

Perspective paper

# Chemical diversity of propolis and the problem of standardization

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## Abstract

Chemical variability of propolis is discussed with respect to the problem of standardization. Several chemical types of propolis are formulated, based on their plant source. Reliable criteria for chemical standardization of different propolis types are needed but such generally accepted criteria do not yet exist. The chemical profile of “poplar” propolis, typical for the temperate zone, can be characterized by the following parameters: total flavone and flavonol content, total flavanone and dihydroflavonol content, and total phenolics content. These parameters correlate better with the biological activity and are more informative than the quantification of individual components. There is still a lot of work to be done to achieve standardization of other propolis types. Working with standardized material will allow scientists to connect a particular chemical propolis type to a specific type of biological activity and formulate recommendations for mainstream practitioners.

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**Keywords:** Propolis; Plant origin; Biological activity; Standardization

## 1. Introduction

Propolis (bee glue) is a sticky dark-colored material that honeybees collect from plants and use it in the hive: they apply it to seal the walls, to strengthen the borders of combs, to embalm dead invaders. Propolis is not only a building material, it is the most important “chemical weapon” of bees against pathogen microorganisms and 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 (Wollenweber et al., 1990), applied for treatment of wounds and burns, soar throat, stomach ulcer, etc.

Because of its popularity in folk medicine, propolis has become the subject of intense pharmacological and chemical studies for the last 30 years. Numerous studies have proven its versatile pharmacological activities: antibacterial, antifungal, antiviral, antiinflammatory, hepatoprotective, antioxidant, antitumor, etc. (Banskota et al., 2001). A significant number of papers dealing with propolis chemistry were also published and researchers began to understand that

its chemical composition was highly variable and depended on the local flora at the site of collection (Marcucci, 1995; Bankova et al., 2000). Although the biological activity of bee glue and especially its activity against microorganisms was always present, in samples from different geographic and climatic zones this activity was the result of completely different chemical composition (Kujumgiev et al., 1999). It turned out that the term “propolis” is not characterizing with respect to the chemical composition, unlike the term “bee venom” for example. The question arises if there was any sense in biological studies carried out with just “propolis” without any chemical characteristic of the material used. Deliberately, it became clear that comparing propolis samples from different regions of the world (e.g. Bulgaria and Brazil) might be the same as comparing extracts of two plants that belong to different plant families. As a result, recently almost every publication on propolis biological activity includes some kind of chemical characterization of the bee glue used (Bankova, 2005). However, in order to be accepted officially into the main stream of the healthcare system, propolis needs chemical standardization that guarantees its quality, safety, and efficacy. And here comes the question.

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## 2. Is it possible to standardize something as inconstant as propolis?

Bee glue is a plant derived product and it has been proved that bees do not change its chemical composition (Bankova et al., 2000). Therefore, it is completely reasonable to approach the problem of propolis standardization in the same way as it is done for medicinal plants. In order to establish relevant quantitative criteria for quality in medicinal plants and extracts therefrom, different concepts have to be followed depending on the available knowledge on the active principle(s) (Bauer, 1998). If the active principles are known and accepted, they have to be quantified using an appropriate analytical method. If the active compounds are not known or still under discussion, the total extract is regarded as the “active principle” and in that case marker compounds must be used for quality control. In the case of propolis, a lot of knowledge has already been gathered on active components and one of the most important active principles was found to be CAPE (caffeic acid phenethyl ester) (Banskota et al., 2001). But how could CAPE possibly be used for standardization if most tropical samples do not contain even traces of it? The same is true for many other active propolis constituents. In such case, is universal chemical standardization possible for a product as changeable as propolis? The obvious answer is no. Is any standardization of propolis possible at all? The answer is yes, if we formulate different propolis types according to their plant source and the corresponding chemical profile.

