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Plant Sources of Propolis: an Update from a Chemist's Point of View

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Dedicated to Professor E. Wollenweber on the occasion of his 65th birthday.

The newest developments in research on propolis plant sources are summarized. Special attention is paid to data based on reliable chemical evidence including comparison between propolis samples and plant material, and on well-documented bee behavior. A number of new proved propolis source plants are listed. Hypothetical sources, suggested as a result of comparison of propolis chemical composition and literature data about particular plants are also discussed.

Keywords: propolis, plant sources, taxonomic markers.

Propolis (bee glue), a honey bee product, has been used since ancient times as a remedy in Europe and the Middle East. It has recently gained popularity in all parts of the world as an alternative medicine, food additive for health amelioration and disease prevention. To produce it, bees collect vegetable material and mix it with wax. It is now generally accepted that bees collect propolis from resinous plant 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, for example mucilages, gums, resins, and latices [1]. In some cases bees also may cut fragments of vegetative tissues to use them in propolis production [2].

In her monograph, Crane [1] gives a long list of plants thought to be propolis sources in different parts of the world. It is important to note that this list is based principally on observations of bee behavior and only in a few cases, on comparative chemical analysis of propolis and plant materials. This list must be regarded only as preliminary, unconfirmed information, because propolis collection is a relatively rare activity of honeybees and often takes place high up in the trees, so is difficult to be observed [1]. Much more conclusive evidence could be found in the chemical composition of propolis by comparing it with plant materials collected in the vicinity of the hives. This approach was introduced by the works of Popravko [3] in Russia and Lavie in France [4], dating back to the 1970s. They analyzed propolis flavonoid composition and compared it to birch and poplar bud exudates, respectively. This line of research proved to be successful: many similar publications followed and now it is generally accepted and chemically proved that in temperate zones (Europe [3, 5 – 8], North America [9, 10], and the non-tropical regions of Asia [11, 12]), the bud exudates of *Populus* species and their hybrids are the main source of bee glue. In the northern regions of Russia, birch buds (*Betula verrucosa*) play the same role [13]. More recently, it was found by chemical studies that in large regions of Brazil, *Baccharis dracunculifolia* could be regarded as one of the main propolis sources [14]. For obvious reasons, in this review we are going to discuss only data based on reliable chemical evidence and comparison of propolis samples with plant material.

It is important to note that there have been some attempts to identify source plants of bee glue, using pollen analysis of propolis samples [15]. In our opinion, this approach is misleading. While remains of vegetative structures may be given credit as consistent evidence for propolis origin, with pollen analysis it is impossible to know with certainty whether the presence of a specific pollen is the result of a visit aimed at collecting resin or just contamination of a material obtained by bees for other purposes [2]. Pollen analysis could be used for identification of geographic origin, but not plant origin of propolis.

When propolis origin is discussed, there is another question that always arises: do bees change chemically the substances they take from plants? Despite many claims in the literature (completely unsupported by proof), the answer is no. There is no chemical evidence whatsoever that bees' enzymes have performed any chemical changes in the compounds taken from plants and used for propolis production. This very fact makes it possible to track the collected material back to the source plant. There is a good reason for this fact: such a change does not seem necessary with respect to the function of propolis in the hive. The sticky materials used by bees are secreted by plants in order to protect their buds, young leaves, and wounded tissues from the elements of weather and from attack by bacteria, fungi, and viruses. Propolis has similar functions in the hive, and it is well known that it is the main chemical defense of bees against infections. Any chemical change would mean unnecessary energy expenses and be disadvantageous from an evolutionary point of view. This specific biological function of propolis precursors is valuable, not only to propolis users (bees and humans), but also to propolis researchers: bees have performed a preliminary "screening" and found, in the course of evolution, plant sources that posses pronounced antimicrobial and antioxidant properties. Human beings have only to identify the parent plants and study their bioactivity and active principles in detail.

Other aspects of the importance of the knowledge of propolis plant sources are the possibility of typification for the needs of standardization and quality control [16], and the fact that access to propolis is important for the health of the colony [17]. Data on propolis plant sources have been reviewed [18] and here we summarize the literature published after this date.

