British Journal of Medicine & Medical Research
8(2): 88-109, 2015, Article no.BJMMR.2015.431
ISSN: 2231-0614

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Use of Propolis in Cancer Research

P. Vit[1,2,3*], F. Huq[2], O. M. Barth[4], M. Campo[5], E. M. Pérez-Pérez[6], F. A. Tomás-Barberán[7] and E. L. Santos[8]

[1]Agrarian and Livestock Research Center, Academic Unit of Agrarian and Livestock Sciences, Universidad Técnica de Machala, Machala, El Oro Province, Ecuador.
[2]Biomedical Science Cancer Research Network, Discipline of Biomedical Science, University of Sydney, 75 East Street, Lidcombe, NSW 1825, Australia.
[3]Apitherapy and Bioactivity, Food Science Department, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida, Venezuela.
[4]Laboratory of Morfology and Viral Morfogenesis, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.
[5]Academic Unit of Chemical Science and Health, Universidad Técnica de Machala, Machala, El Oro province, Ecuador.
[6]Laboratory of Biotechnological and Molecular Analysis, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida, Venezuela.
[7]Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O.Box 164, 30100, Campus Espinardo, Murcia, Spain.
[8]Environmental and Biological Science, Federal University of Grande Dourados, Rodovia Dourados Ithaum, Km 12, 79804-970 Dourados, MS, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Authors PV and FH designed, outlined the review and the anticancer uses of propolis. Author OMB contributed to the botanical origin of propolis and is author of the pollen images, author MC the chemical composition and table, author EMPP organized the tumor microenvironment, author FATB reviewed the flavonoids, and author ELS the immune response. Author FH assessed the English style. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/16216
Editor(s):
(1) Mahin Khatami, National Cancer Institute (ret.), The National Institutes of Health, Bethesda, MD, USA.
Reviewers:
(1) Anonymous, Italy.
(2) Kwang-Huei Lin, Department of Biochemistry, School of Medicine, Chang-Gung University, Taiwan.
(3) Anonymous, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1114&id=12&aid=8888

Review Article

Received 16th January 2015
Accepted 28th March 2015
Published 18th April 2015

**Corresponding author: Email: vitolivier@gmail.com;*

ABSTRACT

Interest to develop new anticancer drugs and to design combination treatments with little or no secondary effects provides new scope for traditional phytochemicals in chemoprevention and therapy. Propolis is a known source of polyphenols, and flavonoids found in them have been widely studied as biochemical markers for botanical origin and in explaining their antioxidant capacity as a key factor in chemoprevention. Antimicrobial, anti-inflammatory and anticancer biological activities of propolis are known. Studies of cancer cells to measure the anticancer effect of propolis are designed with one carefully chosen component, and with extracts applied to cells in culture media. The antitumor effect of propolis and caffeic acid phenethyl ester (CAPE), bioactive compound of propolis extract, is seen to be associated with its ability to initiate apoptosis of cancer cells. Chrysin is a flavonoid of interest to identify signaling molecules related to cancer. As cancer cells develop multidrug resistance (MDR) during chemotherapy, this opens a new avenue of research on cellular mechanisms of propolis components in combined treatments designed to overcome MDR.

Keywords: Anticancer; anti-inflammatory; apoptosis; CAPE; chrysin, cancer; flavonoids; MDR; prevention; phenolics; pollen analysis; propolis.

ABBREVIATIONS

BRP;- Brazilian red propolis CA;- caffeic acid CAPE-; phenetyl caffeate COX;- cyclooxygenase COX-1;- cyclooxygenase-1 COX-2;- cyclooxygenase-2 EEP-; ethanolic extract of propolis GPE;- grape polyphenols HUVEC;- human umbilical vein endothelial cells IFNγ;- interferon γ IgG-; immunoglobulin IL-1β, IL-2, IL-6-; interleukin family, interleukin-2, interleukin-6 iNOS;- inducible nitric oxide synthase LPS;- lipopolysacharide MDR -; multidrug resistance MMPs;- metalloproteinases MoDCs-; monocyte-derived dendritic cells NADPH-oxidase;- nicotin adenin dinucleotide phosphate oxidase NF-kB-; nuclear factor kappa-light-chain-enhancer of activated B cells NO-; nitric oxide OSF-; oral submucous fibrosis RNS-; reactive nitrogen species ROS-; reactive oxygen species STAT3;- cytokine-activated transcription factor in Th17 TNBC-; triple negative breast cancer TNF-α-; tumor necrosis factor TRAIL-; tumor necrosis factor-related apoptosis-inducing ligand TSCCa-; tongue squamous cell carcinoma STAT 3-; signal transducer and activator of transcription 3 Th17-; T helper 17 cell UPLC–qTOF;-MS/MS;- ultra-performance liquid chromatography quadrupole time of flight mass spectrometry VEGF;- vascular endothelial growth factor.

1. PLANT RESINS TRANSFORMED INTO PROPOLIS BY BEES

Propolis is a complex natural matrix that honey bees collect from tree buds, saps and other plant sources. The primordial role of bees as pollinators could be compared to their efficiency as collectors of plant secondary metabolites of diverse botanical origins [1]. Honey bees (*Apis mellifera*) as well as the social stingless honey bee species (Meliponini), need to collect resins from plants in order to elaborate a product used in nest construction and protection. Freshly secreted beeswax scales also contain the characteristic phenolic compounds found in propolis, suggesting that bees themselves can ingest propolis or the plant resins collected for making propolis [2].

The most common European propolis is obtained from resins of *Populus* trees [3], and the Moroccan black propolis is from that of *Euphorbia resinifera* [4]. The Brazilian green propolis of *Baccharis dracunculifolia* was firstly identified by Bastos et al. in 2000 [5]. An exotic red propolis was recognized in Brazil by Daugsch et al. in 2006 [6], with the resin collected from stem exsudates of *Dalbergia ecastaphyllum* (Fabaceae). Brazilian propolis of three *Frieseomelitta* stingless bee species is rich in terpenes [7]. The Atlantic forest of the South and Southeast regions in Brazil acts as the source of large quantity of brown propolis [8]. Venezuelan propolis originates in the flower exudates of *Clusia* species [9]; germacrene D (26.5%) and β-caryophyllene (10.2%) are the major components in the essential oil of xerophyitic propolis [10]. Three main types of Cuban propolis (brown, yellow and red) were characterized based on their secondary metabolites [11]. The brown type, rich in polyisoprenylated benzophenones,

originates from floral resin of *Clusia rosea*. The yellow-type contains triterpenoids belonging to oleanane, lupane, ursane, and lanostane skeletons, suggesting a large distribution of their botanical sources which have not been identified so far [12]. In addition, chalcones, pterocarpans, isoflavans and isoflavones were found to be the main constituents of red propolis, indicating a botanical relation to the *Dalbergia* species [13,14]. Isoflavones were also identified in Mexican propolis, with a 1,3-diarylpropane and 1,3-diarylpropene carbon skeleton, these compounds serve as the network between Mexican propolis and the genus *Dalbergia* [15]. Pollen analysis is a helpful technique in ascertaining regional origins of propolis samples. The sediment of alcohol extracted pieces of clean propolis has to be observed using a microscope, and the obtained pollen spectra characterize the regional vegetation, including that of resinous plants [16]. Frequent pollen types in Brazilian propolis sediments are shown in Fig. 1.

