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Chapter 4

A REVIEW OF THE PLANT ORIGINS, COMPOSITION AND BIOLOGICAL ACTIVITY OF RED PROPOLIS

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ABSTRACT

Propolis is a mixture of various amounts of beeswax and resins collected by bees from plants, particularly from buds and resinous exudates. The composition of propolis varies according to the kind of bee, geographic and plant origin of the samples. Propolis is important for the hive defense and has been used for its medicinal properties since ancient times. Red propolis has been found in the northeastern coast of Brazil, as well as in Cuba, Venezuela, Mexico and China. Among the most frequently cited plant sources are species of the Leguminosae and Clusiaceace families. The composition of red propolis varies both qualitatively and quantitatively, confirming different plant sources. The compounds that have been found in red propolis samples are listed herein. Red propolis has shown diverse biological activities: antimicrobial activity against different bacteria and yeast; against *Leishmania amazonensis* parasites; antioxidant, cytotoxic and potential antitumor activity; antipsoriatic, anti-inflammatory and analgesic activities; anti-obesity and hepatoprotective effects. Many new studies of red propolis are being developed due to the promising therapeutic activity; however, future studies must differentiate between red propolis from diverse geographic origins and chemical profiles.

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INTRODUCTION

The word propolis has a Greek origin: meaning *pro*, in defense of, and *polis*, city; implying a product involved in the defense of the bee community. Propolis is used to strengthen the hive walls and coats the internal walls. It is also used to cover holes and cracks and to repair combs [1]. To prevent ants from invading the hives, bees place a ring of propolis on the entrance to make it narrow and difficult to enter. However, if an insect or small animal comes into the hive, the bees attack and kill it. If the body is too big to throw out of the hive, it is left inside and covered with propolis to prevent putrefaction [2]. As there are many bacterial and fungal species likely to grow in the hive, a layer of propolis reduces the microbial growth on the walls of the hive, also preventing wind and water entry [3].

Bees harvest propolis with their jaws and then manipulate it with the front legs and transfer it to the back legs. The salivary secretion serves as lubricant for the sticky glue texture of propolis. Returning to the hive, other bees separate each particle of the collected resin, mix with wax and the resulting propolis is pasted where it is needed inside the hive [4]. Propolis generally contains 30% beeswax, 5% pollen, 50-55% resinous substances and 15%essential oils [5].

Several species of bees produce propolis, although some have been better studied than others [6]. Different species of *Apis* live in diverse areas depending on the climatic features of each zone. *Apis mellifera* is an aggressive honey bee implicated in propolis collection [7]. This bee has a defensive behavior against intruders in their hives [8] and uses propolis as a defense mechanism. There are studies describing the different specialization levels of the bees to forage and the interaction between them [9]. Honeybee behavior has also been studied to understand better the processes involved in propolis and/or honey production [10]. In order to increase propolis production, Manrique and Egea (2002) studied why some *Apis mellifera* colonies produce more propolis than others, based on the genetic expression, showing it is possible to make a selection of bees to reach this objective [11].

The influence of the seasons on propolis production has been researched mostly in Brazil, as the mild weather permits resin collection throughout the year [12]. Environmental variables such as air temperature, humidity, precipitation, hours of solar radiation and wind speed were studied. Several different plants are the botanical source of propolis throughout the world. Since there are different plant sources for propolis depending on the geographical area of production, there are also different types of propolis. In tropical regions the botanical origin of some types of propolis is still being researched. The knowledge of plant sources of propolis is necessary to permit the chemical standardization of propolis (knowing the composition of the plant exudates, it is possible to know the quantitative composition of the propolis sample) [1].

Propolis has been used for its medicinal properties since ancient times and was used by the Egyptians to embalm cadavers because of its anti-putrefactive effect. The medicinal properties were recognized by Greek and Roman physicians like Aristoteles, Dioscorides, Phynil and Galen, and used by them as antiseptic and mouth disinfectant as well as for healing wounds. These applications of propolis were perpetuated until Middle Ages and spread to Arab physicians too. Incas used propolis as antipyretic and it was first included in the London pharmacopeia as an official drug in the seventh century. Since then, propolis has become very popular in Europe for its antibacterial activity [13]. Scientific proof of the therapeutic properties of propolis began to be studied in the 1950's, although the focus of these first studies was about bees' pollen collection [14].

