Green propolis: Composition and biological properties

SUMMARY
Green propolis derives mainly from vegetative apices of Baccharis dracunculifolia (alecrim plants). However, wide variation detected in the chemical composition suggests contributions from alternative resin plant sources. Prenylated cinnamic acid-derived compounds bearing prenyl groups are predominant and pharmacologically active components of the resin of green propolis and represent its salient chemical features. Terpenoid compounds, such as sesqui, di and pentacyclic triterpenoids, have also been detected in green propolis. Recent studies on green propolis have disclosed potentialities of substances having a wide range of biological (and potentially pharmacological) properties. In fact, several biological activities, such as anti-cancer, anti-oxidant, anti-inflammatory, anti-septic, anti-mycotic, bacteriostatic, astringent, anti-ulcer, choleretic, spasmyloytic and anaesthetic properties have been reported for green propolis and its constituents. In particular artepillin C [3-(4-hydroxy-3,5-di(3-methyl-2-butenyl)phenyl)-2 (E)-propenoic acid] isolated from Brazilian green propolis possesses several well documented activities, such as anti-microbial, apoptosis-inductor, immunomodulator, antioxidant, anti-inflammatory and, more important, anti-tumor properties. On the basis of available scientific literature, naturally occurring polyphenols and "acids" in green propolis are expected to help reduce the risk of various life-threatening diseases. However, standardized quality controls and good design clinical trials are essential before either propolis or its active ingredients can be adopted routinely in any future therapeutic application.

INTRODUCTION
Propolis is a complex resinous material produced by bees having a highly variable physical appearance, color and consistence depending on many factors. Bees use propolis to seal openings in the hive for diverse purposes, to avoid the entrance of intruders, to maintain constant the hive inner temperature and to contribute to the attainment of an internal aseptic environment (1-5). In fact, propolis is used to protect the hive from widespread bacterial infection.

Propolis composition is extremely complex, being formed of beeswax, resin and volatiles. The insects secrete beeswax, while resin and volatiles are obtained from plants, usually taken from secretions or by cutting fragments of vegetative tissues. The biological activity of propolis is strictly related to these plant-derived substances. Hence, although propolis is obviously an animal product, a considerable proportion of its components responsible for biological activities are plant derived. In fact, the resin contains most of the compounds found in alcohol/water or mix extracts consumed by people from many countries as food complements or alternative medicine (1-5). Propolis contains other constituents, and more than 300 known substances have been reported (5,6).

Starting with a gradual upsurge of interest from people from several countries in the mid 1980s, propolis ended up being an important product in complementary and alternative medicine with application in cases of chronic medium and external otitis, pharyngitis, chronic rhinitis, amygdalitis and bronchial asthma, among other ailments (1-5). However, besides its well-known antimicrobial activity, propolis shows a wide diversity of effects, including immune activation, cytotoxicity, and anti-inflammatory, anti-viral, anti-ulcer, local anaesthetic and hepatoprotective effects (4).

BOTANICAL ORIGIN OF PROPOLIS
An important issue in propolis research refers to its botanical origin and the consequent variation in chemical composition (and biological activities) for samples from different locations, but also...
from the same locality (7). Propolis samples produced in Europe and South America share anti-microbial, anti-viral, wound-healing, immune-stimulating, anti-inflammatory and anesthetic activities. However, samples that are quite different from the chemical point of view might be similar in their biological activities because different plants in Europe and South America provide resin for propolis production in the two continents (8). In typical European propolis the major pharmacologically active constituents have long been identified as flavonoids, the most common and abundant being galangin (9,10). This is a consequence of the fact that in Europe bees collect propolis resin mainly from vegetative poplar (Populus nigra, Salicaceae) buds (10). On the contrary, in the Tropics, poplars are seldom cultivated, so alternative plants are sources of propolis resin. For example, the flowers of Clusia minor in Venezuela and of Clusia rosea in Cuba produce resins in which flavonoids are minor constituents, the major compounds being polyprenylated benzophenones (11,12).