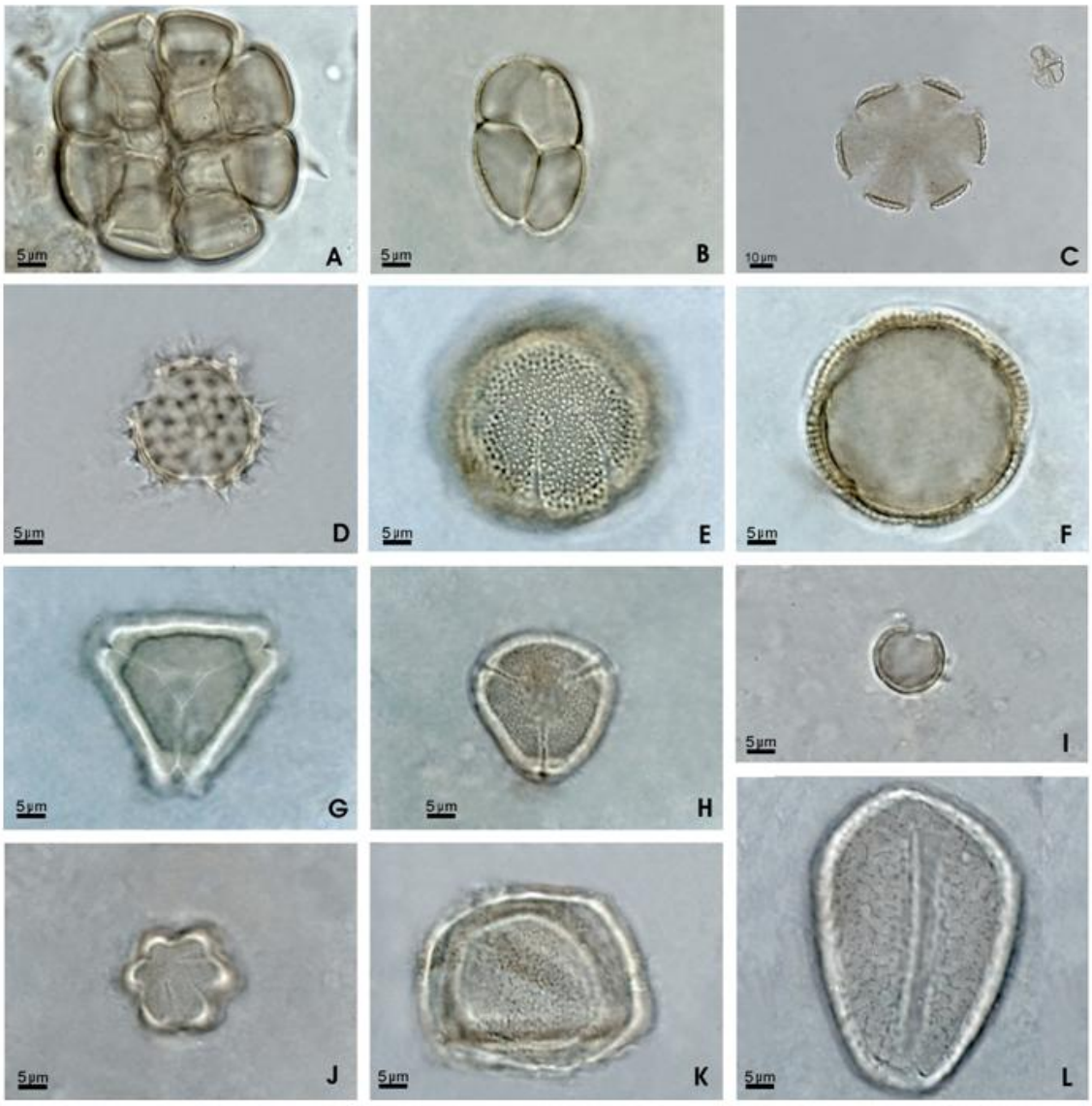


Fig. 1. Frequent pollen types in Brazilian propolis sediments
A *Acacia* (Mimosaceae), **B** *Mimosa verrucosa* (Mimosaceae), **C** *Hyptis* (Lamiaceae), **D** Asteraceae, **E** *Borreria densiflora* (Rubiaceae), surface. **F** idem, optical section, **G** *Eucalyptus* (Myrtacae), **H** *Myrcia* (Myrtaceae), **I** *Piper* (Piperaceae), **J** Melastomataceae, **K** Arecaceae, equatorial view, **L** idem. distal pole view.

Most research on propolis has been carried out with *Apis mellifera* products which are known to have distinctive composition derived from their botanical origin. The most abundant propolis types available in the market are derived from *Populus* and *Baccharis*, with different active components but having similar bioactivity [17]. Although unifloral European honeys produced by *Apis mellifera* have been characterized [18], a similar document on propolis is not yet available, and would be of great interest. Additional to botanical origin, the entomological origin is also found to contribute to the variability of products from the Meliponini hive [19]. For example, hydroalcoholic propolis extract from Brazilian *Scaptotrigona* was found to be associated with reduced Concanavalin A-stimulated nitric oxide (NO) production in mice [20].

