic extract of red propolis was observed against *Trichophyton rubrum*, *Trichophyton tonsurans* and *Trichophyton mentagrohytes* samples, using as controls itraconazole and terbinafine. The data obtained showed that the green propolis alcoholic extract's antifungal activity was from 64 to 1024 μg/mL. The antifungal activity of red propolis alcoholic extract was more efficient than the green propolis alcoholic extract for all three species studied. The antifungal potential of the alcoholic extracts of green and red propolis demonstrated suggest an applicable potential as an alternative treatment for dermatophytosis caused by these species [82, 81]. On the other hand the diterpenes: 14,15-dinor-13-oxo-8(17)-labden-19-oic acid and a mixture of labda-8(17),13E-dien-19-carboxy-15-yl oleate and palmitate as well as the triterpenes, 3,4-seco-cycloart-12-hydroxy-4(28),24-dien-3-oic acid and cycloart-3,7-dihydroxy-24-en-28-oic acid were isolated from Cretan propolis. All isolated compounds were tested for antimicrobial activity against some Gram-positive and Gram-negative bacteria as well as against some human pathogenic fungi showing a broad spectrum of antimicrobial activity [83]. Concerning the antimicrobial activity of propolis phenols, *Candida albicans* was the most resistant and *Staphylococcus aureus*

http://dx.doi.org/10.5772/54003

the most sensitive from Portugal, Braganca and Beja`s propolis. The reference microorganisms were more sensitive than the ones isolated from biological fluids [84]. Tables 4, 5, 6, and 7 show results from *in vitro* antimicrobial activity of ethanolic extract and gel containing Brazilian green propolis. Imaging studies with electron microscopy suggest the rupture of the cell wall of *Candida albicans* as one of the mechanisms of action of Brazilian green propolis (Figure 1) [78].

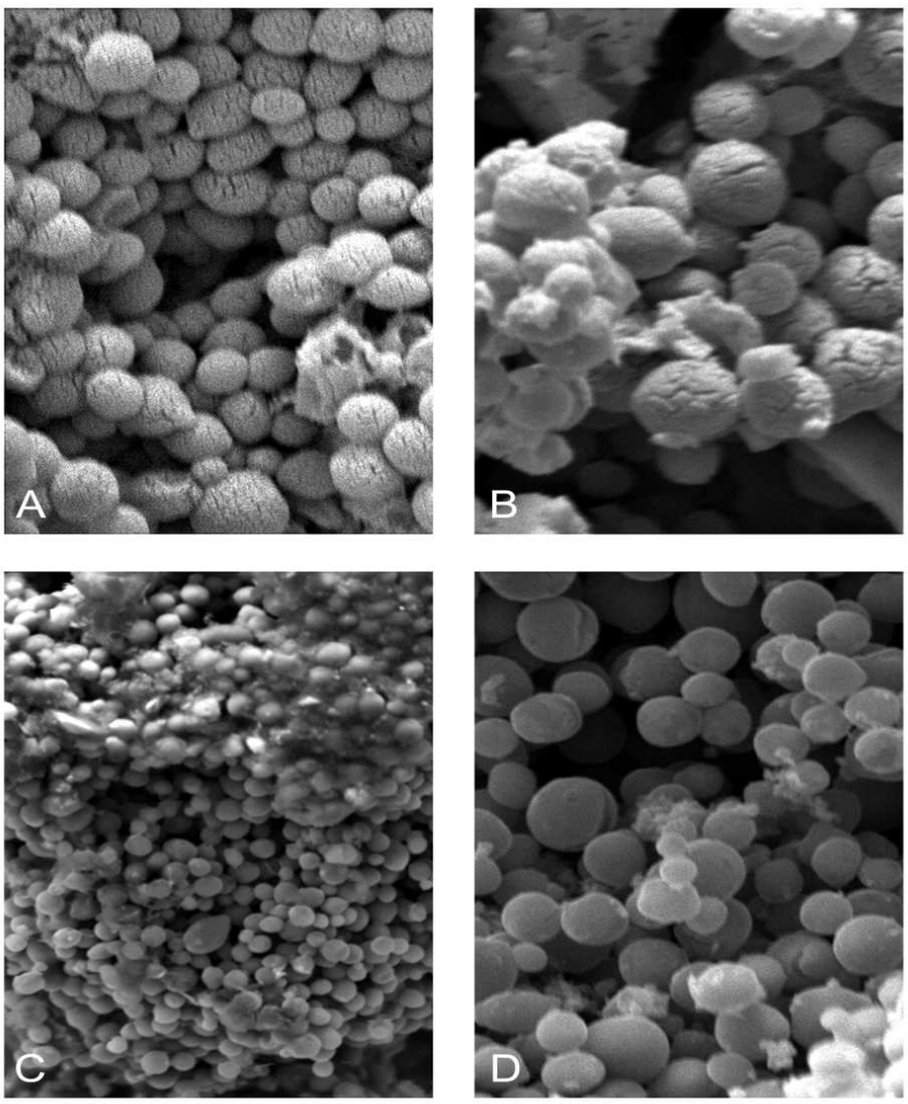

Figure 1. Micrographs showing *C. albicans* treated for 24h with subinhibitory concentrations of Brazilian Green Propolis extract (BGP). Scanning electron micrographs: Treated (panels A, B, and C) and untreated (panel D). A and B: cell wall detachment. C: cell agglomeration. Mello et al. [78].

