Important Developments in Recent Propolis Research

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Introduction

Bees have been in existence for over 125 millions of years, their evolutionary success has allowed them to become perennial species that can exploit virtually all habitats in the world. This success in the animal kingdom is largely because of the chemistry and application of bee products: honey, beeswax, venom, propolis, pollen and royal jelly. Being the most important "chemical weapon" of bees against pathogen microorganisms, propolis has been used as a remedy by humans since ancient times.

For this reason propolis has become the subject of intense pharmacological and chemical studies for the last 30 years. As a result, a lot of useful knowledge was gathered. However, it is important to note that in the last decade the paradigm concerning propolis chemistry radically changed. In the 1960's, propolis was thought to be of very complex, but more or less constant chemistry, like beeswax or bee venom (Lindenfelser, 1967: Kivalkina, 1969). In the following years, the analysis of numerous samples from different geographic regions lead to the disclosure that the chemical composition of bee glue is highly variable. This circumstance was soon understood by seasoned chemists, such as Popravko (1978) and Ghisalberti (1978). Nevertheless, most of the scientists studying biological properties of propolis continued to assume that the term "propolis" was a determinative with respect to chemical composition as the botanical name for a medicinal plant. Numerous studies, carried out with the combined efforts of phytochemists and pharmacologists, led in the recent years to the idea that different propolis samples could be completely different in their chemistry and biological activity.

Chemical diversity of propolis creates problems in its biological studies

To understand what causes these differences, it is necessary to remember where propolis comes from. For "manufacturing" of propolis, bees use 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: lipophilic materials on leaves and leaf buds, gums, resins, lattices, etc. (Crane, 1980). The plant origin of propolis determines its chemical diversity. Bee glue's chemical composition depends on the specificity of the local flora at the site of collection and thus on the geographic and climate characteristics of this site. This fact results in the striking diversity of propolis chemical composition, especially of propolis originating from tropical regions.

Nowadays, it is well documented that in the temperature zone all over the world, the main source of bee glue are the resinous exudates of the buds of poplar trees (genus Populus, section Aigeiros) (Bankova et al., 2000). For this reason European propolis contains the typical "poplar bud" phenolics: flavanoid aglycones (flavones and flavanones), phenolic acids and their esters (Bankova et al., 2002). Poplar trees are common only in the Temperate zone; they cannot grow in tropical and subtropical regions. For this reason in these habitats, bees have to find other plant sources of propolis to replace their beloved poplar. As a result, propolis from tropical regions has a different chemical composition than poplar type propolis. In the last decade Brazilian propolis attracted both commercial and scientific interest. The main source of Brazilian bee glue turned out to be leaf resin of Bacccharis dracunculifolia (Marcucci and Bankova, 1999, Kumazawa et al., 2003). Among the main compound classes found in Brazilian propolis are prenylated derivatives of p-coumaric acid and of acetophenone. Diterpenes, lignans and flavanoids (different from those in "poplar type" propolis) have also been found (Marcucci and Bankova, 1999). However, in Brazil several types of propolis were registered in a recent study by Park et al. (2002) coming from plant sources different from B. dracunculifolia and containing compounds other than the above-mentioned. Recently the chemistry of Cuban propolis awakened the attention of scientists. Its main components are polyisoprenylated benzophenones and this makes Cuban propolis different from European and from Brazilian bee glue. The plant source of this propolis type was detected to be the floral resin of Clusia rosea, where the prenylated benzophenones came from (Cuesta Rubio et al., 2002). There is no doubt that in other ecosystems propolis plant sources and respectively the chemical composition of propolis will continue to surprise scientists.

The distinct chemistry of propolis from different origin leads to the expectation that the biological properties of different propolis types will be dissimilar. However, in most cases this is not the truth! Actually, propolis is the defence of bees against infections and antibacterial and antifungal activity of all samples is not surprising. The similarity in other types of activity is less obvious but it is a fact. Of course, the responsible compounds are different, as it is presented in Table 1.

The only exception seems to be the allergenic property of European (poplar type) propolis. This issue needs detailed investigations. Until now, no studies have been performed to find out if other propolis types have allergenic properties. It is very tempting to search for propolis that causes no contact allergy or causes it much more seldom.

The fact that different chemistry leads to the same type of activity and in some cases even to activity of the same order of magnitude is amazing. Nonetheless, it is important to have detailed and reliable comparative data on every type of biological activity, combined with chemical data, in order to decide if some specific areas of application of a particular propolis type can be formulated as preferable. The biological tests have to be performed with chemically well characterized, and if possible, chemically standardized propolis. Such detailed comparative investigations are a challenge to propolis researchers. The most important recent developments in propolis research are those ones, which are aimed to meet this particular challenge.