Modern herbalists recommend propolis for its anti-bacterial, anti-fungal, anti-viral, hepatoprotective and anti-inflammatory properties, to increase the body's natural resistance to infections and to treat gastro-duodenal ulcers. Applied externally, propolis relieves various types of dermatitis caused by bacteria and fungi. Nowadays, propolis is a popular remedy available in the form of capsules, as an extract, as a mouthwash, in throat lozenges, creams and in powder form as well as part of cosmetic formulations and health food items [13].

GEOGRAPHIC AND PLANT ORIGINS OF RED PROPOLIS

The physical appearance of propolis varies widely, depending on its botanical and geographical origin and on the type of bee involved in the collecting process. Propolis color may be cream, yellow, green, light, dark brown or red [15, 6]. The chemical composition of propolis is variable depending on the biodiversity and the geographical origin of this natural substance [17, 18].

There are various geographical areas where red propolis is found: the northeastern coast of Brazil, including the states of Alagoas, Bahia, Paraiba, Sergipe and Pernambuco, [19]; Cuba (Pinar del Rio), [20]; Venezuela [21]; México (Champoton) [22]; and China [23].

Studies regarding the plant sources of red propolis are very recent, dating back to 2007 and only for red propolis from Northeastern states of Brazil and from Cuba. Determining the botanical origin of propolis is a tricky task and two approaches have been applied: the comparative chemical composition analysis and the palynological analysis.

The chemical composition of red propolis samples was compared to samples of plant exudates. This approach demands detailed bee observation to be able to know which plants are visited by bees for resin collection. This observation is very important in order to determine and collect samples from possible plant sources and compare them to samples of propolis from a beehive located in the same area [17, 19].

For example, Daugsch et al. (2008) observed that bees were collecting the red exudates on surfaces of *Dalbergia ecastophyllum* to produce propolis. By comparison of propolis samples with *D. ecastophyllum,* the authors concluded that this was the botanical origin of the Brazilian red propolis. Brazilian red propolis contains isoflavonoids, and these compounds are present only in few species in the vegetal kingdom, almost exclusively in the Leguminosae family. *D. ecastophyllum* belongs to this family and contains the most important isoflavonoids that were also present in the red propolis samples tested [19]. This confirmed that this plant species is a source of resin for the production of Brazilian red propolis [17].

However, there are some compounds present in the red propolis samples that are absent in the *D. ecastophyllum* sample, suggesting that there are probably other botanical sources. It was observed that in the areas where certain propolis samples were collected, *D. ecastophyllum* was scarce, so bees also collected the exudates from other plants [17]. Piccinelli et al. (2011) detected some polyisoprenylated benzophenones in a Brazilian red propolis sample. These compounds are not present in *D. ecastophyllum*, and this result supports the theory of the contribution of other plant sources, probably from the Clusiaceace family [20].

The same study also analyzed Cuban red propolis, whose composition is similar to the Brazilian red propolis, so the botanical origin might be the same. This theory was confirmed by comparing the composition of the Cuban propolis sample to a sample of *D. ecastophyllum* [20].

Palynological analysis is another interesting approach to to identify the plant origins of red propolis. As with honey samples, the type of pollen found in propolis could be indicative of the plant species responsible for those resins. Barth and Luz (2009), carried out a study of propolis samples from the northeastern states of Brazil by the palynological method; all the data available related to this subject belongs to this single study. After analysis of the samples, 72 types of pollen were recognized and classified by family. According to this study, the red propolis samples showed great similarity with respect to the occurrence of the *Borrelia, Cocos* and *Schinus* pollen. The authors considered the presence of *Schinus* pollen to be characteristic of red propolis. In all sample localities included in this study, *Cocos nucifera* was growing along the coastal areas, which made it one of the possible plant sources. Red propolis sediments frequently contained pollen grains of Mimosa (pollen types of *M. scabrella, M. verrucosa* and *M. caesalpiniaefolia*), Arecaceae (*Cocos nucifera* and others), Cecropia (Cecropiaceae), three pollen types of Borreria (*B. densiflora*, *B. latifolia* and *B. verticillata*, Rubiaceae), *Symphonia globulifera* (Clusiaceae), Myrcia (Myrtaceae), Solanaceae, Tapirira and *Schinus terebinthifolius* (Anacardiaceae) [16].