Microorganisms	MIC (ìg/mL)	MBC (ìg/mL)	Inhibition zones (M±SD =mm)
C. albicans	20-50	100-300	16.3±0.52
C. tropicalis	20-50	100-300	12.3±0.08
C. glabrata	20-50	100-300	15.6±0.50
C. krusei	20-50	100-400	28.3±0.15
C. parapsilosis	20-50	100-400	18.6±0.08
C. guilliermondii	20-50	100-400	12.6±0.57
S. mutans	25-50	200-400	18.3±1.15
S. sobrinus	25-50	200-400	28.6±0.57
P. intermedia	20-50	200-400	17.5±2.50
T. forsythensis	30-60	300- 500	14.0±0.00
B. fragilis	25-50	300-500	15.3±1.15
S. aureus	25-50	200-400	16.3±2.08
P. gingivalis	30-50	200-400	14.0±0.00
F. nucleatum	30-60	200-400	15.2±0.26
F. necrophorum	30-60	200-400	17.3±0.57
A. actinomycetemcomitans	30-60	200-400	14.6±0.57

Table 4. Minimum Inhibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC), Means and Standard Deviation (M±SD) of diameter inhibition zones obtained in agar diffusion test using Brazilian Green Propolis Extract (BGP) against *Candida* spp., Gram positive and Gram negative oral pathogenic bacteria. (Tests in triplicate).Paula et al. [59]

Microorganisms	Propolis MIC (ìg/ml)	Nystatin MIC (ìg/ml)	Chlorexidine MIC (ìg/ml)	Tetracycline MIC (ìg/ml)
C. albicans	14.00	16.00	_	_
C. tropicalis	14.00	16.00	_	_
S. mutans	28.00	_	8.00	1.00
S. aureus	14.00	_	32.00	4.00
A. israelii	1.75	_	32.00	4.00
E. faecalis	7.00	_	- 16.00	2.00
A. actnomycetemcomitans	3.50	_	8.00	1.00

Table 5. Minimum Inhibitory Concentration (MIC) of propolis ethanolic extract and control obtained for each strain tested. Tests in quadruplicates. (Paula et al.) [59].

	Propolis ointment %		Tetracycline 1%							
Bacteria	**48 hs activity**	**7 days activity**	**48h**	**7days**						
	5%	10%	15%	20%	5%	10%	15%	20%		
S. mutans	13.33±3.09	19.00±2.00	19.66±2.08	23.33±1.52	9.66±1.52	15.66±2.58	14.33±2.08	18.33±1.52	14.33±0.57	9.33±1.52
S. aureus	13.00±1.00	17.66±2.08	18.66±2.08	21.66±0.57	9.66±0.57	12.33±1.15	14.00±1.00	15.00±2.00	18.33±2.51	11.00±1.53
A. israelii	12.00±1.00	14.66±2.08	15.00±2.08	21.66±2.51	7.33±1.15	9.66±2.08	11.66±2.08	13.66±1.52	14.00±1.73	10.00±1.00
E. faecalis	14.66±1.15	18.33±0.57	21.00±1.00	24.00±1.00	9.66±0.57	11.00±1.00	12.66±0.57	15.00±1.00	9.66±2.08	7.66±1.15
A.a.	14.33±1.52	18.00±2.00	21.66±2.08	25.33±2.08	8.66±2.51	11.00±2.00	14.33±1.52	13.66±1.52	18.00±2.00	12.00±1.00

Table 6. Susceptibility of oral bacteria to Brazilian propolis adhesive formulation. Inhibition zones values in mm (M ±SD; n=3). Negative control was inactive..A.a. = *A. actinomycetemcomitans* (Santos et al.) [71]

Fungi	**Propolis ointment %**		**Nystatin 5%**							
	48 h activity	**7 day activity**	***48 h***	***7day***						
	5%	10%	15%	20%	5%	10%	15%	20%		
C. albicans	16.33±1.52	21.66±1.57	23.00±1.00	26.00±1.00	12.33±1.52	17.00±1.00	16.66±1.52	20.66±0.57	12.00±2.00	8.66±1.52
C. tropicalis	16.66±2.51	24.33±2.03	23.00±2.00	26.00±2.00	13.33±2.08	19.33±0.57	17.66±0.57	19.00±1.00	14.66±1.52	10.66±1.52

Table 7. Susceptibility of *Candida* species to Brazilian propolis adhesive formulation. Inhibition zones values in mm (M ±SD; n=3). Negative control was inactive. (Santos et al.) [71]

5.2.2. Antiviral activity

There are many reports on the antiviral activity of propolis. In a study performed in Ukraine compared the efficacy of ointment with propolis Canadian ointments acyclovir and placebo (vehicle) in treating subjects with type 2 Herpes applicant. The preparation of propolis containing flavonoids found to be more effective than the other two in wound healing and reduction of local symptoms [98]. The cytotoxic and antiherpetic effect of propolis extracts against HSV-2 was analysed in cell culture, and revealed a moderate cytotoxicity on RC-37 cells. However both propolis extracts exhibited high anti-herpetic activity when viruses were pretreated with these drugs prior to infection. Selectivity indices were determined at 80 and 42.5μg/mL for the aqueous and ethanolic extract, respectively, thus propolis extracts might be suitable for topical therapy in recurrent herpetic infection [99]. Huleihel & Isanu [100] reported potent antiviral activity of propolis against Herpes simplex-1 infection *in vitro* and *in vivo*. They suggested that the propolis can prevent absorption of the virus within the host cells and interfere with viral replication cycle. *In vitro* studies suggest that the green propolis has potent antiviral activity against variants X4 and R5 HIV-1. Similar activity was observed with CD4 + lymphocytes in operation, at least in part, as an inhibitor of viral entry [101,35]. Also, the

antiviral activity of components of propolis, such as esters of cinnamic acids replacements was studied in vitro [5, 9, 102]. The antiviral effect of propolis extracts and selected constituents, e.g. caffeic acid, *p*-coumaric acid, benzoic acid, galangin, pinocembrin and chrysin against herpes simplex virus type 1 (HSV-1) was analysed in cell culture by Schnitzler et al.[103]. The 50% inhibitory concentration IC50 of hydro ethanolic propolis extracts for HSV-1 plaque formation was determined at 0.0004% and 0.000035%, respectively. Both propolis extracts exhibited high levels of antiviral activity against HSV-1 in viral suspension tests, plaque formation was significantly reduced by >98%. Both propolis extracts exhibited high anti-HSV-1 activity when the viruses were pretreated with these drugs prior to infection. Among the analysed compounds, only galangin and chrysin displayed some antiviral activity. However, the extracts containing many different components exhibited significantly higher antiherpetic effects as well as higher selectivity indices than single isolated constituents. Propolis extracts might be suitable for topical application against herpes infection [104]