Most important recent developments in propolis research

Biological studies combined with chemical analyses

More and more publications appear, which combine antimicrobial and other biological tests with chemical analyses, most often GC-MS (Velikova et al., 2000; Keskin et al., 2001, Hegazi et al., 2001; Hegazi et al., 2002; Abd El Hady et al., 2002; Yildirim et al., 2004) or HPLC (Laurentis et al., 2002; Santos et al., 2002). In a recent work, qualitative chemical characterization of the samples tested for antibacterial activity was combined with quantification of the major groups of biologically active substances of the corresponding samples (Popova et al., 2004).

Bioassay guided studies

Studies based on bioassay-guided chemical analysis represent a very promising trend in propolis research. Using this approach, Chen et al. (2003) isolated two new cytotoxic prenylflavones from Taiwanese propolis. Both compounds demonstrated cytotoxic properties on three cancer cell lines and also were potential radical scavengers (DPPH). Banskota et al., (2002) isolated the active components from Netherlands propolis with antiproliferative activity in cancer cell lines: caffeic acid phenethyl ester (CAPE) and several analogues, including two new glyceryl esters of substituted cinnamic acids. The same compounds were found by Nagaoka et al., (2003) to be responsible for the nitric oxide inhibiting activity of Netherlands propolis. Usia et al. (2002) isolated form Chinese propolis a number of compounds with antiproliferative activity. Most of them were known as “poplar propolis” constituents but among them were two new flavonoids: 2-methylbutyrouylpinopbanskin and 6-cinnamychrysin. Banskota et al. (2001) studied Brazilian propolis in order to identify the substances with hepatoprotective activity and those active against Helicobacter pillory. They found that these activities were due mainly to phenolic components but diterpenic acids contributed also to hepatoprotective activity. Several anti-HIV compounds, derivatives of moronic acid, and a new triterpenoid called melliferon were isolated from Brazilian bee glue by Ito et al. (2001). The major component of Cuban red propolis, the prenylated benzophenone nemorosone, was found to possess cytotoxic activity against several tumor cell lines and radical scavenging action (Cuesta-Rubio et al., 2002).

Comparative biological studies of propolis from different origin and chemical composition

Perhaps the most interesting trend in recent propolis research is the comparative study of biological properties of propolis from different geographic location and different chemical composition. The number of this type of study is yet limited. Kujumgiev et al. (1999) compared the antimicrobial (Antibacterial, antifungal, antiviral) activity of propolis from diverse geographic origin. The results presented an unambiguous proof that in spite of the great differences in the chemical composition of propolis from different geographic locations, all samples exhibit significant antibacterial and antifungal (and most of them antiviral) activity. This study clearly demonstrated that in different samples, different substance combinations are essential for the biological activity of bee glue. Trying to develop this comparative approach, Popova et al. (2004b) searched for statistically significant correlation between biological activity and geographic origin of propolis samples. ANOVA was used to compare the antibacterial action of three groups of bee glue: European, Brazilian and from Central America. The results showed that propolis from Europe and Brazil had similar activity despite the drastic differences in chemical composition. Their antibacterial activity was significantly higher than that of Central American propolis. The ANOVA was applied also to compare the toxicity of the same three propolis groups to A. salina nauplii. In this case, there was no significant correlation between geographic origin and potential cytotoxicity. This demonstrates that the search for new promising cytotoxic compounds in new propolis sources is reasonable.

The cytotoxic, hepatoprotective and free radical scavenging activity of propolis from Brazil, Peru, The Netherlands and China was compared by Banksota et al. (2000). They found that propolis from the Netherlands and China possessed the strongest cytotoxic activity; while almost all samples possessed significant hepatoprotective activity. The scavenging activity against DPPH free radicals of all samples was similar, only the Peruvian sample showed weak scavenging activity.

The work of Kumazawa et al. (2004) goes further. The authors compared the antioxidant activity of propolis of various geographic origin (Argentina, Austria, Brazil, Bulgaria, Chile, China, Hungary, New Zealand, South Africa, Thailand, Ukraine, Uruguay, the US and Uzbekistan) and combined this data with chemical analyses. Major constituents of the samples tested were identified by HPLC analysis with photo-diode array and mass spectrometric detection. Seventeen phenolic compounds in 16 kinds of propolis were identified and quantified by HPLC. Propolis with strong antioxidant activity contained antioxidative compounds such as kaempferol and phenethyl caffeate.

Such comparative studies are extremely valuable with respect to propolis standardization and practical applications in therapy. It is our hope that in the near future their number is going to grow significantly. They will enable scientists to connect a particular chemistry to a specific type of biological activity and will help people to efficiently use the valuable bee product propolis.