The variety of plant species found indicates open land vegetation with trees and plants of hydrophilic preference. Samples from different states were found to have common pollen types, noting that some plant species are present in various areas while other are native of specific areas depending on the environmental characteristics.

In this study, in contrast with the research carried out by comparing red propolis samples and resin composition, *Dalbergia ecastophyllum* pollen was not detected, confirming that this is not the exclusive source of red propolis [16]. Furthermore, pollen of Clusiaceae family was found which correlates with the polyisoprenylated benzophenones found by Piccinelli et al. (2011) [20].

COMPOSITION OF RED PROPOLIS

Bees collect plant resins and exudates to produce propolis from different plant species, many of which are still unknown, hence the composition of propolis differs according to its botanical origin. Red propolis samples show differences in the chemical composition determined by the vegetation around the beehive. This raises a few questions that should be taken into account. Are the samples of red propolis from different geographic regions similar? How many plant sources are shared, considering there are samples from quite different geographic origins? Why are all these samples red?

At this point there are no easy answers to these questions. Some compounds have been identified to be part of propolis from some specific areas, while they are not present in red propolis from other regions. The composition not only varies qualitatively but also quantitatively. Some studies refer to a high flavonoid content, which has a direct relation with the plant source (when the resins come from *D. ecastophyllum* from the Leguminosae family), while others have higher percentage of phenolic compounds probably indicating they come from a plant belonging to the Clusiaceae family. The compounds that have been found in red propolis samples are listed in Table1.

Table 1. List of compounds identified in red propolis, divided by chemical classes

Class of compounds	Name	Reference
PHENYLPROPENE DERIVATES	trans-anethol	25
	methyl eugenol	
	trans-methyl isoeugenol	
	elemicin	
	trans-isoelemicin or asarone	
	methoxyeugenol	
	1-(30,40-dihydroxy-20-methoxyphenyl)-3-(phenyl)propane	22
	(Z)-1-(20-methoxy-40,50-dihydroxyphenyl)-2-(3-phenyl)propene	
	estragol	24
	metil-cis isoeugenol	
	1-methoxy-4-(1-propenyl)-benzene	29
	1,2,3-Trimethoxy-5-(2-propenyl)-benzene	
	scrobiculatones A and B (inseparable mixture)	21
	guttiferone E and xantochymol (inseparable mixture)	25
PHENOLIC COMPOUNDS	2,4,6-trimethylphenol	29
	methyl o-orsellinate	
	methylguaiacol	26
	homovanillic acid	
	phenolic acid, ferulic acid	
	caffeic acid-4-O-hexoside	
LIGNANS	pinoresinol dimethyl ether	28
	pinoresinol	
	syringaresinol	
TERPENS	anisylacetone	26
	methyl abietate	29
TRITERPENIC ALCOHOLS	α-amyrin	25
	β-amyrin	
	cycloartenol	
	lupeol	
	the ketone 20(29)-lupen-3-one	
	2,3-epoxy-2-(3-methyl-2-butenyl)-1,4-naphthalenedione	
MONOTERPENIC HYDROCARBONS	p-cymene	24
	limonene	
OXYGENED MONOTERPENS	1,8-cineol (eucaliptol)	24
	linalool	
SESQUITERPENIC HYDROCARBONS	α-cubebene	24
	α-copaene	
	β-gurjunene	
	β-caryophylene	
	α-bergamotene	

Table 1. (Continued)