## 3. Propolis chemical types, determined by its plant origin

The materials available to bees for “manufacturing” of propolis are substances actively secreted by plants as well as substances exuded from wounds in plants: lipophilic materials on leaves and leaf buds, resins, mucilages, gums, lattices, etc. (Crane, 1988). The composition of the plant source determines the chemical composition of bee glue. Combined with the knowledge of active principles, it gives clues to standardization and quality control, allowing the specification of propolis types that have distinct chemical composition. The present knowledge on most important biologically active chemical constituents of propolis from different geographic locations and the corresponding plant sources is represented in Table 1. This table defines chemical types of propolis which have to be regarded as distinct entities in the process of standardization and quality control. It is important to remember that conclusions concerning the biological activity of one of these propolis types can by no means be automatically transferred to another one.

In Table 1, the most studied propolis types are mentioned. Of course, there are many other propolis source plants and corresponding chemical types of propolis. For example, the one found in some Mediterranean regions (Sicily, the Adriatic coast) has as main components diterpenic acids (Trusheva et

Table 1  
Propolis types according to their plant origin and their chemical composition

Propolis type	Geographic origin	Plant source	Main biologically active substances	References
Poplar propolis	Europe, North America, non-tropic regions of Asia	<i>Populus</i> spp. of section <i>Aigeiros</i> , most often <i>P. nigra</i> L.	Flavones, flavanones, cinnamic acids and their esters	Nagy et al. (1986), Greenaway et al. (1990), Bankova et al. (2000)
Birch propolis	Russia	<i>Betula verrucosa</i> Ehrh.	Flavones and flavonols (not the same as in poplar propolis)	Popravko and Sokolov (1980)
Green (alecrim) propolis	Brazil	<i>Baccharis</i> spp., predominantly <i>B. dracunculifolia</i> DC.	Prenylated <i>p</i> -coumaric acids, diterpenic acids	Marcucci and Bankova (1999)
Red (Clusia) propolis	Cuba, Venezuela	<i>Clusia</i> spp.	Polyprenylated benzophenones	Cuesta Rubio et al. (2002), Trusheva et al. (2004)
“Pacific” propolis	Pacific region (Okinawa, Taiwan)	Unknown	C-prenylflavanones	Chen et al. (2003), Kumazawa et al. (2004)
“Canarian” propolis	Canary Islands	Unknown	Furofuran lignans	Christov et al. (1999)

al., 2003); in Brazil, 11 propolis types other than Alecrim (green) propolis have been described, but their occurrence is much more restricted (Park et al., 2002), etc.

Anyway, it is important for researchers studying biological activity of propolis to be aware of the existence of the problem and to be able to distinguish between different propolis types. It is essential 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. Reliable criteria for chemical standardization of different propolis types are needed. However, such generally accepted criteria do not yet exist for any propolis type. Our recent studies were directed towards the possibilities to standardize poplar type propolis.

#### 4. Standardization of poplar type propolis based on biologically active substances

Undoubtedly, poplar type propolis is the most profoundly studied and the best known type of bee glue, both from chemical and pharmacological point of view. It is important for propolis users, such as companies producing propolis preparations, or scientists performing any type of biological studies on propolis, to be able to recognize this propolis type. In our laboratory we developed a simple test for identification of poplar type propolis. Based on present knowledge of the chemical composition of poplar bud exudates (Nagy et al., 1986; Greenaway et al., 1990; Bankova et al., 2000), we chose seven phenolic compounds as markers and developed a rapid TLC procedure allowing us to tell the poplar samples from all the other ones (Popova et al., 2003).

If a particular sample has been identified as one of poplar origin, its main active components are known. However, any attempt to measure the concentration of the active principles faces the fact that more than 25 individual phenolics in poplar propolis were found to possess different types of biological activities (Marcucci, 1995; Banskota et al., 2001). Moreover, as is evident from the literature (especially concerning antimicrobial activity) it is not possible to ascribe the activity solely to one individual component (Kujumgiev et al., 1999). Attempts to correlate the concentrations of individual constituents with the biological activity of poplar propolis failed: Bonvehi et al. (1994), while studying the correlation between antibacterial activity (expressed as minimum inhibitory concentration, MIC, against *S. aureus*) and percentage of various active constituents, found that no individual compound surpassed Pearson–Lee value. We assume, therefore, that quantification of active compounds into groups having the same or close chemical structure is more promising.