5.3. Antioxidative activity

The antioxidative activity deserves special interest because propolis could be topically applied successfully to prevent and treat skin damaged [85, 86, 87]. Phenolic compounds found in high concentrations in Brazilian green propolis, including Artepillin C, have a wide range of biological properties including the ability to act as an anti-oxidizing free radicals and nitric oxide radicals and also the ability to interfere with the inflammatory response through inhibition of iNOS and COX-2 activities [88]. Although studies of propolis ethanol extracts are very common, it is reported that the aqueous extract has good antioxidant activity, associated with high content of phenolic compounds [89,90,91, 92]. Some studies have indicated propolis inhibiting superoxide anion formation, which is produced during autoxidation of â-mercaptoethanol [93,2]. The antioxidative activity of propolis and its main phenolic compounds, caffeic acid, p-coumaric acid, ferulic acid, and caffeic acid phenethyl ester, were investigated in yeast *Saccharomyces cerevisiae*. Yeast cells showed decreased intracellular oxidation, with no significant differences seen for the individual phenolic compounds. Ethanol Extract Propolis (EEP) antioxidative activity was also investigated at the mitochondrial proteome level and changes in the levels of antioxidative proteins and proteins involved in ATP synthesis were seen [94]. Brazilian green propolis is derived of *B. dracunculifolia* and protective effects of *B. dracunculifolia* glycolic extract against oxidative stress in isolated rat liver mitochondria (RLM) were investigated by Guimaraes et al.[95]. So, *B. dracunculifolia* exhibit potent antioxidant activity protecting liver mitochondria against oxidative damage and such action probably contribute to the antioxidant and hepatoprotective effects of green propolis [95]-. CAPE are involved with the renal damage protection induced by Cd (II) owing to its antioxidant capacity and anti-inflammatory effect [96]. Preadministration of Brazilian Propolis Ethanol Extract (50 or 100 mg/kg) to the stressed rats protected against the hepatic damage and attenuated the increased hepatic lipid peroxide and NO(x) contents and myeloperoxidase activity and the decreased hepatic non-protein SH and ascorbic acid contents and superoxide dismutase activity, possibly through its antioxidant and antiinflammatory properties [97].

5.4. Antitumoral activity

Several researchers reported the antitumoral property of propolis *in vitro* and *in vivo* [105,106, 30, 68]. Propolis isolated components showed antiproliferative activity in tumor cells [6]. Artepilin C, the major component of Brazilian green propolis, has antiangiogenic activity. Propolis may suppress tumor growth *in vivo*, but these mechanism effects is not completely understood [107, 39, 60]. Propolis shows antitumor properties, and its anticarcinogenic and antimutagenic potential is promising, but the mechanisms involved in chemoprevention are still unclear [108]. On other hand, CAPE and chrysin may be useful as potential chemotherapeutic or chemopreventive anticancer drugs [42]. However, the human aldo-keto reductase (AKR) 1C3, also known as type-5 17â-hydroxysteroid dehydrogenase and prostaglandin F synthase, has been suggested as a therapeutic target in the treatment of prostate and breast cancers was inhibited by Brazilian propolis-derived cinnamic acid derivatives that show potential antitumor activity, and it was found that baccharin a potent competitive inhibitor (K(i) 56 nM) with high selectivity [109]. There are currently several authors studied the antitumor activity of propolis, especially its components. Some initial studies are, however, some authors already have in-depth evaluation of about the propolis activity onto various animal or human types of tumor cell lines [110-115].

5.5. Immunomodulatory activity

The immunomodulatory activity of propolis is one of the most studied areas in conjunction with its anti-inflammatory property [116-120]. The immunomodulatory action of propolis seems to be limited to macrophages, with no influence on the proliferation of lymphocytes [121]. The inhibitory effect of green propolis (5-100μg/mL) on splenocyte proliferation was observed *in vitro* [122], and previous studies demonstrated that flavonoids have an immunosuppressive effect in lymphoproliferative response [123-125]. Since, propolis contains flavonoids, that may explain the reported effect [6,10]. Another explanation for the inhibitory effect on lymphocyte proliferation from the observation that both CAPE has inhibitory effects on transcription of nuclear factor-êB (NF-êB) (p65) and nuclear factor of activated T-cells (NFAT). Consequently, CAPE inhibited IL-2 gene transcription, IL-2R (CD25) expression and proliferation of human T cells, providing new insights into the molecular mechanisms involved in inflammatory and immunomodulatory activities of this natural component [6]. Green propolis exhibited immuno-stimulatory and immunomodulatory effects on CD4/CD8T cells and on macrophages *in vitro* and *in vivo* mice [126]. Propolis administration to melanoma-bearing mice submitted to stress stimulated IL-2 expression, as well as Th1 cytokine (IL-2 and IFN-ã) production, indicating the activation of antitumor cell-mediated immunity. Also, propolis stimulated IL-10 expression and production, which may be related to immunoregulatory effects indicating its antitumor action *in vivo* [127]. On other hand, Orsatti and Sforcin [128] demonstrated the propolis immunomodulatory action in chronically stressed mice, upregulating TLR-2 and TLR-4 mRNA expression, contributing to the recognition of microorganisms and favoring the initial steps of the immune response during stress. A new line of research involving propolis is the possible application as a vaccination adjuvant, although most commercial vaccines use aluminum salts to this end. A sample of green Brazilian propolis was

tested, together with other adjuvant compounds, to immunize mice against inactivated swine herpes virus (SuHV-1). When administered together with aluminum hydroxide, the propolis extract increased both cellular and humoral responses [103].

