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Review

Recent trends and important developments in propolis research

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The newest developments in propolis pharmacological research are summarized. The problem regarding biological studies, caused by the chemical variability of propolis, is discussed. The most important trends and developments in recent propolis research are outlined: biological studies performed with chemically characterized samples, bioassay-guided studies of active principles and comparative biological studies of propolis of different origin and chemical composition. These types of studies are extremely valuable with respect to propolis standardization and practical applications in therapy. They will allow scientists to connect a particular chemical propolis type to a specific type of biological activity and formulate recommendations for practitioners.

Keywords: propolis – plant origin – bioactive compounds – composition/activity relationship

Introduction

Bees have been in existence for >125 million years and their evolutionary success has allowed them to become perennial species that can exploit virtually all habitats on Earth. This success is largely because of the chemistry and application of the specific products that bees manufacture: honey, beeswax, venom, propolis, pollen and royal jelly. As the most important 'chemical weapon' of bees against pathogenic microorganisms, propolis has been used as a remedy by humans since ancient times. It is still one of the most frequently used remedies in the Balkan states (1), applied for treatment of wounds and burns, sore throat, stomach ulcer, etc.

For this reason, propolis has become the subject of intense pharmacological and chemical studies for the last 30 years. As a result, much useful knowledge has been gathered. However, it is important to note that in the last decade, the paradigm concerning propolis chemistry radically changed. In the 1960s, propolis was thought to be of very complex, but more or less constant chemistry, like beeswax or bee venom (2,3). In the following years, analysis of numerous samples from different geographic regions led to the disclosure that the chemical composition of bee glue is highly variable. This circumstance was soon understood by seasoned chemists, such as Popravko (4) and Ghisalberti (5). Nevertheless, most of the scientists studying the biological properties of propolis continued to assume that the term 'propolis' was as 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 recent years to the idea that different propolis samples could be completely different in their chemistry and biological activity.

The Problem of Chemical Variability of Propolis

To understand what causes the differences in chemical composition, it is necessary to keep in mind the plant origin of propolis. For propolis production, bees use materials resulting from 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, latices, etc. (6). 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

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climatic 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 temperate zone all over the world, the main source of bee glue is the resinous exudate of the buds of poplar trees, mainly the black poplar *Populus nigra* (7). For this reason, European propolis contains the typical 'poplar bud' phenolics: flavonoid aglycones (flavones and flavanones), phenolic acids and their esters (8). 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 from that of 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 the leaf resin of *Baccharis dracunculifolia* (9,10). Among the main compound classes found in Brazilian propolis are prenylated derivatives of *p*-coumaric acid and of acetophenone. Diterpenes, lignans and flavonoids (different from those in 'poplar type' propolis) have also been found (9). However, in Brazil, several types of propolis were registered in recent studies (11,12), that come from plant sources different from *B.dracunculifolia* and containing compounds other than those mentioned above. Recently the chemistry of Cuban propolis caught the attention of scientists. Its main components are polyisoprenylated benzophenones, and this makes Cuban propolis different from both European and Brazilian bee glue. The plant source of this propolis type was detected to be the floral resin of *Clusia rosea*, from whence came the prenylated benzophenones (13). There is no doubt that in other ecosystems, propolis plant sources and the chemical composition of propolis will continue to surprise scientists.

The distinct chemistry of propolis from different origins leads to the expectation that the biological properties of different propolis types will be dissimilar. However, in most cases. this is not true! Actually, propolis is the defense of bees against infections, and the antibacterial and antifungal activity of all samples is not surprising. The similarity in many of the other types of activity is less obvious but it is a fact. Of course, the responsible compounds are different, as shown in Table 1: mainly favanones, flavones, phenolic acid and their esters in poplar type (European) propolis, prenylated *p*-coumaric acis and diterpenes in *Baccharis* type (Brazilian) propolis; prenylated benzophenones in Cuban red propolis, etc.

The only exception seems to be the allergenic property of European (poplar type) propolis. This problem 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 less often.

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 which are aimed at meeting this particular challenge.

Important Trends and Developments in Recent Propolis Research

Biological Studies Performed with Chemically Characterized Samples

More and more publications are appearing which combine antimicrobial and other biological studies with chemical analyses of the tested propolis samples. The most often used techniques for chemical analyses are gas chromatography–mass spectrometry (GC–MS) (18–24) and high-performance liquid chromatography (HPLC) (25–27). 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 (28). The use of chemically characterized propolis samples for biological experiments is the only

Table 1. Compounds responsible for the biological activity of different propolis types