Class of compounds	Name	Reference
	farnesene	
	δ-germacreno	
	α-selinene	
	isocariophylene	
	β-bisabolene	
	δ-cadinene	
OXYGENED SESQUITERPENS	δ-cadinol	24
FLAVONOIDS	isosativan	25
	medicarpin	
	prenylated benzophenones	
	3,4,2,3-tetrahydroxychalcone	
	volkensiflavone	
	5,7,3,4-tetramethoxyflavone	
	homopterocarpin	29
	4,7-dimethoxy-2-isoflavonol	
	chrysin	17
	pinocembrin	22
	arizonicanol A	
	melilotocarpan A	
	melilotocarpan D	
	3-hydroxy-5,6-dimethoxyflavan	
	quercetin	19
	rutin	
	Luteolin	
	formononetin	
	retupsapurpurin B	20
	retupsapurpurin A	
	(6αS,11αS)-6α-ethoxymedicarpan	28
	(6αS,11αS)-medicarpan	
	(6αS,11αS)-3,10-dihydroxy-9-methoxypterocarpan	
	(6αR,11αR)-4-methoxymedicarpin	
	(6αR,11αR)-3,8-dihydroxy-9-methoxypterocarpan	
	3-Hydroxy-8,9-dimethoxypterocarpan	17
	(6aR,11aR)-3,4-Dihydroxy-9-methoxypterocarpan	26
	vesticarpan	20
FLAVONOIDS	alnustinol	26
	(2R,3R)-3,7-dihydroxy-6-methoxyflavanone	28
	(2R,3R)-3,7-dihydroxyflavanone	
	garbanzol	
	(7S)-dalbergiphenol	
	(3R)-4´-methoxy-2´,3,7-trihydroxyisoflavanone	
	(3S)-ferreirin	26
	(3S)-violanone	
	(3S)-vestitone	28
	isoliquiritigenin	19
	2-hydroxy-4-methoxychalcone	26

Class of compounds	Name	Reference
	2´,4´-dihydroxychalcone	28
	4,4´-dihydroxy-2´-methoxychalcone	
	(αS)-α,2´,4,4´-tetrahydroxydihydrochalcone	
	mucronulatol	22
	(3S)-7-O-methylvestitol	26
	(3S)-mucronulatol	
	(3S) - vestitol	
	(3S)-isovestitol	28
	neovestitol	20
	2´-hydroxybiochanin A	28
	pratensein	
	xenognosin B	
	daidzein	19
	dalbergin	
	biochanin A	
	7,4-dihydroxyisoflavone	29
	gliricidin	26
	calycosin	
	(2S)-dihydrooroxylin A	28
	(2S)-dihydrobaicalein	
	(2S)-naringenin	
	(2S)-7-Hydroxy-6-methoxyflavanone	26
	naringenin-C-hexoside	
	Flavanone: 7-hydroxyflavanone	22
	pinobaskin	19
	pinobaskin - 3-acetate	
	liquiritigenin	
	alnusin	28
BENZOFURANS AND BENZOPYRANS	2-(2,4-dihydroxyphenyl)-3-methyl-6-methoxybenzofuran	26
	2-(20,40-dihydroxyphenyl)- 3-methyl-6-methoxybenzofuran	28
	2,6-dihydroxy-2-[(4-hydroxyphenyl)methyl]-3-benzofuranone	
	2H-1-Benzopyran-7-ol	29
ALKANES, ALCOHOLS, KETONES, ALDEHYDES AND ALIFATIC HYDROCARBONS	n-tricosane	26
	n-pentacosane	
	n-heptacosane	
	n-nonacosane	
	n-hentriacontane	
	n-tritiacontane	
	4-hydroxy-4-methyl-heptan-2-one	24
	6-methyl-5-hepten-2-one	
	octanal	
	nonanal	
	n-decanal	

Table 1. (Continued)

Class of compounds	Name	Referenc e
	anisaldehyde	
	n-dodecanal	
	farnesol	26
	butanedioic acid, dimethyl ester	29
	hydroxy-butanedioic acid, dimethyl ester	
	Hexadecanoic acid, methyl ester	
	10-Octadecenoic acid, methyl ester	
	tetradecanoe	24
	pentadecane	
	hexadecane	
AROMATIC HYDROCARBONS	naphthalene	24
	resorcinol	26
	benzoic acid	29
	2,2,6-Beta-trimethyl-bicyclo(4.3.0)non-9(1)-en-7α-ol	

The seasonal variability of red propolis compounds and bioactivity was studied using *Artemia salina* [24]. In this study, propolis samples were collected in the state of Pernambuco, in Brazil, at different times of the year: February, June and October. The authors observed there was a small variation in the volatile compound profile. Some sesquiterpens where only present in the sample collected in October, and some n-alkanes, a sesquiterpen and a monoterpen were only present in the sample collected in June, proving in this way that a seasonal variation in propolis composition exists. However, the main compound present in all the samples was the same (trans-anethol) and also high percentages of α-copaene and methyl-cis-isoeugenol were present.