2. MEDICINAL REPUTATION OF PROPOLIS

Healing properties of bee products are related to the antioxidant, anti-inflammatory, antimicrobial and anticancer activities of flavonoids. However other substances such as organic acids, terpenoids, amino acids, and vitamins, can also contribute to the healing power. Like honey, propolis is considered to be an old remedy for many ailments [21]. Propolis preparations are offered to the consumers in 10-20% ethanolic tinctures, water or oil extracts, powder in capsules, aerosols, mixed with honey or as ingredient of medicinal cough syrups, tablets, tooth paste and candies. Water extracts of propolis are found to be tumoricidal [22]. The edible oil extract of propolis was found to be as effective as the ethanol extract in inhibiting *in vivo* tumor growth [23]. Propolis has antibacterial, antifungal and antiviral action [24], and also antiparasitic action against protozoal parasites (*Plasmodium falciparum, Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania infantum*) [25]. Hence propolis is considered to be an antibiotic agent. Other studies have been conducted against infectious diseases, such as acute cervicitis [26], parasitic vaginal infections [27], dental plaque [28], facial septic injury [29], giardiasis [30], prevention of oral mucositis and candidiasis after radiotherapy on head-neck cancer [31]. Cytotoxicity of propolis from Myanmar [32,33] and Mexico [33] was also investigated. Classical reviews of propolis components listed a series of chemical structural features found in some 300 species [34-36]. New substances are increasingly identified in propolis from diverse botanical, geographical [37-39] and entomological [7] origin. A biological matrix with such compositional complexity should be used with some degree of caution about the risks involved. At the same time, its use in alternative and complementary therapy should be explored based on its medicinal benefit. In agreement with [17], propolis should be considered as a food supplement with functional properties. The anticancer activity of propolis is reviewed here.

3. EFFECT OF PROPOLIS ON CANCER CELLS AND TUMOR

Diverse disciplines of medicine, have demonstrated beneficial effects of propolis based on experimental evidence *in vitro* [40], *in vivo* [41] and clinical trials [30]. Consumers are often seen to ask whether propolis can contribute to cancer cure. Should propolis extracts be taken during chemotherapy? The answers to such questions remain unclear. Putative and potential attributes are ascribed to propolis in traditional medicines and a growing database on cancer related research around the world.

Because of the problems of drug resistance and side effects associated with currently used anticancer drugs and the realization that nature provides a vast resource of tumour active compounds with both chemopreventive and therapeutic attributes, increasing attention is given to natural substances. This article summarizes the biological effects of components of propolis reported to have chemopreventive action against unresolved chronic inflammation such as cancer.

3.1 Antioxidant Activity

It is well established that cellular metabolism generates reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and highly reactive hydroxyl ion (HO^-), as well as reactive nitrogen species (RNS), especially nitric oxide (NO). ROS and RNS are ideal signaling molecules because they are locally generated, highly and rapidly diffusible, and can be neutralized by cellular antioxidants [42]. ROS are usually detoxified by intracellular peptides and enzymes, such as glutathione, superoxide dismutase, and catalase [43]. However, unbalanced production and degradation of ROS and RNS can result in accumulation of these reactive molecules, a condition that is commonly referred to as oxidative stress. Exposure of macromolecules (including lipid, proteins and DNA) to reactive

species results in oxidative modifications with deleterious effects [44].

Antioxidants present in consumable fruits and vegetables rich in vitamins, amino acids and carotenes have received considerable attention in the prevention and treatment of cancer. Propolis extracts have been proposed as a natural antioxidant of plant oils [45]. Interestingly, another group of foods including ginger *Zingiber officinale*, garlic *Allium cepa*, tea *Camelia sinensis*, bee propolis, maca *Lepidium meyenii*, turmeric *Curcuma longa* and other spices has joined the rank of potent anticancer products. In essential and fixed oils, there are a number of compounds that have also demonstrated potent anticancer activity.

Propolis is a potential source of natural antioxidants such as phenolic acids and flavonoids. For various reasons, in today's world increasing attention is given to natural substances towards counteracting the effects of oxidative stress, which underlies many diseases, such as cancer, diabetes and atherosclerosis. It has been reported that the chrysin, galangin, kaempferol and quercetin decrease the production of NO [46]. Significant antioxidant activity is also reported for phenethyl caffeate (CAPE); its neuroprotective effect [47] and anti-inflammatory activity *in vitro* and *in vivo* is due in part to its antioxidant properties [48].

Thirugnanasampandan et al. [49] analyzed the chemical composition and antioxidant activity of hydroalocoholic extract of propolis collected from Coimbatore region, Tamilnadu, India. Ethanol extracts were analyzed by GC-MS, high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), and *in vitro* antioxidant activities were evaluated with DPPH radical scavenging activity and hydroxyl radical scavenging activity. GC-MS, HPTLC and HPLC revealed the presence of fatty acids, alcohols and quercetin. Dose dependent DPPH and hydroxyl radical scavenging activity of hydroalcoholic extract of propolis was calculated to be 16.20 and 34.44 μg/ml. Inhibition of lipid peroxidation was found to be significant and the IC_{50} value was calculated as being 10 and 13 μg/ml, respectively. These results are in agreement with the results of Propolis collected from China [50] and Brazil [51]. The authors suggest that bioactive compounds present in propolis may alleviate many diseases and can be used for better human health. Recently, Pérez-Pérez et al. [52] evaluated the total antioxidant activity (TAA) of ethanolic extracts of propolis produced by *Tetragonisca angustula* from Mérida, Venezuela, through the radical cation ABTS. The TAA value was 190.6 ± 4.7 TEAC/100 g of propolis, and a positive relation between antioxidant activity and polyphenol content (R^2 = 0.987) was found to be true.

The antioxidant capacity of propolis may be related to some of its biological effects, including chemoprevention [53]. The flavonoids in propolis are powerful antioxidants, capable of scavenging free radicals and thereby protecting the cell membrane against lipid peroxidation [54]. Moreover, ROS and RNS, together with other factors, are involved in cellular ageing and death in conditions, such as cardiovascular disease, arthritis, cancer, diabetes, Parkinson's disease, and Alzheimer's disease [55,56]. Diverse compounds from propolis have been described as potent inhibitors of oxidative stress. Although the composition of propolis is highly varied, one of its major components, caffeic acid phenethyl ester (CAPE) is found to block ROS production in several systems [57]. Indeed, CAPE has been identified as one of the major cancer chemopreventive and anti-inflammatory compounds in propolis. *In vitro,* propolis inhibits peroxidation of LDL and nitration of proteins. Moreover, in bovine aortic endothelial cells, propolis was reported to increase NOS expression and inhibit NADPH oxidase (NOX) [58]. *In vivo* studies show propolis can increase antioxidant capacity in animals and humans, leading to decreased lipid peroxidation, which is strongly associated with the risk of cardiovascular disease [59].

