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 arouse 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.
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, latices, 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
characterized by the three parameters: total flavone and flavonol ing.

However, the same or close chemical structure is more promis-

ing. Therefore, that quantification of active compounds into groups

vidual compound surpassed Pearson–Lee value. We assume,

percentage of various active constituents, found that no indi-

imum inhibitory concentration, MIC, against

correlation between antibacterial activity (expressed as min-

dation of propolis, to be able to recognize this propolis type. In

material will allow scientists to connect a particular chemical

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 chem-

cal and pharmacological point of view. It is important for

propolis users, such as companies producing propolis prepara-

tions, 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

test to measure the concentration of the active princi-

ples 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 con-

cerning 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: Bonhehi et al. (1994), while studying the

correlation between antibacterial activity (expressed as min-

imum inhibitory concentration, MIC, against S. aureus) and

percentage of various active constituents, found that no indi-

vidual compound surpassed Pearson–Lee value. We assume,

therefore, that quantification of active compounds into groups

having the same or close chemical structure is more promis-

ing.

The chemical profile of poplar propolis can be charac-

terized by the three parameters: total flavone and flavonol

content, total flavone 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 pro-

cedure 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 pro-

cedure. The procedures were validated by using a model

mixture of 14 compounds representing the poplar propo-

lis composition as found in previous studies. The accu-

racy (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

satisfactorily, as proven by Student’s t-test (Popova et al.,

2004).

Having these validated methods, we analyzed a rela-

tively large number of poplar propolis samples from dif-

ferent 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 opportu-

nity to formulate the characteristics of a “typical poplar

sample”, based on statistics: flavones/flavonols 8 ± 4%,

flavanones/dihydroflavonols 6 ± 2%, total phenolics 28 ± 9%,

MIC 211 ± 132 µg/ml.

Processing the data, we found a significant negative corre-

lation 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 infor-

mative that the quantification of individual components. This

fact supports our concept that measuring the concentra-

tions 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 propo-

lis standardization. There is still a lot of work to be done

by researchers to achieve a reliable standardization of propo-

lis 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 for-

mulate recommendations for mainstream practitioners. This
could help the general public to make more efficient use of the beneficial properties of propolis.

References