In the study carried out by Trusheva et al. (2006), [25] the most abundant compound found was elemicin. Together with Methyl eugenol, Methyl isoeugenol and Isoelemicin, it was deemed responsible for the unusual anis-like odor of the samples they collected. β-amyrin was the main triterpenic alcohol. There was an inseparable mixture of Guttiferone E and Xanthochymol found in this sample, which coincides with the same compounds found in species of *Clusia*. This fact suggests that *Clusia* might be the botanical origin of some resins in this sample that was collected in Maceio, Alagoas State, Brazil. Righi et al., (2011) used a sample from Maceio, Alagoas State, Brazil as well. [26] However, the main compounds found in the sample were a chalcone and the isoflavans (3*S*)-7-*O*methylvestitol and (3*S*)-vestitol and the pterocarpan Medicarpin. They found that the sample was rich in phenolic compounds, corroborating previous studies carried out by Cabral et. al. in 2009 [27]. These compounds vary widely among different red propolis samples. The difference between the results obtained in both studies show that even samples collected in the same area have a different qualitative composition, which might be due to the season in which the samples were collected or the flora directly around the hives.

Daugsch et al. (2008), compared samples from the states of Bahia, Sergipe, Alagoas, Pernambuco, and Paraiba in northeastern Brazil, and the main compounds in all the samples were Isoliquitigenin, Formonetin and Pinocembrin.

The study carried out by Awale et al. (2008) analyzed samples from the South Coast of Paraiba in Brazil [28]. These authors isolated up to 41 compounds from the samples of red propolis, which presented a wide structural diversity among the flavonoid compounds. This study together with the study on red Mexican propolis [22] confirms that plants from the *Dalbergia* genus have many of the same main compounds.

Alencar et al. (2007), studied samples of red propolis collected in a mangrove area in the State of Alagoas, Brazil [29]. Isoflavones such as Homopterocarpin, Medicarpin and 4,7-dimethoxy-2-isoflavonol were the most abundant compounds.

The main compounds in the samples from Marechal Deodoro, Alagoas State, Brazil, were studied by Silva et al. (2008) [17]. The isoflavonoids Medicarpin and 3-Hydroxy-8,9-dimethoxypterocarpan represented more than 60% of the propolis extracts studied. High levels of phenolic compounds were found by Cabral et al. (2009) [27] that also analyzed red propolis from Marechal Deodoro, Alagoas in Brazil. Samples from the same area have also been studied by Oldoni et al. (2011) that reported Vestitol, Neovestitol and Isoliquiritigenin as the bioactive compounds of red propolis. [30]

Regarding Cuban red propolis composition, Cuesta-Rubio et al. (2007), studied samples from different parts of Cuba provided by La Estacion Experimental Apicola [31]. The main compounds turned out to be isoflavonoids. Seven Cuban propolis samples from Pinar del Rio, Villa Clara and Matanzas, showed some quantitative differences but similar qualitative composition. Vestitol, Formononetin and another unidentified isoflavan were always present, although their concentration varied. Once again, quantitative variations between samples collected in areas near to each other suggest that the flora, climate, age and soil can influence the composition of propolis. Isoflavans were also confirmed to be the main components in Cuban red propolis [32]. Another study on Cuban red propolis collected in Pinar del Rio, reported the presence of the isoflavonoids: Medicarpin, Vestitol, Liquiritigenin, Isoliquiritigenin, Formonetin and Biochanin A, as principal components [20]. These results obtained by Piccinelly et al. (2011) agree with other studies on red propolis from Brazil; however they evidenced the presence of polyisoprenylated benzophenones not found in samples from other geographical origins.

The composition of red Mexican Propolis from Champoton, was studied by Lotti et al. (2007) and isoflavonoids were found to be the main compounds, much like red Cuban propolis [22]. The high concentration of Pinocembrin in the sample was noteworthy as it is also found in *D. ecastophyllum* as well as Mucronulatol and Vestitol. Melilotocarpan A and D are pterocarpans that were also found in the red Mexican propolis sample, and these compounds were isolated from another species of *Dalbergia*. These data reveal that the source(s) of red Mexican propolis are from the *Dalbergia* genus.

