

# BEE Informed



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## Determining Quality in Propolis Samples

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Bee propolis, the glue-like substance honeybees manufacture from plant resins, possesses anti-microbial and anti-viral properties as well as being anti-inflammatory and hepatoprotective with biological activities to repel tumors and stimulate the immune system. It is a popular remedy used in alternative and complimentary medicine, such as apitherapy, is a constituent of "biocosmetics," and is consumed as a "health food." Because it is so often used to promote health, it is important that the propolis made available is of high quality.

What does "high quality propolis" mean? This simple question does not have a straight-forward answer, even though propolis has been the subject of detailed chemical and biological studies for more than 50 years. This lack of qualitative information hinders the wide use of propolis as a medicine, and is a problem rapidly being recognized by mainstream medical practitioners. This article is an attempt to give a definitive answer to the question of how to qualify propolis for medicinal and cosmetic use.

A good propolis first of all must be free of all toxic contaminants. Acaricides are used to control bee parasites in many countries and can be found as residues in propolis (Bogdanov et al., 1998); heavy metals may also accumulate in dangerous amounts (Woisky & Salatino, 1998). Thus, an important obligatory parameter in quality control of propolis is the contamination level of all main acaricides and heavy metals.

Propolis must not only be free of toxins, it must also be useful. The percentage of substances inert with respect to biological action, such as wax, insoluble particles, and ash, must be registered. However, if propolis is to be used medically, its most important characteristic is its level of activity. It is essential to characterize the abundance of biologically active substances in a sample, and this is what is most difficult.

This difficulty arises from the botanical origin of propolis: bees collect it from different plant sources depending on the geographic zone and the specificity of the local flora, thus leading to significant variations in propolis' chemical composition and making the choice of compounds to be quantified a difficult task. Currently, many people believe that a propolis sample is of high quality if it contains a high percentage of flavonoids (Bonvehi & Coll, 1994; Park et al., 1998). A brief survey of the literature about the biological action of individual propolis constituents demonstrates that this approach could be wrong, especially with respect to samples of tropical origin.

Since 1960, many investigations have been carried out to identify the antibacterial and anti-fungal substances of propolis. The first results, published by Lavie and his group, pointed to two flavonoid aglycones as antibacterial agents in propolis: galangin and pinocembrin (Villanueva et al., 1964; Villanueva et al., 1970). Further research on European samples supported these results and added a few other phenolics to the list: pinobanksin, pinobanksin-3-O-acetate, benzyl-p-coumarate and caffeic acid esters (Metzner et al., 1979). Pinocembrin and the caffeate mixture were found to be the main anti-fungal substances in propolis, as well (Metzner et al., 1979). Phenolic acids, such as caffeic and ferulic, were also indicated as antibacterial constituents. In recent studies, propolis and some of its cinnamic and flavonoid components were found to uncouple the energy transducing cytoplasmic membrane and to inhibit bacterial motility, which may contribute to the anti-microbial action (Mirzoeva et al., 1997). Recent studies on tropical, especially Brazilian, samples lead to the discovery of new antibacterial compounds. A number of them are phenolics and their

derivatives, although completely different from those found in European propolis. The most important ones are carbon-prenylated derivatives of p-coumaric acid, the 3,5-diprenyl-p-coumaric acid being one of the major antibacterial compounds in Brazilian propolis (Aga et al., 1994). In propolis from the Canary Islands, antibacterial furofuran lignans were found (Christov et al., 1999). Antibacterial substances of non-phenolic nature were isolated from Brazilian propolis, as well; these are diterpenic acids with a labdane skeleton (Bankova et al., 1996). Obviously, flavonoids are not by far the only antibacterial constituents of propolis.

Anti-viral action is another important biological property of propolis. Once again, it is attributed to the phenolic compounds, mainly esters of caffeic and ferulic acids (3-methylbut-2-enyl caffeate, 3-methylbutyl ferulate), caffeic acid itself, and some flavonoid aglycones (luteolin, quercetin) (Amoros et al., 1992, Serkedjieva et al., 1992).

According to the latest investigations, the anti-inflammatory activity of propolis is connected to its radical scavenging activity to a great extent. Natural phenolics are among the substances known as potential radical scavengers. In detailed studies on radical scavenging action of individual components, caffeic acid phenethyl ester, together with the flavonoids galangin, kaempferol, and kaempferid, were identified as active components in exerting propolis' renowned anti-inflammatory activity (Krol et al., 1996). Phenolics were reported to affect the activity of several systems known to be involved in the inflammatory process.

