

REVIEW ARTICLE

# Chemical diversity of propolis makes it a valuable source of new biologically active compounds



Vassya Bankova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.

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\*Corresponding author: Email: bankova@orgchm.bas.bg

## Summary

Propolis, with its diverse pharmacological activities and low toxicity, has attracted the attention of modern scientists for about 50 years. However propolis, or bee glue, has demonstrated remarkable chemical variability, which is a serious obstacle to its standardization and consequently, to its official acceptance into the main stream of our healthcare system. On the other hand, the variation in the chemistry of propolis from different ecosystems has made it a source of new biologically active molecules, mainly antioxidative, antibacterial and anticancer agents. The most important recent findings concerning bioactive molecules isolated from propolis are reviewed and discussed here.

**Keywords:** propolis, caffeic acid phenethyl ester, artepillin C, prenylated benzophenone, propolins

## Introduction

Propolis is not the most popular bee product. Nevertheless, it is one of the most fascinating materials manufactured by bees, both as a building material and a defensive substance. Bees make use of the mechanical properties of this resinous product by employing it in hives for blocking holes and cracks, repairing combs and strengthening the thin borders of the comb. On the other hand, they make use of its biological action by containing the putrefaction of the "embalmed" intruders that have been killed in the hive and are too large to be carried out, and it is responsible for the lower incidence of bacteria and moulds within the hive compared to the atmosphere outside (Ghisalberti, 1978). The action against infections is an essential characteristic of propolis that has been recognized and used by human beings since ancient times. Hippocrates (460-377 BC), who is considered to be the father of modern medicine, prescribed the use of propolis to help heal internal and external sores and ulcers, and this was the first recorded use of propolis as a medicine (Najafi *et al.*, 2007). Propolis has been used as a remedy in Europe ever since. It is still one of the most frequently used home remedies in the Balkan states where it is applied for treatment of wounds and burns, sore throats and stomach ulcers.

Modern science focused on propolis after the 1960s, inspired by the general interest in bee products and natural products.

Numerous studies have revealed versatile and valuable pharmacological activities of propolis: foremost, antibacterial, antimycotic and antiviral, but also many other and less obvious effects, such as cytotoxic, antioxidant, anti-inflammatory and immunomodulation, to mention those most often studied (reviewed by Ghisalberti, 1978; Burdock 1998, Banskota *et al.*, 2001; Bankova 2005a). Chemical studies were performed in order to identify the active principles (reviewed by Ghisalberti, 1979; Walker & Crane, 1987; Marcucci 1995; Bankova *et al.*, 2000, Bankova *et al.*, 2008).

First chemical analyses were performed on European propolis from different locations, and demonstrated the presence of flavonoid aglycones, phenolic acids and their esters, which are the characteristic constituents of poplar bud exudate. The origin of propolis from poplar buds has been well documented by numerous chemical comparisons of both materials from most European countries, including our own studies (Bankova & Kuleva, 1989; Grenaway *et al.*, 1987; Nagy *et al.*, 1986; Tamas *et al.*, 1979). In the 1990s tropical propolis attracted the attention of scientists and after the analysis of a number of samples from different geographic regions it became obvious that the chemical composition of bee glue was highly variable (Bankova *et al.*, 2000, Bankova, 2005b). These findings led to a paradigm change in propolis research, which occurred about the turn of the 21st century. It was realised that unlike beeswax or bee venom, there is no single product that is "just propolis". This resulted in a new approach to research into the

biological activity of propolis because it was understood that it is was not enough to report that experiments were performed with propolis, but that it was necessary to chemically characterise the particular propolis used in experiments (Bankova, 2005b).