Venezuelan propolis collected in a tropical rain forest in Trujillo State was studied by Trusheva et al. (2004) and an inseparable bioactive mixture was identified as Scrobiculatones A and B (polyisoprenylated benzophenones [21]. These compounds were also isolated in red Cuban propolis [31].

BIOLOGICAL ACTIVITY OF RED PROPOLIS

Red propolis has been shown to have diverse biological activities. Since differences in propolis composition exist, depending on its geographical origin and plant source, it is important to keep in mind that these activities may not be true for all samples of red propolis. Therefore, in the future, the origin of a sample should always be stated in studies of composition and activity.

The antimicrobial activity has been studied against different bacteria; *Staphylococcus aureus* [27], *Streptococcus mutans*, and *Actinomyces naeslundii* [30]. Cabral et al. (2009) suggested that the antibacterial activity against *S. aureus* was due to a synergic effect between phenolic compounds and other compounds present in the crude propolis extract [27]. However they didn't assign this activity to a specific compound. Alencar et al. (2007) also associated the antimicrobial activity mainly to the polyphenolic compounds [29].

The antimicrobial effect against *S. aureus* was also verified by Oldoni et al. (2011), who isolated and identified some of the compounds responsible for the antimicrobial activity. Vestitol and Isoliquiritigenin were the two compounds that showed strongest activity against the three species of bacteria they tested, being that Isoliquiritigenin had stronger activity [30]. They realized as well that no isolated compound showed antibacterial effect against A. *naeslundii.* Testing the possible synergetic effect of flavonoids against bacteria, they showed that Vestitol and Isoliquiritigenin were not able to potencialize each other's antibacterial activity [30].

Righi et al. (2011), confirmed the antimicrobial activity of red propolis against Gram-positive bacteria (*Bacillus subtilis*, *Enterococcus faecalis* and *Streptococcus pyogenes*) and Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium* and *Escherichia coli*), but the individual isolated activity of the compounds in the sample was not studied [26].

The effect of red propolis against parasites was studied on *Leishmania amazonensis* by Ayres et al. (2007) and red propolis reduced significantly the infection produced by this parasite, however it did not present direct effect on promastigotes or extracellular amastigotes. Ayres et al. (2007) didn't identify the compounds responsible for this activity [33].

The antifungal activity of propolis has been studied on yeast cells of *Saccharomyces cerevisiae* and on species of *Trychophyton* [34, 35]. Lotti et al. (2011) demonstrated that Propolis could be a source of compounds that could alleviate the multidrug resistance problem in fungi such as *S. cerevisiae*, thus it could be used for the treatment of fungal infections together with azoles. They studied the effect of some isoflavonoids of red propolis, and 7-O-methylvestitol appeared to be the most active. The study carried out by Siqueira et al. (2009) evidenced that red propolis is more efficient than green propolis for the *Trychophyton* species. The study reveals the antifungal activity of red propolis and suggests it as a possible alternative treatment for dermatophytosis caused by these species. Among all the red propolis compounds, Medicarpin, was shown to be an important compound with antimicrobial and antifungal activity [25].

Reactive Oxygen Species, (ROS) are formed in cells as a consequence of biochemical reactions and external factors. Under abnormal conditions, the excess production of these compounds causes cellular damage and thus participates in pathologies such as cancer, neurodegenerative diseases, coronary diseases and others. The antioxidant activity of red propolis was studied by Righi et al. (2011), who showed that phenolic compounds were responsible for this activity because they intercepted the free radical chain oxidation [26]. These authors suggested that chalcones and isoflavonoids might be involved in the process, as electron donors; 7-O-methylvestitol, Medicarpin and 3,4,2',3'-tetrahydrochalcone, were shown to have antioxidant activity. Alencar et al. (2007) had already verified with the Pearson test, that there was a correlation between antioxidant activity and flavonoids [29], and Cabral et al. (2009) also showed a correlation between phenolic compounds and antioxidant activity [27]. As probably all the phenolic and flavonoid compounds participate, at least partially, in the antioxidant activity, studies on isolated compounds with antioxidant activity are very few. Oldoni et al. (2011) analyzed the activity of Vestitol, Isovestitol and Neovestitol, showing that Vestitol had higher antioxidant potential, but that the others also presented this activity [30].