There are many reports of antioxidant activity of propolis and its effect on human health. For example, Turkish propolis was found to inhibit hydrogen peroxide ($H_2O_2^-$) induced damage to DNA in cultured fibroblasts, which may be related to its chemopreventive activity [60]. Red propolis from Cuba was shown to have protective effects in models of alcohol-induced liver damage, most likely due to its antioxidant properties, inhibiting macrophage apoptosis via effects on glutathione (GSH) and the tumor necrosis factors/nuclear factor kappa B (TNF/NF-κB) pathway [61,62]. Brazilian propolis from *Baccharis dracunculifolia* modulated 1,2-dimethlyhydrazine (DMH-) induced DNA damage in colon cells [63]. Isla et al. [64] described the acceptance of propolis candies. Righi et al. [65] attributed the antioxidant effect of Brazilian red propolis to

chalcones and isoflavonoids (including 7-Omethylvestitol, medicarpin, and 3,4,2,3-tetrahydrochalcone) that act as electron donors; furthermore, total flavonoid content in Brazilian red propolis is correlated with its antioxidant activity, suggesting that all the phenolic and flavonoid compounds present contribute to this activity.

3.2 Anti-proliferative Activity

The antitumor activity of propolis in animal models and cell cultures is likely to result from its ability to inhibit DNA synthesis in tumor cells, capacity to induce apoptosis, and ability to activate macrophages to produce factors capable of regulating the function of B, T and NK cells, respectively. The results also suggest that flavonoids from propolis play a protective role against the toxicity of the chemotherapeutic agents and/or radiation in mice, giving hope that they may have similar protective function in humans. Combination with an adjuvant antioxidant therapy may enhance the effectiveness of chemotherapy by ameliorating the side effect on leukocytes, liver, and kidneys and consequently enabling dose escalation [66]. Although many polyphenols have an anti-metastatic activity, CAPE (from poplar propolis) and artepillin C (from *Baccharis* propolis) have been identified as the most potent antitumor agents [36].

Barlak et al. [67] investigated if protein expression profile in PC-3 prostate cancer cell lines could be differentiated when incubated with dimethyl sulfoxide and water extracts of Turkish propolis. Dimethyl sulfoxide and water extracts of propolis of 20 µg/mL were found to reduce the cell viability to 24.5% and 17.7%, respectively. Statistically significant discriminatory peaks between control PC-3 cells and dimethyl sulfoxide extract of propolis-treated PC-3 cells were found to have proteomic features at m/z 5143, 8703, 12661, 20184 and 32794, detected by CM10 Protein Chip, and the peak at m/z 3772, detected by Q10 Protein Chip. It was concluded that dimethyl sulfoxide and water extracts of Turkish propolis may have anti-proliferative activity through modulation of protein expression profile in PC-3 prostate cancer cell lines along with their antioxidant capacity.

In another study, Umthong et al. [68] investigated antiproliferative activity of partially purified *Trigona leaviceps* propolis from Tahiland on human cancer cell lines. It was found that the hexane extract had high *in vitro* antiproliferative activity against the five tested cancer cell lines but low cytotoxicity against the two normal cell lines. Further fractionation of the hexane extract by quick column chromatography using eight solvents of increasing polarity revealed that the two fractions eluted with 30% and 100% (v/v) CH_2Cl_2 in hexane (30DCM and 100DCM, respectively) had a higher anti-proliferative activity. In conclusion, *T. laeviceps* propolis appears to contain compound(s) with antiproliferative activity *in vitro* on cancer but not normal cell lines. Enriched propolis fractions typically revealed a higher antiproliferative activity (lower IC_{50} value). Overall, propolis from Thailand may have the potential to serve as a template for future anticancer-drug development.

In addition to abundant evidence of antiproliferative activity of propolis, there is a general interest for the selection of the most appropriate method for preparation of propolis extracts as potential antioxidant and anticancer agents. Khacha-Ananda et al. [69] evaluated antioxidant activity, total phenolic, total flavonoid compounds and cytotoxicity of propolis extracts from two different extraction methods, against cancer cell lines. Propolis was collected from Phayao province and extracted with 70% ethanol using maceration and sonication techniques. The antioxidant activity was evaluated by DPPH assay. The percentage propolis yield after extraction using maceration (18.1%) was higher than using sonication (15.7%). Nevertheless, antioxidant and flavonoid compounds of the propolis extract obtained by sonication were significantly greater than using maceration. Propolis extract from sonication showed antioxidant activity by 3.30±0.15 mg gallic acid equivalents/g extract. Total phenolic compound was 18.3±3.30 mg gallic acid equivalents/g propolis extract and flavonoid compound was 20.49±0.62 mg quercetin/g propolis extract. Additionally, propolis extracts from two extraction methods demonstrated the inhibitory effect on proliferation of A549 and HeLa cancer cell lines at 24, 48 and 72 hours in a dose-dependent manner.

In general, due of its curative properties, propolis is now gaining popularity in health foods and in cosmetic products. The biological activity of propolis itself derives from high levels of phenolic acids present while flavonoids are thought to be responsible for activity of propolis extracts. Studies have shown that propolis can suppress the IL-6-induced phosphorylation of signal

transducer and STAT3, an essential cytokine-activated transcription factor in Th17 development. Therefore, mechanisms of action of propolis on Th17 differentiation could be instrumental in controlling disturbed cytokine networks in inflammation, autoimmune diseases, and infections. The use of propolis has been proposed in patents such as: WO201363714; CN102885854, WO2013142936, US20130266521, and US20130129808, which are related to the treatment of dental diseases; adjuvant in anticancer treatment; in cosmetic products; as an anti-inflammatory agent and natural antibiotic. Although there are many publications on the efficacy of propolis, its applicability to human health and the mechanisms of its action are yet to be completely understood, giving impetus for further studies [70].