6. Toxicity

It must be emphasized that propolis has the advantage of being a natural product, with a higher molecular diversity. It has many therapeutic substances compatible with the metabolism of mammals in general, which reduces the possibility of causing adverse reactions to oral tissue as compared to industrial products tested [13]. The aqueous and alcoholic extracts of propolis do not cause irritation to the tissues [17] and are considered relatively toxic [7]. Experimental mouthwash solutions containing propolis showed no significant inhibitory activity of microorganisms as effective as chlorhexidine, but found lower cytotoxicity on human gingival fibroblasts; propolis is relatively non-toxic and studies have exhibited a no-effect level in a mice study of 1400 mg/kg weight/day leading the authors to propose that a safe dose in humans would be 1.4 mg/kg weight/day, or approximately 70 mg/day [63]. On other hand, Pereira et al. [29] demonstrated high effectiveness of mouthwash containing propolis in control of dental plaque and gingivitis in humans and not observed no toxic or side effects in the administration of the rinse during 90 days. Propolis is considered safe in small doses. Therefore, adverse effects are common at doses above 15g/day. The most commonly experienced adverse effects are allergic reactions, as well as irritation of the skin or mucous membranes [129]. Caution should be used in the treatment of individuals with asthma and eczema and nettle rash [2].

7. Standardization

A universal chemical standardization of propolis would be impossible. Therefore, a detailed investigation of its composition, botanical origin and biological properties is significant [6]. It was postulated that different propolis may have different chemical and pharmaceutical properties. In this sense, standardization of propolis is required. Most studies on the chemistry of propolis include those directed to the European propolis composed of *Populus* sp. These studies have been conducted by paired with Gas Chromatography Mass Spectrometry (GC-MS). Therefore, due to the lower reproducibility of these methods, the use of High- Performance Liquid Chromatography (HPLC) is currently recommended [22,130,131]. An alternative method, using electro-spray, was recently tested to determine the patterns and content of polyphenolic components of propolis [132]. Nuclear magnetic resonance is one of the best detection methods because it recognizes components sensitive or insensitive to Ultraviolet Light (UL) [133,134]. Standardization can prevent product adulteration. Therefore, the methods used to extract components of propolis require adequate standardization [22, 87,135].

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8. Oral clinical studies

Several clinical studies have demonstrated propolis efficacy in clinical trials, but the majority of studies involve topical application [20, 136-138]. The great diversity and the complexicity of chemical components makes difficult to standardize and to research the mechanisms of action. It is known the propolis anti-inflammatory, anti-microbial, analgesic, antioxidant, and antitumorproperties. Recently, some authors have demonstrated the properties of some components, however, one can not consider when using propolis but as a whole. The antimicrobial activity, for example, may be effective when considering the synergism between the components. Moreover, there was always the concern of several authors to develop oral mouthwashes- based propolis to control oral microbiota [138-140]. Koo et al.[141] demonstrated the effect of a mouthrinse containing selected propolis on 3-day dental plaque accumulation and polysaccharide formation and observed the Dental Plaque Index(PI) for the experimental group was 0.78 (0.17), significantly less than for the placebo group, 1.41 (0.14). On other hand, the experimental mouthrinse reduced the PI concentration in dental plaque by 61.7% compared to placebo ($p < 0.05$). The clinical efficacy of an alcohol-free mouthwash containing 5.0% (W/V) Brazilian green propolis (MGP 5%) for the control of plaque and gingivitis were demonstrated by Pereira et al.[29] (Tables 8, 9, 10, and 11). Twenty five subjects, men and women aging between 18 and 60 years old (35 ± 9), were included in a clinical trial`s phase II study of the patients who had a minimum of 20 sound natural teeth, a mean plaque index of at least 1.5 (PI), and a mean gingival index (GI) of at least 1.0. They were instructed to rinse with 10mL of mouthwash test for 1 minute, immediately after brushing in the morning and at night. After 45 and 90 days using mouthwash, the results showed a significant reduction in plaque and in gingival index when compared to samples obtained in baseline. These reductions were at 24% and 40%, respectively ($P < 0.5$). There were no important side effects in soft and hard tissues of the mouth.

	Baseline	45 days	90 days	Reduction %		
MGP5%	N=22 1.17 (0.20)	N=22 0.64 (0.24)	N=21 0.70 (0.18)	Baseline- 45 days 45*	Baseline- 90 days 40*	45 days – 90 days

Table 8. Mean scores of Gingival Index (DP) and percent reduction between periods (Pereira et al., 2011) [29].∗ Friedman test (ANOVA) $P < .05$.

	Baseline	45 days	90 days	Reduction-%		
MGP5%	$n = 22$ 0.30 (0.17)	$n = 22$ 0.08 (0.06)	$n = 21$ 0.07 (0.03)	Baseline–45 days 73*	Baseline–90 days 77*	45 days–90 days 13 (ns)**

Table 9. Mean scores of Severity Gengival Index (DP) and percent reduction between periods (Pereira et al., 2011) [29]. *Friedman test (ANOVA) $P < .05$. ∗∗Not significant.

	Baseline	45 days	90 days	Reduction-%		
MGP5%	*n* = 22 2.39 (0.69)	*n* = 22 1.77 (0.61)	*n* = 21 1.82 (0.62)	Baseline–45 days 26*	Baseline–90 days 24*	45 days–90 days∗ _____

Table 10. Mean scores of Plaque Index (DP) and percent reduction between periods (Pereira et al., 2011) [29].∗Friedman test (ANOVA) *P* <.05.

	Baseline	45 days	90 days	Reduction-%		
MGP5%	*n* = 22 0.44 (0.19)	*n* = 22 0.26 (0.14)	*n* = 21 0.26 (0.15)	Baseline–45 days 41*	Baseline–90 days 41*	45 days–90 days ____

Table 11. Mean scores of Severity Plaque Index (DP) and percent between periods (Pereira et al., 2011) [29].∗ Friedman test (ANOVA) *P* <.05.