One of the few studies of Chinese red propolis showed that red propolis had a higher antioxidant activity than green propolis [23]. In this study, caffeic acid phenethyl ester (CAPE), which is a compound present in this red propolis sample, showed potent activity as well as other caffeic derivates.

To understand the cytotoxic activity of red propolis, it is important to take into account the physiological base of cancer. Cancer cells proliferate very rapidly and have a high demand of nutrients and oxygen. However cancer cells can acquire tolerance to nutrient starvation and become resistant in this way. Awale et al. (2008) studied the cytotoxic activity of red propolis by creating a nutrient-deprived condition for the cancer cells, and analyzing the cytotoxic activity of the individual isolated propolis compounds [28]. Of all the compounds studied, 3,8-hydroxy-9-methoxypterocarpan displayed the most potent preferential cytotoxicity against the cancer cell line studied. All the pterocarpans present in the sample showed cytotoxic activity, and their decreasing order of activity was : (6aR,11aR)-3,8-dihydroxy-9-methoxypterocarpan, (6aR,11aR)-3,4-dihydroxy-9-methoxypterocarpan, (6aS,11aS)-3,10-dihydroxy-9-methoxypterocarpan, (6aS,11aS)-medicarpan, (6aS,11aS)-6a-ethoxymedicarpan, (6aR,11aR)-4-methoxymedicarpin and (6aR,11aR)-3- hydroxy-8,9-dimethoxypterocarpan. 7-hydroxy-6-methoxyflavanone exhibited cytotoxic activity against melanoma, Lewis lung carcinoma, human lung carcinoma and human fibrosarcoma, while Mucronulatol only showed cytotoxicity against melanoma and Lewis Lung carcinoma cell lines [36]. This compound targets the control of the cell line progression, and thus it exerts cytotoxicity in cancer cells [22].

Alencar et al. (2007) assessed the cytotoxic activity of red propolis on Hela tumor cells, but didn´t identify the isolated compounds responsible for it [29]. Nunes et al. (2007) worked on the bioactivity of red propolis on *Artermia salina* and suggested that a possible antitumor activity of red propolis could be due to its content of phenolic compounds [24]. Red propolis showed strong suppressive effects against vascular endothelial growth factor induced angiogenesis which is a key regulator of pathogenic angiogenesis in diseases such as cancer and diabetic retinopathy [23]. Nevertheless more studies on this topic are still needed.

Other properties of red propolis have been reported, such as its antipsoriatic, anti-inflammatory and analgesic activities [37]. Red propolis presents wound healing activity and was incorporated into collagen-based films [38]. Ledon et al. (2002) didn't find dermal and ocular toxicity on their tests on a red propolis extract, however they found a dose dependent allergic response, evidenced with the production of erythema [39]. The anti-obesity effect of propolis is another studied activity. Propolis enhances the differentiation of adipocytes by activating PPARγ, and is capable of inhibiting TNFα effects on adipocyte differentiation and adiponectin expression, thus it has been put forward as a possible diet supplement for prevention and treatment of obesity and obesity-associated disorders [40]. Red propolis appears to have a hepatoprotective effect and might have activity in the prevention of hepatitis [41].

Many new studies of red propolis are being developed due to the promising therapeutic effects shown above. However, care must be taken to differentiate between red propolis from diverse geographic origins and chemical profiles.

CONCLUSION

The samples of red propolis studied so far have come mainly from the tropical regions in South America, with the exception of one sample from China. In spite of the red color, the chemical composition has been found to vary qualitatively and quantitatively, even between samples from the same region. The plant sources of resins have been investigated by two different methods: the comparative chemical composition analysis and the palynological analysis. Many different plant sources have been suggested, being that the most frequent are plants of the Leguminosae and Clusiaceace families. Further studies are necessary to determine the main chemical markers of red propolis, or if there are different types of red propolis, and how variations in bee, season and region may affect its composition.

Red propolis has shown diverse biological activities: antimicrobial activity; against parasites; antioxidant, cytotoxic; antipsoriatic, anti-inflammatory and analgesic activities; anti-obesity and hepatoprotective effects. These results indicate that this natural product deserves to be better studied for its promising therapeutic effects, and many new studies of red propolis are indeed being developed. However, future studies must differentiate between red propolis from diverse geographic origins and chemical profiles.

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