3.3 Apoptosis

Apoptosis, known as programmed cell death, is an essential process of life used in the removal of unwanted old and injured cells. This mechanism includes morphological changes in cells such as rapid condensation and budding of the cell, formation of membrane-enclosed apoptotic bodies with well-preserved organelles [71] and intrinsic pathway involving caspase and NF-κB [72]. Induction of apoptosis is one of the most important markers of cytotoxic action of antitumor agents and one of the mechanisms proposed for the anticancer therapeutic effects of propolis [73,74] and CAPE, an active component isolated from propolis [75]. Propolis appears to be efficient against different tumor cells both in vitro and in vivo [76]. The antitumor effect of propolis is found to be associated with its ability to initiate apoptosis of cancer cells. Multiple surveys reported that propolis induces apoptotic pathways in cancer cells, such as melanoma cells [77] histiocytic lymphoma cells [73], mammary carcinoma Cells [78,79], pancreatic cancer cells [75], epithelial carcinoma cells [80,69], lung epitelial cells 69], prostate cancer cells [81,82], hepatoma cells [83], colon and epithelial colorectal adenocarcinoma cells [84], breast cancer cells [85], colon adenoma and carcinoma cells [86], showing the potential use of propolis in cancer prevention and therapy.

4. EFFECT OF PROPOLIS ON TUMOR MICROENVIRONMENT

Cellular and animal models, are used for the *in vitro* and *in vivo* studies of biological mechanisms involved in signaling pathway, apoptosis, immune response, anti-inflammatory action, cancer progression and anticancer activity of propolis. The use is considered to enable characterization and increase understanding of the mechanisms of biological action of the propolis.

4.1 Anti-angiogenic Activity

Angiogenesis is the multistep process by which blood vessels are formed. This tightly regulated process involves migration, proliferation, and differentiation of endothelial cells [87]. Regulation of angiogenesis is absent or aberrant in several diseases characterized by persistent, inappropriate blood vessel development. Inappropriate angiogenesis occurs in more than 80 diseases, particularly in many types of cancer and inflammatory diseases as atherosclerosis [88,89]. Green propolis extracts containing artepillin C and CAPE were found to significantly reduce the number of new vessels formed and the expression of metalloproteinases (MMPs) and production of vascular endothelial growth factor (VEGF) from endothelial cells [90].

Different steps of angiogenesis can be affected by propolis and its components. For example, Brazilian propolis and its major component, artepillin C, can inhibit proliferation of human umbilical vein endothelial cells (HUVEC) as well as endothelial cell migration and capillary tube formation in a dose-dependent manner. Moreover, artepillin C can suppress angiogenesis in both *in vivo* and *in vitro* models while CAPE inhibits MMP2, MMP-9, and VEGF activity [91]. Xuan et al. [92] demonstrated that a concentration 12.5 µg polyphenols/ml of ethanol extracts of Brazilian propolis decreases the expression of integrin b4 and p53 and the production of reactive oxygen species. Moreover, Brazilian red propolis at 10 mg/l reduces the migration and sprouting of endothelial cells, attenuates the formation of new blood vessels, and decreases the differentiation of embryonic stem cells into CD31-positive cells [93].

Meneghelli et al. [94] studied the effects of a hydro-alcoholic propolis extract collected in Santa Catarina State (Southern Brazil) in autumn on angiogenesis, using *in vitro* and *in vivo* models. Cultures of human umbilical vein endothelial cells were used to assess the effects of propolis on cell viability, proliferation, and cell migration, as well as capillary tube formation. The autumnal extracts significantly decreased

the cell viability, based on IC_{50} values, which decreased (56%) from 297 to 130 µg/ml in 24 h and 72 h of treatment respectively (cytotoxicity assay). The process of cell proliferation was found to decrease by 81.7 to 48.4% due to exposure (72 h) to 130-180 µg/ml of propolis extract, as compared to control (vehicle). The cell migration was also reduced by 39.6 to 12.6%, respectively (versus control). The treatments performed *in vivo* with administration of 450 mg propolis/kg inhibited both angiogenesis and vasculogenesis by 82.3 and 66.5% in the chorioallantoic and yolk-sac membranes of chick embryos. Furthermore, higher contents of flavonoids and total phenolic compounds with predominance of the flavonol quercetin and the phenolic acids, e.g., gallic acid, protocatechuic acid and chlorogenic acid were found in the propolis hydro-alcoholic extract by means of UV-vis-spectrophotometry, reverse phase-high performance liquid chromatography analysis and 1D and 2D-nuclear magnetic resonance experiments.

Both *in vitro* and *in vivo* studies are increasingly revealing antiangiogenic activity of propolis extracts and their constituents. Further preclinical research is required to determine whether individual compounds or complex mixtures will be more beneficial. A potential advantage of phytochemicals and other compounds found in propolis is that they may act through multiple cellular signaling pathways, and under different pathophysiological conditions while also inhibiting angiogenesis and reducing inflammation. Overall, propolis constituents may be helpful as adjunct therapies for diseases such as cancer as well as cardiovascular diseases, in which angiogenesis must be controlled.

4.2 Immune Response

Knowledge on mechanisms of action of propolis on the immune system has advanced in the last few years. In vitro and in vivo assays have shown that propolis may activate macrophages, increasing their microbicidal activity, enhance the lytic activity of natural killer cells against tumor cells and stimulate higher antibody production. The results have also indicated that inhibitory effects of propolis on lymphoproliferation may be associated with its anti-inflammatory property [95]. Other studies have shown that Brazilian propolis may suppress the IL-6-induced phosphorylation of signal transducer and activator of transcription 3 (STAT3), an essential cytokine-activated transcription factor in T helper 17 cell Th17 development, action mechanisms of propolis on Th17 differentiation could be instrumental in controlling disturbed cytokine networks in autoimmune diseases and inflammation [96,97]. CAPE is a potent inhibitor of T cell receptor-mediated T cell proliferation, via suppression of both IL-2 gene transcription and IL-2 synthesis in stimulated T cells [98] and inhibits cytokine and chemokine production by human monocyte-derived dendritic cells which might be related to the nuclear factor kappa of activated B cells (NF-κB) signaling pathway [40]. Recently, [99] have shown that immunological enhancement activity of propolis flavonoid liposome is due to enhancement of the phagocytic function of macrophages and the release of IL-1β, IL-6, and interferon γ and in vivo by activation the cellular and humoral immune response, including inducing higher level concentrations of immunoglobulin (lgG), IL-4, and interferon γ. These mechanisms together probably contribute to the immunosuppressive and anti-inflammatory effects of propolis.