**ORIGIN OF GREEN PROPOLIS**

Probable sources of Brazilian propolis are Araucaria heterophylla, Clusia major, Clusia minor and species of Baccharis (13). Other possible sources are Araucaria angustifolia, Baccharis dracunculifolia and Eucalyptus citriodora (7). In fact, secretory hairs belonging to Baccharis dracunculifolia (called alecrim in some parts of Brazil) have been found in high quantities in both dry and rainy seasons (14). Identical prenylated cinnamic acids have been found in Baccharis dracunculifolia leaves and in Brazilian propolis. A similar composition has also been observed between alecrim material and propolis from the state of São Paulo. An attempt to classify propolis produced in Brazil according to botanical origin and chemical composition has identified 12 types (15). Five of them correspond to the southern, one to the southeastern and six to the northeastern regions of the country. 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. However, given the widespread occurrence of B. dracunculifolia not only in the southeast but also in the south of Brazil, claims that the origin of the southern propolis are poplar trees may be an oversimplification of the real range of propolis types in that region. It is not realistic to conclude that in Brazil a certain plant species is the sole resin source for a certain propolis type. Indeed there is a higher probability that, depending on the availability of representatives in the field, plants of a certain species prevail to a higher or lower extent as resin sources, while other species may also contribute (sometimes substantially) material for propolis production. The latter possibility is coherent with observations of more or less strong deviations from an expected chemical profile for a propolis type (7).

The characteristic green color of alecrim-propolis is a consequence of its botanical origin, because bees collect young chlorophyll-containing tissues, namely vegetative buds and unexpanded leaves of B. dracunculifolia. Such young leaves contain secretory hairs, probably with volatile and aromatic oils inside, hence the resinous aroma of the typical green propolis.

**CHEMICAL COMPOSITION OF GREEN PROPOLIS**

Since most analyses carried out with European propolis have revealed that flavonoids predominate as resin constituents, researchers have assumed a similar composition also for Brazilian propolis, also considering a similar pharmacological behaviour. However, the first detailed chemical analyses revealed quite distinct profiles for samples of Brazilian propolis. In fact, prenylated phenylpropanoids were shown to be very common and abundant constituents in propolis from Brazil, mainly from the southeastern region. Pharmacologically active prenylated cinnamic acid-derived compounds were identified (Figure 1) bearing a prenyl group making up a heterocyclic ring (chromenes) and quite exclusively present in green propolis. Other cinnamic acid-derived compounds common in green propolis have one or two prenyl groups not involved in ring formation. Prenylated cinnamic acids turned out to be a salient chemical feature of green propolis.

Among the non-chromene prenylated cinnamic acids of green propolis, artepillin C (Figure 1 and Figure 2) has attracted great attention, not only for its anti-microbial action but also for its toxicity to tumor cells (7). Prenylated cinnamic acids may also be present as esters, such as 3-prenyl-cinnamic acid allyl ester (Figure 1). Despite not being major constituents, flavonoids do occur in green propolis (one frequent example being kaempferide) along with benzothenurs possessing cytotoxic properties (16) (Figure 1).

Mono and sesquiterpenes are frequently detected in green propolis, accounting for its characteristic resinous odor and probably contributing to its anti-
microbial activity. For example, famesol, labdane-type diterpenes, such as isocupressic and agathic acid, and clerodane diterpenoids, such as 13-symphyoreticulic acid, have been found in green propolis (Figure 1). Non-volatile sesquiterpenes, such as dehydrocostus lactone, may also occur in green propolis (17).

Triterpenoids, some of them generally widely present in plants, have been found in green propolis, such as two novel esters of long chain fatty acids and the pentacyclic triterpenoid lupeol, procrrims a and b (18) (Figure 1). 3-prenylcinnamic acid allyl ester (Figure 1) has also been reported to be a major constituent of green propolis (19).

Considering the numerous compositional studies, there seems to be a gradual variation in the proportion of mevalonate-derived substances (terpenoids, including sesqui-, di- and triterpenoids) and the typical shikimate-derived (phenolics, prenylated or not) compounds depending on different samples of green propolis (7). The relative amount of triterpenoids may sometimes be evaluated by the physical appearance of the sample, which loses its hardness and intensity of green, turning increasingly cream and pulverulent with increasing levels of triterpenoids and reduction in the amounts of the typical shikimate derivatives.

*Baccharis* is a large genus having many representative species in the Brazilian flora. Species of the genus other than *B. dracunculifolia* are likely providers of propolis resin. Furthermore, very young leaves of *B. dracunculifolia* had a chemical composition similar to the vegetative buds of the same plant, but the composition of successively more expanded leaves showed an increasing deviation from the bud pattern (14). It seems very likely that differences in plant chemistry may be another important effect accounting for divergences in propolis composition.

**BIOLOGICAL ACTIVITIES OF GREEN PROPOLIS**

Several biological activities, such as anticancer, anti-oxidant, anti-inflammatory, anti-septic, anti-mycotic, bacteriostatic, astringent, anti-ulcer, choleric, spasmodyltic and anaesthetic properties have been reported for green propolis and its constituents (20).