During the last five years, a number of publications appeared detailing the hepatoprotective effect of propolis in different experimental systems. Ramirez et al. (1997) suggested that this effect could be because of the anti-oxidative action of propolis extracts. Caffeic and ferulic acids and their esters were found to be the main anti-oxidative components of European propolis; the activity of the flavonoid aglycones was significantly lower (Marinova et al., 1989). From Brazilian propolis, two dicaffeoylquinic acid derivatives were isolated as hepatoprotective agents: 3,4-dicaffeoylquinic acid and its methyl ester (Basnet et al., 1996).

Alcohol extracts of propolis possess local anaesthetic action, according to German authors. The substances responsible are again pinocembrin and a mixture of caffeate esters (Paintz & Metzner, 1979).

Special attention has been directed toward the anti-tumor effects of propolis. Caffeic acid phenethyl ester (CAPE) is the anti-tumor substance from propolis that has received the most attention. Its anti-tumor properties were discovered in a bioassay-guided chemical study of propolis by the research group of Koji Nakanishi (Grundberger et al., 1988) and examined thoroughly. CAPE was found to inhibit human breast carcinoma and melanoma cell lines in culture. Human tumor cells displayed a significantly greater sensitivity to the action of CAPE than the analogous normal lines of non-tumorous cells in that the CAPE was more toxic to the tumor cells than to the normal ones.

Similar results were obtained using other propolis constituents with similar structures: methyl caffeate and phenethyl ester of dimethylcaffeic acid (Rao et al., 1992). Brazilian propolis delivered structurally different anti-tumor agents: carbon-prenylated derivatives of p-coumaric acids, e.g. 3,5-diprenyl-p-coumaric acid and similar molecules showed cytotoxic activity in vitro against human tumor cell lines, as well as in vivo in mice transplanted with human tumor cells (Kimoto et al., 1998). Another group of anti-tumor propolis constituents was isolated from Brazilian samples, too: clerodane diterpenic acids, active against human hepatocellular carcinoma (Matsuno et al., 1997). At present this field is being studied extensively by several research groups in Japan.

This summary of the research literature points out that flavonoids are not the only important active substances in propolis. In fact, it is clear that many other compounds must be considered in assessing the quality of propolis. Present knowledge and understanding about propolis' active principles provides the basis for another important conclusion: it is impossible to apply one standard analytical procedure to all propolis samples to measure the quantity of active substances. This is because, due to the chemical diversity of propolis, the active substances in different samples can have completely different chemical natures even though they have the same type of biological action!

Is there a way out of this puzzling situation? Perhaps, if we try to think like bees and to consider the sources the bees use for propolis. The plant origins of propolis could give clues to standardizing its quality control. Propolis could be categorized easily by plant source, which can be established by simple chromatographic comparison of both materials, using thin layer, high performance liquid, or gas chromatography. This approach can give information about the qualitative composition of the propolis sample in so far as the composition of the corresponding plant material is known. It is

generally accepted and has been chemically proven that in temperate zones the bud exudates of *Populus* species and their hybrids are the main source of propolis. This is true for Europe, North America, and the non-tropical regions of Asia (Bankova et al., 2000). It has been discovered that in New Zealand the source plants are introduced species of Poplar.

When discussing "poplar type" propolis, it is clear that the product is a mixture of flavonoid aglycones, hydroxycinnamic acids, and their esters, and that these are the compounds that must be quantified. In Russia, however, and especially in its northern parts, birch buds (*Betula verrucosa*) are the common source of propolis, and flavonoid aglycones are of interest for quality control (Popravko, 1978). Chemical provings in some Brazilian regions have shown that *Baccharis* species leaf exudate is the main propolis source (Bankova et al., 1999). In this case, the important active constituents are carbon-prenylated derivatives of *p*-coumaric acid, and their percentage should be measured.

Knowledge on active components and plant sources of propolis could lead to the formulation of a range of propolis types based on botanical origin, for example, "European," "North Russian," and varieties of "Brazilian."

Based on these considerations, it can be said that a propolis sample of good quality must have the following characteristics:

1. Be free of toxic contaminants
2. Contain acceptably low percentages of wax, insoluble matter, and ash
3. Be of a defined plant source determining the type of active compounds in it
4. Contain a high percentage of these active compounds

Unfortunately, such a system of quality control still does not exist, partly because of incomplete information about plant sources for propolis in tropical countries, and partly because there is insufficient qualitative data on flavonoid content and phenolic acid esters content in European and North American propolis. This data is needed to define a range of these substances to guarantee the desired biological effect; the formulation of a standard is based on a large number of measurements. The elaboration of a particular criterion for quality control of propolis remains a challenge to propolis researchers all over the world, but it is a challenge that is being met.