4.2.1 Anti-inflammation

Inflammation is a beneficial response activated to restore tissue injury and to control pathogenic agents. After tissue injury, a complex network of chemical signals initiates and maintains a host response to repair the injured tissue. However, if inflammation is unregulated, it can become chronic, inducing cell transformation in the surrounding tissue, resulting in alteration of molecular targets, signaling pathways, apoptosis, increased proliferation rate, and angiogenesis. Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, irritants, and free radicals. Anti-inflammatory activity is a primary effect of the host defense system. The inflammatory process is triggered by several molecules including pro-inflammatory enzymes and cytokines, low molecular weight compounds such as eicosanoids or the enzymatic degradation of tissues [100]. According to several studies the enzyme most related to inflammatory process is cyclooxygenase-2 (COX-2), an isoform of cyclooxygenase (COX), which catalyses the transformation of arachidonic acid to prostaglandin. The other isoform is cyclooxygenase-1 (COX-1), which regulates homeostasis processes [100]. Propolis has inhibitory effects on myeloperoxidase activity, NADPH-oxidase ornithine decarboxylase, tirosine-proteinkinase, and hyaluronidase from guinea pig mast cells [101].

In the last 30 years, many studies have pointed out the anti-inflammatory properties of propolis that are basically due to the presence active flavonoids and cinnamic acid derivatives. The compounds inhibit the development of inflammation provoked by a variety of agents [102,103]. Among the flavanoids are acacetin, quercetin, and naringenin while among cinnamic acid derivatives are caffeic acid phenyl ester (CAPE) and caffeic acid (CA) [101]. Galangin is a flavanoid of particular interest, because of its capacity to inhibit cyclooxygenase (COX) and lipo-oxygenase activity, limit the action of polygalacturonase, and decrease the expression of the inducible isoform of COX-2 [104-106]. Chrysin, another flavonoid present in propolis, also shows anti-inflammatory activity [107,108] and the mechanism of its action is related to suppression of activity of pro-inflammatory COX-2 and inducible nitric oxide synthase (iNOS) [103].

Propolis has been reported to produce anti-inflammatory activity both *in vitro* and *in vivo* by the inhibition of proinflammatory cytokines and increase of anti-inflammatory cytokines [109,110]. It may inhibit the production of nitric oxide, IL-1β, and IL-6 in lipopolysaccharide stimulated RAW 264.7 cells and block the activation of NF-κB in TNF-α-stimulated HEK 293T cells [111]. CAPE, another bioactive compound present in propolis, shows anti-inflammatory activity by inhibiting the release of arachidonic acid from the cell membrane. This leads to the suppression of COX-1 and COX-2 activity and inhibits the activation of the genic expression of COX-2 [112,113]. Márquez et al. [98] have evaluated the immunosuppressive activity of CAPE in human T-cells. It was found that the compound is a potent inhibitor of early and late events in receptor-mediated T-cell activation. Moreover, it was found that CAPE specifically inhibited both interleukin IL-2 gene transcription and IL-2 synthesis in stimulated T-cells. The findings are seen to provide new insights into the molecular mechanisms involved in the immunomodulatory and antiinflammatory activities of this natural compound.

CAPE is known to play potent anti-inflammatory role being able to reduce histamine and leukotrienes (LTs) release from isolated rat peritoneal mast cells [114]. Furthermore, [106] have demonstrated inhibitory effect of CAPE against COX-1 and COX-2 activities in J774 macrophages. CAPE is also found to suppress gene expression of cytokines under inflammatory conditions of hypertrophic adipocyte [115] and reduce the activation of NF-κB and COX2 expression in obese mice [116]. Several studies have confirmed that propolis and CAPE exhibit promising anti-inflammatory activity.

Shi et al. [117] investigated anti-inflammatory properties of fifteen propolis samples collected from different regions of China. Eleven compounds including caffeic, p-coumaric, ferulic, isoferulic, and 3,4-dimethylcaffeic acids, pinobanksin, chrysin, pinocembrin, galangin, pinobanksin 3-acetate, and caffeic acid phenylethyl ester were quantified using a UHPLC whereas 38 compounds were identified by UPLC/Q-TOF-MS. The 15 propolis samples significantly differed in their total phenolic and total flavonoid contents, as well as their phytochemical profiles. The methanol extracts of propolis also showed significant anti-inflammatory effects in LPS-stimulated RAW 264.7 mouse macrophage cells at a concentration of 10 μg propolis extract/ml. More recently, Bueno-Silva et al. [118] evaluated anti-inflamatory activity of ethanolic extract (EEP) of Brazilian redpropolis (BRP), and neovestitol and vestitol isolated from BRP using a neutrophil migration assay. Neovestitol, vestitol, and EEP inhibited neutrophil migration at a dose of 10 mg/kg. Both the isoflavonoids neovestitol and vestitol, are bioactive compounds consistently displaying anti-inflammatory activity at low dose and concentration with promising potential for use in pharmaceutical and food industries. In another study, Mossalayi et al. [119] showed that hematotoxicity-free doses of grape polyphenols (GPE) and propolis extracts (PR) differentially decreased the secretion of pro-inflammatory cytokines from activated human peripheral blood leucocytes. While GPE inhibited the monocytes/macrophage response, propolis decreased both monokines and interferon γ (IFNγ) production. When used together, combined effects lead to attenuation of all inflammatory mediators, evidenced by a significant modulation of the transcriptomic profile of pro-inflammatory genes in human leukocytes. To ascertain *in vitro* data, GPE+PR were tested for their ability in improving clinical scores and cachexia in chronic rat adjuvant-induced arthritis (AA). The extracts significantly reduced arthritis scores and cachexia with the effect being more significant in animals receiving continuous low doses than those receiving five different high doses. Animals treated daily had significantly better clinical scores than corticoid-treated rats. Together, these findings can be

seen to indicate that the GPE+PR combination induces potent anti-inflammatory activity due to complementary immune cell modulation.

4.2.2 Immunomodulation

Some studies have provided information on the influence of propolis on the immunological system [120,121]. Research with poplar propolis demonstrated its action on the immune system [122,123]. Brazilian green propolis showed improvement in innate immunity, contributing to the recognition of microorganisms and activation of lymphocytes by antigen-presenting cells [121]. Furthermore, there has been a significant increase in spontaneous cytokine secretion in peripheral blood leukocytes [124] with CAPE and artepillin C being two potent immunochemicals [125].

Inflammation, aging and cancer are linked together in terms of tumoricidal (growth-arresting) and tumorigenesis (growth-promoting) aspects of immunity. The National Cancer Institute of Health in Bethesda, MD, USA, suggested a 'yin-yang' paradox to interpret acute inflammation as two gears onto apoptosis as a female-receptive 'yin', and wound healing onto male-active 'yang' with oxidative stress being the major driving force [126]. This author also uses the expression 'common denominator' for persistent inflammation as the genesis of age-associated diseases. Possibly 'yin-yang' is a wider concept that cannot be reduced to apoptosis and wound healing in the immune scenario. Also non-age related health disorders have immune components. The fact that propolis have diverse botanical origins illustrates the variable nature of this resin that – like food, may behave as yin or yang.