In this study, the MGP 5% showed evidence of its efficacy in reducing PI and GI. However, it is necessary to perform a clinical trial, double-blind, randomized to validate such effectiveness [29]. Regression of 95% gingivitis and suppuration in all the teeth irrigated with Brazilian Green Propolis gel (BGPg), as well as a pocket depths and all treated patients with the BGPg showed periodontitis/gingivitis regression. This result suggest that 10% BGPG used could be used as an adjuvant therapeutic method assigned for the treatment of periodontal disease (Figure 2) [142]. Ethanol Propolis Extract (EPE) inhibited all the *Candida albicans* strains collected from HIV-seropositive and HIV-seronegative Brazilian patients with oral candidiasis. No significant difference was observed between Nystatin and EPE. But significant differences were observed between EPE and other antifungals. *C. albicans* showed resistance to antifungal agents. This fact suggests commercial EPE could be an alternative medicine for candidosis treatment from HIV-positive patients (Figure 3) [143]. Brazilian commercial ethanol propolis extract, also formulated to ensure physical and chemical stability, was found to inhibit oral candidiasis in 12 denture-bearing patients with prosthesis stomatitis candidiasis association is show in Table 12 and Figure 4 [144]. Also, denture stomatitis presents as a chronic disease in denture-bearing patients, especially under maxillary prosthesis. Despite the existence of a great number of antifungal agents, treatment failure is observed frequently. So, the clinical efficacy of a Brazilian propolis gel formulation in patients diagnosed with denture stomatitis was evaluated. Thirty complete-denture wearers with denture stomatitis were enrolled in this pilot study. At baseline, clinical evaluation was performed by a single clinician and instructions for denture hygiene provided. Fifteen patients received Daktarin® (Miconazole gel) and 15 received Brazilian propolis gel. All patients were recommended to apply the product four times a day during one week. Clinical evaluation was repeated by the same clinician after treatment. All patients treated with Brazilian propolis gel and Daktarin® had complete clinical remission of palatal candidiasis edema and erythema. [77]. Noronha [31] found the efficacy of a Brazilian green propolis mucoadhesive gel (BPGg) in preventing and treating the oral mucositis and candidiasis in patients harboring malignant tumors and receiving radiotherapy. All patients who used the gel applied 24 hours before the first radiotherapy session, three times

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a day, during the whole period(six weeks) of radiotherapy, did not develop mucositis and candidosis over the entire period of radiotherapy.

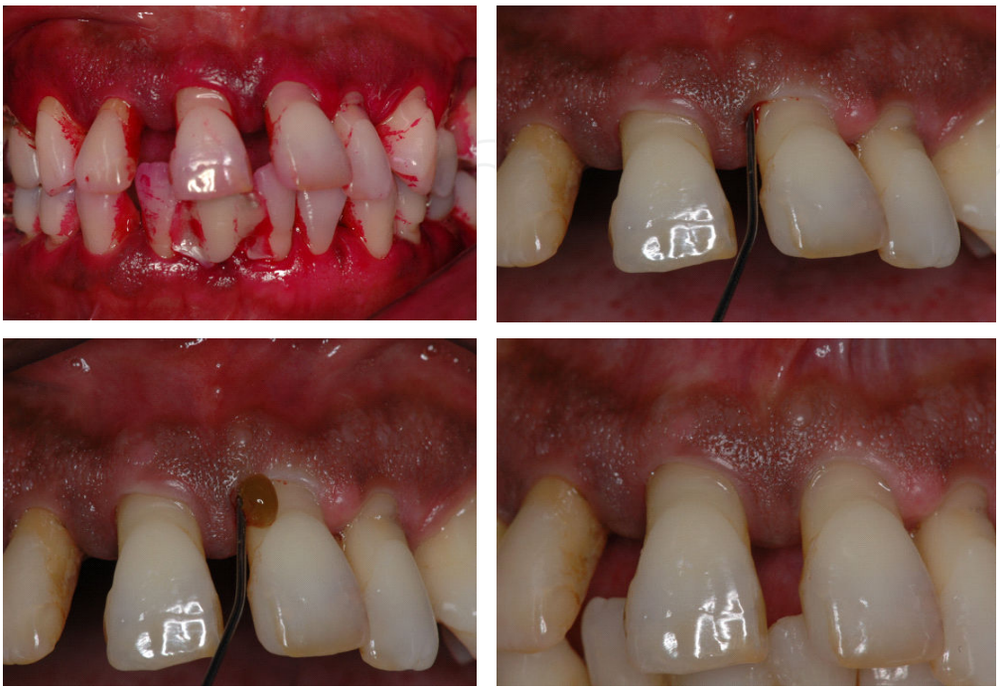

Figure 2. Periodontitis treatment with mucoadhesive green propolis gel. **A**) Evidencing of dental plaque with basic fuchsin. **B**) Confirmation of insertion loss and presence of periodontal pockets with periodontal probe. **C**) Applying mucoadhesive green propolis gel intra-periodontal pocket. **D**) Clinical aspect of the periodontium after treatment with gel containing propolis (Cairo do Amaral et al. [142].

The prevalence of candidosis in denture wearers is as well established as its treatment with antifungal agents (AAs). However, little research has been done regarding the effects of AAs on denture base surfaces. Then, da Silva et al.[150] evaluate the effects of fluconazole (FLU), nystatin (NYS) and propolis orabase gel (PRO) on poly (methyl-methacrylate) (PMMA) surfaces. So, PRO was able to induce changes in PMMA surface properties, such as roughness, which could be related to microbial adhesion [146]. Recurrent aphthous stomatitis (RAS) is a common, painful, and ulcerative disorder of the oral cavity of unknown etiology. No cure exists and medications aim to reduce pain associated with ulcers through topical applications or reduce outbreak frequency with systemic medications, many having serious side effects. Propolis is a bee product used in some cultures as treatment for mouth ulcers. A randomized, double-blind, placebo-controlled study, patients were assigned to take 500 mg of propolis or a placebo capsule daily. Subjects reported a baseline ulcer frequency and were contacted biweekly to record recurrences. Data were analyzed to determine if subjects had a decrease of 50% in outbreak frequency. The data indicated a statistically significant reduction of outbreaks in the propolis group (Fisher's exact test, one sided, $p = 0.04$). Patients in the propolis group also self-reported a significant improvement in their quality of life ($p = 0.03$). This study has shown propolis to be effective in decreasing the number of recurrences and improve the quality of life in patients who suffer from RAS [145].