The remarkable chemical variability of propolis is, of course, due to its plant origin and to the fact that at different geographic locations the source plants might vary with respect to the local flora at the site of collection. To manufacture propolis, bees collect plant material and mix it with wax. It is now generally accepted that bees collect resinous plant materials, produced by a variety of botanical processes, in different parts of plants. These are substances actively secreted by plants, as well as substances exuded from wounds in plants; they include lipophilic materials on leaves and leaf buds, mucilages, gums, resins, and latices (Crane, 1988). In some cases, bees also may cut fragments of vegetative tissues to release the substances needed for propolis production (Weinstein Teixeira *et al.*, 2005). The specificity of local flora is responsible for the chemical composition of propolis. However, there is another important, but often neglected factor - the choices made by bees. It is obvious that bees choose sticky resinous materials because of their physical properties. On the other hand, this material is also their chemical defence against microorganisms, but it is not clear how exactly bees recognise these properties in the collected material. Numerous studies demonstrate that the choice of propolis source is far from random. Understanding this point is important from an academic and practical point of view, because it adds to the knowledge about bee-plant relationships.

The special preference of honeybees to the genus *Populus* has been established from data concerning propolis composition in the temperate zone. In Europe, *Populus nigra*, the black poplar, is the source of choice for bees; in other temperate and subarctic regions, other *Populus* species fulfil this role (Bankova *et al.*, 1992, Popova *et al.*, 2004). Poplar exudates are similar (not identical) in their chemical composition. Yet bees thrive in tropical habitats, where no poplar trees grow. In Brazil, in the states Sao Paulo and Minas Gerais, the preferred source turned out to be *Baccharis dracunculifolia* (Marcucci & Bankova, 1999). One of our investigations into propolis and its potential sources in Brazil demonstrated that the choice of plant source was "purposeful". We analysed by GC-MS propolis and plant secretions from the three species in the vicinity of the hives that were most frequently mentioned as botanical sources of bee glue in Brazil (*Baccharis dracunculifolia*, *Araucaria angustifolia* and *Eucalyptus citriodora*). Based on this chemical evidence, *B. dracunculifolia* was shown to be the main propolis source in the studied area. The antibacterial and antifungal activity of all four materials were also tested, and the most active were found to be propolis and *Baccharis* leaf exudate (Bankova *et al.*, 1999). Bees had made the best choice, but we cannot understand how they did it. This is a question yet to be answered.

It is important to note that although of different chemical composition, propolis always demonstrates considerable biological activity, especially antimicrobial activity (Kujumiev *et al.*, 1999, Seidel *et al.*, 2008). For this reason, the chemical diversity of different propolis samples has the potential to provide valuable leads to active components. The following examples are the most important and prominent cases that confirm and illustrate this aspect of propolis.

### **Caffeic acid phenethyl ester (CAPE)**

The ultimate success story of a bioactive compound isolated from propolis is, without any doubt, that of the caffeic acid phenethyl ester (CAPE). This relatively simple compound has been known for a long time but first attracted attention in 1979, when a mixture of caffeic acid esters, including phenethyl ester, was identified as the main antibacterial and antifungal principle of European propolis (Metzner *et al.*, 1979). Its popularity with researchers increased significantly after a publication reporting on the identification of CAPE as the main cytotoxic compound in propolis with preferential cytotoxicity on tumour cells (Grunberger *et al.*, 1988). The authority of the senior author Nakanishi, and the remarkable activity of the compound were the reason for the ever increasing interest of scientists in this compound in the years since 1988. At present, it is the most studied of all the individual constituents of propolis. In the Scopus scientific database, we found 474 hits for CAPE in title, abstract and/or keywords for Life Sciences and Health Sciences, 183 of them being in Health Sciences. Different aspects of the biological and pharmacological activities of CAPE have been studied, including the mechanism of its action.

The cytotoxicity of CAPE has been intensively studied. It was found to induce apoptosis of human pancreatic cancer cells (Chen *et al.*, 2008) and colon cancer cells (Xiang *et al.*, 2006). It inhibited the growth of C6 glioma cells *in vitro* and *in vivo* (Kuo *et al.*, 2006). Other studies prove its chemopreventive effect (Dorai & Aggrawal, 2004). CAPE has been proved to block the NF- $\kappa$ B activation process (Natarajan *et al.*, 1996). Although the maintenance of appropriate levels of NF- $\kappa$ B activity is crucial for normal cellular proliferation, constitutive NF- $\kappa$ B activation is involved in the enhanced growth properties as seen in several cancers (Bharti & Aggarwal, 2002). Dietary intake of safe and nontoxic chemopreventives like CAPE may thus be beneficial for patients whose tumours express persistently high levels of activated NF- $\kappa$ B, such as non-small cell lung carcinoma, thyroid, colon, breast, stomach, squamous head and neck carcinomas.

