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 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

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...
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 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

| Propolis type   | Antibacterial activity | Antiinflammatory activity | Antitumor activity | Hepatoprotective activity | Antioxidant activity | Allergenic action |
|-----------------|------------------------|---------------------------|--------------------|---------------------------|---------------------|-------------------|
| European (poplar type) | Flavanones, flavones, | Flavanones, flavones, | Caffeic acid phenethyl ester (16) | Caffeic acid, ferulic | Flavonoids, phenolic and their esters (15) | 3,3-Dimethylallyl caffeate (14) |
| Brazilian (Baccharis type) | prenylated *p*-coumaric | prenylated *p*-coumaric | Prenylated *p*-coumaric acids, clerodane diterpenes, benzofuranes (15) | Prenylated *p*-coumaric acids, flavonoids, lignans, caffeoyl quinic acids (15) | Prenylated benzophenones (13) | Not tested |
| Cuban | 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 prenyllavones 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-picrylhydrazyl (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 ‘propolis constituents’, but among them were two new flavonoids: 2-methylbutyroylpinopbanskin and 6-cinnamylchrysin. From Greek propolis, the new flavanone derivative 7-prenylpinocembrin has been isolated, together with totarol and 7-prenylstrobralin, 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 (Crustacea). 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 Banskota 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 microbiidal activity of Brazilian and Bulgarian propolis and analyzed their chemical composition by High Temperature – High Resolution Gas Chromatography – 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.