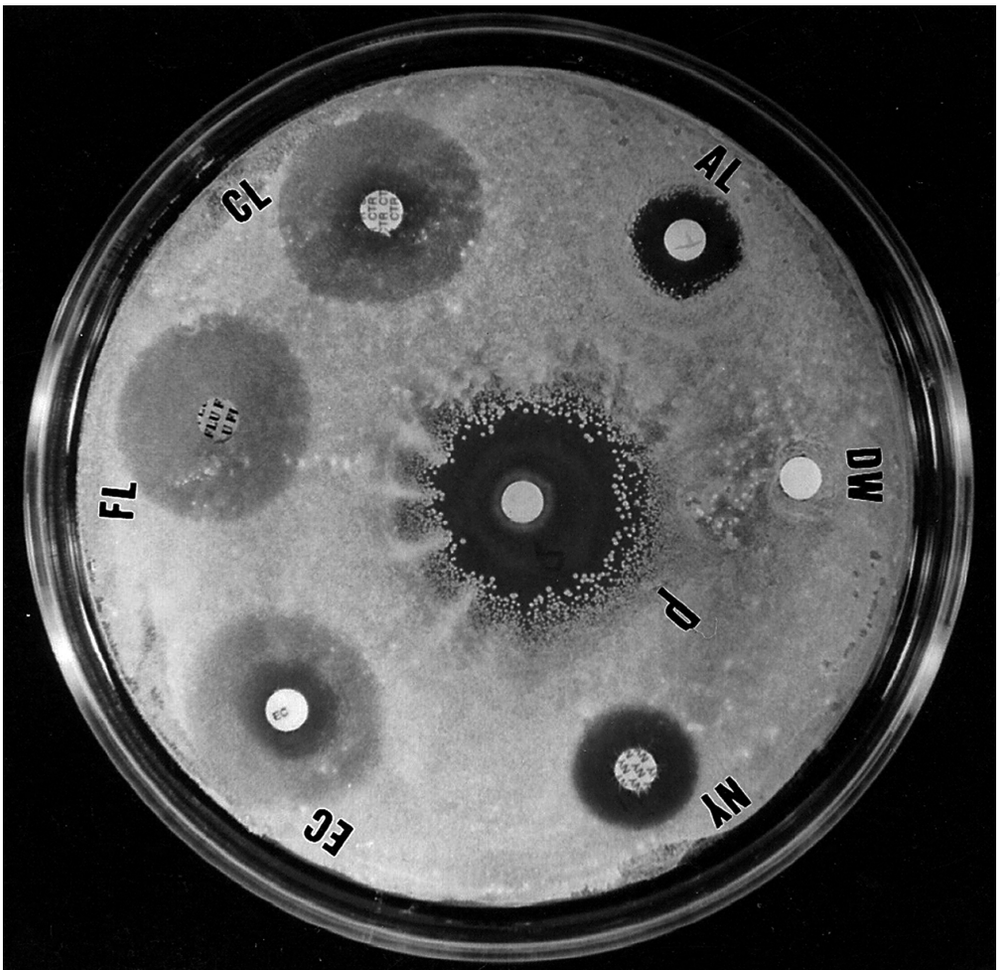

Figure 3. Inhibition zones of in vitro culture of *Candida albicans* collected from HIV-positive patients exposed to Ethanol Propolis Extract (EPE= P), and antifungal agents: CL= clotrimazole; FL= fluconazole; EC= Econazole; NY =Nystatin; AL= Alcohol; DW= Destiled water. (Martins et al., 2002) [143].

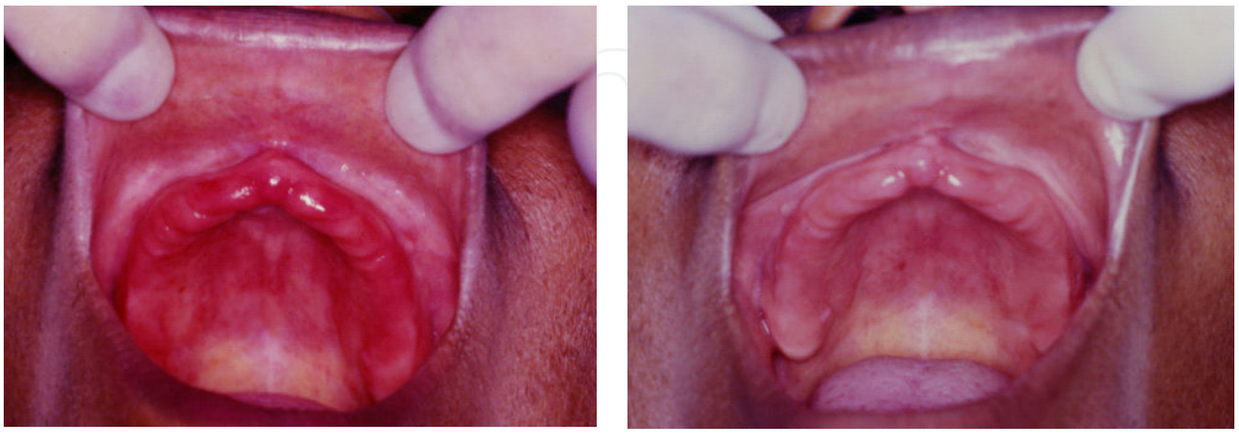

Figure 4. Clinical aspects of oral candidosis in patients with Total Removable Dental Prothesis (TRDP). **A**) Before propolis use. **B**) After propolis use. Source: Prof. Vagner Santos archives (2005) [146].