5. ANTICANCER USES OF PROPOLIS AND ITS COMPONENTS

"In the treatment of malignant neoplasms, any delay is critical" [127]. Cancer involves fundamental biological processes concerning disordered cell replication, death and disorganization of organ structure. Plant products namely podophyllotoxin, vinca alkaloids and taxol have contributed a lot in the development of cancer chemotherapy. Recent studies have shown that modulation of nitric oxide production using a plant derived immunomodulators could provide a cost effective and least toxic alternative to chemotherapy. Nutritional oncologists these days are trying to identify the naturally occurring dietary factors which are or may be anti carcinogenic.

Propolis extracts have shown activity against diverse cancer cell lines such as Hela [80], prostate adenocarcinoma [128], basophilic leukemia [129] and human breast [130], to study activation of caspase, inhibition of mast cell granulation. Selective toxicity of Portuguese propolis extracts was observed between human renal carcinoma and normal cells [131]. Plukenetione was identified for the first time in Cuban propolis induced G0/G1 arrest and DNA fragmentation in colon carcinoma cells. Plukenetione A contributed to the anti-tumoral effect of Cuban propolis mainly by targeting topoisomerase I as well as DNA polymerase [132]. Chinese propolis showed antitumoral activity against breast cancer in MCF-7 and MDA-MB-231 [85]. The average concentration of flavonoids in propolis is 19%, and it varies from 5 to 26%. Besides these high concentrations of flavonoids, synthetic compounds inspired by propolis can be efficient to treat tumors [133]. In Table 1, active anticancer components of propolis are summarized.

Different anticancer mechanisms postulated for propolis were reviewed by Chi Fung Chan et al. in 2013 [125]: 1. Suppression of cancer cells proliferation by anti-inflammatory effects; 2. Decrease of the cancer stem cell populations; 3. Alteration of oncogene signaling pathways; 4. Antiangiogenic effects; 5. Adaptation of the tumoral microenvironment.

Diverse forms of propolis –aerosols, candies, capsules, extracts, ointments, tablets, tooth pastes− are available to the consumer [64,31].

5.1 Phenolic Compounds

Phenolic compounds are organic substances characterized by the presence of hydroxyl groups attached to one or more benzene rings. These secondary metabolites are present in high concentrations in the wine, tea, grapes and in a wide variety of plants [147].

The term phenolic covers a large and diverse group of chemical compounds that have been identified in about a dozen subcategories and can be found from simple molecules to highly polymerized compounds. Furthermore, it may be associated with molecules such as sugars, organic acids and lipids. Some of the groups of

compounds include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, lignans, tannins, and others [147,148]. Phenolics are widely used in human health as antiseptics and in sunscreens to protect the skin. They are also used for their oestrogenic, antioxidant, inhibiting platelet, anti-inflammatory and anticancer activities [147].

Among all the secondary metabolites originated in plants, the phenolic extracts of propolis have fascinated phytochemists and there is growing scientific evidence on the medicinal benefits of propolis. New active compounds of propolis [36], and the factors involved in propolis pharmaceutical applications [149] were reported. A brief overview on the role of flavonoids present in propolis is addressed to explain their anticancer attributes in multiparametric studies.

5.2 Flavonoids

Flavonoids are a group of small molecules (C6-C3-C6, MW ~ 300) widely known to contribute to the colors of flowers and fruits. Five subclasses of dietary flavonoids were considered in selected food: flavones, flavonols, flavanones, flavan-3-ols and anthocyanidins [150]. In this database there is an entry for bee pollen and honey but not for propolis, which is mainly used as a dietary supplement. The flavonoid content in 100 g honey is 0.05 mg apigenin, 0.63 mg luteolin (flavones) and 0.17 mg isorhamnetin, 0.11 mg kaempferol, 1.03 mg myricetin, 0.51 g quercetin (flavonols). Over the past few years, a number of studies have used flavonoid profiles of honey to find botanical markers [151]. Propolis and honey are natural products with great chemical variability and careful selection of active compounds is needed for further anticancer demonstrations. The wide spectra of polyphenols studied to characterize and to differentiate bee products is a valuable background for predictions on what honey and propolis types are better options for anticancer therapies.

5.3 Anticancer Approach

Cancer cells are different from normal cells because they extend their life. They create inflammation, possibly to obtain more nutrients from the surrounding tissues. Apoptosis or programmed cell death is a mechanism necessary for health, induced by some drugs when it is lost in tumors. Signalling molecules of apoptosis and other cellular pathways are measured to assess cancer onset, progress and regression in anticancer studies. These markers have membrane, cytosolic or nuclear related mechanisms. Multiparametric indicators also consider the cell cycle arrest or proliferation of subpopulations of cells, differentiating necrotic from apoptotic states.

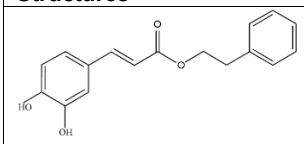
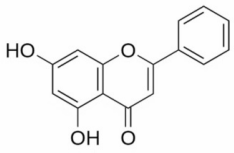
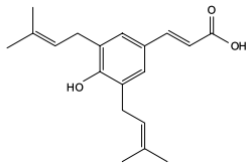
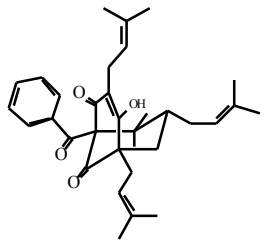
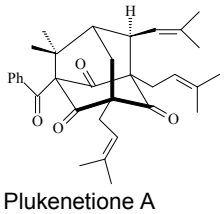
Chemoprevention is an important issue to consider, focusing on dietary components such as polyphenols and their epigenetic action as modulating agents of gene expression [152-155]. Thus flavonoids present in honey and propolis have been studied for their chemopreventive action. More recently, phytochemicals are also studied to overcome multidrug resistance when applied in combination with targeted therapy [156], and a hypothesis on the genotoxic role of honey flavonoids targeting cancer cells, has been proposed [157]. The EEP of Brazilian propolis from Cerrado bioma was antigenotoxic in a *Drosophyla melanogaster* model.