Plant sources of tropical propolis

Plant sources of propolis in South America: In the last 15 years, propolis from South America and especially from Brazil has become a subject of increasing commercial and scientific interest, because Brazil is the only tropical country that exports large amounts of propolis. There are several types of Brazilian propolis [19], but the most popular and abundant one is the so-called "green propolis". It has been suggested, because of the presence of specific taxonomic markers (C-prenylated cinnamic acids), that its source is the Asteraceaen shrub *Baccharis dracunculifolia*, locally called "alecrim" [20 – 22]. Later, direct evidence of the plant origin of green propolis from *B. dracunculifolia,* based on observation and filming of bee behavior and on chemical comparison of plant material and propolis, was provided in the works of Kumazawa et al. [23] and Weinstein Teixeira et al. [24]. They found that bees cut small parts of the vegetative apices of young leaves and buds of alecrim and manipulate (chew) them, liberating resinous substances from trichomes and ducts. Then, the material was transferred to the curbicula and carried to the hive for propolis production. In both articles, the authors registered coincidence of the chemical composition of plant material (young leaves or shoot apices) and propolis from the hive under observation. For chemical comparison, HPLC-MS and GC-MS (after methylation) were used. Weinstein Teixeira et al. [24] supported their findings also with microscopic analysis of propolis and identified leaf fragments of *B. dracunculifolia*: resiniferous ducts, glandular and non-glandular trichomes. This convincing and thorough approach should be followed by everyone who is willing to prove ultimately the origin of propolis from a particular source. It is interesting to note that Weinstein Teixeira et al. [24] emphasize the variability in chemical composition of green propolis, due to variations in the chemical composition of alecrim apices, and to incidental contributions from alternative plant sources.

As already mentioned, green or "alecrim" propolis is not the only propolis type in Brazil. Alencar et al. [25] and Park et al. [19] identified buds and unexpanded leaves of *Hyptis divaricata* as basic plant source of propolis in Bahia State, in the North-East of Brazil. They proved this by comparing the "fingerprint" of propolis with that of plant material from the same location, using GC-MS and HPLC-

DAD profiles. However, no individual taxonomic markers were identified.

In a recent publication, propolis from the Brazilian State of Piaui was found to contain cycloartane triterpenoids (mangiferolic, mangiferonic, and isomangiferonic acids, among others), discovered earlier as constituents of the stem bark of *Mangifera indica* [26]. This finding led to the suggestion that the stem bark of mango trees was the main plant source of this particular type of propolis.

Propolis studies in other regions of South America resulted in the disclosure of new source plants. Most important of them are the trees of the genus *Clusia* of the Guttiferae family. These dioeciuos plants secrete a viscous, hydrophobic resin from glandular tissues in both male and female flowers. This resin is readily gathered by meliponine and euglossine bees, for which it most often serves as the sole pollinator reward [27]. The flower resin possesses the mechanical properties needed as nest building material for stingless and orchid bees and also has proved to be antibacterial [27]. So it is not surprising that *Apis mellifera* bees have discovered this excellent propolis source and make use of it in tropical ecosystems. The *Clusia* flower resin contains a characteristic type of chemical compounds, polyisoprenylated benzophenones. These compounds are unique to the Guttiferae family and thus excellent taxonomic markers. Constituents of this type have been identified as main components in propolis from Cuba and Venezuela. Tomás-Barberán et al. [28] found that in Venezuela, *Clusia* was one of the main sources. The flower resin of two different species, *C. major* and *C. minor*, were analyzed by HPLC and compared to the propolis profile; a perfect match was shown. In a recent study, the chemical composition of several Venezuelan propolis samples indicated that *C. scrobiculata* may be their source, according to the particular benzophenones in it [29]. In Cuba, *C. rosea* (*C. major*) was suggested to be the main propolis source [30, 31], based on chemical comparison.

Recently, isoflavonoids have been found in propolis from Cuba and South-Eastern Brazil [32, 33] and an assumption has been made that some Leguminosae plants may contribute to propolis production in these regions [32]. However, caution should be used in accepting this hypothesis because it is known that a number of non-leguminous plants are able to biosynthesize isoflavonoids [34].

Chemical data have also suggested that in some regions of El Salvador, the Tolu balsam tree, *Myroxylon balsamum*, may be the source of bee glue [35].

Plant sources of propolis in Asia: In the temperate climatic regions of Asia, *Populus* species have been identified as a propolis source [18, 36, 37]. Recently, several chemical studies have been published dealing with Asian propolis from tropical and high altitude (Himalayas) locations that have a different chemical composition to that of poplar propolis.

In Nepalese bee glue, the main chemical components were identified as open-chain neoflavonoids [38]. The major constituents have earlier been found in the wood of *Dalbergia* species and are specific to this genus, thus the authors suggest that the source of this propolis might be plants belonging to the genus *Dalbergia*. Moreover, many plants of various *Dalbergia* species, for example Indian rosewood, *D. sissoo*, grew in the place where propolis was collected.