Recently, CAPE was considered a promising substance for anti-HIV therapy, as it was found to be an effective inhibitor of HIV-1 integrase (Burke *et al.*, 1995, Johnson *et al.*, 2004; Pommier *et al.*, 2005), it inhibited the integration step to a substantially greater degree than the initial cleavage step of the enzyme (Fesen *et al.*, 1993).

CAPE is one of the important antioxidant compounds in poplar type (European) propolis (Russo *et al.*, 2002; Velazquez *et al.*, 2007). It decreases oxidative stress (Serarslan *et al.*, 2007; Ozguner *et al.*, 2005) and has demonstrated neuroprotective properties in different test systems (Wei *et al.* 2008; Altug *et al.*, 2008; Özyurt *et al.*, 2006). The antioxidant properties are also connected to its documented anti-inflammatory activity *in vitro* and *in vivo* (da Cunha *et al.*, 2004). CAPE was found to be a potent modulator of the arachidonic acid cascade (Mirzoeva & Calder, 1996) and to inhibit the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation (Michaluart *et al.*, 1999). CAPE suppressed acute inflammation in a model of carrageenin-induced subcutaneous inflammation (Orban *et al.*, 2000). It also accelerated cutaneous wound healing in rat models (Serarslan, *et al.*, 2007) and demonstrated beneficial effects in the prevention of caustic esophageal strictures in rats.

Antibacterial activity of CAPE has been demonstrated *in vitro* against different microorganisms: *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus* (Kishimoto *et al.*, 2005, Velazquez *et al.*, 2007, Kujumiev *et al.*, 1993). Of special importance is the anti-influenza virus activity of CAPE (Kishimoto *et al.*, 2005, Serkedjieva *et al.*, 1992). In general, the combination of anti-inflammatory, antibacterial and antiviral action is appropriate with respect to the application of propolis preparations containing CAPE for wound healing and for treating of sore throat, common cold.

CAPE has even demonstrated excellent fungicidal properties on fungi infecting tomato; it was found to be more effective than a commercial fungicide and without negative effects on tomato fruit (Ojeda-Contreras *et al.*, 2008).

### **Artepillin C**

Artepillin C is a diprenyl-4-hydroxycinnamic acid derivative first isolated from *Baccharis* species in 1981 (Bohlmann *et al.*, 1981). It was found in Brazilian propolis for the first time in 1994 by Aga and colleagues, who found that it exhibited antibacterial activity. This finding, combined with some structural similarity to CAPE (the presence of the *trans*-cinnamoyl system), led to increased interest in this compound and before long a lot of important pharmacological activities had been found and some studies revealing the mechanisms of action of artepillin C were performed. It was demonstrated to possess antiinflammatory (Khayyal *et al.*, 1993, Paulino *et al.*, 2008), apoptosis-inducing (Matsuno *et al.*, 1997), antioxidant (Hayashi *et al.*, 1999) and anticarcinogenic properties (Kimoto *et al.*, 2001a; Kimoto *et al.*, 2001 b). It was also highly cytotoxic to a variety of malignant human and murine solid tumour cell lines *in vitro* (Konishi *et al.*, 2005). Furthermore, artepillin C was found to inhibit the growth of transplanted solid human and mouse tumours including that of malignant melanoma, in athymic and thymic mice, respectively (Kimoto *et al.*, 1998). Konishi (2005)

reported that artepillin C was mainly permeated across Caco-2 cells by transcellular passive diffusion. It was easily incorporated into hepatic HepG2 cells without conjugation, and suppressed oxidative damage to the cellular membrane and DNA (Shimizu *et al.*, 2006). In addition, it was found to be a highly bioavailable compound (Shimizu *et al.*, 2004).

A recent review on artepillin C refers to 17 articles dedicated to studies on biological activities of the compound (Estrada *et al.*, 2008), and the Scopus Database displays 8 articles containing artepillin C among the keywords for 2008.