References

1. Wollenweber E, Hausen BM, Greenaway W. Phenolic constituents and sensitizing properties of propolis, poplar balsam and balsam of Peru. Bull Groupe Polysynthes 1990:15:112–20.
2. Lindenfelser LA. Antimicrobial activity of propolis. Am Bee J 1967:107: 90–2.
3. Kivalkina BP. Propolis: its antimicrobial and healing properties. PhD Dissertation, Kazan University, 1964 (in Russian).
4. Popravko SA. Chemical composition of propolis, its origin and standardization. In: A Remarkable Hive Product: PROPOLIS. Harnaj, V. (ed) Bucharest: Apimondia Publishing House, 1978, 15–8.
5. Ghisalberti EL. Propolis: a review. Z Naturforsch 2000;52c:82–88.
6. Hernandez NMR, Cuesta Rubio O, Aviles A, Avellanede DLS. Actividad antimicrobiana de Apis melifera. Chem Pharm Bull 2004;52:561–71.
7. Bankova VB, De Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. Apidologie 2000:31:13–15.
8. Bankova V, Popova M, Bogdanov S, Sabatini AG. Chemical composition of European propolis: expected and unexpected results. Z Naturforsch 2002;57c:530–3.
9. Marcusi MC, Bankova VS. Chemical composition, plant origin and biological activity of Brazilian propolis. Curr Top Phytochem 1999:2: 115–23.
10. Kumazawa Sh, Yoned M, Shibata I, Hamasaka T, Nakayama Ts. Direct evidence for the plant origin of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. Chem Pharm Bull 2003;51:740–2.
11. Park YK, Alencar SM, Aguiar CL. Botanical origin and chemical composition of Brazilian propolis. J Agric Food Chem 2002:50:2502–6.
12. Sawaya ACHF, Tomazela DM, Cunha IBS et al. Electrospray ionization mass spectrometry fingerprinting of propolis. Analyst 2004;129: 739–44.
13. Cuesta Rubio O, Fronata-Uriba BA, Ramirez-Apan T, Cardenas J. Polyisoprenylated benzophenones in Cuban propolis: biological activity of nemosorne. Z Naturforsch 2002;57c:372–8.
14. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol 1998:36:347–63.
15. Banskota AH, Tezuka Y, Kadota Sh. Recent progress in pharmacological research of propolis. Phytother Res 2000;15:561–71.
16. Grenburger D, Banerjee R, Eisinger K et al. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. Experientia 1988;44:230–2.
17. Hernandez NMR, Cuesta Rubio O, Aviles A, Avelanede DLS. Actividad antimicrobiana de nemosornea aislada de Clusia rosea. Rev Cubana Farm 2001:35 (Suppl Especial):197–9.
18. Valkova M, Bankova V, Sorkun K, Houcine S, Tsvetkova I, Kujumgiev A. Propolis from the Mediterranean region: chemical composition and antimicrobial activity. Z Naturforsch 2000;55c:790–3.
19. Keskin N, Hazir S, Basar HC. Kurbucoglu M. Antibacterial activity and chemical composition of Turkish propolis. Z Naturforsch 2001;56c: 1112–5.
20. Hegazi AG, Abd El Hady F. Egyptian propolis: 1—Antimicrobial activity and chemical composition of Upper Egypt propolis. Z Naturforsch 2001; 56c:82–88.
21. Hegazi AG, Abd El Hady F. Egyptian propolis: 3—Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. Z Naturforsch 2002:57c:395–402.
22. Abd El Hady F, Hegazi AG. Egyptian propolis: 2—Chemical composition, antiviral and antimicrobial activity of East Nile Delta propolis. Z Naturforsch 2002:57c:386–91.
23. Yildirim Z, Haciyavuzli S, Onur Kutlu N et al. Effect of water extract of Turkish propolis on tuberculosis infection in guinea-pigs. Pharmacol Res 2004;49:287–92.
24. Erdem BG, Olmez S. Inhibitory effect of Bursa propolis on dental caries formation in rats inoculated with Streptococcus sobrinus. Turk J Zool 2004;28:29–36.
25. De Laurentis N, Cafaarchia C, Lai O, Losacco V, Milillo MA. Chemical composition and biological investigation of Apulia region propolis. Riv Ital EPPOS 2002:34:29–41.
26. Santos FA, Bastos EMA, Uzedda M et al. Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. J Ethnopharmacol 2002:80:1–7.
27. Da Silva Cunha IB, Salomao K, Shimizu M et al. Antitrypanosomal activity of Brazilian propolis from Apis mellifera. Chem Pharm Bull 2004;52:602–4.
28. Popova M, Silici S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine 2004. In Press, corrected proof online, 5th November 2004.
29. Chen Ch, Wu, Chi, Shy H, Lin J. Cytotoxic prenylflavonones from Taiwanese propolis. J Nat Prod 2003;66:503–6.
30. Banskota AH, Nagaoka T, Sumioka LY et al. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. J Ethnopharmacol 2002;80:67–73.
31. Nagaoka T, Banskota AH, Tezuka Ya, Midoriakawa K, Matsuhide K, Kadota Sh. Caffeic acid phenethyl ester (CAPE): potent nitric oxide inhibitor from the Netherlands propolis. Biol Pharm Bull 2003;26:487–91.
32. Usia T, Banskota AH, Tezuka Ya, Midoriakawa K, Matsuhide K, Kadota Sh. Constituents of Chinese propolis and their antiproliferative activity. J Nat Prod 2002;65:673–76.
33. Mellieu E, Chinou I. Chemical analysis and antimicrobial activity of Greek propolis. Planta Med 2004;70:515–9.
34. Banskota AH, Tezuka Y, Adnyana IK, Midoriakawa K, Matsuhide K, Kadota Sh. Hepatoprotective and anti-Helicobacter pylori activities of constituents from Brazilian propolis. Phytomedicine 2001;8:16–23.
35. Ito J, Chang F, Wang H., Park YK, Ikegaki M, Kilgore N, Lee K. Anti-AIDS agents. 48. Anti-HIV activity of moronic acid derivatives and the new melliflore related triterprenoid isolated from Brazilian propolis. J Nat Prod 2001;64:1278–81.
36. Kujumgiev A, Tsvetkova I, Serkedjiwe Yu, Bankova V, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis from different geographic origins. J Ethnopharmacol 1999;64:235–40.
37. Popova M, Bankova V, Naydenksy Ch, Tsvetkova I, Kujumgiev A. Comparative study of the biological activity of propolis from different geographic origin: a statistical approach. Macedonian Pharm Bull 2004; 50:9–14.
38. Banskota AH, Tezuka Y, Adnyana IK et al. Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. J Ethnopharmacol 2000;72:239–46.
39. Salomao K, Borba CM, Campos LC, Machado DG, Aquino Neto FR, de Castro SL. Chemical composition and microbioidal activity of extracts from Brazilian and Bulgarian propolis. Lett Appl Microbiol 2004;38: 87–92.
40. Kumazawa Sh, Hamasaka T, Nakayama Ts. Antioxidant activity of propolis of various geographic origins. Food Chem 2004;84:329–39.
41. Hamasaka T, Kumazawa Sh, Fujimoto T, Nakayama Ts. Antioxidant activity and constituents of propolis collected in various areas of Japan. Food Sci Technol Res 2004;10:86–92.
42. Chen Ch, Weng M., Wu Ch, Lin J. Comparison of radical scavenging activity, cytotoxic effects and apoptosis induction in human melanoma cells by Taiwanese propolis from different sources. eCAM 2004;1:175–85.

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