Research was carried out in different parts of the world where it is concluded that propolis actually possess antiproliferative activity against various cancer cells in human and mouse [141]. The mechanism of action by which propolis exert their antiproliferative activity on cancer cells has not been fully described. However studies suggest that their cytotoxic effect may be due to the induction of apoptosis.

Some chemical constituents of propolis are capable of inducing apoptosis, among which the predominant ones are artepillin C [144,158], propolina C [159], and CAPE [75]. The antiproliferative effects of propolis, CAPE or chrysin in cancer cells are the result of the suppression of complexes of cyclins, as well as cell cycle arrest. The results of *in vitro* and *in vivo* studies suggest that CAPE and chrysin present in propolis may inhibit tumor cell progression and may be useful as potential chemotherapeutic or chemopreventive anticancer drugs [74].

CAPE and artepilline C, exhibit anticancer and chemopreventive effect in the treatment of colon cancer metastasis [160-162]. Propolin C, a flavonoid isolated from Taiwanese propolis, induces cytotoxic effect on human melanoma cells [77]. Caffeic acid reduces tumor growth in lung metastatic mammary tumors [152].

Table 1. Structures of propolis components relating to actions in cancer models

Structures	Medicinal action	Reference
 CAPE	Inhibits viability of BxPC-3 (80.4 +/- 4.1%) and PANC-1 (74.3 +/- 2.9%) cells.	[75]
	Inhibits MCF-7 (hormone receptor positive, HR+) and MDA-MB-231 (a model of triple negative BC (TNBC) tumor growth, either *in vitro* or *in vivo* without much effect on normal mammary cells.	[134]
	Exhibits strong antitumor effects in oral cancer cells: fibroblasts from oral submucous fibrosis (OSF), neck metastasis of gingival carcinoma (GNM) and tongue squamous cell carcinoma (TSCCa).	[135]
	Improves apoptosis increasing the activity of caspase-3 or caspase-7 in various types of cancer cells: HL-60, CI41, U937, human ovarian carcinoma SK-OV-3, human lung carcinoma NCI-H358, human hepatocellular carcinoma HepG2, C6 glioma, human cervical cancer ME180, and BxPC-3 cells.	[75,136, 137, 138, 139,140, 141]
 Chrysin	Induces apoptosis in U937 cells by the inactivation of PI3K/Akt signal pathway as well as down regulation of NF-kB and IAP activation. Is a potentially important agent to be used in prevention or therapy of patients with leukemia.	[142]
	Involved in the route of apoptosis in human colorectal cancer cells HCT116, human nasopharyngeal carcinoma cells CNE-1, and human liver cancer cell line HepG2.	[143]
 Artepillin C	Artepillin C was applied to human and murine malignant tumor cells *in vitro* and *in vivo*, it exhibited a cytotoxic effect and the growth of tumor cells was inhibited. The artepillin C was found to cause significant damage to solid tumor and leukemic cells using MTT reduction assay, DNA synthesis assay, and morphological observation *in vitro*.	[144]
	Inhibits the tube formation of human umbilical vein endothelial cells (HUVECs) in a concentration-dependent manner (3.13–50 lg/ml).	[90]
	Artepillin C sensitized the tumor necrosis factor-related to apoptosis-inducing ligand (TRAIL)-resistant LNCaP cells by engaging the extrinsic (receptor-mediated) and intrinsic (mitochondrial) apoptotic pathways. Artepillin C increased the expression of TRAIL-R2 and decreased the activity of NF-κB.	[82,145]
 Nemorosone	Low concentrations of nemorosone elicited growth-inhibitory effects on ERα+ MCF-7 cells.	[146]
 Plukenetione A	Plukenetione A induced G0/G1 arrest and DNA fragmentation in colon carcinoma cells.	[132]

5.4 Signalling Molecules Studied to Understand the Anticancer Action of Chrysin

Chrysin (5,7-dihydroxyflavone) has been studied by several authors for its effects in suppressing inflammation by NF-κB and JNK activations [163], to trigger the response of unfolded endoplasmic reticulum resident protein GRP78 [164], to enhance the apoptosis induced by a ligand [165], p38 and Bax activation [166]. However, in another study, chrysin was reported to inhibit apoptosis induced by the antitumor-drug topotecan believed to be associated with the inhibition of ATP-binding cassette (ABC) transporters [167]. Synthesis of chrysin derivatives prepared by alkylation, halogenation, nitration, methylation, acetylation and trifluoromethylation has also been focused towards the development of anticancer drugs [168], with higher anticancer activity than 5-fluorouracil for some derivatives [169]. The adaptive response of cancer and normal cells to chrysin and derivatives is a mosaic under construction leading to a model for better understanding of interactions of flavonoids with cells and cell components as a chemopreventive and genotoxic agent.

6. CONCLUSIONS

Aerobic life being under constant oxidative stress, antioxidants have an important role to play in maintaining integrity of cells and the life itself. Although some researchers suggest that flavonoids being chemopreventive may not play any useful role in killing cancer cells, the idea is simplistic and does not take into account the fact that programmed cell death is brought about by multiple pathways as are the mechanisms of drug resistance. Recent studies show that combinations of targeted therapies and phytochemicals produce sequence-dependent synergism that may provide a means of overcoming drug resistance. Research with propolis extracts and key flavonoids being at the infancy, it is difficult to state with certainty their role in cancer medicine; this should await results from well-designed, thorough and extensive studies. Integrating Chinese cosmovision in bee product research may be an interesting approach for another review. Important analogies are transmitted to link honey with earth, pollen with wood, propolis with the transmutation element metal, royal jelly with water, and bee venom with fire.

ACKNOWLEDGMENTS

Endeavour Awards from Australia for the 2011 Research Fellowship at The University of Sydney to Prof. P. Vit, during her sabbatical leave from Universidad de Los Andes. Prof. F. Huq's group at The University of Sydney (USYD), BRIG and Cancer Research Donation Account. The supportive environment at the USYD Discipline of Biomedical Science, and at UTMACH Agrarian and Livestock Research Center and Planning Department. Prometeo-Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT) for the 2014-2015 scholarship to P. Vit at Universidad Técnica de Machala, Ecuador. Prof. E. L. Santos was supported by Grants from Foundation to Support Development of Education, Science and Technology of Mato Grosso do Sul State – FUNDECT and Brazilian National Research Council (CNPq), Brazil. To anonymous reviewers who improved the manuscript with their comments. The yin-yang issue was requested by one editor.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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