### **Polyprenylated benzophenones**

Polyprenylated benzophenones were detected for the first time in propolis in 1993 but their importance in the biological activity of bee glue became clear about 10 years later. The pharmacological activities of polyprenylated benzophenones isolated from plants have already attracted some attention: their anti-inflammatory, antitumoral (Díaz-Carballo *et al.*, 2003) and antioxidant properties have been documented (Merza *et al.*, 2004). However, their presence in propolis from Cuba, Venezuela and Brazil resulted in further studies. Antiradical activity was demonstrated for guttiferone E and xanthochymol (Trusheva *et al.*, 2006). A recently isolated new natural compound from Brazilian propolis, plukenetione H, possessed good activity against cariogenic microorganisms (Castro, 2008). More detailed studies revealed valuable pharmacological properties of two prenylated benzophenones in Cuban propolis: nemorosone and plukenetion, which were first isolated from *Clusia* plants but not particularly studied for bioactivities. Nemorosone demonstrated cytotoxic activity against epitheloid carcinoma (HeLa), epidermoid carcinoma (Hep-2), prostate cancer (PC-3) and central nervous system cancer (U251) cell lines (Cuesta Rubio *et al.*, 2002). It also exhibited antioxidant capacity (Cuesta Rubio *et al.*, 2002). Plukenetione A proved to be cytotoxic in a panel of cell lines from various cancer entities in a recent study. Interestingly, this compound did not display cross-resistance in sub-lines expressing MDR1 phenotype. It efficiently inhibited the enzymatic activity of both topoisomerase and DNA polymerase, suggesting them to be major physiological targets. As revealed in cell cycle studies, plukenetione A enforced S-phase depletion and G0/G1 arrest. The aforementioned inhibitory effects were sufficient to induce drastic changes in the cellular biology, for example, DNA damage and the alterations in gene expression patterns of some genes relevant for cell replication and metabolism. The properties of plukenetione A as an inhibitor of replicative enzymes seem to be promising for the application in cancer chemotherapy (Díaz-Carballo *et al.*, 2008).

### **Prenylated flavanones (propolins)**

Prenylated flavonoids are known plant constituents which have recently been found to be the major constituents of propolis from the Pacific islands of Okinawa and Taiwan (Kumazawa *et al.*, 2004; Chen

*et al.*, 2003). These compounds were found to possess antiradical activity against DPPH and antioxidant activity in  $\beta$ -carotene bleaching system (Kumazawa *et al.*, 2007). Individual compounds isolated from Pacific propolis were studied and anticancer properties of many of them demonstrated. Propolin A and propolin B were found to have cytotoxic properties against three cancer cell lines. DNA content analysis and DNA fragmentation indicated that propolin A efficiently induced apoptosis in cancer cell lines, but had no effect on the cell cycle programme. They also showed strong scavenging effects against most types of free radicals. (Chen *et al.*, 2003, 2007). Propolin C induced apoptosis through activating caspases, Bid and cytochrome C release in human melanoma cells (Chen *et al.*, 2004). Propolin H induced G1 arrest in human lung carcinoma cells and may have therapeutic applications (Weng *et al.*, 2007). Propolin G has demonstrated to be a potent caspase-dependent inducer of apoptosis in brain cancer cells. With Taiwanese propolis extract it exhibited a protective effect against oxidative stress in rat cortical neurons (Huang *et al.*, 2007).

## Conclusions

Research into propolis has revealed the pharmacological properties of substances previously known as plant secondary metabolites, but insufficiently evaluated. Analysis of propolis has also led to the discovery of many valuable plant substances that otherwise might not have been found. This is due to the ability of honeybees to find, in whatever environment they inhabit, sources of substances in plants that provide an efficient protection of their hives from infections and from the elements of weather.

Obviously, the study of bee glue and the search for plants used by bees for propolis collection in tropical, subtropical and even northern regions has the potential to uncover new biologically active compounds with important pharmacological effects, especially antibacterial, antioxidant and anticancer substances. For this reason, new types of propolis from unexplored regions will continue to attract growing interest among scientists searching for new bioactive molecules.

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