Patient	Age (years)	Race	Gender	Prosthesis	Local lesions	Antifungal agent	Result
ISS Hard	29	B	F	TRDP	palate/soft palate	Nys	+
SVCL	34	W	F	TRDP	Hard palate	Nys	+
AFF	36	W	M	TRDP	Hard palate	Nys	+
GMR	37	W	M	TRDP	Hard / soft palate	Nys	++
MIC	39	B	F	TRDP	Hard palate	NYS	+
AFS	71	B	F	TRDP	Hard palate	Nys	++
EGSM	29	W	F	TRDP	Hard /soft palate	EPE	+
TMS	31	B	F	TRDP	Hard palate	EPE	++
LMC	33	W	M	TRDP	Hard palate	EPE	+
HL	38	W	M	TRDP/PRDP	Hard palate/ alveolar mucosa	EPE	+
SFS	39	W	F	TRDP	Hard /soft palate	EPE	++
MCTS	43	W	M	TRDP/PRDP	Hard palate/ alveolar mucosa	EPE	+
MJNM	46	W	F	TRDP	Hard palate	EPE	++
	46	B	F	TRDP	Hard palate	EPE	+
HBS	48	B	M	TRDP	Hard palate	EPE	+
JJAF	50	W	F	TRDP	Hard palate	EPE	+
GRA	56	W	F	TRDP	Hard palate	EPE	++
NMBA	63	W	F	TRDP	Hard palate	EPE	++

Table 12. Clinical aspects of patients with oral candidiasis from Clinic of Semiology and Pathology of Dentistry School UFMG participating in this study and Results of *in vivo* patients treatment of oral candidiasis with 20% Brazilian green ethanol propolis extract (EPE) and Nystatin (Nys). Use posology: 4 time/day for 7 days, topic application in local lesion and prosthesis surface F, female; M, male; TRDP, total removable dental prosthesis; PRDP, partial removable dental prosthesis; B, black; W, white. (Santos et al., 2005) [146]

9. Future perspectives

The potential pharmacological activity investigation of natural products, especially antimicrobial activity, has attracted the attention of several researchers. Increase of bacterial resistance to traditional antimicrobial agents and side effects are often seen [147, 28]. Many

mouthwashes with alcohol are used as adjuvants in the control of dental plaque and gingivitis, but undesirable side effects are observed, despite its efficacy. This stimulates the research of alternative products, such as the use of toothpastes and mouthwashes based on natural products, because there is the need for prevention and treatment options that are safe, effective and economical. Mouthwash based on herbal extracts and propolis are for sale in the Brazilian and world market, without, however, have undergone clinical studies proving their effectiveness and documenting possible undesirable side effects. Previous studies have demonstrated the efficacy of propolis extracts as an antimicrobial agent useful for dental caries and periodontal pathogens microorganisms in *in vitro* studies [24,78,59,148,149]. Propolis standardization is necessary and several authors from different countries are involved in the study of pharmacological activity and mechanism of action of various types of propolis. The separation of organic compounds and their mechanism of action on cells may lead to new products that can be important in controlling tumor growth, and infection control. However one should not forget that the effect of synergism observed in raw propolis is responsible for its excellent antimicrobial activity making it a unique product against bacterial and fungal resistance. Moreover, pre-clinical and clinical phase I, II, III studies are necesssary in order to better determine the effect on patients and safety. Several components of propolis have shown efficacy in the growth inhibition of *in vitro* tumor cells and *in vivo* tumors. This may be the way to the discovery of drugs against cancer, however, the clinical confirmations should be prioritized. The diversity of pharmacological properties of propolis may also be extended to studies against autoimmune diseases in order to ameliorate the clinical evolution. Also, studies against systemic diseases that affect largely population world as is the case of diabetes and hypertension. But for that attention should turn to as separation of compounds that can be a great gain for treatment of these diseases.

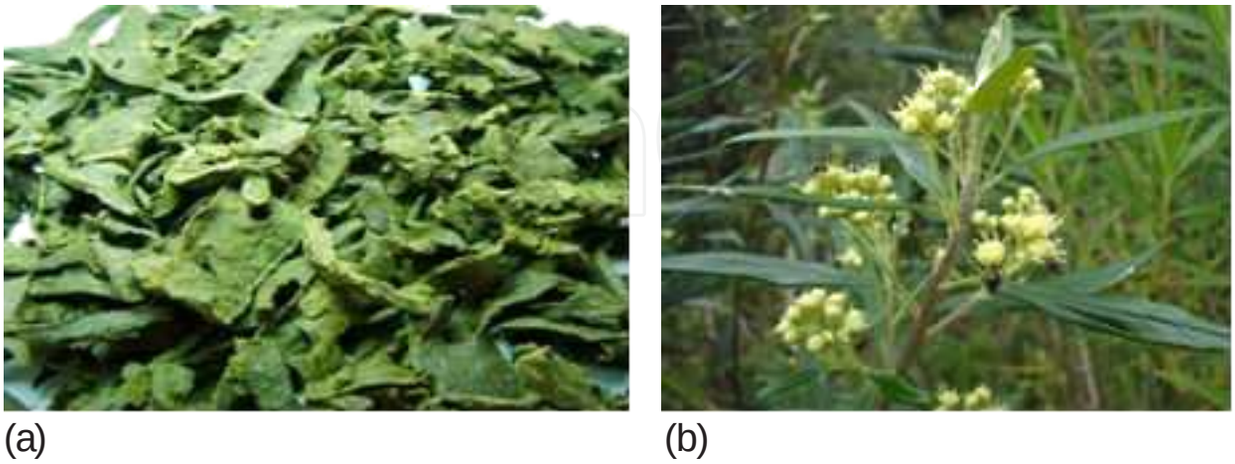

Figure 5. (a) Physical aspect of Brazilian green crude propolis. (b) Plant caracteristic of *Baccharis dracunculifolia*. (Prof. Vagner Santos archives, 2012).