The chemical profile of poplar propolis can be characterized by the three parameters: total flavone and flavonol

content, total flavanone and dihydroflavonol content, and total phenolics content. We developed and validated rapid, low-cost spectrophotometric procedures for quantification of the three main groups of bioactive substances in poplar type propolis (Popova et al., 2004). The spectrophotometric assay based on the formation of aluminium chloride complex was applied for quantification of total flavones/flavonols. Because of the high amount of flavanones and dihydroflavonols in “poplar” propolis, the introduction of a distinct procedure for their quantification was considered of special significance and the colorimetric method with DNP (2,4-dinitrophenylhydrazine) was applied for the purpose. Total phenolics content was measured by the Folin–Ciocalteu procedure. The procedures were validated by using a model mixture of 14 compounds representing the poplar propolis composition as found in previous studies. The accuracy (recovery) varied in the range 84–109%, and the relative standard deviation was 0.5–6.2%. The developed spectrophotometric procedures were applied to real poplar propolis samples. The results were verified independently by a HPLC procedure. The two sets of results agreed satisfactory, as proven by Student’s *t*-test (Popova et al., 2004).

Having these validated methods, we analyzed a relatively large number of poplar propolis samples from different regions of Europe and the Middle East—a total of 114 (Popova et al., 2005), and tested the samples also for their antibacterial activity (MIC against *S. aureus*). The large number of analyzed samples gives us the opportunity to formulate the characteristics of a “typical poplar sample”, based on statistics: flavones/flavonols  $8 \pm 4\%$ , flavanones/dihydroflavonols  $6 \pm 2\%$ , total phenolics  $28 \pm 9\%$ , MIC  $211 \pm 132 \mu\text{g/ml}$ .

Processing the data, we found a significant negative correlation between the concentration of total phenolics in propolis balsam and MIC: the greater the concentration, the lower the MIC ( $P = 0.003$ ). Obviously, the percentage of total phenolics correlates better with the biological activity and is more informative than the quantification of individual components. This fact supports our concept that measuring the concentrations of groups of active compounds instead of that of individual components is the right approach in the case of propolis.

#### 5. Conclusion

Evidently, the approach based on typification according to the plant source gives good results in the field of propolis standardization. There is still a lot of work to be done by researchers to achieve a reliable standardization of propolis types other than poplar type. This is especially important with respect to the reliability of the results obtained in studies on propolis biological activities. Working with standardized material will allow scientists to connect a particular chemical propolis type to a specific type of biological activity and formulate recommendations for mainstream practitioners. This

could help the general public to make more efficient use of the beneficial properties of propolis.