Studies concerning a new tropical type of propolis have appeared in the literature during the last 3 - 4 years. Information about this distinct propolis type comes from Okinawa [39] and Taiwan [40, 41] and it may be called “Pacific” propolis. The specific components for this bee glue are C-prenylated flavanones. Some of these compounds were new, but the known ones had previously been isolated from *Hernandia nimphaefolia*, a small tree of the tropical family Hernandiaceae [42]. This fact indicates *H. nimphaefolia* as the possible plant source, but further research is needed in order to confirm or deny this hypothesis. It is important to note that “Pacific” propolis possesses significant cytotoxicity and radical scavenging activity against DPPH radicals [39, 41], so the study of the presumed source plant seems promising.

Propolis plant sources in the Subtropics

In regions with subtropical climate, poplar trees are rare and bees have to find other plants from which to collect propolis. The diversity of plant sources is reflected by the chemical diversity of Mediterranean propolis from different locations. For example, in several samples from Sicily, the main constituents were found to be diterpenic acids of the labdane type (communic, isocupressic, acetylisocupressic) [43].

The same compounds have previously been found in Brazilian propolis and their source is suggested to be *Araucaria* spp. Their presence in a European sample, originating from a totally different ecosystem where no *Araucaria* grows, is surprising. However, it is important to note that the diterpenic acids of the labdane type are typical oleoresin components of representatives of the family Cupresaceae, to which *Araucaria* belongs. In the Mediterranean region, many Cupressaceous plants are widespread, such as *Cupressus sempervirens* (Italian cypress) and *Juniperus phoeniciana* (Phoenician juniper); both grow on Sicily. One of them might be the plant source of the analyzed samples, which should be the subject of further investigations.

Recently, several publications appeared on the chemical composition of Turkish propolis and it was demonstrated that a significant number of Turkish samples originated from *Populus nigra* [44, 45]. In some regions, however, the participation of some other plants has been suggested because of the presence of some specific taxonomic markers [46]. In a sample from Artvin (Eastern Anatolia), a series of glycerides of *p*-coumaric, ferulic and caffeic acid were identified. This chemical composition has been found to be characteristic of the bud exudate of *Populus euphratica*, one of the poplar species growing in Turkey [47]. Obviously, in the region of Artvin, *P. euphratica* was the main source plant for propolis collection. In another Turkish sample, originating from Southern Anatolia (Adana), together with the typical poplar phenolics, some diterpenic acids were present, which most probably do not come from poplar buds, and a mixed origin was proposed for this specimen [47]. The second source plant remained unknown.

Other subtropical samples of non-poplar origin, for which the source plant remains unknown, have recently been studied in Greece [48]. In these samples, some labdane diterpenes (including cupressic and isocupressic acids) and prenylated derivatives of pinocembrin and pinostrobin were identified. The presence of the diterpenes led the authors to the conclusion that Coniferae trees play a significant role as propolis sources. On the other hand, the presence of prenylpinocembrin and prenylpinobanksin, earlier identified in buds of *Platanus acerifolia*, raises the question of the participation of this plant in propolis of Greek origin.

Propolis plant sources in subarctic regions

The fact that in the northern parts of Russia, from the region where poplars do not grow, birch buds (*Betula verrucosa*) supply bees with resin for propolis production [13] has been known for over 30 years. However, other subarctic regions have only recently been studied in this respect. Propolis from Canadian boreal (Richmond) and Pacific coastal forests (Victoria) was investigated [49] that lay outside the area of distribution of *P. nigra*. In the sample from Victoria, *p*-hydroxyacetophenone, benzyl hydroxybenzoate and cinnamic acid were the major components, accompanied by significant amounts of dihydrochalcones, not characteristic of *P. nigra*, which allowed the identification of its plant source as *Populus trichocarpa*, Section *Tacamahaca*. The sample from Richmond was characterized by large amounts of *p*-coumaric and cinnamic acid, typical for poplars of Section *Leuce*, subsection *Trepidae*. The plant source of the Richmond sample was identified as *P. tremuloides*. Both samples showed good radical scavenging activity against DPPH. Obviously, Northern type propolis is a promising potential source of biologically active substances and deserves further investigation.

Concluding remarks

In the last years, the number of propolis plant sources, positively identified by chemical analysis, has grown rapidly. It is important to note that the ultimate proof that a particular plant is the source of bee glue should be a combination of meticulous and well documented bee behavior observations, and comparative chemical analysis of propolis and plant material, as demonstrated by the works of Kumazawa et al. [23] and Weinstein Teixeira et al. [24].

An important item connected to propolis origin is the leads provided to phytochemists by bees. Propolis research has resulted in the revealing of pharmacological properties of substances previously know as plant secondary metabolites, but never evaluated before, and to the discovery of many valuable plant substances that otherwise would hardly be found. Obviously, the search for plants used by bees for propolis collection in tropical, subtropical and even northern regions has the potential to uncover new biologically active compounds with important pharmacological effects. It is also of importance for better understanding of bee behavior and the interaction between bees and plants.

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