Propolis component	Pharmacological properties	Author/year
Green Propolis extract	Apoptosis and cell propliferation	Giertsen et al, 2011 [153]
Moronic acid	Epstein-Barr virus suppresion	Chang et al., 2010 [154]
Polyphenols	Neurological diseases	Farooqui and Farooqui, 2012 [15]
Red propolis extract	Adipocyte differentiation	Iio et al., 2010 [155]
Caffeic acid phenethyl ester Cardanol, cardol	Antitumoral / anticancer, citotoxicity	Chuu et al., 2012 [156] Sawaya et al., 2011 [39] Chan et al., 2012 [152] Watanabe et al., 2011 [159] Teerasripreecha et al, 2012 [68]
epicatechin, *p*-coumaric acid, morin, 3,4-dimethoxycinnamic acid, naringenin, ferulic acid, cinnamic acid, pinocembrin, and chrysin , 3-prenyl-4-hydroxycinnamic acid	Antioxidant	Guimaraes et al., 2012 [95] Guo et al., 2011 [87] Sawaya et al, 2011 [39]
3-prenyl-4-hydroxycunnamic acid, 2,2-dimethyl-6-carboxyethenyl,2H-1-benzopyran; 3,5-diprenyl-4-hydroxycinnamic acid derivative 4 (DHCA4) 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran (DCBEN	Antiparasitic *Trypanosoma cruzi; Leishmania amazonensis*	Sawaya et al., 2011 [39] Salomao et al., 2008 [160] Salaomao et al., 2011 [161]
Green, red and brown propolis extracts; Artepillin C; Crysin	Anti-inflamatory	Marcucci et al., 2000 [11] Ha et al., 2010 [158] Sawaya et al., 2011 [39] Moura et al., 2011 [57] Orsatti et al., 2012 [128]
Green, Red, Brown propolis extract; p-coumaric acid (PCUM), 3-(4-hydroxy-3-(oxo-butenyl)-phenylacrylic acid (DHCA1); Caffeic acid, caffeoylquinic acid, diterpenic acids, flavonoids	antimicrobial	Martins et al., 2002 [143] Paula et al., 2006 [59]; Santos et al., 2007 [71] Dias et al., 2012 [162]; Mattigatti et al., 2012 [163] Sawaya et al., 2011 [39] Choudhari et al., 2012 [157]

Table 13. Recent advances in propolis components studies.

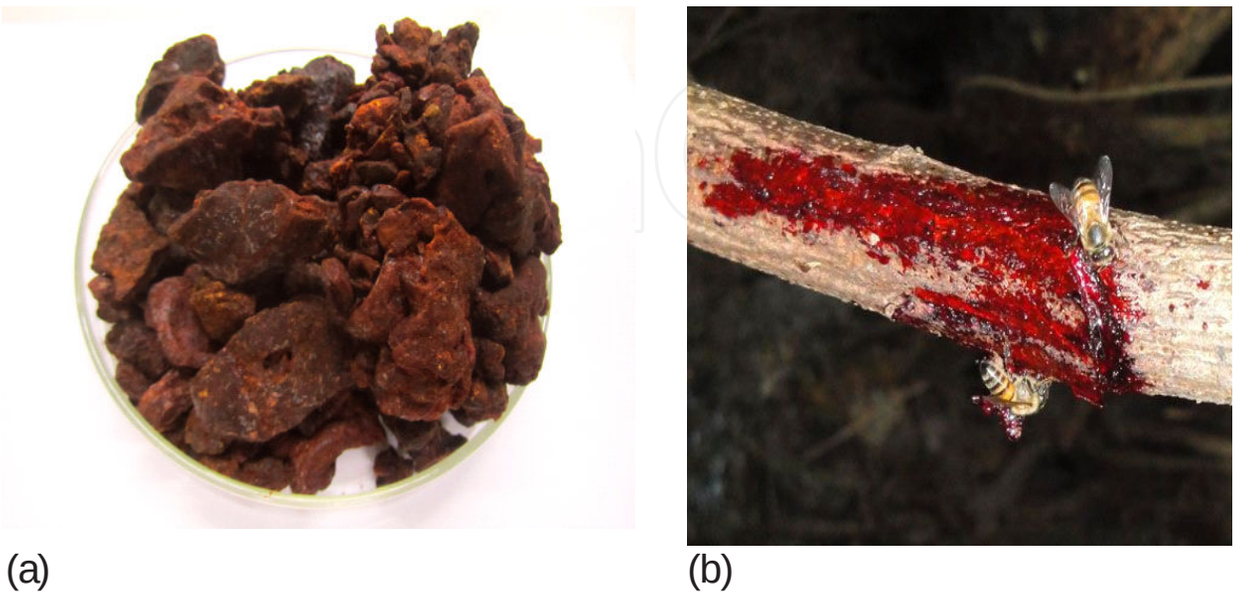

Figure 6. (a) Physical aspect of Brazilian red propolis. (Prof. Vagner Santos archives, 2012) (b) Dalbergia ecastophylum plant aspect. http://www.google.com.br/imgres?q=Dalbergia+ecastophyllum&num=10&hl=ptBR&biw=1280&bih=673&tbm= isch&tbnid=WIUAFEd2jCOSxM:&imgrefurl=http://meliponariojandaira.blogspot.com/2011/02/abelhas-indigenas-sem-ferrao.

Acknowledgements

Research Foundation of Minas Gerais State (FAPEMIG), National Council of Scientific and Tecnologic Development (CNPq) for financial support in all of our research group and also for supporting the publication of this chapter. Special thanks to Gustavo Araujo and Rafael Tomaz.

Author details

Vagner Rodrigues Santos

Address all correspondence to: vegneer2003@yahoo.com.br

Universidade Federal de Minas Gerais, Faculty of Dentistry, Department of Clinical, Pathology and Surgery, Laboratory of Microbiology and Biomaterials, Brazil

http://dx.doi.org/10.5772/54003

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