Propolis type	Antibacterial activity	Antiinflammatory activity	Antitumor activity	Hepatoprotective activity	Antioxidant activity	Allergenic action
European (poplar type)	Flavanones, flavones, phenolic acids and their esters (14)	Flavanones, flavones, phenolic acids and their esters (15)	Caffeic acid phenethyl ester (16)	Caffeic acid, ferulic acids acid, caffeic acid phenethyl ester (15)	Flavonoids, phenolic and their esters (15)	3,3-Dimethylallyl caffeate (14)
Brazilian (*Baccharis* type)	Prenylated *p*-coumaric acis, labdane diterpenes (15)	Unidentified (15)	Prenylated *p*-coumaric acids, clerodane diterpenes, benzofuranes (15)	Prenylated *p*-coumaric acis, flavonoids, lignans, caffeoyl quinic acids (15)	Prenylated *p*-coumaric acis, flavonoids (15)	Not tested
Cuban	Prenylated benzophenones (17)	Not tested	Prenylated benzophenones (13)	Unidentified (15)	Prenylated benzophenones (13)	Not tested
Taiwanese	Not tested	Not tested	Prenylated flavanones (42)	Not tested	Prenylated flavanones (42)	Not tested

meaningful way to study the biological and pharmacological activities of bee glue at the beginning of the third millennium.

Bioassay-guided Studies of Active Principles

Studies based on bioassay-guided chemical analysis represent a very promising trend in propolis research. Using this approach, Chen *et al*. isolated two new cytotoxic prenylflavones from Taiwanese propolis (29). Both compounds demonstrated cytotoxic properties on three cancer cell lines and also were potential radical scavengers – radicals of 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Banskota *et al*. (30) isolated the active components from Netherlands propolis with antiproliferative activity in cancer cell lines: caffeic acid phenethyl ester (CAPE) and several analogs, including two new glyceryl esters of substituted cinnamic acids. The same compounds were found by Nagaoka *et al*. (31) to be responsible for the nitric oxide-inhibiting activity of Netherlands propolis. Usia *et al*. (32) isolated from Chinese propolis a number of compounds with antiproliferative activity. Most of them were known 'poplar propolis' constituents, but among them were two new flavonoids: 2-methylbutyrouylpinopbanskin and 6-cinnamylchrysin. From Greek propolis, the new flavanone derivative 7-prenylpinocembrin has been isolated, together with totarol and 7-prenylistrobopinin, as important antibacterial principles (33).

Banskota *et al*. (34) studied Brazilian propolis in order to identify the substances with hepatoprotective activity and those active against *Helicobacter pylori*. They found that these activities were due mainly to phenolic components, but diterpenic acids also contributed to hepatoprotective activity. Several anti-HIV compounds, derivatives of moronic acid, and a new triterpenoid called melliferon were isolated from Brazilian bee glue (35). The major component of Cuban red propolis, the prenylated benzophenone nemorosone, was found to possess cytotoxic activity against several tumor cell lines and to have radical scavenging action (13).

Comparative Biological Studies of Propolis of 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 locations and different chemical composition. The number of this type of studies is as yet limited. Kujumgiev *et al*. (36) compared the antimicrobial (antibacterial, antifungal and antiviral) activity and chemical composition of propolis from diverse geographic origins. The results presented 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 combinations of substances are essential for the biological activity of bee glue. Trying to develop this comparative approach, Popova *et al*. (37) searched for a statistically significant correlation between biological activity and geographic origin of propolis samples. Analysis of variance (ANOVA) was used to compare the antibacterial action of three groups of bee glue: European, Brazilian and Central American. 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 also applied to compare the toxicity of the same three propolis groups with *Artemia salina* (Crustaceae). 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*. (38). 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 weaker scavenging activity.

Salomao *et al*. (39) compared the microbicidal activity of Brazilian and Bulgarian propolis and analyzed their chemical composition by High Temperature – High Resolution Gas Chromotography – Mass Spectrometry (HT-HRGC-MS), and found that although they were of totally distinct compositions, they were active against *Trypanozoma cruzi* and some pathogenic fungi.

The work of Kumazawa *et al*. (40) is an excellent example of this approach. The authors compared the antioxidant activity of propolis of various geographic origins (Argentina, Austria, Brazil, Bulgaria, Chile, China, Hungary, New Zealand, South Africa, Thailand, Ukraine, Uruguay, the USA and Uzbekistan) and combined these data with chemical analyses. Major constituents of the samples tested were identified by HPLC 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. In the same way, antioxidant activities and chemical constituents of propolis from different regions of Japan were compared by the same research group (41). They concluded that strong antioxidant activity correlated with a high concentration of caffeic acid and phenethyl caffeate. In addition, propolis from Okinawa was found to have antioxidants not seen in propolis from other areas.

Following a similar model, Chen *et al*. (42) compared the radical scavenging activity, cytotoxic effects and apoptosis induction in human melanoma cells of Taiwanese propolis from different locations. Propolins (C-prenylated flavanones) in the samples were detected by HPLC, and the total phenolic content was determined by spectrophotometry. The high concentration of propolins was found to be essential for the apoptosis induction in human melanoma cells and for the antiradical properties.

Conclusion

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 allow scientists to connect a particular chemical propolis type to a specific type of biological activity and formulate recommendations for the practitioners. This could help the general public to make more efficient use of the beneficial properties of propolis with respect to CAM.

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