The anti-ulcer activity of green propolis hydroalcoholic crude extract was evaluated by using models of acute gastric lesions induced by ethanol, indomethacin and stress in rats (21). Green propolis extract displayed an anti-secretory activity, which led to a reduction in the gastric juice volume, total acidity and pH. These findings indicate that Brazilian green propolis displays good anti-ulcer activity, corroborating the popular use of propolis preparations, and contributing to its pharmacological validation.

In a further study (22), green propolis was evaluated for its effect on activated macrophages in mice subjected to immobilization stress. Stressed mice showed a higher hydrogen peroxide generation on the part of the peritoneal macrophages. On the basis of these results, it was suggested that green propolis from *B. dracunculifolia* displays an immunomodulatory action.

![Figure 2 – HPLC-ESI-MS profile of a ethanolic extract of Brazilian green propolis. The artepillin C mass value is reported in the mass extract inset.](image)

Green propolis was evaluated as an immunological adjuvant increasing the percentage of animals with high antibody titers (23). Compounds such as artepillin C and the derivatives of cinnamic acid besides other flavonoid substances were abundant in the propolis extract used, and they could be the main substances with adjuvant action. The effect of the green propolis extract on the humoral immune response can be exploited in the development of new vaccines.

Pharmacological activities of a standard ethanol extract from Brazilian green propolis was evaluated in mouse models of pain and inflammation. This extract having a high content of flavonoids was able to induce, at low concentrations, anti-inflammatory and analgesic effects in mouse models (24).

Brazilian green propolis inhibits the neurotoxicity and apoptosis induced in cultured retinal ganglion cells protecting against oxidative stress (lipid peroxidation). In mice in vivo, propolis intraperitoneally administered was able to reduce the retinal damage (25, 26). These findings indicate that Brazilian green propolis (and caffeoylquinic acid derivatives) have neuroprotective effects against retinal damage *in vitro*, and that these effects may be partly mediated via anti-oxidant effects. Finally, a green propolis-induced inhibition of oxidative stress may be partly responsible for its neuroprotective function against *in vitro* cell death and *in vivo* focal cerebral ischemia (27).

*Streptococcus mutans* triggers dental caries establishment via two major factors: synthesis of organic acids, which demineralize dental enamel, and synthesis of glucans, which mediate the attachment of bacteria to the tooth surface. Green propolis extracts show inhibitory effects on the *S. mutans* cariogenic factors, thus suggesting a potential source for pharmaceutical products employed for this purpose (28). Furthermore, extracts of green propolis display antimicrobial activity related to the effect of several compounds present in the crude extract (29). In particular, green propolis was efficient against *Escherichia coli* showing potential for human and veterinary medicine (30).

A water-soluble derivative of green propolis inhibits angiogenesis in induced rat bladder cancer (31) and the anti-inflammatory/anti-angiogenic effects of propolis are associated with cytokine modulation. As a consequence, Brazilian green propolis (and its caffeoylquinic acid derivatives) may represent candidates for preventive or therapeutic agents against diseases caused by angiogenesis (32).

*In vivo* antileishmanial activity has also been reported for Brazilian green propolis (33) along with its capacity to reduce the parasitemia by *Trypanosoma cruzi* and to increase the survival of the animals (34).

**ARTEPILLIN C: A MAGIC BULLET**

As previously reported, artepillin C [3-{4-hydroxy-3,5-di(3-methyl-2-butenyl)phenyl}-2 (E)-propenoic acid] is
a low-molecular weight phenolic compound isolated from Brazilian propolis. Bees collect exudates from Baccharis dracunculifolia in order to produce green propolis, which contains a large concentration of this compound (35). Artepillin C possesses several well-documented activities, such as antimicrobial, apoptosis-inductor, immunomodulator, anti-oxidant and anti-inflammatory properties (35). In fact, artepillin C shows anti-inflammatory effects mediated, at least in part, by prostaglandin E2 and nitric oxide inhibition through NF-κB modulation (35).

More important, several studies have focused attention on its important anti-tumor capacity. Artepillin C exhibits a cytotoxic effect clearly inhibiting the growth of tumor cells when applied to human and murine malignant tumor cells in vitro and in vivo (36). By this study, artepillin C was observed to activate the immune system and to possess direct antitumor activity (36). When applied to human leukemia cell lines of different phenotypes, lymphocytic leukemia and non-lymphoid non-myeloid leukemia cell lines in vitro, artepillin C exhibits potent cytoidal effects and induces marked levels of apoptosis in all the cell lines. These results suggest that artepillin C, as an active ingredient of Brazilian propolis, has anti-leukemic effects with limited inhibitory effects on normal lymphocytes (37).