## References

- Bankova, V., 2005. Recent trends and important developments in propolis research. *Evidence Based Complementary and Alternative Medicine* 2, 29–32.
- Bankova, V., de Castro, S.L., Marcucci, M.C., 2000. Propolis: recent advances in chemistry and plant origin. *Apidologie* 31, 3–15.
- Banskota, A.H., Tezuka, Y., Kadota, Sh., 2001. Recent progress in pharmacological research of propolis. *Phytotherapy Research* 15, 561–571.
- Bauer, R., 1998. Quality criteria and standardization of phytopharmaceuticals: can acceptable drug standards be achieved. *Drug Information Journal* 32, 101–110.
- Bonvehí, J.S., Coll, F.V., Jorda, R.E., 1994. The composition, active components and bacteriostatic activity of propolis in dietetics. *Journal of the American Oil Chemists Society* 71, 529–532.
- Chen, Ch., Wu, Chi., Shy, H., Lin, J., 2003. Cytotoxic prenylflavones from Taiwanese propolis. *Journal of Natural Products* 66, 503–506.
- Christov, R., Bankova, V., Tsvetkova, I., Kujumgiev, A., Delgado Tejera, A., 1999. Antibacterial furofuran lignans from Canary Islands propolis. *Fitoterapia* 70, 89–92.
- Crane, E., 1988. *Beekeeping: Science, Practice and World Resources*. Heinemann, London.
- Cuesta Rubio, O., Frontana-Urribá, B.A., Ramirez-Apan, T., Cardenas, J., 2002. Polyisoprenylated benzophenones in Cuban propolis; biological activity of nemorosone. *Zeitschrift fuer Naturforschung* 57c, 372–378.
- Greenaway, W., Scaysbrook, T., Whately, F.R., 1990. The composition and plant origin of propolis: a report of work at Oxford. *Bee World* 71, 107–118.
- Kujumgiev, A., Tsvetkova, I., Serkedjieva, Yu., Bankova, V., Christov, R., Popov, S., 1999. Antibacterial, antifungal and antiviral activity of propolis from different geographic origin. *Journal of Ethnopharmacology* 64, 235–240.
- Kumazawa, Sh., Goto, H., Hamasaka, T., Fukumoto, S., Fujimoto, T., Nakayama, Ts., 2004. A new prenylated flavonoid from propolis collected in Okinawa, Japan. *Biosciences, Biotechnology, Biochemistry* 68, 260–262.
- Marcucci, M.C., 1995. Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie* 26, 83–99.
- Marcucci, M.C., Bankova, V.S., 1999. Chemical composition, plant origin and biological activity of Brazilian propolis. *Current Topics in Phytochemistry* 2, 115–123.
- Nagy, E., Papay, V., Litkei, G., Dinya, Z., 1986. Investigation of the chemical constituents, particularly the flavonoid components, of propolis and *Populi gemma* by the GC/MS method. *Studies in Organic Chemistry (Amsterdam)* 23, 223–232.
- Park, Y.K., Alenkar, S.M., Aguiar, C.L., 2002. Botanical origin and chemical composition of Brazilian propolis. *Journal of Agricultural and Food Chemistry* 50, 2502–2506.
- Popova, M., Bankova, V., Butovska, D., Petkov, V., Damyanova, B., Sabatini, A.G., Marcazzan, G.L., Bogdanov, S., 2003. Poplar type propolis and analysis of its biologically active components. *Honeybee Science* 24, 61–66.
- Popova, M., Bankova, V., Butovska, D., Petkov, V., Nikolova-Damyanova, B., Sabatini, A.G., Marcazzan, G.L., Bogdanov, S., 2004. Validated methods for quantification of biologically active constituents of “poplar type” propolis. *Phytochemical Analysis* 15, 235–240.
- Popova, M., Bankova, V., Sabatini, A.G., Marcazzan, G.L., Bogdanov, S., in preparation.
- Popravko, S.A., Sokolov, M.V., 1980. Plant sources of propolis. *Pchelovodstvo* 28/29 (in Russian).
- Trusheva, B., Popova, M., Bankova, V., Tsvetkova, I., Naydensky, C., Sabatini, A.G., 2003. A new type of European propolis, containing bioactive labdabes. *Rivista Italiana E.P.P.O.S.* 36, 3–7.
- Trusheva, B., Popova, M., Naydenski, H., Tsvetkova, I., Rodriguez, J.G., Bankova, V., 2004. New polyisoprenylated benzophenones from Venezuelan propolis. *Fitoterapia* 75, 683–689.
- Wollenweber, E., Hausen, B.M., Greenaway, W., 1990. Phenolic constituents and sensitizing properties of propolis, poplar balsam and balsam of Peru. *Bulletin de Groupe Polyphenols* 15, 112–120.