Moreover, Brazilian propolis and its extract Artepillin C were found to prevent oxidative renal damage and carcinogenesis in mice (38) along with the inhibition of lipid peroxidation and the development of pulmonary cancers (39). Furthermore, artepillin C in Brazilian propolis is able to prevent colon cancer through the induction of cell-cycle arrest and may be a useful chemopreventing factor in colon carcinogenesis (40) along with its capacity to significantly reduce the frequency of colonic aberrant crypt foci and to show colon cancer-preventing activity (41).

In a further study, the anti-angiogenic effects of Brazilian propolis and its main component artepillin C were investigated (42). Artepillin C was found to be, at least in part, responsible for the anti-angiogenic activity of the ethanol extract of Brazilian propolis in vivo proving useful in the development of agents with therapeutic or preventive activity against tumor angiogenesis. Additionally, recent studies suggest that artepillin C-based Brazilian green propolis extracts could be among the first effective neurofibromatosis therapeutics available (43), not to mention the important role of Brazilian green propolis and its bioactive compounds in prostate cancer chemoprevention (44, 45).

Due to its important activities, the total synthesis of artepillin C in water has been reported (46). However, most of the studied and reported activities have been performed on total extracts from Brazilian green propolis. As a consequence, artepillin C activities may be based (or increased) on the synergic effect with other propolis components.

CONCLUSIONS

Reactive oxygen species (ROS) are implicated in a wide range of human diseases. When an imbalance between ROS generation and antioxidants occurs, oxidative damage will spread over most cell targets (DNA, lipids, proteins, etc). Hence, the study of anti-oxidant substances in foods and natural medicinal sources has captured increased interest. Such substances are currently recognized as effective aids for the treatment and prevention of human diseases. Among anti-oxidants, many stemming from plants have one or more phenolic hydroxyls. Phenolic compounds may exert anti-oxidant effects as free radical scavengers, as hydrogen donating sources or as singlet oxygen quenchers and metal ion chelators. Phenolic compounds are known to counteract oxidative stress in the human body by helping to maintain a balance between oxidant and anti-oxidant substances. Flavonoids and phenolic acids are major classes of phenolic compounds, whose structure-anti-oxidant activity relationships in aqueous or lipophilic systems have been extensively reported (1,4-6). In addition to their anti-oxidant activity, many phenolic compounds have been shown to exert anti-cancer or anti-carcinogenic/anti-mutagenic activity to a greater or lesser extent. Their physiological and pharmacological activities may be derived from their anti-oxidant properties, which are related to their molecular structure. Mechanisms of anti-oxidant action may include suppression of ROS formation, removal or inactivation of oxygen reactive species and up-regulation or protection of antioxidant defenses. In this sense, hydrogen transfer from artepillin C to radicals proceeding via one-step hydrogen atom transfer rather than via electron transfer, indicates that artepillin C can act as an efficient anti-oxidant (47).

Overall, green propolis (and propolis) has a wide spectrum of alleged applications including potential anti-infection and anti-cancer effects and many of the therapeutic effects can be attributed to its immunomodulatory functions (48). The composition of propolis can vary according to the geographic locations from which the bees obtained the ingredients. However, two main immunopotent chemicals have been identified as artepillin C and caffeic acid phenethyl ester (CAPE). Propolis, artepillin C and CAPE have been shown to exert a summative immnosuppressive function on T lymphocyte subsets and activate macrophage function. On the other hand, they also have potential anti-tumor properties by different postulated mechanisms such as suppressing cancer cell proliferation via its anti-inflammatory effects, decreasing the cancer stem cell populations, blocking specific oncogene signaling pathways, exerting anti-angiogenic effects, and modulating the tumor microenvironment. The good bioavailability by the oral route and good historical safety profile makes propolis an ideal adjuvant agent for future immunomodulatory or anticancer regimens. In fact, since more than 70% of human cancers, such as breast and prostate cancers, require the kinase PAK1 for their growth, it is quite possible that artepillin C-based Brazilian green propolis extracts could be potentially useful for the treatment of these cancers.

Development and utilization of more effective anti-oxidant, anti-inflammatory, immunomodulatory or anti-cancer extracts (molecules) of natural origin are desired. Naturally occurring polyphenols and "acids" are expected to help reduce the risk of various life-threatening diseases, including cancer and cardiovascular diseases, due to their activities. 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.

REFERENCES

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